

# Life Science Journal

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# Life Science Journal

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## A Teaching Tool for Designing a Programmable System on Chip Using Hardware Description Language

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**Abstract:** This paper aims to design a simulator which is specifically used to design a programmable system on chip (PSOC) for increasing teaching quality and enhancing students' learning effects. The proposed teaching tool could effectively help students to learn MCS51 microcontroller's principle as well as several input/output peripheral applications in one semester. There are four functions provided by the simulator: (1) using PSOC hardware description language designing an application; (2) capable of simulating designed circuits; (3) allowing compiling the hardware description codes; and (4) capable of checking the data sheets of the MCS51 chip and I/O peripheral devices. The designed teaching tool based on Java programming language can support the characteristics of being easily understood and memorized, thereby enhancing students' interests and system integration concepts while taking the microcontroller principle and practice course.

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<http://www.lifesciencesite.com>.

**Keywords:** Teaching tool, and hardware description language.

### 1. Introduction

In the era of e-education, an institution has developed various learning education such as distance learning, on-line learning, and adult learning in their certificate programs, undergraduate programs, and graduate programs. Generally, distance learning is heavily based on skill development and sometimes requires laboratory works which need to interface with computers to facilitate a hands-on learning approach [1]; online learning allows students study at home or anywhere they like via computers and the Internet [2]; adult learning is typically for specific age group above the legal age limits, which is related to their daily life, goals, and career needs [3]. Of all these types of learning education, if the learners did not learn the material well initially, they will not retain it well. Therefore, the need of increasing the availability of particular educational resources in various parts of curriculum materials such as motivational training materials for learners is with great importance to help learners retaining what the program delivers to them. In order to achieve this goal, Information technology (IT) in learning education plays an increasingly central role in institutions; specifically, it is placing new demands on expertise [4].

With the rapid developed automated industry in microcontroller related products, developing the computer simulation software becomes more important and gets more attention in recent year because a simulator can provide users to test the

product before making a practical product, thereby cutting the wrong probability. Hence, microcontroller control is one of the mainstream subjects for people developing an automation product in the field of Electrical Engineering, Mechanical Engineering, Computer Science, and Information Technology. In an educational institution, when a professor teaches microcomputer related course, he/she would introduce its main principle and applications in order to unite the theory and the practice together and increase students designing a practical product capability effectively. Currently, Keil 8051 development tool is nowadays the most popular software teaching tool applied in the microcontroller related course, which allows users to use either assembly or C programming language to make a specific control system.

In the reviewing teaching tool related technology, Davies [5] investigated the opinions on using the simulator as teaching tool in the class for the students majoring in Materials Engineering. After interviewing some students with 2000 questionnaire's survey results, the conclusions are the operation process of the existing simulation software is too complex to understand as well as the students don't have enough time practicing the simulation. References [6-8] developed simulators as teaching tools which can be applied on the Internet. Yang and Altj [6] developed a distribution simulator using the Internet as the transmission media, which allows users to transmit information to end-learner via the

Internet and remote control the end-learner's learning progress. This simulator supports the advantage of learning everywhere. Huang [7] developed an interactive teaching platform for students majoring in psychology. The developed platform named PHYSIO has the advantage for students studying on-line. Esquembre [8], an engineering department teacher, developed a graph-base with simple interface simulator as the teaching tool. Literatures [9-11] are the references using JAVA programming language to build a software simulator. Whaley [9] proposed a virtual machine named Joeq which is specifically designed for scholars working on their research. The Joeq system mainly contains four kinds of virtual simulations, including automatic control distribution, time interval calculation, filtering trash information, and compiler. Kreuzinger et al. [10] developed a special virtual microcontroller named Komodo which is mainly designed for the on-line services of multithreading and multiplexing processes which replace the subroutine functions of the interrupt services. Sánchez and Manzoni [11] developed the ad hoc wireless network simulator, named ANEJOS which is compatible with any router. Moreover, Khare et al. [12] developed a simulator named V-ST to analyze and evaluate the system chip hardware structure. Murray [13] proposed a medical consulting and decision making teaching tool.

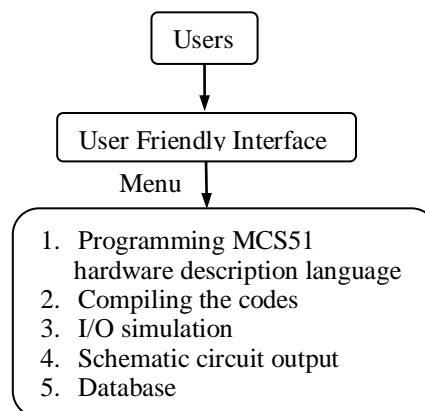
MCS51 microcontroller is one of the most popular single chips taught in an academic in Taiwan. It allows users to use complex set of assembly instructions or C high level programming language to make some peripheral control applications, including LED, DIP Switch, Timer, interrupt, spot matrix, LCM, motor and UART communication. However, there are two drawbacks existing in the current software teaching tool. They are: (1) the syntax of codes provided in Keil without hardware structure characteristics is hard for students to learn well and remember easily; students are therefore lack of a picture of designed control system and brainstorm stimulation on developing a practical product and (2) one semester generally covers only fundamental principle and software simulation, which lacks the coordination of the theory and practice, thereby decreasing students' interest in learning microcontroller applications.

In order to overcome two weaknesses described above, this paper refers to the advantages of microcontroller-based teaching tools [14-16] and develops a simulator which is specifically used to design a programmable system on chip (PSOC) for increasing teaching quality and enhancing students' learning effects. The proposed teaching tool could effectively help students to learn MCS51 microcontroller's principle as well as several

input/output peripheral applications in one semester. There are four functions provided by the simulator, including using PSOC hardware description language designing an application, simulating designed circuits, compiling the hardware description codes, and checking the data sheets of the MCS51 chip and I/O peripheral devices. The designed teaching tool designed by Java programming language supports users with easy understanding and memorizing and enhances their interests while taking the microcontroller principle and practice course.

## 2. MCS51 Teaching Simulation Tool

Figure 1 illustrates the structure of the developed MCS51 teaching simulation tool. A user-friendly interface is built between the user and the menu of the proposed teaching tool. As given in Figure 1, there are five functions provided in the system. They are programming MCS51 hardware description language, compiling the codes, I/O simulation, schematic circuit output, and database. The roles of each unit in the menu are described as follows.



**Figure 1.** Structure of developed MCS51 teaching tool.

### 2.1. Programming MCS51 Hardware Description Language

The proposed MCS51 hardware description language (MCS51 HDL) refers to the syntax of current Very High Speed Integrated Circuit (VHSIC) Hardware Description Language (VHDL) and C programming language. The syntax of MCS51 HDL contains mainly three types of instruction sets as well as some reserved words such as int, print, input, output and so on. One instruction set is related to the mathematic operations, including addition, subtraction, multiplication, division. Another instruction set is related to logical operations, including And, OR, NAND, NOR, XOR, and so on. Loop control is another type of the instruction set, which contains if, if-else, switch-case, for, while, and so on.

Figure 2(a) illustrates the structure of MCS51 HDL program, which basically includes five parts: initialization, input device, output device, hardware pin configuration, and main codes. Initialization defines the fundamental pin configuration to activate an MCS51 chip. As shown in Figure 2(a), pin 9, 12, 19, 20, 31, 40 are the fundamental pin configuration to activate an MCS51. Input and output (I/O) device define what kinds of I/O peripheral devices being used in the application. In this study, fundamental peripheral devices are such as LED, 7-segment buzzer, DIP switch, push button, and 4X4 keypad. Hardware defines the pin configuration of the application project. Main codes define the functions of the application. Figure 2(b) shows an example of MCS51 HDL program, which only uses 8-LED output devices. The example runs 8-LED left shift and right shift, where  $i=1$  and  $i=0x40$  define two different initial conditions.

**2.2. Compiling the Codes**

Figure 3 illustrates the process of compiling MCS51 HDL codes, which includes two passes. One pass called pass 1 aims to check the syntax of MCS51 HDL codes, which will output the location and the name of the instruction if the syntax of the instruction is wrong. The other pass called pass2 aims to generate corresponding machine code (Hex file) for users downloading the MCS51 HDL codes on to the chip.

```

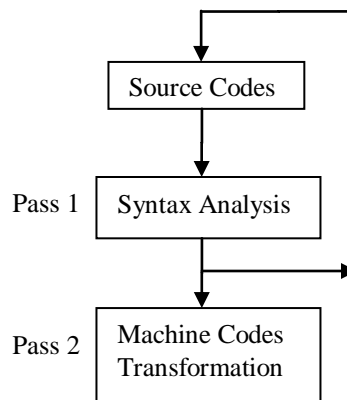
BEGIN
{
  DelayX1ms ( unsigned int ) ;
  int i , loop ;
  be(1)
  {
    i=1 ;
    rrr(loop=0;loop<8;loop++)
    {
      P0 = ~i ;
      DelayX1ms(5000) ;
    }
    i = i << 1 ;
  }
  i=0x40 ;
  rrr(loop=0;loop<7;loop++)
  {
    P0 = ~i ;
    DelayX1ms(5000) ;
    i = i >> 1 ;
  }
}
    
```

(b)

**Figure 2.** (a)Structure of MCS51 HDL program; (b) example of MCS51 HDL program.

<pre> Initialization {   PIN40 VCC(+5)   PIN31 VCC(+5)   PIN20 VSS(GND)   PIN18-19 HZ   PIN9 RESET } Input Device { } Output Device { } Hardware { } BEGIN { }                 </pre>	<pre> Initialization {   PIN40 VCC(+5)   PIN31 VCC(+5)   PIN20 VSS(GND)   PIN18-19 HZ   PIN9 RESET } Input Device { } Output Device {   LED:0..7 ; } Hardware { } BEGIN {   P0[0,7] &lt;= LED[0,7] ; }                 </pre>
---	---

(a)



**Figure 3.** Process of compiling MCS51 HDL.

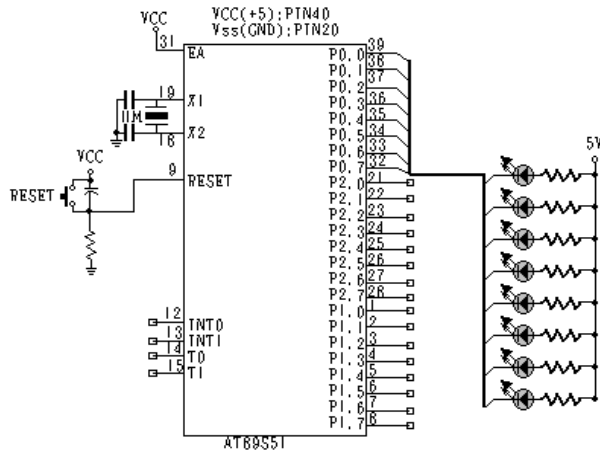
**2.3. I/O Simulation**

A user is allowed to define the input signal on specific pin or port; the system runs MCS51 HDL codes and outputs the results on the corresponding port.



**2.4. Schematic Circuit Output**

The system runs MCS51 HDL codes and outputs the corresponding schematic circuit for users having a picture of designed control system. Figure 4 is the schematic circuit output of MCS51 HDL example given in Figure 2(b).



**Figure 4.** Schematic circuit output of MCS51 HDL example given in Figure 2(b).

**2.5. Database**

The system provides MCS51 datasheet, fundamental peripheral I/O devices' specifications, including LED, 7-segment, buzzer, switch, keypads etc., and the syntax of MCS51 programming codes. The datasheets of the mathematical operation, logical operation, loop control and reserved word instruction sets are listed in Table 1 to Table 4, respectively.

**Table 1.** Mathematical operation instruction sets

Sym	Function	Ex.	Explanation
+	Addition	a+b	If a=4,b=2 then a+b=6
-	Subtraction	a-b	If a=4,b=2 then a-b=2
*	Multipli.	a*b	If a=4,b=2 then a*b=8
/	Division	a/b	If a=4,b=2 then a/b=2
%	Modulation	a%b	If a=4,b=2 then a%b=0
+1	Increase	a+1	If a=4, after a+1, a=5
-1	Decrease	a-1	If a=4, after a-1, a= 3

**Table 2.** Logical operation instruction sets

Symbol	Function	Example
&	AND	F=A&B
#	OR	F= A # B
!	NOT	F = A' = !A
&'	NAND	F = (A & B)' = A&' B
#'	NOR	F = (A # B)' = A#' B

**Table 3.** Loop control instruction sets

Symbol	Function
if(){}	if
if(){ }else{ }	if-else
switch(){case: }	switch-case
for(){ }	for(){ }
while(){ }	while(){ }
do{ } while()	do{ } while()

Symbol	Example
if(){}	if(A==0){B=0;}
if(){ }else{ }	if(A==0){B=0;}else{C=0;}
switch(){case: }	switch(i){case1:out='A';break;
for(){ }	for(i=0;i<256;i++){A=i}
while(){ }	while(i<10){A=i;i++;}
do{ } while()	do{ A=i;i++; }while(i<10);B=33;

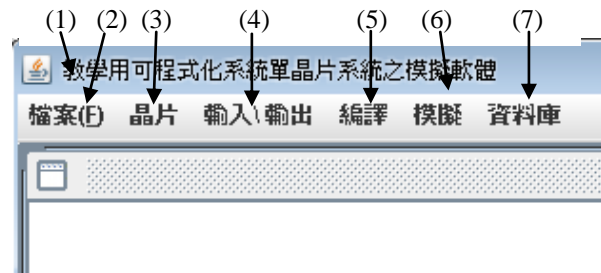
**Table 4.** Reserved words.

Symbol	Explanation
IN:	Input
OUT:	Output
Title:	Title
Value:	Define Value of Device

**3. Experimental Results**

The system is implemented by using JAVA programming language. A user can free download the software via <http://www.java.com/en/download/index.jsp> webpage. Figure 5 shows the interface of the proposed teaching simulation tool, which includes seven items: File, Chip device, Input/Output devices, Compile, Simulation, and Database interface functions. Each function of Figure 5 is introduced as follows

- (1) The title of the proposed teaching simulation tool.



**Figure 5.** Interface of proposed teaching simulation tool.

- (2) File: Including creating new file, open a file, save file, and exit four sub-functions as shown in Figure 6.

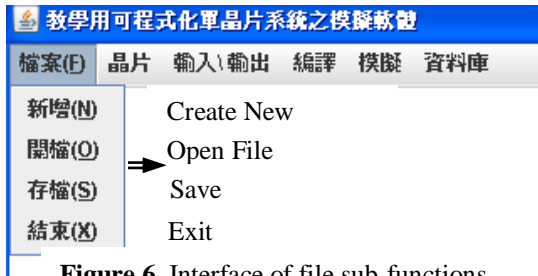
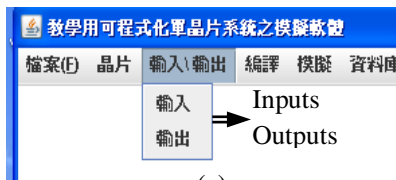


Figure 6. Interface of file sub-functions.

- (3) Chip: It means MCS51 microchip. The contents of this icon illustrate the basic circuitry connections for an MCS51 chip in the working condition.
- (4) Input/Output: It shows input/output devices. As shown in Figure 7, the Inputs devices include push button (SW), 8-pin dip switch (DIP8), 4-pin dip switch (DIP4), and 4 by 4 keyboard. The Outputs devices include color red, green, and blue LEDs, 7 segment display (7SE), and buzzer (SP). A user can check the specifications of input/output devices by clicking the icon on the bottom of the sub-functions.



(a)

符號	說明
SW	按鈕開關
DIP8	8支腳指撥開關
DIP4	4支腳指撥開關
KB4*4	4*4數字鍵盤

(b)

符號	說明
LEDR	紅色LED
LEDG	綠色LED
LEDB	藍色LED
7SE	七段顯示器
SP	蜂鳴器

(c)

Figure 7. Interface of input/output sub-functions.

- (5) Compile: Compiles MCS51 HDL codes.
- (6) Simulation: The system runs MCS51 HDL codes and outputs the results on the corresponding I/O port. Figure 8 gives the example of parallel port 1 status.

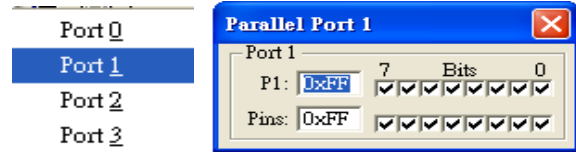
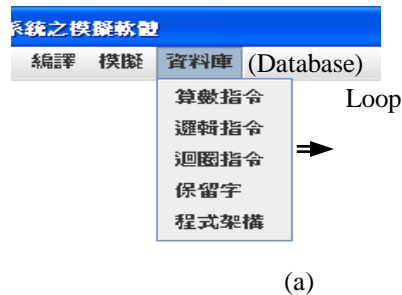


Figure 8. Parallel port 1 status.

- (7) Database: This function includes the introduction of three instruction sets (mathematical logical and loop control operations), reserved words, and program structure, as illustrated in Figure 9. A user can check the specifications of input/output devices by clicking the icon on the bottom of the sub-functions.



(a)

運算元	符號	範例	
加法	ADD	+	F = A + B
減法	SUB	-	F = A - B
乘法	MUL	*	F = A * B
除法	DIV	/	F = A / B
遞增	++	+1	F = A + 1
遞減	--	-1	F = A - 1

(b)

運算元	符號	範例
AND	&	F = A & B
OR	#	F = A # B
NOT	!	F = A' = !A
NAND	&'	F = (A & B)' = A&'B
NOR	#'	F = (A # B)' = A#'B
XOR	##	F = A ## B = A \$ B

(c)

Figure 9. Interface of mathematical and logical Operations of database Sub-functions.

#### 4. Conclusions

This study proposes a simulator which is specifically used to design a programmable system on chip (PSOC) for increasing teaching quality and enhancing students' learning effects. The main contribution of the proposed teaching tool is to demonstrate the advantages of ahead-of-time compilation design concept on developing an automatic control system. Compared to the existing method, the proposed system supports three distinctive advantages. (1) The proposed teaching tool reduces the testing time for developing an application project, thereby enhancing the efficiency of circuit design. (2) The students have another choice using hardware structure based programming language to design their applications, which has characteristics of easy to learn and to remember. This advantage may have them brainstorm stimulation on developing a practical product and cultivate their competition ability. (3) The system supports the output schematic circuit function, which increases the coordination of the theory and practice, thereby increasing students' interest in learning microcontroller applications.

This work can be extended to build a virtual microcontroller laboratory by using the Internet technology for various learning education such as distance learning, on-line learning, and adult learning in their certificate programs, undergraduate programs, and graduate programs.

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12/30/2011

## Constructing Microcontroller-Based 3 by 3 Football Team Sets As an Educational Tool

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**Abstract:** The objective of this paper is to construct microcontroller-based 3 by 3 football team sets for students to learn the knowledge of information and wireless communication technology in microcomputer related class. The constructed microcontroller-based 3 by 3 football team sets includes four parts: a mechanical set, a main control board, a motor driver board, and wireless control module. The mechanical set with two wheels is used as the feet of a robot. The main control board with 89S51  $\mu$ -controller chip is used to control the movement of the robot, such as going forward/back and turning left/right. The motor driver board is used to drive two wheels on the mechanical set. The wireless control module is used to remote control of a robot. Some investigations and evaluations have been done when the students finished the course which adopted the proposed microcontroller-based football team sets as the educational tool. Conclusively, the results indicate that applying the constructed teaching sets in an educational institute has had very favorable feedbacks from the students. The proposed tool sets can attract more students interesting in designing microcontroller-based systems and influence them in preparation for their laboratory project. [Sung-Tsun Shih, Chin-Ming Hsu, Chian-Yi Chao. **Constructing Microcontroller-Based 3 by 3 Football Team Sets As an Educational Tool**. Life Science Journal. 2012; 9(2):7-13] (ISSN: 1097-8135). <http://www.lifesciencesite.com>.

**Keywords:** Microcontroller, adult education, hardware, software.

### 1. Introduction

Adult learning is specifically designed for above the legal age group, which is different from other learning education such as e-learning and distance learning. For effective teaching adult students in an institution, a teacher generally needs to know how they can learn well. As Abedi and Badragheh [1] stated that adult education needs to account for motivation of the learner, reinforcement of the skill, retention of key learning, and transference of what is learn of new situations. In advance, Badragheh and Abedi [2] have also suggested that the ways to strengthen adult education are such as offering a variety of formats, schedules, and approaches, meeting people where they are, promoting participation effectively, and fostering strong leadership. Based on these teaching principles, this study tries to use microcontroller-based 3 by 3 football team sets as an educational tool to let the learners retain the knowledge of information and wireless communication technology in microcomputer related class.

Microcontrollers, programmable devices such as Intel 8951, Motorola 6811, Microchip PIC 16C57, Texas Instruments TMS1000, and Zilog Z80180, have ROM, RAM, ALU, and I/O functions inside the single ship with the advantages of small volume, easy use, and good extendibility. Using these programmable devices for hardware control would be able to simplify system design and implementation.

Therefore, they are globally applied in the electromechanical related products, including automatic washers, microwave ovens, swing control, cameras, digital watches, cellular phone and so on [3]. Currently,  $\mu$ -controller education is one of the most mainstream subjects in the fields of Computer, Electronic, Electrical, and Mechanical Engineering. Specifically in Taiwan, many universities view the programmable devices as necessary education for present and future engineers. For example, at Kao Yuan University (KYU), it is a requirement in Electronic/Electrical Engineering curriculums and an elective in Information Technology and Auto-Mechanical Engineering curriculums. The ultimate teaching goal is to train the students using a single chip to design a practical product. However, with the rapid growth of computer, electronic, and information technology in industries, most current related references and tools used for the  $\mu$ -controller education are not good enough to achieve the teaching goal. Hence, this paper aims to construct a set of teaching tool for students to learn the knowledge of  $\mu$ -controller system designing and debugging techniques.

In the past decade, many universities, such as Uni. of South Carolina, USA [4], Uni. of Penn State, USA [5], Uni. of Zaragoza, Spanish [6], Lunghwa Uni., Taiwan [7], etc., have shared their teaching experience on  $\mu$ -controller related courses. At Uni. of South Carolina and Penn State, the  $\mu$ -

controller courses used the available  $\mu$ -controller evaluation board (EVB), Motorola MC68HC11 EVB, with LCD, keypad, a motor and digital thermometer interfaces for Mechanical Engineering and Electrical Engineering students, respectively. At Lunghwa Uni., it used the published book and the developed Intel 89S51 teaching module board with the integrated development environment software for both Computer Science and Electronic Engineering students. However, using such commercial developed integrated modules as the teaching tool has one disadvantage - The tool is like a black box and it is too expensive for a student to buy it; therefore, students need to rely on laboratory facilities for their works and cannot do the experiments at home. As for Uni. of Zaragoza, it only introduced the software tool of MC68HC11 in the  $\mu$ -controller course. In the reviewing some other references, Gault and Snyder [8] put much effort on solving programming problems. Athani [9], Bray etc. [10], and Freedman etc. [11] indicated that two-semester  $\mu$ -controller courses are needed for students to learn well a system design of hardware and software. One semester is for introductory course with conceptual design goals; the other one semester is for project-oriented course with more hands-on experiences. Furman and Hayward [12], Jeon [13], and Lee [14] also strongly suggested that the  $\mu$ -controller course should emphasize more hands-on and use relatively inexpensive kits of components as teaching tools.

Because of many kinds of  $\mu$ -controllers available in the market, how to choose the "right"  $\mu$ -controller for students learning well is an important issue for teachers [3, 15]. Al-Dhaher [3] pointed out that setting the "right"  $\mu$ -controller teaching tool is a critical decision because it may have impacts on students' reactions to the course. Gupta and Moi-Tin [15] also indicated that the "right"  $\mu$ -controller means the teacher needs to consider some factors, including popularity, availability, architecture, features, prices, tools, education support, easy use, and so on. Specifically, Schultz [16] suggested that teachers might consider 8051 or 68HC11  $\mu$ -controller as a teaching target because they are well supported and have matured to being readily available and reasonable alternative to the expensive high performance processors.

In the reviewing current 8051  $\mu$ -controller related books [17-21], three deficiencies exist: (1) some books cover only fundamental principle and software simulation, which lacks the coordination of the theory and practice, thereby decreasing students' interest in learning  $\mu$ -controller advanced applications; (2) it is impossible for students doing the experiments at home because of without covering the flash downloading hardware/software tools; and

(3) most books are lack of project-based examples, thereby decreasing the brainstorm stimulation on developing a practical product.

In order to overcome the weaknesses described above and achieve the goal of using  $\mu$ -controller to develop a practical product, the hardware and software must be integrated efficiently because  $\mu$ -controller applications are heterogeneity and involve many different programming needs and hardware interfaces. In this paper, the authors propose an Intel 89S51 based teaching tool with the capabilities of understanding its basic principle and software simulation; allowing students to do the experiments at home; and using a remote controlled vehicle system to clarify  $\mu$ -controllers' extendibility. At EE department of KYU, the  $\mu$ -controller courses have been offered as one of requirements for junior students and one of electives for senior students. In the proposed teaching tool, it is recommended that all students can build a simple 8951 I/O board in order to carry out the hardware/software of assignments at home rather than rely on three working laboratory hours per week. In the following, Section 2 describes the proposed Intel 8951  $\mu$ -controller teaching tool. The observations and students' reactions are shown in Section 3. Finally, the conclusions and future works are summarized in Section 4.

## 2. Microcontroller-Based Football Team Sets

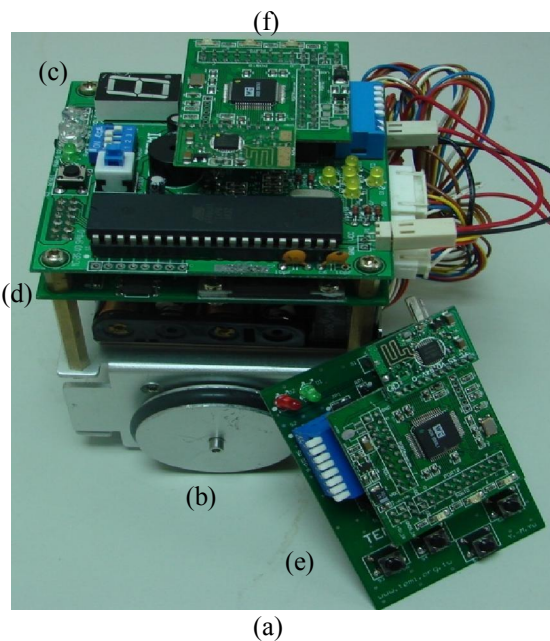
Table 1 gives the syllabus of the microcomputer course. The lectures cover three parts: (1) Introduction to microcontroller-based robot set; (2) Assemble and de-assemble a microcontroller robot set; and (3) Demonstration of the 3 by 3 football team sets, which are described in the following.

Table 1. Syllabus of microcontroller course

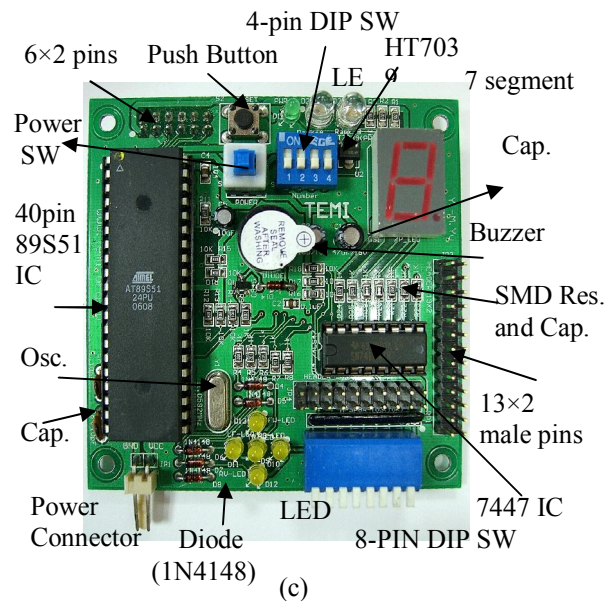
	Week	Course contents
I	1	Intro.to AT89S51 Microcontroller
	2	Intro. to Main Controller Moard
	3	Intro. to Microcontroller Interface
	4	Intro. to Motor Driver Board
	5	Intro. to Mechanical Vehicle Set
	6	Assignments
II	7	Assemble Mechanical Vehicle Set
	8	De-assemble Mechanical Vehicle Set
	9	Assemble a robot system
	10	De-assemble a robot system
III	11	Midterm
	12	Intro. to 3 by 3 Football Game
	13	Intro. to 3 by 3 Football Team Sets
	14	Competition
	15	Competition
	16	Final Examination

### 2.1. Microcontroller-Based Robot Set

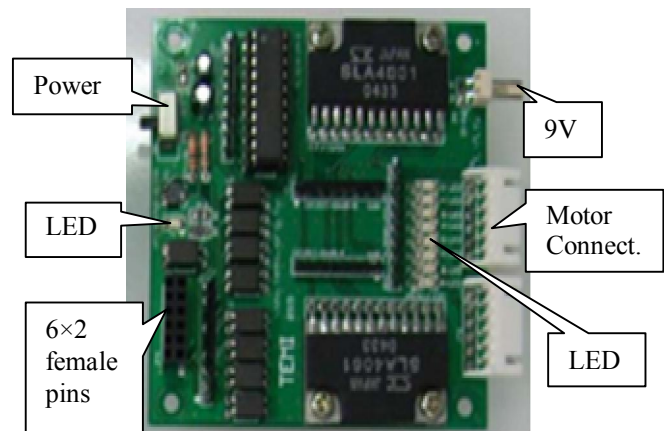
The microcontroller-based robot set, as given in Figure 1(a), includes five parts: mechanical device (b), main control board (c), motor driver board (d), remote control board (e), and 2.4 G Hz RF wireless control module (f). All their detail materials and circuitry schematics can be found at Website of Taiwan Embedded Microcontroller Development Institution (TEMI) [22]. As shown in Figure 1, the mechanical device uses two motors to trigger two wheels for controlling the robot's movement. The main control board including 40-pin AT89S51 IC, power switch, 6x2 male pins, push button switch, 4-pin DIP switch, LED, HT7039 IC, 7-segment display, capacitors, SMD resistors, buzzer, 2x10 male pins, 13x2 male pins, 7447 IC, 8-pin DIP switch, five LEDs for the indication of remote sensing, 1N4148, power connector, and oscillator. The 10x2 and 13x2 male pins are connected to the 2.4 G Hz RF wireless control modules. The main parts of the motor driver board includes two Darlington ICs, two motor connectors, power switch, power connector, LED, and 6x2 female pin connector which is connected to 6x2 male pins of the main control board. As for the remote control board, it includes power switch, power connector, 10x2 male pin connector, 13x2 male pin connector, power light, 8-pin DIP switch, and five push button switches for controlling the movement of the vehicle device. The 10x2 male pin and 13x2 male pin connectors are connected to the 2.4 G Hz RF wireless control modules.



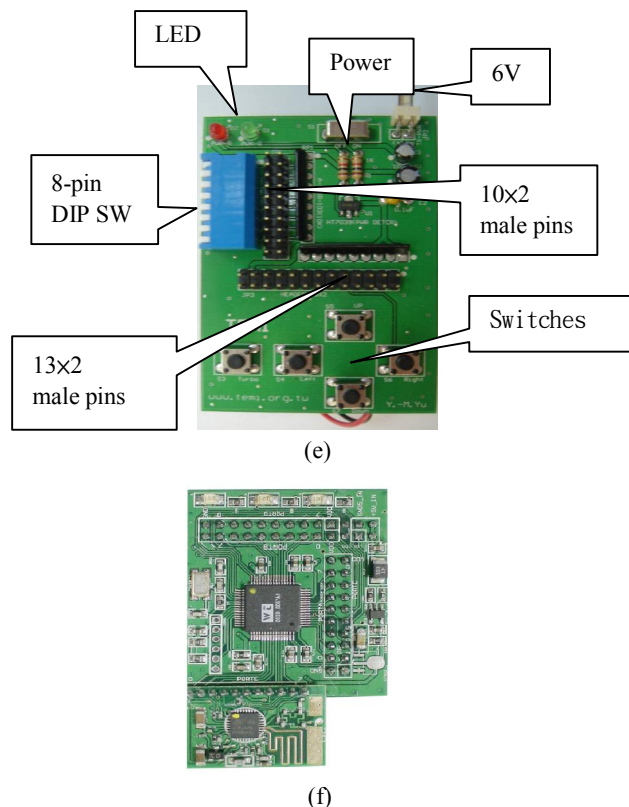
(b)



(c)



(d)



**Figure 1.** (a) Microcontroller robot set; (b) mechanical vehicle device; (c) main control board; (d) motor driver board; (e) remote control board; (f) 2.4 G Hz RF wireless control module.

The following gives the software control procedures in which the programming codes are downloaded into AT89S51 chip for controlling the hardware. The main functions of the software includes controlling the vehicle to go forward and back, turn left and right, and speed up the movement.

Step 1: Main board power detection, if power is “low”, buzzer on, otherwise off.

Step 2: Stepping motor power detection, if power is “low”, buzzer on, otherwise off.

Step 3: Enable external interrupts -- EX0,EX1; INTO

Step 4: Judge the channel set, if it is right, go to step 1, otherwise, go to next step.

Step 5: Judge the push-button pressed

Step 6: Driving motor movement: including forward, back, left turn, right turn... and so on.

Step 7: Go to step 1.

## 2.2. Assemble a Microcontroller-Based Robot Set

Figure 2(a) shows the box of the mechanical set; Figure 2(b) gives the assembled mechanical device and tools. Figure 3 illustrates the procedures of assembling a complete microcontroller-based robot set, as give in Figure 1(a).

Step 1: Fix the step motor in the motor stand base.

First, take out two step motors, the motor stand base eight round-head screws from the mechanical box. Then use eight round-head screws to lock two step motor on the motor stand base, as shown in Figure 3(a) and (b).

Step 2: Fix the tire and the wheel on motor's rotating axes.

First, take out two wheels and tires from the mechanical box. Then, use the screw driver to lock the wheels on motors' axes. Finally, put the tires on the wheel rims, as shown in Figure 3(c), (d), and (e).

Step 3: Finish the mechanical set.

First, take out the battery sustain base and a set of screws and copper columns from the mechanical box. Then, lock the battery sustain base and four copper columns on the motor stand base, as given in Figure 3 (f) and (g).

Step 4: Complete a microcontroller-based robot set.

Figure 3 (j) illustrates the backside of the assembled robot set. After finishing the assemble of a mechanical device, a user need to attached the motor driver board, main control board, and wireless control module on the top of the mechanical device, as shown in Figure (h) and (i). In order to have the assembled robot set work correctly, a user need to prepare one 9V and four 1.5V batteries and stuck them on the battery stand base. Besides, a user has to connect the power transmission lines on motor driver board and main control board, respectively, and connect the transmission lines among the motors, motor driver board, and main control board. As shown in Figure 3 (j), two binding strips are used to fasten the transmission lines on the copper column together.

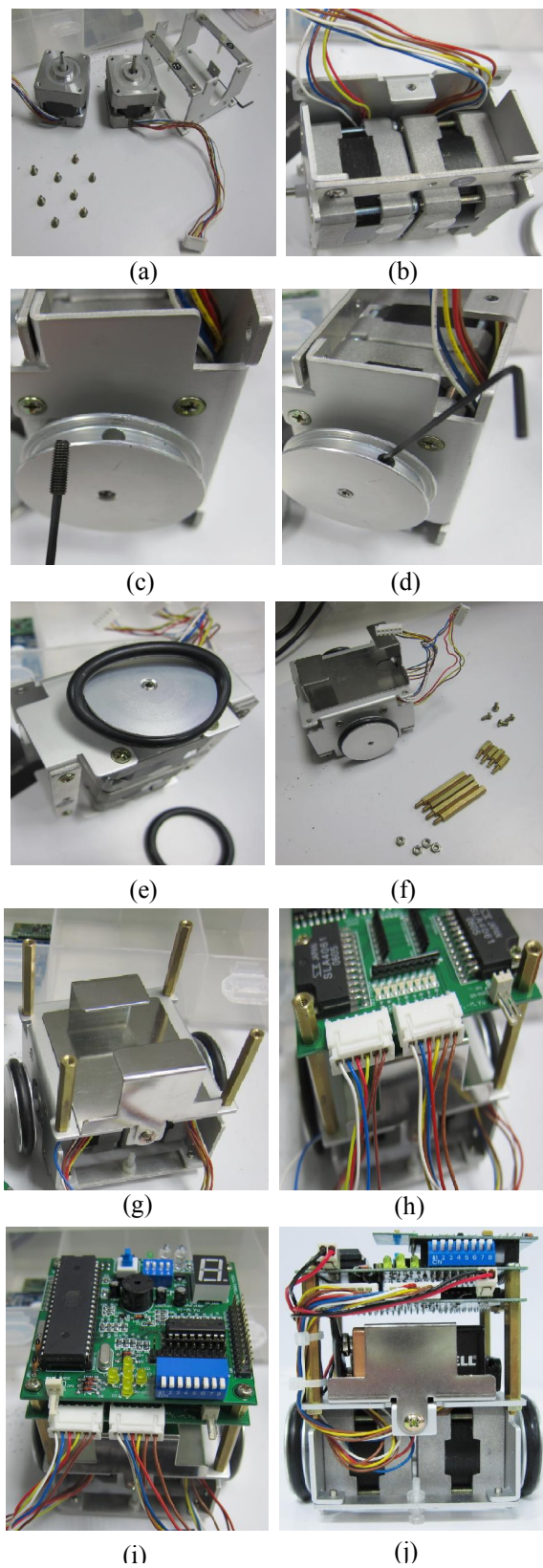


(a)



(b)

**Figure 2.** (a) The box of mechanical set; (b) assembled mechanical device and tools.



**Figure 3.** Procedures of assembling a microcontroller-base robot set.

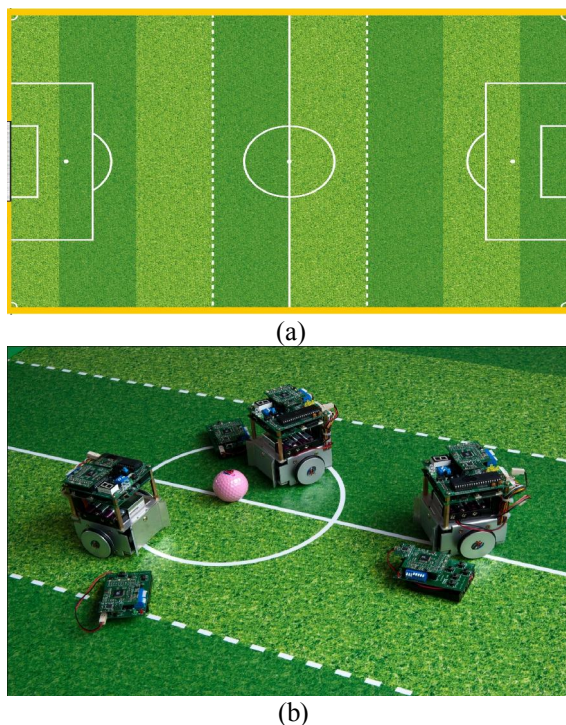
### 3. Experimental Results

At EE department of KYU, there are about 30 sets of microcontroller-based robot facilities provided in the  $\mu$ -controller laboratory for 50 students to debug and run their robot sets. Therefore, a team with two students is issued a developed robot kit with debugging facility. Up to now, the constructed teaching tool has been applied in the course for four years, which trains the students with the debugging techniques and the hardware/software integration technology. Currently, the microcontroller-based robot set is used to examine students' capability on designing  $\mu$ -controller-based project. The students are required to build the hardware, including soldering each part of circuits and assembling all parts of the system, program control codes, and measure the control signals. If a student passes all the tests, he or she can obtain the microcontroller certificate issued by TEMI which is recognized by Taiwan government and local industries.

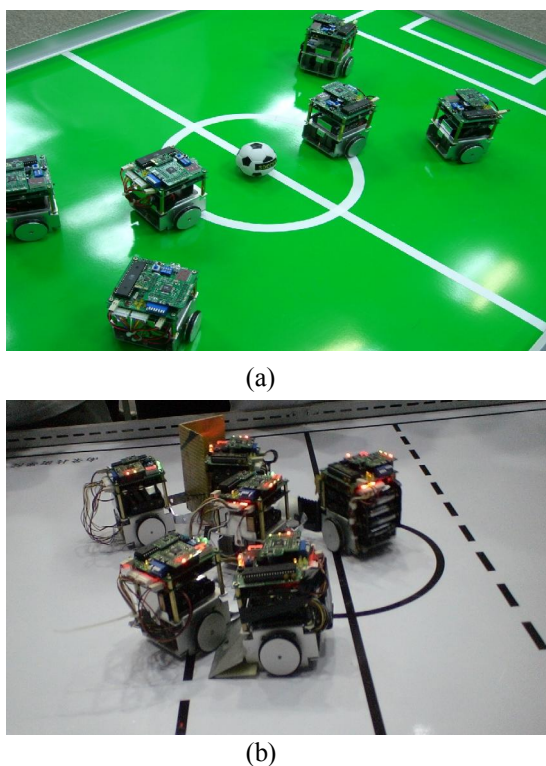
Figure 4(a) illustrates the land of the football game, where the length of the field is 2 meters; the width of the field is 1.15 meters. Figure 4(b) shows a typical 3 by 3 microcontroller-based football team set, where the golf ball is used as the football. Figure 5(a) demonstrates the competition scenario of two 3 by 3 football teams at their initial locations, respectively; Figure 5(b) shows the competition scenario of two football teams with the body appearance changed and the team-number set via the 7-segment different number displays and LED different color displays. In the football game, the time limit for each half-round is 3 minutes. A team wins the game when it kicks more balls into the football gate.

Some investigations have been done when the students finished the course. About 90% of 50 students construct their own I/O control board personally; about 80% of the students successfully complete all assignments, midterm exam, and final exam; about 60% of the students choose the  $\mu$ -controller as their project design topic for their senior laboratory project course. In addition, about 70% of 30 students got the microcontroller certificate issued by Taiwan Embedded Microcontroller Development Institution (TEMI) which is recognized by Taiwan government and local industries. In addition, about 90% of 50 students would like to participate 3 by 3 football game competition. From the students' evaluation of the  $\mu$ -controller course, about 70% of 50 students appreciated the knowledge of hardware/software integration and debugging techniques. Conclusively, these results indicate that applying the constructed 3 by 3 microcontroller-base football team sets as the teaching tool in the  $\mu$ -controller course has had very favorable feedbacks from the students.





**Figure 4.** (a) The land of the football game;  
(b) 3 by 3 microcontroller-based football team set.



**Figure 5.** (a) Competition of two football team sets;  
(b) changing outlook of the football team sets.

#### 4. Conclusions

In this paper, a set of the microcontroller – based 3 by 3 football team sets has been constructed to train students to learn the information technology and communication technology skills effectively. The proposed teaching tool is different from the traditional courses in three aspects: (1) students not only learn the essential contents of  $\mu$ -controller skills but also know the method to design a project by integrating hardware and software of the microcontroller; (2) students have brainstorm training opportunity on microcontroller system design; (3) students are allowed to construct their own robots personally, thereby increasing active participating opportunity. According to the students' feedbacks described in Section 3, using the constructed teaching tool attracts more students interesting in designing the microcontroller-based applications and influences them in preparation for the laboratory project which involves planning, designing and implementing solutions to the project.

This work can be extended to focus on using the microcontroller in various applications and building the interface circuits in modules to increase students' competitions in the real world.

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## Recursive Least Square (RLS) Based Channel Estimation for MIMO-OFDM System

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**Abstract:** Channel State information can be determined by adaptive filtering algorithms for wireless channels. For slow fading channels, simplified channel estimators can be exploited such as Least Square Error (LSE) and Linear Minimum Mean Square Error (LMMSE). But for fast fading channels, the matrix inversion required in case of LMMSE has to be taken recursively which increase the complexity. Under such conditions adaptive filtering algorithms are used to reduce the complexity with better performance. LMS, RLS and Kalman Filtering techniques can be used. But in wireless MIMO channels normally RLS and Kalman Filter are used at the cost of more complexity as compared to LMS which has better computational efficiency and feasibility. For initialization of adaptive filter, the channel can be estimated by LSE or LMMSE initially. In this paper the performance of RLS for both initially estimated LSE and LMMSE channel is compared in terms of Mean Square Error (MSE) and complexity is evaluated in terms of computational time. Optimization of LSE-RLS and LMMSE-RLS is performed as a function of wireless channel taps and Channel Impulse Response (CIR) samples. Monte-Carlo Simulations are carried for RLS channel estimation algorithm.

[Saqib Saleem, Qamar-ul-Islam. Recursive Least Square (RLS) Based Channel Estimation for MIMO-OFDM System. Life Science Journal 2012;9(2);14-19]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 3

**Keywords:** RLS, LMS, MIMO-OFDM, CIR Samples, Channel Taps, LSE, LMMSE

### 1. Introduction

For high performance 4G wireless broadband internet and multimedia services, while providing a comparable quality of service (QoS) to that of existing wire line services, Multiple antennas has been proposed at both Base Transceiver Station (BTS) and subscriber ends. Multiple antennas are used for provision of high range of coverage in NLOS channel conditions (> 90% of the users in the given cell), data transmission with high reliability of 99.9%, high peak data rate for both UL and DL of greater than 1 Mb/s and high spectral efficiency of greater than 4 b/s/Hz/Sector [1]. These system requirements can be achieved by combining MIMO technology with Orthogonal Frequency Division Multiplexing (OFDM) modulation. For high data rates and high delay spreads of time-varying frequency selective channel, OFDM is preferred over single carrier modulation scheme with the advantage of low-complex adaptive equalizers [1].

Since the channel is time-varying, so the transmitter and receiver needs the channel statistics for optimization of the system parameters such as modulation and coding, signal bandwidth, transmission power, channel estimation etc. Channel can be estimated either in time-domain or frequency domain, which can be further categorized into pilot-assisted or decision directed channel estimation. Under fast time-varying channel, a lots of pilots need to be inserted for better channel estimation which comes at the cost of decreased system efficiency [2]. But these pilots can be used for determination of

timing and frequency offset. In frequency domain channel estimators, the correlation of the channel parameters can be exploited which requires the inversion of a large matrix iteratively for decoupling of the inter-antenna interference. To reduce the complexity we use adaptive estimators as compared to simplified estimators i.e. LSE, LMMSE, DFT-CE and DCT-CE. Adaptive filters such as Wiener Filter, LMS and RLS Filters can be used for estimating the time-varying channel. Wiener Filter requires second order channel statistics but LMS and RLS do not require a priori knowledge of channel statistics. The performance of LMS and Kalman Filtering based channel estimator is evaluated in [2], [3], [4] as a function of channel taps and Channel Impulse Response (CIR) Samples. In this paper the performance and complexity of RLS-CE is evaluated for different MIMO techniques. The effect of varying Channel Taps and Channel Length is observed while using the initially estimated channel through LSE and LMMSE.

The rest of the paper is organized as: System Description of MIMO-OFDM is given in Section II, RLS algorithm is discussed in Section III with the simulation results given in Section IV and finally conclusion are drawn in last section.

### 2. MIMO-OFDM System Model

Suppose a MIMO-OFDM system with  $M_T$  transmit antennas and  $M_R$  receive antennas. The

signal to be transmitted at  $k^{th}$  frequency is given as [5]

$$x_k = [x_k^1, x_k^2, \dots, x_k^{M_T}]$$

After passing through MIMO channel, the signal received will be [5]

$$y_k = \sum_{i=0}^L G_i x_{k-i} + n_k$$

Where  $G_i$  represents  $M_R \times M_T$  channel matrix at the  $i^{th}$  delay.

In frequency domain the channel matrix is given by [5]

$$H(e^{j\theta}) = \sum_{i=0}^L h_i e^{-ji\theta}$$

Where  $-\pi < \theta < \pi$ . For  $L=0$ , the channel will be flat-fading and for  $L > 0$ , the channel will be frequency-selective [7].

In case of frequency selective channel, the signals are distorted by Inter-Symbol Interference (ISI), which can be reduced with OFDM modulation. OFDM not only reduces ISI but also makes channel Memoryless [10].

A complete block diagram of MIMO-OFDM system is shown in Figure 1. At a time, the transmitter takes N symbols in form of vectors and arranges them according to the channel matrix. After that IDFT operation is performed, followed by the addition of cyclic prefix. Now the signal is transmitted by  $M^{th}$  transmit antenna after Digital to Analog conversion.

At receiver side first the inserted cyclic prefix is removed before performing DFT operation.

### 3. RLS-Based Channel Estimation

Due to high convergence rate and fast steady-state adaptation, RLS channel estimator is used for time-varying mobile channels. Due to the poor convergence of LMS-CE, RLS is preferred for highly correlated data but for better performance the disadvantage comes in form of increased complexity.

As compared to Gradient Algorithms, RLS algorithm is used to implement simple LS-CE as adaptive estimator. The cost function for LSE initially estimated channel case is given by [8]

$$J_{RLS}[N] = \sum_{i=1}^N \gamma^{N-i} \cdot |E_{m,n}[i]|^2 + \delta \cdot \gamma^N \cdot \|w[N]\|^2$$

Where  $\gamma$  is forgetting factor whose exact value is difficult to be estimated and  $\delta$  is regularization parameter.

The error vector for  $n^{th}$  OFDM symbol at  $m^{th}$  carrier is given by

$$E_{m,n}[i] = H_{m,n}[i] - w^H \hat{H}_{m,n}[i]$$

$\hat{H}_{m,n}[i]$  is the estimated channel, which is determined by LS method at initialization.

Channel up-dating is done by the following steps [9]

- 1- The value of correlation matrix  $\hat{R}_{g_{r,t}g_{r,t}}$  at iteration  $n$  is given by

$$\hat{R}_{g_{r,t}g_{r,t}}[n] = \lambda \hat{R}_{g_{r,t}g_{r,t}}[n-1] + \hat{H}_{r,t}^{RLS}[n] \hat{H}_{r,t}^{H,RLS}[n]$$

- 2- Gain Matrix is given by

$$\hat{R}_{g_{r,t}g_{r,t}}[n] k[n] = \hat{H}_{r,t}^{RLS}[n]$$

- 3- Error vector is

$$E[n] = \hat{H}_{r,t}^{RLS}[n] - \hat{W}^T[n-1] \hat{H}_{r,t}^{RLS}[n]$$

- 4- Conversion Factor at iteration  $n$  is

$$\alpha[n] = 1 - k[n] \hat{H}_{r,t}^{RLS}[n]$$

- 5- After which the error is given by

$$e[n] = \alpha[n] E[n]$$

- 6- After  $n^{th}$  iteration, the up-dated co-efficients are

$$\hat{W}^T[n-1] = \hat{W}^T[n-1] + k[n-1] E^*[n]$$

Now the estimated channel becomes

$$\hat{H}_{r,t}^{RLS}[n] = \sum_{m=0}^{M-1} \hat{W}^T[m] \hat{H}_{r,t}^{RLS}[n-m]$$

The gain vector  $k[n]$  is given by

$$k[n] = \frac{\lambda^{-1} Q[n-1] \hat{H}_{r,t}^{RLS}[n]}{1 + \lambda^{-1} \hat{H}_{r,t}^{RLS}[n] Q[n-1] \hat{H}_{r,t}^{RLS}[n]}$$

and

$$Q[n] = \frac{1}{\lambda} (Q[n-1] - k[n] \hat{H}_{r,t}^{RLS}[n] Q[n-1])$$

Initially the parameter values are

$$Q[0] = [\hat{H}_{r,t}^{RLS}[0] \hat{H}_{r,t}^{RLS}[0] + \delta I]^{-1}$$

and

$$k[0] = Q[0] \hat{H}_{r,t}^{RLS}[0] = \frac{1}{\|\hat{H}_{r,t}^{RLS}[0]\|^2 + \delta} \cdot \hat{H}_{r,t}^{RLS}[0]$$

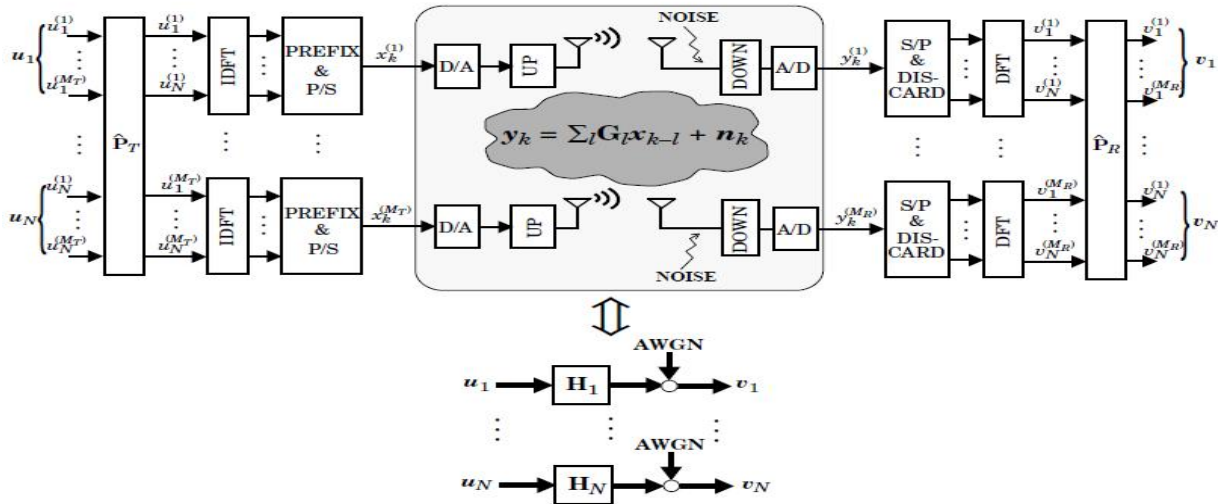


Figure 1. MIMO-OFDM System Model [6]

**4. Simulation Results**

Under different SNR operating conditions, the effect of varying the channel filter length on the performance of RLS estimator is shown in Figure 2. As we increase SNR value, the performance degrades for any channel filter length. For a specific SNR value, the performance degrades as larger length of channel filter is considered. So for better performance, less complexity and less power-consumption, less number of CIR samples are taken for low SNR values. The performance of RLS estimator as a function of SNR and CIR Samples is shown in Figure 3. The complexity of RLS estimator for different channel filter lengths is given in Table 1. By increasing the channel length from 10 CIR samples to 20, the complexity increases by 37%. Further increment of channel filter length to 40 increases the complexity by 93%.

MSE as a function of different channel filter lengths for different MIMO systems is given in Figure 4. Up to filter length of 5, the performance remains same for any MIMO system but as we increase the filter length beyond 5 CIR samples, the performance degrades almost as a linear function of increasing the channel filter length. Figure 4 also demonstrates that as the order of MIMO system is increased the performance also improves and this improvement is observed for all channel filter lengths under consideration. But higher order system gives better performance at the cost of more computational time. For RLS estimator, the initialized channel estimator can be either LSE or LMMSE. The performance comparison for both cases is given in Figure 5. LMMSE-RLS gives the better performance for all channel filter lengths as it exploits the prior knowledge of the channel statistics that is why it has more complexity as given in Table 2. From Table 2,

we note that for  $2 \times 2$  MIMO system the complexity of LMMSE-RLS is 113% greater than that of LS-RLS for channel filter length of 10 but as we increase the channel filter length to 40 CIR Samples then this increment is only 77%. For LS-RLS method, the complexity of  $3 \times 3$  is 71% more than that of  $2 \times 2$  system while for  $4 \times 4$  this increment is about 233%. Similarly in case of LMMSE-RLS approach, as compared to  $2 \times 2$  system the computational time of  $3 \times 3$  is 151% greater while for  $4 \times 4$  case this increment becomes 350%.

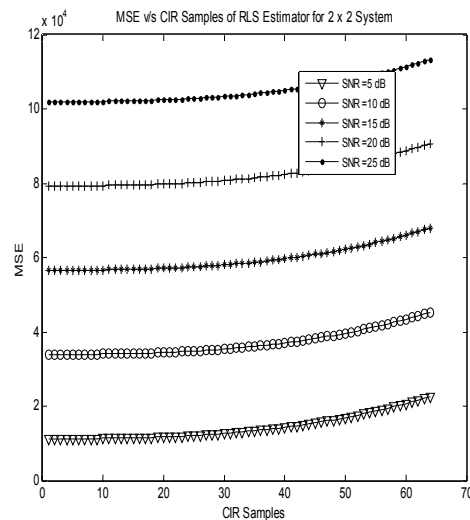


Figure 2. MSE vs CIR Samples of RLS Estimator for  $2 \times 2$  System

The performance of RLS estimator in terms of Mean Square Error as a function of Channel Taps at different SNR operating conditions is shown in

Figure 6. The effect of channel taps is same as that of CIR samples. The performance is better for low SNR values and less number of multi-path channel taps. The effect of channel taps on complexity is shown in Table 3. By increasing the channel taps two time, the complexity increases by 14 % but if the channel taps are made four times, then increment in complexity is 28%.

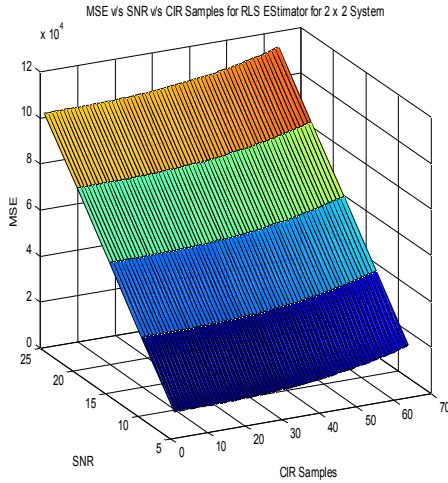


Figure 3. MSE vs SNR vs CIR Samples of RLS Estimator

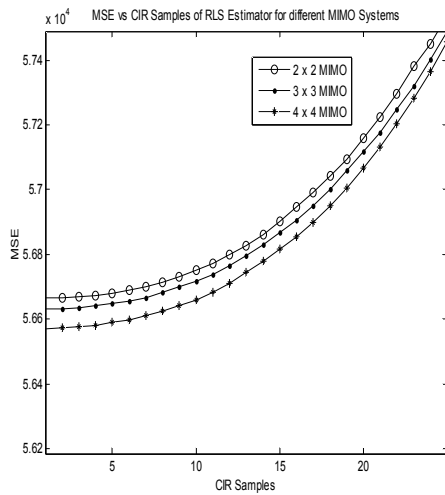


Figure 4. MSE vs CIR Samples of RLS Estimator different MIMO Systems

Table 1. Complexity of RLS as a function of CIR Samples for 4 x 4 MIMO

CIR Samples	Time ( $\mu$ sec)
10	403.2
20	553.15
40	779.34

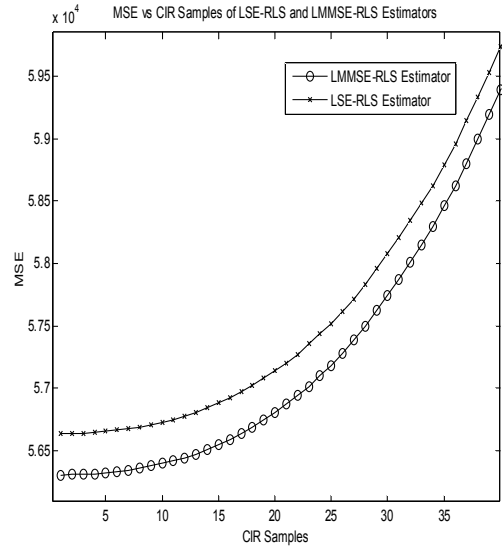


Figure 5. MSE vs CIR Samples of LS-RLS and LMMSE- for RLS Estimator

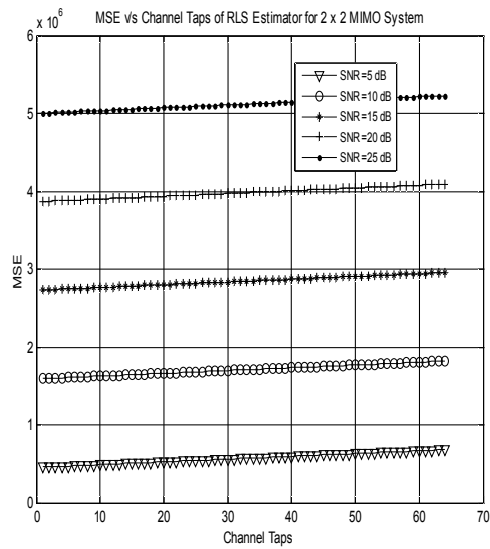


Figure 6. MSE vs Channel Taps of RLS Estimator for 2 x 2 System

The effect of increasing the channel taps is not so significant in case of high order MIMO e.g. 4 x 4, but for low order MIMO systems the effect of increasing channel taps results in degraded performance. For better performance under any channel tap number, higher order MIMO is preferred which gives improved performance at the cost of more computational time. The complexity behavior of both LS-RLS and LMMSE-RLS is given in Table 4. For all values of channel taps, LMMSE-RLS takes 6-7 times more computational time than that of LS-RLS method. The performance comparison of LS-

RLS and LMMSE-RLS is shown in Figure 8. We note that the effect of increasing the channel taps is more significant in case of LSE-RLS than LMMSE-RLS. We also observe that the performance of LSE-RLS for less number of channel taps is same to that of LMMSE-RLS at large number of channel taps. So we can optimize the complexity by considering appropriate value of channel taps for LMMSE-RLS estimator. The combined effect of SNR and channel taps on performance is shown in Figure 9.

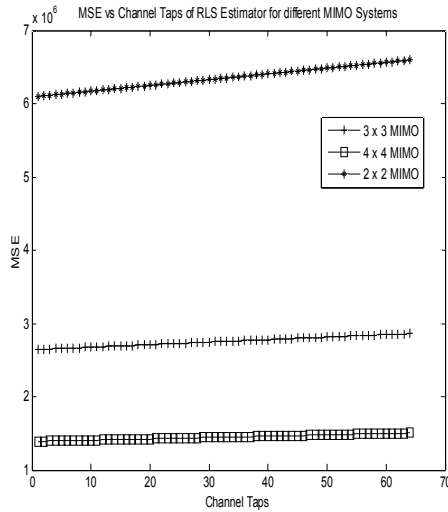


Figure 7. MSE vs Channel Taps of RLS Estimator for different MIMO Systems

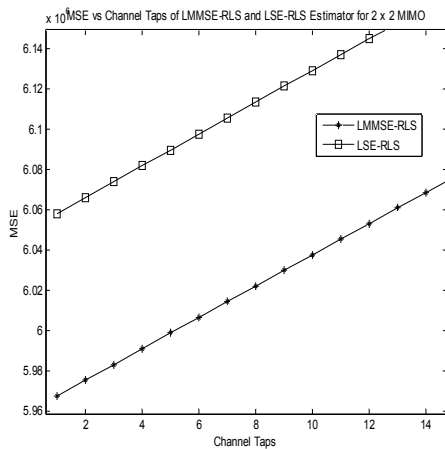


Figure 8. MSE vs Channel Taps of LS-RLS and LMMSE-RLS Estimator

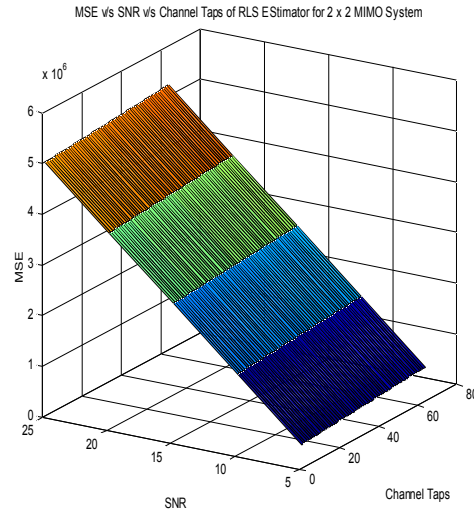


Figure 9. MSE vs SNR vs Channel Taps of RLS Estimator

**5. Conclusion**

In this paper adaptive filtering based channel estimation algorithm, Recursive Least Square (RLS), is optimized for performance in terms of Mean Square Error (MSE) and complexity in terms of computational time. These two parameters are compared for different CIR samples and multi-path channel taps. To make power-efficient communication with better performance less number of CIR samples are used under low SNR values. When the initialized channel estimation is by LMMSE, then RLS gives better performance but with high complexity as LMMSE exploits the second order channel statistics. Higher the order of MIMO system, better will be performance and for any MIMO system, channel filter length of 5 CIR samples is preferred for optimized performance and complexity. Similar behavior is also observed for channel taps as that of channel filter length. For higher order MIMO system, the effect of varying channel taps on performance goes on diminishing so for reduced computational time less number of channel taps are preferred. The optimized channel estimator can also be implemented by using other adaptive filtering techniques such as LMS and Kalman Filtering based channel estimation.

Table 2. Complexity of RLS for different MIMO Schemes

CIR Samples	2 × 2		3 × 3		4 × 4	
	LS-RLS	LMMSE-RLS	LS-RLS	LMMSE-RLS	LS-RLS	LMMSE-RLS
10	104.33	222.62	178.84	559.85	348.15	1000
20	113.74	252.68	236.3	640.61	464	1200
30	170.73	302.92	356.17	700.44	765.6	1400

Table 3. Complexity of RLS vs Channel Taps for  $2 \times 2$  System

Channel Taps	Time ( $\mu\text{sec}$ )
5	252.5
10	850.25
20	323.41

Table 4. Complexity of RLS vs Channel Taps for different MIMO Systems

Channel Taps	$2 \times 2$ ( $\mu\text{sec}$ )		$4 \times 4$ ( $\mu\text{sec}$ )	
	LS-RLS	LMMSE-RLS	LS-RLS	LMMSE-RLS
5	175.53	1400	328	2900
10	201.9	1400	365.5	3100
20	223.4	1600	394.57	3800

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2/2/2012



## Genotoxicity and oxidative stress among spray painter

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**Abstract:** Several organic solvents (OS<sub>s</sub>) are potent carcinogens among population at risk. Their genotoxicity have important implications for cancer production. Genotoxicity could be related to lipid peroxidation with decrease of endogenous body antioxidants. This work included 27 car spray painters exposed to a mixture of mainly aromatic OS<sub>s</sub> and a comparable group of 27 males. For both groups, chromosomal study and assay of serum glutathione peroxidase (GSH-P<sub>x</sub>) were done. It was found that spray painters suffered a significantly higher percentage of chromosomal aberrations (CA<sub>s</sub>%) and lower GSH-P<sub>x</sub> activity specially among smoker ones. Positive correlations were found between CA<sub>s</sub>% and duration of exposure and the lifetime hydrocarbon exposure score (HES). A significant negative correlation was found between GSH-P<sub>x</sub> and duration of work and HES. Finally we recommend following up for workers who are at risk of genotoxicity by periodic examination and regular supplementation with antioxidants.

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**Key words:** GSH-P<sub>x</sub>; chromosomal aberrations; organic solvents; HES; lipid peroxidation; antioxidants.

### 1. Introduction:

OSs have a wide range of applications and exposure to them is an eminent risk factor in occupational and non-occupational environment Kim *et al.*, (2011). Their inhalation can cause injury to several internal organs of human body and weather they are present alone or in a mixture, (Metwally and El-Shabrawy, 2000).

In fact, about 50% synthesized OSs are employed for the production of paints and thinners. Xylene, toluene, styrene, ethylbenzene, acetone and methyl ethylketone are some of most frequently and quantitatively represented solvents in the composition of paints, (Costa *et al.*, 2005).

It is well documented that several OSs are potent carcinogens among population at risk. Their genetic effects have important implications for cancer induction, (Amal *et al.*, 2011).

Genotoxicity could be proposed to be through excessive and persistent formation of reactive oxygen radical species (ROS) inducing lipid peroxidation and decrease endogenous antioxidants in the body such as reduced superoxide dismutase and (GSH-P<sub>x</sub>), (Coskum *et al.*, 2005). There are, also, associated various gene expression changes, some of which may be responsible for oxidative stress (Kim *et al.*, 2011).

The present cross sectional study aimed at assessing the genotoxic effects of chronic OSs inhalation among a group of spray painters. We, also, aimed at evaluating the oxidative stress, through GSH-P<sub>x</sub> assay as a potential indicator of DNA damage and carcinogenic mechanism.

### 2. Subjects and Methods:

#### 2.1. Subjects:

This work included 29 male car spray painters exposed to a mixture of OSs mainly aromatic solvents as xylene, toluene, as well as, pigments and thinners.

They were randomly selected from 3 car painting workshops in Cairo. Spray painting was done either outdoor or in semi-open ill ventilated places for 5 hours/day and most of them used no personal protective devices. The control group consisted of 27 male. They have no past or present current history of exposure to OSs. Both groups were matched for age, sex, smoking habits and socioeconomic standard. Those who were taking regular medications or exposed to any sort of radiation during the last 12 months before sampling were excluded from both groups.

#### 2.2. Methods:

All Participants were interviewed and subjected to a detailed questionnaire including detailed medical and occupational histories. A thorough clinical examination was done for each one.

##### 2.2.1. Study of chromosomal aberration:

The CA analysis was conducted following a standard protocol. A total of 1 ml aliquot of whole blood was cultured in F-10 medium supplemented with 20% fetal bovine serum, 0.5 ml PHA, 5000 IU/ml penicillin and 1000 IU/ml streptomycin. Each

culture was incubated at 37°C for 27 hrs. metaphases were obtained by adding 0.2 ug/ml colchicines to the cultures 3 hrs before harvesting, cells were collected by centrifugation, re-suspended in a pre-warmed hypotonic solution (0.075 M KCL) for 15 min at 37°C and fixed in acetic acid : methanol (1:3 v/v). Chromosome preparations were stained with 3.3% Giemsa. The slides were analyzed at 1000 magnification using a light microscope. One hundred metaphases cells were screened per each individual. Cells with 46 chromosomes were scored for CA. The analysis of CA included chromatid and chromosome breaks, chromatid deletions, chromatid rings and dicentric chromosomes according to Verma and Babu (1989).

### 2.2.2. Activity of GSH-Px:

The activity of GPx in serum was measured spectro-photometrically. The enzyme reaction was initiated by the addition of H<sub>2</sub>O<sub>2</sub> to the reaction medium and the rate of NADPH oxidation was followed at 340 nm. The amount of enzyme that oxidizes 1 mol NADPH per minute was considered to be one unit.

### 2.2.3. Assessment of exposure to OSs:

This was done by estimating the lifetime hydrocarbon exposure score (HES), which equals the product of intensity of exposure (coded 2, 1 and 0.5) and lifetime hours of exposure. The units were, then, arbitrary ones which represent hours weighted by the exposure intensity factor. As a general rule, exposure to hydrocarbons while working indoors without protection was given an intensity factor 2, while working indoors with protection or outdoors without protection was allocated an intensity factor 1. Exposure to outdoor activities with protection was given an intensity factor 0.5. Therefore, the solvent – exposed workers were classified into low exposure group (LEG), and high exposure group (HEG) with the score value of 32500/23501 taken as a cutoff point, (Yaqoob *et al.*, 1992).

### 2.2.4. Statistical analysis:

Data were collected and statistically analyzed. Quantitative data were compared using t-test and for the qualitative data chi-square test x<sup>2</sup> was used. Statistical difference  $p < 0.05$  was considered a significant difference and the  $p < 0.01$  was highly statistically significant. Those with  $p > 0.05$  were not significant.

## 3. Results

Table (1) shows that there was no statistical significant difference between both groups concerning their age and smoking habits. The mean

duration of exposure to spray painters for the exposed group was  $15.2 \pm 7.11$  years.

As noticed in table (2) the mean levels of the different types of CA namely chromatid break, chromosome break, dicentric chromosome, chromosomal deletion and ring chromosome, were higher among exposed groups in comparison to the controls.

**Table 1: characteristics of the studied population**

Parameter	Exposed group	Control	P value
	N=29	N=27	
Age in years Mean± (SD)	43.8 (8.9)	41.86 (9.2)	> 0.05
Smokers No & % Non Smokers No & %	18 (62.07) 11 (37.93)	17 (62.92) 10 (37.08)	> 0.05
Duration of exposure to spray painters (years) Mean±(SD)	15.2 (7.11)		

The mean level of the percentage chromosomal aberrations in general was markedly higher among the exposed group compared to their controls; the differences were statistically highly significant  $P < 0.01$ .

**Table 2: Chromosomal aberrations and serum GSH-Px among the studied groups.**

Group Chromosomal aberration	Exposed N=29	Control N=27	P value
	Mean ±SD	Mean ±SD	
Chromatid break	5.99 ±3.91	5.83 ±1.92	< 0.01
Chromosome break	6.89 ±3.54	4.01 ±1.33	< 0.01
Dicentric chromosome	5.82 ±2.91	1.99 ±0.99	< 0.01
Chromosomal deletion	4.89 ±2.01	1.91 ±0.98	< 0.01
Ring chromosome	1.52 ±1.33	0.29 ±0.38	< 0.01
Total chromosomal aberrations %	25.16 ±9.98	10.11 ±3.11	< 0.01
GSH-Px enzyme (U/mg protein)	15.91 ±5.67	27.19 ±4.99	< 0.01

This table shows that smokers exposed workers had higher frequency of CA and lower mean of GSH-Px compared to those of none exposed. The difference was statistically significant only for the frequency of CA ( $P < 0.05$ ).

**Table 3: Effect of smoking on CAs and GSH-Px among spray painters**

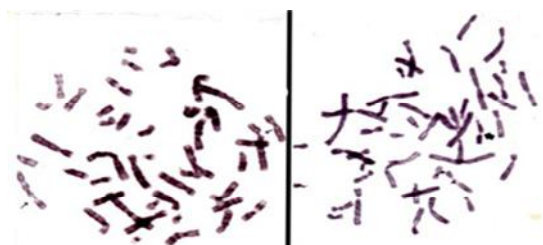
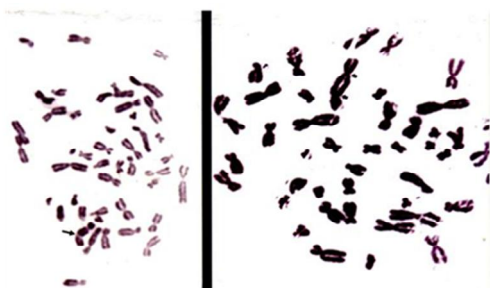
Parameter	Exposed group n = 29 (Mean ± SD)		P value
	Smokers N=18	Non smokers N=11	
CA %	27.66 ±10.99	22.11 ±9.19	< 0.05
GSH-P <sub>x</sub> ( U/mg protein)	14.89 ±5.18	16.99 ±5.32	> 0.05

HES: hydrocarbon exposure score which is calculated by arbitrary units.

**Table 4: Frequency of CAs % and GHS-Px mean Concentration of exposure group in spray painters**

Parameter	Higher exposure group N=13	Lower exposure group N=16	P value
	HES 32501 - 72500	HES 2500 - 32500	
	Mean $\pm$ SD	Mean $\pm$ SD	
CA %	27.88 $\pm$ 7.77	24.11 $\pm$ 7.53	< 0.05
GHS-Px	13.13 $\pm$ 4.17	19.11 $\pm$ 4.11	< 0.05

The mean percentage levels of CAs and GHS-Ps were higher for the first and lower for the letter among the high exposure group with HES (32501 – 72500) compared with those of lower exposure group with HES (2500 - 32500). The differences were statistically significant ( $P < 0.05$ ).

**Fig1:G banding metaphases shows chromosomal breaks in ch.(385)(right) and ch.(13)(left)****Fig2: G-banding metaphases showing dicentric chromosomes**

CAs percentage showed a significant +ve correlation with the duration of work ( $P < 0.05$ ) and highly significant with HES and GHS-Px ( $P < 0.01$ ). GHS-Px showed a significant –ve correlation with the duration of work ( $P < 0.5$ ) and highly significant with HES ( $P < 0.01$ ).

**Table 5 : correlation coefficient of exposure indices and effect indices**

Parameter	Age	Duration of exposure	HES	GHS-P <sub>x</sub>
CA <sub>s</sub> %	0.44	0.58 <sup>x</sup>	0.75 <sup>xx</sup>	-0.71 <sup>xx</sup>
GHS-P <sub>x</sub>	-0.31	-0.56 <sup>x</sup>	-0.69 <sup>xx</sup>	-----

$x = P < 0.05$

$xx = P < 0.01$

#### 4. Discussion:

Several studies have suggested that induction of CAs may play a role in solvent's induced carcinogenesis, (Catalan *et al.*, 2009) . Many epidemiological studies reported that the high

frequency of CAs is a predictive of an increased risk of cancer, (Bonassi *et al.* 2005) .The genotoxicity resulting from occupational exposure can be evaluated using different genetic endpoints, e.g. DNA damage, chromosomal aberrations and micronuclei, (Celik and AKbas 2005). In this work CAs analysis was utilized to evaluate the extent of genome damage in spray painters. CAs are particularly dangerous to the cell ,as well as, the physical discontinuity of the chromosome may cause loss of genetic information and even cell death if a housekeeping gene is involved, (Pasguini *et al.* 2001). In our Work , the mean values of the different types of CAs, namely; chromatid break, chromosome break, dicentric chromosomes, chromosomal deletion, and ring chromosome, as well as, the total chromosomal aberrations percentages were significantly higher ( $P < 0.01$ ) among spray painters compared to their controls (Table2). Similar results were reported by Gonzalez-yebra *et al.* (2009) who found a significant increase in the frequency of different CAs among shoe workers exposed to Oss. These data are, also, in accordance with the findings of kim *et al.* (2008) in workers exposed to OSs in petroleum refinery compared to their controls. Moreover, our results were greatly supported by those of Ihsan *et al.*(2000);Rueff *et al.*(2009) ; Amal *et al.* (2011) on their studies on painters, Our results also, revealed a significant decrease of GHS-Px concentration in spray painters compared to their controls (Table2). GHS-Px is a seleno enzyme responsible for elimination of reactive oxygen species (ROS).

Several studies have implicated oxidative stress as one of the important mechanisms of toxic effects of Oss. They also confirmed that exposure to high concentration of solvents induced lipid peroxidation and decreased endogenous antioxidants in the body such as GHS-Px and superoxide dismutase Farahat and Kamel, 2010).

Cigarette smoking was found to have an additional genotoxic effect (Table3). We found that smokers spray painters reported high frequencies of CAs and lower levels of GHS-Px compared to their controls.

Some studies pointed out the very complex interaction between smoking and occupational exposure to genotoxic agents. Cigarette smoking itself is a well known risk factor for several types of cancer and it is a well known confounding factor influencing the frequency of cytogenetic damage and lipid per-oxidation, (Palus *et al.*, 1998; Rekhadevi *et al.*, 2009) .This might explain the higher frequency of CAs and lower levels of GHS-Px in smoker spray painters,

A dose response relationship was found between the prevalence of CAs , decreased concentration of

GHS-Px and exposure level to spray painting (Table 4). Workers belonging to higher exposure group reported statistically significant higher frequencies of CAs and lower concentration of GHS-Px ( $P < 0.01$ ). Moreover, in the current study, spray painters showed a dose response relationship between exposure indices (age, duration of work and HES) and effect indices (CAs% and GHS-Px) (Table 5).

A significant +ve correlation was found between CAs% and both duration of work and HES, being stronger with HES. On the other hand, significant -ve correlations were found between CAs and levels of GHS-Px which in turn showed significant -ve correlation of duration of work and HES. Our results, therefore, are in accordance with those of other workers, (Amal *et al.*, 2011; Ihsan *et al.*, 2000; Kim *et al.*, 2011; Rueff *et al.*, 2009) who confirmed that heavily exposed workers to OSs and those with longer duration of work reported a significant increased frequency of CAs and decrease levels of GHS-Px supporting the claims of induction of ROS by OSs exposure with depletion of substrate molecules.

Our results were in agreement with those reported by others like catalan *et al.*(2009), who found that workers handling complex chemical mixture and painters whaling paint thinners. Ihsan *et al.*, 2000; Angela *et al.*, 2010) showed higher frequency of CAs and decreased concentration of GHS-Px.

The intimate relation between ROS and DNA damage is well documented in table (5) where a -ve correlation between CAs frequency and GHS-Px ( $r = -0.71$  &  $P < 0.01$ ). Our results were greatly supported by those recorded by Rekhadevi *et al.*(2009) who detected a similar association between decreased superoxide dismutase and GHS-Px on one side and increased frequency of CAs, micronuclei and abnormal comet assay on the other one.

Finally, we conclude that chronic exposure to OSs results in chromosomal damage and lipid peroxidation which might play an important role in activating proto-oncogenes predisposing to transformation to malignancy. Hence, educational programs to educate workers about the potential health hazards of exposure to OSs with stressing on importance of using protective measures should be implemented. We stress on the importance of regular bio-monitoring of genotoxic effects for workers at risk with regular supplementation with antioxidants.

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### Effect of Tumstatin on Hypertrophic Scar in the Rabbit Model

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**Abstract:** This paper aimed to investigate the effect of tumstatin on hypertrophic scar in the rabbit model to explore a new treatment method for hypertrophic scar. An animal model of hypertrophic scar on the rabbit ears was established and was intervened with tumstatin via local injection. The rabbits were randomly assigned into tumstatin group, endostatin group, triamcinolone acetonide group and the saline control group. At 5 days after epithelization, 4 ml of tumstatin, endostatin, triamcinolone acetonide and the saline with concentration of 200 ug/ ml were injected into tissue of scar along the perimeter of the scar in the four experimental groups, respectively. After 30 days of injection, the gross appearance of scar was observed and the blood flow of hyperplastic scar was assessed by laser Doppler flowmetry. The scar tissues on the bilateral ears of each rabbits were resected afterwards. The histological features of scar tissue were examined with HE staining, and the scar thickness was also measured under microscope. The blood flow, vessel density and the scar thickness in the tumstatin group were significantly lower than that in the other three groups, and the difference is statistically significant ( $P < 0.05$ ). Tumstatin may inhibit angiogenesis in hypertrophic scar by means of blocking endothelium proliferation and promoting its apoptosis, have a promising application in the prevention of human hypertrophic scar, which may serve as a promising role in the prevention of human hypertrophic scar.

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**Keywords:** Hypertrophic scar; tumstatin; microvascular endothelial cells; anti-angiogenesis

#### 1. Introduction

Hypertrophic scarring is one of the most common types of pathological scars, and excessive scars form as a result of aberrations of physiologic wound healing and may develop following any insult to the deep dermis, including burn injury, lacerations, abrasions, surgery and piercings. By causing pruritus, pain and contractures, excessive scarring can dramatically affect a patient's quality of life, both physically and psychologically (Bock et al., 2006). Over the past decades, great progress had been made in hypertrophic scars development, but no breakthrough has yet occurred in the specific pathogenic mechanism (Ferrara et al., 2003). At present, it is recognized that scar formation contains an important mechanism, that is, scar angiogenesis, and aberrant vascularization may be a critical factor to the abnormal scar formation. In recent years, the role of angiogenesis in scar formation has been a hot spot in studying mechanism of scar formation (Lakos et al., 2004; Galiano et al., 2004; Takeda et al., 2004). The studies demonstrated that the active angiogenesis was certainly associated with excessive scar

formation, and it might affect wound healing by regulating wound healing process. Therefore, the study of the scar tissue angiogenesis, and exploration on therapeutic and preventive methods based on interrelationship between vascularization and scar formation, may become an important research direction in hypertrophic scars area.

Tumstatin peptide is an endothelial cell-specific inhibitor of protein synthesis and probably one of the most effective angiogenesis inhibitor derived from type IV collagen (Maeshima et al., 2002). Its antiangiogenesis activity is significantly stronger than that of endostatin. The researches found that it exhibited good efficacy in antiangiogenic treatment in tumor, without drug resistance and any side effects. And the studies also revealed that it played an important role in tumor angiogenesis. Currently, many researches on antiangiogenic effect of tumstatin in tumor have been reported. However, the report about the effect of tumstatin on scar angiogenesis has not been found. In the present attempt, the hypertrophic scar formed in the rabbit ear was interfered using tumstatin peptide, whose

efficacy was compared with that of endostatin peptide and triamcinolone acetonide (a conventional medicine), respectively. We observed its anti-angiogenic effect and impact on scar formation, and sought to explore a novel modality for the treatment and prevention of hypertrophic scars.

## 2. Material and Methods

### Main reagents and instruments

New Zealand white rabbits of either sex weighting 2.0-2.5 kg were purchased from experimental animal center of Sichuan University (Chengdu, Sichuan province, China) and were maintained in separate cages. Tumstatin and endostatin peptides were all synthesized by Shanghai Kehua Bio-engineering Co. Ltd (Shanghai, China). Tumstatin peptide (74-98 amino acids) contained 25 amino acids and was referred to as T7 peptide (TMPFLFCNVNDVCNFASTRNDYSYWL). The nucleotide sequence encoding amino acids 1-30 of endostatin termed as peptide 30 was used with amino acid sequence: PGARIFSFDFGKDVLRHPTWPQKSVWHGSDPN.

Triamcinolone acetonide injection was purchased from Shanghai General Pharmaceutical Co., Ltd (Shanghai, China). Moor FLPI laser Doppler flowmetry (Moor, UK) and Motic stereomicroscope (Motic Xiamen) was provided by Experiment Research Center of first affiliated hospital of Chengdu medical college.

### Establishment of hypertrophic scar model in rabbit ears

All experimental rabbits were anesthetized with ketamine (15mg/kg) with administered intravenously via the ear vein, and then fixed on an operating table in the supine position. The routine preoperative preparation was performed and the procedure was conducted under an operating microscope. The full-thickness skin of the inside aspect of each ear was resected, and perichondrium was scraped away. Four 8 mm-diameter round wound model were generated in each rabbit ear. The wound area was exposed to the air after the bleeding was completely stopped. Each rabbit was singly housed under standard caging conditions. The wound exudates and scab was removed every two days. Twenty-five days after surgery, the wound epithelialization occurred and the scab fall off naturally, and an early phases of model of hypertrophic scar was obtained (Kloeters et al., 2007).

### Experiment grouping design and drug delivery methods

The rabbits were randomly divided into four groups: tumstatin group (A), endostatin group (B), triamcinolone acetonide group (C) and the saline control group (D). The ears whose epithelialization time was not consistent with the others was excluded the experiment.

Drug injection was administered posterior to 5 days of epithelialization. Tumstatin (200 ug/ml), endostatin (200 ug/ml), triamcinolone acetonide (20 mg/ml) of total volume of 0.4 ml was injected at multiple points of the scar tissue. After the intralesional injection, the scar turned pale white. The saline control group received saline injection, with the same volume and method of above.

### The gross morphology observation and perfusion detection

The gross morphology, that is, the thickness, color and texture of the scar tissue was observed every other day. The blood flow of the scar tissue was detected 30 days after drug injection. The rabbits were anesthetized 30 min posterior to measurement in the same manner described above. Avoid unnecessary stimulation of the animals. The blood perfusion of the whole ear was assessed using Moor FLPI laser Doppler flowmetry and the quantitative local perfusion was recorded.

### HE staining and scar thickness measurement

The subsequent operation was performed immediately after the perfusion detection, in which the scar tissue was resected, fixed in 10% neutral formaldehyde for 12 hours, followed by decalcification, dehydration, rendering transparency, paraffin embedding, slicing, spreading, drying. It was then stained by hematoxylin solution for 5 min, differentiated in 0.5% acid alcohol for 5 s, rinsed with diluted ammonia for 30 s, counterstained with 0.5% aqueous eosin for 2 min, cleared and mounted in neutral resin. The maximum thickness of the scar in slices was taken as the scar thickness under the Nikon TIS microscope ( $\times 100$  times), which was detected using NIS-Elements D microscope imaging software for real-time measurements.

### Effect of tumstatin on MECs proliferation detected by MTT assay

The subcutaneous tissue was removed from specimens, and then the specimens were cut into narrow strips of 3 mm wide and placed in 20 ml sterile antibiotic bottle, followed by adding of appropriate amount of dispase. After being cultured for 24 h at 4°C, the dermis and epidermis were carefully separated, and the former was put in a new antibiotic bottle. Then the samples were digested

with 1.25 g / L trypsin for 6-20 h at 4°C and washed with PBS. Add 10-20 ml DMEM or PBS to Petri dish, and the MECs were separated by mechanical extrusion method (Wang et al., 2008). The supernatant was removed after centrifugation, and the cells were adjusted to  $2 \times 10^4$  cells/ml. The cells were purified and subcultured, and their proliferative activity was measured by MTT assay.

### Statistical analysis

The paired t-test was performed using SPSS 12.0 software, and analysis of variance was used to compare the mean of several groups with each other. The results were expressed as  $\bar{x} \pm s$ , and a P value of less than 0.05 was considered as statistically significant.

## 3. Results

### Gross morphological changes

Thirty days after drug injection, significant degeneration of the scar tissue was observed in the tumstatin group, whose color was close to the normal color of rabbit ears. It was slightly higher than the surrounding skin with relatively flat surface and soft touch. For the control groups, the scar tissue was light red, and obviously higher than the surrounding skin with hard texture and varied thickness (Figure 1). The results showed significant difference in gross morphology between the tumstatin group and the other three groups ( $P < 0.05$ ).

### Perfusion detection

Microcirculatory perfusion of the tumstatin group was significantly lower compared with the controls ( $P < 0.05$ ), indicating tumstatin can significantly inhibit blood flow of the hypertrophic scar (Figure 2, 3).

### HE staining of the scar tissue

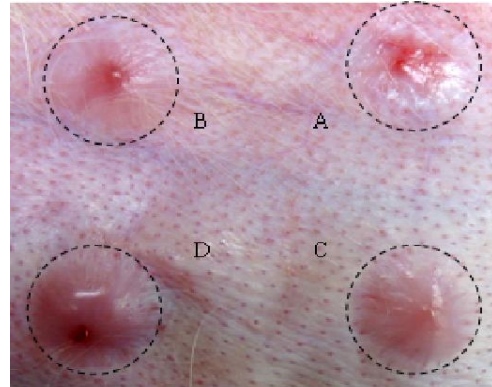
Microscope morphology observation demonstrated that the microvessels and fibroblasts in the tumstatin group were significantly reduced, and the inflammation cells were less infiltrated in the tumstatin group compared with the other groups (Figure 4).

### Scar thickness measurement

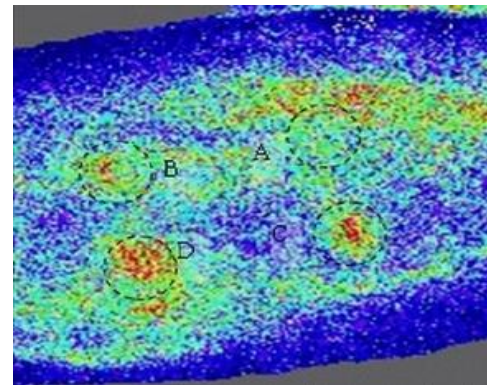
The average scar thickness was 0.39 mm in the tumstatin group, and was 0.43 mm, 0.50 mm and 0.62 mm in the endostatin group, triamcinolone acetonide group and the saline control group, respectively (Figure 5).

### Effect of tumstatin on MECs proliferation detected by MTT assay

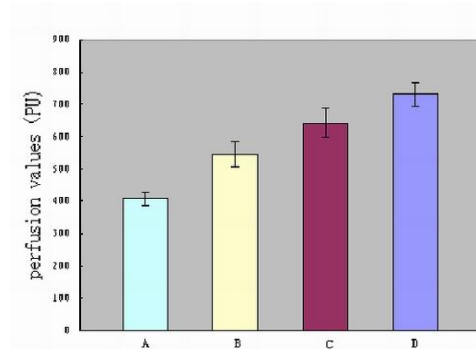
The optical density and OD values are linearly proportional to the number of living cells. As shown in Table 1, the OD value was significantly decreased in the tumstatin group compared with the other three groups, and the difference among them was statistically significant (Table 1,  $P < 0.05$ ). The results suggested that tumstatin can inhibit endothelium cells proliferation in vitro.



**Figure 1. Gross morphological changes of hypertrophic scar.** A: tumstatin group, B: endostatin group, C: triamcinolone acetonide group, D: saline control group.



**Figure 2. Laser Doppler Perfusion Images of the scar tissue.**



**Figure 3. Perfusion values of the scar tissue.**



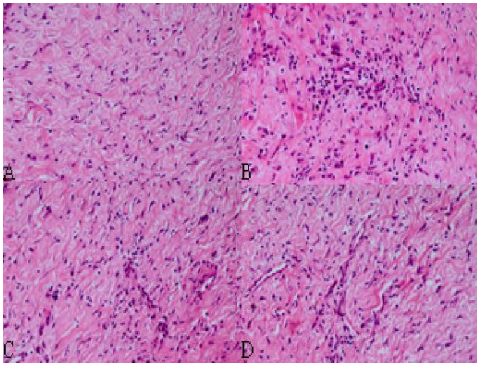


Figure 4. HE staining of the scar tissue.

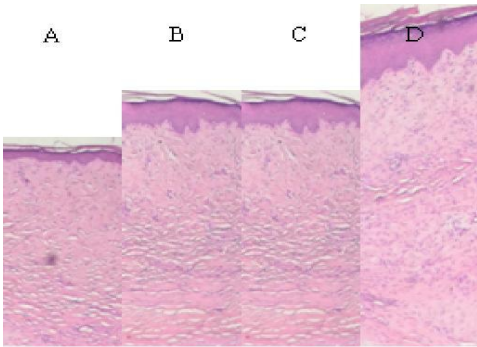


Figure 5. Thickness of the scar tissue.

Table 1. MECs proliferative activity in hypertrophic tissue in each group ( $\bar{x} \pm s$ ).

Grouping	n	OD values
Saline control group	8	0.340±0.048
Triamcinolone acetonide group	8	0.320±0.036
Endostatin group	8	0.265±0.050* <sup>#</sup>
Tumstatin group	8	0.235±0.056* <sup>#</sup>

\* $P < 0.05$ , vs saline control group;

<sup>#</sup> $P < 0.05$ , vs triamcinolone acetonide group.

#### 4. Discussions

Hypertrophic scarring can occur after thermal injury, surgical incision, or other traumatic injury. These lesions are raised, pruritic erythematous scars that may widen, but which remain within the confines of the original wound. Histologically, hypertrophic scars appear hyperplastic and manifest a thickened epidermis, a dermis lacking dermal papillae, and the presence of collagen nodules in an abnormally increased vascular wound matrix (Harunari et al., 2006 and van der Veer et al., 2009). Hypertrophic scarring is a difficult clinical problem, and its management poses a great challenge. Treatments have progressed from rudimentary methods such as gross excision and radiotherapy to less invasive techniques such as intralesional

injections and topical preparations. However, through this evolution of therapies, success rates have not significantly improved (Kelly, 2004 and Chung et al., 2006). Additional research needs to be done to explore more effective treatment method, since there is no universally accepted treatment regimen.

Modern research has lead to an increased understanding of the pathophysiologic process of wound healing and scar formation. Nevertheless, the definite etiology of this abnormal scarring process is, for the most part, unknown. Many researches conducted both at home and abroad have showed that the hypertrophic process of scar is closely associated with development of angiogenesis (Kilarski, 2009 and Jacobi et al., 2006). It is revealed that the average number of blood vessels and blood flow in hypertrophic scar was significantly higher than that in normal skin tissue.

The study on anti-angiogenesis therapy of hypertrophic scarring demonstrated that, the blood vessels were significantly less in hypertrophic scarring (Vranckx et al., 2005). Furthermore, decreased scar volume, fibroblast necrosis and apoptosis were also observed. A large number of experimental results indicated that impeding angiogenesis would inhibit scar formation

Tumstatin is a 28-kilodalton fragment of type IV collagen that displays both anti-angiogenic and proapoptotic activity (Floquet et al., 2004). Because tumstatin interacts with the  $\alpha\beta3$  integrin in two separate locations, it has two distinct functions. The first is to inhibit angiogenesis and the second is to increase the amount of cellular apoptosis, or programmed cell death. By affecting the apoptotic pathway, tumstatin inhibits the proliferation of endothelial cells (Hamano et al., 2003). At present, the role of tumstatin in various cancers has been fully described. It has been suggested that tumstatin can inhibit MECs proliferation and promote their apoptosis, thereby effectively impairing angiogenesis in tumor tissues, which is considered to be the most powerful anti-angiogenic factor. Nevertheless, studies on scar formation have not yet been seen, and thus in our attempt we sought to explore the role of tumstatin played in hypertrophic scarring.

The results obtained revealed that the scar tissue was significantly shrunk with relatively more flat surface after been treated with tumstatin in contrast to the other control groups ( $P < 0.05$ ), suggesting tumstatin can greatly reduce the scar volume. In addition, the microcirculatory perfusion of the tumstatin group was significantly lower compared with the controls ( $P < 0.05$ ), indicating tumstatin can significantly inhibit blood flow of the

hypertrophic scar. Furthermore, decreased microvessels and fibroblasts as well as less infiltrated inflammation cells were observed in the tumstatin group under microscopy. After been interfered with tumstatin, the average scar thickness (0.39 mm) was significantly smaller than that of the controls (0.43 mm, 0.50 mm and 0.62 mm) with statistical significance. Additionally, the OD value was significantly decreased after tumstatin injection compared with the other three groups, and the difference among them was statistically significant ( $P<0.05$ ). The results suggested that tumstatin can markedly inhibit endothelium cells proliferation in vitro.

In conclusion, tumstatin can more effectively, than endostatin and triamcinolone acetonide, inhibit angiogenesis and significantly induce endothelium cells apoptosis, thereby inhibiting the formation of hypertrophic scars.

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## The involvement of amygdala phosphorylated Erk1/2 in the anti-immobility effect of long-term desipramine HCl treatment in a forced swimming test

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**Abstract:** Desipramine (DMI), a tricyclic antidepressant (TCA), is widely used for the clinical treatment of depression. However, the understanding of DMI's mechanism of action is still inadequate. In the present study, we investigated the relationship between the extracellular signal regulated kinase1/2 (Erk1/2) and the effect of DMI treatment. The Porsolt forced swimming test (FST) was used to estimate the anti-immobility effects of DMI. Moreover, the phosphorylated levels of Erk1/2 were assessed using Western blot analysis. There was no obvious difference between treated and untreated rats in their locomotion activity. However, on day 21, the DMI-treated rats showed a significant decrease in the FST immobility time (IMT); there were no changes in IMT after 5 days of DMI treatment. The Erk1/2 phosphorylation of the amygdala was assessed; the Erk1/2 phosphorylation was significantly decreased in the amygdala after 21 days of DMI treatment. Aurintricarboxylic acid (ATA), a nonpeptide MAPK activator, was injected locally into the amygdala regions bilaterally in a group of 21-day DMI-treated rats after the FST; these rats recovered significantly from their immobility behavior. Furthermore, the amygdala Erk1/2 phosphorylation of ATA-treated rats was consistent with the behavioral test. This study is the first to demonstrate that phosphorylated Erk1/2 in the amygdala may be involved in DMI's mechanism of action with long-term treatment but not with short-term treatment.

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**Keywords:** Desipramine, Erk1/2, Amygdala, Forced Swimming Test

### 1. Introduction

DMI, a tricyclic antidepressant, is usually characterized as a norepinephrine reuptake inhibitor [1, 2]. Recent studies have not only shown that DMI's mechanism of action occurs via the acute increase of norepinephrine in the synaptic gap [3,4], but also showed following intracellular molecules to be involved in DMI's mechanism of action: brain-derived neurotrophic factor (BDNF) and Ca<sup>2+</sup>-calmodulin-dependent protein kinase kinase (CaMKK). [5, 6]. However, the involvement of the molecules, Erk1/2, in DMI's mechanism of action is still not well elucidated.

In recent years, the Erk1/2 signaling in the amygdala was revealed to be crucial for the manipulation of fear memory formation or extinction in rats [7, 8], and even revealed to be involved in mood regulation [9]. With the progress of cellular and molecular biology, it is extremely important to assess the certain specific brain region (i.e. the amygdala) that have been found to be involved in the occurrence of the symptoms of depression when antidepressants' mechanisms of action were studied [10, 11]. Therefore, in the present study, we tried to examine the role that Erk1/2 in the amygdala has in

DMI's mechanism of action and to further elucidate DMI's cellular mechanism of action.

The FST, originally described by Porsolt et al. in 1977, is a widely used animal model for the evaluation of antidepressant efficacy. By recording the IMT during the FST in antidepressant-treated rats, the efficacy of antidepressants can be evaluated [12]. A shorter IMT during the FST indicates the dose of the antidepressant that is more effective. Another test, the open field test (OFT), is usually used to assess spontaneous locomotion in animals [13]. Using this test, the effects of DMI on the spontaneous locomotion of rats can also be assessed..

### 2. Material and Methods

#### Animals

This study was conducted in conformity with the policies and procedures detailed in the "Guide for Animal Care and Use of Laboratory Animals". The animal experimental protocols of the "Guide" were approved by the Institutional Animal Care and Use Committee (IACUC) of Chung-Hwa University of Medical Technology. Male Sprague-Dawley rats weighing 130-150 g at the time of testing were

housed in groups at a constant room temperature ( $22\pm 1^\circ\text{C}$ ) and humidity ( $55\%\pm 10\%$  RH) with a 12-h light: dark cycle. Food and water were freely available.

#### **Desipramine HCl treatment protocol**

The rats were treated with DMI (Sigma Company [St. Louis, MO 63195, U.S.A]) or normal saline (saline group) given intraperitoneally. The DMI dosage was 10 mg/kg. The DMI-treated rats received once daily DMI injections for 5 days or 21 days. The 5-day DMI-treated rats were treated from postnatal day (PND) 31 to PND35; the 21-day DMI-treated rats were treated from PND15 to PND35. During the 2-day washout period prior to re-testing, DMI was not given.

#### **Forced swimming test**

Rats after DMI treatments were subjected to the FST 24 hr later. During the FST, the rats were immersed in Plexiglas cylinders (diameter 18 cm, height 38 cm) filled to a depth of 25 cm with water at  $25^\circ\text{C}$ . On the first experimental day, the rats were placed in the water for a 15-min habituation period. After removal from the water, they were dried in a Plexiglas box under a 60 W bulb for 30 min. The next day, they were placed in the cylinders again and observed for 5 min. During this period, the total time that the rats spent making the movements necessary to remain afloat was recorded. After the 5-min test, the rats were removed from the water and sacrificed for immunoreactivity analysis. However, some animals were re-tested; these re-tested animals performed two successive FST at 48-hr intervals.

#### **Open field test**

The rat's locomotion activity was measured using a chamber (length 120 cm, width 120 cm, height 80 cm) made from transparent acrylic. On the first experimental day, the rats were introduced to the center of the chamber for a 60-min habituation period. The next day, they were placed in the corner of the chamber for a 10-min test session during which their locomotion time was recorded.

#### **Western blot analysis**

The rats after the FST were decapitated within 10 min. Then, transverse slices ( $500\ \mu\text{m}$ ) were cut from the brain tissue block using a Vibroslice (Campden Instruments, Silbey, UK). The amygdala regions were examined under a stereomicroscope and were dissected from the brain slices using a scalpel. The minipieces of brain slices obtained from the amygdala were transferred to artificial cerebrospinal

fluid (ACSF) solution bubbled continuously with 95%  $\text{O}_2/5\%$   $\text{CO}_2$  and then prepared to be removed for subsequent analyses.

The amygdala region was sonicated transiently in ice-cold HB-II buffer (50 mM Tris-HCl [pH 7.5], 50 mM NaCl, 10 mM EGTA, 5 mM ethylenediaminetetraacetic acid, 2 mM sodium pyrophosphate, 4 mM para-nitrophenylphosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride [PMSF], 20  $\mu\text{g}/\text{mg}$  leupeptin, and 4  $\mu\text{g}/\text{ml}$  aprotinin). Following sonication, the soluble extract was obtained after pelleting the crude membrane fraction by centrifugation ( $50,000\ \text{g}$ ,  $4^\circ\text{C}$ ). Protein concentration in the soluble fraction was then measured using a Bradford assay, with bovine serum albumin as the standard. Equivalent amounts of protein for each sample were resolved in 8.5% sodium dodecyl sulfate (SDS) – polyacrylamide gels, blotted electrophoretically to Immobilon, and blocked overnight in TBS buffer. To detect the phosphorylated forms of Erk1/2, the blots were incubated with anti-phospho-ERK (anti-P-ERK; New England Biolabs, Beverly, MA, USA) antibody. To control the content of the specific protein per lane, the membranes were stripped with 100 mM  $\beta$ -mercaptoethanol and 2% SDS in 62.5 mM Tris-HCl (pH 6.8) for 30 min at  $70^\circ\text{C}$  and then re-probed with a mouse monoclonal anti-pan-ERK (BD Transduction Laboratories, Los Angeles, CA, USA) antibody. An enhanced chemiluminescence kit (NEN Life Science Products, Boston, MA, USA) was used. The density of the immunoblots was determined by an image analysis system installed with BIO-ID software (Viber Lourmat, Mame-la-Valee, France). To assess the changes in Erk1/2 phosphorylation, the rats' total kinase levels and phosphorylated kinase levels were normalized by the control group levels, and then Erk1/2 immunoreactivity was expressed as a percentage.

#### **Stereotaxic surgery**

The 21-day DMI-treated rats treated with an intra-amygdala reagent (ACSF or ATA) underwent stereotaxic surgeries on PND30. The rats were anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally (i.p.) and then mounted on a stereotaxic apparatus for surgery. Two cannula made of 23-gauge stainless steel tubing were implanted into the LA (lateral amygdala) or the BLA (basolateral amygdala) bilaterally. The coordinates were AP -2.3 mm, ML  $\pm 4.5$  mm, DV  $-7.0$  mm, based on Paxinos and Watson [14]. Three jewelry screws implanted in the skull served as anchors, and the whole assembly

was affixed on the skull with dental cement. A 28-gauge dummy cannula was inserted into each cannula to prevent clogging. After the surgical procedure, the rats were given 6 days (from PND30 to PND35) to recover prior to the FST.

### Drug microinjection

A group of 21-day DMI and stereotaxic surgery-treated rats were subjected to the re-test protocol. In the re-test protocol, aurintricarboxylic acid, a nonpeptide MAPK activator [15], was microinjected into the amygdala regions bilaterally, 10 min prior to the second FST. The ATA dosage was 1  $\mu$ l per side (3  $\mu$ g dissolved in 1  $\mu$ l ACSF), and the injection rate was 0.1  $\mu$ l/min [16, 17].

### Statistics

Results are expressed as mean  $\pm$  SEM. Sample sizes are indicated by n. Comparisons between groups were carried out with a one- or two-way analysis of variance (ANOVA). Differences between two groups were compared using the unpaired Student's *t*-test;  $p < 0.05$  was considered statistically significant.

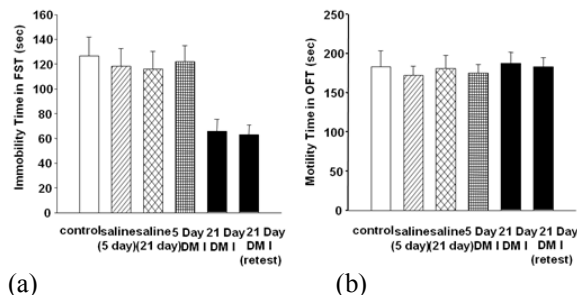
## 3. Results

### The effects of short-term and long-term treatment with DMI on normal rats during the FST and OFT

Figure 1(a) shows the effects of DMI on normal animal behavior. The IMTs of normal rats in the control and the saline groups (5-day and 21-day saline-treated groups) were almost the same (control,  $126.4 \pm 15.6$  sec; saline [5 days],  $118.1 \pm 14.7$  sec; saline [21 days],  $116 \pm 14.2$  sec,  $n=7$  each). However, compared to controls, the 21-day DMI-treated rats had a drastically decreased IMT ( $65.6 \pm 9.8$  sec,  $n=7$ ,  $***p < 0.001$ ), while no significant change was observed in the IMT of 5-day DMI-treated rats ( $122.3 \pm 12.8$  sec,  $n=7$ ,  $p > 0.5$ ). After a 2-day washout period, the IMT of the 21-day DMI-treated group did not return to its original level ( $63 \pm 8.0$  sec,  $n=7$ ).

Figure 1(b) shows the effects of DMI on rat locomotion activity. There was no significant difference among the groups. The motility time (MT) in the control group, saline groups (5-day and 21-day saline-treated groups), 5-day DMI-treated group, and 21-day DMI-treated group were  $183.3 \pm 2.0$  sec,  $172.3 \pm 11.6$  sec,  $180.5 \pm 16.7$  sec,  $175.0 \pm 11.0$  sec, and  $187 \pm 14.6$  sec,  $n=7$  each, respectively. There was no obvious change in the locomotion activity of the

21-day DMI-treated rats after a 2-day washout period ( $183 \pm 12.0$  sec,  $n=7$ ).



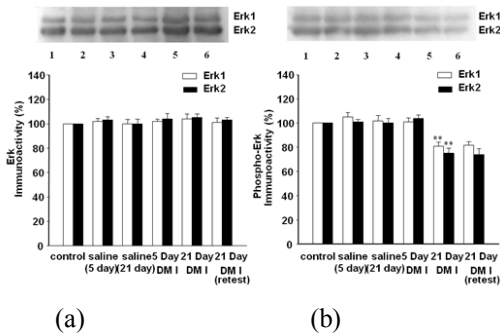
**Fig. 1** The behavioural effects of DMI treatments on normal rats. DMI (10 mg/kg) was given intraperitoneally to normal rats for 5 or 21 days once daily. The IMTs in the control and saline groups (0.9% normal saline treatment for 5 day or 21 days) were almost the same. However, the IMT was significantly decreased in rats after 21-day DMI treatment ( $***P < 0.001$ , compared to the normal or saline control groups). No changes were seen in the IMT performance of 5-day DMI-treated rats. The reduced IMT in 21-day DMI-treated rats did not rebound to initial levels after the 2-day washout period ( $n=7$  in each group). In the OFT, no significant changes in the MT were observed in the 5-day and 21-day DMI-treated rats.

### The effect of DMI on amygdala Erk1/2 phosphorylation

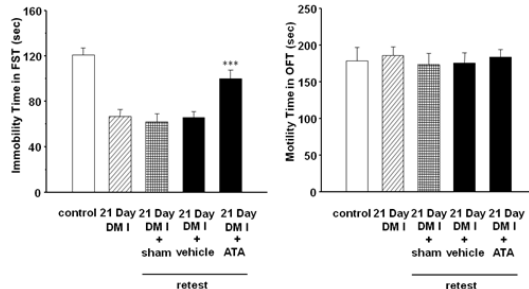
In figure 2(b), it showed that the basal Erk1/2 phosphorylation levels were low in the control groups; nevertheless, the amygdala Erk1/2 phosphorylation levels were also significantly decreased in 21-day DMI-treated rats (Erk1,  $81\% \pm 3.1\%$ ; Erk2,  $75\% \pm 4.2\%$  versus the control or 21-day saline control group [Erk1,  $102\% \pm 4.2\%$ ; Erk2,  $100\% \pm 3.7\%$ ];  $n=5$ ,  $**p < 0.01$ ), while there were no changes in the 5-day DMI-treated group (Erk1,  $101\% \pm 3.1\%$ ; Erk2,  $104\% \pm 2.6\%$  versus the control or 5-day saline control group [Erk1,  $105\% \pm 3.6\%$ ; Erk2,  $101\% \pm 2.2\%$ ];  $n=5$ ,  $p > 0.5$ ). The total amounts of Erk1 and Erk2 were unchanged (Fig. 2(a)). Two days after DMI treatment was stopped, the 21-day DMI-treated rats continued to show inhibitory effects on amygdala Erk1/2 phosphorylation (Erk1,  $82\% \pm 2.6\%$ ; Erk2,  $74\% \pm 5.0\%$ ,  $n=5$ ,  $p > 0.5$  versus 21-day DMI-treated rats).

### Behavioral re-tests for 21-day DMI-treated rats with an intra-amygdala MAPK activator in the FST and OFT

The 21-day DMI-treated rats and the control group rats were studied here. Prior to the FST re-test, the 21-day DMI-treated rats received microinjections of ACSF or ATA, the MAPK activator, into the amygdala regions bilaterally.



**Fig. 2** 21-day intraperitoneal administration of DMI inhibited basal amygdala Erk1/2 levels in rats. (a) There was no change in the total amounts of Erk1/2 in each group. (b) Desipramine was given intraperitoneally for 5 or 21 days to normal rats. The basal amygdala phosphorylated Erk1/2 levels in the 21-day DMI-treated rats were suppressed (\*\*  $P < 0.01$  compared to normal rats) and did not rebound to initial levels after a 2-day washout period. ( $n = 5$  in each group, white bars: Erk1; black bars: Erk2).



**Fig. 3** 21-day DMI-treated rats after intra-amygdala infusion of the MAPK activator, ATA, had a significant restoration in IMT in the FST; there was no effect on the MT in the OFT. The 21-day DMI-treated rats prior to the behavioral re-tests received intra-amygdala ATA. The MAPK activator-treated rats had a significant restoration in IMT on the FST; there was no effect on the MT in the OFT.

Figure 3A shows that there were no changes in the IMT in sham (21-day DMI and cannula-treated) or vehicle (21-day DMI and intra-amygdala ACSF-treated) groups compared to the 21-day DMI treatment only group (21-day DMI group,  $67 \pm 5.8$  sec; sham group,  $62 \pm 7.4$  sec; vehicle group,  $66 \pm 5.3$  sec;  $n = 7$  each, respectively,  $p > 0.5$ ). However, the ATA group (21-day DMI and intra-amygdala ATA-

treated) showed a significant recovery of IMT ( $101 \pm 7.8$  sec,  $n = 7$ ,  $***p < 0.001$ , versus the 21-day DMI-treated group). There were no obvious changes in the locomotion activity among the groups, as shown in figure 3B. The MT of the control group, the 21-day DMI-treated group, the sham group, the vehicle group, and the ATA-treated group were  $179.0 \pm 18.0$  sec,  $186.0 \pm 12.0$  sec,  $174.0 \pm 15.0$  sec,  $176.0 \pm 14.0$  sec and  $184.0 \pm 10.0$  sec, respectively; each group consisted of 7 rats. The infusion cannula tip locations are shown in figure 4.

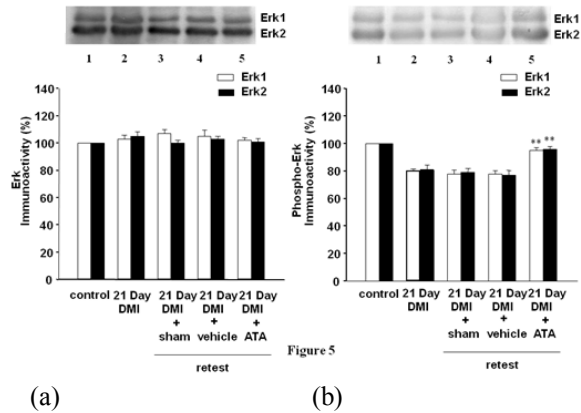


**Fig. 4** The injection sites used for intra-amygdala infusion are shown schematically. The black inverted triangles represent the distribution of the cannula tips in the amygdala of the sham group (21-day DMI-treated rats with cannula only). The white circles and the white triangles represent the distributions of the sites of the cannula tips in the amygdala of 21-day DMI-treated rats infused with ACSF and ATA, respectively.

#### Western blot analysis of amygdala Erk1/2 activities in MAPK activator-treated rats

The effects of ATA on amygdala Erk1/2 phosphorylation were assessed. The amygdala Erk1/2 activities were measured in the control group, the 21-day DMI-treated group (Erk1,  $80\% \pm 1.4\%$ ; Erk2,  $81\% \pm 3.3\%$ ), the sham group (21-day DMI and cannula-treated [Erk1,  $78\% \pm 2.6\%$ ; Erk2,  $79\% \pm 3.0\%$ ]), the vehicle group (21-day DMI and intra-amygdala ACSF-treated [Erk1,  $78\% \pm 2.3\%$ ; Erk2,  $77\% \pm 3.5\%$ ]), and the ATA group (21-day DMI and intra-amygdala ATA-treated [Erk1,  $95\% \pm 2.1\%$ ; Erk2,  $96\% \pm 1.6\%$ ]). In figure 5(b), it can be seen that, other than in the ATA group, the results of all groups were unchanged from the previous values (figure 2). In the ATA group, the amygdala Erk1/2 phosphorylation levels recovered significantly (versus the 21-day DMI-treated group;  $n = 5$ ,

\*\* $p < 0.01$ ). The total amounts of Erk1 and Erk2 were unchanged in all groups (Fig. 5(a)).



**Fig. 5** 21-day DMI-treated rats after intra-amygdala infusion of the MAPK activator, ATA, had a significant restoration in the amygdala phosphorylated Erk1/2 levels. (a) There was no change in the total amount of Erk1/2 in each group. (b) The amygdala phosphorylated Erk1/2 levels in the 21-day DMI and ATA-treated rats showed a significant restoration (\*\*  $P < 0.01$ , compared to 21-day DMI-treated rats). (n=5 in each group, white bars: Erk1; black bars: Erk2).

#### 4. Discussion

Herein we have been the first to demonstrate that amygdala phosphorylated Erk1/2 may be involved in the anti-immobility effect of long-term DMI treatment. The presence of this statement was shown by the following results. First, 21-day DMI-treated rats but not 5-day DMI-treated rats had an obvious decrease in the IMT on the FST. Second, the amygdala phosphorylated Erk1/2 levels were changed only in 21-day DMI-treated rats. Third, the amygdala phosphorylated Erk1/2 levels decreased in parallel with the IMT reduction in 21-day DMI-treated rats. Fourth, no recovery of IMT and no recovery of the amygdala Erk1/2 levels was seen in 21-day DMI-treated rats after a 2-day washout period. Fifth, when given ATA, an intra-amygdala MAPK activator, the 21-day DMI-treated rats recovered the previously decreased IMT on the FST. Lastly, the decreased amygdala phosphorylated Erk1/2 levels of 21-day DMI-treated rats were restored with ATA treatment.

Whether the different ages (day 15 and day 31) of rats receiving the DMI treatment could influence the effects of DMI on rats was a concern. In our additional age-matched control experiment, 15-

day-old rats receiving DMI for 5 days expressed no significant alteration in IMT in FST compared to that of the 21-day-old control group ( $30 \pm 15$  sec versus  $43 \pm 10.0$  sec,  $p > 0.2$ ,  $n = 6$  in each group). The results suggested that 21-day DMI treatment per se was more effective on the reduced immobile behavior of rats in the FST rather than on the early developmental effect of rats.

Einat et al. revealed that the blockade of the Erk pathway in the central nervous system may induce an anti-immobility effect on rats in the FST [14]. Similarly, our previous study also showed that the immobility behavior of rats in the FST may be modulated by the Erk1/2 phosphorylation in the amygdala [18]. The present study, moreover, could also verify the regulatory role of Erk1/2 phosphorylation in the amygdala on the immobility behavior in the FST by the anti-immobility effect of long-term DMI's treatment on rats. From the above results, not only the relationship between the immobility behavior of rats in the FST and Erk1/2 signaling in the amygdala could be identified further, but also Erk1/2 signaling in the amygdala could be a new insight into the mechanisms of antidepressants [4].

In figure 3, the 21-day DMI-treated rats were given intra-amygdala ATA, they recovered from their decreased IMT significantly, although the recovery of the IMT did not reach as well as control levels. In order to confirm that the effects of the recovery of decreased IMT were due to the treatments of ATA on the amygdala region, the location of cannula tips on the lateral or basolateral amygdala were recorded and the data showed that there was no difference between the effects of ATA on the lateral and basolateral amygdala regions (Fig.4). This finding strengthens the correlation between DMI's mechanism of action and Erk1/2. Furthermore, the amygdala Erk1/2 phosphorylation noted in the ATA group provides stronger evidence that DMI's mechanism of action is related to Erk1/2 (Fig.5).

#### 5. Conclusion

Consequently, this study's finding relating to the role that Erk1/2 play in the mechanism of action of DMI does not only provide new information about the pharmacology of antidepressants, but also provides a profound insight into the development of new antidepressants from compounds known to perturb Erk1/2 phosphorylation in specific brain regions.

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## Chemoembolization through lateral sacral artery to treat uterus broad ligament pregnancy

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**Abstract:** The objective was to evaluate the clinical effect of interventional therapy of uterus broad ligament pregnancy. One case of uterus broad ligament pregnancy was performed celioscope exploration then turning to open operation. Because the gestation sac was near the ilio- vessels and the operation risk was very high, it was not disposed at last. By chemoembolization of the blood supply vessel (lateral sacral artery) of gestation sac, embryo was killed rapidly. So interventional therapy was an effective method to treat uterus broad ligament pregnancy.

[ZHANG Jian-hao, GUAN Sheng. **Chemoembolization through lateral sacral artery to treat uterus broad ligament pregnancy.** Life Science Journal. 2012;9(2):36-38](ISSN:1097-8135).<http://www.lifesciencesite.com>.7

**Keywords:** uterus broad ligament pregnancy; lateral sacral artery; chemoembolization

### 1. Introduction

Ectopic pregnancy is one kind of common acute abdomen in obstetric and gynecologic department and it is a kind of main cause of death among gravida and puerpera. Uterus broad ligament pregnancy is quite rare, especially occurred in senior age and kakogenic women. The possible pathogenesis may be that the continuous pregnancy of gestation sac between the two leaves of broad ligament after the rupture of fallopian pregnancy. Most uterus broad ligament pregnancy can be cured by performing laparoscope or open operation for exploration and focal cleaning with high success rate. The application of interventional therapy provides a new approach to treat ectopic pregnancy. The application of uterine artery embolization to cure postpartum hemorrhage and gynecological oncology has a history of decades. In recent years, this technology is adopted to treat cervical pregnancy and has gotten good effectiveness. Interventional therapy can cure uterus broad ligament pregnancy. There is no similar report in China. By chemoembolization of the blood supply vessel of gestation sac embryo can be killed and serum  $\beta$ -HCG descends rapidly. Meanwhile it has some advantages as follows: the risk of hemorrhage is largely reduced; the operation time is shorten; the side effects is minimized; the trauma and anesthetic risks of laparotomy are avoided.

### 2. Materials and methods

A 31-year-old woman presented with menelipsis 58 days, was treated with exploratory laparotomy before 1 day. Her last menstrual period had been more than 1 month earlier, she felt hypogastralgia without incentive and urine HCG was positive. So she went to the local hospital and the transvaginal ultrasonography hint that there was an ectopic pregnancy at the left adnexa and a little hydrops in cavity of uterus. The corporin of serum was 18.1ug/l and  $\beta$ -HCG was

38911mIU/L. “Left tubal pregnancy” was diagnosed and emergent celioscope exploration was performed. A hyacinthine mass with 40×30×20mm in left broad ligment was confirmed and near to the vessels and ureter. The doctors worried about massive hemorrhage during the dissection, so exploratory laparotomy was performed. It was found that retroperitoneal hyacinthine mass was near the ilio- vessels and the operation risk was very high. So they closed the abdomen without disposing the mass.

The patient was taken to our hospital in the way of emergency. Physical examination: vital signs stable; there was a vertical surgical scar of 10cm long covered by surgical dressing with obvious tenderness, without rebound tenderness. The ultrasound of our hospital found: the body of uterus was about 47×45×43mm; the cervix was 31mm long; the echo of muscular layer is uniformity; the separation of uterine cavity was about 8mm; the left ovary was about 34×16mm and there was no obvious abnormal echo; the right ovary was about 40×29mm including a cystic echo mass of 24× 24mm; there was a low level echo area of 36×20mm with abundant blood stream signal including a sac echo about 26×13mm nearby the left ilio-vessels. A 17mm-long embryo bud could be seen in this sac echo without pulse; there was an unregular fluidity dark area as deep as 13mm in cavum Douglasi. The result of serum  $\beta$ -HCG was 30708 m IU/L.

Since the gestation sac was located nearby ilio-vessels and the value of  $\beta$ -HCG was high, it presented that the embryo activity was high. The gestation sac may rupture at any time or invade ilio-sacral vessels causing fetal massive hemorrhage. Emergency abdominal aortography (pictures 1)and bilateral internal iliac arteries angiography were performed. There was a cluster of abnormal staining at the left pelvic cavity according to the left internal iliac angiography, contrast media was dense. We

considered it was an ectopic pregnancy nidus, whose blood supply was from the left lateral sacral artery (pictures 2). The volume of uterus augmented round, supplied by bilateral uterine arteries. So micro-catheter was placed to the left internal iliac artery – lateral sacral artery – gestation sac supply artery for angiography (pictures 3), and MTX 80mg diluted with NS 100mL was perfused slowly, then 350-560um Gelatin Sponge particles was used to embolize the artery until the blood stream was blocked (pictures 4). The patient had no special complaint after the interventional therapy. She was treated with infection prevention, pain relief and symptomatic treatment.

### 3. Results

We rechecked the  $\beta$ -HCG every 3 days. The results were 17265 mIU/L, 12219 mIU/L, 6419 mIU/L, 4577 mIU/L, 2539 mIU/L, 1131 mIU/L, 631 mIU/L, 381 mIU/L, 156 mIU/L, 89 mIU/L, 48 mIU/L, 29 mIU/L (reference value is 0-35 mIU/L). After the value of  $\beta$ -HCG descended to normal, color Doppler ultrasonography showed that: there was low level echo mass with the size of 29×18mm at left ilio-vessels, with a sac echo about 26×11mm, without obvious blood stream signal around. Chinese medicine was taken orally to promote mass absorption, after 45 days  $\beta$ -HCG was 21mIU/L, the patient recovered and discharged.

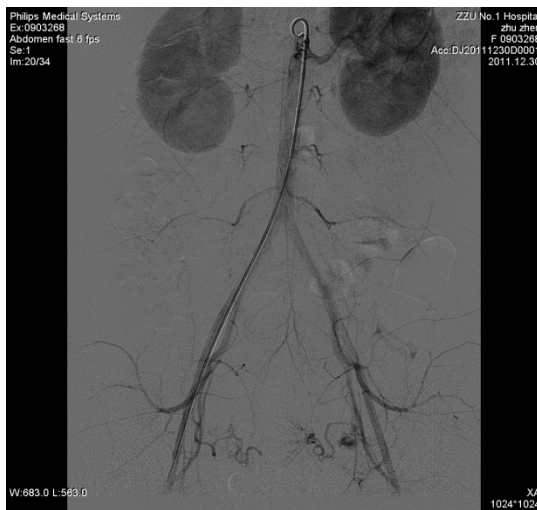


Figure 1. Through abdominal aortography there was a cluster abnormal staining at the left pelvic cavity



Figure 2. Its blood supply was from the left sacral artery

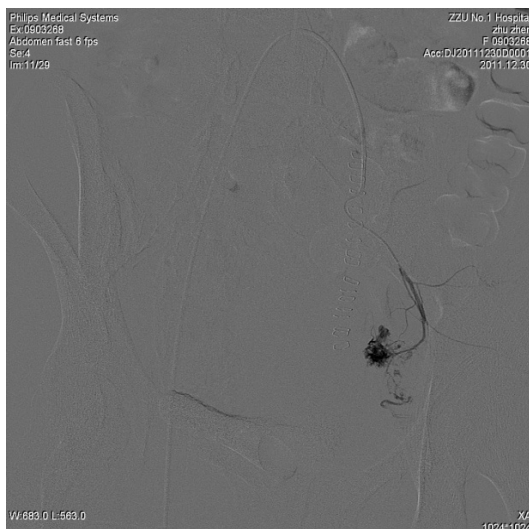


Figure 3. Angiography through micro-catheter before embolization

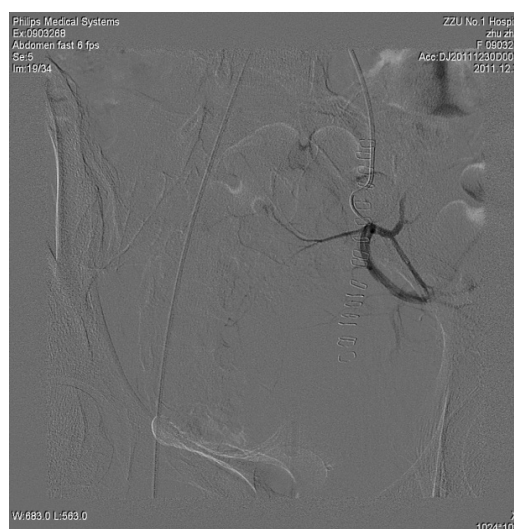


Figure 4. Angiography through micro-catheter after embolization

#### 4. Discussions

Lateral sacral artery is a branch of internal iliac artery, supplying the blood to sacrum. Most lateral sacral arteries are from internal iliac artery directly, some are from superior gluteal artery or inferior gluteal artery. Lateral sacral artery is divided into two branches. The superior branch goes into the first anterior sacral foramina stretching as dorsal branches. The inferior is pachy. It goes to inside and down across the surface of sacral plexus, descending between sacral anterior foramina and sympathetic trunk, extending some branches into the 2-4 anterior sacral foramina, stretching as dorsal branches. Ectopic pregnancy is one kind of common acute abdomen in obstetric and gynecologic department with the incidence of 1/100 (Le Jie, 2008) and it is a kind of main cause of death among gravida and puerpera. Uterus broad ligament pregnancy is quite rare with the incidence of 1/245 of ectopic pregnancy(Wang Shu-zhen, 1987). Most intraligamentous pregnancy can be cured by performing laparoscope or open operation for exploration and focal cleaning with high success rate. The application of interventional therapy provides a new approach to curing ectopic pregnancy. The application of uterine artery embolization to cure postpartum hemorrhage and gynecological oncology has a history of decades. Worldwide scholars are taking this technology into the treatment of cervical pregnancy and have gotten good effectiveness (Yu B et al.,2009) (Liu Y,2004). Interventional therapy cures intraligamentous pregnancy. By chemoembolization of the blood supply vessel of gestation sac embryo can be killed and serum  $\beta$ -HCG descends rapidly.

In this case, celioscope exploration then turning to open operation was routinely performed without the

disposal of focus. The special point was that gestation sac located at the left broad ligament near to ilio-vessels, which had extremely surgical risk. Emergency angiography showed the blood of gestation sac was supplied from the left lateral sacral artery. So chemoembolization was performed, avoiding massive hemorrhage caused by gestation sac rupture.  $\beta$ -HCG was decreasing to normal level and it achieved the effect of clinical cure.

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**Psychological Studies for Women and Men with Sexual Dysfunction**Peimaneh Nemati<sup>1\*</sup>, Soori H.<sup>2</sup>, Seyedreza Haghi<sup>3</sup>,Fahimeh Fallahzadeh Tafti<sup>4</sup><sup>1</sup> Department of Psychology, Mashhad Branch, Islamic Azad University, Mashhad, Iran<sup>2</sup> Department of Epidemiology Safety Promotion and Injury Prevention Research Center-Shahid Beheshti University, Tehran, Iran<sup>3</sup> Department of Management, Mashhad Branch, Islamic Azad University, Mashhad, Iran<sup>4</sup> Department of Psychology, Mashhad Branch, Islamic Azad University, Mashhad, Iran  
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**Abstract:** The prevalence of female and male sexual dysfunction is high and it may significantly affect self-esteem and quality of life. Even sexual dysfunction of short duration can create frustration and anguish. When chronic, it may lead to anxiety and depression, harm relationships, and cause problems in other aspects of life. The goal of the present research is to study the effect of cognitive-behavioral counseling on the level of anxiety in women and men with sexual dysfunction. In this research, Cognitive behavior therapy during 8 individual weekly sessions and 4 group therapy weekly sessions used for subjects group and it focused on cognitive restructuring, modification of cognitive distortions and training of behavioral techniques. Spilburger's Anxiety questionnaire was used as the pre-test and post-test for subject group. Finally data analysis will be shown that the cognitive behavior therapy has significantly effect on reduction of anxiety. The mean scores of anxiety in post-test of subject group were significantly lower than mean scores in pre-test. Cognitive counseling as a therapeutic method can have a significant role in improvement of women and men suffering from anxiety which is resulted from sexual dysfunction.

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**Keywords:** Sexual Dysfunction, Anxiety, Cognitive Behavior Counseling

**1. Introduction**

Female sexual dysfunction (FSD) is defined as persistent or recurring decrease in sexual desire, persistent or recurring decrease in sexual arousal, dyspareunia and a difficulty in or inability to achieve an orgasm (Basson, 2000). The prevalence of female sexual dysfunction is high, ranging from 43% to 88% (Dennerstein, 2002). It may significantly affect self-esteem and quality of life. Even sexual dysfunction of short duration can create frustration and anguish. When chronic, it may lead to anxiety and depression, harm relationships, and cause problems in other aspects of life (Dennerstein, 2005). Several factors, including interpersonal, psychological, physiological, medical, social and cultural variables, have been shown to correlate with sexual dysfunctions (Bradford, 2007). Anxiety disorders can preclude women's ability to attend to sexual stimuli and to be lost in the moment (Meston, 2004). The anxiety resulting from sexual functioning put people in trouble psychologically. Instead of focusing on sexual arousal stimuli, one involves in a sense of anxiety concerning sexual functioning (Adams, 1985). The results of a comprehensive research by Holvorsen and Metz on various methods of treating sexual disorders showed that the most prevalent

methods for psychopathic treatment of sexual malfunctioning that have been in practice from 1996 onward include sensory focus, CBT (Cognitive Behavior Therapy), relaxation practice, hypnosis, and group therapy; the results also showed the above-mentioned treatments have achieved considerable results in improvement of different sexual disorders like sexual idiosyncrasy in women (Kabakci, 2003). Cognitive behavior therapy focuses on decreasing anxiety and promoting changes in attitudes and sexual thoughts, which increase the ability to achieve orgasm and to gain satisfaction from orgasm (Soykan, 2005). The goal of the present research is to study the effect of cognitive-behavioral counseling on the level of anxiety in women and men with sexual dysfunction. The assumption was based on the fact that the method can alleviate the anxiety which is one of the co-morbidities of sexual dysfunction.

**Basic Definitions:**

Sexual dysfunction or sexual malfunction refers to a difficulty experienced by an individual or a couple during any stage of a normal sexual activity, including desire, arousal or orgasm. To maximize the benefits of medications and behavioral techniques in the management of sexual dysfunction it is important

to have a comprehensive approach to the problem. A thorough sexual history and assessment of general health and other sexual problems (if any) are very important.

Assessing (performance) anxiety, guilt (associated with masturbation in many Indian men), stress and worry are integral to the optimal management of sexual dysfunction. When a sexual problem is managed inappropriately or sub-optimally, it is very likely that the condition will subside immediately but re-emerge after a while this cycle continues, it strongly reinforces failure that eventually make clients not to access any help and suffer it all their life. So, it is important to get a thorough assessment from professionals and therapists who are qualified to manage sexual problems. Internet-based information is good for gaining knowledge about sexual functioning and sexual problem but not for self-diagnosis and/or self-management. Sexual dysfunction disorders may be classified into four categories: sexual desire disorders, arousal disorders, orgasm disorders and pain disorders.

**Sexual desire disorders** or decreased libido are characterized by a lack or absence for some period of time of sexual desire or libido for sexual or of sexual fantasies. The condition ranges from a general lack of sexual desire to a lack of sexual desire for the current partner. The condition may have started after a period of normal sexual functioning or the person may always have had no/low sexual desire. The causes vary considerably, but include a possible decrease in the production of normal estrogen in women or testosterone in both men and women. Other causes may be aging, fatigue, pregnancy, medications (such as the SSRIs) or psychiatric conditions, such as depression and anxiety. Loss of libido from SSRIs usually reverses after SSRIs are discontinued, but in some cases it does not. This has been called PSSD; however, this is not a classification that would be found in any current medical text. While a number of causes for low sexual desire are often cited, only some of these have ever been the object of empirical research. Many rely entirely on the impressions of therapists.

**Sexual arousal disorders** were previously known as frigidity in women and impotence in men, though these have now been replaced with less judgmental terms. Impotence is now known as erectile dysfunction, and frigidity has been replaced with a number of terms describing specific problems with, for example, desire or arousal.

For both men and women, these conditions can manifest themselves as an aversion to, and avoidance of, sexual contact with a partner. In men, there may be partial or complete failure to attain or maintain an

erection, or a lack of sexual excitement and pleasure in sexual activity.

There may be medical causes to these disorders, such as decreased blood flow or lack of vaginal lubrication. Chronic disease can also contribute, as well as the nature of the relationship between the partners. Unlike disorders of orgasm, as the success of Viagra attests, most erectile disorders in men are primarily physical conditions.

**Erectile dysfunction or impotence** is a sexual dysfunction characterized by the inability to develop or maintain an erection of the penis. There are various underlying causes, such as damage to the nervi erigentes which prevents or delays erection, or diabetes, which simply decreases blood flow to the tissue in the penis, many of which are medically reversible.

The causes of erectile dysfunction may be psychological or physical. Psychological impotence can often be helped by almost anything that the patient believes in; there is a very strong placebo effect. Physical damage is much more severe. One leading physical cause of ED is continual or severe damage taken to the nervi erigentes. These nerves course beside the prostate arising from the sacral plexus and can be damaged in prostatic and colorectal surgeries. Due to its embarrassing nature and the shame felt by sufferers, the subject was taboo for a long time, and is the subject of many urban legends. Folk remedies have long been advocated, with some being advertised widely since the 1930s. The introduction of perhaps the first pharmacologically effective remedy for impotence, sildenafil (trade name Viagra), in the 1990s caused a wave of public attention, propelled in part by the news-worthiness of stories about it and heavy advertising. The Latin term impotentia coeundi describes simple inability to insert the penis into the vagina. It is now mostly replaced by more precise terms.

**Orgasm disorders** are persistent delays or absence of orgasm following a normal sexual excitement phase. The disorder can have physical, psychological, or pharmacological origins. SSRI antidepressants are a common pharmaceutical culprit, as they can delay orgasm or eliminate it entirely.

**Sexual pain disorders** affect women almost exclusively and are known as dyspareunia (painful intercourse) or vaginismus (an involuntary spasm of the muscles of the vaginal wall that interferes with intercourse). Dyspareunia may be caused by insufficient lubrication (vaginal dryness) in women. Poor lubrication may result from insufficient excitement and stimulation, or from hormonal changes caused by menopause, pregnancy, or breastfeeding. Irritation from contraceptive creams and foams can also cause dryness, as can fear and anxiety

about sex. It is unclear exactly what causes vaginismus, but it is thought that past sexual trauma (such as rape or abuse) may play a role. Another female sexual pain disorder is called vulvodynia or vulvar vestibulitis. In this condition, women experience burning pain during sex which seems to be related to problems with the skin in the vulvar and vaginal areas. The cause is unknown.

**Uncommon sexual disorders in men,** Erectile dysfunction from vascular disease is usually seen only amongst elderly individuals who have atherosclerosis. Vascular disease is common in individuals who have diabetes, peripheral vascular disease, hypertension and those who smoke. Any time blood flow to the penis is impaired, erectile dysfunction is the end result. Hormone deficiency is a relatively rare cause of erectile dysfunction. In individuals with testicular failure like Klinefelter's syndrome, or those who have had radiation therapy, chemotherapy or childhood exposure to mumps virus, the testes may fail and not produce testosterone. Other hormonal causes of erectile failure include brain tumors, hyperthyroidism, hypothyroidism or disorders of the adrenal gland. Structural abnormalities of the penis like Peyronie's disease can make sexual intercourse difficult. The disease is characterized by thick fibrous bands in the penis which leads to a deformed-looking penis. Drugs are also a cause of erectile dysfunction. Individuals who take drugs to lower blood pressure, use antipsychotics, antidepressants, sedatives, narcotics, antacids or alcohol can have problems with sexual function and loss of libido.

**Priapism** is a painful erection that occurs for several hours and occurs in the absence of sexual stimulation. This condition develops when blood gets trapped in the penis and is unable to drain out. If the condition is not promptly treated, it can lead to severe scarring and permanent loss of erectile function. The disorder occurs in young men and children. Individuals with sickle-cell disease and those who abuse certain medications can often develop this disorder.

## 2. Material and Methods

The subjects included 20 persons men and women aged 25-45 years old with sexual dysfunction who had referred to a Hospital in Tehran, the capital of IRAN. First the demographic questionnaire, together with Spilburger's Anxiety questionnaire, were filled by the subjects in order to measure their level of anxiety. This questionnaire was presented by Spilburger et al. in 1970, and was renewed in 1983. The questionnaire measures the anxiety in two scales of situation and trait. The Chronbach's Alpha coefficient in the scale of situation was reported

0.92%, and the corresponding coefficient for trait was 0.90 %. The questionnaire includes 40 questions, and questions 1-20 assess the anxiety of situation. Each question is followed by four options- *never, sometimes, often, very often*. Questions 21-40 deal with anxiety of trait consisting of four options: *almost never, sometimes, most often, and almost always*. The scores of 20-30 signify low level of anxiety, and scores 31-45 denote medium level of anxiety, and eventually the scores above 46 indicate high level of anxiety.

After conducting the test, subjects group underwent cognitive-behavioral treatment (CBT), which consisted of 4 groups and 8 individual sessions. The sessions were decided to be twice a week, and each session lasted one-and-a-half hours. Throughout the session the focus was mainly on cognitive restructuring, modification of cognitive distortions, and training of behavioral techniques such as relaxation education.

Following the counseling sessions, they sat a post-test, and SPSS software, version 18, and Chi-Square test together with T-test were used to analyze the data.

**Protocol of implementation of cognitive-behavioral therapy:** First session of group counseling: the aim of this session was introduction, and assessing the level of the subjects' awareness of sexual behavior.

Second session of group counseling: this session aimed at teaching sexual behavior and giving information, and focused on teaching the relaxation skill in order to reduce their anxiety in intercourse. Third session of group counseling: this session focused on analyzing the wrong images as well as suppositions of the subjects by themselves, and learning some skills and doing some assignments. Fourth session of group counseling: in this session all the subjects' questions were answered, and all the previous subjects were reviewed.

Following the group counseling sessions, since they did not feel free to put forward some of their problems, 8 individual counseling sessions were organized with the following goals:

The first session focused on individual interviews, assessment of their manner of intercourse, and determining the problem. In the second session, false negative views and thoughts that often lead to the expression of negative feelings towards sexual issues were discussed. The purpose of the third session was further cognitive reconstruction in the subjects. In the fourth session, the main objective was sensual focus type II, as well as training the Kegel exercises. During the fifth session, penetration without orgasm, and self-stimulation was practiced, and in the sixth session, reaching orgasm was

practiced in the presence of their spouse, and some other assignments. The aim of the seventh session was individual counseling, intercourse, and orgasm; and eventually, in the last session, all the material covered during the previous sessions were reviewed and conclusions were drawn. The subjects were categorized and assigned to each level of the counseling process depending on the nature of their problems.

### 3. Results

Considering the results gained from demographic questionnaire, the average age for the subjects was determined 32years. 60% of the subjects group had middle school education; 25% of them had high school diploma, and 10% of them had bachelor degree. 5% in subjects group had primary level of education. Also, 60% of subjects group were housewives, while 35% in subjects groups were office employees, and finally, 5% of them were retired. Regarding their economic status, 60% in subject group had an average economic situation; 30% of them had bad economic situation, and 10% of them, had a decent economic state. The results can be seen in the following tables 1 and 2.

**Table1:** Distribution frequency scores demographic data in subjects group

	Variables	Frequency	Percent
Education	Primitive	1	%5
	High school	12	%60
	Diploma	5	%25
Occupation	Bachelor	2	%10
	Housewife	12	%60
	Employee	7	%35
Economy	Retired	1	%5
	Bad	6	%30
	Moderate	12	%60
	Good	2	%10

**Table2:** distribution frequency and compare mean scores situational anxiety and trait anxiety before and after CBT in subjects group

Groups	No	Mean	STD	T	DF	Sig
Situational-pre	20	62.9	7.226	0.09	38	0.93
Situationalpost	20	35.11	6.189	13.12	38	0.000
Trait-pre	20	62.60	7.598	0.243	38	0.81
Trait-post	20	35	8.349	11.07	37	0.000

As it can be seen from the table2, the average Pre-test score for situation anxiety for subjects group was 62.9, and it was high; however, the average score for the Post-test concerning the situation anxiety was 35.11, which means there has been a considerable difference between the pre-test and the Post-test ( $P < 0.05$ ) in subjects group. The difference in figures, in fact, denotes a decrease in anxiety in subjects group and effectiveness of the interference. Also, it was concluded that the average Pre-test score for Trait Anxiety was 62.60 and they had a high level of Trait anxiety. In contrast, the average Post-test scores for

Trait Anxiety were quite different: 35 in subjects group. This implies a significant difference between the pre-test and the Post-test ( $P < 0.05$ ) and a reduction of anxiety as well as effectiveness of interference.

### 4. Discussions

There are many factors which may result in a person experiencing a sexual dysfunction. These may result from emotional or physical causes. Sexual dysfunction may arise from emotional factors, including interpersonal or psychological problems. Interpersonal problems may arise from marital or relationship problems, or from a lack of trust and open communication between partners, and psychological problems may be the result of depression, sexual fears or guilt, past sexual trauma, sexual disorders among others.

Sexual dysfunction is especially common among people who have anxiety disorders. Ordinary anxiousness can obviously cause erectile dysfunction in men without psychiatric problems, but clinically diagnosable disorders such as panic disorder commonly cause avoidance of intercourse and premature ejaculation. Pain during intercourse is often a co-morbidity of anxiety disorders among women.

Sexual activity may also be impacted by physical factors. These would include use of drugs, such as alcohol, nicotine, narcotics, stimulants, antihypertensive, antihistamines, and some psychotherapeutic drugs. For women, almost any physiological change that affects the reproductive system—premenstrual syndrome, pregnancy, postpartum, menopause—can have an adverse effect on libido. Injuries to the back may also impact sexual activity, as would problems with an enlarged prostate gland, problems with blood supply, nerve damage (as in spinal cord injuries). Disease, such as diabetic neuropathy, multiple sclerosis, tumors, and, rarely, tertiary syphilis may also impact on the activity, as would failure of various organ systems (such as the heart and lungs), endocrine disorders (thyroid, pituitary, or adrenal glandproblems), hormonal deficiencies (low testosterone, estrogen, or androgens), and some birth defects.

Since in many men the cause of sexual dysfunction is related to anxiety about performance, psychotherapy can help. Situational anxiety arises from an earlier bad incident or lack of experience. This anxiety often leads to development of fear towards sexual activity and avoidance. In return evading leads to a cycle of increased anxiety and desensitization of the penis. In some cases, erectile dysfunction may be due to marital disharmony. Marriage counseling sessions are recommended in this situation.

Lifestyle changes such as discontinuing smoking, drug or alcohol abuse can also help in some types of erectile dysfunction. Several medications like Viagra, cialis and Levitra have become available to help people with erectile dysfunction. These medications do work in about 60% of men. In the rest, the medications may not work because of wrong diagnosis or chronic history.

Another type of medication that is effective in roughly 85% of men is called intracavernous pharmacotherapy — used by companies such as Boston Medical Group, Performance Medical Centers and independent doctors — and involves injecting a vasodilator drug directly into the penis in order to stimulate an erection. Although there are no approved pharmaceuticals for addressing female sexual disorders, several are under investigation for their effectiveness. A vacuum device is the only approved medical device for arousal and orgasm disorders. It is designed to increase blood flow to the clitoris and external genitalia. Women experiencing pain with intercourse are often prescribed pain relievers or desensitizing agents. Others are prescribed lubricants and/or hormone therapy. Many patients with female sexual dysfunction are often also referred to a counselor or therapist for psychosocial counseling.

A manual physical therapy, the Wurn Technique, which is designed to reduce pelvic and vaginal adhesion, may also be beneficial for women experiencing sexual pain and dysfunction. In a controlled study, *Increasing orgasm and decreasing intercourse pain by a manual physical therapy technique*, twenty-three (23) women reporting painful intercourse and/or sexual dysfunction received a 20-hour program of manipulative physical therapy. The results were compared using the validated Female Sexual Function Index, with post-test vs. pretest scores. Results of therapy showed statistically significant improvements in all six recognized domains of sexual dysfunction. A second study to improve sexual function in patients with endometriosis showed similar statistical results.

Results of the research concerning the persons who referred to a Hospital in Tehran showed in subject groups, the level of anxiety was high and acute; also the group and individual counseling sessions offered to them had significant effect on reduction of anxiety for both Situation and Trait.

Planning the details of the method of intercourse, and also discussion around fears, anxieties and concerns, coming over the sense of guilt, existing misunderstandings, as well as correcting the misconceptions about sexual behavior, and finally the radical alteration of women's view to

sex and sexual act are among the many issues that justify the effectiveness of this therapeutic method.

The findings of the present research corresponds with the results of another study implying that those who enjoyed this type of counseling experienced a significant drop in their level of anxiety (Kabakci , 2003). It also corresponded with the results of another research concluding the effectiveness of cognitive-behavioral treatment for sexual disorders in Vaginistic women and specific phobia of female diseases, and anxiety (Crespo, 2004). In another research, cognitive-behavioral counseling was conducted to promote the sexual intercourse, and reduce the anxiety and fear of sex act, the results of which corresponds with the present study (Turkuile , 2007).

In the studies conducted by Mehrabi, Jaberi and Mehryar on assessing the level of effectiveness of cognitive-behavioral treatments concerning the women inflicted with the sex-phobia disorder, as well as studying the sex intercourse that was conducted, the results showed that as a result of cognitive-behavioral treatment, the level of anxiety in the subjects reduced considerably, and their efforts to have more intercourse was successful. The results of the research also corresponded with the present study (Mehrabi, 1999). Therefore, it is recommended that longer similar therapeutic methods and more number of sessions be organized and conducted, and in order to monitor the consistency of the treatment effects, follow-up tests be performed at various intervals, following the termination of the therapeutic interference. Since the subject who referred to the hospital was limited, there any kind of generalization must be cautioned.

As it was mentioned, sexual disorder has had high prevalence among women and caused several problems in their personal life including anxiety and depression, as well as in their inter-personal relations, and as it was noticed, individual cognitive counseling as a therapeutic method can have a significant role in improvement of people suffering from anxiety which is resulted from sexual dysfunction.

**Table 1:** Distribution frequency scores demographic data in subjects group.

	Variables	Frequency	Percent
<b>Education</b>	Primitive	1	%5
	High school	11	%55
	Diploma	6	%30
	Bachelor	2	%10
<b>Occupation</b>	Housewife	15	%75
	Employer	4	%20
	Retired	1	%5
<b>Economy</b>	Bad	7	%35
	Moderate	12	%60
	Good	1	%5



**Table2:** distribution frequency and compare mean scores situational anxiety and trait anxiety before and after CBT in subjects group

Groups	No.	Mean	STD	T	DF	Sig
<i>Situational-pre</i>	20	63	7.226	0.089	38	0.929
<i>Situational-post</i>	20	35.10	6.189	-13.116	38	0.000
<i>Trait-pre</i>	20	62.60	7.598	0.243	38	0.809
<i>Trait-post</i>	20	34.15	8.349	-11.074	37	0.000

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1/26/12

## Design and Development of Semantic Web Information System (WIS) for Virtual University of Pakistan

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**Abstract:** Semantic web is an extension of current web which defines the meaning of information in such a way so that it can be understandable by the machines and thus machines can process the information and perform reasoning on that information. With the current web, the meanings or semantics of information on the web is only understandable to the human beings whereas the basic purpose of semantic web is to make web pages not only human as well as machine understandable. The need for design methodology was considered at a time when semantic applications were developed in an ad-hoc manner with no systematic approach or methodology used to add semantics at implementation level either using manual or automatic approach. In this paper, we have critically reviewed existing design methodologies of semantic web applications and compared these methodologies based on various attributes and found out their strengths and weaknesses. In this paper, we have proposed a design methodology for semantic web information system and validate it by using Virtual University web application as a case study.

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**Keywords:** semantic web, methodology, hypermedia, Ontology, web information system

### 1. Introduction

The semantic web is an extension of current web which defines the meaning of information in such a way so that it can be understandable by the machines and the information is defined such that it remains usable not only for machines but also for human beings. The major difference between current web and semantic web is that the current web is a huge distributed hypertext system which is a collection of interconnected documents whereas semantic web is a huge distributed knowledge based system as the information becomes knowledge after adding semantic annotations.

The development of complex web applications and the acceptance of internet have driven the ongoing demand for new and better way for the design and development of web applications. The development of complex web applications requires a disciplined approach which uses software engineering principles. Such disciplined approach is called design methodology. The design methodology tells in a systematic way how to do each steps of software development life cycle model whereas SDLC lists only the steps required to develop an application. These design methodologies integrate semantic annotations into web engineering method by defining semantic annotations at design level. The need of design methodology was considered at a time when semantic applications were developed in an ad-hoc manner with no systematic approach or methodology and used to add semantics at implementation level either using manual approach

or automatic approach. Using a design methodology, modification and maintenance of an application requires less effort and time.

The paper is organized as follows. Section 2 briefly explains some of the existing design methodologies of semantic web applications. The strengths and weaknesses of these design methods have also been given in same section. The comparison of these design methods based on various parameters is given in Section 3. Our proposed design methodology is given in Section 4. In Section 5, our proposed design methodology is validated via a case study. Finally, results, conclusion and future work has been given in Section 6.

### 2. Literature Survey

In the literature survey, we have found three types of approaches for the design and development of semantic web applications: Manual approach, Automatic approach and Web engineering approach/Design Methodology (Ambler, 2002). The earlier annotation systems such as SHOE, MindSwap and CREAM were based on manual approach and used to add semantics to HTML pages manually but this manual approach leads to many syntax errors by human beings and it was a tedious and cumbersome task. Then automatic approaches were introduced which uses graphical user interface to add semantic annotation to web sites (Marcos, 2006). These graphical user interfaces are SHOE Knowledge Annotator, SMORE (Semantic Markup, Ontology and RDF editor) and CREAM. From these GUI,

SHOE Knowledge Annotator and SMORE are suitable for annotating static web sites whereas CREAM is suitable for dynamic web sites. Both of these approaches (manual and automatic) had disadvantage that semantic annotations were defined at implementation level and it requires considerable effort to generate semantic contents after the web site is fully implemented. This problem can be solved by

integrating the annotation process into web engineering method which defines the annotation at design level (Jaeger et al., 2005). Web engineering approach or design method is a disciplined and systematic approach which uses engineering principles for the development, deployment and maintenance of web applications.

**Table 1: Comparison of existing Semantic-web applications design methods**

	<b>WSDM</b>	<b>HERA</b>	<b>SHDM</b>	<b>OntoWeaver</b>	<b>OntoWebber</b>	<b>SEAL</b>
Phases/Modules	5	2	5	4	5	9
Methodology	Audience driven Web-engineering	Model driven Web-engineering	Model driven Web-engineering	Model driven Web-engineering	Model driven Web-engineering	Web-engineering
Extension	WSDM (Web Site Design Method)	NA	OOHDM (Object-oriented hypermedia design)	NA	NA	NA
Number of layers supported if a Layered Architecture	×	3	4	×	4	×
Suitable for web application	Localized websites	Semantic WIS / Customized web applications	Semantic WIS	Customized data-intensive web applications	Customized web applications/ data intensive applications/ semantic web community portals	Semantic web portals, Information Retrieval system
Supports localization of web site	√	×	×	×	×	×
Semantic Annotation process starts from	Object chunk (a data model which models the necessary information that are needed to fulfill the requirement of that elementary task)	RDF	UML like class diagram which are later mapped to RDF/XML format	RDF	RDF	RDF generator which generates RDF statements from the internal knowledge warehouse
Semantic web languages	OWL	RDF, RDF(S), RQL	ORM, DAML+OIL, OWL, RDF, RDFS, RQL	RDF	RDF, DAML+OIL	RDF
Supports different types of user	√	×	√	√	√	×
Classification of users	√	×	×	×	×	×
Supports personalization of presentation based on user preferences a design level	×	√	×	√	√	√
Support pre-defined customization or static customization	NA	√	√	×	√	NA
Support dynamic customization	NA	×	×	√	×	NA
	NA	NA	NA	√	√	NA
	NA	NA	×	NA	√	NA
	NA	NA	×	NA	NA	√
	NA	NA	×	NA	NA	√
	NA	NA	Sesame, BOR	JESS	NA	OntoBroker

### 2.1 WSDM (Web Site Design Method)

WSDM was one of the first web design method developed in 1998 by De Troyer and Leune. This methodology is an extension of WSDM (Web Site Design Method) of traditional web-based applications. It is an audience driven design methodology for the development of semantic web applications as it takes into account requirements of different types of users. The main objective of WSDM design method was to create different versions of a web site for different community or locality in order to attract more customers as different localities and communities have their own languages, standards and cultural attributes (Mark and Kevin, 2007).

### 2.2 SHDM (Semantic Hypermedia Design Method)

SHDM is a model-driven design method to develop semantic web application. It is an extension of OOHD (Object-oriented design method) therefore uses object oriented paradigm and then uses ontologies to add annotation (semantic content) to the web application (Bruijn et al., 2006). It has five different phases: Requirement gathering, conceptual design, navigational design, abstract interface design and implementation.

### 2.3 HERA

Hera is a design methodology for the design of semantic WIS (Web Information System). WIS is an information system which uses web technologies to retrieve information from the web and deliver it to users or other information system. It is a model driven design methodology which retrieves data from different data sources and presents the retrieved data in different format to different types of users based on their preferences as it supports customization of web sites (Hepp et al., 2006).

### 2.4 OntoWeaver

OntoWeaver is an ontology-driven design methodology for creating and maintaining customized web applications. Customization of web site means presenting the contents of a web site according to needs or preferences of users and different types of devices used. It is a model-driven methodology which explicitly specifies different site specification at conceptual level and then uses JESS inference engine which performs inferencing on site models to create web site in desired format according to the preferences of users at run time. The declarative nature of site specification enables designer to manage and maintain web application at conceptual level (Bruijn et al., 2006).

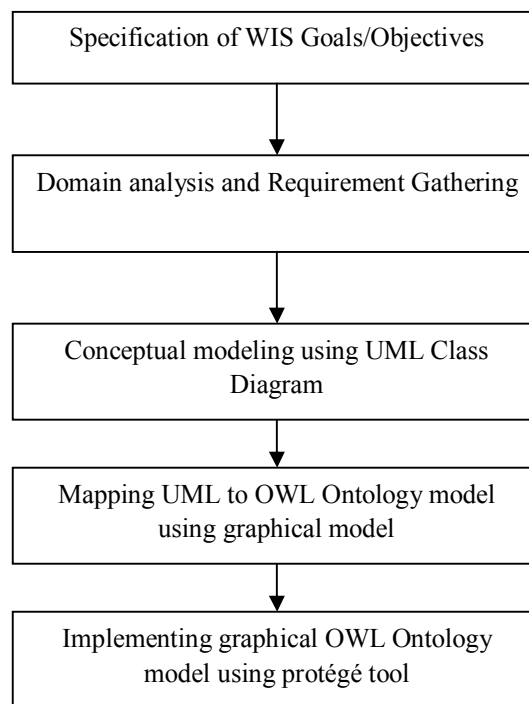
### 2.5 OntoWebber

OntoWebber is a model-driven ontology based design methodology for building data-intensive web site

and web portal. In most of the previous method, design was hard-coded in HTML, ASP, JSP, etc but this method uses re-usable components such as ontologies to design web site making the integration and maintenance of heterogeneous data sources more manageable than other methods. OntoWebber uses layered architecture consisting of 4 layers.

### 2.6 SEAL (Framework for SEMantic portAL)

SEAL is a framework for developing semantic web portals using ontologies and information retrieval concepts such as semantic browsing and semantic ranking for semantic sharing of knowledge on the web portal between human and software agents. The architecture of SEAL consists of various modules such as knowledge warehouse, OntoBroker, RDF generator, Template module, Navigation module, Query module, Semantic personalization module, semantic ranking and Web server. It supports three types of agents: software agents, community users and general users (Murthy et al., 2006). The software agents process information on the web portal using RDF Crawler.



**Fig. 1: Phases of proposed design methodology**

## 3. Proposed Design Methodology

### 3.1 Phases of proposed design methodology (Fig. 1)

- i) Specification of WIS (Web Information System) Goals/Objectives
- ii) Domain Analysis and Requirement Gathering
- iii) Conceptual modeling using UML Class Diagram

- iv) Mapping UML to OWL Ontology model using graphical model
- v) Implementing graphical OWL Ontology model using protégé tool

**i) Specification of WIS (Web Information System) Goals/Objectives:** This is the first phase of our proposed design methodology. The purpose of this phase is to specify Goals/Objectives of web application. For example, we can formulate specification of a web application as given below. As an example, we have taken Virtual University web site.

**Goals/Objectives:** “The purpose of this web information system is to provide a virtual class environment to registered student through which they can view course contents, take online video lectures, post their queries on discussion board, received answers of their queries from instructors, view uploaded assignments given by instructor, upload their solution, view grade book, view latest announcements by university”.

**ii) Domain Analysis and Requirement Gathering:** In this phase, detailed analysis of domain is performed and requirements will be gathered from user which will help in building conceptual model which will be given in next phase.

In this phase, different types of users interacting with WIS are identified and for each type of user, the different requirements are formulated. The requirements are formulated informally in natural language statements.

In our example web site, there are different types of users such as Students, Faculty members, Visitors and IT support peoples.

**iii) Conceptual modeling using UML Class Diagram:** This is the third phase of proposed design methodology. In this phase, we identify different classes, attributes of classes and relationships that exist between different classes and then construct the UML class diagram. Domain analysis and requirement gathering phase helps in identifying all constructs of UML model. UML is a graphical representation model and is used to model the domain of interest.

**iv) Mapping UML to OWL Ontology model using graphical model:** In this phase we have given the rules for transforming UML model to OWL Ontology model.

The following Table 2 gives the equivalent OWL terms/concepts and constructs of UML.

There are some additional constructs and concepts of OWL which are not present in UML.

### 3.2 Properties Restrictions

- a) Value restriction: owl:hasValue (defines a specific value a property must have)
- b) Universal Quantifier: owl:allValuesFrom (all values of this property must come from this class only)
- c) Existential Quantifier: owl:someValuesFrom (at least one value of this property must come from this class, other values can come from other classes)

**OWL Ontology Model:** The following OWL ontology model is constructed after applying rules on UML model to transform it into OWL ontology model (Fig. 2).

**Table 2: UML concepts vs. OWL concepts**

UML concepts	OWL concepts	OWL constructs
Classes	Classes/Concepts	owl:Class
Inheritance/Hierarchy	Taxonomy (subClassOf)	rdfs:subClassOf
Properties/Attributes	Data properties	owl:DatatypeProperty
Relationships among Objects/Individuals (association, composition, aggregation)	Object properties	owl:ObjectProperty
Classes for which relationship exists such as composition and aggregation	Domain of property	rdfs:domain
Classes for which relationship exists such as composition and aggregation	Range of property	rdfs:range
(Multiplicity/Cardinality)	Cardinality constraints Max cardinality Max cardinality Exact cardinality	owl:minCardinality owl:maxCardinality owl:cardinality

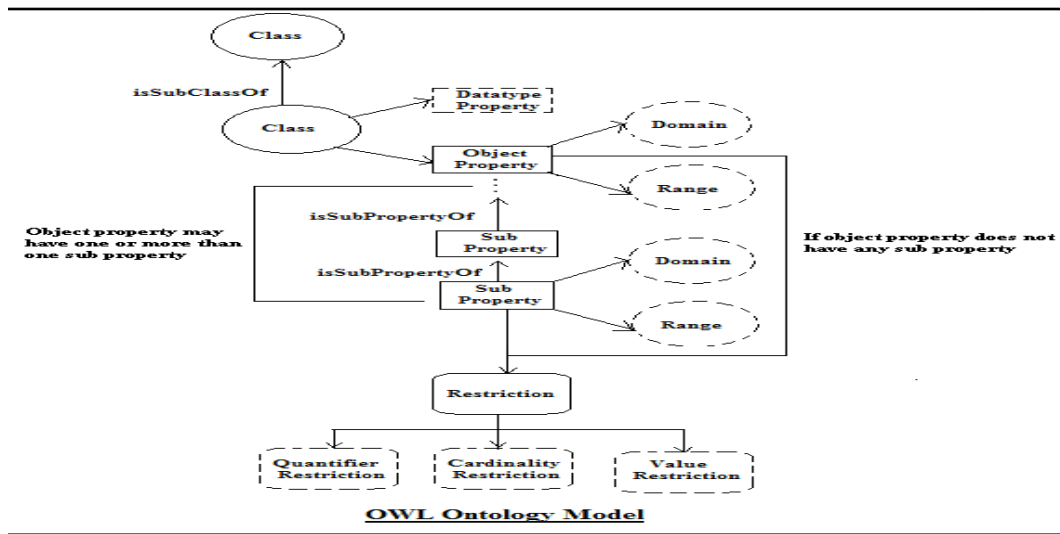


Fig. 2: OWL ontology model

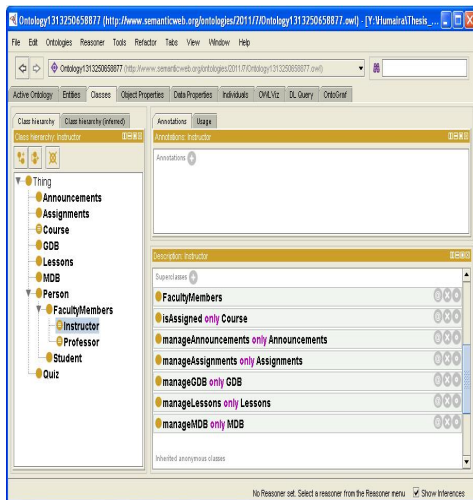


Fig. 3: Ontology class construction

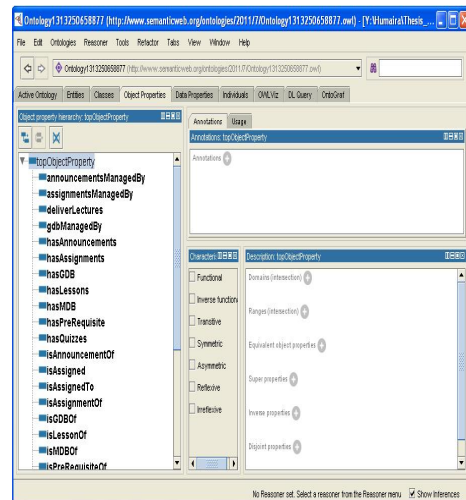


Fig. 5: Ontology object properties construction

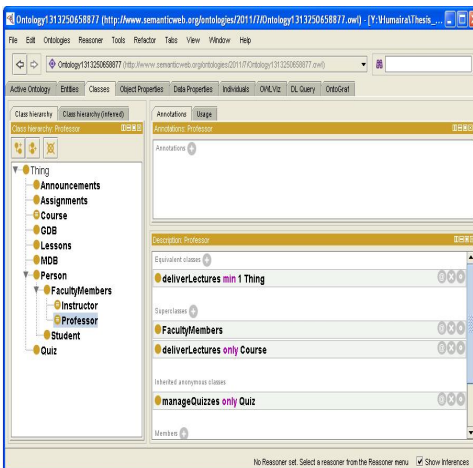


Fig. 4: Ontology class constraints

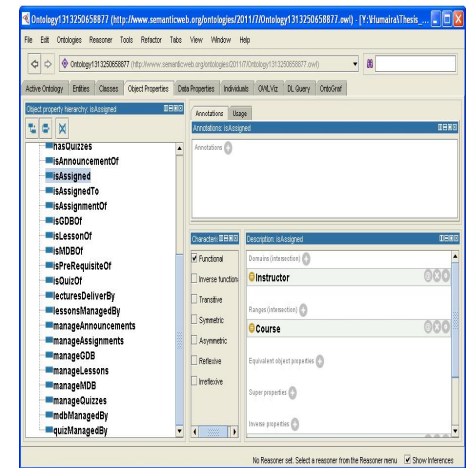


Fig. 6: Ontology object properties constraints

#### 4. Case Study

In this section, we have used Virtual University web site as a case to demonstrate the capability of design method to the design and development of semantic web information system for Virtual University of Pakistan.

The purpose of this web information system is to provide a virtual class environment to registered student through which they can view course contents, take online video lectures, post their queries on discussion board, received answers of their queries from instructors, view uploaded assignments given by instructor, upload their solution, view grade book, view latest announcements by university".

We have identified different classes, their attributes and relationships that exist between these classes which will help in developing domain model. Some screen snaps are given below (Fig. 3 – Fig. 6).

#### 5. Results: Discussions and Analysis

In Our case study, we have applied our design methodology on VULMS (Virtual University Learning Management System) of Faculty members. Faculty members have access rights to the course assigned to them. They are responsible for managing all activities of assigned course such as managing assignments, quizzes, MDB (Moderated Discussion Board), GDB (Graded Discussion Board), announcements, lessons. After developing the system, it has been validated through W3C, Validation service. Through results we are confirmed the correctness and completeness of the system.

#### 6. Conclusion and Future Work

Various design engineering methodologies have been proposed for the design and development of semantic web applications. These methodologies differ from the design methods of traditional web-based application as the design methods for semantic web applications focus on addition of semantics to web applications by enabling machines so that they can understand and process the information. Most of the design methods for semantic web applications are the extensions of traditional methods of web-based applications. These design methods have no guarantee that all software development problems will be solved but they attempt to design and develop semantic web application by applying design techniques and rules. The different methodologies that we reviewed are WSDM, HERA, SHDM, OntoWebber, OntoWeaver, SEAL, SFrameWeb, WebML, OO-H, WESSA, UWE and SW-ODDM.

#### Acknowledgment

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## **Biological Pathway based Design and Implementation of GUI Based Network Management System to Manage Live Network Nodes**

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**Abstract:** This work specifies a set of tools for the network administrators to solve specific, but common problems of LAN, focusing on management issues like fault, configuration and performance. The system is capable of remotely configuring, monitoring and managing live network nodes and serves as a platform for enhancements in order to develop a feature rich yet affordable management system. The proposed system for network management may helpful to minimize the fast intensifying network's cost and complexities by offering improved set of simple tools to the network managers or supporting workers to rapidly resolve or identify network problems. Since it is a significant requirement for the network and personnel resources management to control and manage network problems from a central locality.

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**Keywords:** LAN; GUI-based; Managing; Efficiency; Networks

### **1. Introduction**

#### **1.1 What Is Network Management?**

In literature several definitions of network management exist (Bruce, 1996) and (Gavalas et al., 2002). Today's ever growing large networks can't be handled and examined by human's effort alone. The complication of such a system motivates the need of some networks management tools which will be fully automated. The importance for having such tools is augmented and the complexity of supplying such tools is also boosted, if the networks involve stuff from multiple vendors. There are several network management tools available in the market (Partridge et al., 1988), companies like Cisco Systems, HP, Paradyne Networks and many others have developed systems of this nature but these solutions are generally very expensive with prices in several thousand dollars.

In this work, we have designed and implemented a GUI based Network Management System to manage live network equipment. The system is capable of remotely configuring, monitoring and managing live network nodes and to serve as a platform for enhancements in order to have a feature rich yet affordable management system.

The Management System for Networks is a set of tools for network examining and controlling i.e., integrated in the following meanings:

- An interface controlled by one person having a strong but easy to manage and integrated sets of command for doing all or most of network managements task.
- A few numbers of divided tools i.e., most of the software & hardware essential network

managements are integrated into the available user equipments.

#### **1.2 Why Management is Needed?**

Networks management is necessary to observe and control the operations of the networks and to react to adjusting user requirement.

Network Managers are not only responsible for not only planning, designing, and implementing networks, their job is also to keep the network running smoothly and efficiently, with little or no down time. Generally speaking, the functions of a manager include installing and configuring devices, diagnosing and troubleshooting problems, controlling access to services, etc.

These functions fall into five main categories as follows:

- Capable to configure management
- Ability to boost management performance
- To handle fault management
- Capability to solve security issues for management
- Efficient accounting mechanism for management

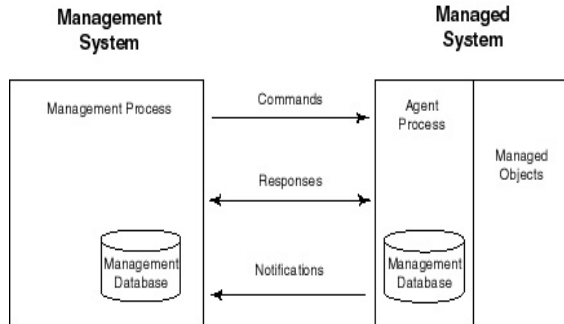
Each of these network management functions will be described in later sections.

#### **1.3 Components of the Networks Management System**

Question is that how does the networking manager knowing, what user is answerable for and then control such a system? Answering to such queries, we have to know the networks management system architecture and that how it performs and achieves its goals. This system is also known as the



agent/manager model, which is further divided into a system (i.e., fully managed), an information database for management, a system manager and the networks protocols (see Fig. 1) (Case, et al., 1988), (Bruce, 1996).



**Fig. 1: Network Manager/Agent Relationships**

As Fig. 1 presents, the managed system includes the managed objects and the agent process. The network management operations are performed by the agent process.

**Project Scope:** There is always scope for improving technical software products, and the Networks Management System (NMS) are no exception. This work focuses on the following:

- Performance Management
- Configuration Management
- Fault Management

Each problem area is examined separately and a solution is sought for each of the problems identified like services management, processes management and event tracking. As a result, a set of tools is developed to solve these problems using a number of different network management techniques like SNMP (Case et al., 1990) and CIM implementation (Cerf, 1988).

## 2. Proposed System Architecture

We have proposed the system after detailed study and analysis of the various technologies used in network management. Currently almost all of the network management systems (NMS) have been developed using standalone network management architecture like SNMP (Simple Network Management Protocol) (Case et al., 1990) or IEEE Network Management Model etc. We have proposed a system which will be a merger of SNMP technology (Uebele, 2007) and CIM (Common Information Model). DMTF (Desktop/Distributed Management Task Force) proposed CIM as an industry standard. The information management functionality to get information about any system on the network will be accomplished using SNMP while other management activities will be performed using

CIM implementation. It will help the network administrators to detect and solve problems of remote machines from a central location and they will also be able to perform effective processes and services management and troubleshooting activities.

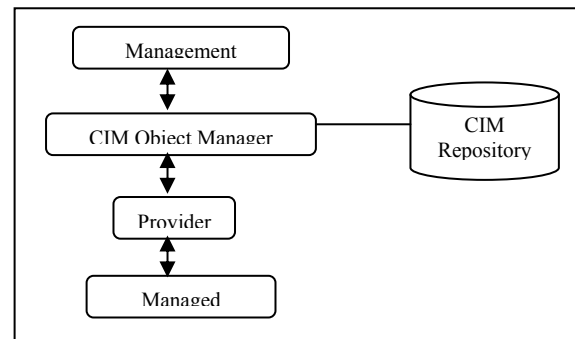
In the subsequent sections, we will explore the advantages by explaining and comparing the technologies used for the proposed system.

### 2.1 Overview of CIM-Common Information Model

DMTF proposed CIM-Common Information Model, initially as part of the WBEM program. CIM is a technique (based on the object-oriented paradigm) to systems, software, devices, systems, user managements and the networks. It presents an integrated intangible frameworks and standards for expressing logical and physical objects into unified managed environments. CIM describes a numbers of objects for providing the general frameworks concerning the basic sets of classification, class and association.

It consists of the following information approaches:

From an architectural point of view Fig. 2 presents the CIM's three-layers approach that includes its CIMOM (Objects Managers), Providers and Management Applications.



**Fig. 2: CIM Architecture**

### 2.2 Proposed System Advantages

**2.2.1 Security:** The security vulnerabilities of SNMP architecture will be removed by using CIM implementation and the proposed system will provide enhanced level of security to access machines remotely.

**2.2.2 Reliability:** The proposed system will be more reliable than others as it will provide a connection-oriented service. SNMP based network management systems provide connection-less service because it works on UDP. The proposed will provide more reliable service for the management tasks.

**2.2.3 Bandwidth:** The proposed system will consume less bandwidth according to the architecture

specified because the nature of the proposed system will be connection-oriented so it will consume less bandwidth.

**2.2.4 Latency Time:** As the proposed system will consume less bandwidth and requests can be mapped to available different protocols, the latency time or round trip time for the request will be less than other network management systems.

**2.2.5 Efficiency and Performance:** In the light of above stated characteristics, the proposed system will be able to perform network management tasks with efficiency and reliability. The information retrieval and other network management activities performed by the proposed system will be fast due to reduced cycle time. Hence, the overall performance and efficiency of the proposed system will be improved.

### 3. Results and Discussions

The nature of the system is distributed and based on client/server architecture. The GUI is designed in a very user friendly manner. This section gives a bird's eye view of application through the snapshots.

#### 3.1 Main Switchboard

When user starts the system following Main form will appear on the screen as shown in Fig. 3. This main form provides three menu options: File, Management Panel and Utilities. Each of these menu options provides further sub-options.

#### 3.1.3 Management Panel

- System Information
- Services Management
- Process Management
- Track Services
- Track Processes
- Event Log

#### 3.3.2 Utilities

- Ping
- Trace Route
- IP/Host Name Resolution
- Reboot Machine

Clicking on any of above menu options, system will enable network administrator to perform his desired task.

#### 3.2 System Information

By clicking on system information option following form shown in Fig. 4 will be appear on screen, containing options to customize the information required about any machine on the network.

To access information of remote machine, following information is required.

- Host Name or IP address
- User ID
- Password

To Access information about local host or local system, just select option button for local system as shown in Fig. 4. Different buttons are available on the form to get information according to requirement. Further to access information about number of Drives, free space, total space etc. select Drive list from the tab menu (Fig. 5). As we will select remote system option button, the text fields for host name, user id and password will become enable and after providing necessary fields values, we will be able to retrieve information.

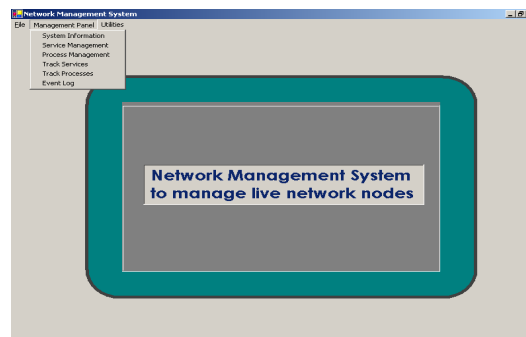


Fig. 3: Management Panel and Utilities

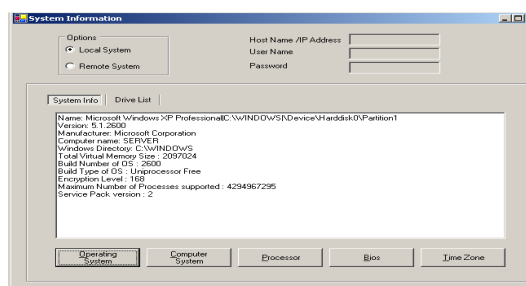


Fig. 4: System Information

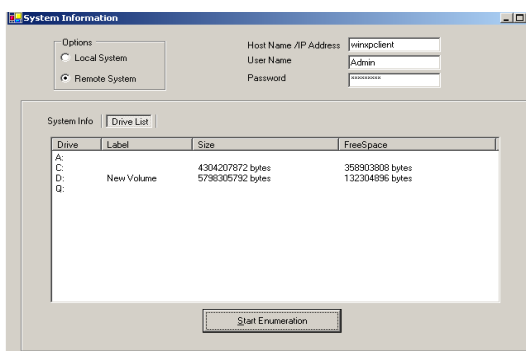


Fig. 5: System Information Drive Listing

#### 3.3 Service Management

Service management option is included in Management Panel menu. This system option will enable network administrators to perform service management effectively. According to the requirement, different services can be stopped and

started for necessary troubleshooting and performance upgrading. By clicking on this option, a management panel form will be appeared on screen and selected option from the top tab menu will be Service management. On this tab of service management, there are four buttons available to perform service management effectively named as Load/Refresh, Toggle Service Mode, Start Service, and Stop Service as shown in Fig. 6.

### 3.4 Process Management

By clicking Process Management, Process Management form will be appeared. On process management from, we can view processes on local or remote machine by giving IP address or Host Name. Further we can Start Process or kill processes according to requirement by providing process name and executable path as shown in Fig. 7. There are three buttons available on this form named as Load/Refresh, Terminate Process and Start Process. Following fields are necessary to fill in case process management of remote machine.

### 3.5 Track Services and Processes

With the help of Track Services option, user can view and track that which services are being initiated and which services are being are stopped. The facility is available on local or remote machine of the network as shown in Fig. 8. With the help of Track Processes option, user can view and track that which processes are being initiated and which services are being stopped.

### 3.6 Event Log and Ping Utility

The system will enable user to view event log of any computer system on the network by specifying its machine name or IP address (Fig. 9). Ping utility is provided to check the physical connectivity between machines. It is a useful utility to troubleshoot the problems regarding network. Host Name or IP address is required to check the physical connectivity. The other features of the system are: Host Name/IP Resolution, Reboot Machine panels.

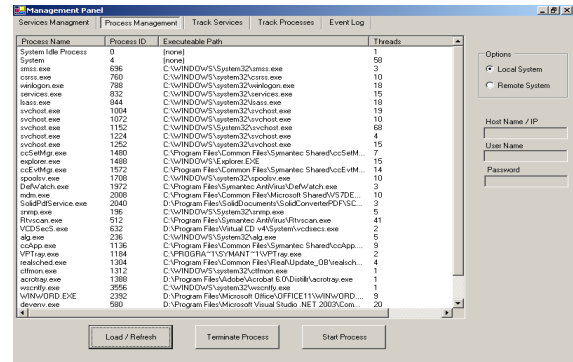


Fig. 7: Process Management

Table 1: Process Management Parameters

Field Name	Description	Remarks
Host Name/IP Address	Name of the remote Host or IP Address to access the machine over the network	User has to choose remote system option
User Name	Name of user which is used for system use	User has to fill this field
Password	Password of user. This is displayed in decrypted form	User has to fill this field

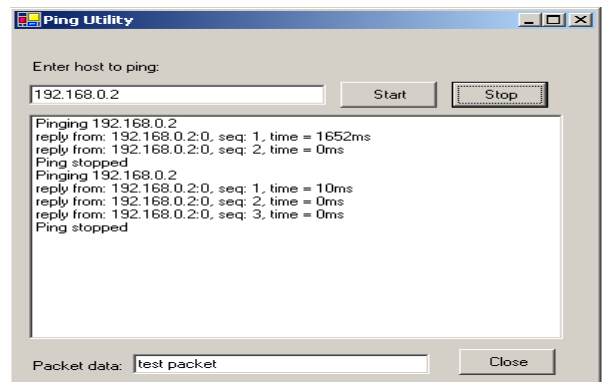
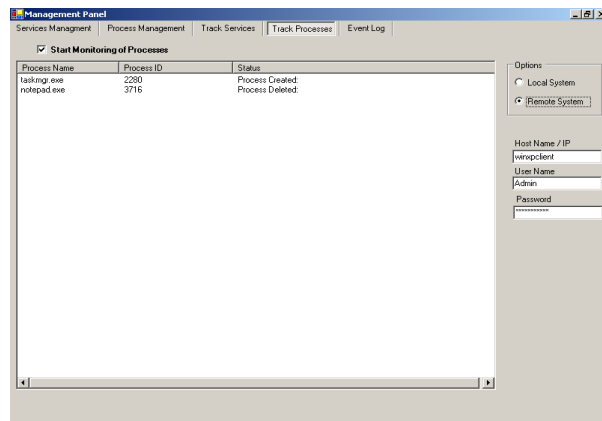


Fig. 8: Track Services and Processes

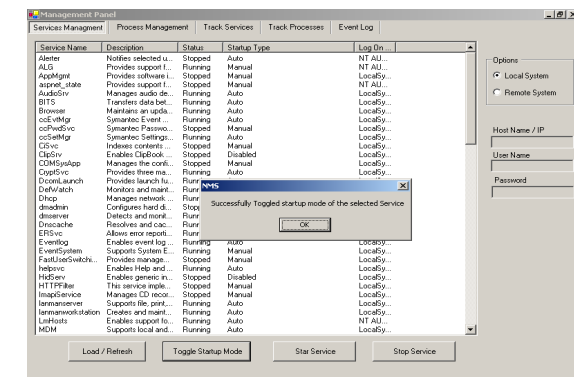


Fig. 6: Services Information and Management

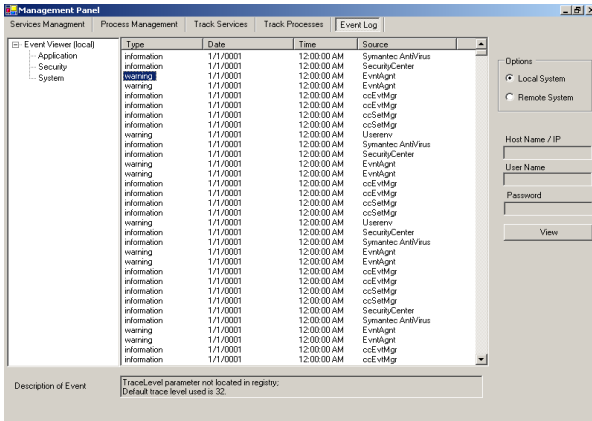


Fig. 9: Event Log and Ping Utility

3.7 System Testing

In this section, some tests are performed to check the proposed system expected behavior. Such testing technique is not related with how input is changed into output. Since the internal implementation detail of the system is not noticeable to the tester. This provides inputs through an interface that the system offers and tests the outcomes. If the output match with the estimated result, system is okay if not a fault is indicated (Patrick, 2007). Here are two sample test cases for this project to perform black box testing.

Table 2: Testing Results  
Project Name: NMS; Module Name: Track Processes

Test case ID: TC 1	
<b>Objective:</b>	To test the functionality of “Track Processes” method.
<b>Inputs:</b>	<ol style="list-style-type: none"> <li>Select local machine for monitoring, click check box of monitoring.</li> <li>Select remote machine for monitoring and enter values of machine name or IP Address, User name and password. Click check box to start monitoring</li> </ol>
<b>Result expected:</b>	<ol style="list-style-type: none"> <li>Monitoring of local machine should start and list of processes, which are being initiated or terminated, will available to user.</li> <li>The system will connect to the remote machine using credentials provided and monitoring details of processes will be visible to the user.</li> </ol>
<b>Outputs:</b>	<ol style="list-style-type: none"> <li>The monitoring of processes is started successfully of local machine.</li> <li>The monitoring of processes is started successfully of remote machine.</li> </ol>
<b>Result:</b>	<b>Test Passed.</b>

4. Conclusions

“Network Management System to Manage Live Network Nodes” is developed to provide a user-friendly environment that could help Network

Administrators and Network Managers to perform necessary management tasks from central point. This system does various functions as of the OSI Networks Managements Functional Area, those being, Configuration, Fault and Performance.

A main objective of the system is to retrieve information about any remote machine on the network quickly and instantly. Network Administrators can perform effective services management and processes management. Further the system is capable to check physical connectivity and name resolution tasks and perform network management activities according to the requirement. Different network management techniques have discussed in detail.

SNMP and CIM model (DMTF industry standard) is used to develop the system. All the basic requirements have been achieved and different utilities have been added to the system to make it more efficient and reliable. Event Tracking and monitoring has been deployed effectively to enhance the overall network performance, as a whole system is working properly to fulfill all the functional requirements.

Table 3: Testing Results  
Project Name: NMS; Module Name: Service Management

Test case ID: TC 2	
<b>Objective:</b>	To test the functionality of “Service Management” method.
<b>Inputs:</b>	<ol style="list-style-type: none"> <li>Choose local machine or remote machine for process management</li> <li>For remote machine also provide, machine Name, User Name, Password.</li> <li>Click load /refresh button to view list of processes</li> <li>Click Toggle startup mode button to change startup type of service.</li> <li>Click / press start button to start selected service</li> <li>Click/Press stop button to stop the selected service</li> </ol>
<b>Result expected:</b>	<ol style="list-style-type: none"> <li>Local machine option button or remote machine option button will be selected.</li> <li>For remote machine connection, necessary data fields will be accepted.</li> <li>A list of services will be displayed</li> <li>The selected service will be started.</li> <li>The selected service will be stopped.</li> </ol>
<b>Outputs:</b>	<ol style="list-style-type: none"> <li>Remote machine / local machine is selected</li> <li>Connection to remote machine is established via credentials provided.</li> <li>A list of services is successfully displayed.</li> <li>The selected service is started successfully.</li> <li>The selected service is stopped successfully.</li> </ol>
<b>Result:</b>	<b>Test Passed.</b>

### Acknowledgments

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2/2/2012

## Multi-Agent System to Suggest Daily Commodities on Social Networking Websites

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**Abstract:** Multi agent system is a rapidly growing field of distributed artificial intelligence that has gained significant position because of its ability to solve complex real world problems. Researches on social networks have received increasing attention in multi agent systems due to the popularity of social networking sites. In this paper, we are going to draft a blueprint of Multi agent architecture for social networking websites to suggest daily life commodities/items, based on general trends and interests. Usually websites show their advertisements or user interest information in the form of pop ups. This information is generally tied up with the most recent searches the user has made on the web browser. These ideas provide may or may not be related with his personal interests. So, he becomes frustrated at some times. But our system is capable of handling the situations intelligently. It extracts the required information from profiles and associates the results with it; providing up-to-date and significance information and making the web search a fascinating experience.

[M. Aslam, M. Junaid Arshad, Amjad Farooq, Syed Ahsan, M. Shahbaz. **Multi-Agent System to Suggest Daily Commodities on Social Networking Websites**, Life Science Journal. 2012;9(2):57-60] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 11

**Keywords:** Multi-agent; Social Networking; Artificial Intelligence

### 1. Introduction

Our world has become a global village. Technological development has brought people closer. Initiation of social websites provides a virtual community for people to share their daily activities with their friends and family. By virtue of which they can share their interests as well as increase their circle of acquaintances. Social Networking Sites (SNS) have attracted millions of users. Some of these sites provide services to different audiences, while others draw attention based on their common interests, languages or cultural, religious or nationality based identities.

While SNSs have provided a wide variety of technical features, their strength is to display the profiles of formulated lists of friends who are the users of the system. Usually users have to create their profiles containing information about themselves. They can also upload their pictures, post blog entries for others to read, search for other users with similar interests, and compile and share lists of contacts. To protect user privacy SNSs usually have control that allow users to choose who can view their profile, contact them, and so on. Recently, it has also become common for an organization to create profiles to advertise their products and services.

An analysis of the most current business models for SNSs suggests that websites should become more active and personalized. Intelligent agents can provide the possibility to their users to enhance the features of SNSs according to their interests as mentioned in their profiles. Like if a person as adores a personality anything new related

with him is automatically provided to the user. Or if he has an interest in a specific field say fashion, all the news related with the new trends is provided without any wastage of time as well energy in searching and browsing the internet.

Generic Models can be used as a template or as patterns for variety of agents as well as application domains. Frances, Jonker, Treur in their paper (Brazier et al., 2002), present an agent model GAM that provides a well-defined conceptual structure for agents. They basically used a compositional development method DESIRE to design it. In (Brazier et al., 1998), they described the compositional multi-agent system development. Furthermore a multi-agent architecture based on a generic broker agent model is also presented in (Jonker et al., 1999). A well-defined comparison of a multi-agent and a single agent has been practically identified in (Balaji and Srinivasan, 2010).

Many Computer-assisted systems are available that help their users to select various commodities that are helpful to them in daily life including giving information about the latest trends or helping them to select their makeover (Mehra and Nissen, 2011) (Sjoblom and Zingmark, 2007). A computer-assisted color selection system bases on aesthetic measure for color harmony and fuzzy logic theory suggests clothing by matching skin color (Hsiao et al., 2008). In the similar way, a Web based application i.e. the online car Selection using a decision support system allows the users to access the web to get information and helps the potential buyer to make the best decision on selection of a desired car

(<http://www.library.utm.edu.my>, 2011) However, none of these systems uses SNS for suggesting daily life items to their users. So in this paper we design and develop an agent-based system which can be incorporated within the SNS sites for intelligently suggesting daily commodities to its users. The generic agent based architecture for intelligent websites presented here offer these possibilities. This architecture not only provides the enhanced features or multiple agents working together in an environment but also polishes their properties of reactivity, pro activeness and social interaction. Our goal is to provide the user with the information on the basis of their interests, trends, norms, culture and so on.

## 2. Material and Methods

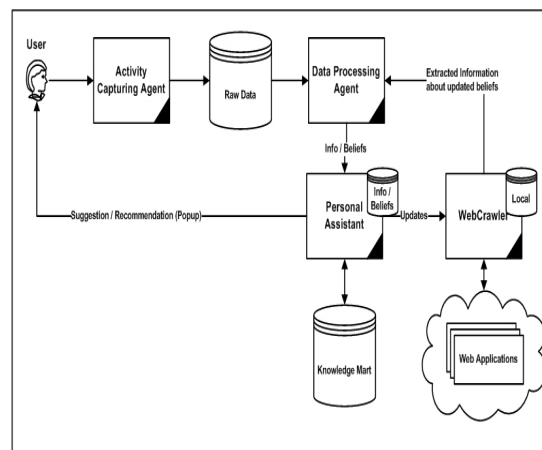
In this section, we sketch out structural design of Suggestion System and put some light on its different components.

Figure 1 shows the architecture of the system that consists of activity capturing agent, a temporary storage, data processing agent, personal assistant, web crawler that interact with the outside world which consists of number of web applications and one knowledge mart. The multi-agent structure of this system relies on two major components that is personal assistant and website crawler. Based on their role in the system, each agent is given a specific name. The Role, each agent is playing, is illustrated in this section.

The application domain, to illustrate the architecture, addresses the design of an active, intelligent Social Website that suggests daily life commodities to its users. The system gives an idea and up to date information about new items, events, trends, happenings according to the user's interests and needs.

**Activity Capturing Agent:** This agent is capable to capture/monitor activities performed by the user. Once it has captured a specific amount of information/data, it performs initial data filtering and cleaning. It then pushes the initially filtered data/facts into a temporary data repository for further crackdown.

The temporary data storehouse holds data for a very short period and then is flushed out. The data can be in the form of text files/ statements. For Example, if a user is performing activity related to search of Air conditioner (AC) from section of home appliances. The capturing agent captures information like the model, made and range of AC.



**Figure 1: System Architecture Diagram**

### 2.1 Data Processing Agent

The presence of data alone does not guarantee that decisions can be carried out smoothly. There is a compelling constraint for the data to be carrying great weight or, in other words, data quality is of utmost importance. The major role of this agent is to monitor short-term data repository for recently arrived data. The data provided to this agent will be in the form of flat files and raw facts. The initial cleaning of data is performed; so on the initially captured/filtered data low level cleaning is performed. This agent will eliminate any irrelevant material from the data and transform the data into something meaningful.

This information is further processed to generate meaningful patterns which are consumed by the Personal Assistant agent and helps to update beliefs and information. The identified patterns are accountable for processing local information repository of Personal Assistant.

The processing agent is further subdivided in three major methods. One of them is data cleaning agent which has discussed above. The Second is used for communication between PA and user. It is responsible for monitoring the information captured by the Activity Capturing Agent which will collect information like norms, trends, fashion, profile information etc.

The last method is consumed for communicating outside world with PA. It is responsible for monitoring information that is tailored by the web crawler when crawling is initiated by personal assistant once the new activities are performed by the user. The crawled information is further processed to generate meaningful patterns that are consumed by personal Assistant.

## 2.2 Web Crawler

This agent is responsible for crawling or spidering information from websites. It uses a means of providing up-to-date data. It is mainly used to create a copy of all the visited pages for later processing by a search engine that will index the downloaded pages to provide fast searches. It is also used for automating maintenance of information or tasks on a Web site.

It starts with the list of patterns or information provided or initiated by the personal assistant. It crawls or sniffs outside world on the basis of beliefs and trends identified through patterns. This collected information is further preprocessed for the refinements of facts to be consumed by the Personal Assistant.

Different web Agents are assigned to different type of websites including related to different categories such as fashion, music, videos, computer hardware and software, food, clothing, books ,magazines, music, household accessories, and so on. Each of these web Agents has autonomy to a large extent; they gather the information about their related areas and then further aggregate into useful information. The web crawler asks the related web Agents about information and then extracts the related information and further sends it back to the Personal Assistant through Processing Agent.

Viewed from outside the basic agent behaviors autonomy, responsiveness, pro-activeness and social behavior such as discussed, for example in [1] provide a means to characterize the agents. Moreover, the following Website agent concepts to define

- Interaction with the world (observation, action performance)
- Communication with other agents

## 2.3 Personal Assistant

This agent is responsible for performing foremost measures of the system. It interacts with the user and outside world through data processing agent. It uses two methods for the communication. One method is responsible for extracting beliefs from meaningful patterns and other is responsible for retrieving information that might be required to update beliefs or action items of agent.

The other component of personal assistant is responsible to communicate with permanent repository. One method is responsible to update beliefs and other is accountable for the storage of latest information. The method for gathering latest information is used to assist agent in suggesting commodities. This method is responsible to gather required information from latest copy. If information

is not latest web crawler is initiated to collect latest patterns.

Once beliefs are update and latest patterns are identified the agent is responsible to display suggestion to the user on the basis of his interest, norms and daily trends

## 3. Results and Discussion

Let's consider a case study a, a user registers himself on a social website say, Social Diaries. First of all while creating an account he provides information about himself in the profile section. We have included option that asks the user if he wants to use the services of a web agent System to Suggest Daily Life Commodities on this site. If he clicks the radio button another option showing Interests is shown. In which the user identifies his interests say he chose Fashion. Furthermore he is asked about the updates if he wants daily updates or monthly etc., as shown in Figure 2.

Figure 2: Social Diaries Profile information

Figure 3: Home Page of the proposed multi agent social website (Social Diaries)





**Figure 4: Home Page showing the agent with new trends in the market**

After finishing the account information when the user visits his wall he finds an agent indicating the areas related to his interest, like shown in Figure 3.

When the user clicks say new trends he is shown with the new trends in the market as shown in the following Figure 4.

Similarly, the agent shows other features of interest as mentioned by the user.

#### 4. Conclusions

The world is moving from web to semantic web. An applications like multi agents helps in making the web more intelligent. By using the multi agent system in our social websites helps the user to gain up to date information related to his interest as soon as he login; truly making a social website a site that not only keeps its user socially connected but also proves to be an information provider about the daily life commodities and interests.

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2/12/2012

## CALIBRATION OF NEW SOFTWARE WITH CONE BEAM C.T FOR EVALUATION OF ITS RELIABILITY IN DENSITOMETRIC ANALYSIS AROUND DENTAL IMPLANTS

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**Abstract:** *Aim:* assessment of the validity and reliability of Idrisi Kilimanjaro software for densitometric analysis around dental implant to evaluate osseointegration by calibration with CBCT. *Material and Methods:* 42 clinically osseointegrated dental implants; 28 immediate (17 mandibular and 11 maxillary) and 14 delayed implants (5 mandibular and 9 maxillary) were inserted in 28 patients of both sex (9 males and 19 females) with average age of 31.7 years. All the implants were radiographed using CBCT 1day and 4 months postsurgically, densitometric analysis was performed around dental implant on CBCT image at these 2 time intervals. Another radiodensitometric analysis was performed on the same radiographic image taken by CBCT and at the same time intervals using new computer software called "IDRISI Kilimanjaro". "Microstat 7" for windows statistical package, paired "t" test, Pearson's correlation and Coefficient of variation of both techniques were used for statistical analysis of the results. *Results:* There was a very high positive correlation between bone density values measured by CBCT and IDRISI techniques while there was no statistically significant difference between coefficients of variation of both readings in both techniques that indicates the reliability of IDRISI Kilimanjaro software for densitometric analysis around dental implants. There was also a statistically highly significant difference between both readings in each technique indicating a highly significant osseointegration of dental implants involved in this study. *Conclusions:* IDRISI Kilimanjaro software is a valid and reliable way in densitometric analysis around dental implants for assessment of osseointegration procedure when it is calibrated with CBCT and it is as accurate as CBCT.

[Khaled A. Elhayes; Mahmoud A. Gamal Eldin. **CALIBRATION OF NEW SOFTWARE WITH CONE BEAM C.T FOR EVALUATION OF ITS RELIABILITY IN DENSITOMETRIC ANALYSIS AROUND DENTAL IMPLANTS.** Life Science Journal. 2012;9(2):61-67] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 12

**Keywords:** CBCT, IDRISI Kilimanjaro, Osseointegration, Dental implant.

### 1. Introduction:

There are two ways of achieving connection between an endosteal implant and the host tissue. Anchorage may be achieved through a non-mineralized zone or connective tissue, which is claimed to be a pseudo-periodontium. The fibro-integration was attributed to the surgical technique and early over loading, *Garetta et al., (1995)*. The other way of direct contact between bone and implant is called osseointegration, *Albrektsson et al., (1981) and Skalak (1983)*. Osseointegration was defined as the apparent direct attachment or connection of osseous tissue to an inert alloplastic material without intervening has been demonstrated as biochemical bonding of living bone to the surface of a hydroxyapatite coated implant, *Rieger et al., (1989)*. In implants dentistry, this phenomenon called osseointegration has become the accepted standard for success in dental implants. Yet, Failure of these devices associated with impaired healing, infection, and over load are well recognized, *Ratner (2001)*. Because osseointegration is essentially a wound healing process, factors that interfere with healing may contribute to implant failure. Hence, conditions shown to adversely affect wound healing may decrease the potential for successful

osseointegration, *Elsubeihi and Zarb (2002)*. Cone Beam Computed Tomography (CBCT) is a relatively new medium for maxillofacial conditions, it was developed in 1998 and has been commonly used commercially since 2000, and it offers less radiation than computed tomography (CT) in 3D image construction, *Benninger et al., (2012)*. CBCT was introduced into the U.S. market in 2001; it has been shown to be a precise imaging modality and is a valuable tool for use in dental applications, *Hatcher (2010)*. It was also used to measure bone density with dental implants, *Lai et al., (2010)*. This imaging technique is now used worldwide; it is a reliable and credible alternative to CT scan for dentomaxillofacial imaging; radiation exposure may vary from one machine to another, but it remains definitely lower than CT dose levels; this technique is compatible with dental implant software. The major diagnostic advantage is the exquisite spatial resolution while the main pitfall is the poor contrast of face and neck soft tissues, due to a lower density resolution compared to CT scan, *Hauret and Hodez (2009)*. CBCT can provide identical information to multislice computed tomography (MSCT), with a considerable dose reduction when panoramic radiography is not

sufficient in the study of the teeth and jaw bones, *Carrafiello et al., (2010)*. It was suggested that voxel values of mandibular cancellous bone in CBCT could be used to estimate bone density, *Naitoh et al., (2009)*. This bone density assessment was performed by measurement of Hounsfield Units (HU) using cone beam computed tomography (CBCT), *Aranyarachkul et al., (2005) and Marquezan et al., (2011)*. In a clinical investigation for measuring bone density in healing periapical lesions by using CBCT; the results of this study supported the use of this CBCT to measure bone density, *Kaya et al., (2012)*. Furthermore, a linear relationship can be used to determine the density of materials (in the density range of bone) from the HU values of a CBCT scan. This relationship is not affected by the object's location within the scanner itself, *Lagravère et al., (2008)*. Preoperative CBCT for bone density measurements may be helpful as an objective diagnostic tool, *Fuster-Torres et al., (2011)*. It is important in presurgical imaging for dental implant treatment, *Naitoh et al., (2009)*. It allows accurate assessment of the entire volume of a proposed implant site, *King et al., (2007)*. In a study of bone mineral density (BMD) measurement, it was concluded that there was a positive correlation between BMD of total bone block measured by Dual-Energy X-ray Absorptiometry (DEXA) and that measured by (CBCT), *Marquezan et al., (2011)*. Radiographic bone density (RBD) assessed by CBCT has also a strong positive correlation with bone volumetric fraction (BV/TV) assessed by micro-CT at the site of dental implants in the maxillary bones and so, pre-operative estimation of density values by CBCT is a reliable tool to objectively determine bone density, *González-García and Monje (2012)*. It was suggested that the trabecular bone volume per total tissue volume BV/TV obtained using CBCT images can be used to evaluate the density of mandibular cancellous bone in dental implant treatment, *Naitoh et al., (2010)*. The bone quality was objectively assessed with density values obtained from CBCT to determine the correlations between bone density and primary stability of dental implants, *Isoda et al., (2011)*. CBCT was also used to evaluate bone quality changes surrounding the apical portion of immediate implants placed under higher insertion torque utilizing an undersized drilling technique, *González-Martín et al., (2011)*. Furthermore, CBCT is a useful approach for evaluating bone density changes around teeth induced by orthodontic treatment, *Chang et al., (2011) and Hsu et al., (2011)*. It is a good choice for analyzing bone mass, *Hohlweg-Majert et al., (2011)*. In recently introduced CBCT, magnification of images did not occur, allowing it being used for accurate clinical procedures, *Yim et al., (2011)*.

CBCT imaging allows remarkably lower radiation doses and thinner acquisition slices compared with medical computed tomography, *Kim et al., (2007)*. IDRISI Kilimanjaro software facilitated image restoration, enhancement, and densitometric analysis and so it was used for image analysis. This software provided a unique facility in comparison to other densitometric measuring software programs, as it facilitated monitoring the changes in bone density at two zones around implant images. The first zone represented the osseointegration zone which was located just adjacent to the implant borders, along the bone-implant interface. On the other hand, the second zone was located just around the first zone and represented the bone surrounding the interface, *Becker (1999) and Radwan (2005)*.

**Aim** of this study was to assess the validity and reliability of Idrisi Kilimanjaro software for densitometric analysis around dental implant to evaluate osseointegration; by calibration of this software with CBCT.

## **2. Material and Methods:**

### **2.1. Material:**

#### **2.1.1. Sample of the study:**

42 clinically osseointegrated dental implants were selected to be involved in this study, all of them were performed by the same operator, they were inserted in 28 patients of both sex (9 males and 19 females) aged 23 – 47 years old with average age of 31.7 years; according to the technique of implant insertion, they were 28 immediate dental implants (17 mandibular and 11 maxillary) and 14 delayed implants (5 mandibular and 9 maxillary).

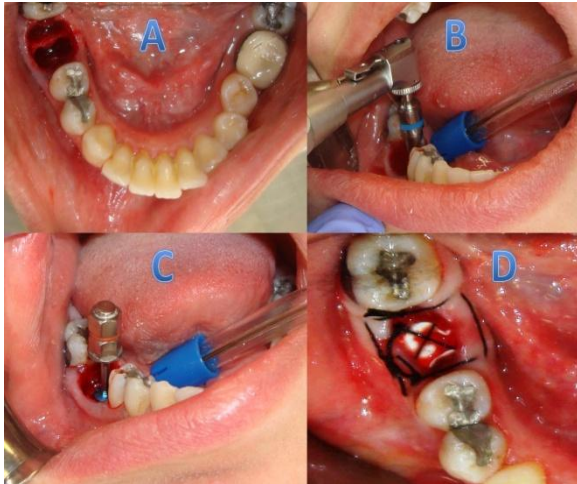
### **2.2. Methods:**

#### **2.2.1. Clinical procedure for dental implants:**

After extraction of the non-restorable tooth that would be replaced by dental implant (in case of immediate implant), adjustment of the extraction socket was performed using the suitable drillers under copious irrigation till reaching the size of proposed dental implant, then, implant was inserted and screwed to its final position in the prepared socket and finally, a non resorbable membrane was placed over the inserted implant to cover it completely over which figure 8 suturing was performed to secure membrane in position. (*Figure 1*).

#### **2.2.2. CBCT procedure for densitometric analysis:**

All the implants involved in this study were radiographed by CBCT 1day and 4 months postsurgically. Exposure was performed using "Scanora3D", Sorredex- Finland, at 15 mA, 85 KV and at a proper field of view. Image reconstruction was performed using special software called



**Fig.1:** Clinical steps for placement of immediate dental implant

**A:** Extraction of non-restorable tooth

**B:** Drilling to widen the socket

**C:** placement and screwing of dental implant

**D:** suturing over the membrane

“Ondemand3D” version 1.0.9, Cybermed, Korea. Standardization during imaging was achieved through adjusting the patient positioning and lights as follows: The seat height was adjusted to position the region of interest (ROI) vertically within the field of view (FOV). The upper light beam indicated the top of the FOV and the lower light beam indicated the bottom of the FOV. The sagittal light (vertical front light) was positioned in the center of the FOV from sagittal direction so that it is in the center of the ROI. The lateral light (vertical side light) was positioned in the center of the FOV in the lateral direction so that it is in the center of the ROI. The patient was instructed not to move during the duration of exposure. Densitometric analysis was performed around dental implant on CBCT image at these 2 time intervals using this “Ondemand3D” software supplied with the previously mentioned machine. The layer thickness to be analyzed was standardized to 1 cm to be sure that it contains all the implant inside; the reading of mean density outside the implant was taken as the final analysis. This analysis gives the actual bone density around the immersed dental implant that proves the process of osseointegration (Figure 2).

### **2.2.3. IDRISI procedure for densitometric analysis:**

Another radiodensitometric analysis was performed on the same radiographic image taken by CBCT and at the same time intervals using new computer software called IDRISI Kilimanjaro that facilitated image restoration, enhancement, and densitometric measurements. { IDRISI 14.01 (Kilimanjaro) is a



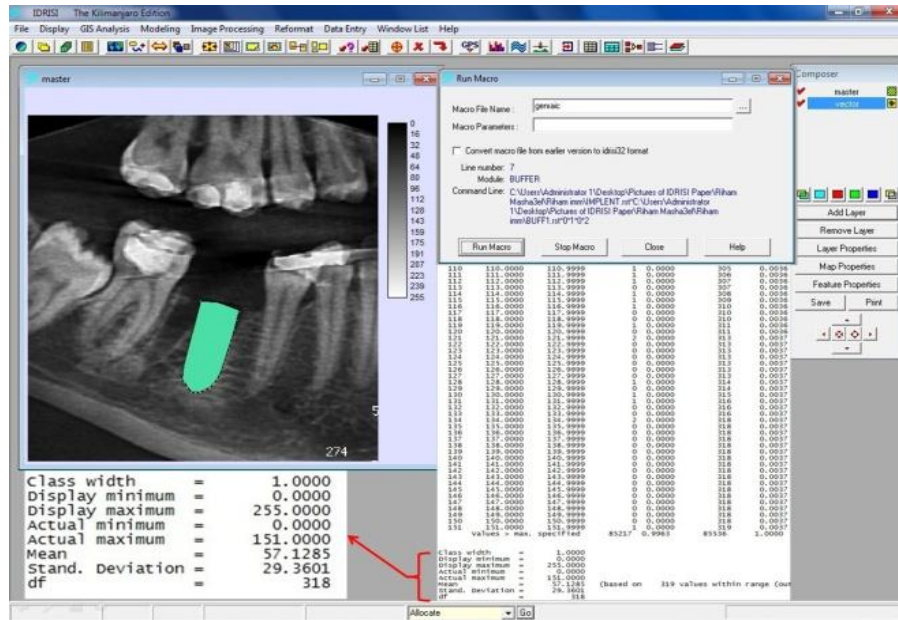
**Fig.2:** Densitometric analysis procedure around dental implant using CBCT

software product of Clark Labs, Clark University, Main Street, Worcester, MA.01610-1477, USA}. IDRISI assessed the density of the bone surrounding implant in the digitized radiographs by dividing it into two zones with standardized width. First zone (zone 1) was located just adjacent to the implant and represented osseointegration zone (implant-bone interface).The second zone (zone 2) was located just around the first one and represented the bone surrounding implant. The software analyze the images through the following steps; image restoration, image enhancement and density measurements. Image restoration technique allows for both radiometric and geometric correction of images. The procedure is followed by image enhancement technique which allows contrast adjustment regarding all the images, then implant edge enhancement, followed by subtracting the implant from the background image (surrounding bone). Finally, the density measurements are calibrated by quantifying the image on 256 grey-scales. Zero scale are given to the totally black regions, 256 for totally white regions and the values in between represent shades of grey, Gonzalez et al., (1992), Bernd (1993) and Dawoud (2009); (Figure 3).

### **2.2.4. Statistical analysis:**

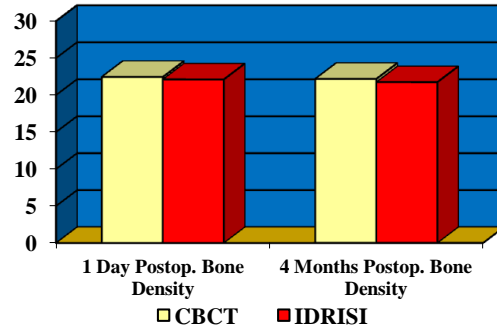
All data were collected and statistically analyzed using “microstat 7” for windows statistical package and Paired “t” test for comparison between both readings in each technique. “Pearson's correlation” was also used for correlation between reading 1 in both techniques and reading 2 in both techniques too (*r value*). Coefficients of variation of both techniques were compared using “Z score” test to compare percentages of variations.

**Fig. 3:**  
Densitometric analysis procedure around the same dental implant using IDRISI



**3. Results:**

All the statistical results considered to be significant at  $P \text{ value} \leq 0.05$ . There was a very high positive correlation between bone density values measured by CBCT and IDRISI techniques ( $r$  value = 0.94), indicating that IDRISI Kilimanjaro software is a reliable technique for densitometric analysis around dental implants (Table 1). There was no statistically significant difference between coefficients of variation of both readings in both techniques that also indicates that IDRISI is a reliable technique for bone density measurement around dental implants (Table 2, Figure 4). Using paired  $t$  test, there was a statistically highly significant difference between both readings in each technique indicating that there was a highly significant formation of bone around dental implants by time that means highly significant osseointegration of these dental implants. (Table 3 & Figure 5). The raw data of densitometric analysis for the 42 dental implants measured by both CBCT and IDRISI Kilimanjaro softwares were tabulated for the previous statistical analysis (Table 4).



**Figure 4:**  
Comparing Coefficient of variation of the same reading in both techniques

**Table 1:** Correlation between bone density values by CBCT and IDRISI

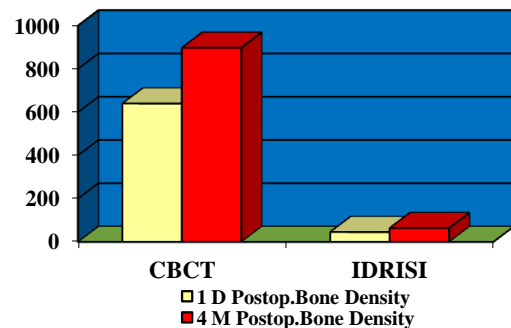
BONE DENSITY	" r " value	Prob
1 day postoperative	0.94	0.000001
4 months postoperative	0.94	0.000001

**Table 2:** Coefficient of variation in both techniques

BONE DENSITY	CBCT	IDRISI	Prob
1 day postoperative	22.44%	22.11%	0.473 (NS)
4 months postoperative	22.18%	21.74%	0.482 (NS)

**Table 3:** Paired  $t$  test comparing both readings in each technique

Technique	BONE DENSITY		"t"	Prob.
	1 day postoperative	4 months postoperative		
CBCT	639.47 ± 137.13	897.44 ± 199.02	19.605	0.000001
IDRISI	45.21 ± 10.00	63.28 ± 13.76	21.974	0.000001



**Figure 5:**  
Comparing mean values of bone density measured by each technique in two time intervals

**Table 4:** Bone density readings for all implants by both techniques

PATIENTS		CBCT	IDRISI	PATIENTS		CBCT	IDRISI
Implant 1	Read 1	465.94	32.6677	Implant 22	Read 1	593.75	46.0271
	Read 2	667.17	46.7801		Read 2	829.18	64.2438
Implant 2	Read 1	721.33	48.3572	Implant 23	Read 1	681.39	50.8517
	Read 2	1089.20	74.3733		Read 2	992.32	71.1838
Implant 3	Read 1	389.99	25.7098	Implant 24	Read 1	726.76	53.0559
	Read 2	617.39	40.0044		Read 2	1034.65	75.6557
Implant 4	Read 1	584.43	41.7491	Implant 25	Read 1	498.66	36.2398
	Read 2	857.94	58.4487		Read 2	744.57	53.8600
Implant 5	Read 1	687.49	43.8665	Implant 26	Read 1	398.98	30.8092
	Read 2	958.70	61.2288		Read 2	580.99	44.0465
Implant 6	Read 1	812.36	48.6959	Implant 27	Read 1	728.64	55.5564
	Read 2	1153.55	70.0490		Read 2	946.32	73.1488
Implant 7	Read 1	514.86	36.0041	Implant 28	Read 1	691.46	50.6415
	Read 2	720.80	51.8062		Read 2	951.23	68.6810
Implant 8	Read 1	632.59	42.4557	Implant 29	Read 1	709.23	54.5565
	Read 2	988.80	64.7364		Read 2	984.15	76.1524
Implant 9	Read 1	823.66	49.9187	Implant 30	Read 1	826.41	63.0318
	Read 2	1234.17	74.2390		Read 2	1187.09	88.9460
Implant 10	Read 1	437.56	29.1706	Implant 31	Read 1	693.45	50.7977
	Read 2	611.97	41.1305		Read 2	920.13	67.5514
Implant 11	Read 1	558.04	29.6092	Implant 32	Read 1	741.36	54.7585
	Read 2	653.73	43.2205		Read 2	999.83	75.7121
Implant 12	Read 1	526.90	45.8630	Implant 33	Read 1	456.32	32.3104
	Read 2	709.03	53.6445		Read 2	634.10	44.4387
Implant 13	Read 1	375.80	52.1134	Implant 34	Read 1	798.46	61.9316
	Read 2	465.99	63.0572		Read 2	1126.65	86.7043
Implant 14	Read 1	619.87	57.1285	Implant 35	Read 1	357.15	29.7657
	Read 2	737.64	67.9829		Read 2	705.49	38.3844
Implant 15	Read 1	497.21	27.1044	Implant 36	Read 1	951.35	63.9821
	Read 2	565.98	28.2222		Read 2	1318.19	88.4434
Implant 16	Read 1	485.34	33.1811	Implant 37	Read 1	645.91	45.2676
	Read 2	650.35	46.4535		Read 2	905.07	63.3580
Implant 17	Read 1	538.76	36.4786	Implant 38	Read 1	831.64	58.0713
	Read 2	795.20	52.2081		Read 2	1273.45	86.9213
Implant 18	Read 1	533.99	35.3252	Implant 39	Read 1	597.31	46.3778
	Read 2	807.55	52.9807		Read 2	761.79	60.2866
Implant 19	Read 1	624.83	39.6334	Implant 40	Read 1	671.82	49.0550
	Read 2	924.74	61.5744		Read 2	887.54	64.6009
Implant 20	Read 1	701.01	46.7414	Implant 41	Read 1	493.71	34.9398
	Read 2	908.50	60.0486		Read 2	590.29	42.3157
Implant 21	Read 1	583.69	46.0323	Implant 42	Read 1	746.36	53.4637
	Read 2	774.52	62.4331		Read 2	879.67	63.2797

Read 1 = 1 day postoperative bone density & Read 2 = 4 months postoperative bone density

#### 4. Discussion:

CBCT low sensitivity to metallic artifacts makes it the technique of choice in the follow-up of cochlear implants, *Hodez et al., (2011)*. In implant imaging,

CT delivers the highest radiation dose to the salivary glands, whereas the CBCT system delivers the lowest dose. Irrespective of imaging modality, during implant imaging, salivary glands receive most

radiation, *Chau and Fung (2009)*. CBCT imaging has the advantage of allowing thinner acquisition slices compared with medical computed tomography, *Kim et al., (2007)*. Furthermore, in recently introduced CBCT, magnification of images did not occur, allowing it being used for accurate clinical procedures, *Yim et al., (2011)*. CBCT has also the potential to reduce the size and cost of CT scanners, this emerging technology produces images with isotropic sub-millimeter spatial resolution with high diagnostic quality, short scanning times of about 10-30 seconds, and radiation dosages of up to 15 times lower than those of conventional CT scans, *Bangbose et al., (2008)*. For all these previous reasons, CBCT was the radiographic technique of choice to be used in our study of dental implants for densitometric analysis and assessment of osseointegration. In our study, CBCT was selected as a standard measure for calibration of other ways of densitometric analysis as there was a positive correlation between total bone mineral density measured by CBCT and by dual-energy x-ray absorptiometry (DEXA) which is the most accurate technique for measuring real bone density as stated by *Marquezan et al., (2012)*. IDRISI Kilimanjaro software was selected at this study to be calibrated for densitometric analysis and osseointegration assessment around dental implant as it is available cheaper way than CBCT and more accurate tool than DIGORA for that purpose, it gives the mean bone density around dental implant with the standard deviation and the degree of freedom in an accurate way. This was in agreement with Radwan who designed a pilot study that compared the results elaborated by IDRISI Kilimanjaro software with those by DIGORA software and stated that no significant difference was found between the records of both softwares, but IDRISI Kilimanjaro software proved to be more accurate tool for densitometric analysis, *Radwan (2005)*. The very high positive correlation seen in our present study between bone density values measured by CBCT and IDRISI techniques at different time intervals; and the insignificant difference between coefficients of variation of both readings in both techniques indicate that IDRISI Kilimanjaro software is as much reliable as CBCT technique for densitometric analysis around dental implants. Furthermore, the highly significant bone density seen at 4 months postoperatively that means a highly significant formation of bone around dental implants by time obviate the highly significant osseointegration of dental implants involved in our study and supposed that IDRISI Kilimanjaro software could be a dependable sole way for assessment of osseointegration of dental implants. This is in agreement with *Dawoud (2009)* who used this

software as a sole way for evaluation of osseointegration of immediate dental implants in two groups of patients and stated that “accelerating bone deposition means enhancement of osseointegration and the results of that study revealed sustained increase in bone density throughout the follow-up periods; this increase indicated progressive osseointegration and increase bone density of variable degree within the two groups.”

**In conclusion**, IDRISI Kilimanjaro software could prove its validity and reliability in densitometric analysis around dental implants for assessment of osseointegration procedure when it is calibrated with CBCT and it is as accurate as CBCT for this purpose.

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## Remote sensing and evaluation of natural resources in Iran

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**Abstract:** Remote sensing technology using satellite access has been increasingly helpful in performing natural resources mapping and management. This includes processes that cannot be done manually or might take many years to complete when you are covering vast areas of land such as satellite imaging, accuracy assessment, image processing, classification, and geometric or radiometric corrections. It is evident that any nation's economic development is largely supported by the richness of its water and land resources. The management capability and mapping tools use to monitor these resources are crucial to raise the economic development of specific regions. Accuracy is a general requirement in managing delicate land and water resources for sustainable development. The remote sensing using satellite based approach in generating data ensures updated cost effective natural resources monitoring and management in Iran. This research will demonstrate the need to maintain remote sensing for mapping and managing natural resources in Iran as well as enhancing and supporting the decision making capabilities of the government regarding the use of its natural resources.

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### 1. Introduction

Remote sensing applications are the best approach in carrying effective sustainable management and evaluation of natural resources in Iran. Informing and supporting top government people about their natural resource management decisions requires precise, straight forward, accurate, current evaluation and information of the condition of Iran's land and water natural resources (Brewer, 2007). Although better information may not automatically mean being able to make better decision, limited access and limited information on the other hand, may limit decision space and quality of results. Forest management strategies need accurate, current, and comprehensive data for evaluation of its condition and developing practical sustainable solution that could address major issues in appropriate scales.

The ecosystem is always in a state of temporal and spatial scales of change mostly brought by its ever changing environment and socio-economic factors. This implies that better use of natural resources, implementation of policy, and management of natural resources especially forestry, agriculture, and freshwater farming shall be given higher importance. Surveys for natural resources directly impact the economic planning of Iran. This is especially true if the local government is keen on measuring and evaluating the annual freshwater

withdrawals such as evaporation losses and finding alternative renewable resources. Recent accurate data are required to support and develop strategic planning and management decisions.

There is a need to maintain remote sensing equipments and other computer assisted satellite supported digital remote sensing data collection and processing for accurate data analysis, strategic decision making, and fast updated extraction as well as collection of information concerning natural resources. This covers land use, soil, geology, topography, vegetation, and many other related large tasks that could not be possibly completed and updated without remote sensing. The innovative, accurate, convenient, and fast collection of data is a powerful logical approach for effective and systematic management of natural resources. Suitable image processing and enhancements would facilitate interpretation of collected information.

Better use of natural resources may increase food production and help in proper utilization of natural resources to provide better living standards for the people in Iran. Inaccurate data could lead to improper use and exploitation of natural resources. This may result to disastrous management, planning, and decision making. The remote sensing technique using the satellite or plane for geophysical survey has brought new hope and approaches in supporting structural geomorphologic interpretations. Spatial

data are important for sustainable natural resources management, evaluation, and development. Mapping out the natural resources to monitor the environment is attainable by using satellite images, aerial photos, and accurate information on land topographic maps. Accurate and fast evaluation of ecological studies for decision making can facilitate economic growth and reveal the potential of the specific region of study.

Remote sensing is the best model for monitoring the environmental conditions in Iran where the most recent technology in resource identification are being used for accuracy in topographic maps interpretation. Satellite images and aerial photographs produce clearer spatial information, which makes rebuilding and correcting natural resources planning and management easier. In today's world, where human activities include utilization of natural resources, it is crucial to take measures and avoid ecological destruction initiate renovation of resources, and increase awareness about overall conservation of environment. The aim of this research is to provide reasonable methods that could help preserve and enhance the potentials of Iran's natural resources. Inattentive use combined with unwise application of images and photographs could lead to disastrous planning over limited and vulnerable resource.

## 2. Theoretical basis of the research:

Regarding the above-mentioned cases as well as various spatial, environmental, social and economical conditions, the historical breadth, growth rate, and the development of the countries, there have been raised some different theories and viewpoints concerned the rural well being that the most important of them are referred here; from a morphological and philosophical viewpoint, of course, rural well being is as important as justice, equality, freedom, and citizen rights, that in some cases, they are considered as the complements of it and sometimes they are regarded as the bases for it or even as an aim or a tool for achieving it. From an economical point of view, as we know, the western countries economy has been influenced by the classical schools of thought derived from some thinkers' theories such as Adam Smith, David Ricardo, and Malthus, during two hundreds years ago. In this kind of the schools, the social rural welfare, in a wide sense, is influenced by the individual values and satisfaction.

The framework of sustainable natural resources management involves maintaining the balance of the country's social demands, economic needs, and ecosystem requirements (Kharazipour, 2009). Evaluating the three factors would help government leaders and economist accurately match policy against economic and social demands with a

list of scientific focus. Remote sensing applications technically strengthened the local as well as international competitiveness of Iran with its satellite and aerial systems imaging capabilities. The technology provides useful applications for system advancement, change management, and natural resources policy besides the collection, processing, and archiving of satellite images. This could substantially shape the economic success of Iran. The risk of the application lies on the skill and knowledge of the operator of the remote sensing equipment about on-orbit coordinates and launch timings. The expense involved in the program cost and schedule is relatively huge. One big risk is cost containment. Iran needs to train people and pursue exchange of knowledge to successfully operate a full satellite imaging system into the orbit.

Technical expertise is a prerequisite for accuracy and cost containment. There is a need to provide information that could be used in making management decisions and shaping the choices for policy, licensing, competitiveness, and national security in a rapidly changing climate and global marketplace. Nurturing technology to better support government derivatives for resource management and national security requires substantial financial funding and openness to foreign systems. Resource management is best managed by remote sensing capabilities regardless if the goal is commercialization or traditional government programs. Remote sensing and image interpretation provide digitally based systems and accurate analysis techniques to obtaining information using electromagnetic radiation in direct contact with the large area for evaluation.

Remote sensing has occupied a very important role acquiring information about land cover, vegetation, seabed topography, and water quality (Jong & Meer, 2006). Remote sensing has also taken significant role in the disciplines of agriculture, oceanography, geology, forestry, geography, meteorology, civil engineering, and zoology. The ecological system of Iran needs consistent monitoring, management, and evaluation for accurate gathering of data about various environment processes that could help determine and increase the understanding of the users. Measuring and evaluating the differences of the past and present conditions of the natural resources help define the complex dynamics of the ocean circulation as well as the complex pattern of detecting water and nutrient deficits (Jong & Meer, 2006). The application provides accurate changes in the advancing and withdrawing of the desert sand and collects information necessary to monitor and maintain Iran's forest or nature reserves. This is also important in

classifying and recognizing types of vegetation, determining disease of vegetation, and identifying stressed or damaged vegetation.

Techniques such as the GPS or global positioning system, GIS or geographic information systems, environmental monitoring, SFAI or small format aerial imagery, and mapping require skills and knowledge about remote sensing, landsat, and optical mechanical scanning systems. The techniques are necessary to perform thematic mapping and inventory of natural resources. Aerial photography could potentially reduce cost by at least 35% in performing mapping, planning, and inventory (Paine & Kiser, 2003). Approaches to interpretation are necessary to extract information using visual inspection of digital images and computerized quantitative analysis.

### 3. Material and Methods

This study uses statistical analysis, literature review, and field observations. The features and topographic characteristics of the desert in Iran reflect the region's severe thermal climatic situation. The knowledge about any surface type diurnal temperature patterns, trend of surfaces, calculation of correlations between surface types and climatic elements, and behavioural pattern during any hour of the day of the natural resources in Iran provide researchers and top government officials the most accurate data that would lead them to develop thermal characteristics models, evaluate natural resources, and predict behaviour in terms of economic relevance. Iran's natural resources biological systems, temperature and behavioural patterns, and physical systems are important in maintaining and interpreting the relationship of man's economic activities to the ecosystem. Thermal remote sensing application has been increasingly making people aware of its role in the country's economy. Remote sensing images facilitate environmental impact prediction. Time series satellite imageries studies were reviewed to evaluate how the application helps regional natural vegetation development.

Qualitative prediction models are important in identifying suitable sites for possible groundwater artificial recharge. Remote sensing helps carry out the task for environmental inventory and valuable information for vegetation cover, topography, and habitat destruction. Quantifying regional habitat could be difficult without the appropriate tools and applications. This study will gather important data to prove that remote sensing is better and more cost effective than traditional data collection method. Intensive literature and related researches about Iran's drought, desertification, soil brining, surface

water reduction, desert area expanding, pasture ruination, and underwater surface loss were performed to identify the role of remote sensing in maintaining and improving the ecosystem in Iran.

### 4. Results and Conclusions

A satellite aerial photograph taken from an altitude of 250 km can cover an area of 190 km x 130 km. A false colour infrared aerial photograph taken from an altitude of 760 m can cover an area of 1.1 sq. km. A thermal infrared image taken from an altitude of 620 km can cover 70 km x 100 km. The infrared image comes out with colour coding such as blue for lowest and red for highest radiation emission. Data may be taken using three band colour composite passive microwave to monitor sea-ice concentrations on specific regions using vertical polarisation. Vegetation index and other important topographic images could be analyzed using the remote sensing technique. Remote sensing allows researchers to collect information using images and electromagnetic radiation, ultraviolet or radio frequencies, without the need for physical contact. However, the enormous opportunity offered by the remote sensing applications has been heavily pulled down by the cost of acquiring the data.

GIS raw data could be transformed into useful information such as forest and renewable resources inventories, mapping, and assessment of urban growth. The sales of GIS raw data, including hardware and software, amount to \$2 billion per year (Williamson, 1993). Only the GIS hardware and software has the capability to carry and perform spatial data from several formats to usable computer files. The Land sat satellites and its systems cost about \$320 million. Land sat 7 cost from \$440 to \$640 million (Williamson, 1993).

The best way to evaluate the value of remote sensing is to identify the opportunities that it offers. One of the tangible advantages in using the application is its ability to obtain data from vast area of land surface or large volume of the atmosphere in a very short period of time using instantaneous snapshots (Rees, 2001). The other tangible advantage is the fact that the application generates digital information that is calibrated and ready for feeding into the computer for further analysis without long conversions and other processes. The power of remote sensing includes measuring environmental variables such as wind velocities, gas concentrations, ozone, temperature, and clouds (Rees, 2001). Remote sensing can measure land surfaces topography, soil moisture content, land cover type, reflectance, temperature, and tectonic motion. Ocean and water surfaces such as ocean tides, temperature, current, and topography could be measured and taken

accurately using remote sensing. The snow, glaciers, icebergs, and ice sheets condition, behaviour, and distribution could be monitored using the application. Remote sensing could be applied to a large number of opportunities and disciplines especially pollution monitoring, land and water resource mapping, and soil characterization. Most spaceborne observations are possible only using the remote sensing applications.

The cost involve in maintaining remote sensing often relate to the idea of achieving and balancing potential gains. The user needs to create proper mission planning with full consideration of the total cost that goes with data collection and processing. The remote sensing application detects radiation and active naturally occurring systems for analysis (Rees, 2001). Iran needs to develop a conceptual design of low orbiting satellite with remarkably low cost and weight that may accommodate an easy to remove mounting panels located at the interior as well as a removable hull. This low cost low orbiting satellite is smaller and allows Iran to place more satellites into the space with shorter turn around times. This reduces production cost on developing large expensive satellites. The LOFTS or Low Orbiting Fourier Transform Spectrometer Satellite design could cost less than \$3 million. Each additional reproduction of the original copy would give at least 25% to 50% savings on cost (Miau & Holdaway, 2000). The LOFTSS design is to carry the FTS or Fourier Transform Spectrometer. The FTS is an instrumentation package used for remote sensing application that weighs only 8 kg. FTS can absorb infrared and visible regions. It can also operate in emission mode even within an infrared region and perform a variety of applications related to remote sensing in terms of climate change, meteorology, atmosphere, and planetary exploration.

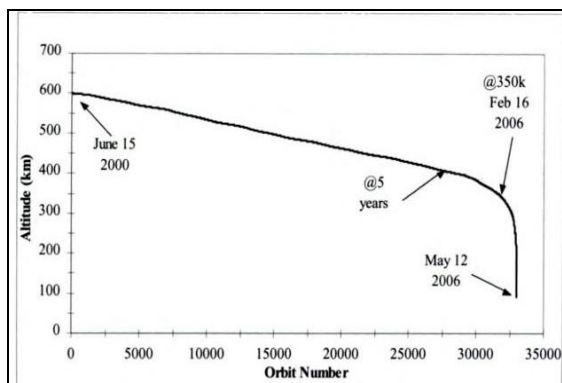


Figure 1. Lifetime analysis of LOFTSS ((Miau & Holdaway, 2000))

The LOFTSS yields a lifetime of 5.9 years bearing an altitude of 600 km (Miau & Holdaway, 2000). The altitude should be lowered to 405 km after 5 years which is still above the minimum required altitude of 350 km. Orbit decay rate is 1.5 km per day from an altitude of 407 km. Composites use would cost at least \$1,000 for the propulsion system, which is about 3% of the total costs.

The cost breakdown of the LOFTSS is cheaper than producing large satellite. The LOFTSS design meets most of the goals for remote sensing the natural resources in Iran. The likelihood of failure is only 4.2% (Miau & Holdaway, 2000). Most of the operations involving space borne remote sensing are national or international programs (Rees, 2001). Their costs are not made public for security reasons.

Risk Factor	1994 Perceived Risk	2001 Perceived Risk (relative to 1994)
Technical	Low	Higher
Market	Low	Higher
Policy & Regulatory	Medium	Lower
Foreign Competition	Medium	Higher

Figure 2: Perceived risks of using commercial satellites in 1994 compared to 2001

Failures may be attributed to users' lack of knowledge, technical expertise, resources, and management skill that can place fully operational remote sensing satellite into the orbit (O'Connell, 2001). This technology may adopt new business models to come up with new information formats they can use for management decisions and collection of imagery data. There were evidences indicating large scale remote sensing systems were not generating enough revenues from their collection of raw data to even cover the cost of building and operating the satellites. In 1990, constructing and launching two Landsat satellites ranged from \$0.75 billion to \$1 billion (The Congress of the United States Congressional Budget Office, 1993). The amount came up to an annual cost of about \$85 million to \$110 million in terms of cost of capital, insurance, and maintenance.

## 6. Discussion

The cost of building large satellites could cripple the budget of Iran only if they cannot find market for the raw data they collected using remote sensing. Even though it has its own local use for predicting natural hazards and evaluating the condition of Iran's

natural resources, it is important that the government could translate some images into commercial market value to cover cost. Reducing the impact of natural disasters is possible with the use of remote sensing applications. Sandstorm or landslide inventories could be made by performing susceptibility mapping that would lead to identification of hazard zones. Setting up a remote sensing application and satellite system for Iran actually means also considering market distribution and commercial value of collected raw data. To be able to continuously operate the facility, Iran should choose the low cost LOFTSS satellite and hire a marketing planning consultant for the raw data translation to commercial value.

It is important to derive revenues from its application in order to cover operational cost. There could be improved methods in segregating top secret profiles and raw data from commercial raw data. The marketing aspect should be presented along with the cost of building the remote sensing satellite to be able to evaluate how much cost should the government handle each year. However, improved market prospects largely depends on the speed, capability, and processing of the remote sensing satellite system. Continued development of the GIS software and hardware is one major consideration to make it possible for the raw data available to a wider audience. Planning on how to market the raw data should support the mission of the remote sensing satellite.

The problem with the remote sensing application besides skills and knowledge is actually the market value conversion of the raw data. This happens to be more important in evaluating the feasibility of the project. Enhanced data and translation of its commercial value will greatly contribute to the maintenance and operational costs of the remote sensing satellite system. Choosing the LOFTSS is more feasible and attainable than the large satellites. This could generate Iran profits if the marketing aspect is handled well. The coordination between marketing and government operations will enhance and direct the flow of the project. The lack of clarity and segregation of the categories and use of raw data may impede market transformation of the

information. Market growth could reduce operational cost. This means finding more market for the raw data, segregating the raw data, and determining pricing policies as well as alternative low cost aerial imaging. This idea will enable Iran to solve financing and technical problems. The systems could be improved if there is a defined market. Commercialization may not be a good idea but it helps carry the burden of the cost of building and operating remote sensing. If Iran can gain profits from transformation of raw data to commercial data, then the focused now would be the process of segregating top secret data from commercial data.

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## Role of Activated Protein C and Soya-bean in Experimental Lung Toxicity in Adult Male Albino Rat: Histological and Immunohistochemical Studies

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**Abstract: Background:** Amiodarone (AM) is a potent antiarrhythmic drug that is limited in clinical use by its adverse effects, including potentially life-threatening AM-induced pneumotoxicity. The resemblance of the morphologic changes in amiodarone-treated rats lungs to pulmonary toxicity in humans suggest that this form of chemical injury may serve as useful model of this disease entity. **Aim of the Work:** Studying the effect of amiodarone on the lung of rat histologically and immunohistochemically as well as the role of concomitant use of soybean and activated protein C (APC) in ameliorating pulmonary toxicity (pneumotoxicity or parenchymal lung disease) induced by amiodarone. **Material and Methods:** Thirty adult male albino rats were used for the present study. The animals were divided into four groups: group I (control), group II (each rat was amiodarone orally treated for 6 weeks), group III (each animal was given soybean and amiodarone for 6 weeks) and group IV (each animal was given activated protein C and amiodarone for 6 weeks). The lung was then examined histologically and immunohistochemically. **Results:** Amiodarone caused increased interalveolar thickness with increased cellularity and mononuclear cellular infiltrations. Hypercellularity involved macrophages with large vacuolated cytoplasm and hyperplastic type II pneumocytes in group II. There was congested blood capillaries with some extravastion of red blood cells. By electron microscope, the hypertrophied type II pneumocytes showed multiple lamellar bodies and small inclusions with dilated rough endoplasmic reticulum. Macrophages with eccentric nuclei and prominent lamellar bodies with increased lysosomes were detected in group II. Also, increasing collagen fibers were detected. Rats treated with APC and soybean showed attenuation of these changes. There was a significant increase in plasminogen activator inhibitor-1 (PAI-1) immunoreaction in group II with nonsignificant change in group III and group IV.

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**Key Words:** Activated Protein C, Soya-bean, Lung Toxicity, plasminogen activator inhibitor-1 (PAI-1), lamellar bodies.

### 1. Introduction:

Pulmonary toxicity is a complex, chronic illness that may result from a variety of acute and chronic lung diseases (*Martin and Rosenow, 1988*). The excessive accumulation of matrix proteins, mainly produced by fibroblasts and myofibroblasts, is responsible for impairment in alveolar walls, loss of elasticity and development of rigid lung (*Massey et al., 1995*). The progression of pulmonary fibrosis is closely related to a complicated network consisting of many chemical mediators, coagulation abnormalities and endothelial cells (*Blake and Reasor, 1995*). Procoagulant signalling mechanisms as a member of this network, contributes to the development of lung inflammation and fibrosis in acute lung injury and fibrotic lung disease (*Idell, 2003*).

Amiodarone is a potent antiarrhythmic drug whose use has been limited due to numerous side effects (*Waerhaug et al., 2009*). One of the more serious side effects is the development of pulmonary toxicity (*Massey et al., 1995*). As the use of amiodarone increases, the occurrence of its side

effects will likely increase accordingly. Despite a great deal of information regarding the mechanisms of amiodarone-induced lung injury, management of this lung disorder is frequently difficult, and unfortunately therapeutic approaches to inhibit the development of amiodarone pulmonary toxicity are scanty (*Padmavathy et al., 1992*).

Emerging evidence that amiodarone administration results in interstitial alveolar inflammation and reports from earlier studies mentioned that coagulation abnormalities with oxidative metabolic reactions in lung injury are compelling reasons to use APC and soybean in suppressing amiodarone induced pulmonary toxicity (*Padmavathy et al., 1992*).

Several studies suggest that oxidant-antioxidant imbalances in the lower respiratory tract play a critical role in the pathogenesis of pulmonary injury. For example, pulmonary inflammatory cells of patients with pulmonary injury generate higher levels of oxidants than those in control patients (*Padmavathy et al., 1992*).

Soybean has been a food in china for thousands of years. It is an abundant, economic source of protein. No other nation has acquired the same taste for soybean as the Chinese and Japanese, but the bean has become an important raw material for the international food industry (*Liechtenstein, 1998*). Attention has recently focused on the possible role of soybean in the diet for the prevention and treatment of degenerative diseases (*Messina, 1999*). Several studies documented the hypocholesterolaemic, anticarcinogenic effects of soybean as well as its role in lowering the risk of osteoporosis (*Ishimi et al., 1999*).

The health benefits of soybean and its products have been documented (*Mitchell et al., 1998*). The active components of the soybean products responsible for these effects have yet to be defined. It is believed that isoflavones, genistein and daidzein are responsible for these observed benefits (*Malencic et al., 2007*). Soy-bean isoflavones may play a beneficial role in preventing hormone-related disorders such as breast cancer and autoimmune diseases. Soybean isoflavones, exhibited in vitro, strong antioxidant potency in decreasing the risk of coronary heart disease, osteoporosis and chronic renal disease (*Mahwah, 1996*). Several studies have shown that supplementations with either purified isoflavones (genistein and daidzein) or 80% methanol extract (containing isoflavones) from several soy food including tofu, inhibited reactive nitrogen species-induced oxidation that cause many chronic diseases such as interstitial lung disease (*Silva et al., 2002*). Soybean polyphenol content may act also as antioxidant, thereby reducing the risk of pulmonary injury and atherosclerosis (*Su et al., 2000*). As most of the previous studies reported the effect of soybean on lung injury biochemically, the current study evaluated the role of soybean on lung-induced toxicity histologically and immunohistochemically.

There is increasing evidence that the activation of the coagulation cascade with the resultant extravasation of active coagulation proteinases into alveolar tissue may play an important role in the pathogenesis of lung toxicity (*Chambers, 2008*).

The protein C (PC) pathway is one of the most important regulators of the blood coagulation system (*Dhainaut et al., 2004*). The zymogen PC is converted into its active form, activated PC (APC) via thrombomodulin-thrombin complex on the phospholipid surface of endothelial cells, monocytes and platelets (*Jian et al., 2005*). Recent studies have demonstrated that, in addition to its regulatory function on the coagulation system, APC may also regulate the inflammatory responses (*Looney et al., 2009*). APC is decreased in many pathological states, e.g. endotoxin induced pulmonary vascular injury and

Escherichia coli in experimental animal models of sepsis (*Richard et al., 2007*). Therefore, we hypothesized that APC may improve lung histology and function in an animal model of lung induced toxicity using light and electron microscopy.

## 2. Material and Methods:

### Chemicals:

Amiodarone: hydrochloride-aniodinated benzofuran derivative was obtained from Sanofi Pharmaceuticals Company, France. Each 1 mg was dissolved in 10 mL of distilled water. It was given to the animals orally by gastric tube at a single daily dose of 29 mg/kg body weight.

Soybean: water soluble soybean fiber (WSSF) was prepared in Department of Nutrition in the National Research Center. It was prepared from defatted soybean, its molecular weight is 50000. it was added in a dose of (50 gm/kg of diet) to the standard diet (*Maksimovic et al., 2005*).

Activated protein C: a natural anticoagulant was obtained from Eli-Lilly Company. Each 1 mg was dissolved in 1 ml isotonic sodium chloride solution. It was injected to the animals interaperitoneal (I.P.) at a single daily dose of 5 mg/kg body weight (*Leeder et al., 1994*).

### Animals groups:

The present study was carried out on 30 adult male albino rats weighing 180-200 grams each. All animals were kept under standard laboratory conditions with free access to food and water throughout the study period. They were kept for one week for acclimatization before the experiments in clean properly ventilated cages. The animals were divided into four groups:

**Group I (Control group):** It was further subdivided into 3 subgroups:

- a. Subgroup **I(a)**: Included 5 animals that received no treatment.
  - b. Subgroup **I(b)**: Included 5 animals that received (WSSF) as the previously mentioned doses, route of administration, daily for 6 weeks.
  - c. Subgroup **I(c)**: Included 5 animals that were injected I.P. at single daily dose by activated protein C 5 mg/kg for 6 weeks.
- **Group II:** Included 5 rats which were received amiodarone at a single daily dose of 29 mg/kg body weight orally for 6 weeks.
  - **Group III:** Included 5 rats which received amiodarone and (WSSF) as the previously mentioned doses, route of administration, daily for 6 weeks.
  - **Group IV:** Included 5 rats received amiodarone

and activated protein C as the previously mentioned doses, route of administration, daily for 6 weeks.

At the appropriate time, all animals were sacrificed and thoracotomy was done. The lungs were dissected after intracardiac perfusion and intratracheal injection with 4% paraformaldehyde in 0.1 M phosphate buffered solution (pH 7.4) containing 2.5 glutaraldehyde solution to inflate the lungs. The trachea was ligated just caudal to the larynx and the thoracic contents were removed as one unit, then both lungs were excised.

For light microscopy, the lung specimens were fixed by immersion in 10% buffered formalin solution for 24 hours, dehydrated in a graded ethanol series, cleared in xylene and then embedded in paraffin wax. Sections (5µm thick) were stained with haematoxylin and eosin (H and E), with Mallory's trichrome stain for demonstration of collagen fibres (*Fraire et al., 1993*).

For Immunohistochemistry, paraffin sections of the lung specimens were deparaffinized, hydrated, washed, heated in buffer citrate and incubated for 30 minutes with rabbit serum. Then the slides from each group were incubated with anti-plasminogen activator inhibitor-1 (PAI-1) mouse monoclonal antibodies (Catalogue Numbers 3785, American Diagnostica, Stamford, CT) at a 1:200 dilution for 22 hours at room temperature. On the next day, all slides were washed in buffered phosphate solution, incubated with biotinylated anti-mouse antibody dilution 1:200 for 1 h (Vector Laboratories, Burlingame, Calif., USA). Then, they were incubated in avidin-biotin-peroxidase complex (Patts, Glostrup, Denmark) chromogen for few minutes. Finally all lung sections were counterstained with Mayer's haematoxylin catalogue number (TA-060-MH). The specificity of the immune reactions was tested by replacing the primary antiserum with phosphate buffer saline as a negative control (*Jessurun et al., 1998*).

For electron microscopy, thin slices of the lungs were fixed in 2.5% phosphate buffered glutaraldehyde solution (pH7.4) for 2 hours and postfixed in 1% osmium tetroxide solution in phosphate buffer for 2 hours. Subsequently, the specimens were dehydrated in a graded series of ethanol, treated with propylene oxide and embedded in Epon. After heat polymerization the sections were cut using an ultramicrotome. Ultrathin sections were double-stained with uranyl acetate and lead citrate to be examined by a JEOL transmission electron microscope (JEM 1010, Japan), at 80 KV in the Faculty of Science, Ain Shams University.

### Morphometric Study:

The morphometric data were obtained using Leica Qwin 500 UK, image analyzer computer system in Histology Department, Faculty of Medicine Cairo University. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units.

Using the interactive measuring menu the thickness of the interalveolar septa were measured in haematoxylin and eosin-stained sections at a magnification of 400. In each section ten readings were obtained from ten randomly-chosen non-overlapping fields and the mean values and standard deviations were calculated automatically by the image analyzer. Using the measuring field menu and by the color detecting method the area % of positive plasminogen activator inhibitor-1 immunostained area as well as the collagen fibers were measured in plasminogen activator inhibitor-1 immunostained and Mallory's trichrome stained sections. The measurements were done at a magnification of 400. Ten readings from ten randomly-chosen non-overlapping fields were obtained. The thickness of the interalveolar septa and the area % of + ve plasminogen activator inhibitor-1 Immunostained area as well as collagen fibers, all data were expressed as mean (X),  $\pm$  standard deviation (SD) and student's test was used to compared between different groups. P-value was calculated using Minitab program and  $P < 0.05$  was considered non-significant while  $P < 0.001$  was highly significant.

### 3. Results:

#### I- Light Microscopic Results:

Examination of sections stained with H and E from the control animals (Group I) revealed that all subgroups showed normal histological structure of the lung. The interalveolar septa were seen to be thin and the alveoli and alveolar sacs appeared clear and patent (fig. 1).

Mallory's trichrome-stained sections showed fine collagen fibers around the bronchioles and to a lesser extent in the interalveolar septa (fig. 2). Moreover, PAI-1 immunoreactivity of lung sections of control group revealed negative immune reaction to PAI-1 almost in all fields (fig. 3).

As regards amiodarone-treated animals (group II), in Hx. and E.- stained sections, the changes were variable among the animals. Complete loss of normal lung architecture was observed with loss of continuity of alveolar epithelium (figs. 4, 5, 6).

Meanwhile, there was extensive thickening and distortion of the interalveolar septa with dilatation and congestion of the pulmonary blood vessels (figs.



4,5). Focal areas of overexpansion, areas of attenuated intrapulmonary air passage and alveolar lining epithelium with destroyed inter-alveolar septa were observed (figs. 5, 7).

Homogeneous eosinophilic material with perivascular and peribronchial cellular infiltration and in the interstitial tissue were observed with vacuolated macrophages inbetween them (figs. 4, 5, 6, 7, 8).

Injury of the lining epithelium of some alveoli and bronchioles was observed with sloughing of some cellular debris and some pneumocytes type II appeared vacuolated with dark nuclei (figs. 6,7, 8).

Mallory's trichrome staining showed an apparent increase in the content of collagen fibers in the perivascular area and in the thickened inter-alveolar septa (fig. 9). Perivascular and peribronchial deposition of coarse collagen fibers was observed (fig.10). Regarding PAI-1 immunoreactivity, sections revealed strong immunopositivity for PAI-1 and a statistically highly significant increase in the area % of the reaction appeared as brownish cytoplasm (figs. 11,12,13).

Examination of sections stained with Hx. and E from amiodarone and soybean-treated animals (group III) showed decrease in the interalveolar thickness while some areas with thickened septa were observed (fig. 14). Moreover, animals treated with amiodarone and activated protein C (group IV) showed preservation of normal architecture, nearly similar to the control group (fig. 15).

However, the increase in collagen fibers was still observed in group III (fig. 16), but group IV was appeared similar to the control group in (fig. 17).

On the other hand, the lung section of group III showed moderate decrease in the area % of the immune reaction to PAI-1 (fig. 18). However, negative to moderate immune reaction to PAI-1 in the area % was observed in group IV (fig. 19).

## II- Electron microscopic results:

Ultrastructural examination showed the two types of pneumocytes lining the alveoli. Type I pneumocytes, were flattened in shape with a flat large nucleus and smooth surface. Type II pneumocyte were cuboidal and had central nuclei with few short scattered microvilli on their cell surfaces. Type II pneumocytes were characterized by the presence of many electron-dense concentric lamellae of secretory materials (fig. 20).

In amiodarone-treated animals (group II), type II pneumocyte showed degenerative changes of its lamellar bodies, nearly devoid of its lamellation and numerous cytoplasmic vacuoles and dense bodies (figs. 21, 22, 23, 24). The interstitium showed extravasations of RBCs, many mononuclear cellular infiltration and transversely oriented collagen fibers (figs. 22, 23, 24).

Thickening of the inter-alveolar septa and alveolar macrophage were observed with vacuolated cytoplasm and lamellar inclusions (figs. 22, 25). Examination of group III showed improved picture as compared to group II as some lamellar bodies in type II pneumocyte filled with parallel lamella (fig. 27). Sections of group IV revealed that, most of the observed changes in group II were decreased in both severity and frequency. Type II pneumocytes appeared nearly similar to the control (fig. 28).

Morphometric and statistical analysis showed non-significant changes ( $P>0.05$ ) in the means of inter-alveolar thickness in subgroups Ib and Ic and a high significant increase ( $P<0.001$ ) in group II as compared with subgroup Ia. Regarding group III and IV there was a decrease in the thickening of the interalveolar septa as compared to group II but there was a non-significant change ( $P>0.05$ ) as compared with subgroup Ia (Table 1).

**Table (1): The mean thickness of the interalveolar septa in different groups as compared to the control subgroup I-a by student's t-test.**

Group	Mean $\pm$ S.D.	T-value	P-Value	Significance
I-a	1.83 $\pm$ 0.35			
I-b	1.84 $\pm$ 0.42	0.03	>0.05	N.S.
I-c	2.10 $\pm$ 0.39	0.98	>0.05	N.S.
II	19.01 $\pm$ 5.11	7.01	<0.001	H.S.
III	2.60 $\pm$ 0.41	1.91	>0.05	N.S.
IV	1.98 $\pm$ 0.52	1.96	>0.05	N.S.

There was a non-significant change ( $P>0.05$ ) in the mean area of percentage of collagen in subgroups Ib and Ic as compared to subgroup Ia. There was a highly significant increase ( $<0.001$ ) in mean area of percentage of collagen content in animals of group II

as compared to the control subgroup Ia. Regarding group III and IV there was a non-significant change ( $P>0.05$ ) in the mean area of percentage of collagen as compared to the control group Ia (Table 2).

**Table (2): Mean area percentage of collagen content/field in different groups compared to the control subgroup Ia by student's t-test.**

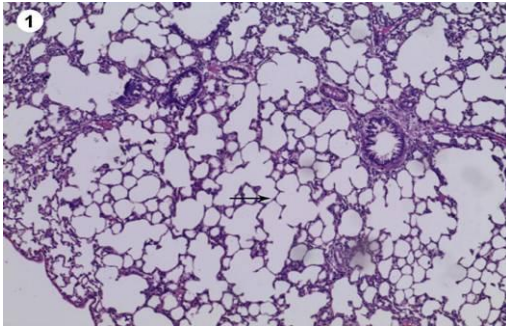
Group	Mean $\pm$ S.D.	T-value	P-Value	Significance
I-a	4.11 $\pm$ 1.01	-		
I-b	4.13 $\pm$ 0.52	0.27	>0.05	N.S.
I-c	4.25 $\pm$ 0.31	0.44	>0.05	N.S.
II	37.5 $\pm$ 4.11	8.10	<0.001	H.S.
III	8.15 $\pm$ 2.16	6.06	>0.05	N.S.
IV	8.12 $\pm$ 2.13	6.03	>0.05	N.S.

As regards the means of area percentage of PAI-1/field, there was a non-significant change in subgroups Ib and Ic, as compared to the control subgroup Ia ( $P > 0.05$ ), a highly significant increase

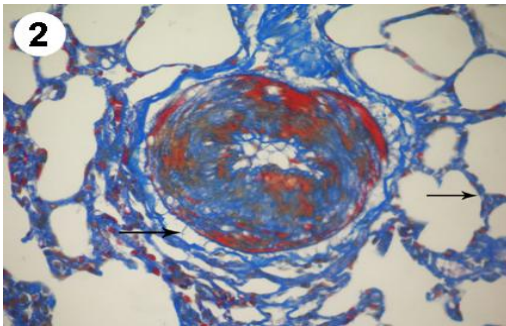
(<0.001) in the means of area % of PAI-1/field in animal of group II and a non-significant increase (>0.05) in animals of groups III and IV as compared with the control subgroup Ia (Table 3).

**Table (3): Mean area percentage of PAI-1/field in different groups compared to the control subgroup I-a by student's t-test.**

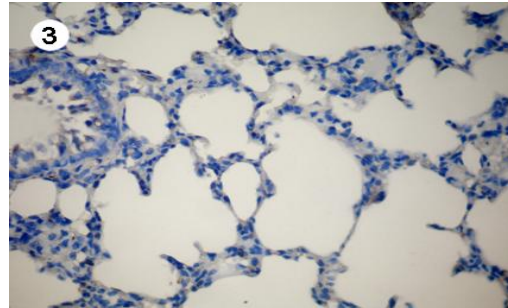
Group	Mean $\pm$ S.D.	T-value	P-Value	Significance
I-a	8.19 $\pm$ 3.01	-		
I-b	8.56 $\pm$ 1.97	0.32	>0.05	N.S.
I-c	8.01 $\pm$ 2.04	0.04	>0.05	N.S.
II	27.22 $\pm$ 3.96	8.99	<0.001	H.S.
III	14.85 $\pm$ 8.72	3.11	>0.05	N.S.
IV	14.81 $\pm$ 8.53	3.15	>0.05	N.S.



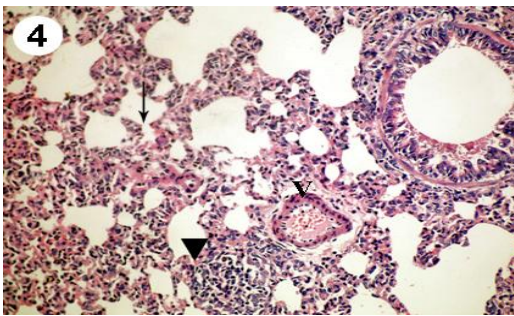
**Fig. 1:** A photomicrograph of rat lung of the control group (Group I) showing normal lung architecture with thin inter-alveolar septa (arrow). Notice the normal clear alveoli and alveolar sacs. Hx. & E.; X 200



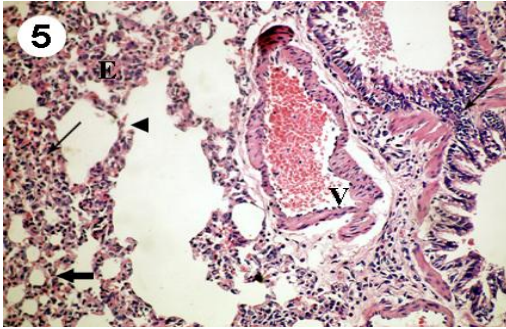
**Fig. 2:** A photomicrograph of rat lung of the control group (Group I) showing few scanty delicate collagen fibers in the inter-alveolar septa and surrounding the bronchioles (arrow). Mallory's trichrome; x 400



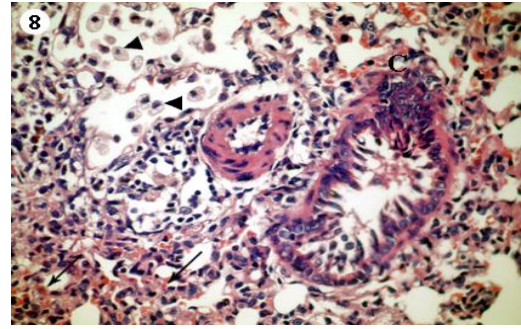
**Fig. 3:** A photomicrograph of rat lung of the control group (Group I) showing negative immune reaction to plasminogen activate inhibitor-1 (PAI-1) in the inter-alveolar septa, alveolar cells, peribronchial areas and bronchial epithelium. Immunostaining & Hx. counterstain.;x 400



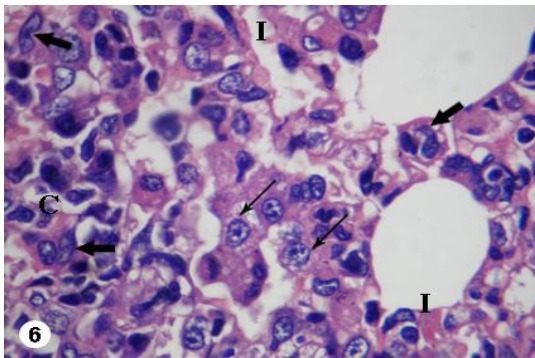
**Fig. 4:** A photomicrograph of rat lung from amidarone-treated group (Group II) showing thickened inter-alveolar septa, loss of continuity of alveolar epithelium (arrow), congestion and dilatation of the pulmonary blood vessels (V) with perivascular and interstitial mononuclear cellular infiltration in the form of nodules (arrow head). Hx. & E.; x 200



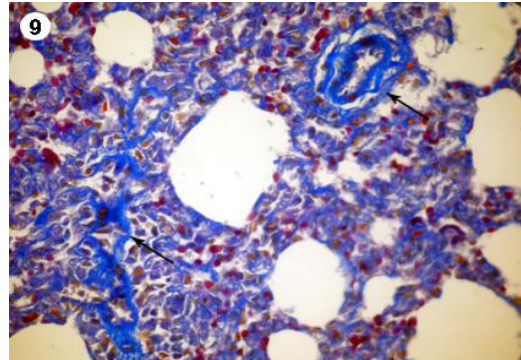
**Fig. 5:** A photomicrograph of rat lung from amiodarone-treated group (Group II) showing a congested blood vessel (V), interstitial and peribronchiolar mononuclear cellular infiltration (thin arrow), with focal areas of overexpansion with destroyed interalveolar septa (head arrow). Notice the presence of eosinophilic material in thickened interalveolar septa (E) and the collapsed alveolar spaces (thick arrow). Hx. & E.; x 200



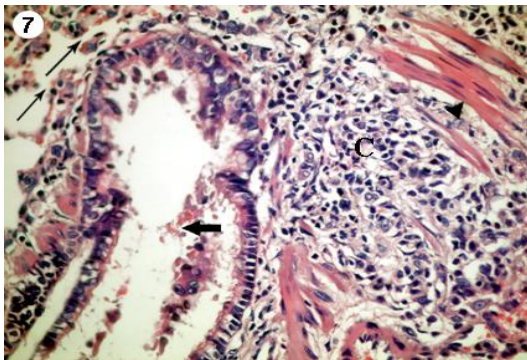
**Fig. 8:** A photomicrograph of rat lung from amiodarone-treated group (Group II) showing loss of normal architecture and subepithelial cellular infiltrate encroaches on the bronchiolar lumen (C), sloughing of vacuolated pneumocytes type II with dark pyknotic nuclei inside alveoli (arrow head) and extravasation of blood in alveolar spaces with marked collapse of many alveoli (arrow). Hx. & E.; x 400



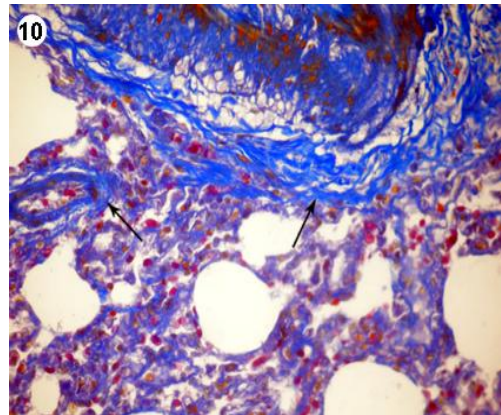
**Fig. 6:** A photomicrograph of rat lung from amiodarone-treated group (Group II) showing apparent increase in both size and number of type II pneumocytes with highly vacuolation of their cytoplasm (thin arrow). Notice homogenous eosinophilic material in the interstitial tissue (I), cellular infiltration (C) and macrophages in between them (thick arrow). Hx. & E.; x 1000



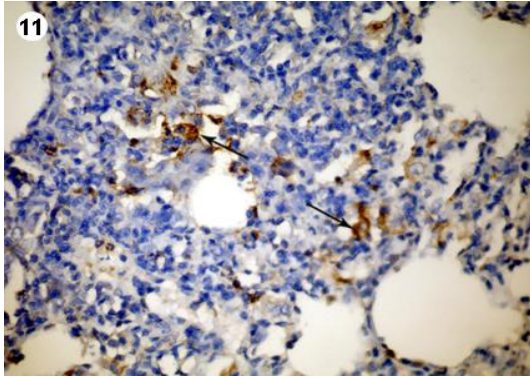
**Fig. 9:** A photomicrograph of rat lung from amiodarone-treated group (Group II) showing increase in the collagen fibers perivascular and in the thickened inter-alveolar septa (arrow). Mallory's trichrome; x 400



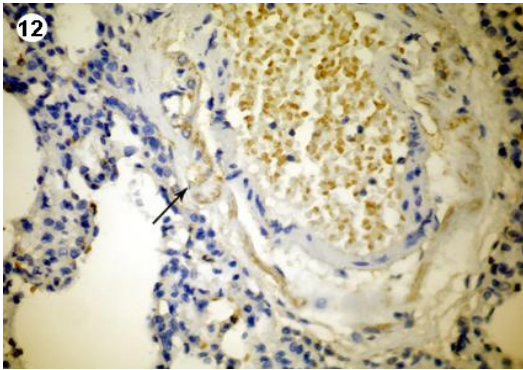
**Fig. 7:** A photomicrograph of rat lung from amiodarone-treated group (Group II) showing loss of lung architecture with extensive infiltration by mononuclear inflammatory cells (C), areas of destroyed inter-alveolar septa (thin arrow) and area of bundles of collagenous fibres in inter-alveolar septum (arrow head). Sloughing of bronchiolar lining epithelium (thick arrow) were also noticed. Hx. & E.; x 400



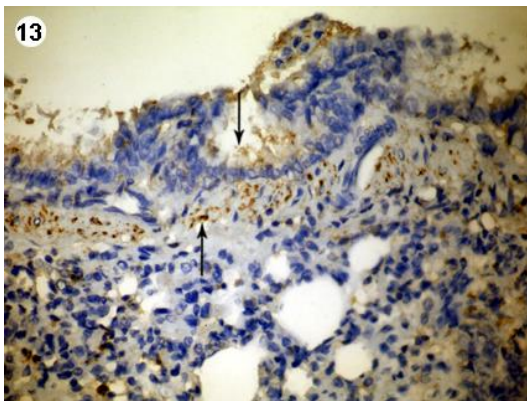
**Fig. 10:** A photomicrograph of rat lung from amiodarone-treated group (Group II) showing coarse collagen deposition (arrow) in perivascular and in peribronchiolar areas. Mallory's trichrome; x 400



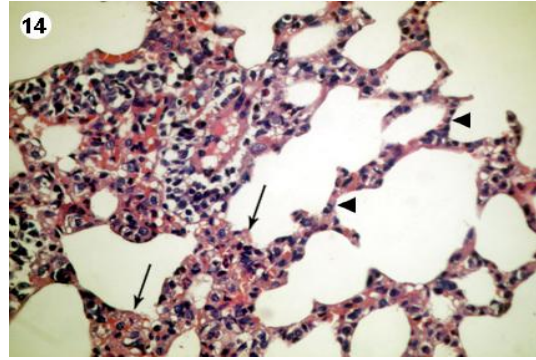
**Fig. 11:** A photomicrograph of rat lung from group II showing positive immune reaction to PAI-1 in some alveolar cells and thick inter-alveolar septa (arrow). Immunostaining & Hx. counterstain.;x 400



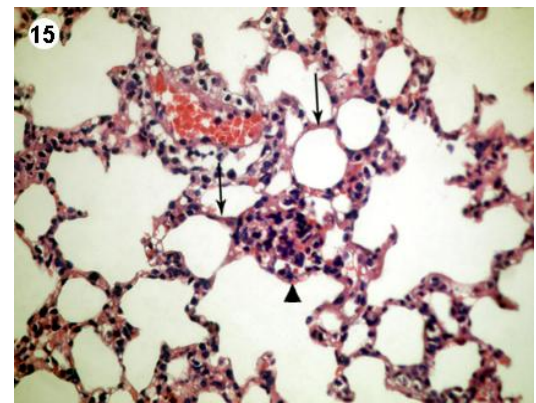
**Fig. 12:** A photomicrograph of rat lung from group II showing positive immune reaction to PAI-1 in the perivascular area (arrow). Immunostaining & Hx. counterstain.;x 400



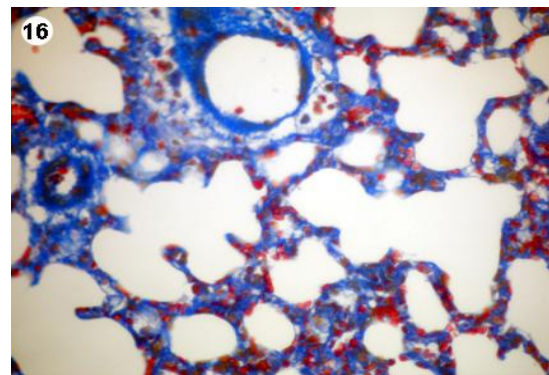
**Fig. 13:** A photomicrograph of rat lung from group II showing positive immune reaction to PAI-1 in the peribronchiolar areas and bronchiolar epithelium (arrow). Immunostaining & Hx. counterstain.;x 400



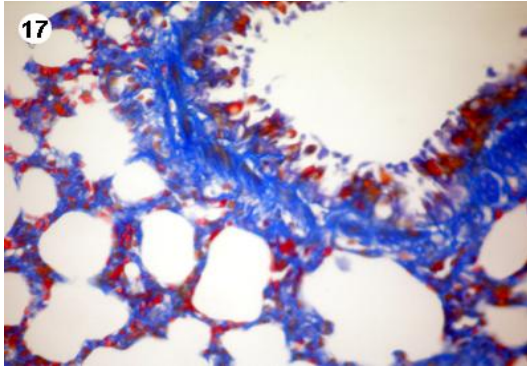
**Fig. 14:** A photomicrograph of rat lung from amiodarone and soybean treated group (Group III) showing decrease in the inter-alveolar thickness (arrow head). Some areas with thickened septa were observed (arrow). Hx. & E.; x 400



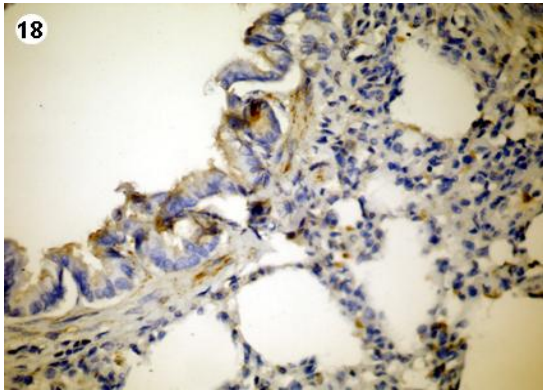
**Fig. 15:** A photomicrograph of rat lung from amiodarone and activated protein C-treated group (Group IV) showing preserved normal architecture with decrease in the inter-alveolar thickness to be more or less as control group (arrows) but with few thickened infiltrated parts (arrow heads). Hx. & E.; x 400



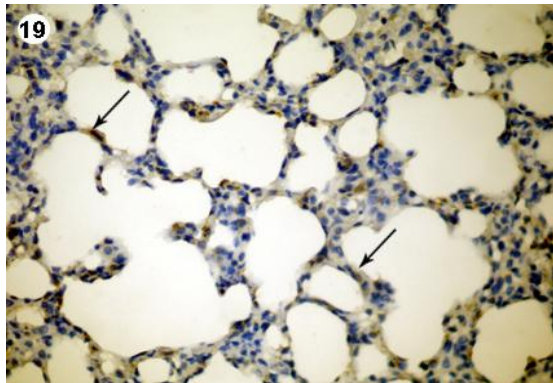
**Fig. 16:** A photomicrograph of rat lung from group III showing decrease in the collagen fibers in the inter-alveolar septa and in the peribronchiolar areas. Mallory's trichrome; x 400



**Fig. 17:** A photomicrograph of rat lung from group IV showing decrease in the collagen fibers in the thin interalveolar septa and peribronchiolar areas to be more or less similar to control group. Mallory's trichrome; x 400



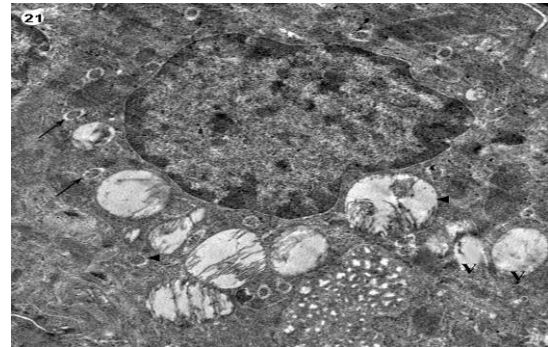
**Fig. 18:** A photomicrograph of rat lung from group III showing moderate immune reaction to PAI-1 in the interalveolar septa, peribronchiolar area and in bronchiolar epithelium. Immunostaining & Hx. counterstain.;x 400



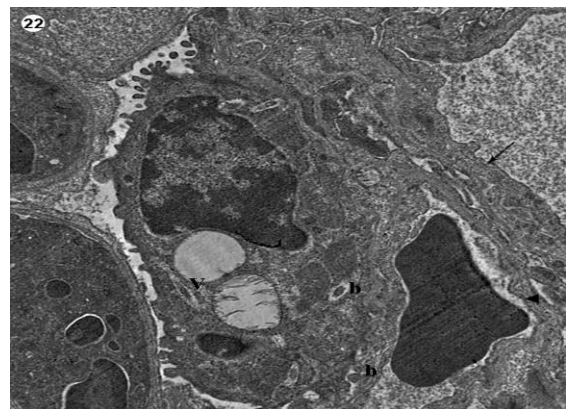
**Fig. 19:** A photomicrograph of rat lung from group IV showing negative to moderate immune reaction to PAI-1 in most alveolar cell walls and in the inter-alveolar septa (arrows). Immunostaining & Hx. counterstain.;x 400



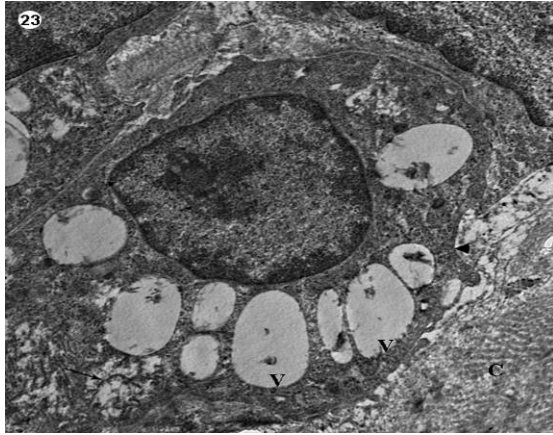
**Fig. 20:** An electron micrograph of rat lung from control group showing type I pneumocyte containing flat nucleus (N) and type II pneumocyte with many electron dense concentric lamellae of secretory material (thin arrow) and central nucleus with euchromatin (arrow head) Notice the microvilli on the surface of type II pneumocyte (thick arrow).



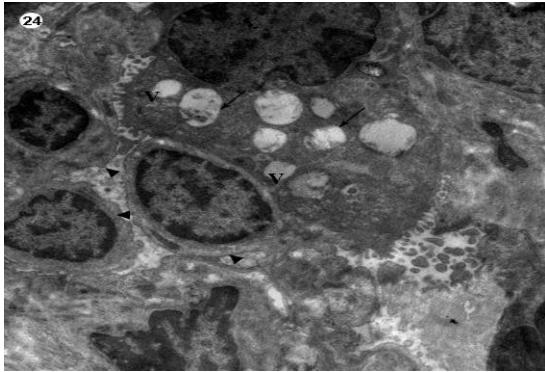
**Fig. 21:** An electron micrograph of rat lung from group II showing type II pneumocyte with degenerative changes of its lamellar bodies (arrow head), many cytoplasmic vacuoles (V) and numerous dense bodies (arrow). Notice absence of microvilli. X 3,000



**Fig. 22:** An electron micrograph of rat lung from group II showing type II pneumocyte with numerous cytoplasmic dense bodies (b) and vacuoles (V), thickening of interalveolar septa (arrow) with congested blood vessels (arrow head). x 2,500



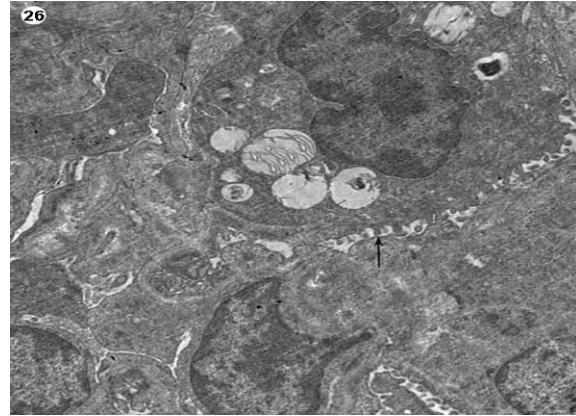
**Fig. 23:** An electron micrograph of rat lung from group II showing type II pneumocyte nearly devoid of the secretory material in its lamellar bodies with presence of many large vacuoles (V), rarefaction of the cytoplasm (arrow) and absence of microvilli (arrow head). Notice transversely oriented collagen fibers (C). x 3,000



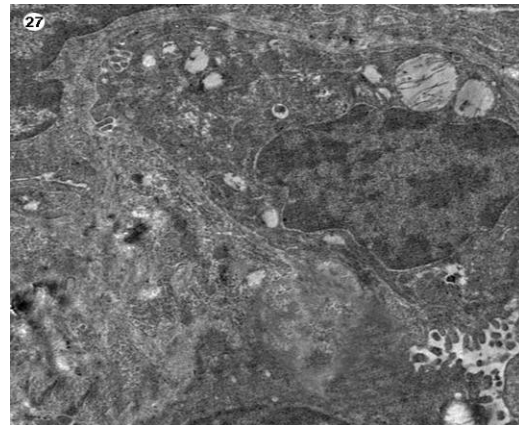
**Fig. 24:** An electron micrograph of rat lung from group II showing type II pneumocyte with vacuolated cytoplasm (V) and numerous disorganized lamellar bodies (arrow) that lose their lamellar appearance. Notice many mononuclear cellular infiltration (arrow head). x 2,000



**Fig. 25:** An electron micrograph of rat lung from group II showing an alveolar macrophage with its eccentric kidney shaped nucleus (N), irregular outlines and vacuolated cytoplasm (V) with occasional lamellar inclusions (thin arrow) and lysosomes (L). Notice, marked thickening of inter-alveolar septa (thick arrow). x 3,000



**Fig. 26:** An electron micrograph of rat lung from group III showing type II pneumocyte with many lamellar bodies, some of them filled with parallel lamella. Notice microvilli (arrow).x 2,500



**Fig. 27:** An electron micrograph of rat lung from group IV showing type II pneumocyte has decreased number of lamellar bodies with normal parallel lamella, rounded central nucleus with euchromatin and has microvilli. x 3,000

#### 4. Discussion:

The antiarrhythmic drug amiodarone produces lung toxicity in humans and animals. Some data suggested a role for oxidative stress in amiodarone-induced lung toxicity. Amiodarone inhibits mitochondrial complex I and II respiration and produces mitochondrial dysfunction in lung epithelial cells and macrophages. Also, amiodarone increases the levels of reactive oxygen species (ROS) and oxidized glutathione. Further studies have revealed that amiodarone is metabolized to an early radical that may give rise to other ROS (Massey *et al.*, 1995). Both catalytic and scavenger antioxidants have been shown to attenuate amiodarone-induced lung injury. In addition, phenolic antioxidants were effective in limiting amiodarone-induced ROS and lung injury. The resemblance of the morphological changes in amiodarone-treated rats lungs to pulmonary injury in

humans suggests that this form of chemical injury may serve as useful model of this disease entity (Esmon *et al.*, 1997).

The etiology of amiodarone pulmonary toxicity is unknown. However, several causes have been proposed including direct toxicity, the involvement of hypersensitivity, elevated oxidant involvement and drug-induced alterations in membrane properties. It has been postulated that the cause is complex and multifactorial, possibly involving several of these mechanisms (Shimizu *et al.*, 2003).

Several mechanisms have been postulated to explain the suppressive effect of APC on lung injury. One mechanism is the ability of APC to suppress inflammation. The anti-inflammatory activity of APC is a reflection of its ability to suppress the secretion of inflammatory cytokines, such as TNF- $\alpha$  and monocyte chemoattractant protein-1 from the inflammatory cells and to inhibit the activation and extravasation of leukocytes. APC also may inhibit the lung injury by binding to and inhibiting plasminogen activator inhibitor-1 leading to increased activity, of plasminogen activator and a net enhancement of fibrinolytic function (Vangerow *et al.*, 2007). APC, as anticoagulant activity block interalveolar fibrin deposition and thrombin generation. APC is able to activate pro-matrix metalloproteinases and in that way suppresses the deposition of extracellular matrix in the lung (Jian *et al.*, 2005).

A further antifibrotic activity of APC is its ability to inhibit the expression of platelet-derived growth factor (PDGF) on macrophages, bronchial epithelial cells and pulmonary artery endothelial cells. PDGF is a potent mitogenic factor for fibroblasts. Moreover, inhibition of PDGF secretion by APC blocks the development of lung injury (Emmons and Peterson, 2001).

Previous studies mentioned that, soybean water soluble fiber ameliorated the lung toxicity changes induced by amiodarone and attributed this effect to its compounds. These compounds include a protease inhibitor, trypsin inhibitor, isoflavones (genistein and diadzein), saponins, inositol (hexaphosphate phytic acid) and sitosterol (Emmons and Peterson, 2001). Polyphenols compounds are often chain-breaking antioxidants and some have been used in the food industry as preservative. In general, they require larger doses or concentrations to produce antioxidant effects in model system because their lower rates of reaction with ROS and their limited ability to be recycled endogenously. Also, another study found positive correlation between the content of total isoflavone and genistein with antioxidant capacity (Silva *et al.*, 2002).

A recent study showed that isoflavones might have their effects on the pathways by inducing the

gene expression or modifying the enzyme activity rather than acting directly as free radical scavenger. On the other hand, other researchers found that all tested soybean isoflavones and glycosides appeared to be much weaker antioxidants than green tea and  $\alpha$ -tocopherol (Bolt *et al.*, 2001). Other research works showed that the active components of soy products responsible for these effects rather than isoflavones including phospholipids, lecithin, low but considerable content of linolenic acid (7.8%) and  $\alpha$ -tocopherol (Card *et al.*, 2003).

The amiodarone induced pulmonary toxicity manifested by many criteria included a mixed cellular exudates in the interstitium, protein exudates in air spaces with or without leukocytes, proliferation of lining epithelium, gradual progression to fibrosis, continuing evidence of cellular reaction and diffuse distribution (Massey *et al.*, 1995). The present work showed that amiodarone could induce pulmonary injury in the form of hyperplasia of type II pneumocytes, large foamy macrophages with mononuclear cellular infiltrations in lung parenchyma and thickened interalveolar septa. These coincide with other workers who observed amiodarone pneumotoxicity after 4 weeks of therapeutic dosage manifested by congested blood capillaries with perivascular cellular infiltration (Dhainaut *et al.*, 2004).

The current experiment revealed a remarkable mononuclear cellular infiltration peribronchiolar, perivascular and in the interstitial tissue. Hypersensitivity and immunological reactions could be important in the pathogenesis of amiodarone-induced interstitial pneumonitis, where injured epithelial lung cells release increased amounts of inflammatory cytokines in response to amiodarone (Jian *et al.*, 2005). These cytokines initiate the inflammatory reaction and attract inflammatory cells that were observed in the current study. The presence of red blood corpuscles in the extravascular tissue can also initiate the inflammatory reaction and attract the infiltrating cells to the site of congestion.

The present study showed many areas of destruction of the interalveolar septa and connection of many alveoli together with formation of large irregular air spaces. These morphological changes are compatible with emphysematous lung and similar findings described by other researchers (Looney *et al.*, 2009). Fragmentation of the alveolar septa could be due to proteolytic destruction of the lung parenchyma by the proteolytic enzymes released from the inflammatory cells. Moreover, in the present study dark pyknotic nuclei indicated the occurrence of apoptosis of alveolar wall or endothelial cells and is sufficient to cause pulmonary emphysema, even without the accumulation of inflammatory cells.

Disruption of the balance between apoptosis and replenishment of structural cells in the lung might contribute to the destruction of lung tissue leading to emphysema (Esmon *et al.*, 1997). Areas of collapsed alveoli and thickened interalveolar septa which were observed in our study were present side by side with the emphysematous ones. These alterations could be attributed to combined endothelial and epithelial injury that leads to basement membrane denudation, resulting in collapse of alveolar septa.

Congested blood vessels in our study was explained to be due to direct vasodilator effects of amiodarone by blocking alpha receptors and calcium channels inhibitory effects. EM study of capillary endothelial cells appeared swollen with vacuolization in their cytoplasm. This is explained by earlier study as amiodarone increased production of free oxygen radicals and capillary permeability. The capillary endothelial cells showed cellular swelling and detachment from underlying basement membrane, vascular congestion and thickening of the wall of arterioles were also reported (Oyama *et al.*, 2005).

The morphometric study in the present work revealed a highly significant increase in the thickness of the interalveolar septa in amiodaron-treated rats as compared with the control ones. This might be due to cellular infiltration in the septa and increased collagen content. This coincides with a previous study that showed hypercellularity and edema of interalveolar septa in amiodarone-treated group (Pitsiavas *et al.*, 1997). The original hypothesis for the pathogenesis of pulmonary fibrosis is that chronic inflammation stimulates the ability of fibroblasts to migrate, proliferate, and produce the extracellular matrix, thereby resulting in parenchymal fibrosis. A new hypothesis for the pathogenesis of pulmonary fibrosis suggesting that the inappropriate regeneration of the sequentially injured epithelium is sufficient to stimulate fibroblasts, resulting in irreversible parenchymal fibrosis (Taylor *et al.*, 2003).

As regards to type II pneumocytes, the current work also showed a highly significant increase in number of type II pneumocytes and an apparent increase in their size denoting cellular hyperplasia and hypertrophy suggesting that the type II cells are attempting to proliferate to regenerate the injured epithelium, particularly if there is destruction of the sensitive type I pneumocytes. Similar changes of the epithelial lining were previously reported by many researchers. Proliferation of type II pneumocytes was shown to be the most sensitive pathological indicator of alveolitis. Moreover, the density of type II pneumocytes could be a useful index for evaluating alveolar damage even in mild alveolitis. As regards to EM examination, type II pneumocytes showed degenerative changes of lamellar bodies in their

cytoplasm in amiodarone experimental group. This observation is in agreement with a previous work which explained that these lamellar bodies were the source of pulmonary surfactant (Yasuda *et al.*, 1996).

Some cells contain numerous dense bodies. Our present work is also in agreement with a previous research that suggested that dense bodies were due to a secondary lipid storage disorders induced by amiodarone. On the other hand, an earlier report found that chronic amiodarone therapy inhibited lysosomal phospholipases leading to phospholipids accumulation that appeared as dense bodies (Sunderji *et al.*, 2000).

In group II, the macrophages showed with lamellar bodies and pale vacuolated cytoplasm. This is in agreement with another study that suggested the increase in number was due to increased production of surfactant by type II pneumocytes (Sunderji *et al.*, 2000). As regards to the vacuolated, foamy appearance of macrophages, earlier research attributed it to secondary phospholipidosis caused by amiodarone. Another study explained that the cause for these inclusions in the macrophages was due to ingestion of surfactant and foreign particles as the macrophages played an important role in the lung defense (Lafuente-Lafuente *et al.*, 2009). They added that amiodarone inhibited phospholipase enzyme of lysosomes leading to the increase of phospholipids in lysosomes and appearance of lamellar bodies in macrophagy.

The collagen fibers content of the interalveolar septa was increased. This finding is attributed to immune and inflammatory mechanisms that leads to fibroblast stimulation and collagen deposition (Massey *et al.*, 1995). The increased amount of lung collagen detected in the experimental rats and its deposition in an abnormal locations indicated occurrence of interstitial fibrosis. This fibrosis was reported by an earlier work to be the end result of alveolar damage which may occur as a sudden acute incident or as a slowly developing process (Wang *et al.*, 1992). After concomitant use of APC, there was a decrease of collagen fiber content. This is in agreement with previous report who stated that APC was able to attenuate lung injury in rats. The density of neutrophil increased significantly with segmented nuclei and highly condensed chromatin in the lung interstitium (alveolar spaces). Also, eosinophils with bilobed nuclei could be observed in the alveolar wall and dilatation of rough endoplasmic reticulum that was attributed to disruption of protein sorting pathway (Nicolescu *et al.*, 2007).

Previous studies indicate that activation of the coagulation cascade may play an important role in the pathogenesis of lung injury (Jian *et al.*, 2005). In the present experiments, we examined the effect of APC



and soybean on amiodarone-induced lung toxicity. Histological examination showed that both agents alleviated inflammatory cell infiltration and progression of lung inflammation. The pulmonary reaction following the administration of intraproteineal amiodarone in the experimental animals has been extensively used the pathogenesis of the inflammation is the subject of further investigation. In the present study we found that dietary supplementation with soybean was effective in decreasing pulmonary toxicity induced by AM in vivo. The histological picture of lungs of rats given the soybean-enriched diet was near the control values at 36 days following AM administration. The soybean-enriched diet with APC decreased the AM-induced damage to the lung so that their appearance was similar to the control.

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**Anatomical Studied on the Cranial Nerves of *Liza Ramada* (Family: Mugilidae) Nervus Glossopharyngeus**Dakrory, A.I.<sup>1</sup>; Ali, R.S.<sup>2</sup>. and Issa, A.Z.<sup>1</sup>.<sup>1</sup>Department of Zoology, Faculty of Science, Cairo University<sup>2</sup>Department of Zoology, Faculty of Science, Helwan University[Dakrory2001@yahoo.com](mailto:Dakrory2001@yahoo.com)

**Abstract:** This study deals with the nervus glossopharyngeus of *Liza ramada*. The microscopic observations showed that, the nervus glossopharyngeus arises by one root and leaves the cranial cavity through its own foramen. It gives visceromotor fibres for the first levator arcualis branchialis muscles. It has single extracranially located epibranchial (petrosal) ganglion. The ramus pretrematicus carries general viscerosensory fibres for the epithelial lining of the pharynx and special ones for the taste buds. The ramus posttrematicus carries both general viscerosensory fibres for the epithelial lining of the pharynx and special ones for the gill filaments, as well as visceromotor fibres for the first adductor arcualis branchialis and the first obliquus ventralis muscle.

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**Key Words:** Nervus glossopharyngeus- *Liza ramada*.

**1. Introduction:**

The study of the cranial nerves is important because their distribution is correlated with the habits and habitats of animals and also because they show an evolutionary trend among animals of the same group. The cranial nerves connect the brain with all the important centers of perception of the outer surface of the head, as well as the inner surface of the buccopharyngeal and other visceral regions, so that they seem to be important in determining the animal's behavior (Shaheen, 1987).

The sensory systems (receptors, their nerves as well as their canters) play a major and sometimes a decisive role in many fish behavioral patterns (feeding, defense, spawning, schooling orientation, migration, etc..).

Although there are several classical studies on the cranial nerves of fishes, yet they are still useful to the investigators. The most valuable works from these early ones were those carried out by Allis (1897, 1903, 1909 & 1922) and Herrick (1899 & 1901).

Several authors published their works on the cranial nerves of bony fishes. The most recent of them are the studies of Northcutt and Bemis (1993) and Piotrowski and Northcutt (1996) on *Latimeria chalumnae* and *Polypterus senegalus*, respectively and Dakrory (2000) on *Ctenopharyngodon idellus*. Other works on the nerve or group of nerves were performed. De Graaf (1990) studied the innervation of the gills in *Cyprinus carpio* by dissection. Also, Song and Northcutt (1991) gave a detailed description on the morphology, distribution and innervation of the lateral line receptors in the holostean, *Lepisosteus platyrhincus*. Dakrory (2003)

studied the ciliary ganglion and its anatomical relations in some bony fishes.

It is quite evident from the above historical review that there are numerous works on the cranial nerves of fishes, but a few studies have been made concerning the cranial nerves of some species belonging to Mugilidae. Although the previously mentioned studies of different authors may throw light on the subject of the cranial nerves of fishes, yet it cannot be stated that the cranial nerves of a Mugilidae is similar to other fishes; and what are the differences if present? Thus it was suggested that a detailed microscopic study on the glossopharyngeal nerve in *Liza ramada* will be very useful.

The main and fine branches of this cranial nerve, its distribution, its relation with other nerves and with the other structures of the head, their analysis and the organs they innervate are studied thoroughly, hoping that it may add some knowledge on this important subject and also to the behaviour and phylogeny of this group of fishes.

**2. Material and Methods**

The species under investigation of the marine water bony fish *Liza ramada* belongs to family Mugilidae.

*Liza ramada* is a diurnal fish inhabiting shallow areas. It feeds on epiphytic algae, detritus and small benthic or planktonic organisms, pelagic eggs and larvae. It is an economically important fish as a source of proteins.

The fully formed larvae of this species were collected from the coast of the Mediterranean Sea at Kafer El-Sheykh Governorate, during August 2006. The heads of the fully formed larvae were fixed in

aqueous Bouin for 24 hours. After that the heads were washed several days with 70% alcohol. Decalcification was necessary before cutting and staining *in toto* for this bony species. This was carried out by placing the heads in EDTA solution for about 40 days, changing the solution every 3 days.

The heads were sectioned transversely (10 microns in thickness), after embedding in paraffin. The serial sections were stained with Mallory's triple stain (Pantin, 1946). The serial sections were drawn with the help of the projector. From these sections, an accurate graphic reconstruction for the glossopharyngeal nerve was made in a lateral view. In order to show the position of the nerve, and its relations to the other different structures of the head, several serial sections were photomicrographed.

### 3. Results

In *Liza ramada* studied the nervus glossopharyngeus arises from the ventrolateral side of the medulla oblongata by a single root (Figs. 1 & 2, RO.IX). Directly after its origin, this nerve runs posteriorly and ventrolaterally passing dorsal and then dorsolateral to both the saccular ramus of the nervus octavus and the sacculus and ventromedial and ventral to the utricle. At the end of this course, this nerve passes outside the cranial cavity through its own foramen (Fig. 3, F.GPH) which is located on the ventrolateral side of the auditory capsule penetrating the exoccipital bone (Fig. 3, EXO).

Extracranially, the nervus glossopharyngeus joins directly the head of sympathetic trunk forming a common nerve (Fig. 1, N.COM). This nerve extends forwards lateral to the auditory capsule and dorsomedial and then medial to the internal jugular vein. After a considerable course in this position, it becomes ventromedial to the latter vein. Here, the common nerve separates into the head sympathetic trunk dorsomedially and the nervus glossopharyngeus ventrolaterally (Figs. 1 & 4, N.IX). Shortly forwards the nervus glossopharyngeus carries the petrosal "Epibranchial" ganglion (Figs. 1 & 5, G.EB.IX). From the anterior end of the ganglion the nervus glossopharyngeus (Fig. 1) originates and continues forwards passing dorsomedial and then medial to the first levator arcus branchialis muscle. It gives off a lateral branch for the latter muscle (Fig. 1, N.LB.I). More forwards, the nervus glossopharyngeus turns its course ventrally and posteriorly to enter the first holobranch. Here, it gives off a lateral motor branch for the first levator arcum branchialis muscle (Fig. 1, N.LB.I). At its entrance the first holobranch, it runs ventral to the second levator arcus branchialis muscle dorsolateral and dorsal to the first efferent branchial vessel and medial to the first levator arcus branchialis

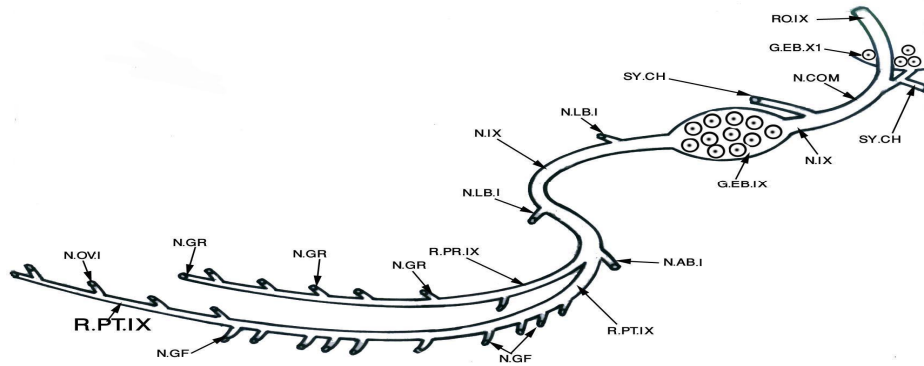
muscle. Here it gives off a motor branch for the latter muscle (Fig. 1, N.LB.I). The main nerve runs dorsolateral, lateral and then ventral to the first efferent branchial vessel and lateral to the epibranchial bone. Here, the nervus glossopharyngeus (Fig. 1, N.IX) divides into a medial pretrematic and lateral posttrematic rami.

#### Pretrematic ramus

Directly after its separation from the posttrematic ramus, the pretrematic one (Fig. 1, R.PR.IX) extends anteroventrally passing lateral and then ventral to the epibranchial bone and ventromedial to the ramus posttrematic. The ramus pretrematic continues anteroventrally passing ventral and ventromedial to the epibranchial bone and dorsomedial to the ceratobranchial bone (Fig. 6, R.PR.IX). After a short forward course, it gives off a ventral branch for the gill rakers (Fig. 1, N.GR) and their covering epithelium. This ramus continues forwards dorsal and medial to the ceratobranchial for a long course giving off numerous twigs for the gill rakers and epithelium covering these rakers (Fig. 1, N.GR).

#### Posttrematic ramus

The posttrematic ramus of the nervus glossopharyngeus of *Liza ramada* (Figs. 1 & 7, R.PT.IX) extends lateroventrally passing ventrolateral to the first efferent branchial vessel and lateral to the epibranchial bone. Here, it gives off a fine nerve for the epithelium of the upper gill filaments (Fig. 1, N.GF). At the point of the articulation of the epibranchial and ceratobranchial bones of the gill arch, the posttrematic ramus turns anteriorly to run ventrolateral to the latter bone and dorsal to the efferent branchial vessel. Thereafter, it continues forwards extending ventrolateral to the ceratobranchial, lateral to the first efferent branchial vessel and dorsal to the gill filament (Fig. 1, R.PT.IX). During this course it gives off numerous branches for the muscles and epithelium of the filament. More forwards, the posttrematic ramus extends ventral and ventromedial to the ceratobranchial bone and dorsal and then dorsomedial to the first efferent branchial vessels. Reaching the point of the attachment of the first holobranch with the isthmus, this ramus continues anteromedially passing ventromedial to the first oblique ventral muscle and medial to the first afferent branchial vessel. Here, it gives off a branch for the latter muscle (Fig. 1, N.OV.I). Anterior to the articulation between the hypobranchial and ceratobranchial, the ramus posttrematic continues anterodorsally passing lateral to the hypobranchial to ramify and end in the epithelium and the pharyngeal taste buds of the roof of the isthmus.



**Fig. 1:** Graphic reconstruction of the glossopharyngeal nerve in a lateral view. G.EB.IX: Epibranchial ganglion of the nervus glossopharyngeus; G.EB.X1: Epibranchial ganglion of the first vagal branchial trunk; N.AB.I: Nerve to the first adductor arcus branchialis muscle; N.COM: Common nerve; N.GF: Nerve to the gill filament; N.GR: Nerve to the gill rakers; N.LB.I: Nerve to the first levator arcum branchialis muscle; N.OV.I: Nerve for first obliquus ventralis muscle; N.IX: nervus glossopharyngeus; R.PH.X1: Ramus pharyngeus of the first branchial vagal trunk; R.PR.IX: Pretrematic ramus of the glossopharyngeal nerve; R.PT.IX: Posttrematic ramus of the glossopharyngeal nerve; RO.IX: Root of nervus glossopharyngeus; SY.CH: Sympathetic chain.



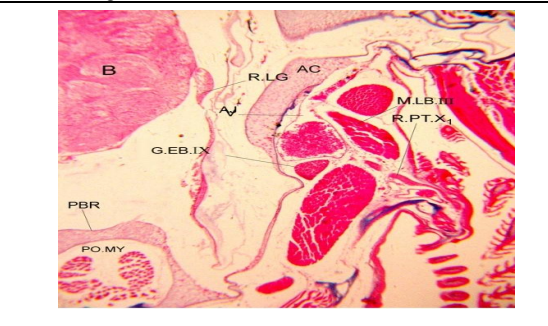
**Fig. 2:** Photomicrograph of a part of transverse section passing through the otic region showing the root of both the nervus glossopharyngeus. AC: Auditory capsule; ALLN: Anterior lateral line nerve; B: Brain; G.EPB.X1: Epibranchial ganglion of the first vagal branchial trunk; R.LG: Lagenar ramus; R.PH.X1: Ramus pharyngeus of the first branchial vagal trunk; R.PT.X1: Posttrematic ramus of the first vagal branchial trunk.



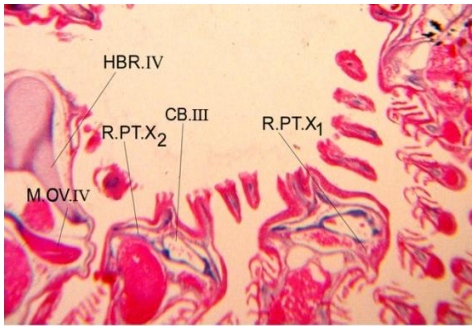
**Fig. 3:** Photomicrograph of a part of transverse section passing through the otic region showing the glossopharyngeal foramen in the exoccipital bone, the rami posttrematic, pharyngeus as well as the epibranchial ganglion of the first vagal branchial trunk; AJV: Anterior jugular vein; ALLN: Anterior lateral line nerve; B: Brain; EB.II: Epibranchial of second holobranch; EXO: Exoccipital bone; F.GPH: Glossopharyngeal foramen; G.EB.X1: Epibranchial ganglion of the first vagal branchial trunk; N.IX: Nervus glossopharyngeus; R.AM.PO: Ramus ampullaris posterior; R.LG: Lagenar ramus; R.PH.X1: Ramus pharyngeus of the first branchial vagal trunk; R.PT.X1: Posttrematic ramus of the first vagal branchial trunk.



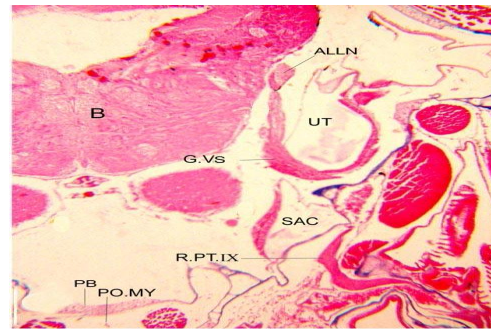
**Fig. 4:** Photomicrograph of a part of transverse section passing through the otic region showing the position of the glossopharyngeal nerve. AC: Auditory capsule; AJV: Anterior jugular vein; B: Brain; N.IX: Nervus glossopharyngeus; M.LB.I: First levator arcus branchialis muscle; PSC: Posterior semicircular canal; R.AM.PO: Ramus ampullaris posterior; R.LG: Lagenar ramus



**Fig. 5:** Photomicrograph of a part of transverse section passing through the otic region showing epibranchial ganglion of the glossopharyngeal nerve and the posttrematic ramus of the first branchial vagal trunk. AC: Auditory capsule; B: Brain; G.EB.IX: Epibranchial ganglion of the nervus glossopharyngeus; M.LB.III: Third levator arcus branchialis muscle; PBR: Prootic bridge; PO.MY: Posterior myodome; R.LG: Lagenar ramus.



**Fig. 6:** Photomicrograph of a part of transverse section passing through the otic region showing the pretrematic ramus of the glossopharyngeal nerve and the posttrematic rami of the first and second branchial vagal trunks. CB.III: Third ceratobranchial; HBR.IV: Fourth hypobranchial cartilage; M.OV.IV: Fourth oblique ventralis muscle; R.PT.X1: Posttrematic ramus of the first vagal branchial trunk; R.PT.X2: Posttrematic ramus of the second vagal branchial trunk.



**Fig. 7:** Photomicrograph of a part of transverse section passing through the otic region showing the posttrematic ramus of the nervus glossopharyngeus. ALLN: Anterior lateral line nerve; B: Brain; G.VS: Ganglion visceralis; PB: Prootic bone; PO.MY: Posterior myodome; SAC: Sacculus; R.PT.IX: Posttrematic ramus of the glossopharyngeal nerve.

#### 4. Discussion

The nervus glossopharyngeus of *Liza ramada* arises from the medulla oblongata by a single root; a case which is found in several bony fishes. The nervus glossopharyngeus, however, arises by two roots in *Ameiurus* (Herrick, 1901), *Polyodon* (Norris, 1925), *Mastacembelus armatus* (Maheswari, 1965) and *Amphipnous cuchia* (Saxena, 1967). On the other hand, Norris (1925) recorded four or five groups of rootlets for the nervus glossopharyngeus in *Scaphirynchus*.

Among cartilaginous fishes, a single root for the nervus glossopharyngeus was recorded in *Dasyatis rafinesque* (Chandy, 1955) and *Pteroplatea altavela* (Mazhar, 1979). However, in *Rhinobatus halavi*, the nervus glossopharyngeus arises by means of three rootlets, which soon unite into one root (Dakrory, 2000).

In Agnatha, there is a single postotic nerve (glossopharyngeal-vagal nerve) which issues from the vagal lobe and passes to the pharynx and gills (Matsuda *et al.*, 1991). This is the case found in the lamprey *Ichthyomyzon unicuspis* (Wicht, 1996) and the hagfishes *Eptatretus stoutii* and *Myxine glutinosa* (Braun, 1998). On the other hand, separate glossopharyngeal and vagal nerves were mentioned by Jollie (1968) and Fritzsich and Northcutt (1993) in lampreys and by Kuratani *et al.* (1997) in embryos of *Lampetra japonica*.

Among Amphibia, the nervus glossopharyngeus arises separately from the medulla oblongata as a single trunk and enters the anterior part of a large ganglion shared by the nervi glossopharyngeus and vagus in *Salamander salamandra* and *Plethedon cinereus* (Wake *et al.*, 1983) and *Bufo regularis* (Shaheen, 1987). In *Bufo viridis* (Soliman and Mostafa, 1984), however, the nervi glossopharyngeus

and vagus arise together by three roots, which enter a common ganglion.

In *Liza ramada*, there is a separate foramen in the exoccipital bone, for the glossopharyngeal nerve. This is also the case found in *Lampanyctus leucopsarus* (Ray, 1950), *Cyprinus carpio* (de Graaf, 1990), *Gnathonemus petersii* (Lazar *et al.*, 1992), *Polypterus senegalus* (Piotrowski and Northcutt, 1996), *Clarias gariepinus* (Adriaens and Verraes, 1998), *Ctenopharyngodon idellus* (Dakrory, 2000), *Tilapia zillii* (Ali, 2005; Dakrory and Ali, 2006) and in *Mugil cephalus* (Hussein, 2010).

Among cartilaginous fishes, the nervus glossopharyngeus passes through a glossopharyngeal canal, which is communicated with the cavity of the auditory capsule. This was confirmed by Allis (1923) in *Chlamydoselachus anguineus*, de Beer (1931) in *Scyllium canicula* and El-Toubi (1949) in *Acanthias vulgaris*. This canal was also found by Hamdy (1960), El-Toubi and Hamdy (1959 & 1968), Hamdy and Hassan (1973), El-Satti (1982) and by Dakrory (2000). Hence, this canal appears to be a common feature for the Chondrichthyes while it is absence in bony fishes.

In the amphibians so far described, the nervi glossopharyngeus and vagus pass outside the cranial cavity through the jugular foramen (Sokol, 1981; Soliman and Mostafa, 1984; Shaheen, 1987; Haas, 1995; Reiss, 1997; Hall and Larson, 1998). Thus, the presence of a single foramen for the exit of the nervi glossopharyngeus and vagus appears to be a general rule in amphibians.

Concerning the case in Reptilia, the nervus glossopharyngeus passes outside the cranial cavity through an apertura medialis recessus scalae tympani, and enters the latter recessus which represents the anterior part of the fissura metotica. Then, it leaves the recessus through the apertura lateralis recessus scalae

tympani (de Beer, 1937; Dakrory, 1994). In such case, this nerve passes outside the cavity of the auditory capsule.

In the present study, there is only one glossopharyngeal ganglion, the petrosal (epibranchial) ganglion, which is located extracranially. Among bony fishes, the nervus glossopharyngeus has a single extracranial petrosal ganglion in *Polypterus senegalus* (Lehn, 1918), *Lampanyctus leucopsarus* (Ray, 1950), *Trichiurus lepturus* (Harrison, 1981), *Cyprinus carpio* (de Graaf, 1990), *Ctenopharyngodon idellus* (Dakrory, 2000), *Tilapia zillii* (Ali, 2005; Dakrory and Ali, 2006) and in *Mugil cephalus* (Hussein, 2010).

On the other hand, a medial sensory intracranial ganglion, in addition to the lateral extracranial petrosal (epibranchial) one, were found for the nervus glossopharyngeus in *Ameiurus* (Herrick, 1901), *Scorpaena scrofa* and *Polypterus* (Allis, 1909, 1922, respectively), *Parasilurus asotus* (Atoda, 1936), *Latimeria chalumnae* (Northcutt and Bemis, 1993) and *Polypterus senegalus* (Piotrowski and Northcutt, 1996).

Among cartilaginous fishes, a single, extracranially located petrosal ganglion was found in *Squalus acanthias* (Norris and Hughes, 1920) and *Dasyatis rafinesque* (Chandy, 1955).

Among Amphibia, Wake *et al.* (1983) mentioned that only one ganglion is present for the nervi glossopharyngeus and vagus in *Salamandra salamandra* and *Plethodon cinereus*. This was also the case described by Norris (1908) in *Amphiuma means*, Paterson (1939) in *Xeopus laevis*, Soliman and Mostafa (1984) in *Bufo viridis*. In this respect, Northcutt (1992) stated that, in *Ambystoma trigrinum* and other salamanders, the glossopharyngeal ganglion fuses with all the other sensory ganglia of the postotic cranial nerves, forming postotic ganglionic complex. On the other hand, Shaheen (1987) stated that each of the glossopharyngeal and vagal nerves has its own separate ganglion.

Dealing with reptiles, the nervus glossopharyngeus has a well distinct petrosal ganglion, as described by many authors. In addition to this ganglion, a root ganglion (ganglion superius) may be present in some forms. Such ganglion superius was observed in *Cerastes vipera* (Hegazy, 1976), *Agama pallida* (Soliman *et al.*, 1990).

In the present study, the nervus glossopharyngeus, proximal to the petrosal ganglion is connected with the cranial sympathetic nerve. This is the case found in *Tilapia zillii* (Ali, 2005; Dakrory and Ali, 2006) and in *Mugil cephalus* (Hussein, 2010). On the other hand, in *Cyclothone acclinidens*, Gierse (1904) stated that the nervus glossopharyngeus fuses intracranially with the root of the posterior lateral line nerve and, probably, anastomoses with the ramulus ampularis posterior. Similarly, Allis (1909, 1922) found a communicating

branch arising from the intracranial ganglion cells of the nervus glossopharyngeus to the root of the nervus vagus in *Scorpaena scrofa*, and from the root of the former nerve to that of the latter one in *Polypterus*, respectively. This was confirmed by Lehn (1918) and Saxena (1967) in *Polypterus senegalus* and *Amphipnous cuchia*, respectively. On the other hand, Handrick (1901) stated that, the roots of the nervi glossopharyngeus and vagus join and emerge together through the same foramen, but without apparent interchange of fibres in *Argyropelecus hemigymnus*. Dakrory (2000) also found a connection between the nervus glossopharyngeus and the middle lateral line nerve in *Ctenopharyngodon idellus*.

In *Liza ramada*, two rami arise from the petrosal ganglion; the rami pretrematic and posttrematic. The same was recorded in *Mugil cephalus* (Hussein, 2010). Herrick (1899) stated that in *Menidia* the nervus glossopharyngeus shows a considerable reduction in its peripheral branches; there is no ramus pharyngeus and the pretrematic ramus is reduced to a tiny remnant. Still further reduction for the ramus pretrematicus was found by Gierse (1904) in *Cyclothone acclinidens*. Moreover, this nerve, in *Gnathonemus petersii*, is small both peripherally and centrally (Lazar *et al.*, 1992). On the other hand, the pretrematic ramus is completely lacking in *Esox* and *Silurus* according to Stannius (1849). Moreover, *Ctenopharyngodon idellus* has two rami only, the rami pharyngeus and posttrematicus emerge from the nervus glossopharyngeus (Dakrory, 2000). The nervus glossopharyngeus has all the rami of a typical branchial nerve in *Amia calva*, *Scomber scomber* and *Scorpaena scrofa* (Allis, 1897, 1903, 1909, respectively), *Polycentrus schomburgkii* (Freihof, 1978), *Trichiurus lepturus* (Harrison, 1981), *Cyprinus carpio* (de Graaf, 1990) and *Polypterus senegalus* (Piotrowski and Northcutt, 1996).

Among cartilagenous fishes, three rami arise from the petrosal ganglion; the rami pharyngeus, pretrematic and posttrematic. This is the typical condition found in elasmobranches as reported by Norris and Hughes (1920) in *Squalus acanthias*, Chandy (1955) in *Dasyatis rafinesque* and Dakrory (2000) in *Rhinobatus halavi*.

In *Liza ramada*, there is no connection between the nervus glossopharyngeus and the nervus facialis; i.e. there is no Jacobson's anastomosis. The same was recorded in *Tilapia zillii* (Ali, 2005, Dakrory and Ali, 2006) and in *Mugil cephalus* (Hussein, 2010). On the other hand, a Jacobson's anastomosis was found in *Amphipnous cuchia* (Saxena, 1967) and *Trichiurus lepturus* (Harrison, 1981). However, another Jacobson's anastomosis was found between the ramus pharyngeus of the nervus glossopharyngeus and the posterior palatine ramus of the nervus facialis in *Menidia* and *Ameiurus* (Herrick, 1899, 1901), *Polycentrus schomburgkii* (Freihof, 1978) and

*Ctenopharyngodon idellus* (Dakrory, 2000). In this respect, Piotrowski and Northcutt (1996) described a connection between the ramus pharyngeus of the nervus glossopharyngeus and the rostral pole of the facial ganglion in *Polypterus senegalus*.

Among Amphibia, the ramus hyomandibularis facialis is communicated with the nervus glossopharyngeus in *Xenopus laevis* (Paterson, 1939), *Bufo viridis* (Soliman and Mostafa, 1984) and *Bufo regularis* (Shaheen, 1987). In *Amphiuma means* (Norris, 1908), however, the nervus glossopharyngeus anastomoses with the ramus alveolaris (chorda tympani).

In Reptilia, Jacobson's anastomosis is carried either through the medial cranial sympathetic ramus, which connects the nervi glossopharyngeus and vagus (Willard, 1915; Soliman *et al.*, 1974; Dakrory, 1994), or through the lateral cranial sympathetic ramus (Soliman, 1969).

In the present study, the ramus posttrematicus of the nervus glossopharyngeus is not divided into two parts anterior and posterior. It is also found in both *Tilapia zillii* (Ali, 2005; Dakrory & Ali, 2006a) and *Mugil cephalus* (Hussein, 2010). On the other hand, this ramus is divided into pars anterior and pars posterior in all the ray-finned fishes (Norris, 1925), *Latimeria chalumnae* (Northcutt and Bemis, 1993) and *Polypterus senegalus* (Piotrowski and Northcutt, 1996) and the shark, *Squalus acanthias* (Norris and Hughes, 1920).

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## Prevention of Ultraviolet B-induced Lens Oxidative Damage in Mice by *Dunaliella salina*, A Carotenoids-Rich Alga

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**Abstract:** The present study examined the protective effects of *Dunaliella salina* (*D. salina*) on ultraviolet B (UVB) radiation-induced lens oxidative damage in male ICR mice. Lens oxidative damage was induced by exposure UVB radiation. Animal were orally administered (gavage) *D. salina* at doses of 0, 123 and 615 mg/kg body weight/day for eight days. Lens glutathione (GSH) and malondialdehyde (MDA) levels as well as the activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) in lens were measured to monitor lens injury. The results showed that UVB irradiation caused significant damages to lens, including decreased the activities of SOD, catalase and GSH-Px, and GSH content in lens whereas increased lens MDA content, compared with control group. Treatment with *D. salina* could significantly ( $p < 0.05$ ) ameliorate lens oxidative damages, as evidenced by increased the activities of SOD, catalase and GSH-Px, and GSH content and decreased the MDA content in lens when compared with UVB-treated group. Those results demonstrate that *D. salina* exhibits potent protective effects on UVB radiation-induced lens oxidative damage in mice, likely due to both the increase of antioxidant enzymes activities and the inhibition of lipid peroxidation.

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**Keywords:** Carotenoids; *Dunaliella salina*; Lens; Oxidative damage; Ultraviolet B.

### 1. Introduction

Ultraviolet (UV) irradiation is the most frequent cause of radiation injury to the eye, which is affected by oxidative stress due to its physical and metabolic characteristics. Particularly, the lens is most affected by oxidative damage caused by UVB radiation, because it is an avascular structure and has a constant and spare production of lenticular proteins [1]. Many studies reported that antioxidants can effectively prevent and cure UVB-induced protein oxidation and photoperoxidation of lipids in lens [2-3]. A major defense mechanism for prevention and treatment of lens oxidative damage comprises reducing the production of reactive metabolites by raising the levels of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px), and decreasing lipid peroxidation [1, 4].

*Dunaliella salina* (*D. salina*) is a type of unicellular biflagellate green alga from Chlorophyceae gender without a rigid cell wall structure and can yield three major valuable products, glycerol,  $\beta$ -carotene and proteins [5-8] *D. salina* is safe and good for human health, because *D. salina* contains abundant  $\beta$ -carotene and other carotenoids including lutein, zeaxanthin and  $\alpha$ -carotene [9-10]. Recently,

our group demonstrated that *D. salina*, which contains abundant carotenoids and xanthophylls, is efficient antioxidants against a variety of oxidative stress *in vitro* and *in vivo* [8, 10]. It is well know that the major precursor of vitamin A is  $\beta$ -carotene, which quenches excited sensitizer molecules and singlet oxygen. Additionally, lutein and zeaxanthin are found in high concentrations in some ocular tissues, such as the macula, retina and lens [11-12]. Several studies have demonstrated that high dietary xanthophylls intake is associated with reduced cataract prevalence [13-14].

In consideration of excellent antioxidant activities of *D. salina*, we hypothesized that administration of *D. salina* may enhance the antioxidant defense system and thus provide against UVB-induced lens oxidative damage in mice. In the present study, male ICR mice were orally treated with *D. salina* daily accompanied by UVB exposure for a period of 8 days. Lens GSH and MDA levels, as well as SOD, catalase and GSH-Px activities in lens tissues, were also measured to monitor lens injury.

### 2. Material and Methods

#### *D. salina* material

Commercially available spray-dried preparations of *D. salina* paranooids cultured in the outdoor cultivation pool at GONG BIH Enterprise Co., Ltd (Yunlin City, Taiwan) were suspended in distilled water prior to use. The quality of *D. salina* powder was described and provided by the company. The carotenoid contents in the *D. salina* were measured as described previously [10].

### Animals

Male ICR mice ( $20 \pm 2$  g) were obtained from the Animal Department of BioLASCO Taiwan Company and were allowed to quarantine and acclimate for a week prior to experimentation. The animals were handled under standard laboratory conditions of a 12-h light/dark cycle in a temperature and humidity controlled room. Food and water were available *ad libitum*. Our Institutional Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for in accordance with the institutional ethical guideline. All procedures were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

### Treatment

The animals were randomly divided into four groups each consisting of ten mice. Group I served as the normal control and was given olive oil by gavage daily for a period of 8 days. For inducing lens damage in mice, eyes of animals in Groups II, III and IV were exposed to UVB irradiation by the method of Tanito and colleagues [18] with slight modification. After anaesthesia was induced by intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight), both of their eyes were exposed to  $560 \mu\text{W}/\text{cm}^2$  of UVB light (UVLS-26; UVP Inc., Cambridge, UK) for 180 seconds in a darkroom. During irradiation, the mice were confined in an adjustable retaining cage that protected most of the animal, except the head from the UV light. The wavelength of the light source peaked at 312 nm (range, 280-315 nm). The energy output was measured with a UV detector (USB4000) with a sensor (CC-3-UV-S; both from Ocean Optics, Inc., FL, USA). The entire UVB irradiation course was completed in a consecutive 5-day period. After UVB light exposure, Group II served as the UVB control and was orally administered olive oil daily for a period of 8 days. Groups III and IV were orally administered the *D. salina* dissolved in olive oil at doses of 123 and 615 mg/kg, respectively, daily for a period of 8 days. At the end of the experiment, all animals were sacrificed by  $\text{CO}_2$  for euthanasia. Eye samples were dissected out and washed immediately with ice-cold saline to remove as much blood as

possible, and immediately stored at  $-70^\circ\text{C}$  until further analysis.

### Measurement of lipid peroxidation

The quantitative measurement of lipid peroxidation was done by measuring the concentration of TBARS in liver using the method of Berton and colleagues [15]. The amount of malondialdehyde (MDA) formed was quantitated by reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation. In brief, samples were mixed with TBA reagent consisting of 0.375% TBA and 15% trichloroacetic acid in 0.25N hydrochloric acid. The reaction mixtures were placed in a boiling water bath for 30 min and centrifuged at 1,811g for 5 min. The supernatant was collected and its absorbance was measured at 535 nm. The results were expressed as nmole/mg protein using the molar extinction coefficient of the chromophore ( $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ ).

### Measurement of SOD, catalase, GSH-Px and GSH in lens

Lens homogenates were prepared in cold Tris-HCl (5 mmol/L, containing 2 mmol/L EDTA, pH 7.4) using a homogenizer. The unbroken cells and cell debris were removed by centrifugation at 10,000 rpm for 10 min at  $4^\circ\text{C}$ . The supernatant was used immediately for the assays for SOD, catalase, GSH-Px and GSH. The activities of all of these enzymes and GSH levels were determined following the instructions in the Randox Laboratories Ltd kit (Antrim, United Kingdom).

### Statistical analysis

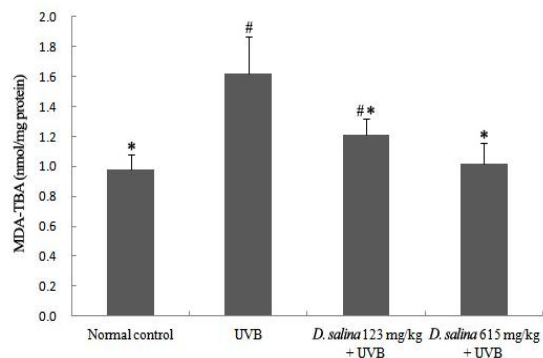
All values are expressed as the means  $\pm$  SDs. Comparisons between any two groups were performed using one-way analysis of variance (ANOVA), followed by the Munnnett multiple comparison test. The calculations were performed with SPSS software (Drmarketing Co., Ltd. New Taipei City, Taiwan). Statistically significant differences between groups were defined as  $p < 0.05$ .

## 3. Results

### 3.1 Effect of *D. salina* on lipid peroxidation after UVB exposure in the lens

MDA level is widely used as a marker of free radical mediated lipid peroxidation injury. The results of the MDA assays in the lens are shown in Fig. 1. MDA levels in the UVB-treated group ( $1.62 \pm 0.25$  nmol/mg protein) were significantly higher than that in the control group ( $0.98 \pm 0.10$  nmol/mg protein,  $p < 0.05$ ). However, MDA levels in the *D. salina* treated group ( $1.21 \pm 0.11$  and  $1.02 \pm 0.14$  nmol/mg protein at dose of 123 and 615 mg/kg, respectively) were significantly lower than that in the UVB-treated

group ( $p < 0.05$ ). These findings indicated that the free radicals being released in the lens were effectively scavenged when treated with *D. salina*.



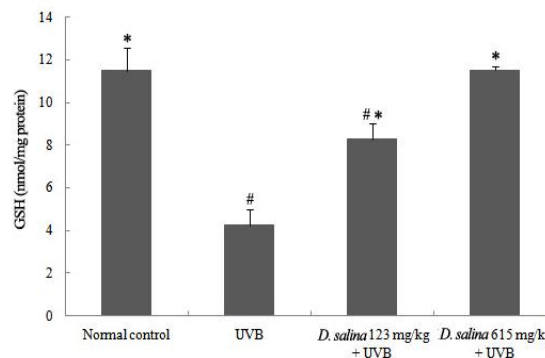
**Fig. 1.** Effects of *D. salina* on lens MDA-TBA level in UVB-induced lens oxidative damage in mice. Data are mean  $\pm$  SD of all the animals in a group; #  $p < 0.05$  compare with normal control; \*  $p < 0.05$  compare with UVB-treated group.

### 3.2 Effect of *D. salina* on GSH level after UVB exposure in the lens

GSH, a nonenzymatic antioxidant in the detoxification pathway, is abundant in the lens that reduces  $H_2O_2$ , hydroperoxides (ROOH) and photo-oxidation. The effects of *D. salina* on GSH levels are summarized in Fig. 2. UVB irradiation caused a significantly decrease on the GSH levels in the lens ( $4.25 \pm 0.74$  nmol/mg protein) as compared to the normal control group ( $11.49 \pm 1.10$  nmol/mg protein). In contrast with UVB-treated group, mice treated with *D. salina* at dose of 123 and 615 mg/kg showed a significantly increase the levels of GSH upto 170% (Fig. 2). These results suggested that *D. salina* provides protection against UVB-induced GSH depletion in the lens.

### 3.3 Effect of *D. salina* on antioxidant enzyme activities after UVB exposure in the lens

To further elucidate the reduction of MDA accumulation and GSH depletion in the UVB-exposed lens, we examined the status of antioxidant enzymes, SOD (Fig. 3A), catalase (Fig. 3B) and GSH-Px (Fig. 3C), in the lens. The activities of lens SOD, catalase and GSH-Px in the UVB-treated group were significantly decreased by 36%, 33% and 54%, respectively, when compared with the normal control group. In contrast, there was a significant increase ( $p < 0.05$ ) of SOD, catalase and GSH-Px activity in the *D. salina*-treated groups at a dose of 123 and 615 mg/kg, respectively, as compared to the UVB treated group. These findings indicated that administration of *D. salina* effectively restored/maintained the activities of lens SOD, catalase and GSH-Px, that was decreased by UVB exposure in the lens.



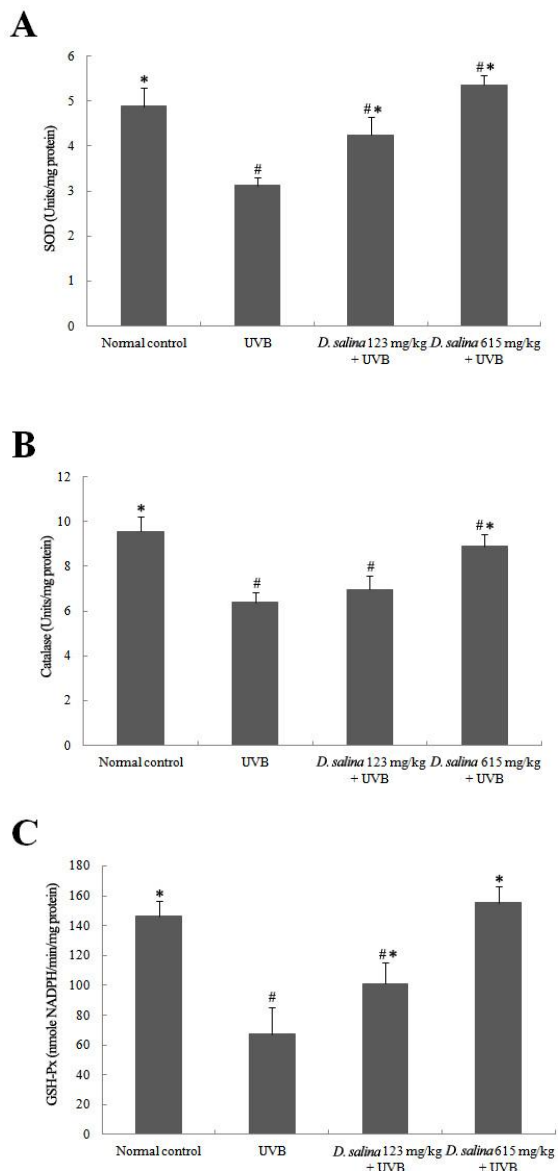
**Fig. 2.** Effects of *D. salina* on lens GSH amount in UVB-induced lens oxidative damage in mice. Data are mean  $\pm$  SD of all the animals in a group; #  $p < 0.05$  compare with normal control; \*  $p < 0.05$  compare with UVB-treated alone group.

## 4. Discussion

Previous study, we observed that *D. salina*, which contains abundant carotenoids and xanthophylls, is efficient antioxidants against a variety of oxidative stress [10]. Various epidemiological studies have indicated that dietary lutein and zeaxanthin play a crucial role in the prevention of the oxidation of lens proteins [16-18]. Moreover, lutein and zeaxanthin were also identified in the human lens [10-11]. Therefore, we considered that *D. salina* may be useful in the prevention of various damages induced by oxidative stress. In the present study, the capability of *D. salina* to protect against UVB radiation-induced oxidative damage in the lens was investigated.

Earlier studies have demonstrated the harmful effects of UVB radiation from sunlight in the lens [19-20]. UVB-induced reactive oxygen species, such as hydrogen peroxide, singlet oxygen, superoxide anions and hydroxyl radicals, are reported to initiate peroxidation [21] and react to proteins or lipids, leading to membrane lipid peroxidation and finally cell necrosis [20]. The main ROS generation occurs in the lens due to high exposure to UVB radiation. Lipid peroxidation by the generation of ROS is one of the principal mechanisms of UVB radiation-induced lens injury [1]. Moreover, the initiation of oxidative stress related to various tissue injuries, cell death and the progression of many acute and chronic diseases is generally believed to be induced by increased lipid peroxidation [22-23]. The capacity of an antioxidant to scavenge reactive oxygen species has been recognized as an important factor that contributes to protective effects in the lens. Indeed, considerable experimental animal models have reported that several antioxidant agents, such as ascorbic acid [24-25], vitamin E [2],  $\alpha$ -tocopherol [26] and *N*-acetylcysteine [27], reduced UVB

radiation-induced phototoxicity effects by prevention of lipid peroxidation. In the present study, UVB radiation-induced oxidative damage caused a significantly increase in MDA levels in the lens. Treatment with *D. salina* significantly reversed these changes. The administration of *D. salina* caused a significant decrease in MDA levels compared to the UVB radiation-induced oxidative damage group.



**Fig. 3.** Effects of *D. salina* on lens antioxidant enzymes (A) SOD, (B) catalase and (C) GSH-Px activities in UVB-induced lens oxidative damage in mice. Data are mean  $\pm$  SD of all the animals in a group; #  $p < 0.05$  compare with normal control; \*  $p < 0.05$  compare with UVB-treated alone group.

Previous studies on the mechanism of UVB radiation-induced lens damage showed that GSH is an extremely efficient intracellular antioxidant for oxidative stress and it reduces  $H_2O_2$ , hydroperoxides (ROOH) and photooxidation cause by UVB radiation exposure [1]. GSH is easily oxidized to GSSG by xenobiotic compounds, and there may additionally be reaction with any of the selenium-containing GSH-Px isozymes that may subsequently result in the reduction of GSH levels. GSSG is either rapidly reduced by glutathione reductase and NADPH or utilized in the protein-folding process in the endoplasmic reticulum [28]. Because of these recycling mechanisms, GSH is major defense mechanism against photo-oxidation in the lens [29]. The oxidation-reduction cycle of this tripeptide links dietary antioxidants such as ascorbate, riboflavin, carotenoids and tocopherols with the prevention of photo-oxidation [4]. The level of GSH in the lenticular epithelium is as high as that in the liver and its concentration decreases by exposure to UV radiation [30]. In the present study, the content of GSH in the lens was significantly decreased in UVB radiation-exposed mice compared with control mice. Conversely administration of *D. salina* to UVB radiation-exposed mice significantly elevated GSH content in the lens compared to the untreated group, indicating that *D. salina* can protect against the UVB radiation-induced GSH depletion in the lens.

The intracellular antioxidant status is always maintained at equilibrium in mammalian cells. Dietary supplementation with extra natural antioxidants mainly help the intracellular antioxidant defense system that includes non-enzymatic antioxidants (e.g., GSH, bilirubin, and vitamins E and C) and enzymatic antioxidants such as SOD, catalase and GSH-Px in protecting cells and organs against ROS-induced oxidative damage [31]. Moreover, oxidative stress associated with increased ROS is known to hasten cataract formation and lens oxidative damage in laboratory rodent models [32]. Therefore, the enzymatic antioxidant activities and/or the inhibition of free-radical generation are important in terms of protecting the lens from UVB-induced oxidative damage [1, 26].

Each of these enzymes catalyzes the reduction of a particular type of ROS. Superoxide anions are catalyzed in most tissues of the body, including the lens, into hydrogen peroxide ( $H_2O_2$ ) by SOD, which is an exceedingly effective defense enzyme.  $H_2O_2$  can become highly toxic because it produces the hydroxyl radical. This toxicity is prevented by catalase and GSH-Px. Catalase is a haemeprotein in all aerobic cells that metabolize  $H_2O_2$  to oxygen and water. GSH-Px is a selenoprotein that catalyses the reduction of  $H_2O_2$  and hydroperoxides to non-toxic

products [1, 33]. These antioxidant enzymes protect the lens against UVB radiation-induced lipid peroxides or free radicals. Therefore, these antioxidant enzymes are easily deprived of their activity by lipid peroxides or free radicals, resulting in their decreased activities in UVB radiation exposure [4, 34]. In the present study, SOD, catalase and GSH-Px levels were significantly elevated by administration of *D. salina* to UVB radiation-damaged lens, suggesting that it has the ability to restore and/or maintain these enzymes' activities in UVB radiation-induced oxidative damaged in the lens.

### 5. Conclusion

The results of this study demonstrate that *D. salina* was effective for the prevention of UVB radiation-induced oxidative stress in the lens. Our results show that the protective effects of *D. salina* may be due to both an increase in the activity of the antioxidant defense system and an inhibition of lipid peroxidation. The inhibitory effects of a dietary *D. salina* may be useful as a protective agent against UVB radiation-induced lens damage *in vivo*.

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## Relationship between Polymorphisms of Angiotensin-converting Enzyme Gene Insertion/Deletion, Endothelial Nitric Oxide Synthase Gene Intron 4 VNTR and Risk for Cervical Cancer

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**Abstract:** Background and Aims: Angiotensin-converting enzyme gene (*ACE*) and endothelial nitric oxide synthase gene (*eNOS*) have been reported to be associated with risk for a wide range of cancers. This study evaluated the relationship between polymorphisms of *ACE* insertion/deletion (I/D), *eNOS* intron 4 variable number of tandem repeats (VNTR) and risk for cervical cancer. Methods: Polymorphisms of *ACE* I/D and *eNOS* intron 4 VNTR were analyzed by PCR in 147 cervical cancer cases and 167 healthy control subjects. Statistical analyses were performed with SPSS for Windows. Results: We found that the D/D genotype of *ACE*, compared with the I/I and I/D genotypes, was significantly associated with an increased risk for cervical cancer (D/D vs. I/I: OR = 0.41, 95% CI= 0.18-0.94; D/D vs. I/D: OR = 0.34, 95% CI=0.15-0.78); the 4a/4b genotype of *eNOS*, compared with the 4b/4b genotype, was not associated with increase in the risk for cervical cancer (4b/4b vs. 4a/4b OR = 0.58, 95% CI= 0.31-1.08). The rare variant named 4c allele in *eNOS* intron 4 VNTR was encountered in one patient and one control subject. Conclusions: Our results provide that the *ACE* I/D polymorphisms is associated with the risk for cervical cancer, and there is no enough evidence to show the significant association between the polymorphisms of *eNOS* intron 4 VNTR and the risk for cervical cancer. Further studies with more subjects in diverse ethnic population are necessary to confirm the general validity of our findings.

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**Key Words:** Polymorphisms, angiotensin-converting enzyme gene, endothelial nitric oxide synthase gene, cervical cancer

### 1. Introduction

Cervical cancer is the second most common cancer among women worldwide [1-2]. Previously, many risk factors have been reported to be associated with the occurrence of cervical cancer. Among these factors, human papilloma virus (HPV) is regarded as a prime etiologic factor in cervical cancer. And abortion too many times, several sex partners, and proliacy in early marriage are also identified as risk factors for the development of cervical cancer [3]. Even though the crucial roles of these factors play in the development of cervical cancer, the underlying cellular and molecular mechanisms in the etiopathogenesis of it remain largely unknown.

Renin-angiotensin-aldosterone system (RAS) is an important circulation endocrine system in human body, which can adjust blood pressure by action on

vascular tension, kidney blood flow dynamics and electrolyte balance, and is closely related with vascular endothelial proliferation and interactions of many cytokines. Angiotensin I-converting enzyme (ACE) is key enzyme of RAS, and can convert angiotensin I (Ang I) of no active T-peptide to angiotensin II (Ang II) of highly vascular activity and stimulating aldosterone secretion and degrade bradykinin. The gene encoding ACE is located on the long arm of chromosome 17 (17q23) that is composed of 26 exons and 25 introns with coding sequence long 4.3 kb. It has been found that the *ACE* polymorphisms, involves the presence (insertion [I]) or absence (deletion [D]) of a 287-bp sequence of DNA, occurs in intron 16 of the gene. The plasma concentration of ACE in the same individual is constant, but different individual has a lot of difference, the difference in large measure depends on the *ACE*

polymorphisms. Previous studies have shown that the D/D genotype has an increased risk for the development of cancer and the other reproductive system disease, including breast cancer, gastric cancer, prostate cancer and recurrent miscarriage [4-11]. According to these reports, the *ACE* I/D polymorphisms affect the plasma concentrations and activity of Ang II by affecting the plasma concentrations and activity of ACE. The Ang II can promote cell proliferation of human breast cancer and vascular smooth muscle through combination with angiotensin II receptor 1 (AT1R), this could be involved in the mechanism of breast cancer. The combination of Ang II and AT1R may also induce increased formation of nitric oxide (NO) and cell adhesion molecules both of which are relevant to tumor growth and metastasis.

Nitric oxide (NO) synthesized with L-arginine and oxygen as the substrates by nitric oxide synthase (NOS) is multifunctional small molecular gas material and has the high free radical activity, and participates in and adjusts multiple physiological and pathological physiology processes. The NOS has 3 kinds of isoforms that comprises endothelial (e), inducible (i), and neuronal (n) NOS. Studies in recent years have found that the expression of eNOS is not only exists in normal tissues, but also in tumor tissues widely, and adjusts endothelial cell damage and bone marrow endothelial progenitor cells mobilization and homing, and participates in the formation of blood vessels through NO generation, which can affect the growth and metastasis of tumors [12]. The gene that encodes eNOS is located on chromosome 7q36 and contains 26 exons and 25 introns with an entire length of 21 kb in humans, and has a 27-bp VNTR polymorphism in intron 4 (intron 4b/a). Studies have shown that the polymorphisms in *eNOS* intron 4 VNTR is associated with the risk for cancers, including breast cancer, prostate cancer and colon cancer [13-16], and the other female reproductive system disease such as recurrent miscarriage [17].

Several epidemiological studies evaluated polymorphisms of *ACE* I/D and *eNOS* intron 4 VNTR in human cancers, but the biologic mechanisms are unexplored. Therefore, in this study, we evaluated the relationship between the polymorphisms (*ACE* I/D, *eNOS* intron 4 VNTR) and the risk for cervical cancer in the population of the Han nationality from southwest China.

## 2. Material and Methods

### 2.1 Study population

A total of 314 subjects, comprising 147 patients with cervical cancer (mean age 42.44±8.20) and 167 female control subjects (mean age 48±7.37) who were

healthy check-up people, were recruited for this study. These subjects in both groups were unrelated ethnic Han Chinese selected from Chengdu City or the surrounding regions in the Sichuan Province between February, 2009 and June, 2010. Cervical cancer patients were diagnosed by histopathological confirmation in the West China Second University Hospital (Table 1). All participants gave the written informed consent for their participation, and the research protocol was reviewed and approved by the Ethics Committee of Sichuan University.

**Table 1.** Characteristics of the 147 cervical cancer patients

Characteristics	Patients, n	Patients, %
Clinical stages		
Carcinoma in situ	33	22
Non- carcinoma in situ	114	78
Pathological type		
Squamous cell carcinoma	83	56
Adenocarcinoma	7	5
Gland squamous cell carcinoma	4	3
Other types	6	4
Not classification	47	32

### 2.2 Genotyping

All subjects of controls and 68 patients with cervical cancer donated approximately 2ml venous blood immediately following admission to the hospital, and were interviewed to obtain demographic and clinical information. Blood samples were collected in sterile tubes with EDTA-Na<sub>2</sub> anticoagulants, and stored at -20°C. 79 patients with cervical cancer donated tumor tissue from the surgery department, and stored at -70°C. Genomic DNA was extracted from the stored blood and the tumor tissue by using a commercial extraction kit (Bioteke Corporation, Beijing, China) according to the manufacturer's instructions.

Polymorphisms of *ACE* I/D and *eNOS* intron 4 VNTR were identified on the basis of PCR amplification of the respective fragments. Primer sequences [18] determine polymorphisms of *ACE* I/D were tested by PCR. The PCR procedure was denaturation at 94°C for 5 minutes, then 35 cycles of 94°C for 30 seconds, 70°C for 90 seconds, 72°C for 90 seconds, and finally by 10 minutes at 72°C for final extension. The genotypes were identified as follows: I/I, a single band of 597 bp; I/D, two bands of 319 and 597 bp; and D/D, a single band of 319 bp. The D allele in heterozygous subjects was preferentially amplified, therefore this maybe misclassify the I/D genotype as the D/D genotype. For fear of this misclassification, a second independent PCR was done with a primer pair that recognized insertion-specific sequences [19] and the identical PCR conditions, because this reaction yields a 335 bp fragment just in the presence of the I/I and I/D genotype but not in the D/D genotype. The

27-bp intron 4 VNTR polymorphisms of *eNOS* was analyzed by using primers as previous research published [20]. The thermocycling procedure was consisted of initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 40 seconds, extension at 72°C for 40 seconds, and a final extension at 72 for 5 minutes. We detected two variants that the intron 4b ( five 27-bp repeats) and the intron 4a (four 27-bp repeats) in individuals carrying genotype 4b/4b, 4a/4b or 4a/4a. In this study, we also discovered a rare variant named 4c (six 27-bp repeats) in the *eNOS* in one patient carrying 4a/4c and one control subject carrying 4b/4c [12].

The PCR products were distinguished on 5% polyacrylamide gel and visualized by silver staining to determine the genotypes. The PCR products of the two loci with different genotypes were confirmed by the direct sequencing method.

### 2.3 Statistical Analysis

Genotype and allele frequencies of *ACE* I/D and *eNOS* intron 4 VNTR were obtained by Modified-powerstate standard edition software. Hardy-Weinberg equilibrium was tested with a goodness of fit chi-square test (with one degree of freedom) to compare the observed genotype frequencies among the subjects with the expected genotype frequencies. Genotype and allele frequencies

of the two loci were compared between cervical cancer patients and control subjects by the chi-square test, and odds ratios (ORs) and 95% confidence intervals (CIs) were used to evaluate the relative risk conferred by a particular allele or genotype. The demographic and clinical data of the groups were compared by the chi-square test and the Student's *t*-test. Statistical significance was assumed at the  $P < 0.05$  level. All data were analyzed by the SPSS for Windows software package version 13.0 (SPSS Inc., Chicago, IL).

### 3. Results

The genotype and allele frequencies of *ACE* gene I/D and *eNOS* gene intron 4 VNTR in the cervical cancer patients and control subjects are shown in Table 2 and Table 3. The genotype frequencies of the two loci in the control subjects were all in agreement with the Hardy-Weinberg equilibrium. We found that the D/D genotype of *ACE*, compared with the I/I and I/D genotypes, was significantly associated with an increased risk of cervical cancer (D/D vs. I/I: OR = 0.41, 95% CI= 0.18-0.94; D/D vs. I/D: OR = 0.34, 95% CI=0.15-0.78); the 4a/4b genotype of *eNOS*, compared with the 4b/4b genotype, was not associated with increase in the risk of cervical cancer (4b/4b vs. 4a/4b OR = 0.58, 95% CI= 0.31-1.08). A new variant named 4c allele in *eNOS* intron 4 VNTR was encountered in one patient and one control subject.

**Table 2.** The genotype frequency of *ACE* gene I/D and *eNOS* gene intron 4 VNTR in cervical cancer patients and controls

Gene	Genotype	Patients, n (%)	Controls, n (%)	OR (95% CI)	<i>P</i> value
<i>ACE</i>	D/D	9 (6.1)	25 (15.0)	1.00 (reference)	
	I/I	60 (40.8)	68 (40.7)	0.41 (0.18-0.94)	0.032
	I/D	78 (53.1)	74 (44.3)	0.34 (0.15-0.78)	0.009
<i>eNOS</i>	4b/4b	117 (80.1)	145 (86.8)	1.00 (reference)	
	4a/4b	28 (19.2)	20 (12.0)	0.58 (0.31-1.08)	0.081
	4a/4a	0	1 (0.6)		
	4b/4c	0	1 (0.6)	-	-
	4a/4c	1 (0.7)	0	-	-

Abbreviations: OR=odds ratio; CI=confidence interval

**Table 3.** The allele frequency of *ACE* gene I/D and *eNOS* gene intron 4 VNTR in cervical cancer patients and controls

Gene	Allele	Patients, n (%)	Controls, n (%)	OR (95% CI)	<i>P</i> value
<i>I/D</i>	I	198 (67.3)	210 (62.9)	1.00 (reference)	
	D	96 (32.7)	124 (37.1)	0.82 (0.59-1.14)	0.24
<i>eNOS</i>	4b	262 (89.7)	311 (93.1)	1.00 (reference)	
	4a	29 (9.9)	22 (6.6)	1.57 (0.88-2.79)	0.13
	4c	1 (0.3)	1 (0.3)	1.19 (0.07-19.07)	0.90

Abbreviations: OR=odds ratio; CI= confidence interval

### 4. Discussion

There are many research papers about the relationship between polymorphisms of *ACE* I/D, *eNOS* intron 4 VNTR and the risk for human cancers. No study concerning the effect of the polymorphisms (*ACE* I/D, *eNOS* intron 4 VNTR) on cervical cancer

has been reported. In this study, we analyzed the influence of the polymorphisms (*ACE* I/D, *eNOS* intron 4 VNTR) on individual susceptibility to cervical cancer. The alleles frequency of the two loci in the population of the Han nationality from southwest China was similar to those reported in previous studies, the

frequencies of the D allele (37.1%) and 4b allele (93.1%) were nearly identical to those observed among the Chinese women in control subjects [6, 21-22].

We found that the D/D genotype of *ACE* was significantly associated with an increased risk for cervical cancer (D/D vs. I/I: OR = 0.41, 95% CI= 0.18-0.94; D/D vs. I/D: OR = 0.34, 95% CI=0.15-0.78). This positive result was similar to the most previous studies that the D/D genotype of *ACE* has an increased risk for the development of cancer, including breast cancer, gastric cancer and prostate cancer [4-10]. The possible mechanism is that due to the absence of a 287-bp sequence of DNA, the D/D genotype of *ACE* gene loses the regulation of the *ACE* gene expression, increasing the plasma concentrations and activity of *ACE* and leading to the plasma concentrations and activity of AngII increase, which can promote the development of tumor. And the results may show that the *ACE* polymorphisms may exist in populations with enriched genetic susceptibility and may contribute to human cancer susceptibility and progression.

The eNOS is a key enzyme for synthesizing NO and plays an important role in tumor growth and angiogenesis [23]. NO is an important endothelium-derived relaxing factor. Studies have indicated that NO participates in the development and transfer process of tumorigenesis and plays a dual role, on the one hand NO has promoting tumor effect through regulating the cell proliferation related gene expression and promoting the formation of tumor blood vessels, on the other hand NO plays antitumor function through inducing tumor cell apoptosis [24]. Although no significant association of the polymorphisms in *eNOS* intron 4 VNTR and the risk of cervical cancer was observed (4b/4b vs. 4a/4b: OR = 0.58, 95% CI= 0.31-1.08), some researches suggested that the 4a genotype of *eNOS* has an increased risk for the development of cancer, including prostate cancer and colon cancer [16-17]. This may be relevant to the different ethnic population or the regional difference, and be relevant to the distribution and express of the eNOS in cervical cancer by the other polymorphisms in *eNOS* (eg. G894T, T786C) [25-26], and also be relevant to the relatively small subjects in this study. A rare variant named 4c allele in *eNOS* intron 4 VNTR polymorphisms was encountered in one patient and one control subjects, which indicates that the complex relationship exists between the polymorphisms in *eNOS* and cervical cancer, and further studies with more subjects in diverse ethnic populations are necessary to confirm the general validity of our findings.

There may be some limitations in this study. First, the study subjects were nearly from ethnic Han Chinese, and the results should not be extended to other ethnic populations because of genetic heterogeneity.

Second, study materials in previous researches about polymorphisms on human cancers are nearly blood samples, some of our samples were the tumor tissues besides blood. To our knowledge, genomic instability may, and most commonly, result from gross chromosomal changes, such as translocations or amplifications, which leads to chromosomal instability. Genomic instability may also present itself through alterations in the length of short repeat stretches of coding and non-coding DNA, resulting in microsatellite instability (MSI). The MSI often refers to simple repeat sequence (1-4bp) increase or lost. In this study the two loci we selected do not belong to the MSI, and the sample differences between tumor tissues and blood can be neglected to affect our results.

In conclusion, the *ACE* I/D polymorphisms is associated with the risk for cervical cancer in the population of the Han nationality from southwest China, which indicates that it may be used as a genetic marker for cervical cancer. There is no enough evidence to show the significant association between the polymorphisms of *eNOS* intron 4 VNTR and the risk for cervical cancer. These findings should be further validated by larger, preferably prospective, studies with more diverse ethnic groups.

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## Adoption of Decision Support Systems to Supplement Organizational Decision Making

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**Abstract:** Decisions support system is used to assist and refine managerial decision making. Any Business or organization consists of Marketing, R & D, Financial Management and planning. These interrelated resources must have a coherent and effective combination for a sustainable organization growth. For all these resources to integrate effectively management decision making plays a very important role. At present, the process of decision making in most local organizations, is done without any aid of technology, and based solely on the experience of the decision maker. This study focuses on the effectiveness of decision support systems in assisting the process of decision making for organization.

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**Key Words:** Decision Making, DSS; Decision Support Systems

### 1. Introduction

In the modern era technologies play a vital role in simplifying and often easing out day to day managerial activities by helping the organizational decision making. An information system that helps organizational decision making is considered a decision support system. It helps solving problems by supporting different business models.

Decision support system focuses on the following:

- inventories of information assets (including legacy and relational data sources, cubes, data warehouses, and data marts),
- comparative sales figures across time, and;
- projected revenue based on sales assumptions

Such systems can be build on any knowledge domain with real practical applications. The DSSs have numerous benefits including; improved personal efficiency and speedy decision making; increasing organizational control.

Modern technology has simplified and benefited every part of our work lives and has helped us perform things that were unthinkable in the past. The DSS is no exception; as it is a vital part of any organization's decision making portfolio, and implementing it properly helps the organization's top management make effective and informed decisions that aid the organization in the long run to succeed in achieving its mission and goals.

### 2. Literature Review

Decision support systems effectively support future planning that can be implemented at many intervals. Bonczek et al. (1980), refers to Decision Support Systems as a computer-based system consisting of:

1. Language system - communication between the user and DSS components
2. Knowledge system, and;
3. Problem-processing system--the link between the above two components

Various forms of DSS exists including model driven, communication based, data oriented, document focused and knowledge base. Knowledge is accumulated over time and plays a crucial role in the decision making process. DSSs may be designed in various ways to incorporate all the possible factors under consideration which, in real life, make the system very complicated.

Keen (1980) expressed DSS as applicable "to situations where a 'final' system can be developed only through an adaptive process of learning and evolution". Figure1 shows the a typical DSS which consists of human decision making and computer systems.

DSS is not just a system of hardware and software which is a popular misconception.

According Simon(1960), "*An unstructured (or semi-structured) decision cannot be programmed because of its precise nature and structure that is elusive and complex*". The objective if the DSS is to support and facilitate the human decision making process rather than replacing it completely.

DSS is distinguished from a Management Information System (MIS) with respect to effectiveness focus opposed to efficiency in decision processes (facilitating decision processes).

Supporting all phases of the decision makes an important performance objective of DSS (Sprague and Carlson 1982).

Figure 2 shows the main research areas which are the focus of DSS.

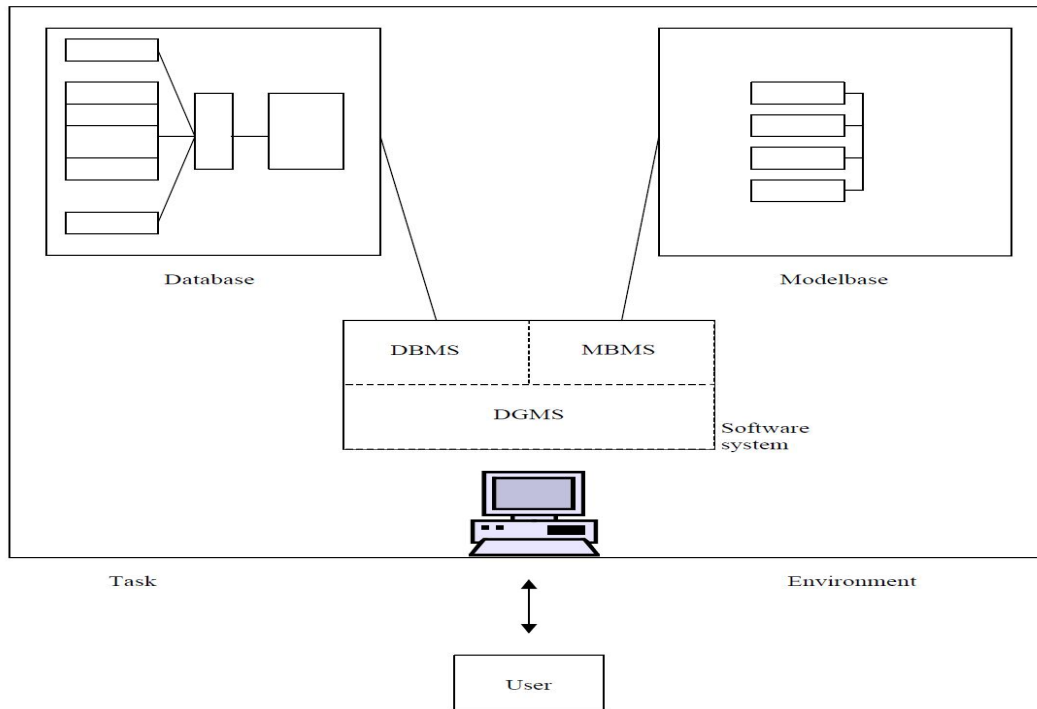


Figure 1 DSS Components (Sprague and Carlson, 1982)

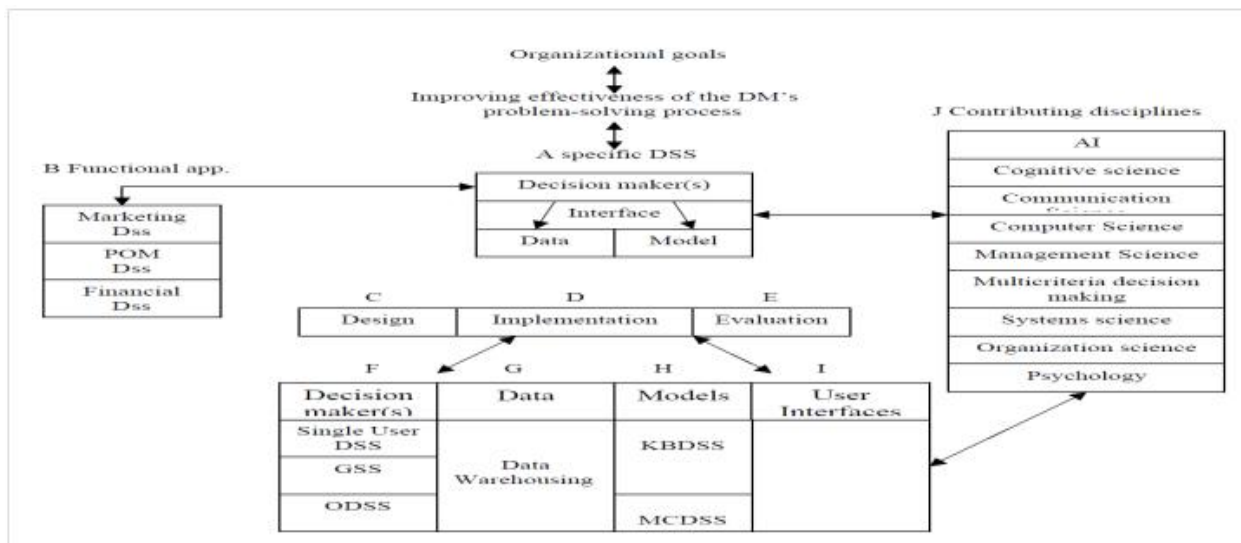


Figure 2 DSS Applications and Disciplines (Sean, 2001)

### 3. Research Methodology

The aimed to measure the extent to which DSSs help in the organizational decision making and the impact of DSS in the organizational working environment with implications for Pakistan, notorious for its lack of use of technology. The hypothesis was therefore to find out whether the Decision Support System would benefit decision making in an organization especially in Pakistan.

The target population included individuals working in different organizations and at various levels of career path. It was assumed that the sample would understand decision making thoroughly and would be able to answer the questionnaire. The sample size was 20 to get in depth inputs on understanding of DSSs from the sample and made a suitable size. The chosen sample belonged to a diverse working background having a working knowledge of how decisions were undertaken

in their respective organizations. A qualitative assessment was conducted using a self administered questionnaire consisting of a mix of both open and closed ended questions. The open ended questions were included to extract comments and personal opinions from the subjects, while the close ended questions were included to assess various statements regarding the DSS implementation in their organizations. The research instrument was composed of 15 questions with ten closed ended type and the remaining five open ended type. Some close ended were based on a five point Likert-type scale. The aim here was to measure the DSS knowledge and implementation among different organizations. The scale required the respondents to provide a mark of 1 for strong disagreement with a particular statement to a mark of 5 strong agreement, while other close ended questions were based on Multiple Choices out of which the respondents chose responses. Open ended questions required the respondents to describe their experiences of using DSS if implemented. In cases of non DSS usage the reasons were inquired including working environment and costs

etc. The aim was to gather information on the awareness of DSS in Pakistani work culture, its implementation and the benefits that could be derived through proper implementation of DSSs. The questionnaire was distributed electronically using e-mail asking respondents to fill in the questionnaire and mail it back.

The research took on an exploratory paradigm; DSS in Pakistan is a relatively new concept and has less awareness in organizations. The research aimed to achieve ways to increase awareness levels of decision support technology and its implementation in Pakistan.

**4. Results**

The purpose of the study was an identification of the differences in decision making activities with and without DSSs, an assessment of DSSs’ general awareness levels in Pakistan and implementation in organizations. Responses from twenty respondents were condensed into ten random responses with a 1-10 scale measuring agreement to various identified domains. The results are shown in Figures 3 and 4.

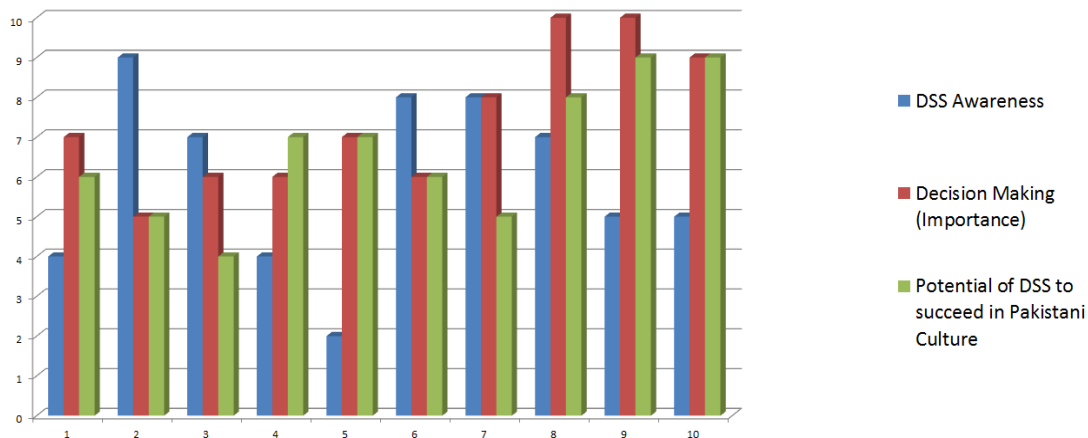


FIGURE 3: COMPARISON OF VARIOUS DSS FACTORS

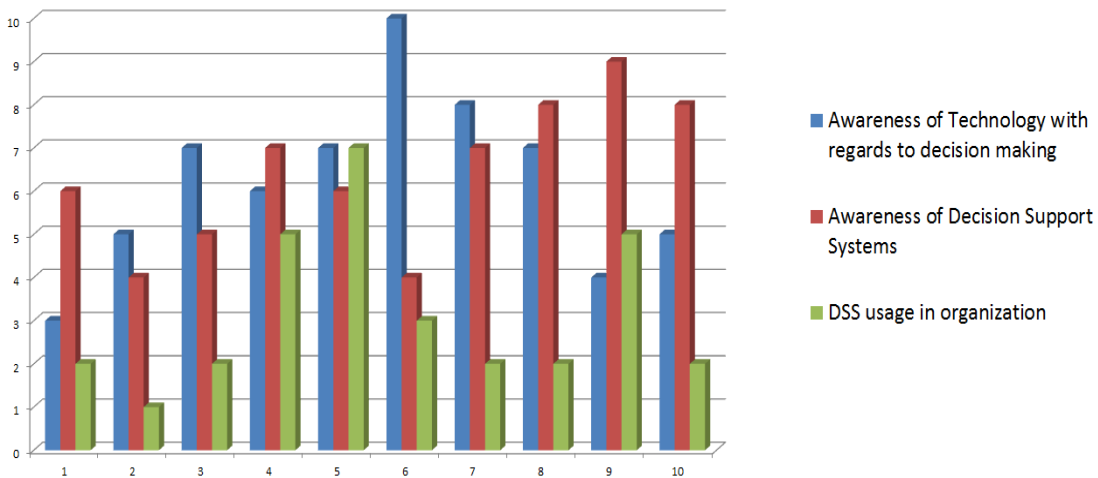


FIGURE 4: COMPARISON OF VARIOUS DSS FACTORS



## 5. Conclusion and Recommendations

The most important factor in the DSS implementation is awareness of Decision support technology. From the survey, it could be reflected that awareness of technology and decision support systems is not present at desired levels in Pakistani work environment both private and public. Awareness is a vital step in its successful implementation, for that fact that lesser knowledge of a technology can in no way lead to full scale implementation. Organizations using DSS are very few in Pakistan and most are not ready to accept this new technology for decision making. The behavior results from companies not having sufficient knowledge about the working and results of decision support technology. The potential for success of the DSS in Pakistan is the directly proportional to its knowledge, awareness, technical training and directly measurable decisional consequences leading to effectiveness and efficiency.

It may be assumed that experts in a domain are not subject to judgmental biases and approach optimality in decision making. Empirical evidence shows that experts are more accurate than novices within an area of expertise; however, experts also are vulnerable to the same judgmental biases as novices and demonstrate apparent errors and inconsistencies in judgment. Seminars and awareness workshops may be arranged to highlight the basics, costs, and benefits of using DECISION SUPPORT technology to help creating its awareness and promoting its use in Pakistan. Decision support systems over the last few years have gained much popularity due to its powerful integration of scientific methods which helps taking complex decision. DSS are very helpful when a decision is based on precision and accuracy. These systems minimize human deficiencies by effectively taking complex decisions with accuracy. Decision support systems have many attractive features which are very likely to succeed in the long run. DSS will never replace human analytical capabilities but will be very useful tools in aiding and supporting such capabilities. For an effective implementation of such systems user interface should be the focus of the developers as it plays very vital role in determining how accurate the decision process is and measures its effectiveness.

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## Effectual Dynamics and Prolific Usage of Knowledge Management & Engineering in Health Care Industry

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**Abstract:** Knowledge management is about managing and sharing knowledge. Knowledge management includes areas such as organizational behavior, IT, leadership, training and strategy which is very important for both organizational and individual's health. This study aims at applying this concept of knowledge management to the health care industry. The health care industry is one of the most important industry in any economy. Although this industry is showing growth trend in many countries but still there are many medical errors that are occurring. Errors are even occurring in developed countries. In order to reduce these errors, this study has recommended a model which involves "The ministry of health" and "NADRA" of Pakistan. These ministries will help in record keeping (Record of patients and best practices). This model will in return improve the quality and reduce the errors of the health care industry.

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**Keywords-** Knowledge Management, Health, Leadership, Pakistan, Organization, Strategy, NADRA1. INTRODUCTION

The objective of this study was to investigate if the implementation and integration of knowledge management shall improve the performance of the health care industry or not. The present health care industry of Pakistan has been evaluated in this study. The main aim was to determine the main benefits that knowledge management has to offer to the healthcare industry of Pakistan.

*"Knowledge management is the name of a concept in which an enterprise consciously and comprehensively gathers, organizes, shares, and analyzes its knowledge in terms of resources, documents, and people skills"* (Angus and Patel, 1998).

Knowledge management is generally concerned with sharing and transferring of knowledge. This knowledge makes the basis of best practices in health care industry. These best practices can then help in improvement of performances (Angus and Patel, 1998). This study will help in determining what positive effect knowledge management will bring to the health care industry of Pakistan.

It is a known fact that the sharing of knowledge is most critical in the health industry. This is because human life is involved and the information is very personal. So this study focuses on the improvement in performance in health care industry that can be brought about by the implementation of knowledge management and knowledge engineering.

### Problem Statement:

Analyzing the factors responsible for effective implementation and integration of knowledge management and engineering in health care industry of Pakistan.

The objective of this research is to design a knowledge management framework for the health care industry. This study has been done to identify through the use of knowledge management framework whether the performance of health care industry can be improved. The performance of the health care industry depends highly on knowledge sharing. Knowledge sharing is critical in the health care industry, since the underlying value is of human life and privacy. There are many countries that spent heavily on their health care. This is a critical and sensitive industry for any country. Yet there are many errors happening in this industry. The occurrence of these errors increases the operating cost instead of reducing them.

In this paper the main aim was to come up with a knowledge management model along with other management tools that helps in reducing these errors, sharing of knowledge, and saving more human lives. The proposed model (given by this study) will help the health care professionals in making better decisions and hence improves the quality of the health care industry.

## 2. Knowledge Management

Different organizations have different meaning and purpose of knowledge management and depend on the type of initiative. Managing knowledge is almost the heart of everything that happens in the health sector today. There is a strong urge and need to have fast access to wide range of sources for accurate information to help and deliver the best possible services for users and (Beverly, 2007). Knowledge management is a practice and not just a technology or a set of methodologies. Three main components of knowledge management are people, process and technology. For any health sector to have a good practice in 21st century it should make sure that these three components are in

place and functioning properly to achieve the best practice. From the health sector point of view the knowledge management can be thought of ensuring the right information is available to the right people and practiced by the right people at the right time (Beverly, 2007). As health sector is very much interconnected and interrelated so a successful initiative in one department can be easily extended to practice in other departments. According to Beverly (2007) "Knowledge management is concerned with creating, managing and sharing explicit knowledge (e.g. reports, policy statements, procedures, practice guidelines, books, journals articles), as well as tacit knowledge (i.e. encouraging employees to share knowledge that they have gained through experience).

According to Boss (2002), knowledge management consists of the following four processes:

- Knowledge creation
- Knowledge structuring
- Knowledge dissemination
- Knowledge application

Figure 1 presents the four processes that are involved in the knowledge management cycle as proposed by Boss (2002).

Most health care organizations are poor in knowledge management and information. Although health sector is knowledge and information rich sector but there is not much quality information available to use effectively for the best practice.

According to Rector (2001) "Getting the right information to the right people at the right time in the right form to make a difference is still the exception rather than the rule".

There are lot of restrictions in place for information and knowledge sharing in the health sector which results in inefficient use of resources, loss of many resources and clinical errors that leads to serious financial and health problems for people and government respectively.

Health care industry consists of hospitals, clinics, pharmacies and customers that are connected in information and knowledge sharing activities leading up to the improvement of the quality care and best practice.

Boss (2002) and (Ruggles, 1997) believe that to implement the knowledge management environment of four processes several categories of knowledge management capabilities needs to be supported by organizations.

### 3. Industry Analysis

#### 3.1 Business strategies related to knowledge management

The business activities that are linked with knowledge management according to Newman (1991) are:

- Change management
- Best practices

- Risk management
- Benchmarking

The advantage derived from knowledge management is that a record of the best practices is kept. If a person comes up with an even better way of doing things then that is recorded. Then that process is followed. The information can be of anything; corporate asset to business strategies and policies. Knowledge management helps in reducing the risk factor by not repeating the same mistakes again.

In knowledge management emphasis is given to creativity, creating new knowledge value while other programs emphasize leveraging existing knowledge. (Sveiby, 1996)

According to the United Nation the health care industry includes, (ISIC Rev.4)

- 1) hospital activities,
- 2) medical and dental practice activities,
- 3) other human health activities

The people that are involved in this industry are innumerable, (ISIC Rev.4)

Nurses, midwives, physiotherapists, scientific or diagnostic laboratories, pathology clinics, ambulance, nursing home, or other para-medical practitioners in the field of optometry hydrotherapy, medical massage, music therapy, occupational therapy, speech therapy, chiropody, homeopathy, chiropractic, acupuncture, etc.

No one can neglect the importance of the world health industry (Economy Watch, 2005). This is why a general growth trend is seen in the health care industry. Many different countries such as China, Turkey, Indonesia, Russia, India, Brazil, and Mexico are showing a growth trend. These countries compromise approximately 1/5th of the world health care sales.

It is also seen that the developing countries are also going for the health care industry. This is because it helps in generating higher revenues and contributes a lot to the GDP. According to the statistics of the U.S. the health care industry of U.S. alone is raising 13.4 million job opportunities. It is also expected that the job opportunities will further rise by 19% by the year 2014. (Economy Watch, 2005)

The providers of the healthcare look at quality as cost efficiency, whereas the patient sees the quality as cost efficient and accurate diagnosis. To improve the quality of the healthcare further the providers must anticipate the requirement of the patient. If the providers try to cut cost in improving these services they will significantly increase their costs. This is because it might increase the duration of the patients stay (Stewart, 2003). The healthcare providers must look into the TQM practices. This will bring forth many advantages. A learning culture will be created, new ways to solving problems will be adopted, there will be improvement in service, team work will be promoted, and the service will be market oriented (Ovretveit, 2001).

### 3.2 Rate of medical error in advanced countries

Health care is very expensive. A lot of countries invest a significant percent of GDP on their health care. USA spends the highest amount on its health care then UK and Canada. Still there are many errors that are occurring (A Research Center of the University of Sheffield and CITY Liberal Studies, 2005).

According to a research done by Kaiser Family Foundation (2008), almost 7000 patients die in USA due to errors. In another research it was said that one out of every twenty five patients die due to medical errors (AHRQ, 2000). These medical errors cost the USA almost \$37.6 billion. It is said that 54 percent of these errors were preventable (IOM, 1999).

In a report according to National Health Services of the UK 72,000 patients have died due to medical errors where as in Canada 24,000 patients have died because of the same reason (CBC, 2004).

### 3.3 Data, Information, Knowledge and KM

Data is like raw information. In the healthcare system this raw information does not provide complete details. It consists of the name of the patient and doctors name along with the treatment he is about to receive basically general information (London hospital, 2001). Information is when a doctor makes a decision or decides a treatment for a patient by looking at the previous information provided to the doctor. This means using the data to make it into a useful form. The information systems have been made so that the collection and analyzing of data is made easy (Herring, 1992). Whereas knowledge is an integration of know-what, know-how, know-where, know-who and know-why processed from information (White, 2000). KM is where knowledge is creatively, effectively and efficiently applied to benefit patients (Prince, 2000).

The aim of KM is to improve the learning with in the healthcare system. To ensure such a system a knowledge storage space will be required, this knowledge space needs to be accessible, and the knowledge should also be convertible and understandable (Sharma, Wickramasingha & Gupta, 2005). This whole process of data turning into Knowledge can be summarized below in figure1.

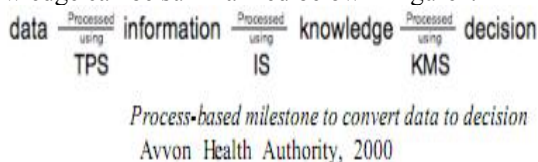


Figure 1 Data transformation into knowledge

### 4. Health Care Industry in Pakistan

The healthcare of Pakistan is in a bad state. It is not well funded. The healthcare is said to receive only 0.75% of the GDP (Economic Survey of Pakistan,

2005-06) where as the USA receives about 25% of the GDP (WHO, 2005). The expenditure till now is as follows.

#### 1) Federal Government Spending on Health (2007-08)

Development Expenditure	Rs. 14.272 billion
Current Expenditure	Rs. 3.791 billion

Source: Ministry of Health, 2007

Despite these challenges the health ministry has initiated many programs. These programs are to generally fight against diseases such as Cholera, Crimean Congo Hemorrhagic fever, Dengue Hemorrhagic Fever, Hepatitis C, HIV, Swine Flu, Tuberculosis and leishmaniasis (Akram and Khan, 2007). The medical force that is present to overcome these diseases are,

#### 2) Human Resources (Registered, 2007)

Doctors	107,835
Doctors registered as specialists	19,623
Dentists	7446
Dental specialists	433
Nurses	43,646
Midwives	2,788
Lady Health Visitors	3,864
Lady Health Workers	95,000
Lady Health Supervisors	3,385
Population per doctor	1,475
Population per dentist	21,362
Population per nurse	3,644

Source: Ministry of Health, 2007

Despite having such a strong medical force and good health programs, not having sufficient funds catches up. This can be shown in the following table.

#### 3) The Mortality Rate per Disease

Infant Mortality Rate (IMR) (per 1000 persons)	76.7
Maternal Mortality Rate (MMR) (per 100,000 live births)	350
Under -5mortality rate (per 1000 persons)	101
Parasite Incidence of Malaria (per 1000 persons)	0.75
Incidence of TB (per 100,000 persons)	181

Source: Ministry of Health, 2007

The health care industry comprises of the following health service providers.

#### 4) Health Services Providers (2006-07)

Total Health Facilities	13,937
Hospitals	965
Dispensaries	4,916
Basic Health Units	4,872
Rural Health Centers	595
MCH Centers	1,138
TB Centers	371
First Aid Points:	1,080
Beds in hospitals & dispensaries	105,005
Population per bed	1,515
Population to health facility ratio	11,413

Source: Ministry of Health, 2007

The three main stake-holders in any health sector are people, administration and government. They all are involved in knowledge and information sharing activities. For a successful knowledge management in any health sector all the three stake-holders should adopt a learning approach in creating, transforming and retaining knowledge. For example, for people i.e. patients to have the best facilities available in hospital, for administration i.e. doctors to have the best environment for patient care by capturing and sharing knowledge and for government to be able to operate efficiently and effectively in any health care initiatives. And for the above stakeholders to be able to measure and evaluate learning outcomes for the best practice, open system architecture capable of using shared resources and serving all the three stake holders might be a solution for best practice.

### 5. Research Methodology

The research methodology that has been adopted is interpretive and analytical. The theory of knowledge management was studied in great detail before this research was carried out. This was done in order to understand the impact that knowledge management has in the health care industry. Inductive reasoning was used with the aim to determine the possible advantages that knowledge management has to offer.

Interview method was used and in these interviews the main interviewees were doctors working in management areas. These doctors were selected randomly. Doctors from different hospitals of Islamabad both public and private were interviewed. The sample size taken into consideration was 40. All those interviewed were health industry or health industry related professionals.

Qualitative data was gathered in order to find out the potential benefits that knowledge management has to offer the health care industry of Pakistan. In order to do so, semi structured interviews were conducted. On the basis of this data the knowledge management adaptation and integration issues were determined.

The 40 health care professionals that were interviewed were informed about knowledge management. They were given the basic back ground information of knowledge management. This was done so that they could prepare themselves for the interview, so that they could gather their thoughts.

- The basic information about knowledge management.
- The benefits knowledge management can offer.
- The present status of their IT.
- The potential of their extension into IT.
- The budget allocated for the extension of IT.

After giving the interviewee the information a second interview was conducted. By this time the

interviewee had gained considerable information. The interviewee's had also aligned their thoughts by then. This time the discussion was based on the proposal of implementing knowledge management. Their response was then recorded. The second interview recorded information such as:

- The cost of implementing knowledge management.
- Comprehending the advantages that knowledge management would bring to health care industry.
- Describing the knowledge management system in foreign countries.
- How knowledge management will enable hospitals to improve patient care.
- Possible drawbacks and limitations associated with such implementations.
- Current management resistance to this change

The information gathered from all sources was carefully recorded. The careful recording was done in order to assure the authenticity of the information. It was also being done to make sure that quality and accurate information was gathered.

### 5.1 Data Interpretations and Findings

In this research the primary data was collected through interviews. Therefore the data is mainly qualitative. The response of the interviewees was recorded and analysis of data gathered is as follows.

**Table 1:** The present status of their IT

Satisfaction Level of Doctors with Current IT Status		
	Satisfied	Unsatisfied
Public Sector Doctors	10%	90%
Private Sector Doctors	80%	20%

Most of the hospitals in private sectors are advanced in the equipment and IT systems. They have good record keeping systems. The new patients when they enter the hospital for treatment, they are given a reference number and in this reference number the patient records are kept. These are the records of the treatment that a patient receives in that particular hospital.

Mostly the information system in these private hospitals is good as long as the patient keeps on taking treatment from that hospital. If ever the same patient cannot find a particular treatment available in that hospital then he/she would have to go to another hospital which would not be aware of the prior problems of the patient.

The public sector hospitals in Pakistan totally lack in IT structure. They have no proper reporting systems, record keeping and communication. The technology that is used in these hospitals is mostly obsolete. There

are no computers used, hence there is no communication/record keeping.

During our interview with the doctors of the private sector, we found out that they are mostly satisfied with their current IT systems. 80% of the doctors in private sector are satisfied with their current IT status. Whereas the other 20% are the senior doctors that find operating on the systems a little confusing. This is mainly the only reason for their dissatisfaction.

**Table 2**

	Extension in Current IT Status	
	Agreed	Non-Agreed
Public Sector Doc	70%	30%
Private Sector Doc	20%	80%

The doctors in private sector say that they are very much satisfied with their current IT status. They say that the only extension would be to bring in more advanced medical technology that could help them in treating patients more efficiently and accurately. Where as 20% of the doctors felt that there should be some more advancement in their hospitals. They want that the communication between departments should be improved further.

The public sector doctors are highly unsatisfied with their almost non existent IT status. They say that they are already lacking in medical technology, so it is highly unlikely to expect any extension/improvement in their IT status.

When we asked about the budget allocation they have for IT in private sector hospitals, different doctors came up with different figures. This is because the doctors interviewed were from different hospitals and of different sizes. The figure when averaged was about 30%.

The doctors in the public sector were asked this same question, they remarked we are already very much underfinanced and the hope for the extension in IT is near to zero.

**Table 3**

	Satisfaction with Current Management to handle Changes	
	Satisfied	Not Satisfied
Public Sector	30%	70%
Private Sector	80%	20%

80% of the doctors in administration of private sector feel that their management staff can handle the change. This is because the staff is already used to the advance operating systems present in the IT area. They are educated enough to learn new things.

In the public sector, doctors have some reservations about handling the change because the staff of public sector hospitals are not educated enough to learn the

new things of technology. And they are resistant to accepting the change.

Electronic patient record (EPR) and the electronic health record (EHR) are the way forward for the Health care industry (Razzaque and Jalal-Karim, 2010).

So in order to implement the EPR and EHR system the whole health care system should be made electronic consisting of secure computer systems. This should be done so that the hospital computers should communicate with each other. So if a doctor of one hospital happens to come across a patient of another hospital can get access to his past records. These electronic records will help the health care professionals make better decisions and save more lives (Razzaque and Jalal-Karim, 2010)

**5.2 MODELS**

**MODEL 1**

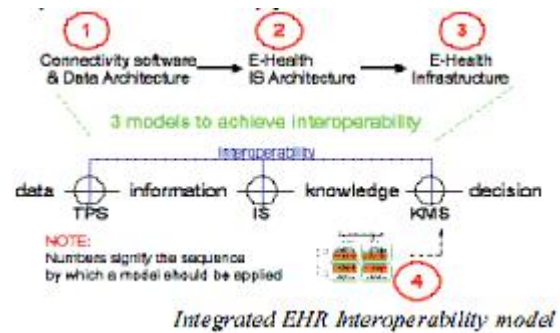
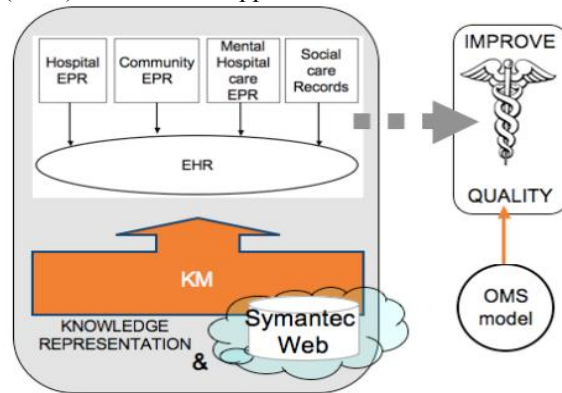


Figure 2 Integrated EHR Interoperability Model  
Source: Razzaque and Jalal-Karim, 2010

**MODEL 2**

The HC KM model to improve the quality of healthcare was proposed by Razzaque and Jalal-Karim (2010). This model supports the EPR and EHR.



HC KM facilitator model to improve quality of healthcare  
Adopted from – (Jalal-Karim and Balachandran, 2008).

Figure 3 HC Facilitator Model to improve quality of Health Care

Source: Jalal-Kari and Balachandran, 2008

In this model knowledge representation is referred to as the storing and the processing of information and knowledge so that it can be used by other applications. The Semantic web is referring to the web site where this information will be available (Wickram et al., 2009).

## 6. Recommended Model

### 6.1 Proposed Model to improve healthcare industry & government

Model that is recommended is based on the data that was analyzed from interviews conducted in hospitals by the team. The model has been designed according to the requirements of Pakistani hospitals and health care industry. The health care knowledge management (HC KM) will significantly improve the quality (Wickram et al. (2009, p. 1).

The proposed model is not going to only help Pakistan but may also be used and implemented in other developing countries. The application of this model will improve functioning of many health care departments including hospitals, health care centers and governmental departments in Pakistan.

There are 4 steps that are proposed by this model they are:

Step 1 (A): NADRA should add a memory chip to the Pakistani ID card. This chip should have the ability to store up to 1 MB to 10 MB of data. This will not be that difficult a task because UBL (united bank limited, pakistan) already is providing a credit card that possesses a chip. This task will increase the little cost of production for NADRA, this is because the chip will cost an extra 100 Rs (Naaptol.com, 2010). The existing charges of NADRA are,

Ser	Category	Normal (Rs)	Urgent (Rs)	FastTrack (Rs)	FastTrack with Home Delivery* (Rs)
1	Fresh CNIC	0	200	1000	1100
2	Modification	150	200	1000	1100
3	Renewal	75	200	1000	1100
4	Duplicate	150	200	1000	1100

Source NADRA, 2010

Now what we suggest is that NADRA should reduce the validity of the ID card to period of 2 years and make a contract with the chip producers and order in large bulk to minimize the cost of the memory chips. NADRA can then increase the charge per ID card by 50 Rs. or can also be subsidized by the government. After a period of 2 years when the ID card is supposed to be renewed the chip from the old ID card can be reused.



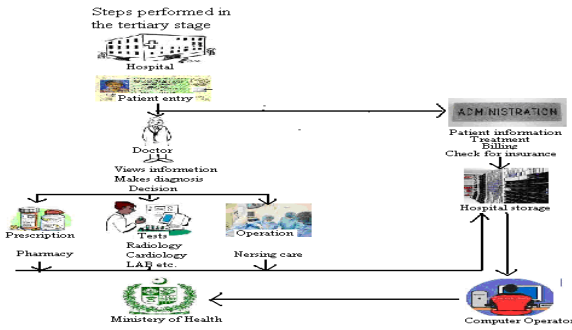
ID card  
with  
memory  
chip

This chip will be used for the purpose of storing the medical record of the individual. The record in this chip will be stored in 5 sections.

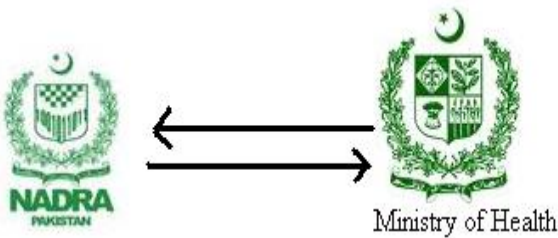
1. The Primary information: This will contain information about the general physician and the diagnosis he makes along with prescription.
2. The Secondary information: This will possess the information prescribed by the specialist. It will also possess the prescription.
3. The Tertiary information: This will possess the information provided by the hospital. This portion will require the most memory space. This is because it will contain the test results, the nursing care, and the progress report.
4. The fourth section called "General" will contain general information about who to contact in case of emergency. If the patient has medical insurance then that will be also available in this section.
5. This fifth section called "Allergies" will contain information such as the medicine the patient is allergic to and other common medicine that doesn't suit the patient.
6. The sixth section may contain the old history of patients that shall help the doctor to understand patient and about his history.
7. The seventh section may also help the government that each chip in ID card may also contain patient's legal history.

The ID card will act like an ATM. The health information will only be displayed if the right pin number is given by the right person. The other advantage of this model can be that if the ID card is lost then the finger print recognition can be used to find out the identity and medical history of the patient.





Step 1 (B): Now when the expiry period comes the individual is to turn in his old ID card. The ID card will be destroyed but the chip will be erased of all information and then reused. Now this time when the ID card goes to the NADRA it will need to be verified. This is the step 2 of NADRA they call it Verification and Clearance from NADRA Data Warehouse. When this is being done the new chip will get information from the Ministry of Health data base



Step 2: The Ministry of Health's budget shall also be increased to implement this project. For the proposed model the health ministry will have to open another department that will maintain a data base. This data base will be the back up of the patient information. The updates to the patient information will come through the hospitals.

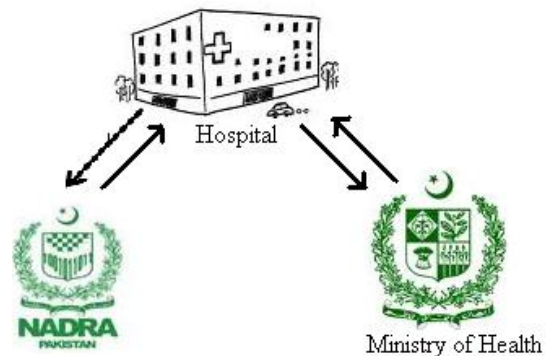
Step 3: An operating system is required to be made. This operating software should be user friendly. The government should come up with such software or it should be sponsored by the government. This software should be adopted by all healthcare providers. Moreover, the locally developed module can also be installed with the compatibility of windows operating system. If the software is a little difficult to understand then training campaigns should be set up, so

that people can learn how to operate such a system. These trainees can also be hired by the hospitals to maintain the patient record.

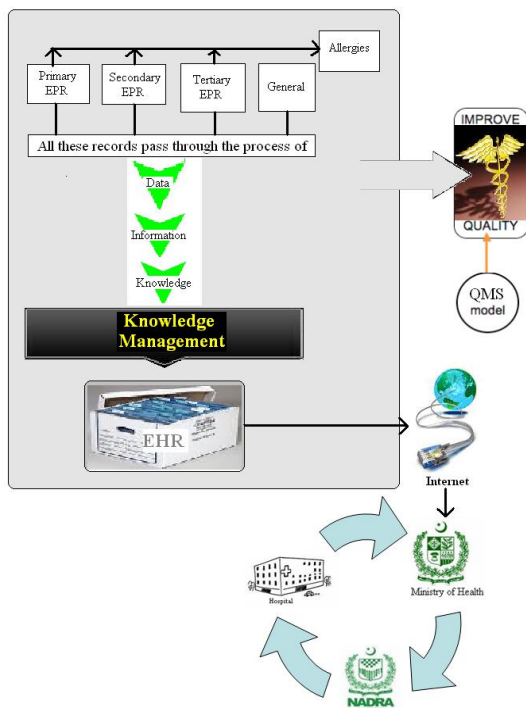
Step 4: All the providers of health care must have online facilities. This is so that data can be updated in the ministry of health where the data base is being maintained. The hospitals should possess some good storage facility where all this information about their patients is maintained.

The need for online is that when a patient receives some treatment a record of the patient changes. This change will have to be recorded in his permanent file that is being maintained at the data base by the ministry of health. So in the event if a person loses his ID card a record will be present in at least two locations, the hospital where the patient receives most of his treatment and the ministry of health.

At times it is seen that there has been an accident. The person that is injured is unknown. His wallet is stolen and the contact person is not known. Due to this reasons the individual dies because they do not know who to contact. Now in this situation the hospital should take a finger print and send it to NADRA. NADRA will then run it through the finger print scanner. When the identity is known then the information will be sent to the hospital. The hospital will then establish contact with the Ministry of Health and withdraw his medical record. In this record his contact person will also be mentioned.



MAIN MODEL



## 7. Conclusion

Knowledge Management is about sharing and managing the knowledge. Many factors have been identified, discussed and recommended for rapid improvement of health care sector in Pakistan. Moreover, the same information can also be used by the government in security and tracing issues. For effective use of knowledge and information in health sector, necessary skills needs to be developed to store, retain and share knowledge. As described earlier people are the most important part in any knowledge management strategy. But for an effective KM strategy, technology and people should be supported by appropriate technology.

Knowledge management can improve the performance of an organization because sharing of knowledge is the most critical in the health industry. If the proposed model is followed then all the record keeping system will change and the files will be made electronic. This will make the records be easily available and improve the communication, coordination and quality that the health care has to offer.

This model can offer great success to the health care industry by reducing the number of medical errors. There are also some additional merits of this system. Fake medical centers, doctors and nurses can also be caught which are playing a quite important role in killing the patients by given them wrong injection or medicine. This model can also be modified to help insurance companies.

Knowledge management should be considered a business model to coordinate and collaborate efforts to improve the organizational performance by creating, sharing, retaining and applying the knowledge. It is also believed that organizational productivity can be enhanced by reusing knowledge across organizations

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1/26/12

## Study of the relationship of glyated hemoglobin levels and neurological impairment and three months prognosis in patients with acute ischemic stroke

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**Abstract:**Objective:To explore the relationship of glyated hemoglobin levels (HbA1c) and neurological impairment and three months prognosis in patients with acute ischemic stroke. Method:180 patients with acute ischemic stroke within twenty four hours after onset were included in the present analysis from the Department of Neurology of the First Affiliated Hospital of Zhengzhou University.Three milliliter of Venous blood within twenty four hours after admission to test the blood HbA1c.The patients were divided into three groups based on the glyated hemoglobin (HbA1c). These three groups designated as: Normal group (HbA1c<5.7%), Intermediate group (5.7%-6.5%) and Elevated group (HbA1c≥6.5%). Evaluation of neurological functional impairment with NIHSS scores within twenty four hours after admission, and to evaluation of the prognosis with MRS score after three months. Result:As for patients, in age, sex, hypertension, hyperlipidemia, smoking history aspects, the difference was not statistically significant among three groups (P>0.05). As for the history of diabetes, blood glucose on admission, NIHSS scores, three months MRS score, the difference was statistically significant among three groups (P<0.05). The blood HbA1c and NIHSS score and three months MRS score of 180 patients were respectively analyzed using Pearson correlation analysis, the results showed that the blood HbA1c content and NIHSS score and three months MRS score in patients with ischemic stroke were positively correlated (P <0.001). Conclusion: Different HbA1c levels in patients with acute stroke has different neurological impairment on admission and three months prognosis are also different, showing that a higher blood HbA1c levels has a more serious neurological impairment and the prognosis is worse after three months. HbA1c levels maybe is an important predictors to evaluate the neurological impairment and three months prognosis in patients with acute ischemic stroke .

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**Keywords:** glyated hemoglobin; ischemic stroke; neurological impairment

### 1. Introduction

Acute stroke is a limited or comprehensive brain function deficit syndrom caused by acute cerebral circulation disorder. Its incidence,morbidity and mortality rates are high[1-3], it is a serious threat to human's health. At present,Cerebrovascular disease has been one of the three diseases that seriously threaten human's health. In some areas in China,Stroke has been ranked in the first place of the fatal diseases.The existing studies [4-6] have shown that the main cause of ischemic stroke is the lesions of the vascular wall, and atherosclerosis is the most common cause which leads to the lesions of the vascular wall[7-8]. As we all know,Diabetes is one of the major risk factors of atherosclerosis,which can accelerate the process of the vascular lesions.Glycosylated hemoglobin(GHb) is a variety of non -enzymatic glycation reaction products generated by thehemoglobin in the role of continuous glucose,and their formation is irreversible,its synthesis rate is proportional to the blood glucose concentration. Its main form is

HbA1c , which represents the average blood glucose levels in 2-3 months. Studies have shown[9]that the blood HbA1c levelcan be as one of the important predictors of occurrence, development and prognosis of patients with ischemic stroke. The new guidelines issued by the American Diabetes Association (ADA) [10] in 2010 takes HbA1c ≥ 6.5% as one of the diagnosti criteria for diabetes,and takes HbA1c ≥ 5.7% as one of screening criteria of the diabetes.However,at present, the Correlated study of the relationship of blood HbA1c levels and neurological impairment and three months prognosis in patients with ischemic stroke has been reported little.

### 2. Materials and Methods

#### 2.1Object of study

Over a period of one year from July 2010 to September 2011, From the Department of Neurology of the First Affiliated Hospital of Zhengzhou University, 180 patients with acute ischemic stroke

within twenty four hours after onset were included in the present analysis. Including 97 males and 83 cases of women, ages ranging from 45 to 81 years old, the average age was  $60.5 \pm 8.65$  years old. Diagnostic criteria: All cases were in accordance with the diagnostic criteria of World Health Organization (WHO) 1976 [11]. Inclusion criteria: (1) Cases within the diagnostic criteria. (2) Stroke attack in twenty four hours. (3) Head CT to exclude hemorrhage. Exclusion criteria: exclusion of patients with diseases that lead to ischemic stroke, such as arteritis, blood system diseases, heart disease et al. Exclusion of patients who were accompanied with atrial fibrillation, cancer, generalized infection, autoimmune diseases and liver, kidney or cardiac failure.

## 2.2 Research methods

Three milliliters of venous blood was taken to test blood glucose, blood lipids and glycated hemoglobin (HbA1c) in the next day morning after admission. Age, sex, past history (hypertension, diabetes), smoking history of selected patients were recorded. The patients were divided into three groups based on the glycated hemoglobin (HbA1c). These three groups designated as: normal group (HbA1c  $< 5.7\%$ ), intermediate group ( $5.7\% - 6.5\%$ ) and elevated group (HbA1c  $\geq 6.5\%$ ). The degree of neurological impairment (NIHSS score) was evaluated within twenty four hours after admission. The prognosis (MRS score) was evaluated after three months, by a neural physician who was trained. According to NIHSS score, the patients were divided into three groups: mild ( $< 4$  score), moderate ( $4 \sim 15$  score), severe ( $> 15$  score). According to MRS score, the patients were

divided into two groups: independence ( $0 \sim 2$  score) and dependence ( $3 \sim 5$  score and death).

## 2.3 Statistical Methods

All continuous variables were expressed as mean  $\pm$  standard deviation. All data were analyzed using one-way ANOVA or the  $X^2$  test by the spss 13.0 software package. The blood HbA1c and NIHSS score and three months MRS score of 180 patients were statistically analyzed using Pearson correlation analysis,  $P < 0.05$  was statistically significant.

## 3. Result

### 3.1 The clinical data of three groups

As for patients, in age, sex, hypertension, hyperlipidemia, smoking history aspects, There was not statistically difference ( $P > 0.05$ ) among three groups. As for the history of diabetes, blood glucose on admission, NIHSS scores, three months MRS score, The difference was statistically significant ( $P < 0.05$ ) among three groups. In diabetes aspects, elevated group accounts for 48 cases (90.57%), intermediate group accounts for 21 cases (38.18%), normal group accounts for 12 cases (16.67%), elevated group is higher than the intermediate group and normal group. In the same time, in NIHSS score on admission, elevated group was  $8.60 \pm 3.27$  points, it was higher than the intermediate group ( $6.71 \pm 3.29$  score) and normal group ( $4.09 \pm 3.14$  score). In three months MRS score aspects, elevated group ( $3.79 \pm 0.97$  score) was higher than the middle group ( $2.97 \pm 1.36$  score) and normal group ( $2.81 \pm 0.87$  score), as shown in the Table 1.

Table 1 General characteristics of three groups

Characteristics	Normal group (n=72)	Intermediate group (n=55)	Elevated group (n=53)	$X^2/F$	P value
Age (Years)	$59.68 \pm 3.868$	$61.26 \pm 1.281$	$60.264 \pm 4.073$	0.590	0.556
Hypertension (case)	40	35	37	2.707	0.258
Hyperlipidemia (case)	23	25	21	2.460	0.292
Diabetes (case)	12	21	48	68.848	$< 0.001$
Smoking history (case)	28	21	23	0.3680	0.832
NIHSS (score)	$4.090 \pm 3.140$	$6.710 \pm 3.290$	$8.600 \pm 3.270$	18.190	$< 0.001$
MRS (score)	$2.810 \pm 0.870$	$2.970 \pm 1.360$	$3.790 \pm 0.970$	13.350	$< 0.001$
Glycated hemoglobi (%)	$5.270 \pm 0.303$	$6.180 \pm 0.390$	$7.950 \pm 0.640$	536.300	$< 0.001$
Blood glucose (mmol/l)	$5.610 \pm 0.780$	$6.690 \pm 1.940$	$7.650 \pm 3.250$	524.410	$< 0.001$

### 3.2 Comparison of the severity of neurological impairment and three months prognosis of three groups

The severity of neurological impairment on admission and three months prognosis of three groups of patients were analyzed respectively by the  $X^2$  test, the difference was statistically significant ( $P < 0.05$ ). In neurological impairment aspect on admission, severe patient of elevated group accounted for 26.40%, that is higher than intermediate group (12.70%) and normal group (5.55%). In three months prognosis aspects, dependent patients of elevated group accounted for 33.96%, that is higher than the intermediate group (21.82%) and normal group

(13.89%). The blood HbA1c and NIHSS score and three months MRS score of 180 patients were respectively analyzed using Pearson correlation analysis. The results showed that the blood HbA1c content and NIHSS score in patients with acute ischemic stroke was positively correlated ( $r = 0.384$ ,  $P < 0.001$ ), the blood HbA1c content and NIHSS score in patients with acute ischemic stroke was also positively correlated ( $r = 0.326$ ,  $P < 0.001$ ). That is to say: a higher blood HbA1c levels has a more serious neurological impairment and the prognosis is worse after three months, as shown in the Table 2, Table 3, Figure 1, Figure 2.

Table 2. Comparison of the severity of neurological impairment of three groups

Group	Case (n)	Mild (%)	Moderate (%)	Severe (%)
Normal group	72	37 (51.39)	31 (43.06)	4 (5.55)
Intermediate group	55	23 (41.80)	25 (45.50)	7 (12.70)
Elevated group	53	21 (39.60)	18 (34.00)	14 (26.40)

Note:  $X^2 = 11.74$ ,  $P = 0.019$

Table 3. Comparison of three months prognosis of three groups

Group	Case (n)	independent (%)	dependent (%)
Normal group	72	62 (86.11)	10 (13.89)
Intermediate group	55	43 (78.18)	12 (21.82)
Elevated group	53	35 (66.04)	18 (33.96)

Note:  $X^2 = 7.12$ ,  $P = 0.028$

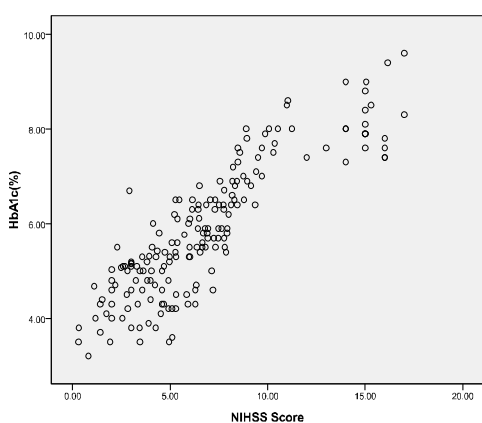


Figure 1. Scatter chart of HbA1c and NIHSS score

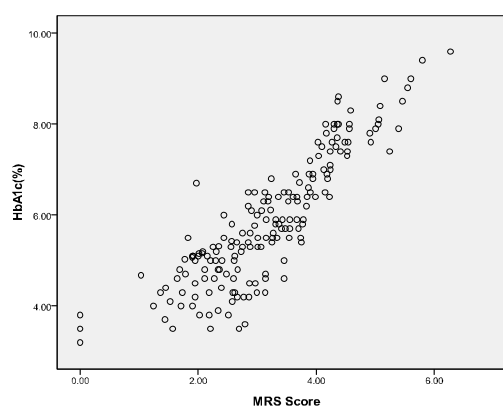


Figure 2. Scatter chart of HbA1c and 3 months prognosis score

#### 4. Discussion

Studies[12] have shown that HbA1c rise in the blood and HbA1c continually accumulate in the vessel wall, which lead to increase of thromboxane A<sub>2</sub> and protein kinase, which lead to excessive collagen fibers cross-link by reducing release of nitric oxide, this course results in hardening of the blood vessel wall and decline of artery compliance, and a higher content of HbA1c allows oxygen dissociation curve to the left, resulting in oxygen dissociation barrier, nerve tissue ischemia and hypoxia, myelin loss, nerve degeneration, dysfunction and necrosis.

Kizer et al[13-16]. studied the relationship glycated hemoglobin (HbA1c) and stroke (average follow-up of 9.2 years), in 1691 cases of patients with diabetes. The results showed that after adjusting age, gender, smoking, blood lipids and other variances, HbA1c and stroke risk was significantly associated. They emphasized that strict control of glycated hemoglobin (HbA1c) might be benefit for stroke prevention for the patients with diabetes. Our study showed that in the history of diabetes, blood glucose on admission, NIHSS scores, three months MRS score, among three groups of patients, the difference was statistically significant ( $P < 0.05$ ). The blood HbA1c content and NIHSS score in patients with acute ischemic stroke was positively correlated ( $r = 0.384$ ,  $P < 0.001$ ), the blood HbA1c content and NIHSS score in patients with acute ischemic stroke was also positively correlated ( $r = 0.326$ ,  $P < 0.001$ ). That is to say, a higher blood HbA1c levels has a more serious neurological impairment, and the condition might be more serious. So, HbA1c levels on admission might be an important predictors to evaluate the neurological impairment in patients with acute ischemic stroke.

The results also showed that the severity of neurological impairment on admission and three months prognosis of three groups of patients were analyzed respectively by the  $X^2$  test, the difference was statistically significant ( $P < 0.05$ ). In neurological impairment aspects on admission, severe patients of elevated group accounted for 26.40%, that is higher than intermediate group (12.70%) and normal group (5.55%). In three months prognosis aspects, dependent patients of elevated group accounted for 33.96%, that is higher than the intermediate group (21.82%) and normal group (13.89%). and a higher blood HbA1c levels has a more serious neurological impairment on admission and the prognosis is worse after three months. The mechanism might be associated with long-term high blood glucose and high blood HbA1c, which lead to lesions of large blood vessels and microangiopathy and which lead to oxygen dissociation curve to the

left, resulting in oxygen dissociation barrier, nerve tissue ischemia and hypoxia, that is not benefit for the recovery of neurological function, and the prognosis is worse. This result is in line with the result of Kamouchi et al[17], who studied 3627 patients, the result showed that neurological improvement is lower relevant to age and sex and is higher relevant to the blood HbA1c level on admission. namely, a higher blood HbA1c levels has a more serious neurological impairment and the prognosis is worse in three months.

In summary, Our study suggests that blood HbA1c levels on admission may influence severity in patients with acute ischemic stroke when stroke attack and may predict three months prognosis. So, HbA1c levels maybe is an important predictors to evaluate the neurological impairment and three months prognosis in patients with acute ischemic stroke, Therefore, effectively lowering blood HbA1c levels may reduce the severity of neurological impairment in patients with acute ischemic stroke, and maybe can improve the life quality of patients with acute ischemic stroke.

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## Gender Differences and Construct of the Early Adolescent's Emotional Intelligence

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**Abstract:** The emotional intelligence construct is a rather novel concept with little empirical support, particularly, in relation to the link between gender and the early adolescent's emotional intelligence. Hence, the specific objective of this research is to determine the relationship between gender and early adolescent's emotional intelligence (EQ). EQ is a set of abilities such as conception, emotion appraisal and expression, emotion management and regulation, and emotion utilization of emotion. The present study was carried out among 234 Iranian students in the second and grades of guidance schools (age 12-15) in Tehran, Iran. The students (girls and boys) were clustered through random and multistage sampling. Data were collected using the Schutte's (1998) Emotional Intelligence Scale. T-test indicates that there is a statistical significant difference between emotional intelligence among boys and girls. [Fataneh Naghavi, Ma'rof Redzuan, Arezoo Asgari, Mojgan Mirza. **Gender Differences and Construct of the Early Adolescent's Emotional Intelligence**. Life Science Journal 2012;9(2):124-128]. (ISSN:1097-8135). <http://www.lifesciencesite.com>. 21

**Keywords:** Early Adolescent's Emotional Intelligence, Gender Differences, Emotional Quotient (EQ), Gender and Emotional Intelligence.

### 1. Introduction

Emotional intelligence refers to understanding the feelings of oneself and of others, is related to people, and one's ability to adapt to coping with the immediate surroundings to be more successful in dealing with environmental demands (Bar-On & Parker, 2006). According to new study, there are several notable differences between male and female in term of their emotional intelligence. Male are seen to have significantly stronger interpersonal skills than their female counterparts do, and male also appear to have a stronger sense of self and arrangement better among stress. Goleman (1995) suggests counter views of emotionally intelligent female and male; by contrast, male who are high in emotional intelligence are socially poised, outgoing and happy, and they are also not prone to fearless or worried rumination. Moreover, they have a considerable capacity for commitment to people or causes, for taking responsibility, and for having an ethical outlook, and they are also sympathetic and caring in their relationships. Their emotional life is rich, but more properly, they are relaxing with themselves, others, and the social universe they live in. According to this point of view, female are more attentive of their feelings and those of others, relate better interpersonally, and are considerably more socially responsible than male. On the other hand, male appear to have stronger self-regard and manage better

with immediate problems of a stressful nature than female (Stein, 2004).

According Goleman, the particular sub-components in which female scored superior than male consist of:

- Interpersonal, which refers to the capacity to set up and maintain mutually satisfying relationships that are characterized by intimacy and giving and receiving affection.
- Empathy, which refers to the capacity to be attentive of, to realize and to appreciate the feelings of others.
- Social responsibility, which refers to the capacity to demonstrate one's self as a cooperative, contributing and constructive member in one's social group.

On the other hand, the particular sub-components in which male scored higher than female consist of:

- Stress tolerance, which refers to the capacity to withstand adverse events and stressful situations without falling apart.
- Self-regard, which refers to the capacity to respect and accept one's self as principally good (1995).

Petrides and Furnham (2000) have found the relationship between gender and emotional intelligence among two hundred and sixty

predominantly white participants who had completed a measure of trait emotional intelligence (EI) and assessed their scores. The findings indicated that the woman scored higher than the man on the “social skills” factor of measured trait emotional intelligence. However, it was demonstrated that the man believed they had higher emotional intelligence than the woman. Most of the correlations between the measured and self-estimated scores were significant and positive, thereby indicating that people have some insights into their emotional intelligence. The correlations between the measured and self-estimated scores were generally higher for the man than those of the woman, and a regression analysis indicated that gender is in fact a significant predictor of self-estimated emotional intelligence.

Brudy and Hall (2000) showed in their study entitled, “Sexuality and emotion”, that male and female learn different lessons in managing their emotions. Parents mostly talk to their girls, rather than boys, about their emotions (except for anger). Compared to boys, parents provide more information about feelings to their girls. Since female get mastery over language faster than male, this causes them to become more experienced at precisely expressing their feelings and more skilful in using words to name emotional reactions and replace words for physical reactions than male. Male, for whom emotions expression has not been emphasized, are probably unaware of their and others’ emotional states to a large extent.

Similarly, Hagan, Simpson and Gillis (1985) found that at the age of 10, the percentage of female who show open aggression, like male, when they are angry is almost the same. At the age of 13 years, nevertheless, there is a significant difference between the two genders. Compared with male, female obtain more skills in artistic aggressive techniques, such as collective banning, revengeful gossiping, and indirect avenging.

In their research on gender difference in relation to emotional intelligence, Katyal and Awasthi (2005) used 150 students whom they had selected randomly for estimation of gender differences. The data were collected through standardized “Emotional Intelligence Scale” and the findings showing that the majority of male, female and the total sample had good, followed by a low emotional intelligence. In particular, female were found to have higher emotional intelligence than that of the male. Conversely, the difference touched only 0.10 levels, and hence, the findings were just suggestive of the trend.

There are wide individual differences in early adolescent’s emotional intelligence and research regarding these individual differences which were

viewed as an important and necessary extension of past research that focused mainly on normative development (Denham, 1989; Denham, Zoller & Couchoud, 1994; Sarni, 1999; Sroufe, 1996; Steele & Aronson, 1999). Since their young age, females have been found to report, and sometimes, demonstrate more empathy and sympathy than their male counterparts.

The gender of the early adolescent should also be considered in any attempt to understand the possible differences in a family’s emotion socialization practices. For example, anger reactions are more tolerated in male than in female (Condrey & Ross, 1985). In addition, anger responses in female are more likely to be followed by negative emotional reactions from their mothers, whereas the anger responses of male receive more empathic maternal reactions (Malatesta & Haviland, 1982). According to Naghavi & Ma’rof the important point is that, today in the Iran, Iranian families have started to take on roles vastly different from family of previous generations. Moreover, family takes on ever more responsibility for raising their early adolescents than in the generations that preceded them (2012). Family of female anticipates more emotional management and the use of more sophisticated emotion regulation strategies than family’s male (Banerjee & Eggleston, 1993). These findings led us to expect that family’s female would report expressing more emotions than family’s male.

In addition, family’s female were accepted to express more positive emotion and sadness than those of male. Female are also socialized to be more aware to the sadness and hurt feelings of others compared to male (ZahnWaxler, Cole, & Barrett, 1991). According to Naghavi & Ma’rof (2011) Gender differences in EI can be glimpsed from infancy due to the differential teaching given to boys and girls. It is expected that this research would identify different between boys and girls have influences on early adolescents’ emotional intelligence. Although a body of relevant research literature is available, the findings of such research studies which investigated the effects of gender on early adolescents’ emotional intelligence were derived mainly from western-based samples that are socially and culturally different from the Iranian sample.

## 2. Materials and Methods

The purpose of this study was to examine the relationship between family functioning, alexithymia and sub-components of the early adolescent’s emotional intelligence among Iranian guidance schools students in Tehran, Iran. The schools were chosen based upon their location and programs of study. The population of research involved in this

study consisted of all the Iranian students who enrolled in guidance schools of Tehran (234 students, academic year 2010-2011).

The data were collected using (Schutte, 1998) Emotional Intelligence Scale for assessing early adolescence's emotional intelligence. To identify the difference between emotional intelligence and gender of early adolescents.

The emotional intelligence scales used to assess emotional intelligence, i.e. Schutte's Emotional Intelligence Self-measuring Scale (introduced by Schutte and her colleagues in 1998 and Mayer and Salovey's original emotional intelligence model, 1990), was used to measure emotional intelligence, which includes emotional conception and appraisal, emotion regulation and emotion utilization. This scale includes 33 self-report items. Some examples of the items included in the scale are:

- A. I can easily identify my emotions and feelings.
- B. I can persuade myself by imagining success in work.
- C. I admire others when they do something good.

The subject selected his/her degree of agreement or disagreement by any of these sentences in a five-point Likert scale, from strongly disagreed = 1 to strongly agreed = 5. In this study, the reliability for the emotional intelligence test was obtained by using Crombach's alpha,  $\alpha = 0.84$ .

Considering the question and research hypothesise, the following statistical method is used to analyze data: Descriptive statistics was provided to show the variation in the estimated means and standard deviations for each of the dependent and independent variables across the sample. After normality test, In this study, t-test was used for determine the difference between the mean score of emotional intelligence of the groups of boy and girl as the dependent variables.

### 3. Results and Discussion

#### Description of the participants

The study was among 7150 girls and boys Iranian students. After determining the sample gathering, 4 regions selected random among Tehran's 19 educational regions. Then, among the guidance schools of each region, 2 schools are selected by simple random method: one girls' school and one boys' school. In each school, pupils are selected from grade 3 and grade 2 by simple random method. The sample (234) consisted of the guidance schools pupils (12-15 years old). The respondents (234) for this study were the early adolescence with 116 boys and 118 girls.

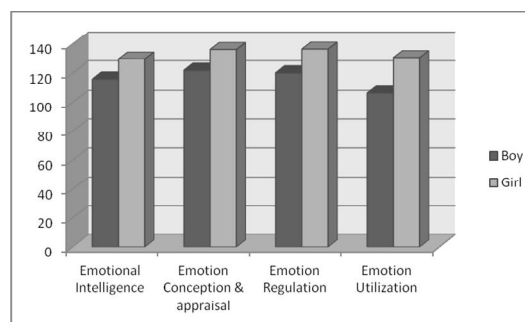
**Table 1: A summary of samples for early adolescents by demographic variables**

Demographic Variables	Frequency	Percentage (%)
Sample	234	100.00
Early Adolescent's		
Gender		
Male (boy)	116	49.6
Female (girl)	118	50.4
<b>Total</b>	<b>234</b>	<b>100</b>

**Table2: Descriptive information on emotional intelligence of the early adolescents**

Variables	Number	Mean	SD	Skewness	Kurtosis
Emotional intelligence	234	122.82	8.58	0.09	-0.03
Emotional conception & appraisal	234	121.84	9.96	0.09	-0.05
Emotional regulation	234	128.23	8.75	0.07	-0.03
Emotional utilization	234	118.39	7.71	0.10	-0.04

This research studied the effects of gender on the early adolescents' emotional intelligence. Hence, a descriptive analysis of early adolescents' emotional intelligence with respect to gender was obtained. Table 3 presents the descriptive information of the early adolescent's emotional intelligence, according to their gender.



**Figure 1. The mean value of the early adolescents' emotional intelligence and its subscales in terms of gender**

**Table 3. Descriptive information of the early adolescence's emotional intelligence and its factors in terms of gender**

Variables	Number		Mean		SD	
	Girl	Boy	Girl	Boy	Girl	Boy
Emotional intelligence	118	116	129.52	115.96	5.51	4.97
Emotion conception & appraisal	118	116	136.12	121.84	5.41	4.51
Emotion regulation	118	116	136.36	120.10	4.78	5.01
Emotion utilization	118	116	130.47	106.31	6.02	3.99

The mean value score of the early adolescents' emotional intelligence, with respect to gender (Table 3 and Figure 1) show a rank between the sub-scales of emotional intelligence for both girls and boys. This particular rank includes emotional intelligence, emotion conception and appraisal, emotion regulation and emotion utilization. Table 3 shows that the

average of the early adolescents' emotional intelligence and its factors are higher in girls than boys. In addition, the scores dispersion of emotional intelligence, emotion conception, and emotion utilization factors are also higher in girls than in boys.

In relation to gender, a comparison carried out for the mean of the two groups in this study (boys and girls, as presented in Table 4 ) indicated that the emotional intelligence of girls is higher than that of the boys. In addition, the t-test technique was also used to test the difference between emotional intelligence of the two groups, with boys (M=115.96, SD=4.97) and girls (M=129.52, SD=5.51), statistically. The statistical finding indicated that there was a noticeable difference between emotional intelligence of the boys and that of the girls ( $t=19.77$ ;  $p<0.01$ ). Table 4 presents the statistical information on early adolescents' emotional intelligence in terms of gender. Table 5 shows a summary of the t-test results for the early adolescents' emotional intelligence in terms of gender.

**Table 4: Descriptive and statistical information on the early adolescents' emotional intelligence in terms of gender**

Gender	N	Mean	SD	SE Mean
Girl	118	129.52	5.51	0.51
Boy	116	115.96	4.97	0.46

Table 4 indicates that the emotional intelligence of girls (M=129.52; SD=5.51), which is higher than the emotional intelligence of boys (M=115.96; SD=4.97). In addition, the findings of the previous study on the comparison between the emotional intelligence of the samples according to their gender have also indicated that girls are more aware of their feelings and those of others and can relate better interpersonally compared to boys. Nonetheless, boys seem to have stronger self regard and cope better with immediate problems of stressful nature than girls (Goleman, 1995, Stein, 2004).

**Table 5: A summary of the t-test results for early adolescents' emotional intelligence by gender**

		t-test for equality of means		Sig	Mean difference
EI	Equal variances assumed	T	Df		
	Equal variances assumed	19.77	232.00	0.000	13.57
	Equal variances not assumed	19.79	230.29	0.000	13.57

Note:  $P<0.01$

The result indicated that there is a significant difference in the early adolescents' emotional intelligence based on gender. In other words, girls have significantly higher emotional intelligence than

boys. It seems that further to individual differences of men and women, the expectations of the society and also the people around them, especially the parents, are different in terms of children's sexuality. Culturally, girls are mostly expected to be more expressive of feelings, whereas abstaining from feelings expression in boys is strengthened as a manly model. The fact that girls develop verbal skills earlier than boys means that they are more skilled at articulating their feelings and have greater expertise in the use of words. Hence, girls have more information about the emotional world and they therefore speak more about their emotional aspects and use emotional terms more often than boys (Brody & Hall, 1993; Fivush, 1991). In addition, the findings of the previous study on the comparison the emotional intelligence of the samples according to their gender also indicated that girls were more aware of their feelings and those of others and could therefore relate better interpersonally than boys (Stein, 2004). Meanwhile, Petrides and Furnham (2000) have demonstrated that boys self believed that they have higher emotional intelligence than girls. With respect to gender, however, the differences in the scores for emotional intelligence are still being developed (Schutte *et al.*, 1998) and the result of test of analysis of differences between emotional intelligence of boys and girls confirmed some findings of the previous study.

## Conclusion

Based on the findings of the current research, girls were found to have greater propensity for emotional intelligence (Goleman, 1995; Katyal & Awasthi, 2005). This study found a greater percentage of boys who scored in a trend of the lower category of emotional intelligence than girls. According to Salopake (1998), emotional intelligence is a learned ability and it tends to improve as people mature. So, regarding to this finding understanding the emotional intelligence levels of early adolescents, specifically among different genders may be useful to practitioners to identify areas for other steps of their life and for early adolescents' social activities that are crucial for their achievements in life. For this reason, gender education of mutual learning would be provided to help students learn how to appropriately deal with people.

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## Lag in optimal optical correction of urban elementary school students in Taiwan

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**Abstract:** The occurrence of the lag in optimal optical correction was investigated in three geographically different urban elementary schools in Taiwan. The results showed that among the schools in Tamsui (N=606), Taichung (N=702), and Tainan (N=586), Tainan had the highest proportion of students with correction (47.9% of all students), followed by Taichung (26.2%), and Tamsui (20.8%). Tamsui appeared to have the most lag in optimal correction: 55.3% needed further correction, yet only 20.8% had been corrected, or a lag of 34.5%. In contrast, the lag in Tainan and Taichung was 16.3% and 28.9%, respectively. Based on linear regression analysis, the lag or unmet needs could result in the worsening of myopia. To reduce the lag, follow up with the parents after student vision screening was supplemented with public health education or direct provision of any optical devices to the students.

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**Keywords:** vision screening, school myopia, spherical equivalent, lag in correction, optical correction

### 1. Introduction

Annual vision screening is a mandated physical exam for Grades 1 through 6 in all elementary schools in Taiwan. It is usually conducted in a basic mode, i.e., measurement of visual acuity, often supplemented with color vision test and body height and weight determinations. The screening is performed by school nurses and assisted by teachers. The results are then communicated directly to the parents with recommendation for further action, if warranted.

Indeed, the principal purpose of vision screening is for a timely correction of significant refractive error. It is not known, however, as to what extent this has been accomplished. There is now evidence from studies conducted in China of an unmet need for vision correction in both urban and rural areas [1-4]. The barriers included parental unawareness, neglect, and economical hardship. Even in low myopia prevalence areas, there is also an unmet need, and the rate is higher among urban than rural children [5-8], made worse by a long-held mistaken belief that spectacle-wear can further cause myopic progression [9].

To examine the extent of the lag, its prevalence, and the possible underlying factors, a survey of three geographically separate urban elementary schools in Taiwan was conducted, and the results reported here.

### 2. Material and Methods

#### 2.1 Methodology

Students from three urban schools from the north to the south of Taiwan were selected. The location of these schools and the number of participating students (N) were Tamsui: 25°00'40"N; 121°26'45"E, and N=606; Taichung: 24°8'35"N; 120°40'37"E, and N=702; and Tainan: 23°1'29"N; 120°10'5"E, and N=586. These schools belonged in the same educational system with similar didactic and physical education hours, and vacation times.

Prior to the start of the study, informed consent was obtained from the parents. The mode of current myopia correction, including spectacles, contact lenses, and night-wear orthokeratology lenses, was reported in a questionnaire, also completed by the parents. This information was further verified on site during screening.

Vision screening was performed and completed within 4-5 school days in each school with the methodology established previously [10,11]. Relevant to the present study, the following were conducted: (1) for preliminary testing, non-cycloplegic refraction with an autorefractor (Nikon, Speedy-1, Japan) recorded in the minus cylinder form; (2) lensometric determination of current spectacle correction; and (3) confirmation of current refractive error with (i) distance retinoscopy followed by (ii) subjective refraction - all in the minus cylinder form. These procedures were performed by 3rd and 4th year optometry students under the supervision of faculty members.

Myopia that needed correction was defined as having a refractive error of -1.00D or worse based on

the best correctable spherical equivalent (defined as the spherical correction plus  $\frac{1}{2}$  of the minus cylindrical correction) from the final subjective refraction. Previous refractive error at the time of screening was determined from current optical correction and a difference of equal to or worse than -1.00D spherical equivalent was regarded as that needing further, new correction.

## 2.2 Statistical analysis

Linear regression analysis of the unmet needs for correction and the best correctable spherical equivalent was performed with SPSS 18 package.

## 3. Results

### 3.1 Characteristics of current correction:

First, the three schools showed a disparity in the current modes of correction, with the school in Tainan having the highest use of optical correction in all three categories: spectacles, contact lenses, and orthokeratology lenses. Not surprisingly, spectacles remained the principal corrective device followed by orthokeratology lenses and conventional contact lenses. The results are shown in Table 1.

These results also represented the rate of optical correction before the vision screening. It appeared that the school in Tainan had the highest proportion of students with correction (47.9% of all students), followed by Taichung (26.2%), and Tamsui (20.8%).

Table1 : Modes of current myopia correction in the three elementary schools in Taiwan.

Location	Correction mode				Total
	None	Spectacles	Contact lens	Ortho-k lens	
Tamsui	N 480	122	0	4	606
	% 79.2	20.1	0	0.7	100
Taichung	N 518	179	2	3	702
	% 73.8	25.5	0.3	0.4	100
Tainan	N 305	252	6	23	586
	% 52.0	43.0	1.0	3.9	100
Total	N 1303	553	8	30	1894
	% 68.8	29.2	0.4	1.6	100

### 3.2 Unmet corrective needs:

After the screening and based on the -1.00D spherical equivalent threshold, students were divided into two categories: (1) those with no need for correction or with already adequate current correction; and (2) those who required correction or

new correction. The results are summarized in Table 2.

Table2 : Unmet needs for optical correction in the three schools in Taiwan.

Location	No further needs	Correction needed	Total
Tamsui	N 271	335	606
	% 44.7%	55.3%	100.0%
Taichung	N 315	387	702
	% 44.9%	55.1%	100.0%
Tainan	N 210	376	586
	% 35.8%	64.2%	100.0%
Total	N 796	1098	1894
	% 42.0%	58.0%	100.0%

The school in Tamsui appeared to have the most lag in optimal correction: 55.3% of students needed either correction or further correction, yet only 20.8% of them had been corrected (cf Table 1), or a 34.5% lag. A similar lag was also found in the other two schools; much less so in Tainan (64.2% needed further correction vs 47.9% already corrected, or a 16.3% lag) and slightly less, a 28.9% lag in Taichung (26.2% already corrected vs 55.1% still needed further correction).

### 3.1 Linear regression analysis

Furthermore, the needs for correction and additional correction could predict the increase in best correctable spherical equivalent, or worsening of myopia, of up to 47.2% (Tamsui), 43.9% (Taichung), and 47.7% (Tainan) (see Table 3, Adjusted  $R^2$ ):

Table3 : Linear regression of corrective needs and best correctable spherical equivalent.

Location	R	$R^2$	Adjusted $R^2$
Tamsui	0.687	0.473	0.472
Taichung	0.663	0.439	0.439
Tainan	0.691	0.477	0.477
Location	Standard error of estimation	F Change	Sig of F Change
Tamsui	1.17148	541.152	0.000
Taichung	1.42149	548.510	0.000
Tainan	1.45918	533.572	0.000

## 4. Discussion

These results suggest that the lag of optimal correction or the unmet need for correction in the three schools located in geographically different

areas in Taiwan (Tables 1 and 2) is representative of the widespread extent of this lag. In addition, it also raises the issue that the purpose of routine vision screening, i.e., for a timely correction, was, in fact, not achieved.

The goal of a full 100% correction rate at any given time is impossible to reach, owing to the nature of school myopia which progresses at different rates for individual students. Other factors may contribute to the lag, chief among them maybe economical concern and lack of understanding of myopization.

It is possible that since the least lag was observed in the more affluent school in Tainan, as evidenced by the high rate of orthokeratology correction, economic concern can be important. However, it does not seem that this is universally true, as the lag was also seen in other well-supported societies in different parts of the world [12-14].

More important, the parents must be educated to dispel the commonly accepted myths, some of which are, for example, that spectacle-wear further worsens myopia [9], that deliberate under-correction can retard the progression, and that, by extension, less wear time is beneficial for the control of myopia. Our analysis has shown otherwise; in fact the unmet needs may further cause an increase in myopia (Table 3), in support of previous other studies [15,16].

To reduce the lag, some measures can be readily implemented. For example, the screening results can and must be followed up, after informing the parents, to ensure the remedial action is, in fact, taken. The most effective way of maximally reducing the lag maybe a direct provision of spectacles and/or contact lenses to the students through a system of school-based vision care providers.

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## Prevention of shivering during regional anaesthesia: Comparison of Midazolam, Midazolam plus ketamine, Tramadol, and Tramadol plus Ketamine

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**Abstract:** Shivering is frequent during the post-anesthetic recovery period, and there is no clear consensus about the best strategy for its treatment. Post spinal shivering is very distressing for patients and may induce a variety of complications. In this prospective randomized, comparative, placebo controlled study, the efficacy of each of Midazolam, Midazolam plus ketamine, Tramadol, and Tramadol plus Ketamine for prophylaxis of post-spinal shivering was evaluated and compared to each other. One hundred ASA status I and II patients, who were undergoing elective orthopedic surgery under spinal anaesthesia, were included in the study. Patients randomly assigned to one of five groups; group C (n=20) received saline as a control, group M (n=20) received Midazolam 75 µg /kg, group MK (n=20) received Midazolam 37.5 µg/kg plus Ketamine 0.25 mg/kg, group T (n=20) received Tramadol 0.5mg/kg and group TK (n=20) received Tramadol 0.25mg/kg plus Ketamine 0.25mg/kg. All of these drugs were diluted to volume of 5 ml and was given as an I.V. bolus immediately after intrathecal injection. The incidences of shivering in groups C, M, MK, T and TK were 55%, 45%, 5%, 30% and 15% respectively (p-value was 0.003). The differences between group MK and groups C, M and T were statistically significant (p-value was <0.001, 0.004 and 0.046 respectively) while difference between group MK and group TK was not significant (p-value was 0.302). Group TK also showed a statistically significant lower incidence of shivering when compared to group M, but when compared with group T, the difference was not statistically significant. The incidence of shivering in group T was less than its incidence in groups C and M but this was not statistically significant. The difference between groups C and M was not statistically significant, so we concluded that I.V. midazolam plus Ketamine or Tramadol plus Ketamine is better than Midazolam or tramadol for prophylaxis of post spinal shivering. Midazolam plus Ketamine is superior to tramadol plus Ketamine.

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**Keywords:** N-Methyl- D-Aspartate (NMDA), SpO<sub>2</sub> Peripheral O<sub>2</sub> saturation, ICP intracranial pressure.

### 1. Introduction:

Post-anesthetic shivering is spontaneous, involuntary, rhythmic, oscillating, tremor-like muscle hyperactivity that increases metabolic heat production up to 600% after general or regional anesthesia (Ozaki et al; 1994).

Post anesthetic shivering may cause discomfort to patients, and aggravate wound pain by stretching incisions and increase intracranial and intraocular pressure. Shivering may increase tissue oxygen demand by as much as 500% and accompanied by increase in minute ventilation and cardiac output to maintain aerobic metabolism. This may be deleterious in patients with impaired cardiovascular reserve or a limited respiratory capacity. Shivering also may interfere with the monitoring of patients by causing artifacts of the ECG, blood pressure, and pulse oximetry (Honarmand A. and Safavi M. R., 2008).

Core temperature is maintained within a normal range during exposure to a cool environment because of sympathetically mediated

vasoconstriction. Regional anesthesia produces vasodilatation, which facilitates core-to-peripheral redistribution of heat and the cool periphery is warmed at the expense of the core compartment. Thus, hypothermia from epidural anesthesia results from redistribution of heat from the core to the periphery (Hynson, et al; 1991).

Various opioid and non opioid agents were used to prevent and treat shivering, but they are not without side effects like hemodynamic instability, respiratory depression, nausea and vomiting. Variety of physical agents (radiant heat, space blanket) were also used to prevent post anesthetic shivering, but those cumbersome and with limited success (Glosten B. et al; 1993). Tramadol which is a centrally acting analgesic drug with µ-opioid agonist has been found effective in the prevention and treatment of shivering with less side effects than other µ-opioid agonists (Mohta et al., 2009). Midazolam is one of the benzodiazepines. It was found that it may decrease the incidence of shivering. Ketamine which is a competitive N-Methyl- D-Aspartate (NMDA)

receptors antagonist, has been found to be effective in preventing and treating post anesthetic shivering via central effects or via its effect on the hemodynamics of the cardiovascular system (Pitoni et al;2011)

## 2-Patients and methods

After obtaining institutional approval and written consent from all patients, this prospective, randomized, comparative and placebo controlled study was carried out in Tanta University hospital from November 2009 to July 2010 on one hundred ASA status I and II, patients between the ages of 21-60 years who were undergoing elective orthopedic surgery under spinal anesthesia.

### Exclusion criteria:

Patients with thyroid diseases, cardiopulmonary diseases, neuromuscular diseases or psychological disorders were excluded from the study. Also, patients on narcotics, sedatives or any medication likely to alter thermoregulation. Patients with recent history of febrile illness and those with history of malignant hyperthermia.

Routine preoperative investigations including complete blood picture, renal function testes, liver function testes and coagulation profile.

**Anesthetic technique:** All patients did not receive any pre-medication. On arrival to the operating theatre, all patients had a venous cannula inserted. IV fluids in the form of lactated Ringer's solution were infused at a rate of 10 ml/Kg/h over 30 minutes before spinal anesthesia then the rate was reduced to 6 ml/Kg/h. fluids were not warmed. The ambient temperature was maintained at 22-24 °C.

All patients had spinal anesthesia where 15 mg hyperbaric Bupivacaine 0.5% was instituted at either L3/L4 or L4/L5 using a 22 G quince spinal needle under complete aseptic conditions.

**The patients were allocated randomly to one of five groups:**

**Group C** (n=20): Received saline as a control.

**Group M** (n=20): Received midazolam 75 µg/Kg.

**Group MK** (n=20): Received midazolam 37.5 µg/Kg plus Ketamine 0.25 mg/Kg.

**Group T** (n=20): Received Tramadol 0.5mg/Kg.

**Group TK** (n=20): Received Tramadol 0.25mg/Kg plus ketamine 0.25 mg/Kg.

All of these drugs were diluted to volume of 5 ml and was given as an bolus immediately after intrathecal injection. Supplemental Oxygen was given via a face mask at a rate of 5 L /min during the operation. All patients were covered with one layer of surgical drapes over the chest thighs and calves during the operation and one cotton blanket over the entire body after the operation. After intrathecal injection the sensory and motor block were assessed with pinprick test every 5 minutes. When spinal

anesthesia was established the presence of shivering was observed and graded by using a scale similar to that validated by Tsai and Chu where :

0= No shivering.

1=Piloerection or peripheral vasoconstriction but no visible shivering.

2= Muscular activity in only one muscle group.

3= Muscular activity in more than one muscle group but not generalized.

4= Shivering all over the body.

If shivering occurred, it was graded and recorded and if the grade was 3 or 4 after 15 min from the administration of the tested prophylactic drug, it was considered severe shivering and rescue treatment in the form of IV 25 mg of pethidine was given.

Heart rate, respiratory rate, mean arterial blood pressure, peripheral oxygen saturation (SpO<sub>2</sub>) and tympanic membrane temperature were recorded using standard non invasive monitors at 10 minutes intervals during the pre-and the postanesthesia period.

The degree of sedation was assessed according to a five-point scale where:

1=fully awake and oriented.

2=Drowsy.

3= Eye closed but responds to commands.

4=Eye closed but responds to mild physical stimulation.

5= Eye closed and not responding to mild physical stimulation.

Any other side effects was recorded and properly treated e.g. hypotension,nausea, vomiting and hallucination.

### Statistical analysis:

Statistical presentation and analysis of the present study was conducted, using SPSS statistics (V.17.0;SPSS Inc., Chicago,IL, USA ).

## 3-Results

This study was conducted after patients approval and consent on 100 patients presented for orthopedic surgery using spinal anesthesia. Part of the research was during anesthesia and surgery including clinical data such as the heart rate, mean arterial blood pressure, peripheral O<sub>2</sub> saturation, respiratory rate and core temperature.

The other part of the research was a trial to evaluate the prophylactic use of Midazolam ,Midazolam plus Ketamine ,Tramadol and Tramadol plus ketamine on the incidence of post spinal shivering ,where the patients were closely observed for detection of shivering and its grade. Also the

Patients were closely monitored for detection of any side effect.

Comparison of patients' demographic data showed that the differences among the five groups were not statistically significant as regard age, weight, BMI, sex, ASA status and duration of surgery (Table 1).

There was statistically insignificant differences among the five groups as regard the mean tympanic membrane temperature base value ( $p$ -value = 0.067) while there were statistically significant differences among five groups at all the time intervals; 10, 20, 30, 40, 50 and 60 of post-anesthesia period ( $p$ -value was <0.001, <0.001, <0.001, 0.001 and <0.001 respectively).

The change in the mean tympanic membrane temperature in group MK was statistically significant ( $P$ -value was <0.05) when compared with group C, group M and group T at all time intervals. However that change in temperature was not statistically significant ( $P$ -value was >0.05) when compared with group TK at any time.

The change in the mean tympanic membrane temperature in group TK was statistically significant ( $P$ -value was <0.05) when compared with group C and group M at all time intervals. However that change in temperature was statistically significant ( $p$ -value was <0.05) when compared with group T till 20 minutes of the post-anesthetic period, then at 30, 40, 50 and 60 minutes the change in temperature was statistically insignificant ( $P$ -value was 0.05).

The change in the mean tympanic membrane temperature in group T was not statistically significant when compared with group C and group M at any time of the post-anesthetic period ( $P$ -value was >0.05).

The change in the mean temperature in group M was not statistically significant ( $P$ -value >0.05) when compared with group C at any time. (Figure 1)

In our study MAP and heart rate values were not significantly different between the groups at any time of the post anesthesia period.

There was significant difference among the five groups as regard the incidence of shivering in the post-anesthesia period ( $p$ -value was 0.003). So, multiple 2x2 Fischers exact test were done to compare each to groups.

Group MK showed significant low incidence of shivering (5%) when compared with other groups, that incidence is less than that occurred in group TK (15%) but it was not statistically significant. Group TK also showed a statistically significant lower incidence of shivering when compared to group C and group M ( $P$ -value was

0.009 and 0.041 respectively), but when compared with group T, less incidence of shivering was not statistically significant ( $p$ -value was 0.225). The incidence of shivering was less in the group T than group C and group M but was not statistically significant ( $p$ -value was 0.100 and 0.257 respectively). There was no statistically significant difference between the group C and group M ( $P$ =0.376) (Table 2).

The number of patients suffered from each grade of shivering was compared: No statistically significant differences were found among the groups as regard the grade of shivering (Fig 2).

No patients showed severe shivering in group MK that was statistically significant when compared with group C where 6 patients (30%) suffered from severe shivering ( $P$ -value was 0.010). When comparing groups; group MK with group M (10%), group T (5%) and group TK (5%), no statistically significant differences were found ( $p$ -value was 0.243, 0.500 and 0.500 respectively). The incidence of severe shivering in group T was equal with that of group TK and low when compared with group C that was statistically significant ( $p$ -value was 0.045 for each group). The difference between group C and group M was not statistically significant in spite of the lower incidence in group M. Also the differences between group M and each of group T and group TK were not statistically significant (Fig 3).

Statistical analysis showed that no significant differences among the groups as regard the incidence of hypotension, hallucinations, nausea and vomiting ( $p$ -value was 0.0681, 0.240, 0.832 and 0.456 respectively) (Fig 4).

Also in this study the number of patients suffered from each grade of sedation were compared, the median sedation score was significantly higher in group M (3) than group C (1), group MK (2), group T (1) and group TK (1.5) where  $P$ -value was <0.001, 0.027, 0.001 and 0.001 respectively. Group MK showed statistically significant higher median sedation score than group C and group T ( $P$ -value was <0.001 and 0.009 respectively) but not statistically different when compared with group TK ( $p$ -value was 0.167).

No statistically significant difference was found between group T and group TK though the higher median sedation score in group TK as  $P$ -value was 0.153. (table 3)

#### 4-Discussion

Shivering is a frequent complication in the postoperative period. Shivering may occur as an adverse effect of surgery and anesthesia. It may be associated with an increase in oxygen consumption,

intraocular or intracranial pressure, and wound pain (Macintyre *et al.*, 1987 and Sessler *et al.*, 1994). Thus, both the prevention of shivering and the treatment of established shivering should be regarded as clinically relevant medical interventions in the perioperative period. The mechanism which leads to shivering after regional anaesthesia is not very clear, but the probable mechanisms could be decrease in core body temperature secondary to sympathetic block; peripheral vasodilatation; increased cutaneous blood flow, which leads to increased heat loss through skin; cold temperature of operation theatre; rapid infusion of cold IV fluids; and effect of cold anaesthetic drugs upon the thermo sensitive receptors in the spinal cord (Chaturvedi S *et al.*, 2002). The relative efficacy of pharmacological interventions to prevent this phenomenon is not well understood. Therefore, the present study was designed to detect the efficacy of each of midazolam, midazolam plus ketamine, tramadol and tramadol plus ketamine for prophylaxis of post spinal shivering.

In this study it was shown that I.V. midazolam (37.5 µg/Kg) plus ketamine (0.25mg/Kg) or tramadol (0.25mg/Kg) plus ketamine (0.25mg/Kg) is better than midazolam (75 µg/Kg) alone or tramadol (0.5 mg/Kg) alone for prophylaxis of post spinal shivering, whereas the midazolam plus ketamine combination is superior to tramadol plus ketamine combination as the former provides higher median sedation score. Kose *et al.* 2008 and Gecaj-Gashi A. *et al.* 2010 found that Ketamine 0.5-0.75 mg/kg is more rapid than meperidine (25 mg) for the reduction of postoperative shivering, but the side effect profile may limit its usefulness. Ketamine produces undesirable psychological reactions termed emergence reactions. The common manifestations are vivid dreaming, extracorporeal experiences (sense of floating out of body), hallucinations and illusions. However in the current study the incidence of hallucinations in patients receiving ketamine was very low (10% in group TK and 5% in group MK) that was not significant when compared to the control group. This can be explained by the use of low dose of ketamine in the present study (0.25 mg/kg). This supported by previous studies by Honarmand *et al.*, 2008 and Sagir *et al.*, 2007 where similar dose of ketamine was used with no incidence of hallucinations.

Tramadol activates the monoenergetic receptors of the descending neuraxial inhibiting pain pathway. The anti-shivering action of tramadol is probably mediated via its opioid or serotonergic and noradrenergic activity or both (Mathews *et al.*, 2002 and Bilotta *et al.*, 2002). Tramadol 2 mg/kg had the best combination of antishivering and analgesic efficacy without excessive sedation and thus

appeared to be a good choice to be administered at the time of wound closure to provide antishivering effect and analgesia without significant side effects in the postoperative period (Mohta M *et al.*, 2009). Tramadol is an opioid analgesic with opioid action preferably mediated via  $\mu$  (mu) receptor with minimal effect on kappa and delta binding sites.

Kurz, *et al.*, 1995 studied the effect of midazolam on thermoregulation and found that reduction in heat production after administration of midazolam is less than that after induction of anesthesia with clinical doses of volatile anesthetics, propofol, and opioids. Also, they reported that midazolam, even in plasma concentrations far exceeding those used routinely, produces minimal impairment of thermoregulatory control. This explains the lower incidence of shivering observed in our patients receiving midazolam. However, in another study by Grover *et al.*, 2002 they showed that administration of midazolam towards the end of the anesthetic procedure doesn't prevent shivering but it subsides earlier in the postoperative period. In the present study, post-spinal shivering occurred in 55% of patients of group C. In group M shivering occurred in 45% of patients which was lower when compared with group C. Also the incidence of severe shivering (score  $\geq 3$ ) was not significantly different in both groups. This incidence was consistent with the failure of midazolam to prevent or minimize the core hypothermia. The incidence of shivering in group T was 30% of patients which was significantly lower than that of the control group C. Adding small dose ketamine to midazolam or tramadol enhanced their anti-shivering effect, the incidence is lowered to 5% in group MK and 15% in group TK. The median sedation score was significantly higher in group M than group C, group MK, group T and group TK. Group MK showed statistically significant higher median sedation score than group C and group T but not statistically significant difference was found between group T and group TK. In this connection, the patients of group M and group MK have more preoperative comfort than other groups.

It is clear from the present study that adding Ketamine to midazolam or Tramadol; enhanced their anti-shivering effect. This suggests that Ketamine has a synergistic anti-shivering effect when combined with any of the two drugs. So, further studies are needed to find out the exact mechanism of interaction.

In conclusion: I.V. midazolam plus ketamine or tramadol plus ketamine is better than midazolam or tramadol for prophylaxis of post – spinal shivering, whereas the midazolam plus ketamine combination is superior to tramadol plus ketamine combination.

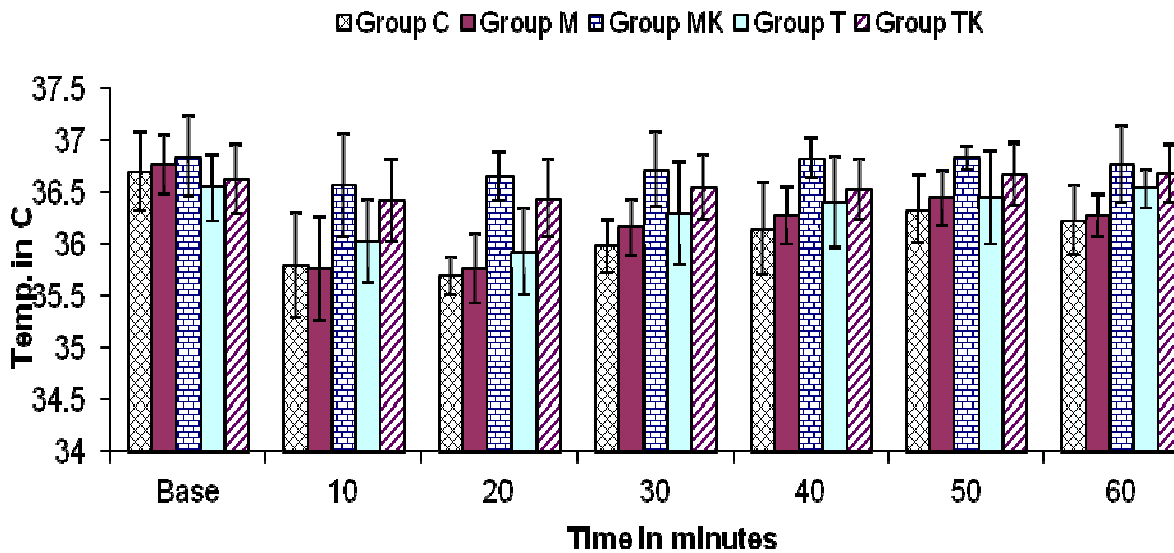
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**Table 1: patients' demographic data, ASA status and duration of surgery.**

	GROUP C	GROUP M	GROUP MK	GROUP T	GROUP TK	P-VALUE ANOVA
Age (years)	44.04±14.90	42.944±14.07	35.36±13.99	41.86±14.23	46.17±9.07	0.126
Weight (Kg)	76.53±13.72	75.66±11.36	4.75±10.66	73.94±13.13	76.94±12.86	0.951
BMI(Kg\m2)	29.64±4.28	28.61±7.46	28.89±5.73	27.67±6.70	30.28±5.84	0.710
Duration of surgery/min	94.09±12.58	95.12±10.47	86.39±10.57	87.95±11.15	89.72±14.31	0.096
Sex (m/f)	10/10	12/8	13/7	11/9	12/8	Chi-squared $\chi^2$ 0.899
ASA(I/II)	19/1	20/0	20/0	18/2	20/0	0.240

BMI = Body mass index



**Figure1: Changes of tympanic membrane temperature in the five groups**

**Table2: overall incidence shivering in the five groups.**

	GROUP C	GROUP M	GROUP MK	GROUP T	GROUP TK	Chi-square	
Shiverers	11 (55%)	9(45%)	1(5%)*	6(30%)	3(15%) ¥	$\chi^2$	P
Non-Shiverers	9 (54%)	11(55%)	19(95%)	14(70%)	17(85%)	16.190	0.003

Value are expressed as number of patients and percent (%)

\*Significant in comparison with group C, group M and group T (P-value <0.05).

¥ Significant in comparison with group C, group M (P-value <0.05).

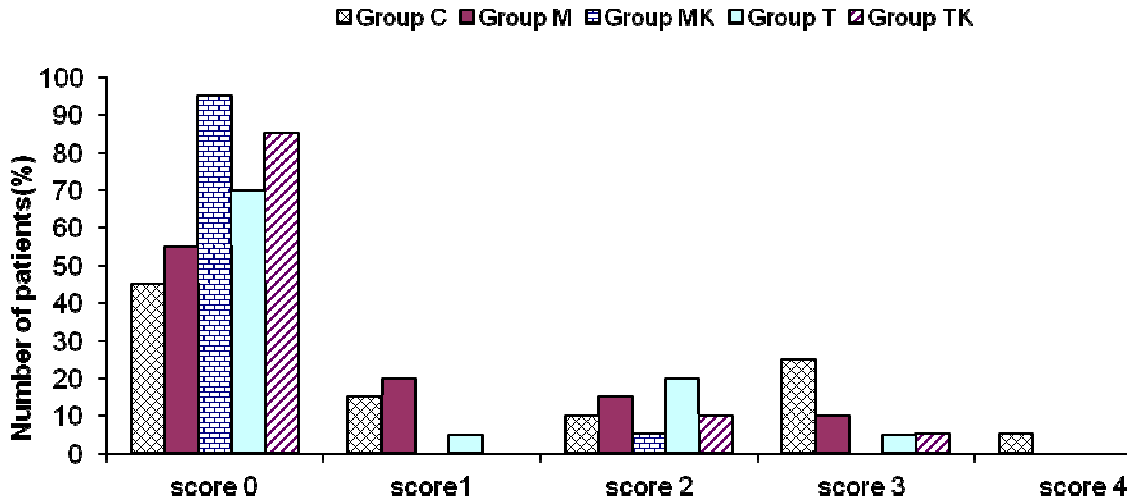


Figure2: Shivering score of all patients in all five groups

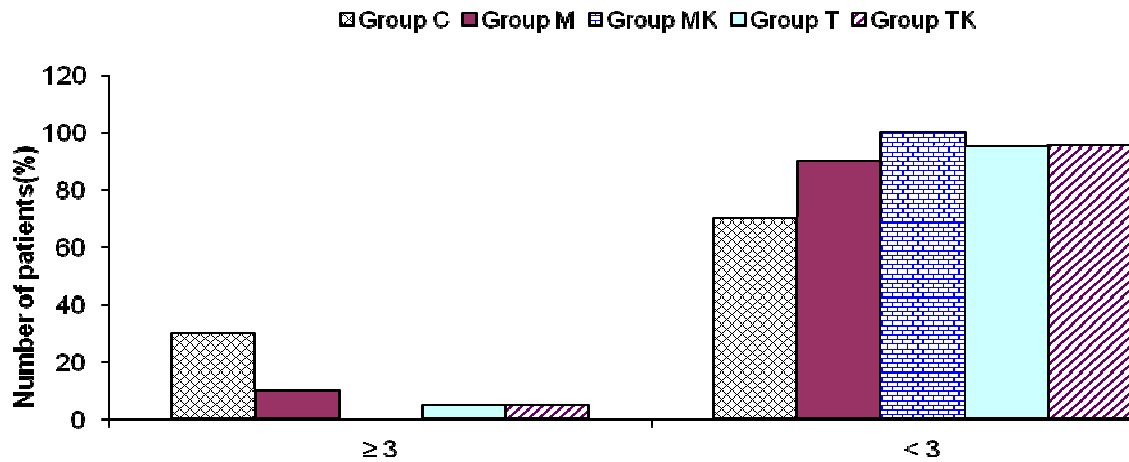


Fig 3: Incidence of severe shivering (score ≥ 3) in the five groups. Values are expressed as number of patients (%)

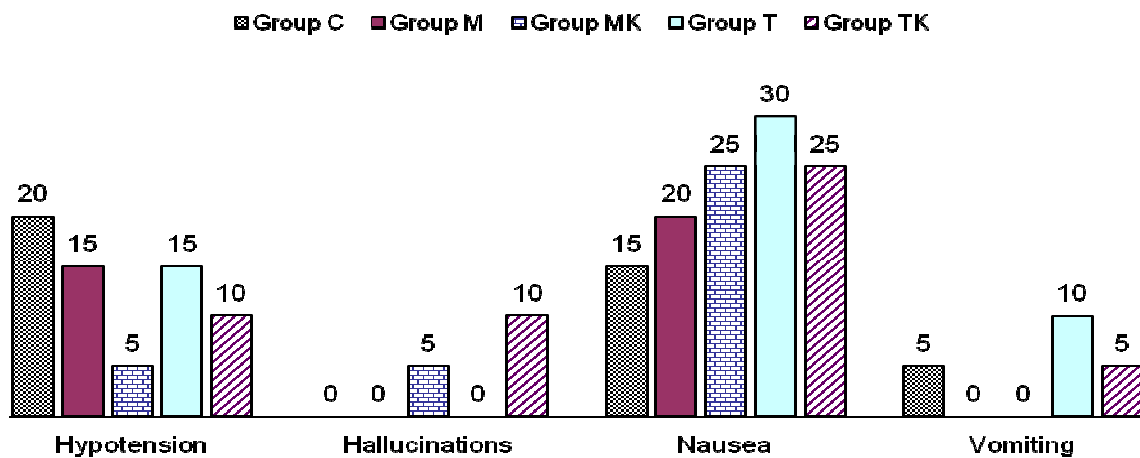


Figure 4: Incidence of complications in the five groups, values are expressed as number of patients (%)

**Table 3: Sedation score of all patients in the five groups**

Sedation score	GROUP C N=20	GROUP M N=20	GROUP MK N=20	GROUP T N=20	GROUP TK N=20	Chi-square	
						$\chi^2$	P
1	20 (100%)	3 (15%)	7(35%)	14(70%)	10(50%)	34.380	<0.001
2	0	4(20%)	7(35%)	6(30%)	8(40%)	10.667	0.031
3	0	9(45%)	5(25%)	0	2(10%)	21.875	<0.001
4	0	4(20%)	1(5%)	0	0	12.632	0.013
5	0	0	0	0	0	Not tested	
Median (range)	1(1-1)	3(2-3)*	2(1-3) ¥	1(1-2)	1.5(1-2)		

Value are expressed as number of patients and percent (%)

\*statistically significant when compared with other groups (P-value <0.05).

¥ Statistically significant when compared with group C and group T (P-value <0.05).



## Feeling of Depression and loneliness among Elderly people Attending Geriatric Clubs at Assiut City

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**Abstract:** People over the age of sixty-five are a national problem that requires more attention and research because the suicide rate is more prominent in this age group than in any other. The study **aimed** to assess feeling of depression and loneliness in elderly persons and identify the relation between the old age and feeling of depression and loneliness. Interviews were conducted individually with elderly people Convenience **sample of the study** were 50 elderly persons aged 60 years and above from Geriatric club in Legitimate Assembly and Geriatric Club at Assiut cultural center. **Tools** were used in this study UCLA Loneliness Scale (Version 3), It will be used to assess the feeling of loneliness among elderly people, and Beck Depression Inventory Scale. **The results** revealed that there were statistically significant difference between depression and age. There were statistically significant highly correlation between depression and loneliness. **Conclusion**, depression and loneliness significantly increase with age.

[Nadia Ebraheem Sayied; Hussnia Shehata Mohamed and Reda Abd El Aal Thabet. **Feeling of Depression and loneliness among Elderly people Attending Geriatric Clubs at Assiut City**. Life Science Journal 2012; 9(2):140-145]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 24

**Key Words:** Depression, Loneliness, Elderly

### 1. Introduction:

Many people experience loneliness and depression in old age, either as a result of living alone or due to lack of close family ties and reduced connections with their culture of origin, which results in an inability to actively participate in the community activities. With advancing age, it is inevitable that people lose connection with their friendship networks and that they find it more difficult to initiate new friendships and to belong to new networks. (Singh, and Misra, 2009).

Depression is the most common mood disorder in later life. It may be associated with serious consequences, including; disability, functional decline, diminished quality of life, increased mortality and increased service utilization. Moreover it is undiagnosed in about 50% of cases (Charney et al, 2003). World health organization (WHO) considered that the age of 65 is the beginning of aging, but in Egypt, the age of 60 is still considered the beginning of aging according to the retirement age for most of people (Sheriff, 2000).

World Health Organization is predicting that by the year 2020, depression will become the second leading cause of disability, so it is considered as a major public health problem (Finley et al, 2002 & Ustun et al, 2004). The risk of depression in the elderly increases with other illnesses and when ability to function becomes limited. Estimates of major depression in older people living in the community range from less than 1 percent to about 5 percent, but rises to 13.5 percent in those who require home

healthcare and to 11.5 percent in elderly hospital patients – Hybels and Blazer (2003).

Depression in the elderly is a widespread problem that is often not diagnosed and frequently under treated. Many older people will not admit to the signs and symptoms of depression, for fear that they will be seen as weak or crazy, **Screening for Depression Recommendations and Rationale (2002)**. A study by Max *et al.* (2005) revealed that the presence of perceived loneliness contributed strongly to the effect of depression on mortality. Thus, in the oldest old, depression is associated with mortality only when feelings of loneliness are present. Depression is a problem that often accompanies loneliness. In many cases, depressive symptoms such as withdrawal, anxiety, lack of motivation and sadness mimic and mask the symptoms of loneliness.

### Significance of the Study:

One of the main features of the Egyptian population over the last few decades is the gradual increase in the absolute and relative numbers of older people. The percent of older people, defined as 60 years of age and more, was 6.1% of the total population in 1996 and reached 7.2% in 2006 according to the last Egyptian census. The expected percentage of older people may reach 8.9% in 2016 and 10.9% in 2026. About 79%. These demographic trends are important for the future patterns of health care and disease. This reflects the importance of providing health care for older people in Egypt, **Egypt Demographic & Health Survey (2005, 2008)**

**Aim of the study**

- 1- To assess feeling of depression and loneliness in elderly persons.
- 2- Identify the relation between the old age and feeling of depression and loneliness.

**2. Subjects and methods:**

**Research Design:** A descriptive research design was used in carrying out this study.

**Research setting:**

The study was carried out at two setting which affiliated in the West Assiut City (geriatric club in legitimate assembly) and East Assiut City (geriatric club in Cultural Center). Participation of Geriatric club in Legitimate assembly was yearly and the elderly paid 40 pounds every year, also the participation the same in club of cultural center but the elderly paid 10 pounds every year.

The elderly participate in both clubs, these clubs provide many services for elderly such as, internal and external trips, birth day party, and library for reading books (cultural and religious books), and also there is a religious guider. There is visitant physician in geriatric club of legitimate assembly for follow up of elderly with diabetes and hypertension, the physician come to club every Saturday and Tuesday for measuring blood pressure and sugar level in blood

**Sample**

Convenient sample of elderly people attending to the previous setting were included in this study, their number were 50 elderly person aged 60years and above. All of them from urban area .

**Tools of data collection:**

**1- Sociodemographic data questionnaire:** -Which include, elderly name, age, sex, marital status, level of education, occupation, and living with whom, residence.

**2- UCLA loneliness scale:** - It will be used to assess the feeling of loneliness among elderly people. UCLA Loneliness Scale (Version 3). It Developed by **Russell (1996)**. It consists of twenty items which assess feeling of loneliness in elderly people. The subjects responds on a 4-point (1=indicate never, 2=rarely, 3=sometimes, 4=often). The scoring system of this scale as the following from 15 and 20 are considered a normal experience of loneliness. Scores above 30 indicate a person is experiencing severe loneliness. 9 items are revised scoring. This scale was translated into Arabic language. Both the Arabic and English items were submitted to five experts from the English section, Faculty of Art, Assiut University to be reviewed for its translation. A jury of five experts in the psychiatric field examined the content validity, And tested for its validity.

**3- Beck Depression Inventory Scale (BDI) (1961):**

Arabic modified version by Ghareeb, (1990). It consists of 13 items. It will be used to assess the feeling of depression. Each item is containing four statements ranked in order of severity and measured on a likert scale of four points (0-3). The scoring system ranged from 0-9 not depressed, 10-15 mild depressed, 16-24 moderate depression, 25-39 severe depression.

**Methods of data collection****1. Preparatory phase and administrative design**

An official approval was obtained from the Dean of faculty of nursing, Assiut University to the director of geriatric club in legitimate assembly and director of geriatric club in cultural center to obtain their vital assistance and necessary approval to conduct the study. This letter includes permission to carryout the study and explains the purpose and nature of the study

**Ethical consideration**

The purpose of this study was explained for every interviewed individual that includes directors of the studied clubs, elderly men and women.

The elderly has ethical right to agree or refuse participation in the study; consent to participate in the study was secured orally and informed that the information obtained will be confidential and used only for the purpose of the study.

**2- Implementation phase:-**

After getting official approval for performing the study from pertinent authorities, the process of data collection was started. Interviews were conducted individually with elderly people. Data collection was done from 4.00 p M to 6.00 P M, two days / week each interview took about 30 minute. Data collection took about three months from the beginning of May 2011 to July 2011.

**Pilot study**

A pilot study was conducted at the beginning of the study. It included 10% of the total sample to investigate the feasibility of data collection tools and their clarity. Subjects included in the pilot study were excluded from the total studied sample.

**3. Results**

Table (1) shows that, sociodemographic characteristics of the studied group, as regards elderly age 62 % of the studied group aged less than 70 years, according to elderly sex 80%of them were females and 20%were males. according to level of education about 42% of the studied group were secondary level of education, in relation to occupation 60% of them were retired, according to

living with whom about 68 % of them living with first degree relatives, while 22% of them living alone and 10% of them living with second degree relatives.

Table (2) shows that, there were statistically significant difference between loneliness and age (P value = 0.030), as well as loneliness and level of education (P value = 0.033).

Table (3) illustrates that, there were statistically significant difference between depression and age (P value = 0.024), as well as depression and level of education (P value = 0.013) of the studied group.

Figure (1) shows that, about 72% of studied group had severe loneliness, while 26% of them had moderate loneliness.

Figure (2) indicates that, there were 60% of the studied group didn't have depression; on the other hand 26% of them had moderate depression

Figure (3) shows that there were statistically significant highly correlation between depression and loneliness of the studied group ( $r=0.709$ ,  $p=0.000$ ).

Figure (4) shows that there were statistically significant correlation between age and depression of the studied group ( $r=0.334$ ,  $p=0.018$ ).

Figure (5) indicates that there were statistically significant correlation between age and loneliness of the studied group ( $r=0.319$ ,  $p=0.024$ ).

**Table (1): Sociodemographic characteristics of studied group (n=50)**

	No. (n= 50)	%
<b>Age: (years)</b>		
< 70	31	62.0
≥ 70	19	38.0
Mean ± SD (Range)	68.2 ± 6.8 (60 – 85)	
<b>Sex</b>		
<b>Male</b>	10	20%
<b>Female</b>	40	80%
<b>Level of education:</b>		
Illiterate	12	24.0
Basic education	9	18.0
Secondary	21	42.0
University	8	16.0
<b>Occupation:</b>		
Retired	30	60.0
House wife	20	40.0
<b>Person who living with:</b>		
First degree relatives	34	68.0
Second degree relatives	5	10.0
Alone	11	22.0

**Table (2): Relation between loneliness and sociodemographic characteristics of studied group (n=50)**

	Mean ± SD	Range	Test	P-value
<b>Age: (years)</b>			T	
< 70	38.0 ± 11.8	19 – 57	5.02	0.030*
	47.1 ± 16.8	23 – 74		
			F	
≥ 70	46.5 ± 16.6	22 – 74	3.74	0.017*•
<b>Level of education:</b>	48.8 ± 11.4	31 – 66		
Secondary	40.0 ± 12.8	21 – 64		
University	29.4 ± 10.9	19 – 53		
<b>Occupation:</b>			T	
Retired	40.0 ± 14.1	19 – 68	0.74	0.393
House wife	43.6 ± 15.0	21 – 74		
<b>Person who living with:</b>			F	
First degree relatives	39.1 ± 13.7	19 – 68	0.52	0.230•
First degree relatives	48.0 ± 19.2	27 – 74		
Alone	45.8 ± 13.8	21 – 63		

Independent samples t-test

• ANOVA test

\* Statistical significant difference ( $P < 0.05$ )

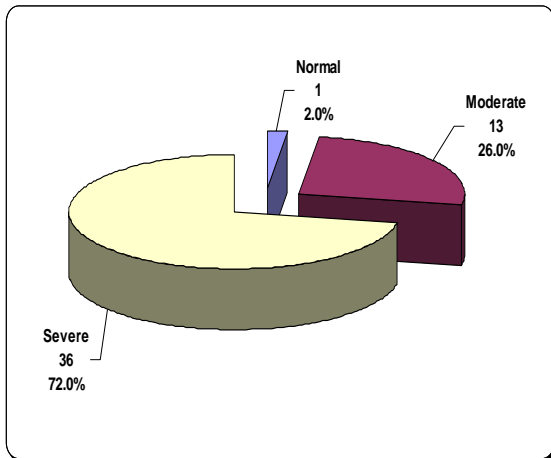
**Table (3): Relation between depression and sociodemographic Characteristics of studied group (n=50)**

	Mean ± SD	Range	Test	P-value
<b>Age: (years)</b>			T	
< 70	8.1 ± 5.5	0 – 21	5.41	0.024*
≥ 70	12.5 ± 7.9	1 – 21		
<b>Level of education:</b>			F	
Illiterate	12.8 ± 7.4	3 – 22	3.73	0.018*•
Basic education	12.8 ± 5.9	7 – 22		
Secondary	8.7 ± 6.7	1 – 21		
University	4.5 ± 2.3	0 – 7		
<b>Occupation:</b>			T	
Retired	9.3 ± 6.9	0 – 22	0.36	0.552
House wife	10.5 ± 6.7	1 – 22		
<b>Person who living with:</b>			F	
First degree relatives	9.5 ± 6.9	0 – 22	0.43	0.655•
First degree relatives	12.4 ± 7.8	4 – 21		
Alone	9.2 ± 6.3	1 – 21		

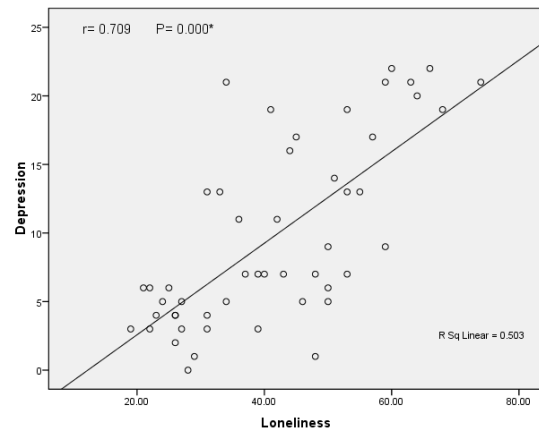
Independent samples t-test

• ANOVA test

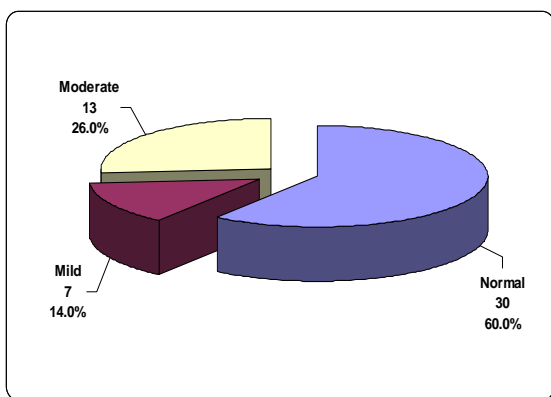
Statistical significant difference (P < 0.05)



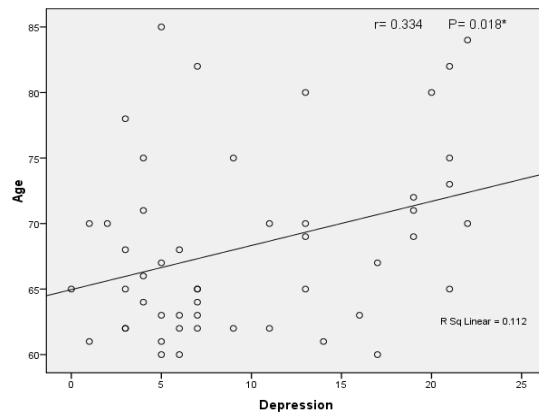
**Figure (1) Distribution of the studied group according to loneliness (n = 50)**



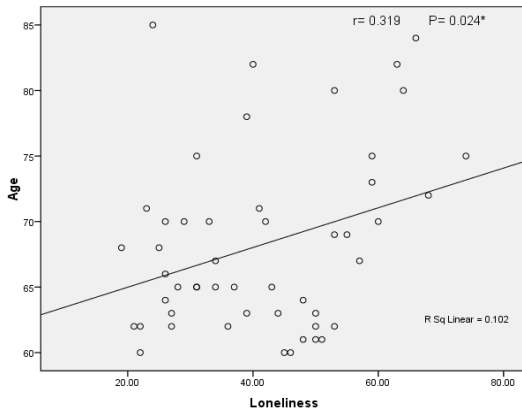
**Figure (3) Correlation between depression and loneliness of the studied group (n=50)**



**Figure (2) Distribution of the studied group according to depression (n = 50)**



**Figure (4) Correlation between age and depression of the Studied group (n=50)**



**Figure(5): Correlation between age and loneliness of the Studied group (n =50)**

#### 4. Discussion

Depression is a common and disabling psychiatric disorder in later life. It is well known that depression increases mortality and has a negative impact on the well-being and daily functioning of the elderly **Beekman et al., (2002)**, **Rovner et al., (1993)**; **Wells et al., (1989)**. In particular, institutionalized elderly people seem to be at increased risk of developing depressive symptoms due to frequently occurring chronic physical illness, a factor that is closely related to depression in old age (**Godlove et al., 2000**; **Gurland et al., 1979**; **Henderson et al., 1993**; **Parmelee et al., 1992**; **Stek et al., 2003**).

In the present study about two thirds of the studied group aged less than 70 years. This finding is supported with **Singh, et al., (2009)**, who stated that prevalence of depressive symptoms increase with age 67years. Also **Segal, et al (2007)** reported that, according to the National Institutes of Health, of the 35million American aged 65or older, about2 million of them suffer from full blown depression. Another 5 million suffer from sever form of the illness.

As regard level of education and occupation about 42% and two thirds of the studied sample were secondary level of education and retired. This study agree with, **Asnani, etal, (2010)** who stated that, Depression was significantly associated with unemployment, ( $p < 0.001$ ), whereas unemployment ( $p: 0.002$ ), and lower educational attainment were significantly associated with loneliness. Also this may be attributed to the fact that all the elderly belonged to the working group were employed in government jobs before retirement.

As regard living with whom about two thirds of them living with first degree relative and one quarter of them living alone these finding agree with, **Singh and Misra (2009)**, most of the elderly were staying with their children and grandchildren, which did not allow them to stay lonely for long time.

**Jongenelis, (2003)**, illustrated that, factors associated with depression, the following risk indicators were found: age below 80 years, lack of social support, and loneliness. This result consistent with the present study that indicates there was significant difference between loneliness and depression and elderly age, education and social support. On the other hand, this disagrees with **Kaasa, (1998)** who stated that there is no significant difference in the percentage of lonely people in the various age groups over 80 years. Also **Park, (2009)** ,reported that People can experience loneliness for many reasons and many life events are associated with it, like the lack of friendship relations during childhood and adolescence, or the physical absence of meaningful people around a person are a few causes for loneliness .

The present study revealed that about 72% of the studied group had severe loneliness. This is consistent with **Singh, and Misra., (2009)** who stated that, despite the elderly being sociable, they experienced increased feelings of loneliness. Possible explanation for this may be that feeling lonely not only depends on the number of connections one has with others but also whether or not one is satisfied with his life style. An expressed dissatisfaction with available relationships is a more powerful indicator of loneliness. This may be due to the loss of a significant person in one's life or as a result of dysfunction of communication .

In the current study about 60% didn't have depression and 26% of the studied group moderate, while 14% of them had severe depression. This finding is similar to that reported by, **Abdo, et al,( 2011)**, Mild to moderate depression was observed in 75.6% of the depressed group, while 24.4% of them had severe depression. Also (**Shin et al., 2008**) who reported that the percentage of mild to moderate depression among their study subjects was 76.3% and about 23.7% of them had severe depression .

**Blazer, (2000)** stated that, depression in old age was found to be strongly associated with feelings of loneliness .This results agree with the present study that revealed that, highly correlation between loneliness and depression .Also **Max et al (2005)**, reported that depression with feelings of loneliness differs from depression without feelings of loneliness. Depression with feelings of loneliness leads to more pronounced motivational depletion and serious consequences, including social isolation, reduced self-care, decreased mobility and poor diet. In this respect, **Chou & Chi (2004)** stated that loneliness has been identified as a risk factor for depressive symptoms in cross-sectional and longitudinal studies of older adults.

**Conclusion** It can be concluded from this study by there was statistically significant highly correlation between depression and loneliness as well as

depression and loneliness significantly increase with age.

**Recommendations**, Based on the results of this study, the following can be recommended that providing psychiatric nurse in these clubs to encourage elderly people to ventilate or express feeling. Training to geriatric clubs personal about how to deal with the elderly and help them to express their feeling.

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## Definitive Radiation Therapy for Early Glottic Carcinoma

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**Abstract: Background:** Definitive radiation therapy (RT) for early laryngeal glottic carcinoma offers an excellent probability of cure and it is associated with persistent disease or tumor recurrence in a minority of patients. The objective of treatment controlling the malignant tumor and preserving a functionally useful voice is best optimized by the use of radiotherapy in early stages. Although surgery can offer equal good results, it is best reserved in the event of radiation failure. **Aim of the work:** The aim of the present retrospective study is to identify the treatment outcomes and different prognostic variables affecting early (T1N0M0 and T2N0M0) laryngeal glottic squamous cell carcinoma (SCC) treated with definitive radiation therapy. **Methods:** The medical records of 81 patients with T1N0M0 and T2N0M0 glottic invasive SCC treated with definitive radiation therapy throughout the period from January, 2002 to September, 2010, at Clinical Oncology Department, Tanta University Hospital, were reviewed. All patients had at least 12 months of follow-up. These patients were evaluated for response to RT, local control, disease specific survival (DSS) rates and treatment toxicity. The different prognostic factors affected the local control and DSS rates were also statistically analyzed. **Results:** The median follow-up period was 45.9 months. Eleven patients (13.58%) received <65 Gy as a total irradiation dose and 86.42% received > 65 Gy. Complete disappearance of the laryngeal glottic lesion (CR) was achieved in 91.36% of patients. Five-year local control rates were 89.1% in T1 and 64.96% in T2 tumor stage. Multivariate analysis revealed that anterior commissure involvement ( $P=0.048$ ) and overall treatment time ( $P=0.002$ ) are adversely affected local control rates with statistical significance. Seven (9.46%) patients failed locally and 2 (2.7%) had developed distant failure. Five-year DSS rates were as follows: T1, 89.81% and T2, 70.76%. Severe early radiation reaction involving skin, larynx & pharynx were rare (1.2%, 3.7% and 6.2%, respectively), and severe late reactions was recorded in 1.2% of patients. **Conclusion:** Definitive RT cures a high proportion of patients with T1N0M0 and T2N0M0 glottic SCC and has a low rate of severe complications. The anterior commissure involvement and overall treatment time were adversely affected local control rate. [Alaa Maria; Mohamed El-Shebiny and Omnia Abd El-Fattah. **Definitive Radiation Therapy for Early Glottic Carcinoma.** Life Science Journal 2012; 9(2):146-153]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 25

**Key words:** Radiation therapy, larynx, early glottic carcinoma.

### 1. Introduction:

The treatment of choice for early glottic cancer (T1N0 and T2N0) is controversial. Radiotherapy produces an excellent cure rate and results in near-normal to normal voice restoration. Voice quality achieved with RT is generally considered superior to that obtained with cordectomy or hemilaryngectomy. Transoral laser excision (TLE) produces excellent cure rates and post-treatment voice quality comparable to RT in selected patients<sup>(1)</sup>.

The local control rate with definitive RT treatment was reported to be in the range of 82% to 94% for T1<sup>(2-5)</sup> and 65% to 80% for T2 disease<sup>(2, 4, 6)</sup>. However, 5% to 40% of the patients will need salvage surgery (total or partial laryngectomy) for persistent or recurrent disease<sup>(7-9)</sup>.

The purpose of our study is to assess the treatment outcomes of definitive RT for early-stage SCC of the glottic larynx, with local control rate is considered endpoint in this study. Radiotherapy results have been analyzed according to the different clinical, pathologic, and therapeutic factors, aiming at

a more precise definition of the factors predicting for better treatment results.

### 2. Patients and Methods

Between January, 2002 and September, 2010, a total of 81 previously untreated patients with T1N0M0 and T2N0M0 biopsy-proven invasive SCC of the laryngeal glottis were managed by definitive radiation therapy at the Clinical Oncology Department, Tanta University Hospital. Those patients were evaluated for response to RT, local control and DSS rates. Patients' data were collected from their treatment and follow up sheets.

All patients had follow up evaluation after treatment on a monthly base in the first year, every two months in the second year and thereafter every six months with median follow-up 45.9 months (7-96 months). Patients were clinically staged according to the 2010 American Joint Committee on Cancer (AJCC)<sup>(10)</sup>.

All patients were treated with megavoltage photon beam (Cobalt-60) in supine position using 2 lateral opposed wedge fields and the neck was

immobilized using neck pads and head thermoplastic mask.

Patients were treated to total doses ranging from 61 to 70 Gy at 1.8 to 2.25 Gy per fraction once daily, five fractions per week, over 39 to 80 days (median, 53 days). The main reason for protraction of overall treatment time was time gaps because of machine failure and/or acute toxicities necessitating treatment interruption. Twenty (24.69%) patients of the studied population had a treatment gap time > 8 days.

The treatment target was the entire larynx with limited safety margins and sparing the spinal cord from the start of radiation course. The field sizes in 68 patients was ranged from 6 x 6 cm to 7 x 7 cm and was more extended in 13 patients who received elective neck node irradiation to the internal jugular lymph nodes, depending on the discretion of the attending physician.

Typical field borders for T1 extended superiorly from the top of the thyroid notch, inferiorly to the lower border of the cricoid cartilage, with 1 to 1.5 cm falloff anteriorly. Posteriorly the field stops at the anterior edge of cervical vertebral bodies (prevertebral fascia). Frequently the collimator is rotated slightly so that the field of irradiation is parallel to slope of the neck. Both lateral fields were treated daily. No elective neck node irradiation was given.

Fields were enlarged for T2 cancers depending on the extent of the tumor. The fields were not routinely reduced in size at any point during the treatment. For patients who received elective nodal irradiation the upper border was 2 cm above the angle of the mandible.

The treatment volume was identified by the use of simulator. The dose was specified to an isodose line that covered the lesion and was 95% of the maximum dose. The dose was calculated in the mid-plane point on the central axis of the beam using 2D treatment planning system.

Treatment response was evaluated by indirect laryngoscopy. Persistent disease was considered by finding of tumor within two months after completion of radiation treatment course. Local control or complete response defined as freedom of persistent disease, while local recurrence was defined by finding malignancy in the larynx or regional lymph nodes "radiological and/or biopsy-proven" after complete response to therapy. Salvage for persistent or recurrent disease was consisted with partial or total laryngectomy.

Acute complications during RT were defined as severe if an unplanned treatment break was necessary. Late complications were defined as severe if they necessitated a surgical procedure such as

placement of a gastrostomy or tracheostomy tube, or if the complication resulted in death. Acute and late toxicities are graded according to toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC).<sup>(11)</sup>

#### **Statistical Analysis**

The Kaplan-Meier method provided estimates of local control and disease specific survival<sup>(12)</sup>. Local control was calculated as the time from the pathologic diagnosis to development of failure in the larynx or regional lymph nodes or to the last date of follow-up. Disease specific survival was defined as the time from pathologic diagnosis until either death or last follow up for patients without events. Patients were censored if they died with no evidence of cancer at the time of death.

The log-rank test was used for univariate comparison between patient's, tumor and treatment parameters including age, gender, smoking, stage, anterior commissure involvement, grade, overall treatment time and radiation dose. Cox regression model was used for multivariate analysis<sup>(13)</sup>. Statistical analysis was performed using Statistical Package for Social Sciences software (SPSS v-12). Significance was prespecified as  $P < 0.05$ .

### **3. Results**

Patients' age ranged from 36 to 79 years (median, 58.6 years). The frequency of the laryngeal glottic SCC was greatly higher in males than females (male to female ratio was 9:1). Also it was significantly higher in smokers versus non-smokers (70.37% and 29.63%). Seventy percent of the patients had T1 disease stage and 29.63% of patients had T2 disease stage. Anterior commissure involvement of the vocal cord was encountered in 34.57% of the whole patients. Most of patients 64.20% had well and moderately differentiated SCC. The patients, tumor and treatment characteristics are listed in Table 1.

#### **Local control rate**

With definitive RT, complete disappearance of the laryngeal glottic lesion was achieved in 91.36% (74 patients). The remaining 7 patients had residual tumor and were referred to be salvaged surgically. Three patients underwent successful total laryngectomy, while the other 4 patients were not operated. Out of 74 patients who had achieved complete response, 7 patients developed local recurrence with a median time of the local recurrence was 14.1 months (10-21 months). They underwent salvage surgery (two partial laryngectomy and five total laryngectomy), later on three of them had developed regional neck failure.



**Table (1): Patients, tumor & treatment characteristics**

	Patients (n=81)	%
<b>Age</b>		
< 60	39	48.15%
≥60	42	51.85%
<b>Gender</b>		
Male	73	90%
Female	8	10%
<b>Smoking</b>		
Yes	57	70.37%
No	24	29.63%
<b>Stage</b>		
T1	57	70.37%
T2	24	29.63%
<b>AC involvement</b>		
Yes	28	34.57%
No	53	65.43%
<b>Tumor Grade</b>		
G I	19	23.46%
G II	33	40.74%
G III	29	35.80%
<b>Total dose</b>		
<65 Gy	11	13.58%
>65 Gy	70	86.42%
<b>Dose/fraction</b>		
1.8 Gy	11	13.58%
2 Gy	61	75.31%
2.25 Gy	9	11.11%
<b>Overall treatment time</b>		
≤53 days	55	67.9%
>53 days	26	32.1%
AC: Anterior Commissars		

#### **Local tumor control by different prognostic variables**

Univariate analysis of factors affecting local control revealed that patients age ( $P=0.026$ ), disease stage ( $P=0.012$ ), anterior commissure invasion ( $P=0.0001$ ), pathologic differentiation ( $P=0.003$ ) and overall treatment time ( $P<0.0001$ ) significantly impacted this end point. Multivariate analysis demonstrated that anterior commissure involvement ( $P=0.048$ ) and overall treatment time ( $P=0.002$ ) are significantly influenced this end point (Table 2). The 5-year local control rate for the whole patients after RT was 82% (Figure 1).

Distant failure occurred in 2 patients (2.47%), the first one developed brain metastasis 55 months after therapy and received palliative cranial irradiation and the second patient developed pulmonary metastasis 58 months after therapy and treated with chemotherapy.

#### **Disease specific survival by different prognostic variables**

Univariate analysis of factors affecting the DSS rate revealed that; patients age ( $P=0.005$ ), disease stage ( $P=0.036$ ), anterior commissure invasion ( $P=0.0009$ ), pathologic differentiation ( $P=0.001$ ) and overall treatment time ( $P<0.0001$ ) significantly impacted this end point. Multivariate analysis demonstrated that age ( $P=0.009$ ), anterior commissure invasion ( $P=0.011$ ) and overall treatment time ( $P=0.0002$ ) significantly influenced this end point (Table 3). The 5-year DSS rate for the whole patients was 84.63% (Figure 2).

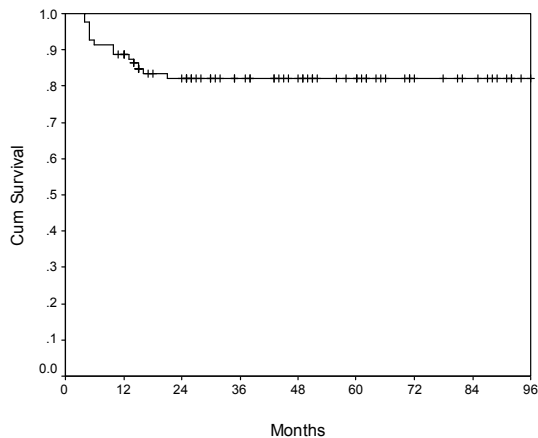
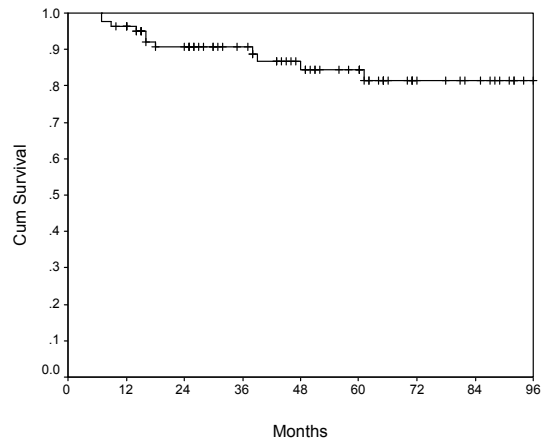
#### **Radiation toxicity**

Grades 3 & 4 acute radiation toxicities in this study including skin, larynx and pharynx were rare (1.2%, 3.7% and 6.2% respectively), Table 4. However, 9 (11%) patients suffered acute toxicities necessitating unintended treatment breaks. The common late radiation reactions recorded were mild. One patients (1.2%) required tracheostomy after RT because of persistent laryngeal edema.

**Table (2): 5-year local control after radiation therapy for 81 patients.**

	Event/ patients	5- year local control	Univariate analysis <i>P</i> -value	Multivariate analysis <i>P</i> -value
<b>Age</b>				
< 60	3/39	91.27%	0.026	NS
≥60	11/42	73.12%		
<b>Gender</b>				
Male	13/73	81.62%	0.742	---
Female	1/8	87.50%		
<b>Smoking</b>				
Yes	12/57	78.09%	0.184	---
No	2/24	91.67%		
<b>Stage</b>				
T1	6/57	89.10%	0.012	NS
T2	8/24	64.96%		
<b>AC involvement</b>				
Yes	11/28	58.33%	0.0001	0.048
No	3/53	94.04%		
<b>Tumor Grade</b>				
G I & II	4/52	91.93%	0.003	NS
G III	10/29	64.51%		
<b>Total dose</b>				
<65 Gy	3/11	71.59%	0.351	---
>65 Gy	11/70	83.77%		
<b>Dose/fraction</b>				
1.8 Gy	3/11	71.59%	0.618	---
2 Gy	10/61	83.00%		
>2 Gy	1/9	88.89%		
<b>Treatment time</b>				
≤53 days	1/55	98.18%	<0.0001	0.002
>53 days	13/26	46.13%		

AC: Anterior commissure; NS: non-significant

**Fig (1): Local control for the whole patients after definitive RT (81 patients)****Fig (2): Disease specific survival for the whole patients (81 patients)**

**Table (3): 5-year DSS for the whole group (81 patients)**

	Event/ patients	5-year DSS	Univariate analysis <i>P</i> -value	Multivariate analysis <i>P</i> -value
<b>Age</b>				
< 60	1/39	96.88%	0.005	0.009
≥60	10/42	72.83%		
<b>Gender</b>			0.982	---
Male	10/73	83.28%		
Female	1/8	100%		
<b>Smoking</b>			0.286	---
Yes	9/57	78.87%		
No	2/24	95.83%		
<b>Stage</b>			0.036	NS
T1	5/57	89.81%		
T2	6/24	70.76%		
<b>AC involvement</b>			0.0009	0.011
Yes	9/28	65.68%		
No	2/53	96.23%		
<b>Tumor Grade</b>			0.001	NS
G I & II	3/52	93.86%		
G III	8/29	56.70%		
<b>Total dose</b>			0.632	---
<65 Gy	2/11	80.81%		
>65 Gy	9/70	85.10%		
<b>Dose/fraction</b>			0.813	---
1.8 Gy	2/11	80.81%		
2 Gy	8/61	85.65%		
>2 Gy	1/9	88.89%		
<b>Treatment time</b>			<0.0001	0.0002
≤53 days	1/55	96.43%		
>53 days	10/26	62.20%		

AC: Anterior commissure; NS: non-significant

**Table (4): Radiation toxicity in 81 patients with glottic carcinoma**

	G1-2	G3-4
<b>Acute toxicity</b>		
<b>Skin</b>	80 (98.8%)	1 (1.2%)
<b>Larynx</b>	78 (96.3%)	3 (3.7%)
<b>Pharynx</b>	76 (93.8%)	5 (6.2%)
<b>Late toxicity</b>		
<b>Skin</b>	11 (13.6%)	0
<b>Larynx</b>	10 (12.3%)	1 (1.2%)
<b>Pharynx</b>	9 (11.1%)	0

#### 4. Discussion

The goals of treating T1–T2N0 glottic cancer with RT are to cure the disease, preserve the larynx, maintain good voice quality, and minimize RT-related complications. Local control is arguably the most important end point and, if successful, predicts favorable disease-free survival.<sup>(14)</sup>

In the present study, univariate analysis of the studied patients, tumor and treatment characteristics

showed that, patients age ≥60, T2 stage, involvement of the anterior commissure, histologic grade III and increased overall treatment time had poor predictive influence on the local control rate at 5 years, while with the multivariate analysis, anterior commissure involvement and overall treatment time had poor influence on the local control rate at 5 years. Also, by the multivariate analysis the patients age, anterior commissure involvement and overall treatment time were the prognostic variable that significantly influenced the 5-years DSS rate.

Many authors had reported a numbers of factors that adversely affect local control rates of early laryngeal carcinoma treated with radical RT such as, longer overall treatment time, poorly differentiated histology, smaller fraction size, subglottic extension, treatment delays/interruptions, age, smaller field sizes, lower total dose, gender, stage, higher beam energy, anterior commissure involvement, pretreatment hemoglobin levels, and impaired vocal cord mobility<sup>(3, 4, 6, T5-22)</sup>.

In our study, 5-years local control rate was 82% and 5-years disease specific overall survival rate was 84.63%. **El-Assal et al.**<sup>(23)</sup> reported, 10-years local control rates were 84% and 70% while the 10-years survival were 76% and 69% for T1 and T2 lesions respectively. **Le et al.**<sup>(20)</sup> reported 10-years local control of 81% for T1 and 61% for T2 glottic carcinoma. **Dinshaw et al.**<sup>(24)</sup> achieved 82% and 70% 10-years local control rates for T1 and T2 tumors. **Sakata et al.**<sup>(25)</sup> reported 10-years recurrence free survival of 74% for T1 and 62% for T2 tumors. **Warde et al.**<sup>(4)</sup> reported survival of 91% for T1 and 69% for T2 lesions.

In our study, the 5-years local control rates were 89.1% and 64.96% while 5-years DSS were 89.81% and 70.76% for T1 and T2 lesions respectively. **Cellia et al.**<sup>(3)</sup> reported that, 831 T1 glottic with histologically confirmed SCC were classified as T1N0, 3-, 5-, and 10-year OS was equal to 86%, 77%, and 57%, respectively. Corresponding values for local control were 86%, 84%, and 83% and for DSS 96%, 95%, and 93%, taking into account surgical salvage of relapsed cases. **Frata et al.**<sup>(6)</sup> reported that, among 256 T2N0 glottic cancer patients, 3-, 5-, and 10-year OS rates were, respectively, 73%, 59%, and 37%. Corresponding values for cumulative local control probability were 73%, 73%, and 70% and for DSS, 89%, 86%, and 85%, taking into account surgical salvage of relapsed cases.

The significant effect of anterior commissure involvement on the local control rates of early laryngeal carcinoma treated with definitive RT, have been controversial, some investigators failed to show a correlation between commissure involvement and increased local control rate and survival and others found a significant decrease in local control.<sup>(26, 27)</sup> The concern is that, because the anterior commissure is only approximately 1 cm from the skin of the anterior neck, tumors in this location could be under-dosed by low energy photon beam.<sup>(28)</sup> Besides, modification of the RT treatment technique like adding anterior field/anterior oblique field can be considered to combat under-dosage at anterior commissure.<sup>(29)</sup> Moreover, the anterior commissure may represent a weak point with regards to tumor spread because it is here that Broyles' ligament penetrates the thyroid cartilage, and this disruption of its continuity might induce susceptibility to tumor invasion.<sup>(23)</sup>

Although there is a general recommendation that delay in starting or completing of RT -especially when definitive- should be avoided, review of the published literatures reveals controversial data of the prognostic impact of the time gap and overall treatment time on definitive RT treatment outcomes

of early glottic carcinoma. **Voet et al.**<sup>(30)</sup> emphasized the negative impact of prolonged overall treatment time on the local control rate, but **Fein et al.**<sup>(31)</sup> reported that overall treatment time had only borderline significance in a multivariate analysis model and no significance in univariate analysis. In the study of Van den **Bogaert et al.**<sup>(32)</sup> a negative impact of overall treatment time was found in patients treated by a split course protocol, irrespective of side effects, even so in the subgroups of T1N0M0 patients, overall treatment time did not influence local control rate.

In the present study the prognostic effect of technical factors such as total RT dose and dose per fraction was not significant. All our patients were treated with Cobalt-60 photons beam as the low energy linear accelerator was not available at the time of study. However, there is extensive published data regarding management of early glottic carcinoma treated by RT with Cobalt-60 or 2-4 megavoltage photons beam, with local control rates ranging from 85% to 94%.<sup>(4,19,28)</sup> The reported treatment outcome of early glottic carcinoma by primary irradiation with 6 MV photons is limited and conflicting. Some authors reported comparable results with lower energies whereas others raised concern about a poorer outcome.<sup>(33-36)</sup>

In the present study field sizes ranging from 6 x 6 cm to 7 x 7 cm and RT doses ranging from 1.8 to 2.25 Gy per fraction were applied without significant impact on the local control or survival rates. Small field sizes are favored by the results of the randomized study authored by **Teshima** and coworkers.<sup>(37)</sup> **Cellai et al.** had reported that, the use of doses in excess of 65 Gy (2 Gy/fraction) and of field sizes of 36–49 cm<sup>2</sup>, along with modern treatment planning procedures and immobilization devices, is probably the best technical choice available, aiming at the best local control probability with the least incidence of damage.<sup>(3)</sup> **Kim et al.** had reported that, local control rate was higher with doses of 2.0 to 2.25 Gy per fraction than with 1.8 Gy per fraction given to a similar total dose.<sup>(38)</sup> **Le et al** reported that total dose, fraction size, and overall time contribute to lower control rates for T2 but not for T1 glottic carcinomas.<sup>(20)</sup>

In our study, seven out of seventy-four (9.46%) patients had a local recurrence at 5-years follow up. In a retrospective study reported with **Chera et al.**<sup>(22)</sup>, the medical records of 585 patients with T1N0 to T2N0 invasive SCCs of the glottic larynx treated between 1964 and 2006 were reviewed revealing that, thirteen local recurrences (2%) which were likely second glottic primary cancers occurred over 3 years after RT. A total of 67 (80%) of the local

failures were isolated, and 17 (20%) were associated with simultaneous neck failures.

In the present study, while 8 patients had undergone total laryngectomy, only two patients were surgically salvaged with partial laryngectomy making unavailability to statistically comparing the outcome of the type of salvage surgery. In general, surgical salvage is often performed by total laryngectomy because the belief of increased complications that associated with partial laryngectomy of irradiated cartilage and the negative tumor margins are difficult to achieve in a fibrotic edematous larynx. Five-year local control rates with salvage total laryngectomy have been reported at 65% to 85% for T1 and T2 glottic tumors.<sup>(39)</sup> Salvage partial laryngectomy is possible in select patients who do not progress with therapy or who experience recurrence with early-stage disease. Five-year local control rates following salvage partial laryngectomy have been reported at 66% to 96%.<sup>(40-42)</sup>

In conclusion, with definitive radiation therapy, a 5-year local control probability of 82% can be expected, with an incidence of 1.2% of severe late effects; therefore, this type of treatment could be considered as a standard treatment for T1-T2N0 glottic cancer. Anterior commissure involvement and overall treatment time are prognostic factors that adversely affecting the disease local control rate.

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