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Acta Zhengzhou University Oversea Version

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CONTENTS

Micro Vascular Free Tissue Transfer Surgeries : Impact of a Designed Teaching Protocol on Nurse's Performance for Reduction or Prevention of Post Operative Flap Failure

Samia Y. Sayed[*1], Hala M. Ghanem[1], Warda Y. Mohamed[2] and Tarek A. El-Gamal[3]

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Abstract: Introduction: Micro vascular transplants, also known as a free flap or free tissue transfer, involves transplanting nonessential donor tissue from one part of the body to another to restore form or function using microsurgical techniques. The transplanted tissue must have a single blood supply with an artery and draining vein that are both adequate to sustain circulation and life in the transplant. The free flap is anastomosed (blood vessels connected) to the recipient vessels and blood flow is re-established. Aim of the study is 2-fold: first: to design a teaching protocol for nurses working with patients undergoing microvascular free tissue transfer surgeries, and second: to evaluate the effect of implementing the designed teaching protocol on nurse's performance for reduction or prevention of post operative flap failure. Quasi-experimental research design has been utilized in this study. Subjects and Methods: A study was conducted in Assiut University Hospitals. A sample of convenience including all nurses working in Reconstructive Microsurgical Unit (10) & Traumatology Care Unit (20) in addition to (30) patients with free tissue transfer surgeries. Tools utilized were:-a) Nurses performance regards care of patients undergoing free tissue transfer surgeries questionnaire sheet to assess nurse's knowledge in addition to some sociodemographic data.b) Nurses performance regards care of patients undergoing free tissue transfer surgeries observation checklist sheet to assess nurse's skills. c) Flap observation checklist sheet among patients undergoing free tissue transfer surgeries to monitor postoperative free tissue transfer surgeries complications , D) Patients assessment sheet for free tissue transfer surgeries to assess flap failure that might develop among all patients admitted to reconstructive microsurgery. Results: A sharp improvement in the mean knowledge and practice scores were found after the application of the teaching protocol. The flap failure decreased from 20% pre-protocol to 3.3% after protocol implementation. A positive correlation was found between nurse's knowledge and practice scores immediately and 2 months after application of the teaching protocol. A significant relationship was found between flap failure and other complications as regards hyperthermia, pain, venous and arterial obstruction. Venous and arterial obstructions are significantly correlated with ischemia time. Conclusions and Recommendations: Patients with free tissue transfer surgeries are at high risk for postoperative complications, which in turn increase the development of the flap failure. These complications include hyperthermia, pain, infection, venous and arterial obstructions and need effective measures to prevent/reduce this considerable profound problem. Improving nurses' knowledge and practice can favorably affect the incidence and outcome of flap failure. Continued nursing education and in-service training programs on reconstructive microsurgery should be well organized within Assiut University Hospital and equipped with the necessary educational facilities and materials necessary to upgrade the knowledge and skills of practicing nurses, which will be reflected on better outcome and service for inpatients.
[Samia Y. Sayed, Hala M. Ghanem, Warda Y. Mohamed and Tarek A. El-Gamal. **Micro Vascular Free Tissue Transfer Surgeries : Impact of a Designed Teaching Protocol on Nurse's Performance for Reduction or Prevention of Post Operative Flap Failure.** Life Science Journal. 2011;8(2):158-170] (ISSN:1097-8135). http://www.lifesciencesite.com.

Key ward: Micro vascular free tissue transfer surgeries, flap failure and nurse's performance.

1. Introduction

The term “flap” originated in the 16th century from the Dutch word “flappe” meaning something that hung broad and loose, fastened only by one side .The flap as defined a unit of tissue that is transferred from one site (donor site) to another (recipient site) while maintaining its own blood supply. Flap classifications based on the type of tissue include: skin and fascia (cutanous flap) e.g. radial forearm flap, muscle (muscle flap), bone (osseous flap) and visceral (colon, small intestine, omentum), skin and tendon (tendocutaneous) and sensory innervated flaps (dorsalis pedis flap with deep peroneal nerve) Martins and Montero, (2007) .

Briggs, (2001) mentioned that successful free tissue transfer begins with proper patient selection. There are a number of patient characteristics which can additively increase likelihood of failure, these characteristics include the followings: age, diabetes and other systemic illness, microvascular diseases

(delay healing revascularization between the flap and surrounding tissue) decreased flap perfusion, hypercoagulation and obesity.

Most common complications of free flaps include loss of arterial supply which may cause necrosis (death) of the flap or loss of venous return which may cause congestion and also loss of the flap. We should differentiate between arterial and venous compromise. Risk of arterial occlusion increases within 24 hours postoperatively and venous congestion between 24 and 72 hours. Recognition of ischemia is important in preventing subsequent flap necrosis thus flap failure (Hitchinson and Williams, 2003, Stroch and Rice, 2005, and Woodberry, 2003)

The perioperative nurse must know the risk factors, creates and maintains a safe, effective environment, and classify the surgical wound. A complete assessment of the patient and surgical procedure is necessary .The preoperative nurse must inspect the micro-instruments and micro-scope to ensure proper function. Also she does a quick visual check of the room before set up and opening of sterile supplies. Three parameters of the operating room environment must be controlled to inhibit the growth of microorganisms. Humidity maintained at 30-60%, the rooms kept cooler at 21 ^{O}C: 24 ^{O}C and ventilated through high-efficiency filters at a rate of 20-25 total room air exchanges per hour (Halvorson, 2002, Jones and Mayou, 2001 and John, 2003).

Preoperative teaching includes instructions in breathing and leg exercises used to prevent postoperative complications, such as pneumonia and deep vein thrombosis. One goal of preoperative nursing care is to teach the patient how to promote optimal lung expansion and consequent blood oxygenation after anesthesia. The patient assumes a sitting position to enhance lung expansion. The nurse then demonstrates how to perform breathing and coughing exercises (Potter and Perry, 2001).

Hassan, (2002) mentioned that it is advisable to observe patient after free tissue transfers surgery in a special high dependency unit or an intensive care unit (ICU), where the staff are well acquainted with the problem of this surgery. Postoperative monitoring includes the following: peripheral circulation (capillary return, skin temperature), pulse oximetry SaO2 greater than 90%, urine output greater than 1 ml/Kg/h. systolic blood pressure greater than 100 mmHg or mean arterial blood pressure greater than 75% mmHg, hemoglobin around 10 g/dl or hematocrit 25: 30 % (checked every 6 hours for 24 hours).

Briggs (2001) reinforced that proper care after free tissue transfer surgery requires personnel who understand the basic principles of free tissue transfer. Supplemental oxygen and humidified air will cool a superficial flap and inhibit its blood flow. Hemodynamics and blood volume must be monitored closely. It is important to inform the intensive care unit personnel to avoid transfusing these patients without notifying the surgeon. Close surveillance for hematoma formation is necessary to avoid the deadly consequences of vascular compression. Blood pressure should be maintained appropriately.Close monitoring of the flap both by nurses and the surgeon is important postoperatively to monitor for this. If caught early, loss of blood supply may be corrected either medically or surgically.

Monitoring circulation of flaps post operatively is critical to success in free tissue transfer. Changes in perfusion need to be recognized quickly to correct any treatable problems. Disruption of perfusion to a flap can result in partial or complete flap loss. Monitoring the free flap during the postoperative phase is critical to ensure flap survival. When recognized early and managed promptly (<6 h), compromised flaps have a 75% salvage rate when taken back to the operating room. (Microsuregeon organization, 2005 and Holze, et al., 2006).

Hitchinson and Williams, (2003) designed an observation chart for free flap. This chart include: type and location of flap, color, temperature, capillary refill, and turgidity. As regards color if the flap is an external flap, check with the donor site for original color but if it is an internal flap, monitor for extremes of color. To measure temperature of the flap, an internal or external flap should always feel warm to the touch but if the flap feels cool or cold, the medical assessment should be sought immediately. As regards capillary refill, it should be timed in second if timing is not possible and an alteration in perfusion is suspected, the medical assessment should be sought immediately. The flap should usually feel spongy not hard or flaccid in relation to turgidity.

Punder, (2005) added that; all these observations should be recorded on a flap assessment chart .Flap should be observed ¼ hourly for the first 6 hours, ½ hourly for the subsequent 18 hours, hourly for the subsequent 48 hours. Reassess individually according to ongoing clinical needs. These timings have been developed following an in depth literature review and the authors' previous clinical experiences within the plastic surgical setting frequent and regular monitoring in the early post-operative phase allows for early detection and increased salvage ability of the flap. There is little justification to continue intensive flap monitoring after the first 3 days. These timings may alter if there is a change in flap perfusion. It must be emphasized that medical assessment should be sought immediately if there is any change in flap appearance.

Significance of study:

Reconstructive microvascular surgeries are recently introduced in Assiut university hospitals as a new surgical branch. This type of surgery is considered a critical one as the patient is threatened by flap failure especially during the first 48 hours postoperatively. Literature review cited many causes for flap failure, some could be classified as preoperative causes, and others can be classified as an intra and/or postoperative causes, in addition to the patient's related factors.

In addition to the scarce national researches performed into this area this research could be an attempt to equip this group of nurses with needed knowledge and practices that could contribute to flap success. As well it may provide some findings that could be helpful to patients, nurses and other health professionals to gain more knowledge about this clinical problem. It will also provide a data base about this specialized type of surgery. It is hoped also that this effort will generate attention and motivation for further investigation in this topic.

The aim of the study:

The aim of the present study is 2-fold: first is to design a teaching protocol for nurses working with patients undergoing microvascular free tissue transfer surgeries, and the second is to evaluate the effect of implementing the designed teaching protocol on nurse's performance for reduction or prevention of post operative flap failure

2. Subjects and Methods

Research design:

Quasi-experimental research design.

Sample:

A sample of convenience including all nurses working in Reconstructive Microsurgical Unit (10) and Traumatology Care Unit (20) who are willing to participate in the study and all patients admitted for free tissue transfer surgeries for at least six months after application of the designed teaching protocol.

Setting:

The study was conducted at the Reconstructive Microsurgical & Traumatology Care Units in Assiut University Hospital.

Tools:

Data pertinent to the study were collected, and utilizing the following four tools:

Tool I- Nurses performance regards care of patients undergoing free tissue transfer surgeries questionnaire sheet:

It was translated and modified by researcher to assess their knowledge about care of patient undergoing free tissue transfer, then a pilot study on five patients was done. According to the results of the pilot study, subjects were included in the study as the changes performed were minimal. It was used prior to implementation of the teaching protocol to measure the exact knowledge level of nurses about free tissue transfer surgeries. It was used immediately after the implementation of the teaching protocol (immediate post-test) in addition to two months later to evaluate the gain in knowledge after the intervention. It consists of (5) main parts:

- Demographic variables of biosocial characteristics of study sample (30 nurses), including age, residence, marital status, educational level, and duration of experience. It included (8) items .
- Nurses' knowledge about microsurgery and types of flap, which included (26) questions .
- Nurses' knowledge about nursing care before free tissue transfer surgeries, which included(17) questions.
- Nurses' knowledge about nursing care during free tissue transfer surgeries, which included (16) questions.
- Nurses' knowledge about nursing care after free tissue transfer surgeries, which included (33) questions.

The questionnaire sheet was administered by researcher to the nurses for answering all its components then collected. The total number of questions was (92).

Scoring system: each right answer was given one score. The total scores were (92). Those who obtained less than (50%) were considered having unsatisfactory level. From (50% to 70%) were considered having satisfactory level. While those who obtained above than (70) were considered having good level.

Tool II: Nurses performance regards care of patients undergoing free tissue transfer surgeries observation checklist sheet:

It was developed and modified by researcher to assess the learnt skills. This tool was used before and immediately after the implementation of the teaching protocol as well as two months later to evaluate the impact of the training teaching protocol on nurses' practice. It consists of the following (5) main items:

- General care of patient with free tissue transfer surgeries which includes (16) items.
- Specific care which includes(47) items as regards the following:

1. Activities to maintain body heat
2. Activities to keep bed rest

3. Activities to reduce pain & swelling.
4. Manage postoperative hypothermia.
5. Measures to reduce arterial or venous insufficiency.

- Postoperative monitoring of vascular viability of flap as regards color, temperature, turgor, capillary refill, dermal bleeding, and activities to perform pin-prick test. It includes (17) items.
- Care of wound and donor site which includes (19) items .
- Instructions about home care after microsurgery which includes (13) items.

The observation checklist was applied by researcher to evaluate the nurses' practice as regard identification, prevention and management of causes of flap failure. Scoring system: Each item was observed, categorized and scored into either 'done correctly =1, or not done =0. The total score for all items was 112. Those who obtained less than (50%) were considered having unsatisfactory level. From 50% to 70%)were considered having satisfactory level. While those who obtained above than 70% were considered having good practice level.

Tool III: Flap observation checklist sheet among patient undergoing free tissue transfer surgeries:

It was developed and modified by researcher to monitor postoperative free tissue transfer surgeries complications, which covers the following areas (color, temperature, turgor, capillary refill, dermal bleeding and pin prick test).

It consists of (33) items which covers the following:

1. Color of the flap which includes (4) items.
2. Temperature of the flap which includes (3) items .
3. Turgor of the flap which includes (3) items .
4. Capillary refill of the flap which includes (3)items .
5. Dermal bleeding which includes(4) items: (2) items to assess color of bleeding and (2) items to assess site of bleeding recipient or donor .
6. Pin prick test which includes (3)items
7. Vital signs which include (4) items blood pressure, temperature, pulse, respiration .
8. Laboratory investigations which includes(4) items hematocrit, prothrombin time& concentration, blood sugar, hemoglobin .
9. Intake and output which includes (2) items negative, positive .
10. Occurrence of flap failure which includes (2) items failed or not
11. Flap was observed ¼ hourly for the first 6 hours, ½ hourly for the subsequent 18 hours, hourly for the subsequent(48) hours. Reassess individually according to ongoing clinical needs. This was done every day until the patient discharged from traumatology care units.
12. As regards this tool, the healthy flap was differentiated from unhealthy according to the following table. In which identification of arterial insufficiency and venous congestion will help in early detection of flap failure):

Table 1: Healthy and compromised flap:

Observation	Healthy flap	Arterial Insufficiency	Venous Congestion
Skin Color	Similar to that of donor area	Pale	Purple/ blue
Turgidity	Soft	Spongy, prune-like	Stretched, swollen
Temperature	Warm	Cold	Cold
Capillary Refill	2-3 Seconds	Absent/ Sluggish > 6 Seconds	Brisk < 3 Seconds
Pin-prick test	1:2 drops of blood	No blood	Rapid exit of dark red blood

This table adopted from Hitchinson and Williams, (2003)

Tool IV: Patients assessment sheet for free tissue transfer surgeries:

It was used to assess flap failure that might develop among all patients admitted to reconstructive microsurgical unit until six months after implementation of the teaching protocol. The assessment sheet includes (31) items and covers the following areas:-

- Socio demographic data: patients age, sex, residence, marital status, occupation, educational level, and date of admission. It includes (8) items.
- Flap data covering the following (23) items: Medical diagnosis, site and cause of injury, recipient and donor site, type of tissue used, ischemia time, hours of surgery, intraoperative and postoperative complications related to flap failure. Signs of arterial or venous insufficiency,

and immediate measures to reduce them. The patient is assessed by researcher and nurses by daily flap assessment sheet.

Methods

The study tools and teaching protocol of work were formulated after a review of current and related literature about flap failure and assessment of nurse's knowledge and practice in this regard. The content validity of the tool and teaching protocol was checked, revised by expert professors in fields of medicine and nursing and correction was carried out accordingly.

A pilot study was implemented on five nurses and five patients to test the feasibility, the ability of the tools to elicit the desired and test information, to estimate the time needed to fill out the tools. Analyses of the pilot study revealed that minimal modifications are required. To facilitate the implementation of the teaching protocol about flap failure, researcher prepared the training places, teaching aids and media (pictures, videotapes and handouts). This was followed by arranging for the teaching protocol schedule based on the contents of protocol, number of staff involved, time availability, shifts as well as the resources available.

An official permission to proceed with the proposed study was granted from the head of the Reconstructive Microsurgical and Traumatology Care Units as well as the hospital nursing director. Names of nurses included in the study were obtained from the head nurses of the two selected units (reconstructive microsurgical unit and Traumatology care unit). Nurses and patient were informed of the purpose and nature of the study. The investigator emphasized that the participation is voluntary and confidentiality and anonymity of the subjects will be assured through coding all of data.

At initial interview, the researcher introduces herself to initiate line of communication, explain the nature and purpose of the teaching protocol and fill out the questionnaire sheet. Also she scheduled with them the teaching sessions for both theory and practice and the nurses were divided into small groups, each group contains two to four nurses. Each group of nurses was given the freedom to choose their optimal time for receiving the teaching protocol whenever they have minimal workload.

The teaching protocol has been implemented for nurses in terms of sessions and teaching on the spot during their official working hours. There was a total of nine sessions. These nine sessions were repeated 10 times to each group. Number of nurses in each session ranged between two- four nurses. The duration of each session was an hour, including 15 minutes for discussion and feedback. Each session usually started by a summary of what has been taught during the previous sessions and the objectives of the new topics. Feedback and reinforcement of teaching were performed according to the nurses needs to ensure their understanding. Giving recognition to the interested nurses was emphasized for motivation during the teaching protocol implementation.

An Arabic version of flap observation checklist sheet was used. It was fully explained to nurses (contents and how to apply), then it was distributed to nurses by the researcher immediately after the application of the teaching protocol in order to identify how the nurse uses this sheet for early detection of patients at risk of flap failure. Also the researcher explained the nature and purpose of the teaching protocol to the selected patients who are willing to participate in the study and filled out the patient assessment sheet. Each patient was handed a list of instructions about home care after microsurgery.

It was done to evaluate the effect of implementing the designed teaching protocol on nurse's performance for reduction or prevention of post operative flap failure. Each nurse obtained a copy of the teaching protocol booklet that included all the training contents. Immediately after protocol implementation as well as after 2 months, the nurses' knowledge and practices have been evaluated by the researcher through filling the tools. Also the patients and free flap were assessed for early detection of flap failure using flap observation checklist sheet (¼) hourly for the first (6) hours,(½)hourly for the subsequent (18) hours, hourly for the subsequent (48) hours and then every 4 hours for the next days until the patient was discharged from Traumatology Care Units, as well the researcher filled the patient assessment sheet, The whole period for teaching protocol implementation was 1 year.

An official letter was issued from the Dean of the Faculty of Nursing to the Head of the Reconstructive microsurgical and Traumatology care units as well as the Head of Nursing Service Administration soliciting the necessary approval to conduct the present research. Meetings with nursing supervisors and physicians of these two units to explain the objectives and contents of the teaching protocol and the methods for applying the teaching protocol to gain their cooperation and to allow the release of nurses to attend the teaching protocol during minimal workload activities.

Limitation of the study:

1. Since the researcher was the only data collector, this study did not include patients monitoring for 24 hours. So, it was impossible to be sure if flap observation checklist sheet were properly applied.

2. Investigation findings are limited to one geographical area in Arab Republic of Egypt (Assiut University Hospitals).
3. Limited number of patients. Patients flow was little.
4. As results of small number of nurses working in this unit researcher was obliged to include nurses working with similar patient in other units inside the hospital.
5. Literature and nursing researches in this area were inadequate.

Statistical Design:

Data were analyses using SPSS. The following tests for significance were used, Frequency, Percentage, Means and Standard Deviation. Chi square (with yets correction). Correlation coefficient, ANOVA and t-test for comparison of means. A probability level of 0.05 was adopted as a level of significance for testing the research hypothesis.

3. Results

Frequency distribution of socio demographic characteristics of nurses showed that, the majority of the nurses (63.3%) their age ranged from 20 to 25years (mean: 24.63±2.65); 70% of them were single, living in urban area, and have baccalaureate degree. All nurses (100%) had no in-service training courses related to microsurgery. Their experiences were mostly more than 24 months (56.7) with mean duration of 29.97±23.02 months.

Table (2): showed that, a significant statistical difference between nurse's knowledge in relation to total and subtotal mean knowledge scores with p-value of <0.01 in all items except in postoperative care knowledge items between immediately post protocol and after 2 months.

Table (2): Two by two t-test for the mean knowledge score obtained by nurses Pre-, Immediately post and 2 months after application of the teaching protocol (n=30).

Knowledge	Pre -protocol	Immediately post-protocol
Total:		
Immediately post-test.	37.90**	
2 months post-test.	36.60**	9.70**

** Significant at <0.01 N.S= non significant

Table (3): showed that, a significant statistical difference between nurse's practice in relation to total and subtotal mean practice scores with p-values of <0.01 in all items.

Table (3): Two by two t-test for the mean total and subtotal practice scores obtained by nurses Pre-, Immediately post and 2 months after application of teaching protocol (n=30).

Practice items	Pre-protocol	Immediately post-protocol
Total (Maximum score =112):	291.26**	4.54**
Immediate post-test	66.18**	
2month post-test		

** Significant at <0.01

Table (4): showed that, a positive correlation between nurse's knowledge and practice scores in immediately and 2 months after application of the teaching protocol with p-value <0.01. Thus fourth hypothesis was supported

Table (4): Correlation between nurse's knowledge and practice scores before and after application of the teaching protocol (n=30)

Knowledge	Practice					
	Pre- protocol		Immediately post - protocol		2 months post-- protocol	
	r-value	p-value	r-value	p-value	r-value	p-value
Pre-test	0.11	ns	0.67	**	0.46	**
Immediately	0.01	ns	0.41	**	0.36	**
post-test	0.07	ns	0.55	**	0.35	**
2 months post-test						

** Significant <0.01 ns= not significant

Table (5): showed that, age was positively correlated with total and subtotal knowledge scores especially during the 2 months post-test. However nurse's experience was found to be positively correlated with total and subtotal knowledge's scores all through the study period.

Frequency distribution of socio demographic characteristics of patients showed that, more than half of the patients (60%) their age ranged between 12 to 36 years with mean of (14.6+8.9). The majority of patients were male, living in rural area, single, have secondary education, and have no work in percentages of (70%, 73.3%, 90%, 46.7%, and 63.3% respectively).

Table (5): Correlation between duration of experiences and age of nurses with total and subtotal knowledge scores obtained pre, immediately post, and 2 months after the application of the protocol (n=30).

Sociodemographic variables / Knowledge items	Age		Duration of experience	
	r-value	p-value	r-value	p-value
Total:				
Pre-test	0.137	ns	0.090	*
Immediately post-test	0.187	ns	0.042	**
2 month post-test	0.360	*	0.052	**

** Significant at <0.01 * significant at < 0.05
ns= non significant

Table (6): Frequency distribution of the study sample as regards relationship between flap failure and other complications

Other complications	Flap failure	
	X^2	p-value
Hyperthermia	12.22	**
Infection	2.49	ns
Postoperative shivering	3.15	ns
Pain	8.57	**
Venous obstruction	12.71	**
Arterial obstruction	17.01	**

** Significant <0.01 ns=not significant

Table (7): showed that, a gradual improvement in flap condition reached to the maximum improvement before discharge. A significant statistical differences was found between normal, venous, and arterial flap during 1st, 2nd, 3rd, and before discharge in relation to color, temperature, and turgor with p-value < (0.01).

Table (7): Frequency distribution of the study sample as regards flap observation in first, second, and third postoperative days as well as before discharge (n=30)

Flap observation	1st day		2nd day		3rd day		Before discharge		X^2value P-value
	No.	%	No.	%	No.	%	No.	%	
Color:									
Normal (similar to that of donor area)	26	86.7	23	76.7	26	86.7	29	96.7	
Venous (Purple)	1	3.3	6	20	3	10	0	0.0	12.09
Arterial (white)	3	10	1	3.3	1	3.3	1	3.3	*
Temperature:									
Normal (warm)	20	66.7	27	90	29	96.7	29	96.7	
Venous (cold)	5	16.7	2	6.7	0	0.0	0	0.0	17.17
Arterial (cold)	5	16.7	1	3.3	1	3.3	1	3.3	**
Turgor:									
Normal (soft)	20	66.7	26	86.7	28	26.7	29	96.7	
Venous (hard and swollen)	9	30	3	10	1	3.3	0	0.0	16.8
Arterial (spongy)	1	3.3	1	3.3	1	3.3	1	3.3	**
Capillary refill:									
Normal (2:3 second)	29	96.7	26	86.7	29	96.7	29	96.7	
Venous (<3 second)	0	0.0	3	10	0	0.0	0	0.0	9.23
Arterial (>6 second)	1	3.3	1	3.3	1	3.3	1	3.3	ns
Pin prick test:									
Normal (1:3 drops of bright red blood)	30	100	26	86.7	28	26.7	29	96.7	
Venous (rabid exit of dark red blood)	0	0.0	3	10	1	3.3	0	0.0	
Arterial (no bleeding)	0	0.0	1	3.3	1	3.3	1	3.3	7.30 ns

** Significant p <0.01 *Significant p<0.05 ns=not significant

Table (8) showed that a significant relationship between most of complications and socio demographic characteristics as regards sex, site of injury, cause of injury, and type of transferred tissue. Venous and arterial obstructions were found to be significantly correlated with ischemia time with p-value <0.05.

Table (8): Relationship between socio demographic characteristics of patients and development of complications (n=30)

Sociodemographic characteristics	Complications											
	Hyperthermia		Infection		Shivering		Pain		Venous obstruction		Arterial obstruction	
	X^2	P-value	X^2	P-value	X^2	P-value	X^2	P-value	X^2	P-value	X^2	P-value
Age	0.4	ns	1.35	ns	0.87	ns	1.82	ns	0.23	ns	0.9	ns
Sex	0.71	ns	5.40	*	4.45	*	15.26	**	2.13	ns	0.44	ns
Residence	0.51	ns	2.06	ns	1.67	ns	0.34	ns	1.21	ns	0.37	ns
Marital status	1.5	ns	0.15	ns	2.16	ns	0.84	ns	2.59	ns	0.51	ns
Education	0.43	ns	1.16	ns	3.4	ns	0.81	ns	2.92	ns	1.78	ns
Occupation	3.4	ns	2.84	ns	0.94	ns	0.16	ns	0.72	ns	0.68	ns
Site of injury	4.45	*	1.22	ns	0.04	ns	5.16	*	8.57	**	1.79	ns
Cause of injury	11.27	**	4.39	*	0.87	ns	8.7	**	0.01	ns	1.18	ns
Type of transferred tissue	16.7	**	1.42	ns	0.71	ns	1.5	ns	0.51	ns	11.01	**
Ischemia time	0.87	ns	0.9	ns	1.04	ns	0.45	ns	6.66	**	17.5	**

**= Significant at 0.01 *= Significant at 0.05 ns=Not significant

4. Discussion:

Microsurgery is surgery that is performed on very small structures of 1 to 5 millimeters, such as blood vessels and nerves, with specialized instruments under a microscope. It is frequently used because of their many advantages such as reliable vascularity, less infection, better postoperative function and wider resection of advanced lesions. Therefore application is increasing in various areas of tissue defects and now more than 100 microsurgical procedures are carried out annually in Assiut University Hospitals. This type of surgery is considered a critical one as the patient is threatened by flap failure especially during the first 48 hours postoperatively (Mark, 2003).

Based on the results of the present study, the majority of the nurses were adults. All of them have no in-service training courses related to microsurgery. More than half of them their experiences were mostly more than 24 months. So it had been concluded that nurses are not properly prepared prior to their graduation and starting to serve patients with free tissue transfer surgeries. Their real experiences were gained while working in reconstructive microsurgical unit only. However the researcher was imagining that there should be a perfect training program designed for a selected group of nurses and other health team member a head of time to prepare team capable of dealing with such group of patients.

In the same line, Georgiade, et al, (1999) mentioned that a trained nursing staff in microsurgery unit as part of plastic or orthopedic ward is central and necessary for adequate postoperative care. It is necessary to prepare nurses to handle such specialization area of care at the postgraduate level by enrolling in a specially or continuing education program. As well Stroch and Rice, (2005) reinforce on the principles that promote flap success which is a team approach including specialist nursing and high quality of nursing care provided to the patient and the wound. Nahabedian, (2006) and Goldner, and Urbaniak, (1999) documented that, intensive care and reconstructive microsurgical units should be provided by nurses, especially trained in the monitoring vascular viability of the flap as well as early detection of flap failure.

The current study revealed a great lack of knowledge and practices as regards to free tissue transfer surgeries before the application of teaching protocol as all nurses had an unsatisfactory knowledge score levels. This reflects the lack of scientific preparation in these specialized surgeries. This might be related to the fact that, reconstructive microsurgery is recently introduced in Assiut university hospitals as

a new surgical specialty. So it is to be concluded that studied nurses were not properly prepared prior to their working and dealing with such patients. In this respect, Stroch and Rice, (2005) and Kneal and Davis (2005), reported that nurses have a very dominant role in clinical monitoring, observations, clinical responses, and educating other clinicians regarding the postoperative monitoring of free flap.

Moreover, Shell, (1999) and Change, (2006), mentioned that the use of microvascular surgery has placed demands on the perioperative nurse to remain current on perioperative implications of this type of surgery. Nurses must be able to expand their knowledge of this area through ongoing education, journals, and seminars. Consequently, teaching programs for nursing staff constitute an important part. These programs are urgently designed to assess nursing staff in developing and enhancing the skills needed to provide high standards of care to their patients. As well, Billing (1991) and Dunning (1993) agreed that those programs are urgently needed to provide up-to-date knowledge and improve nurse's competency and skills.

After implementation of the teaching protocol, nurses' knowledge score levels regarding free tissue transfer surgeries were significantly improved. This improvement might be related to the fact that all nurses were in young age this age might have good readiness for learning new things as most of them are also single i.e. they might have less responsibilities and more capacity of learning. These results are in agreement with those of Meyer and Elliott (1999) who noted that nurse's knowledge scores were higher among younger and newly graduated nurses who are attending a training program By time, i.e. after 2 months post-test, the percentages of knowledge were slightly reduced as the majority of nurses were having satisfactory and good levels in all items of knowledge. This indicates that the improvement in knowledge was partially lost 2months after implementation of teaching protocol. This result might be explained by the fact that, knowledge retention is usually affected by time. This effect is toward the reduced level of retention.

Broomfield (1996) conducted a research with the intention of testing the retention of certain nursing skills and knowledge of registered nurses. There has been an initial improvement after performing the training program, but there has been a significant decrease in retention of knowledge 10 weeks later ($P<0.0001$). The findings of his research reflect similar results to previous research works, suggesting that retention of skills and knowledge quickly deteriorates with time if not used or updated regularly. He recommended refresher courses on regular basis.

In this regards, Mehany (1999) and Abd-Alla (2000), found a direct relation between memory loss and length of time that lapses after a certain educational event. Also they reported that nurses who had poor levels of knowledge and/or skills before the exposure to a training program underwent a significant improvement after the implementation of the program.

The current study revealed a great improvement in the practice score levels obtained by nurses after the application of the teaching protocol in all items. This has been concluded by the presence of significant differences between results of pre-tests and post-tests. These significant differences mostly remained two months after the application of the teaching protocol. This finding indicated that skills can be easily improved, especially if linked with their relevant scientific base of knowledge.

In this respect, Sherwood (1996) reported an improvement in nurses' practice after the attendance at continuing nursing education sessions. Research findings indicated that continued nursing education programs increase both knowledge and performance and can also improve attitudes. As well, Bayoumy (1999) and Abd-Alla (2000) documented that the inservice training program has a beneficial effect in improving the nurses' knowledge and skills. They also recommended that educational programs should be organized according to the needs of nurses with continuous evaluation.

In this study the researcher measure nurse's knowledge and practice in different intervals to measure their knowledge retention. Mehany (1999) and Abd-Alla (2000) mentioned that periodic follow up enhances the audience's ability to retain information and improve their skills. As well McCorkel and Grant, (1994) stated that many educational programs use a comparison of the participants pre-test and post-test scores as an indicator for the effectiveness of the program.

As regards incidence of flap failure, the current study found that only 3.3% of patients developed flap failure after application of the teaching protocol as compared to 20% before application of the teaching protocol one year ago. In the same line, a study that was done by Cheung, (1998) reported that the success rate of microvascular free flap surgery was 93.6%. Complete losses of free flaps were found in three patients (one had a radial forearm flap for forehead basal cell carcinoma, one had a transverse rectus abdominis (TRAM) flap for breast cancer, and the third had a lateral arm flap for foot ulceration.

In this respects, Bunche, (2003) reported that, vascular compromise is a frequent complication and ranges from 5% to 26%. Also Stroch and Rice, (2005) stated that in a major flap and reconstruction

surgery, arterial spasm, arterial and venous flow compromise (Thrombosis) and subsequent loss of flap are distinct and high risk.

The current study revealed that 13.3% of studied patients developed an infection after free tissue transfer surgeries. It was found that infection is significantly correlated with the site and cause of injury; in which three patients out of the four who developed infection had severe crush injuries in lower limb. In this regard researchers at the University of Texas Anderson Cancer Center found that patients undergoing free tissue transfer have significantly higher incidence of flap complications such as flap loss and infection (Jansen, 2002). In the same line Green (2000) reported that, deep infection caused flap failure in patients who had severe crush injuries and incomplete wound excision. This endangers flap viability and increase the risk of infection

The present result agrees with Porter, (2004) who reported that, 8-20% of patients undergoing free tissue transfer develop infection. In this respect a study was done on 52 patients with open tibial fractures by (Bunche, 2003) who reported that a seventy-five percent of bone flaps, attempted in patients who eventually achieved soft tissue coverage, failed because of infection.

The current study showed that 10% i.e. three patients only out of thirty developed venous obstruction and 3.3% i.e. one patient out of thirty developed arterial obstruction. Arterial obstruction occurred 18 hours postoperatively, and the patient was returned to operating room for flap salvage but it failed. As regards those patients who developed venous obstruction, the signs and symptoms of venous obstruction appeared in the second postoperative day and no one failed. This might be due to early detection, which helped in early intervention and increased success rate of flap salvage.

In this regards Russell, et al., (2000) and Change, (2006) reported that flap failure is best detected by regular observation of the flap by experienced nursing staff. If there is any doubt the patient must be rapidly returned to theatre and the microvascular anastomosis inspected. Prompt re-exploration will usually salvage an early detected failed flap. As well Nahabedian, (2006) stated that monitoring the free flap during the postoperative phase is critical to ensure flap survival. When recognized early and managed promptly (<6 hours); compromised flaps have a 70% salvage rate when taken back to the operating room. Studies have demonstrated that venous thrombosis alone is more common than either arterial or combined arterial and venous thrombosis. Thrombosis typically occurs within the first two days in 80% of patients. Thus all personnel responsible for flap monitoring must be knowledgeable of the appearance and evaluation of the healthy and compromised flap.

A significant relationship was found between flap failure and other complications as regards hyperthermia, pain, venous obstruction, and arterial obstruction. i.e., other complications are considered as risk factors of flap failure. However the researcher found that the flap failure was due to arterial obstruction.

Regarding flap observation, it was found a gradual improvement in flap condition reached to the maximum improvement before discharge. A significant statistical difference was found between normal, venous, and arterial flap during first, second, third day and before discharge in relation to the color, temperature, and turgor. This means that no abnormal changes were found after the seventh postoperative day. These results are in agreement with those reported by Janezic, et al, (2000) and Storch and Rice, (2005) who reported that the most critical time for flap failure was the first 4 days post operatively. No microvascular complication occurred later than the seventh day.

In the present study only one flap was characterized by white color, cold, spongy, capillary refill more than 6 seconds, and no bleeding with pin prick test. This flap was detected in the 1st day and failed due to arterial obstruction. In the same line Hitchinson and Williams, (2003), Porth, (2000), Woodberry, (2003) Kelly, et al., (2004) and Storch and Rice, (2005), denoted that flap failure occurs primarily as a result of both arterial occlusion and venous congestion. Risk of arterial occlusion increased within 24 hours postoperatively and venous congestion between 24 and 72 hours. Recognition of ischemia is important in preventing subsequent flap necrosis thus flap failure.

The current study showed a significant correlation between the development of flap failure and socio demographic characteristics of patients as regards sex, site of injury, cause of injury, and type of transferred tissue. These results are in agreement with the study of Janezic. et al (2000), they found that, age and sex, site, cause and mechanism of injury, occurrence of thrombi were potential survival factors after free tissue transfer.

There was a significant correlation between the development of venous and arterial obstruction and ischemia time. It was found that the ischemia time was between 2.5 - 3.5 hours in about 60% of patients. In the same line a study was conducted by Nahabedian, (2006), entitled as "Flap, free tissue transfer", which revealed that the flap ischemia time, does not contribute to flap demise if the ischemia time is less than three hours or less than the time for no-reflow to occur.

Janezic. et al (2000) found that the success rate for free flap was 66% and the most frequent cause of failure was thrombosis. Storch and Rice, (2005), found that, regular and objective vascular inspection of the flap by specialist nurses with expertise in flap monitoring and reconstructive surgical wound management are required for the first 48-72 hours. This might necessitate the presence of a specialist nurse to undertake such observations. The nursing care is vital to maintain the survival of the flap, and help in early detection of these factors which potentially contribute to flap failure (Martins and Montero, 2007).

Early diagnosis of flap failure is a precondition for flap salvage. Postoperative monitoring of microvascular transplants is an absolute necessity to further increase the success rate of this procedure. Moreover, the time interval needed for re-establishing vascular flow is the decisive factor for a successful revision. Clinical observation is still the normal standard for free tissue transfer monitoring. Researches cited above pictured the flap failure as an existing profound problem which necessitates an effort and concern to reduce or prevent its occurrence by early detection. In this regards, Futran, et al., (2000) reported that monitoring strategies have been developed to address the issue of early detection of postoperative flap compromise in an effort to permit intervention and flap salvage.

Finally, it can be concluded that, the teaching protocol for nurses working with patients undergoing microvascular free tissue transfer surgeries had achieved its objectives by improving nurses' knowledge and practice about nursing management to reduction or prevention of post operative flap failure. Furthermore, the teaching protocol showed its impact on early reduction or prevention of flap failure. Porth, (2000); and Storch and Rice, (2005), stated that professional nurses have a large role to play in the minimization and prevention of local thrombosis and should be clinically well versed in all aspects of the condition, current strategies to address risk minimization and prevention management and advocates for patient safety.

5. Conclusions:

Based on the result of the present study, it can be concluded that: patient with free tissue transfer surgeries are at high risk for postoperative complications which in turn increase the development of the flap failure. These complications include hyperthermia, pain, infection, venous and arterial obstruction as previously mentioned in the literature and need effective measures to reduction or prevention of post operative flap failure. Nurse's knowledge and practices regarding free tissue transfer surgeries in Reconstructive Microsurgical Unit at Assiut university hospital are inadequate. Nurses are potentially capable to improve their knowledge and practice after exposure to teaching protocol. Application of teaching protocol about care of patients undergoing free transfer surgeries shows a significant improvement in nurses' knowledge and practice. Improving nurses' knowledge and practice can favorable affect the incidence and outcome of flap failure.

Recommendations

Based on results of the present study the following can be recommended:

Continued nursing education and in-service training programs on reconstructive microsurgery should be well organized within Assiut University Hospital and equipped with the necessary educational facilities and materials necessary to upgrade the knowledge and skills of practicing nurses, which will be reflected on better outcome and service for inpatients. Nurses should add to their routine obligations the regular reading of up-to-date references (periodicals, textbooks, etc.). They should always be encouraged to attend scientific meetings and conferences to keep pace with the rapidly growing wealth of knowledge and practice necessary for proper nursing service. Periodic monitoring of nurses knowledge and practice to evaluate the level of nurses. Replication of the study on a larger probability sample acquired from different geographical areas in Egypt to figure out the main aspects of this problems. Newly employed nurses in Reconstructive Microsurgical Unit are required to successfully complete a test of basic knowledge and skills before assuming independent responsibility for patient care. A continuing educations program be planned for and offered on regular basis to nurses in the Reconstructive Microsurgical Unit. Use of flap observation check list sheet as a routine nursing care of patients in Reconstructive Microsurgical Unit and nurses should be adequately trained on how to use it and what to report and when. It is recommended that similar studies should be replicated on longitudinal bases till one year as a minimum time period for follow up. Patients undergoing free tissue transfer surgeries should be exposed to physical therapy practices to avoid/prevent problems that may occur.

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Effects of livin over-expression on myocardial ischemia reperfusion injury in rats

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Abstract: To evaluate the effects of livin over-expression on myocardial ischemia reperfusion injury. Rats were subjected to 30 min of left coronary artery occlusion followed by 120 min of reperfusion with treating the rats by retroviral vector expressing livin 24h before left coronary artery occlusion. Both caspase-3 and livin mRNA expression were detected by real time PCR and the caspase-3 protein was detected by immunohistochemical study; Cardiomyocyte apoptosis was evaluated with TUNEL assay. Myocardial infarction size were detected by TTC dyeing mehod. Caspase-3 mRNA expression increased during IR and decreased significantly after the transfection of retroviral vector expressing livin. Meanwhile the apoptosis index and MI size were increased in IR group and decreased significantly in livin group. Livin overexpression could down-regulate the expression of caspase-3, attenuate myocardial apoptosis, and decrease myocardial infarction size.

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Key words: myocardial infarction; acute; livin; apoptosis

1. Introduction

Myocardial reperfusion after acute myocardial infarction(AMI) could reduce the mortality, improve myocardial function and attenuated cardiac arrhythmia. However, the occurrence of myocardial ischaemia reperfusion injury(IRI) following the reperfusion treatment will result in myocardial dysfunction, arrhythmia, myocardial stunning, re-occlusion of coronary arteries, even when revascularization was successful. Some reperfusion injury may occur that transiently impairs myocardial function or results in sudden cardiac death. Therefore, treatment should not only be directed towards the restoration of myocardial blood flow but should also to prevent or alleviate the consequences of myocardial reperfusion injury.

The mechanisms of myocardial ischemia reperfusion injury remaine unclear. Several factors such as impairment of cardiomyocyte construction, disorder in cardiac energy metabolism, thrombosis and vasospasm were considered as the causes of IRI. Various strategies to deal with IRI have been developed, but no significant effect was seen in clinical settings. Recent studies reported that apoptotic cardiomyocyte increased in myocardial infarction. This phenomenon suggested that cardiomyocyte apoptosis may play a important role in myocardial ischemia reperfusion injury , and IRI might be attenuated if the cardiomyocytes apoptosis was suppressed.

Apoptosis depends on the activation of caspase-3. Livin is one of the inhibiors of apoptosis protein which could combine with caspase-3 and suppress apoptosis. But no data indicated that whether livin could protect herart from ischemia reperfusion injury or not. The present study, we constructed retroviral vector expressing livin and transferred it into rat myocardium, is to investigate the changes of Caspase-3 and livin mRNA expression during cardiac ischemia reperfusion and the effects of livin over-expression on the expression of caspase-3 and cardiomyocytes apoptosis in myocardial ischemia reperfusion process.

2. Materials and methods

(1) Materials

Retroviral vector $pLNCX_2$ and RetroPack™ PT67 cell line were from Clontech Company. Colibacillus strain JM109 was from Takara Co. Caspase-3 antibody was bought from Santa Cruz Company in USA.

(2) Livin gene clone and subclone

Livin RNA from mice MA782 cell was extracted according to QIAGEN RNA extraction procedure, the cDNA was obtained by reversible transcription polymerase chain reaction (rt-PCR) technique, livin gene was amplificated (primer, 5’ ACTCGAGATGGGGCCTGAGAGTAGGGCCAG 3’ XhoI, and L2 5’ AAAGCTTTTAGGACAGGAATGTGCGTACAC 3’ HindIII) and first cloned into pGEM-T easy plasmid then digested by XhoI/HindIII and identified by PCR. The pGEM-T-livin was subcloned into the retroviral vector pLNCX2. The pLNCX2-livin was transferred into packaging cell PT67 after XhoI/HindIII digestion and identified by PCR. A pure packaging cell line pLNCX2-Livin cells expressing livin was cultured and freezed at -80 ℃.

(3) Transfection rat myocardium

All the animal experiment and care were approved by the guidelines on the Use of Laboratory Animals in Zhengzhou University Animal Care Committee(China, Zhengzhou). Eighty healthy SD rats were divided into Control group, IR group, empty vector group and Livin group. Rats were subjected to 30 min of left coronary artery occlusion followed by 120 min of reperfusion with treating the rats with 50μl(3.9×10^7IU/ml) pLNCX2-Livin (livin group) or with 50μl(3.7×10^7IU/ml) pLNCX2 (empty vector group) by intramyocardium injection 24h before left coronary artery occlusion. Rats in IR group underwent the IR process but no treating with vectors. The samples were isolated and freezed in -196℃ liquid nitrogen.

(4) Livin and caspase-3 mRNA detection by real time PCR

Rat myocardium RNA was extracted in trizol method and the cDNA was obtained by reversible transcription with catalysis in AMV and the primer was OLIGO hexamer: livin, up, 5' atggggcctgagagtagggccag 3', 169 bp; down, 5' GACAGAGGCCGAAGCTGGCC 3'. Caspase-3, up 5' gtcgatgcagctaacctcag 3' ,156 bp, down 5' GACTTAGAATCACACACAC 3' .β-actin, up, 5' atggatgacgatatcgctgcg 3', 247 bp, down 5' TCCaTatCGTCCCagttggtg 3'. According to real time PCR kit procedure, reaction system was 50μl. Livin gene amplification was compared with β-actin amplification. The pGEM-T-β-actin and pGEM-T-livin standard curve in different dilution were set up. The relative level of livin expression was calculated by the ratio of livin expression and β-actin expression.

(5) Myocardial apoptosis detected by TUNEL assay:

The infarcted and ischemical myocardium was fixed in formaldehyde and embeded in paraffin and then cut into 4 μm slices along the long axis of left ventricle. The apoptotic myocardial cells were detected in TUNEL method. The samples in control group experienced the same procedure but without Tdtase. Three slices from one sample were observed for 10 fields under microscope of 10×40 to count the positive apoptotic myocardial cells and total myocardial cells. Apoptotic index(AI) was the ratio of positive apoptotic myocardial cells and the mean of myocardial cells in 10 fields.

(6) MI size was evaluated by 2, 3, 5 triphenyltetrazolium chloride (TTC) dyeing method: Evan's blue 3ml (2 g in 100 ml) was injected into left ventricle after the IR, then 5 ml KCL (10 g in 100 ml) was injected into ventricle. The heart was isolated and cut into 5 slices, then put the ischaemic and infarcted myocardium into TTC (1 g in 100 ml) at 37～40℃ for 1O min. The infarcted regions was negative but the risk and normal regions were stained brick red by TTC.

(7) Statistics:

The *P* values for the experiments were calculated using the unpaired Students *t* test. The statistical software was SPSS Version 13.0. Data were indicated as SD±s, α=0.05.

3. Results

(1) Livin amplification results

Electrophoresis result: bright strap at 1000bp is accordance with theoretical value 816bp, seen in figure 1A.

(2) Electrophoresis results of recombinant plasmid pGEM-T-livin amplification production. Positive clone at 992bp (Fig 1B).

(3) Electrophresis results of recombinant plasmid pGEM-T-livin digested by XhoI and HindIII : 2 straps were seen at 816bp and 3027bp (Fig 1C).

(4) Recombinant clone pLNCX2-Livin identified by enzyme digest(Fig 1D).

(5) Positive recombinant clone pLNCX2-Livin identified by PCR:positive clone pLNCX2-Livin, Fig 1E.

(6) Real time PCR amplification results: Livin mRNA expression increased significantly in livin group compared with those in IR group, control group and empty vector group. Caspase-3 mRNA expression increased during IR, but decreased significantly in livin group (Table 1).

(7) Results of Caspase-3 protein expression: The levels of caspase-3 protein expression was 91.39 ± 4.82 in control group, 103.39 ± 8.24 in IR group, 97.43 ± 11.15 in livin group and 102.28 ± 7.83 in empty vector group, respectively. The expression levels of caspase-3 protein were increased significantly in IR group and in empty vector group compared with that in control group. And the caspase-3 protein decreased after transfecting retroviral vector expressing livin (Fig 2, Fig 3).

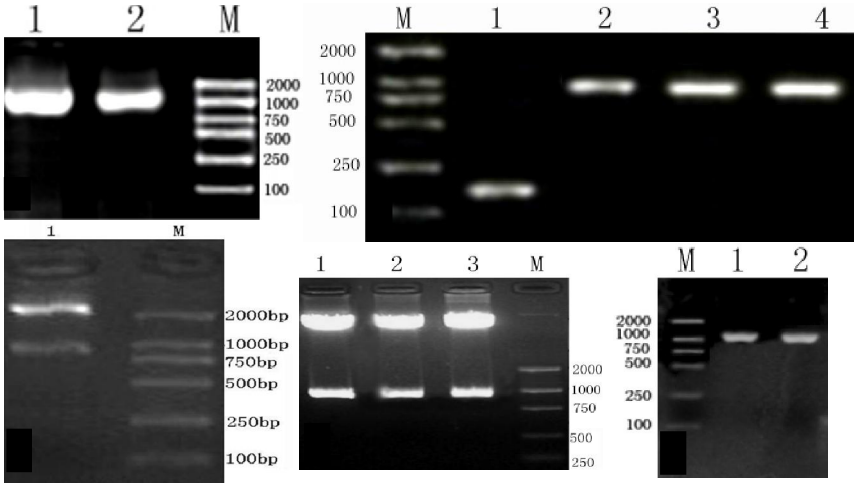

Fig 1A. Livin amplification results 1,2:livin amplification production ; M:Marker
Fig1B. Electrophoresis results of recombinant plasmid pGEM-T-livin amplification production.
M ：DNA marker；1: pGEM-T；2-4: positive clone pGEM-T-livin.
Fig 1C：Electrophresis results of recombinant plasmid pGEM-T-livin digested by XhoI and HindIII:1：XhoI and HindIII digested pGEM-T-livin；M：DNA Marker.
Fig 1D: Recombinant clone pLNCX2-Livin identified by enzyme digest:
M：DNA Marker; 1-3： Electrophresis results of recombinant clone pLNCX2-Livin digested by XhoI and HindIII enzyme.
Fig 1E: Positive recombinant clone pLNCX2-Livin identified by PCR: M：DNA Marker;
1-2：positive recombinant clone pLNCX2-Livin identified by PCR (primier: Pln1/Pln2).

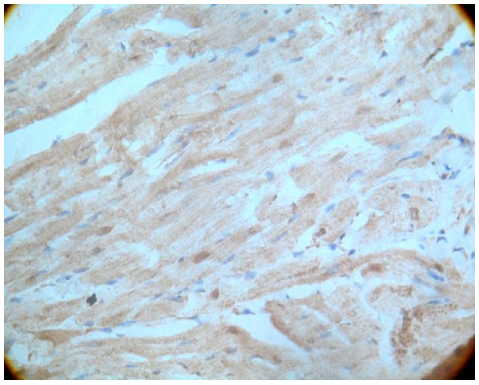

Fig 2. expresssion of caspase-3 in IR group.

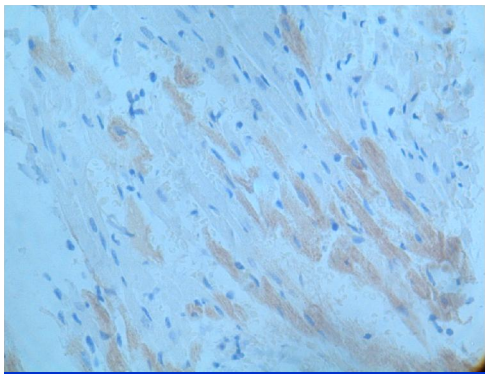

Fig 3. expresssion of caspase-3 in livin group. (10) Myocardial apoptosis: The apoptosis index (AI) was （1.1±0.42）% in control group, （10.35±3.34）% in IR group, （1.7±1.57）% in livin group, and (9.62±3.68)% in empty vector group respectively. AI increased significantly in IR group but decreased after transfecting the retraviral vector expressing livin.

(11) MI size: The MI size was 27.82%± 9.77% in IR group and 19.55% ±2.82% in livin group. The MI size decreased significantly in livin group.

Table 1. Livin and Caspase-3 mRNA expression

group	n	Livin mRNA ($\times 10^{-3}$)	Caspase3 mRNA ($\times 10^{-3}$)
Control group	20	8.41±1.39	5.12±2.11
IR Group	20	7.82±3.22	92.1±34.6 *
Livin Group	20	145±89 * #	56.2±21.1 * #
Empty vector Group	20	7.45±2.51	94.1±18.1*

* compared with control group $P < 0.01$; # compared with IR group $P < 0.01$.

4. Discussion

Acute myocardial infarction was frequently followed by myocardial ischemia reperfusion injury (IRI) because of the opening of occluded coronary arteries[1~3]. The mechanisms of IRI have not been elucidated. Recent studies reported that cardiomyocyte apoptosis increased in ischemia reperfusion(IR)[4]. We detected the caspase-3 mRNA expression in normal rat myocardium and in ischemic reperfusion model. There was a significantly increase of caspase-3 expression in rat heart after 30 minutes ischemia and followed by 2 hours reperfusion compared with that in the control conditions.This supported the report that cardiomyte apoptosis play an important role in the myocardial ischemic reperfusion injury[5~7].

Apoptosis is a process whereby cells undergo programmed death. The mechanism that apoptosis taken place is unclear yet. Apoptosis relies on activation of distinct signalling pathways. The activation of caspase-3 has been found in many models of apoptosis and it may play a pivotal role in a downstream event of caspases cascade and the occurrence of apoptosis[8-10]. Inhibitor of apoptosis protein(IAP) can combine to caspase and suppress caspase-3. Livin was one of IAP family discovered recently. Livin contain a baculovirual inhibitor of apoptosis repeats(BIR domain) and a ring zinc finger domain (RING). BIR domain is the functional area by which livin could combine with caspase and suppress its activity and suppress apoptosis[11,12]. Livin was seen in tumour tissue and was very few in myocardium. It is unknown that whether livin could suppress myocardium apoptosis in rat or not so far.

We measured the expression of livin mRNA in normal myocardium and in ischemic reperfusion myocardium in rat. The results indicated that there was little livin expression in normal rat heart and the livin expression did not increase during the IR process.

Retroviral vector is a stable and effective gene expression vector. In the present study we constructed and produced a retroviral vector expressing livin and transferred it into rat myocardium. By real-time PCR detection we found that the transfection livin vector into rat myocardium was successful. And caspase-3 expression decreased significantly after the transfection of retroviral vector expressing livin. These indicated that livin overexpression could suppress the expression of caspase-3.

Using TUNEL assay we evaluated the changes of myocardial apoptosis during IR. The results indicated that apoptosis index increased during the IR process but decreased significantly in livin group. This suggested that livin overexpression could suppress myocardial apoptosis during IR process and this might be actualized through the suppression of caspase-3.

Myocardial ischemia reperfusion injury is complex and hard to cure. Methods used in present clinical such as vasodilation, thrombolysis and so on were ineffective. Anti-apoptosis is a new idea to prevent the IRI and its effection is worth to expect.

In conclusion, the present study provided a clear evidence that cardiomyocytes apoptosis during myocardial ischemia reperfusion is related to the myocardial ischemia reperfusion injury. Livin overexpression could suppress the activation of caspase-3 and then downregulate cardiomyocyte apoptosis. These indicate that transfecting retroviral vector expressing livin might be useful for the treatment of myocardial ischemia reperfusion injury.

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Selected Ventilatory Functions Response to Closed and Open Kinematic Chain training of the arm in elderly

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Abstract: Aging is associated with pulmonary alterations; these changes culminate in a decrease in muscle strength, lower level of endurance and impairment of mobility. Fortunately, increasing the level of physical activity may affect the declines of these parameters. The present work aimed to investigate the effect of closed vs. open kinematic chain exercises on ventilatory functions in elderly subjects. Thirty elderly subjects (13 female and 17 male) participated in the study their age ranged from 60 to 75 years. They were divided into two study groups equal in number. Group I comprised of 15 subjects received a training program of closed kinematic chain "supported arm exercise" and group Π received a training program of open kinematic chain "unsupported arm exercise". Hand held Spirometer was used for measuring ventilatory functions. Arm ergometer, was used for closed kinematic chain (supported arm exercise group). Both groups were trained for 8 weeks, three times a week. The results showed that the vital capacity, the forced expiratory volume in 1st second, and the maximum voluntary ventilation were significantly improved in both groups but the percentage of improvement was significantly higher in group I of closed kinematic chain training. It is concluded that the outcomes of this study may help to outline the most effective, curative and safety type of arm exercise to be included in training programs for pulmonary and orthopaedic problems in elderly.
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Key words: kinematic chain, mechanics of shoulder elevation, pulmonary functions

1. Introduction

Aging is associated with diminished physical ability as a result of a decrease in muscle strength, endurance, flexibility and neuromuscular coordination. In addition, aging is accompanied by the development of many disabling diseases. Although the chronological criterion that is presently used for identifying the old has been set at 65 years, yet the onset of some of the health problems of elders may occur as soon as they enter their early 50. (Guccione, 2000)

As aging progress, the respiratory system undergoes a measurable decline in the physiologic function. The thoracic cage stiffens with advancing age; with the increased possibility for increased kyphosis coupled with increased work demand of the respiratory muscles thus increases the work of breathing. There are significant changes in the functions of the pulmonary system including: decreased FEV1, FVC and VC, increased RV, and increased FRC. The most important consequence of age-related changes that occur is the reduction in the physiologic reserve of the respiratory system (Robergs and Roberts, 2000).

Previous studies have showed that scalenes are not accessory respiratory muscles as commonly considered, but are active during quiet inspiration. On the other hand , the accessory muscles which are silent in normal breathing are recruited as the ventilation increases. The accessory muscles include: - sternocleidomastoid, extensors of the vertebral column, pectoralis minor, trapezius and serratus muscles. Many of these muscles reverse their usual origin/insertion and help to expand the chest, provided the arms and shoulder girdle are fixed by grasping a suitable support; closed chain (Lumb, 2000).

The performance of many everyday tasks requires use of not only the hands, but also other muscle groups that are used in upper torso and arm positioning. Some of these muscle groups serve respiratory as well as postural functions, so arm exercise can improve ventilation. If the arms are trained to perform more work, or if the ventilatory requirement for the same work is decreased. Moreover, the capacity to perform activities of daily living could improve with parallel increase in strength and endurance of respiratory muscles and so decreased oxygen uptake at the same workload (Celli, 1998).

Although it is possible that arm activities are limited by weak shoulder and arm muscles, it is likely that the ability of patients with chronic

obstructive arm diseases to sustain arm exercises is determined not only by the strength & endurance of arm muscles, but also by the influence of the arm position itself on ventilatory mechanics (Dolmage *et al.,* 1993). The circulation of blood to the tendon also depends on muscle tension. In the tendon, circulation will be inversely proportional to the tension. At very high tension levels, circulation may cease completely. Recent studies have shown that the intramuscular pressure in the supraspinous muscle can exceed 30 mm Hg at 30 degrees of forward flexion or abduction in the shoulder joint. Impairment of blood circulation occurs at this pressure level. Since the major blood vessel supplying the supraspinous tendon runs through the supraspinous muscle, it is likely that the circulation of the tendon may even be disturbed at 30 degrees of forward flexion or abduction in the shoulder joint. Moreover, Arm exercises includes motor control hypothesis that varies according to testing or training conditions (Brindle *et al*., 2006)

Closed kinematic chain training such as lifting free weights or dowels and stretching elastic "Thera" bands, in contrast to open kinematic chain exercise such as arm ergometer and shoulder wheel results in improved upper extremity function. As improvement in unsupported arm activity is likely of greater clinical significance. A program of simple unsupported arm training is the ideal format to achieve this. (Martinez *et al.,* 1993; Lareau *et al.,* 1999).

Simple open kinematic chain arm elevation like a trivial task result in significant increase in the metabolic and ventilatory demands in normal subjects' .This is associated with an increased contribution of the diaphragm to ventilation (Couser *et al.,* 1993). Open and closed kinetic chain exercises appear to be equally effective in improving shoulder joint reposition sense which suggest that shoulder joint reposition sense can be enhanced with training in healthy subjects (Rogol *et al.,* 1998).

Concerning the possible difference between training of the arm and leg on pulmonary functions , results varies .Minute ventilation at peak leg exercise was significantly higher than at peak arm exercise in normal subjects and in mild and moderate cystic fibrosis patients, as well as at peak exercise, the workload for leg exercise were significantly higher than for arm exercise (Alison *et al.,*1998).

Musculoskeletal changes with aging result in common problems such as degenerative changes in intervertebral disk (cervical spondylosis) and frozen shoulder (due to stiffness in peri-articular connective tissue). For which upper limb exercises are commonly considered for inclusion as a main part in many physical therapy programs for those persons (Guccione, 2000).

Previous authors demonstrated a variable difference in the ventilatory response to supported and unsupported arm exercises in patients with chronic obstructive pulmonary disease. It was found that within COPD group, peak work level, peak VO2 and VE were significantly lower for unsupported arm exercise than for both leg and supported arm exercise (Mckeough *et al.,* 2003a).

In a study conducted by Morrissey *et al.* (2000) comparing the open kinematic and closed kinematic chain in knee surgery. The authors concluded that there are no clinically differences in the functional improvement resulting from the choice of OKC and CKC exercises in the early period of rehabilitation. The findings were delimited because of the short period of supervised rehabilitation (2-6 weeks)

This study might be the first one applied to investigate the effect of open and closed kinematic chain arm exercises on the ventilatory function in healthy elderly subjects.

Hypothesis:

It was hypothesized that there were non statistical significant differences in ventilatory response for closed vs. open kinematic chain arm exercises in elderly subjects.

2. Subjects, Materials, and Procedures

Subjects:

Inclusion criteria:

- Thirty elderly right handed subjects (13 female and 17 male) participated in the study.
- Anthropometric measurement: Their ages ranged between (60-75) years with a mean of 65.83 ± 5.08 years. Their weight ranged between (57- 95) Kg with a mean of 81.2 ± 9.14 Kg. While their height ranged between (155-180) cm with a mean of 168.47 ± 6.19 cm.. Body mass index mean is 22.46 ± 4.28
- Subjects selected, with mild to moderate level of activity as most of them were employee; some were teachers while most of females were house wives.
- All of the elderly did not receive any physical therapy programs related to respiratory training before they were participating in the study.

Exclusion criteria:

All non smokers' participants were examined by the responsible physician to exclude sever renal disease, liver disorders, obese and diabetic or sever hypertensive subjects, neurological dysfunction as cerebral stroke, parkinsonism, neuropathy or psychological or mental impairments. In addition severe chronic cardiac problems as heart failure, ischemic heart disease, and coronary artery by pass graft. Chronic chest disease as chronic obstructive lung

disease, restrictive lung disease or chronic chest infection, fibrosis or suppuration or other problems that may interfere with movement as musculoskeletal deformities as scoliosis, kyphosis and kyphoscoliosis, chronic inflammatory orthopedic disorders and rheumatoid arthritis were also excluded.

Materials:

A. Evaluation Equipment:

Electronic Spirometer:

Futuremed Discovery Hand held Spirometer made in Germany fig(1) used for measuring ventilatory functions with disposable mouth piece and nasal clips. It is a computerized apparatus with an electronic memory allowing on a single forced exhalation, the forced vital capacity & the maximum voluntary ventilation values.

Body weight and height scale:

Health made in china, used to measure the subject's weight and height & calculate the body mass index (BMI) according to the formula:

BMI = body weight in kilograms/ height in meter squared (**Barreto *et al.*, 2003**)

Mercury sphygnomanometer & pulsoxemeter:

Speidel, used to measure blood pressure before and after each session.

Fig. (1) Futuremed Discovery Hand held Spirometer

B. Training Equipment:

Arm Ergometer:

Monark Rehab Trainer, Model 881E, made in Sweden, used for closed kinematic chain training (supported arm exercise group). It has the ability for individual calibration according to each subject. Crank arms are individually adjustable both horizontally and vertically according to the subject height in order to be comfortable starting at horizontal level arm position 90 degrees shoulder elevation. It's graduated scale in Watts, showing the workload in 50 r/min & its electronic readouts showing; pedal r/min, total pedal revolutions and time.

Wooden Bars of different weights that were designed with hand grip in the middle third of the bar were used for comfortable grasping during the exercise (Mckeough *et al*., 2003a).

Procedures:

The subjects were assigned into two groups equal in number:

The first group trained in a closed kinematic chain "supported arm exercise group" SAE group: trained with the ergometer. The second group trained as open kinematic chain "unsupported arm exercise group" UAE: include subjects who received a training program with wooden bars.

Participants of both groups received a thorough explanation of the procedures and duration

before starting the study. A written consent form was obtained from each subject before participating in the study. Both groups were trained for 8 weeks, three times a week

The study procedures were carried out at Outpatient Clinic of the Faculty of Physical Therapy, Cairo University.

A. Evaluation procedures:

After the subjects were carefully chosen by physician, all of the subjects underwent several evaluation steps including the following:

1) Preliminary assessment:

It was undertaken by a physical therapist. Careful history was taken for any pervious chest diseases, smoking, any old fractures or traumatic insults to upper limbs and any neurological problem that may hinder the movements of the arm or cause pain during the exercise. Postural examination while the subject was in standing position (Thomas and Strandberg, 2004). Range of motion examination & gross muscle testing for all the joints & muscles of the upper limb of both sides were examined to insure comfortable and complete joint motion during exercises & insure normal muscle strength and endurance (Kinser and Colby, 1990; Bickley, 2003). Vital signs ;Blood pressure, respiratory rate and heart rate were measured and recorded initially then before and after each session through the training program, to insure stable hemodynamic condition. Then BMI was calculated to insure normal average.

2) Ventilatory functions test:

Subject preparation:

- The subjects were required to:
  1) Avoid eating a heavy meal just before the test
  2) Avoid performing any excess effort for 6 hours before the test.
  3) Wear loose clothes that don't restrict breathing in any way.
- While the subject was sitting on stool with trunk unsupported, he was asked to place the nasal clip around the nose. Then the mouth piece was firmly put into his mouth. The subject was asked to breathe few times before starting procedures. After that he was asked to inhale fully and exhale as slowly and as completely as possible, then breath normally again till test ends to examine the VC. For MVV manoeuvre, the subject was asked to put a disposable mouth piece in his mouth tightly, inhale and exhale fully as completely, as fast as possible for 12 seconds. Each manoeuvre was repeated for three successive times and the greatest reading was obtained and recorded. All the previous measures have been recorded and stored then repeated again at the end of the study period (eight weeks).

3) Determination of Target Heart Rate:

Target heart rate (THR) was calculated using Karvonen formula (Quoted and adopted from Sullivan and Schmitz, 1994).

4) Determination of Exercise intensity for closed kinematic chain "SAE "group:

Subject preparation:

The subject was seated with back completely supported and his feet rested on the ground for 10 min to gain homodynamic stabilization. The resting pulse rate, respiratory rate and blood pressure were then measured and recorded. Then the probe of the pulse meter was applied to the subject's ear to measure the heart rate during the exercise.

Instrument preparation:

The arm ergometer machine was placed on an adjustable table so that the fulcrum of the handle was at the level of the subject's shoulder. The cycling speed was adjusted at 50 revolutions per minute (Martin *et al.,* 1991; Regnis *et al.,* 1997; Alison *et al.,* 1998).

Exercise test protocol for group I:

The exercise started by warming up for 10 min with no resistance (0 watt) & terminated with cooling done for 10 min. During the conditioning phase the resistance was increased by 5 watts every one minute until the subject reached calculated target heart rate (THR) this level of resistance obtained is recorded for analysis (Martinez *et al.,* 1993; Mcardle *et al.,* 2001).

The exercise test was repeated after four weeks in the same steps to readjust the intensity of exercise.

5) Determination of Exercise intensity for open kinematic chain " UAE" group:

Subject preparation:

The same preparation steps as closed kinematic chain SAE group. The different sizes wooden bars were put on the table near to the subject.

Exercise test protocol:

The subject started the exercise test with warming up for 10 minutes, using 300g bar, and flex his shoulders as high as he can without moving trunk forward from his waist to the horizontal arm position (with extended elbows) and back to the waist. After the warming up the subject lifted the different sized bars and moved each one from the waist to the 90 degrees shoulder flexion for 10 repetitions while notice the respiratory rate, heart rate, breathing pattern and the possibility for dyspnoea.

This procedure was repeated using bars with different weights, until the subject reached the maximum weight, according to his calculated target heart rate. This maximum obtained weight is recorded for analysis. After that the subject performed cooling down for 10 minutes exactly like warming up procedure using 300g bar(Mckeough *et al.,* 2003a).

The exercise test was repeated after four weeks in the same steps to readjust the intensity of exercise.

B. Training procedures:

1. Exercise session for SAE group:

After warming up exercise for 10 minutes with no resistance (0 watts), the subject started pedaling at the level of resistance obtained from exercise test (60% of THR) for 15 minutes. Then ,the resistance is decreased to 0 watt and he terminated the session with cooling down for 10 min (Martin *et al.,* 1991; Martinez *et al.,* 1993; Regnis *et al.,* 1997; Alison *et al.,* 1998; Mcardle *et al.,* 2001).

2. Exercise session for UAE group "open kinematic chain":

After warming up using 300g bar for 10 min in the same pattern as he previously trained. Then he was trained with the 60% of the weight recorded from exercise test for 15 min. At the end he performed cooling down by using 300g bar for 10 min (Mckeough *et al.,* 2003a).

3. Results:

Concerning the subject characteristics

This study compromised 30 healthy elderly subjects who were selected from Dar Hediet Barakat in Giza for nursing home. Their age ranged from 60 to 75 years, their height ranged from 155 to 180 cm and their weight ranged from 57 to 95 Kg (Table 1).

Table (1): Anthropometric characteristics of all subjects.

Item	Mean ± SD
Age(year)	65.83±5.08
Weight(Kg)	81.2±9.14
Height(cm)	168.47±6.19
BMI	22.46 ± 4.28

Kg: kilogram. Cm: centimeter. BMI body mass index. SD: standard deviation.

Anthropometric Data in both Groups:

From table (2): we can notice that there was no significant statistical difference between groups in Age, Height, Weight and Body mass index as P. value > 0.05.

Table (2): Comparison between the two Study Groups in Age, Height, Weight and BMI.

Item	Group I	Group Π	t-value	Level of significance
Age(year)	67.47 ± 4.49	64.2 ± 5.25	1.83	P. value >0.05
Height(cm)	170.33 ± 5.46	166.6 ± 6.48	1.71	P. value >0.05
Weight(kg)	79.47 ± 11.09	82.93 ± 6.58	1.04	P. value >0.05
BMI	22.46 ± 4.28	23.97 ± 2.9	1.87	P. value >0.05

BMI: body mass index P.value > 0.05 is non significant.

Concerning the gender distribution of both groups there was no significant statistical difference in gender distribution in the studied groups as P. value >0.05, as shown in table (3).

Table (3): Comparison between the Percentages of Male to Female subjects in both groups.

Item	Grou p 1	Grou p 2
Male	10 66.7 %	7 46.7 %
Female	5 33.3 %	8 53.3 %
x^2 value	1.18	
Level of significance	P. value >0.05	

X^2: Chi-squared test P.value > 0.05 is non significant

Comparison between the Pre& Post Study mean of Forced Expiratory volume in 1st second values in both Groups:

As shown in table (4) the pre-study mean values of FEV1 was 1.77 ± 0.63 L/S in group I and 1.45 ± 0.54 L/S in group Π. This indicated a non-significant statistical difference of FEV1 between group I and group Π before closed & open KC arm exercise with P. value >0.05 L/S. While the post-study mean values of FEV1 2.56 ± 0.44 L/S in group I and 1.83 ± 0.55 L/S in group Π. This indicated highly significant increase of FEV1 after supported arm exercise (CKC) than unsupported (OKC) arm exercise with P-value <0.01 L/S.

Table (4): Comparison between the Pre& Post Study mean values of Forced Expiratory volume in 1st second values in both Groups.

Item	Pre-study		Post-study	
	Group I	Group Π	Group I	Group Π
Mean	1.77	1.45	2.56	1.83
± SD	0.63	0.54	0.44	0.55
t value	1.54		3.98	
Level of significance	P. value >0.05		P. value <0.01	

SD: Standard Deviation -value >0.05 is non-significant t: paired t-test P-value <0.01 is highly significant.

Comparison between the Pre & Post Study mean values of Vital Capacity in both Groups:

As shown in table (5), the pre-study mean values of VC was 2.12 ± 0.29 L in group I and 1.88 ± 0.38 L in group Π. This represented a non-significant difference between the both groups in VC before the study with P. value >0.05 L. While the post-study mean values of VC was 2.85 ± 0.36 L in group I and 2.29 ± 0.55 L in group Π. This showed a highly significant improvement of VC with favour for supported arm exercise group with P. value <0.01.

Table (5): Comparison between the Pre & Post-study mean values of Vital Capacity in both groups.

Item	Pre—study		Post-study	
	Group I	Group Π	Group I	Group Π
Mean	2.12	1.88	2.85	2.29
± SD	0.29	0.38	0.36	0.55
t value	1.94		3.29	
Level of significance	P. value >0.05		P. value <0.01	

SD: Standard Deviation P-value >0.05 is non-significant tp: paired t-testP-value <0.01 is highly significant

As illustrated in table (6) the pre-study mean values of MVV was 56.67 ± 18.02 L/min in group I and 50.43 ± 13.35 L/min in group Π. This indicated a non-significant difference of MVV between both groups before the study with P. value >0.05 L/min. On the other hand the post-study mean values of MVV were 97.11 ± 16.07 L/min in group I and 64.69 ± 16.06 L/min in group Π. This revealed a highly significant increase of MVV after CKC than OKC arm exercise with P. value <0.01 L/min.

Table (6): Comparison between the Pre-study mean values of Maximum Voluntary Ventilation in both groups.

Item	Pre-study		Post-study	
	Group I	Group Π	Group I	Group Π
Mean	56.67	50.43	97.11	64.69
± SD	18.02	13.35	16.07	16.06
T value	1.08		5.53	
Level of significance	P- value >0.05		P- value <0.01	

SD: Standard Deviation P-value >0.05 is non-significant tp: paired t-test P-value <0.01 is highly significant

From table (7) it can be noticed that the mean difference of VC between both groups shown a non-significant statistical difference as P. value > 0.05.

Table (7): Comparison of the mean difference between ventilatory function readings in both groups.

Item	Mean Difference		t-value	Level of significance
	Group 1	Group 2		
FEV1	0.782	0.383	2.86	P. value < 0.01
VC	0.731	0.504	1.66	P. value >0.05
MVV	40.44	14.26	6.24	P. value < 0.001

FEV1: forced expiratory volume in 1st second
VC: vital capacity
t-value: student t-test
P. value < 0.01 or < 0.001 is highly significant
MVV: maximum voluntary ventilation
P. value > 0.05 is non-significant
P. value <0.05 is significant

From table (8) one can notice that in both groups there was a high significant improvement in all ventilatory functions

Table (8): Comparison between the percentages of improvement of ventilatory function readings in both groups:

Item	Group I	Group Π	Level of significance
FEV1	55.39 %	30.53 %	P. value < 0.01
VC	36.14 %	36.01 %	P. value >0.05
MVV	83.11 %	29.46 %	P. value < 0.001

FEV1: forced expiratory volume in 1st second
VC: vital capacity MVV: maximum voluntary ventilation
P. value > 0.05 is non-significant. P. value < 0.01 or < 0.001 is highly significant
P. value <0.05 is significant

Correlation between the percentage of improvement and Age in group I:

As shown in table (9), the correlation coefficient value between the percentage of improvement of, FEV1, MVV and age were 0.14 & 0.06 respectively, which means non-significant positive correlation as p-value >0.05. While the correlation coefficient value between the percentage of improvement of VC and age was -0.18, that means non-significant negative correlation as p-value >0.05.

Table (9): Correlation between the percentage of improvement of all variables and Age in group I.

Relative variable	r-value	P-value	Level of significance
FEV1 & age	0.14	p-value >0.05	r-value <0.48
VC & age	-0.18	p-value >0.05	r-value <0.48
MVV & age	0.06	p-value >0.05	r-value <0.48

r-value: Correlation coefficient value.
FEV1: forced expiratory volume in 1st second
VC: vital capacity MVV: maximum voluntary ventilation

Correlation between the percentage of improvement and Age in group Π:

As illustrated in table (10), the correlation coefficient value between the percentage of improvement of FEV1, MVV and age were 0.01 and 0.36 respectively, which means non-significant positive correlation as p-value >0.05. While the correlation coefficient value between the percentage of improvement of VC and age was -0.19, that means non-significant negative correlation as p-value >0.05.

Table (10): Correlation between the percentage of improvement of all variables and Age in group II.

Relative variable	r-value	P-value	Level of significance
FEV1 & age	0.01	p-value >0.05	r-value <0.48
VC & age	-0.19	p-value >0.05	r-value <0.48
MVV & age	0.36	p-value >0.05	r-value <0.48

r-value: Correlation coefficient value. r-value <0.48 is non-significant FEV1: forced expiratory volume in 1st second
VC:vital capacity MVV:maximum voluntary ventilation

4. Discussion

This study measured the effect of different types of arm exercises on the ventilatory functions in the elderly subjects. Arm exercises include two types; closed kinematic chain "supported" arm exercise like using arm ergometer and pen kinematic chain "unsupported" arm exercise using free weights as a base.

Thirty healthy elderly subjects (13 female and 17 male) enrolled in this study. The evaluation of ventilatory functions including VC, FEV1 & MVV were recorded before the training program & repeated at the end of the 8th week of training program for each subject. The selected measurements used in this study are considered as indicators for the integrity of respiratory system, elastic recoil of the lung and airway resistance as well as strength and endurance of the respiratory muscles (Talavera *et al.,* 1998).

All the participants were assigned into two studied groups. The first group "SAE" group has performed supported arm exercise using arm ergometer at 60% of THR. While the second group "UAE" group has performed unsupported

arm exercise using wooden bars with 60% of the weight at THR weights (Mcardle *et al.,* 2001; Mckeough *et al.*, 2003a).

Analysis of the result of the present study indicated that supported arm exercise in the form of arm ergometer can increase VC. This improvement of VC may be due to improvement of the mechanical behaviour of thoracopulmonary system. The arm exercises result in mobility of rib cage in addition to change and improvement of posture of head, neck and shoulders thus increasing chest wall compliance (Plekonen *et al.,* 2003)

Concerning the dynamic lung volumes and flow rates recorded in this study; forced expiratory volume at first second and maximal voluntary ventilation showed significant improvement after closed kinematic chain training "supported arm exercise". The increment of MVV may be referred to the improvement in the respiratory muscles strength and endurance as well as improved co-ordination. In supported arm exercise, the arms are supported thus increasing the ventilatory contribution of the inspiratory muscles of the neck and rib cage decreasing the contribution by the diaphragm thus decreasing the ventilatory demand as well as oxygen demands allowing longer time for exercise (Martinez *et al.,* 1993).

Since the subjects in the present study didn't suffer from any respiratory illness except the normal physiological changes of aging. The resultant improvement in FEV1 may be referred to increased respiratory muscle strength and better coordinated use of all musculature in expelling the air with greater response to closed kinematic chain in supported arm exercise indicating better response to this type.

With regard to the open kinematic chain "UAE" group by using wooden bars the results demonstrate increased VC as well. This improvement of VC may be due to increase in thoracic expansion during the exercise as when the arms are elevated the chest is placed in an inflated position in addition to improvement of thoracic mobility. When the arms are elevated above 90 degrees, some muscles as pectoralis will expand the rib cage by passive stretching, whereas others, such as serratus anterior will do so by active contraction (Mckeough *et al.,* 2003b).

The dynamic lung volumes including FEV1 and MVV have showed a significant improvement after unsupported arm exercise. The improvement of MVV may be referred to increase in inspiratory and expiratory muscles power and endurance capabilities as well as improved compliance of the lung-thorax system and so the ability of respiratory muscles to contract and relax rapidly and deeply is enhanced (Plekonen *et al.,* 2003).

The FEV1 is increased as a consequence of enhancement of strength and endurance of respiratory muscles, in particular the diaphragm, which is reflected on the increase in the lung volumes and leads consequently to increase in the flow air out of the lung.

Although the statistical analysis between the pre and post study values in both groups showed significant improvement in most of variables, on comparing the result of both groups regarding the percentage of improvement one can notice some difference between both groups.

In the open kinematic chain "UAE" group, the performance of such training may displace the respiratory functions of the scapular belt muscles to a more antigravity function, thus increasing the work done by the diaphragm and the ventilatory demand. In addition, exercises with the arms elevated and unsupported keeps the arm muscles under high tension and decrease the arm blood flow due to the increase in adrenergic vasomotor tone. This response seems to be more pronounced in small muscle groups, thus causing early muscle fatigue and shortening the length of time for any arm activity. These in turn explain the more improvement in the respiratory muscles strength and endurance and improved coordination in "SAE" group (Velloso *et al.,* 2003).

However, in closed kinematic chain training "SAE" group, the arms are supported thus decreasing load on muscles of shoulders and upper torso and so increasing their contribution to the ventilatory demand. These explain increased respiratory muscles strength and endurance and improved coordination in the "SAE" group.

In a comparison between these two modes , Electromyographic activities of infraspinatus, posterior deltoid, anterior deltoid, pectoralis major, and supraspinatus muscles are reported to be reduced during supported than unsupported training of shoulder joint. This reduction was probably due to an unloading effect as supporting the limb helps to diminish the weight of the arm, thereby decreasing the demand on the shoulder musculature so decreasing oxygen consumption and improving muscle efficiency (Wise *et al.,* 2004).

It is important to understand possible CNS control strategies behind movements with various types of peripheral feedback and under a variety of

conditions. The addition of an external load in a closed or open chain is typical examples. The shoulder including scapular movement together with the knee joint reflect the change in mechanics with the shift from open to closed kinematic chain including forces (Dillman *et al.,* 1994).

It has been reported that, to establish equilibrium at the glenohumeral joint at any position, a minimum of three forces is required: (1) the weight of the arm, (2) the working musculature, and (3) the resultant of the former forces. Thus, by decreasing one component, for example, the weight of the arm, the other two components must decrease to maintain equilibrium that occurred during "SAE, in which part of the arm weight was carried or supported by the handles of the arm ergometer (Apreleva *et al.,* 2000).

In the present study, during closed kinematic chain "SAE", the subject performs forward elevation of the arms from 70-75 degrees to about 90-100 degrees as the fulcrum is positioned at shoulder level. While during open kinematic chain "UAE", the subject perform flexion from 0-10 degrees to about 130-140 degrees. So the difference between both groups can be explained on biomechanical basis as during each exercise several muscles are working with different levels of activity according to degree of arm elevation.

Studying the electrical activities of shoulder girdle and trunk muscles during varying level of shoulder elevation showed difference in activities. It has been found that EMG activity of deltoid (anterior, middle, posterior) increases throughout elevation to about 100 degrees with the greatest amplitude occurring for anterior deltoid and then plateaus. The same occurs for supraspinatus, but its activity reaches peak at 80-100 degrees and then decreases. The pectoralis major assists anterior deltoid in elevation with the calvicular portion being the more active. While for trapezius muscle; the upper fibers in addition to serratus anterior are active throughout arm elevation with gradual increasing activity, reaching peak at maximal elevation. Secondly; little activity was observed in the lower trapezius until after 90 degrees of elevation, with sharp rise in activity thereafter, reaching peak at latter stages. (Gray *et al.,* 1989; Diver, 2000)

From the above, it is clear that the activity of trapezius (upper, middle, lower), serratus anterior, rhomboids, subscapularis, and teres minor muscles were higher in unsupported than supported arm exercise while for deltoid and supraspinatus muscles, the activity was the same in both modes of exercise.

On studying FEV1, which reflect a volume-time relationship measured from the FVC, depending on both diaphragmatic force and upper airway flow showed increase in response to open kinematic chain in the "SAE" group by about 55.39% while in the "UAE" group it increased by about 30.53% as a response to closed kinematic chain training.

In agreement with other previous studies, Dolmage *et al.*(1993) have reported that oxygen consumption, carbon dioxide production and minute ventilation were significantly greater during unsupported arm elevation than during supported arm elevation by a customized sling. These changes are attributed to the increase in metabolic activity of the active arm muscles that maintain this position.

One of the recent studies that support our results was applied by Mckeough *et al.*(2003b) who concluded that, unsupported arm position affect static lung function and altered lung mechanics in chronic obstructive lung pulmonary disease and healthy subject. Functional residual capacity is (FRC) significantly increased while inspiratory capacity (IC) and total lung capacity (TLC) are significantly decreased with arms above 90 degrees shoulder flexion when compared with both arms below 90 degrees shoulder flexion. With the arms above 90 degree, the chest wall is already in an inspiratory position so that relatively less chest wall expansion. In addition the stretch on latissimus dorsi muscle causes it to act like a tight band around the rib cage restricting complete expansion.

These findings and explanation was confirmed also by Mckeough *et al.*(2003a), who found that peak Vo2 and peak VE were significantly lower for the unsupported arm exercise test than for both leg exercise and the supported arm exercise tests. Mechanical constraint to ventilation during unsupported arm exercise test would have resulted from restriction to chest wall expansion when arms were positioned above head in addition the chest wall muscles act to position and stabilize the arms and torso.

On the other hand, the results of this study contradict the early study of Couser *et al.*(1992) who investigated the respiratory response to unsupported arm elevation for 2 minutes and down at the sides. They showed no significant change of FEV1 and FVC. The difference of the results between the present study and that study

may be due to different protocol they studied as they applied a test to specific position for two minutes and wide age group ranged from 22 to 72 year.

Some of the findings of Lake *et al.*(1990), and Couser *et al.*(1993) , were contradicted with the results of the current study as they found that the VC decreases significantly during arm elevation with no change in the pulmonary functions including FEV1,. This may be due to different sample characteristics & differences in the exercise protocol concerning the weight used and duration of the training.

In conclusion it was recommended that close kinematic chain mode of exercise "SAE" is the exercise of choose for training elderly with any musculoskeletal or pulmonary problems considering efficiency and safety.

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Cultural & social effects of rural women's financial self-reliance

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Abstract: Rural women are among those major groups at society who previously were considered less by planners, due to specific reasons in the past. And this problem is more observable at developing countries. If rural women can work through receiving credits , loan and others finance facilities at favorite jobs and live through earned income (as it called "self-reliance and independence"), so undoubtedly we would see changes in social, economic and cultural relations of village.
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Introduction:

Women form great part of total workforce that needed for agriculture part at universe, as one of the intangible factors at agriculture economy. So, statistics that was represented in relation to extent of women's activity is very lower than real extent. Because in this statistics, mostly, seasonal jobs, part time job, no wage job and their housekeeping activities, aren't considered. rural women, have different roles and duties such as husband, mother, crops producer , participate at ranching activities , planting ,maintaining, harvesting, processing, marketing and preparing food. Rural women maybe venturing to culture cash products, while cultivating subsistence products and if they have no farm land, they have to work for others instead receiving wage. We can consider such women as agriculture propagator, production expert and even in some case as policy maker. Other than activity at agriculture field, women's participation at rural development is critical and is considered in order to supply adequate and needed food (Lahsaeizadeh, 2000).

Importance of women issue at Iran especially rural area, at one side face with fast population growth and mass of unemployed at process of access to rural growth and development, and at other side with limitation of facilities and productive resources. Rural women at all production level of agriculture products and livestock productions work alongside men and generally, development is multidimensional process and contains different economic, social, cultural and political dimensions. Women's participation at this process is active and affective participation, and main aspect of this participation was its economic dimension for rural women. Rural women have key role as a producer at agriculture activities, rural sources and services at rural area. rural women most efficient women of society and among people who are active at productive occupations, so it is obvious that attention to rural women as a strong arm at rural development can follow positive and undeniable affects, in this purpose (Lahsaeizadeh, 2000).

Rural women are considered either directly by producing livestock and agriculture products and rural industries and either by help to agriculture part as workforce and their share at third world countries is far more than other countries. Usually statistics about women's share at agriculture productions is less than real extent because largely, at these statistics seasonal job, part time job, no wage and housekeeping activities sere not considered. Nevertheless, they are forces for creating revolution and potential resources to progress rural economy and increase growth rate of food production (Nawab Akbar, 1997).

Having investment (capital) independency enforce people to think about economic from different angles. He should study the ways for using capital, he must consult with authority and experienced people and he will investigate about relevant markets. Such things will help him to be authoritative & independent. But how rural women can get such independency? Are the women created inherently for housekeeping, parenting and working or is there any opportunity for rural women to show their skills in economic & social development?

It seems that experiences which are obtained from performing financial programs in some villages in the developing countries could answer clearly to such questions(Bakhshoodeh and Salami, 2005).

A glimpse to previous planning about rural development in the world shows that from 1950 many developing countries understood that the main reason for making their economic growth (development) slowly in their countries is the weakness of investment in the agriculture part. Although many countries by patterning from developed societies have proceeded to improve & develop their industrial agriculture part and by this action not only had irreparable damages to many traditional farmers but also the main problem (the lack of capital sources) is also remained in the rural regions (Rahimi, 2001).

Increasing Suffrage, lack of relying on vast patriarchal families, increasing cultural acknowledgment, relation with newer institutions, having intellectual independence, making decision for marrying, occupation, emigration and etc are those rights that they gain. gaining aforementioned rights by women in context of cultural and social framework followed some changes that maybe lead to disfunctions and even create disorders and abnormalities at traditional , familial and kinship relations that dominated on villages (Fakhraee 2002).

What that performing credits programs, has made in recent years, was on broad outlook with purpose to access to same results as above findings.

Thus, in one inclusive outlook , it is possible to use micro-credits programs to solve those issues which involved with rural women's economic limitations, so that lead them toward social empowerment, in the context of economic growth (Rahmani andalibi, 2001).

Most women, especially in developing countries are working three shifts in a day indeed, but, instead for their exhausting activities, they receive: less health care, less literacy and fewer wages. Compensation for them is vast sex discrimination that exists all over the worlds in various forms. For example in India, Pakistan and Bangladesh, about 1million girls die, due to lack of proper health care (Emadi, 2001).

World Health Organization estimated that women work 2times more than men averagely (Bahar, 2001). In United Nation researches, except Australia, Canada and US, women in all countries work more hours than men. But major problem here is that, work means everything that leading to financial income. So, in government statistics, women are considered as unemployed and few of female employees are counted as productive and employed forces (Fani, 2009).

In all communities, rural women are considered as an important factor in achieving rural development goals and in fact are half of the manpower needed for rural development. However, in the rural community of Iran, there are gaps between the ruling class (capital owners) and villagers, between literate and illiterate, and between men and women. Especially in villages women have fewer possibilities in terms of investment and less power and credit (Khazaie, 2001). Role of rural women, over of men, is more influenced with different economic, social, cultural and ecologic factors. Rural women are considered as a noticeable potential in the community either directly (crops production, livestock, handicrafts, cottage industries) or indirectly by helping the agricultural sector (as labor). About 5.6 million women are involved in agricultural production, and activities related to planting... harvesting, preparation of animal food, and taking care of livestock and poultry and some certain activities related to trading and marketing are all different fields of rural women's role and participation. Based on current statistics, women in rural area participate about 50% in conversion industries, 22% in producing crops and livestock, 75% in handicrafts and in areas related to planting…harvesting , respectively, 25, 24 and 4.26. And also in activities related to livestock, they handle 23% of livestock grazing, 42% of animal care and 100 percent of total poultry in the village. Therefore their role in achieving food security is undeniable. But, like most developing countries, this crucial role in society and in process of rural development, is not obvious. In Iranian rural community, about 80% of women work, but they are mostly considered as housewives, unpaid employment, domestic workers, family workers, or independent employers. The statistics often do not take into account seasonal, part-time, unpaid employment, and housekeeping activities. In economics and social sciences, those of women's activities that have emerged out of house and affected national economy, are the ones to be noticed. In most research and statistics men are known as the heads of household and they are also the owners of lands and fields. That only 1% of the rural lands are belonging to women does confirm such matter (Varzgar and azizi, 2001).

Rural women empowerment:

The empowerment is equality that women for financial self-reliance and self-sufficiency can obtain by controlling their emotional decisions. The empowerment can be defined as an evolution and development of activity through private organizations that guides empowerment in the society toward economic improvement.

Empowerment is a process through which people can do activities to conquest on development obstacles that enable them to assign their destiny.

The word empowerment is not the meaning of overcome to main in equalities so it is different with the word self-reliance. (Ruhal amin, 2010).

Empowerment enable person to overcome any difficulties by a suitable management. Finally we can say empowerment provide energy to conquest on mental problems & outer difficulties.

On conclusion we can give a suitable definition to women's empowerment as this: the process of realization of women about themselves (and also the men's realization about them) for the thing they want or have to do.

It should be reminded that the main point should be attended in women's ability is the omission of subjective & social problems and providing economic & social communion for women in all aspects. The mean of women communion is their presence in all of village affairs such as making decision, presence in organization & councils that includes their communion in all economic & social aspects (Araghzadeh, 2002).

Cultural & social effects of rural women's financial self-reliance

As it mentioned before the traditional culture in villages was the reason for weakening women rights and made them oppressed, it is possible that women's self-reliance & financial independency in villages make some crudities (malformation) in the family and village for a short a short time, but we can't disregard it's positive outcome in the social & cultural occasions in the long time, here we will discuss about some of these outcomes (Goetz and Sengupta, 2003).

1- Preferment of women role and their social place:

Women's financial self-reliance can increase the women's social role & place in the villages. In the new condition some of their assignment roles could change to acquisitive roles. The women should use of all their power & energy for doing their acquisitive roles. Thus they can find active view to different functions. The people & groups could increase their social place in the village with improving their social role. If their role and social place preferment be accompanied with the increasing of social intelligence & knowledge, it can have more effect culturally. (Amiri, 2000).

2- Increasing self-confidence:

Self-reliance in different life aspects can increase people's self-confidence. Rural women who are financially independent can live peacefully. With decreasing their problems in life, their self-confidence will increase. And self-confidence is one of personality & mentally condition for being success in life.

3- Family consistency:

At the first, it seems that rural women's financial independency is not acceptable by their husband and this causes some gaps in their family's relations. But little by little these problems will be solved by increasing the rural people's knowledge. Usually poverty is one of the reasons which will destroy or decrease family's consistency. Women by working and having income can help their husband & family. (Fakhraee, 1381).

4- Change in family's relation:

The rural women with having a job and financial independency can change the viewpoint of people who live in villages and cities and they will not look at the rural women as a weak and dependent people. But also their title and place will increase among their families. So by changing people's view to the women, gently we can see some changes in their family's relation which will have respect to the women's right. By increasing women's knowledge and by introducing new rural institution which give financial & authority service to the women, their stimulus (motivation) for reaching their social rights will increase and they try more than before (Amiri, 2000).

5- Making patriarchy weak in the family:

Gently, with changing family's relation in the villages and by increasing rural people's knowledge, we can make the men and women's right equal and also we wont have patriarchy in the family, although patriarchy has historic and olden root in our villages but with improving women's position and increasing their cultural and social know ledge we can destroy patriarchy in the rural families.

6- Population and family adjustment:

The practitioner women's view about the number of the children is different; studies show that practitioner women are interested to fewer children to the house keeper women.

By decreasing families in the village and women's financial independency we are more hopeful to adjust family's population in the future because villages have important role in the population increase in Iran.

Conclusion & discussion:

If rural women could provide a job for them by getting credits, loan and other financial convenience, through their income they can get self-reliance or financial independency and we will see social, cultural & economic change in village. The

question here is that if these changes have positive or negative aspects in the village? It's natural that every change in social phenomenon has both positive and negative aspect, but which is Important here is that which aspect is more than the other and it depends to different condition in various societies. In our rural society there is an especial social & cultural kind that it's outcome maybe different and in some case inconsistent. With these actions rural women could be in idealistic economic condition and they could live with out dependency to their husband's income. In most of the villages in Iran there is patriarchy in the families which is not acceptable for the most of the rural people and groups. When rural women became financially independent, it's acceptable to see its cultural & social outcomes.

Hashemi and others (2004) found that joining to Gramin Bank, has meaningful positive effects on controlling women, and helps to family income.

In researches that conducted by Nanda (2004) became clear that women participation in credits programs had positive effects on their demand about health care. Fiona Steele and et al (2008) in researches that conducted as called " influences of credits programs on empowering women at Bangladesh , found that women who joined to credits programs , have participated in more educational programs and have married with more educated men and also they have saved more and they had more cash.

Ellen and her colleagues (2009) used approach called it "credits and education at Bolivia, Ghana, Honduras, Mali and Thailand". This approach looks for empowering women through financial services with education. In this approach, women get familiar with importance of credits through education and extension and also familiar with ways to access it through establishing different groups.

Shahnaj and chaudhury (2009) in research as "credits and its role on empowering women " concluded that there is meaningful relation between attending in credits programs and empowering women , at economical dimensions.

Ruhal amin and others (2010) found that those who joined credit funds had more ability rather than those who didn't.

Jameela (2010) presented that credit programs has shown lot of affects on empowering women so that has increased their social, politic and economic ability. Thus it is obvious that credits programs and its educational and empowering programs can be affective on social, humane and economic development or rural society, if it be associated with proper and gradual practices and base on reciprocal communications principles and apply opinion of local society. Maybe the main challenges that threaten credits associations , is lack of necessary emphasizes on social dimensions and on reinforcing their basics , that practically cause that this social foundations lose its efficiency soon and practically changed to unsuccessful institution.

In order to overcoming dominant consideration, experts believe that we should consider following in protection process of these social institutions:

- Relating public established institutions with each other and networking established institutions
- Emphasis on stability and self reliance of management system of credits institutions from financial and economic dimensions
- Efforts to gain local confidence and credibility among contacts
- Effectiveness of costs and economic and financial efficiency inside established institutions

Also following suggestions has been offered:

- providing extension educations for men in order to believe economic role of their women , and give them chance of corporation on all economic , credits fields
- Since that base of credit association, forms base on People Corporation, so it's good chance to use these communities to expand extension-education activities. so it is better to consider special programs on different extensional filed such as agriculture, ranching, family health, housekeeping economy and other fields accordance to condition of region and rural women's needs.

Giving the right that women make decision, independency to their family, increasing the cultural knowledge among them& making relation with new institutions, having independency in making decision about marriage, occupation, migration & something like this are the right that women have got it.

Women by getting these rights can make change in the rural cultural & social issues which make disfunction & crudity in their family's relation. However, rural women's self-reliance has caused improvement in the economic, social & cultural issues. For solving women's self-reliance problems we can do these activities:

- Giving promotional services for increasing rural women's skills in various fields.
- Giving promotional instructions to men for believing their women's economic role &

their women opportunity to participate in all economic, authority & … aspects.

- Increasing rural women's knowledge in all social, political, cultural & economic fields.

- Making use of micro-credits programs to motivate & support women for doing economic affairs better & finally to make women self-reliance.

Its result is that, exploiter can't access to desirable condition of production efficiency at first. Secondly, he would incapable for loan repayment. Third, his activity doesn't contain consistency. Fourth, remarkable part of provided credits would exit from production cycle due to exploiter's incapability and lack of skill in exploiter. His technical and occupation skill would improve, if credit is being provided for exploiter as a credit program. and he knows and can applies loan properly and well timed for production and activity, so condition of production and level of income, level of life and would improve.

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The Immune Function as Response to Level and Source of Protein in Pre-Mature and Mature Male Rats.

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Abstract: Dietary protein plays a significant and site-specific role in the developmental expression of the secretary immune system. In this sense, the aims of this study were as follows: 1) to evaluate in rats the severity effect of a protein-deficient diet (4%) on non specific cellular immune response (phagosytosis, killing and lymphocytes transformation index), immunoglobulins (IgG and IgM) and cytokine (IL-6). 2) to assess these parameters in rats re-fed on normprotein diet (20% casein) and rats re-fed on faba bean (20% raw bean) as alternative sources of protein. 3) to compare between premature and adult rats under the various levels and source of dietary protein. Forty eight rats were used in the present study, premature aging 40 days (weighted 85±5 gm) and adult aging 120 days (weighted 170 ±10 gm). Each animal age was divided into two groups, control (n=8) and experimental groups (n=16). Control group fed on a normoprotein chow, while experimental group fed on a diet having 4% protein (diet 1), for 3 weeks. After then, the experimental group of each age was divided into two groups. The first group received a normoprotein diet containing 20% casein (diet 2) and the second group received a diet containing 20% faba bean (diet 3) as alternative source of protein. The experiment was lasted for six weeks and the animal mortality and body weight were regularly recorded. At the end of experiment, the blood samples were collected through cardiac puncture from all animals under light anesthesia. The blood of each animal was split into two essay tubes, one heparinzed for the determination of the complete phagocytosis, killing and lymphocyte transformation index and the other wasn't; to obtain the serum for subsequent analyses of IgG, IgM and IL6 activity, total protein, albumin and thyroxin activity (T3 and T4). The results revealed that the body weight was reduced in protein-deficient rats as related to age associated with appearance of some cases of mortality. The rats re-fed on normoprotein chew (diet 2) showed increase in body weight more than the animal received diet 3 (20% faba bean) and this increase was more pronounced in premature rats. As a function of circulating levels of total protein, albumin and thyroxin, hypoproteic diet induced significant decrease in total protein content as well as T3 activity in both ages but the albumin and T4 level showed insignificant decrease. Nutritional recovery by diet containing 20% casein (diet 1) decreased the activity of T3 in premature rats. Regarding to rats re-fed on either diet 1 or diet 3, they showed significant increase in protein, albumin, T3 and T4 levels as compared to protein-deficient rats but this recovery was more pronounced in rats re-fed on a diet 1. With respect to immune function, low dietary protein induced disorder in the activity of phagocytosis, killing, lymphocytes transformation index (TL index) as well as IgG, IgM & IL6 in premature than in mature rats. But, these remarks of immune function were improved in both ages rat re-fed on diet 1 more than rats re-fed on diet 3. From the present results, it could be suggested that poor protein nutrition or inclusion of faba bean as the only source of protein in diet especially, young rats bring about a reduction in growth as well as impairment of undeveloped immune system because the absence of essential amino acids will comprise the ability of tissue to grow, be repaired or be maintained.

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Key words: hypoprotein diet, non cellular immune cells, immunoglobulin, cytokine, thyroxin, total protein.

1. Introduction

The scientific and technologic development of the biological and health areas taking place these last years has been trying to favour, directly or indirectly, the longevity of the human being. Protein malnutrition has long been recognized as a common problem, especially in children in the developing countries, whose inadequate nutritional intake is deficient for socio-economic reasons (Akinola et al. 2010). Within this scope it is important to consider that dietary protein deprivation during early life is known to have adverse effects on brain anatomy, physiology and biochemistry and even permanent brain damage (Torún & Chew, 1993) and also retards the differentiation of the morphological, metabolic, and contractile characteristics of skeletal muscle fibres in growing stage (Alves et al. 2008). Likewise, apart from protein undernutririon, deficiencies of iron and vitamin A which are together account for more than 75% of the deaths of infants and young children in some developing countries (Ou, 2002). Moreover, the impairment of immune function occurrence as a

result of protein malnutrition correlated with the incidence of infection and the high morbidity of children younger than 5 years in developing countries (Santos et al. 1997). Generally, protein malnutrition disorders included growth failure, hypoproteinemia, oedema, fatty liver and reduced the antioxidant enzymes as well as hampered the immune defence in humans and animals (Sawosz et al. 2009).

Thus, the need for alternative protein sources has recently gained focus. Among the possible protein sources, lupins, peas and faba beans were successfully used in ruminants and non ruminants (Vandoni et al. 2007). Faba beans (*Vicia Faba*), in particular, show a high content of tannins, particularly in the coats, whilst the trypsin inhibitor activity is low in comparison with many other legumes (Volpelli et al. 2010). The nutritional value of beans has been traditionally attributed to it high protein content, which ranges from 25 to *35%*, despite the imbalance in sulphur amino acids. Most of these proteins are globulins (60%), albumins (20%), glutelins (15%) and prolamins. The chemical analysis of this legume revealed low saturated fat content and high in complex carbohydrates, fibres, micronutrients and phytochemicals such as anthocyanins, a variety of phenolic compounds, protease inhibitors, phytic acid, and saponins (Volpelli et al. 2010). Epidemiological (Brown et al. 1999), clinical (Fung et al. 2001) and animal (Rosa, 1998) studies proved that beans reduce blood cholesterol and the high amounts of resistant starch as well as high amounts of soluble and insoluble fibres contents in bean leads to favourable fermentation and possibly inhibits colon cancer (Hangen and Bennink, 2003). Greater consumption of beans by children in developing countries would significantly reduced morbidity and mortality in this age group.

Because qualitative or quantitative protein malnutrition suppresses the specific immune system (Chandra, 1983) and causes severe lymphopenia, while qualitative malnutrition could result in neutrophilia (Balkaya, 1999), the role of the non-specific immune system would be more important in this respect (Biyik et al. 2005). Therefore, the present study was focused on the effect of dietary protein deficiency (4%) followed by re-feeding on a diet containing normoprotin or faba bean as alternative sources of protein on some physiological markers such as animals survival, body weight, circulating total protein, albumin and thyroxin concentrations as well as on some immunological markers such as phagocytosis, killing, lymphocytes transformation index, IgG, IgM, IL6, in premature and adult rats.

2. Experimental methods

This experiment was carried out on forty eight male albino rats from two ages, premature aging 40 days (weighted 85±5 gm) and adult aging 120 days (weighted 170±10 gm) were used in the current study. All rats were kept in metabolic cages under controlled temperature (25°C±5) and light/dark cycles (12/12 hrs). During the whole experimental period water and chow were supplied *ad-libitum.* Each age was randomly allotted to two groups: control (n=8) and experimental group (n=16). The control group was fed with normoprotein chow (20 % casein, diet 1). This chow was bromatologically analyzed and from the results the components were calculated to be added so as to decrease the protein level to 4% casein (diet 2) or 4% casein and 16% faba bean (diet 3) while keeping the vitamin and mineral balance. The experimental animals fed on the prepared hypoproteic diet 2 for three weeks and then they divided into two groups. The first received basal diet 1 containing 20% casein and the second received diet 3 containing 4%casein and 16 % faba bean for other 3 weeks. Mortality and body weight of all groups were recorded throughout the experiment. At the end of experiment, the rats from both control and experimental groups of two ages were fasted for about 12 h and then the blood samples were collected from each animal through cardiac puncture under light anesthesia. The blood of each animal was divided into two essay tubes, one heparinzed and the other wasn't; the serum in this last tube was obtained through centrifugation at 3000rpm for 15 min., the obtained serum was kept at –20°C for subsequent analyses of IgG, IgM and IL6 activity using ELISA (Life Diagnosis, Inc.) according to Salauze et al. 1994 , total protein and albumin concentration according to Gornall et al. (1949) and Doumas et al.(1971) methods in addition to thyroxin activity (total T3 and T4) by radioimmunoassay kit purchased from (Institute of Isotopes Ltd. Budapest) according to Ratcliffe et al. (1974) technique. The non-clotted blood collected was used for the determination of the complete phagocytosis, killing according to **(**Woldehiwet and Rowan, 1990) and lymphocyte transformation index (Boyum, 1968 and Burrels and Wells, 1977).

Statistical analyses

All values were expressed as mean ± SE. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Duncan's test using SPSS program 17.0. *P* values < 0.05 were considered to be statistically significant.

Table (1): Percent composition of the chow offered to control and experimental groups.

Composition (g/100g)	Diet 1	Diet 2	Diet 3
casein	20	4	-
Faba bean	-	-	20
Fat	10	10	10
Carbohydrates	62.5	78.5	62.5
Cellulose	3	3	3
Salt mix.	3.5	3.5	3.5
Vitamin mix.	1	1	1

Source: Percent composition carried out by Laboratory of nutrition analyses of Analysis and Designer Department of Nutrition- National Research Centre, Cairo, Egypt.

3. RESULTS:

The present results showed a gradual decrease in body weight of the premature rats which received hypoproteic diet as compared to control (table 2). When the animals received alternative dietary casein or faba bean, their body weight returned to increase particularly, in the rats re-feeding normoprotein as compared to corresponding re-feeding on faba bean. It is worth noting that diminished daily food consumption from dietary casein in premature rats was more relative to daily consumption from dietary bean and such phenomena was probably the reason for mortality of 25% of the animals in this group. The loss in the body weight was overcome when the animals thereafter fed on dietary casein more than dietary faba bean.

Table (2): Effect of variable levels and sources of dietary protein on mortality and percentage of body weight change of premature rats.

	Control						Hypoproteic diet			Alternative diet					
										Casein			Faba bean		
Weeks	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**4th**	**5th**	**6th**
% of mortality	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**12.5**	**12.5**	**12.5**
% of b.wt. Change	**+1.4**	**+2.7**	**+11.3**	**+15**	**+20.3**	**+32**	**-11.8**	**-11.5**	**-14**	**+42.7**	**+44**	**+50.**	**+26.5**	**+32**	**+25**

The results obtained in table (3) showed loss in the body weight of animals received hypoproteic diet, but when these animals received dietary casein or bean protein, the rate of body weight gain increased to reach its maximum level at the end of experiment. Although the weight gain of survived animals which consumed dietary casein was more than weight gain of animals consumed dietary faba bean, the mortality in this group was more.

Table (3): Effect of variable levels and sources of dietary protein on mortality and percentage of body weight change of adult rats.

Groups	Control						Hypoproteic diet			Alternative diet					
										Casein			Faba bean		
Weeks	1st	2nd	3rd	4th	5th	6th	1st	2nd	3rd	4th	5th	6th	4th	5th	6th
% of mortality	-	-	-	-	-	-	-	-	12.5	12.5	12.5	12.5	-	-	25
% of b.wt. Change	+11.9	+20.6	+9.2	+17.4	+22.5	+40.9	-0.03	-1.1	-6.3	+11.1	+19.2	+10.5	-0.8	-7.1	-1.3

Serum total protein level showed significant decrease ($p<0.05$) associated with insignificant decrease in albumin level in both ages of rats subjected to low dietary protein. But the protein malnutrition recovered when the animals re-fed on normal dietary casein more than the animals re-fed on dietary faba bean and this recovery was more pronounced in adult rats as shown in fig. (1&2).

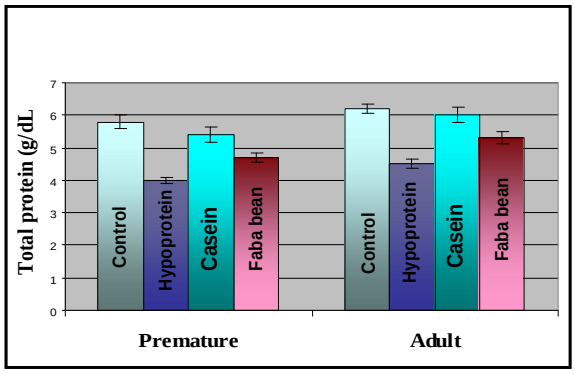

Fig.1. total protein level in premature and adult rats under effect of variable levels and sources of dietary protein

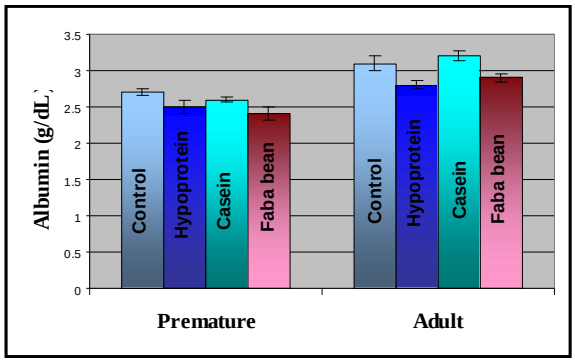

Fig.2. albumin level in premature and adult rats under effect of variable levels and sources of dietary protein

The activity of circulating T3 and T4 significantly ($P<0.05$) decreased in animals fed low-protein diet, returned to normal level by subsequent consumption of dietary casein or faba bean and the subsequent consumption of dietary casein or dry bean caused significant increase in T3 level particularly in adult rats re-fed on dry bean (Figs.3 & 4).

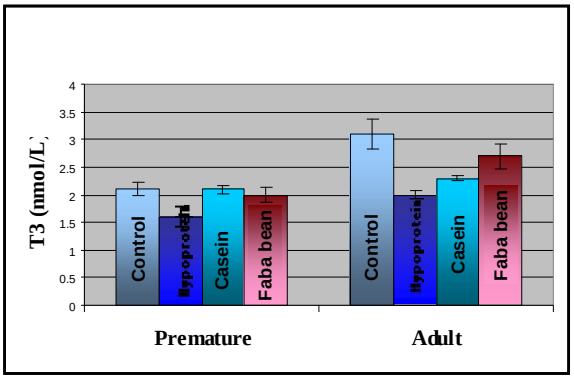

Fig.3. T3 in premature and adult rats under effect of variable levels and sources of dietary protein

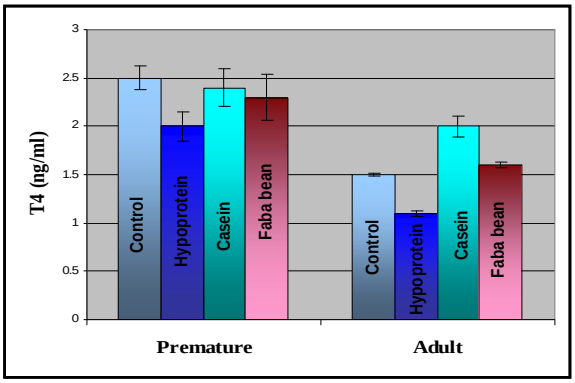

Fig.4. T4 level in premature and adult rats under effect of variable levels and sources of dietary protein.

As shown in figs. (5,6 and 7), hypoproteic diet (4%) induced significant decrease in the activity of phagocytosis and killing process as well as TL- index in premature rats as compared to corresponding adult or control rats. However, the following consumption of dietary casein had significant ($p<0.05$) effect on these non specific cellular immune functions more than the effect of dietary bean consumption and this effect was more pronounced in adult rats.

Figs. (8, 9 and 10) clarified that intake of hypoproteic diet 4% caused significant (P< 0.05) decrease in IgG, IgM and IL6 activity in premature and adult rats but the alternative dietary casein or faba bean modulated this decrease particularly in the adult rats which re-fed on dietary casein.

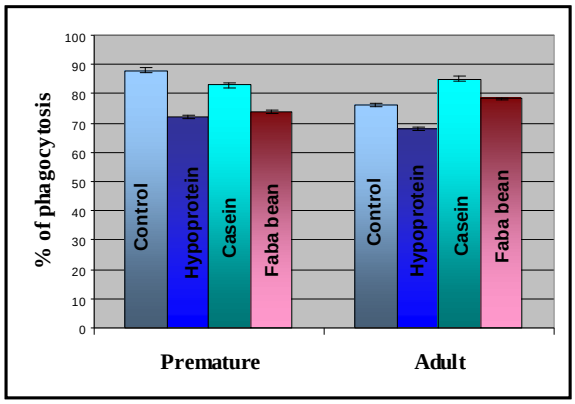

Fig.5. phagocytosis activity in premature and adult rats under effect of variable levels and sources of dietary protein

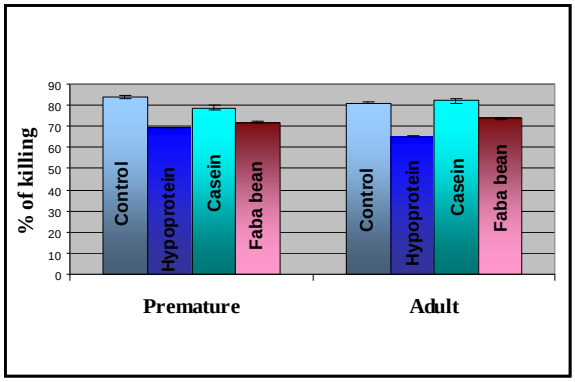

Fig.6. killing activity in premature and adult rats under effect of variable levels and sources of dietary protein

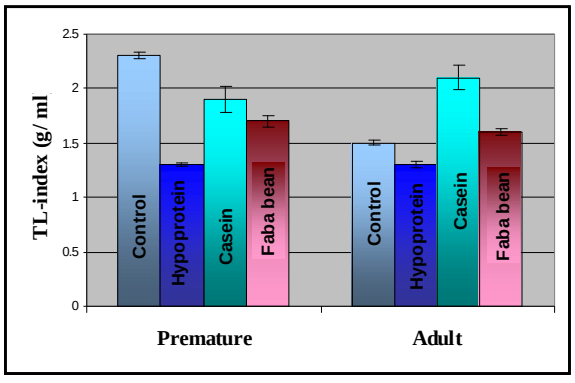

Fig.7. TL-index in premature and adult rats under effect of variable levels and sources of dietary protein

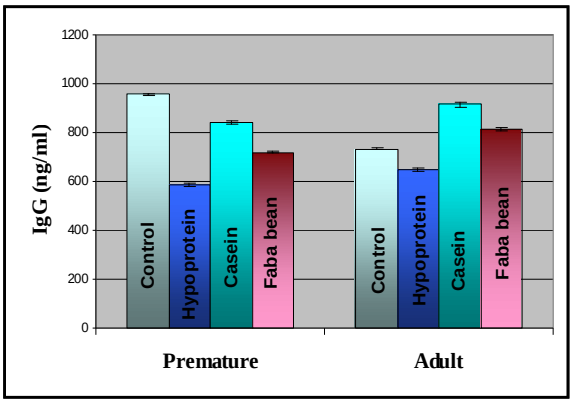

Fig.8. IgG concentration in premature and adult rats under effect of variable levels and sources of dietary protein

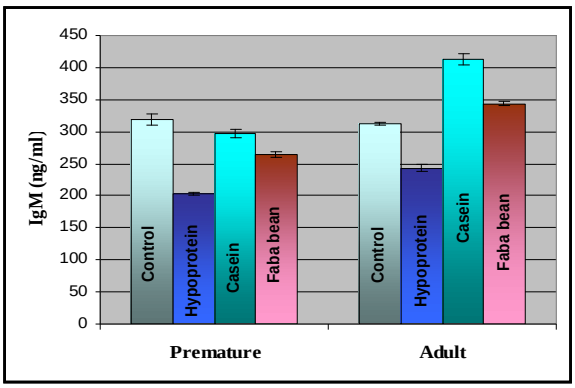

Fig.9. IgM concentration in premature and adult rats under effect of variable levels and sources of dietary protein

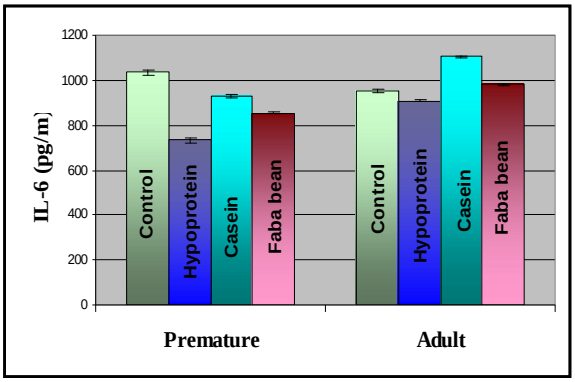

Fig.10. IL-6 concentration in premature and adult rats under effect of variable levels and sources of dietary protein

4. Discussion:

Protein deficiency is prevalent in underdeveloped countries and caused excessive morbidity and mortality in particular children during

first years of life (Underwood, 2002). The earlier the malnutrition, the more severe the effects, resulted in greater severity and extent of damage to a range of organs and systems (Crogan and Pasvogel, 2003). Since, the sequential changes of malnutrition are altered cellular metabolism, impaired physiologic function, significantly affects growth and differentiation of tissues and cells and finally loss of body tissues (Briet et al. 2004). In the present study, hypoproteic diet 4% caused reduction in the body weight of premature and mature rats by 14% and 6.3% respectively, as compared to corresponding control. The significantly lower body weight of these groups was a result of a poor quality protein in the experimental diet due to the deficit in essential amino acids resulted in lower quantity of food consumed by the rats (Hernández et al. 2008). As expected, the effects of protein deficiency on body weights were more pronounced for the premature rats than for the adult rats because they able to select a protein diet appropriate to their bodily needs resulted in retardation in the differentiation of the morphological, metabolic, and contractile characteristics of their skeletal muscle fibres (Alves et al. 2008).

When the rats re-fed on the normal diet (20% casein) for three weeks, the increase in their body weight observed at the last phase of experiment suggesting that these animals recovered from malnutrition. In contrary, the animals allocated on faba bean diet as alternative source of protein showed decrease in their body weight at the end of experiment as compared to rats kept on normal diet and compared to rats re-fed with 20% casein. It was well noticed that survival of rats was correlated with percentage of increase in their body weights as well as the quantity of food consumed in both ages group. On other wise, the weight gain of adult rats was less than weight gain of premature and the weight gain of re-fed casein rats was more than rats re-fed on faba bean. Such impairment of growth in the animals fed on diets containing *Vicia faba* has been reported in rats (Martínez et al. 1987), mice (Martínez et al. 1992). This is because the digestibility and absorption of carbohydrates and proteins is adversely affected by the inclusion of faba bean in the diet (Sobrini et al. 1983). The possible involvement of several antinutritional factors such as polyphenols, tannins, protease inhibitors and lectins as well as the deficiency of sulphur amino acids in *Vicia faba* in the processes of metabolism and nutrient utilization has been the main reason of growth retardation (Larralde and Martinez, 1991). Since, dietary fibre may decrease digestibility of protein and amino acids by stimulating the production of bacterial protein, through adsorption of amino acids and peptides onto the fibre matrix, and by increasing the excretion of endogenous protein (Schulze et al. 1994). The mechanism by which bean exerts its effect in growing rats could be by substance present in it, which might either alter the electrolyte membrane permeability decrease the capacity of carrier system for the substrates (Santidrian, 1981). In this context, Goena et al (1988) established that feeding growing animals with faba bean as source of protein leads to a significant impairment of growth, which must be mainly attributed to a decrease in muscle mass (representing about 40% of body weight in mammals) achieved by a fall in muscle protein synthesis rather than to changes in protein breakdown. The mechanism seems to involve a reduction in muscle RNA activity associated with an increase in liver protein synthesis, which could be indirectly mediated by the flux of amino acids coming from the skeletal muscle (Kurpad, 2006). These facts emphasize the importance of nutritional recovery at early phases of life, in order to achieve normal growth and development (Gigante et al. 2007).

The dynamic balance among the serum thyroxin, circulating protein contents and the body weight was manifested in the present study in protein deficient rats by the depression in T3 and T4 activity associated with reduction in protein content and animal body weigh. Such relationship was depended on the stage of age and was considered as another remarkable feature of animals subjected to low dietary protein (Heard et al. 1977). The albumin was not significantly altered as well because the rats fed on low dietary protein for short run (Obatolu et al. 2003) and it may be decreases when the same diet employing with rats of the same strain, in the long run (Sant' Ana et al. 2001). The most intense effect of the hypoproteic diet employed in the current study was may be the effect on the activities of the complex enzymes of electron transport chain, which may be due to changes in mitochondrial protein synthesis, breakdown, or both (María & Carolina, 2007) or lack of vitamins B complex supplementation in the chow of the experimental group (Guyton & Hall, 2002) and/or association of malnutrition with concomitant sign of intestinal free radical damage and altered protein transport suggesting that oxidative stress is partly responsible for the intestinal dysfunction and depression of metabolic rate observed in malnutrition (Akinola et al. 2010). As related to last suggestion, Sidhu et al. (2004) indicating that malnutrition possibly caused alterations in antioxidants enzymes activities and increase lipid peroxidation. It has been proposed that free radical mediated tissue damage may be involved in malnutrition mainly because of the inadequate protective and repair mechanisms in protein-deficient animals or human. On the other hand, investigations of enzymes responsible for maintaining

glutathione in the reduced state and studies in response to oxidative stress found increased activity of G6PD and showed that impaired antioxidant status and decreased proportions of red cell phospholipids were found in different types of malnutrition (Akinola et al. 2010). In spite of rats allocated on alternative dietary faba bean showed increase in serum protein and albumin levels, it was less than the level in rats re-fed on normal dietary casein. Such finding could be attributed to the tannins constituent in faba beans which increase the secretion of proline-rich proteins by the rat's parotid glands (Jansman et al. 1993) and interacting with both dietary and endogenously secreted proteins in the intestinal tract result in enhancement of fecal excretion of both sources of protein (Jansman et al. 1995). Moreover, the quality of faba bean protein appeared to be limited by the low content in sulfur-amino acids, tryptophan, valine, isoleucine, and threonine (Baudet and Mose, 1980). But the casein re-feeding, restored the activities of mitochondrial in the gastronomies muscle due to increased amino acid availability which important in restoring the activities of mitochondrial complexes (Briet & Jeejeebhoy, 2001).

As far as the relationship between thyroxin hormones (T3 and T4) and metabolic rate is concerned since, the thyroxin stimulated the transcription of genes that influence the metabolic rate of all cells, thus the greater the level of these hormones, the larger the metabolism (Guyton & Hall, 2002) depended on the protein content of the diet (Ahrens et al. 1990). Perhaps the decrease in thyroxin was due to adaptation of thyroid to smaller substrates availability in low dietary protein (Waterlow, 1996). Knowing that T4 is capable of increasing the rate of protein synthesis when there are adequate amounts of carbohydrates and lipids (Guyton & Hall, 2002), this essential cellular process should be impaired in the animals from hypoproteic group, especially in organs of greater cellular and/or protein turnover, due to the low level of the free hormone and also due to the presumptive lack of available amino acids caused by the hypoproteic diet (Araújo et al. 2005). On the other hand, the thyroxin level in both rats ages re-fed on dietary casein restored more than the rats re-fed on faba bean because some of the essential amino acids needed for bio-activity of thyroxin hormone not constituent in feba bean. It is of interest for future research to know how vegetable proteins raise levels of thyroid hormones. We are not aware of data showing that amino acids affect thyroid function, particularly plasma arginine and serine, which correlated best with thyroxin concentration.

The impairment in the immune function particularly, in children has been interpreted as a secondary outcome of inadequate dietary protein (Menezes et al. 2003). The aspects of immunity most consistently affected by protein malnutrition are cell-mediated immunity, phagocyte function, the complement system, secretary antibody, and cytokine production (Weller, 2001). In the present study, consumption of low dietary protein 4% induced significant decrease in processes of phagocytosis, killing and TL index as well as IL6, IgG and IgM in both age groups relative to control but this inhibitory effect was more pronounced in premature rats than in adult rats. Such results considerably attributed to a greatest lymphocyte blastogenesis in children than in adults, resulting from immaturity of immune system (Sasai et al.1981). In addition to that the time span of using protein-free diet played a key factor for the safety of this treatment (Sawosz et al. 2009). Hence, the experimental period of this study was not enough to reduce phagocytic activity clearly in peripheral blood of the adult malnourished rats. In this view, Borelli et al. (1998) have been reported that protein deficiency tended to decrease the number of lymphocytes and functions of T-helper cells, natural killer cells and peritoneal macrophages. Consequently, the low lymphocyte transformation rates in rats consumed hypoprpteic diet therefore indicated impairment of lymphocyte function. On the other hand, deficient of protein influenced the concentration of body potassium, an important intracellular ion which may play a role in contributing to the lymphocyte abnormality (Sellmeyer et al.1972). As a function of IL6, some experimental and clinical studies have suggested that protein malnutrition affect circulating IL-6 level (Agüero *et al*. 2006), which can have anti- and pro-inflammatory function (Lyoumi *et al*, 1998) and others established that protein malnutrition induces a low-grade inflammatory state in rats, as evidenced by elevated serum levels IL-6 and reduced serum levels of albumin (Suzuki et al. 2002 and Ling et al. 2004). Likewise, it was suggested that protein deficient animals present a failure in the regulatory mechanisms of the IgG and IgM resulted in decreased their total levels (Malavé and Layrisse, 1976). Abnormal low levels of IgG and IgM were demonstrated in children as a response to protein deficiency due to specific inhibition of their synthesis by dietary components (Kenrick and Smith, 1970).

Thus, it is reasonable to consider that decreased level of IL6, IgG and IgM observed in the present study, at least in part, reflected the Immunosuppressive effect consequent to a protein-deficient diet that exerted on cells mediated immunity to reduce its products.

In this way, other investigators suggested that protein deprival increased the intensity of oxidative burst in neutrophils. Reactive oxygen species released by neutrophils in an extended amount scan lead to

cell, tissue, and organ damage and also may induce inflammation (Mitra and Abraham 2006). It seemed that deficit of protein-originated antioxidants, especially from sulphuric amino acids, might be a key factor explaining the present results. The increase of oxidative burst in neutrophils might be caused by reduced antioxidant capacity, resulting from deficiency of glutathione and other amino-derivative antioxidants as a sequence of low dietary protein (Sawosz et al. 2009).

The functional of the immune system competence was reduced in rats re-fed on faba bean diets for both cellular and humoral-mediated responses. It is well known that the response of the immune system has been widely recognized as an adequate index for the evaluation of the nutritional value of a diet (Stinnett, 1987), because it is sensitive to legume feeding (Sissons et al. 1988). Since zinc bioavailability is apparently reduced in animals fed on faba bean by the occurrence of phytates as indicated by the reduction in plasma zinc concentration (Martínez et al. 1986) and zinc is apparently involved in some immune mechanisms. The functional competence of the immune system was reduced in rats fed on faba bean diets for both cellular and humoral-mediated responses (Macarulla et al. 1989). Legume proteins contain considerable quantities of phytic acid, dietary fiber and other organic compounds, which may affect mineral bioavailability from the diet (Martínez et al. 1985). Zinc affects gene regulation within lymphocytes. This can dys-regulated intracellular killing, cytokine production, and phagocytosis (Shankar and Prasad, 1998). The macrophage, a pivotal cell in many immunologic functions, is adversely affected by zinc deficiency (Menezes et al. 2003). Also, zinc is crucial for the normal function of cells which mediate non-specific immunity, such as neutrophils and natural killer cell, B lymphocyte development and antibody production, particularly immunoglobulin G, is compromised by zinc deficiency (Larralde and Martinez, 1991).

5. Conclusion:

It could be shown for the first time that introduction of dietary proteins after weaning is important for a critical period in which both local and systemic aspects of the immune system undergo maturation. Also, the effects of early undernourishment are time-dependent and may cause irreversible changes in the regulation of metabolism. However, dietary protein depletion results in malnutrition at the whole body levels, indicated by reduction in body weight associated by impaired in circulating thyroxin hormone and decreased in total protein and albumin as well as suppression on the immune responses. In the light of present data, the reduction in immunological activity in these rats could be rather explained by a direct effect of the reduction of stimulation by dietary antigens. Consequently, it could be predict that protein malnutrition at an early age may have an unsuspected immunological impact in the children. Beans are not typically fed to the children, however, appropriate combinations of beans in adequate amount consumed with other sources of protein, will prevent protein malnutrition.

Further studies are needed to demonstrate whether protein supplementation can reverse the changes, at all health levels, induced by protein malnutrition.

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Molecular cloning and sequence analysis of *sglt1* and tertiary structure prediction of deduced protein in *Cyprinus carpio* L.

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Abstract: Na^+/glucose cotransporter (Sglt1) plays an important role in transporting Na^+ and glucose and maintaining the adjustment of metabolism. The aim to study *sglt1* is to further understand the regulation mechanism of *sglt1* gene in fish. In this study, the full-length cDNA of Na^+/glucose cotransporter gene was cloned in intestine of *Cyprinus carpio* L. using RT-PCR and RACE methods, which included 2856 bp involved in 113 bp 5'-untranslated region, 766 bp 3'-untranslated region, and 1977 bp open reading frame (ORF) which encoded 658 amino acids. The predicted amino acid sequence was the highest similar with that of *Danio rerio* (90.70%), and the lowest similar with that of rabbit (71.40%). Fourteen transmembrane domains were predicted in the 3-D protein model using comparative protein modeling program SWISS-MODEL. The structural core was comparative of 5 TM helices (TM2-TM6 and TM7-TM11) with the inverted repeat. It was demonstrated that Glucose might be bounded in the center of the structural core, and a possible Na^+-binding site was located at the intersection of TM2 and TM9. Thereby, the functional roles and regulation mechanism of Sglt would provide unique opportunities to investigate the biochemical processes in intestine of *Cyprinus carpio* L., and lay the foundation for artificial culture of the species involved.

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Keywords: *sglt1*; cDNA sequence analysis; Protein tertiary structure; *Cyprinus carpio* L.

1. Introduction

Na^+/substrate cotransport (or symport) is a widespread mechanism of solute transportation across cytoplasmic membranes of prokaryotic and eukaryotic cells, which is mainly performed by cotransport proteins. Based on sequences similarities, the members of these proteins are classified into different families (Reizer *et al.*, 1994). Proteins of these families generally utilize electrochemical sodium gradient to drive transportation of substance in reverse concentration like sugars, amino acids, vitamins, ions, myoinositol, phenyl acetate, urea and water (Reizer *et al.*, 1994; Frank *et al.*, 1998). Among them, human sodium-glucose transporter (hSglt1) is one of the best characterized members of the Solute Sodium Symporters Families (SSSF). In addition, the sodium-iodide transporter (NIS) and the sodium-proline transporter (PutP) in *E. coli* and sodium-galactose symporter (*vsglt*) in *Vibrio parahaemolyticus* also were described in publishments (Frank *et al.*, 1998; Schwan *et al.*, 1998; Zeuthen *et al.*, 2001; Hirayama *et al.*, 1997). Sglt1 and NIS catalyze the uptake of substance with a 2:1 sodium-substrate stoichiometry while the value of sodium-proline transported by PutP is 1:1 (Zeuthen *et al.*, 2001; Hirayama *et al.*, 1997; Eskandari *et al.*, 1997). In addition, it was also reported that Sglt1 could couple the uptake of two sodium and one sugar with the transportation of 264 water molecules (Wegener *et al.*, 2000).

The high-affinity Na^+/glucose contransporter (Sglt1) (Martin *et al.*, 1996) is an important member of the sodium: solute symporter family (SSSF) (Ernest *et al.*, 2004) with more than 700 different sequences (Wright *et al.*, 2004; Turk *et al.*, 1997). Sglt1, belonging to the homologous family 5 (SLC5), is abundantly expressed in small intestine(Balen *et al.*, 2008; Hirsh *et al.*, 1998) , while at a lower level in kidney (Ikeda *et al.*, 1989). The major function of Sglt1 is to accumulate sugar in intestinal or kidney epithelial cells adverse concentration gradient. Since the driving force of Na^+-coupled transporters is provided by the Na^+ electrochemical potential gradient across the plasma membrane. It serves as the principal uptake pathway for glucose derived from diet. Mutations of *sglt1* could result in the dysfunctional of this pathway, which would affect intestinal glucose/galactose absorption (Martin *et al.*, 1996). Recently, Sglt1 has been studied as a target protein for diabetes treatment (Ikumi *et al.*, 2008; Sabino-Silva *et al.*, 2010). Ryuichi *et al.* findings indicate that Sglt1 serves as the intestinal glucose sensor for glucose-induced incretin secretion and that a noncalorigenic Sglt1 substrate ameliorates hyperglycemia by stimulating incretin secretion (Ryuichi *et al.*, 2009).

The crystal structure of the *Vibrio parahaemolyticus* sodium/galactose symporter (vSglt) had been reported (Faham *et al.*, 2008). So far, few studies on Na^+/glucose cotransporter were reported in the freshwater fishes. In this study, *sglt1* gene with the high affinity was first cloned in intestine of *Cyprinus carpio* L. and subsequently used to obtain new insights into the molecular mechanism of Na^+/glucose cotransporter. The secondary structure and tertiary structure of Sglt1 protein in *C. carpio* have been predicted with several computational algorithms.

2. Material and Methods

Fish acclimation

In this study, *C. carpios* with body weight of (12.6±0.38) g were used as experimental animals, which were acclimated in a 200-L tank filled with dechlorinated water with constant aeration (DO: 6.2±0.2 mg /L) and a 12/12 h light/dark photoperiod. Water temperature was controlled at (26.8±0.68)℃. During the period of acclimation, the fishes were fed for four times each day (8:30am, 11:30am, 14:30pm and 17:30pm) with commercial pellet feed. After the acclimation, ten fishes were randomly arrested to be used as experimental fishes. Subsequently, the intestines of which were obtained by fish dissection respectively after general anesthesia, and scissored immediately into intestine, and the contents in guts were cleared rapidly. All the operations were conducted under aseptic condition on the ice.

Total RNA extraction and 5' and 3' RACE

The prepared guts were quickly frozen in liquid nitrogen and used to extract the total RNA with TRIzol reagent (purchased from Invitrogen) respectively. The total RNA (5mg) was used to synthesize the first-stand cDNA using AMV reverse transcriptase (from Shanghai Sangon) and oligo-p $(dT)_{18}$ Primer (from Shanghai Sangon) in a 20μL reaction, according to the manufacturer's instruction. The *sglt1* cDNAs were then amplified by PCR in a total volume of 50μL, containing 10mM Tris-HCl(pH9.0), 50mM KCl, 1.25mM $MgCl_2$, 0.2mM dNTPs, 1 units of Taq polymerase (from Takara, Japan), 40pmol primer for each one and 5μL template cDNA (Table 1). A initial reaction of 3 min at 94℃ was followed by 35 cycles (denaturation at 95℃ for 35 s, annealing at specific temperature for 35 s and extension at 72℃ for 2 min), and a final extension for 10 min at 72℃. The annealing temperatures of PCR reaction were depended on the *sglt1* to be amplified (Table I). The PCR product was resolved on 1% agarose gels via electrophoresis. Photographs of the gels stained with ethidium bromide are show in an inverted black/white format.

Table 1. Sequences of oligonucleotide primers used for PCR and rapid amplification of cDNA ends (RACE)

Names	Oligonucleotide sequence (5'→3')	Length (bp)
3' GSP1 *sglt1*	GGTGGATTTGAATGGAATGCTCT	23
3' GSP2 *sglt1*	ACCTCTCCGTGCTCTCCCTGTTT	23
3' RACE outer	TACCGTCGTTCCACTAGTGATTT	23
3' RACE inner	CGCGGATCCTCCACTAGTGATTTCACTATAGG	32
5' GSP1 *sglt1*	ATCCCACGACCATGATGATTGTC	23
5' GSP2 *sglt1*	CTGTGTGGCAGCGTTTGGAGGAG	23
5' RACE outer	CATGGCTACATGCTGACAGCCTA	23
5' RACE inner	CGCGGATCCACAGCCTACTGATGATCAGTCGATG	34

Note: The primers were based on the *sglt1* sequences of Zebrafish and other animals deposited in GenBank.

Cloning and sequencing of *C. Carpios* intestion cDNAs

The amplified bands corresponding to *sglt1* cDNAs were accurately excised from the 1% agarose gel and purified using the Gel extraction kit (from Takara, Japan) respectively. The purified *sglt1* cDNAs were ligated into the pGEM-T Easy vectors (from Promega, USA), and the resultant recombinant plasmids were transferred into competent *Escherichia coli* strain JM109. For each cDNA, 4-6 plasmid clones containing *sglt1* cDNAs were sequenced by ABI3730 using M13+/-universal primers (from Takara, Japan).

3. Results

Isolation of the *C. Carpios sglt1* cDNA by RACE

The primer was originally designed from highly conserved regions of *sglt1* based on the sequence alignment of zebrafish, spiny dogfish, mouse, rat, human, bat, horse, bovine and rabbit *sglt1* cDNA from GenBank. Employing the RACE strategy,

the full-length *sglt1* of *C. Carpio* were cloned. The 5'-RACE and 3'-RACE results were sequenced and spliced to obtain the full-length cDNA (Figure 1). The complete coding sequence of the *C. carpio sglt1* cDNA with 2856 nucleotides comprised coding sequence region with 1974-bp open reading frame (ORF), a 113-bp 5'-untranslated region and a 766-bp 3'-untranslated region including poly (A), encodes a putative protein of 658 amino acids (Figure 2).

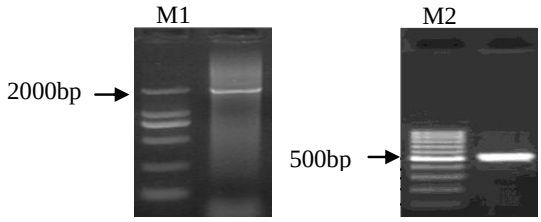


Figure 1. The results of RACE-PCR on *sglt1*. A, the result of 5′-RACE, the acquired gene was about 2000bp fragment; B, the result of 3′-RACE, the acquired was about 500bp fragment; M1, 2000bp DNA ladder; M2, 100bp DNA ladder.

```
-113                                                                                                  gagaacagtcagtc
      acacacctgcaggtgagcgtctgatcc aggtatatagaccgtcgtgtcagagagggaaacagtgtgctgccccaccggccccctttgaaaaccatcaaa
    1 ATG GGT GAA GAA TAT TTT GGA TTC TCC TGG GTT CGC AAT GAA AAC AGA AAA AAC GTC ACC ATA TAT GTT AAC AAT
    1  M   G   E   E   Y   F   G   F   S   W   V   R   N   E   N   R   K   N   V   T   I   Y   V   N   N
   76 CCA GCG GAC ATC TCA GTG ATA GTT ATA TAT TTT CTG GTG GTT CTG GCT GTG GGA ATA TGG GCA ATG GTA CGG ACC
   26  P   A   D   I   S   V   I   V   I   Y   F   L   V   V   L   A   V   G   I   W   A   M   V   R   T
  151 AAT CGA GCC ACA GTA GGA GGC TTT TTC CTG GCT GGC AGA AGT ATG GTG TGG TGG CCG ATT GGA GCC TCT CTT TTT
   51  N   R   A   T   V   G   G   F   F   L   A   G   R   S   M   V   W   W   P   I   G   A   S   L   F
  181 GCT AGT AAC ATC GGA AGT GGG CAT TTT GTG GGA ATA GCA GGA ACT GGA GCA GCT GCT GGT ATT GCT ATC GGA GGA
   76  A   S   N   I   G   S   G   H   F   V   G   I   A   G   T   G   A   A   A   G   I   A   I   G   G
  301 TTT GAG TGG AAT GCT CTT GTT GTA GTG ATT ATT CTG GGA TGG CTC TTC GTG CCC ATC TAC ATT AAG GCT GGG ATC
  101  F   E   W   N   A   L   V   V   V   I   I   L   G   W   L   F   V   P   I   Y   I   K   A   G   I
  376 GTG ACC ATG CCA GAG TAC CTG AAG AAA CGA TTT GGT GGG CAG CGG ATC CGT ATC TAC CTC TCC GTG CTC TCC CTG
  126  V   T   M   P   E   Y   L   K   K   R   F   G   G   Q   R   I   R   I   Y   L   S   V   L   S   L
  451 TTT CTC TAT GTG CTC ACA AAA ATC TCT GCG GAC ATG TTT GCA GGA GCC ATT TTC ATC AAC CAG GCT CTG GGA CTA
  151  F   L   Y   V   L   T   K   I   S   A   D   M   F   A   G   A   I   F   I   N   Q   A   L   G   L
  526 AAC ATT TAC CTG GCC GTA ATT ATT CTG CTG CTC ATT ACA GCT CTT TAT ACA GTA ACA GGT GGT CTA GCT GCA GTC
  176  N   I   Y   L   A   V   I   I   L   L   L   I   T   A   L   Y   T   V   T   G   G   L   A   A   V
  601 ATC TAC ACC GAC ACA CTC CAG ACC ATC ATC ATG GTT GTG GGT TCA TTC ATT CTT ATG GGC TTT GCG TTC AAT GAG
  201  I   Y   T   D   T   L   Q   T   I   I   M   V   V   G   S   F   I   L   M   G   F   A   F   N   E
  676 GTG GGA GGC TAT GAG AAC TTC AAA GAC CGA TAC ATG ACT GCG ATC CCG TCA GTG GTG GGT GTG AAC ATC AGT GAA
  226  V   G   G   Y   E   N   F   K   D   R   Y   M   T   A   I   P   S   V   V   G   V   N   I   S   E
  751 GAG TGC TAC ACT CCT CGT GCA GAC TCC TTC CAC ATC TTC AGA GAC CCC CTC AGT GGC GAT CTG CCC TGG CCT GGA
  251  E   C   Y   T   P   R   A   D   S   F   H   I   F   R   D   P   L   S   G   D   L   P   W   P   G
  826 CTG ATC TTT GGT CTT ACT ATC CAG GCT GGC TGG TAC TGG TGC ACT GAC CAG GTG ATT GTG CAG CGC TGT CTG TCT
  276  L   I   F   G   L   T   I   Q   A   G   W   Y   W   C   T   D   Q   V   I   V   Q   R   C   L   S
  901 GCC AAG AGC CTG TCT CAT GTG AAA GCA GGC TGC ATC CTG TGT GGT TAC CTC AAA CTT CTG CCC ATG TTC CTC ATG
  301  A   K   S   L   S   H   V   K   A   G   C   I   L   C   G   Y   L   K   L   L   P   M   F   L   M
  976 GTT TTC CCT GGC ATG ATC AGC AGA GTT CTT TAT CCA GAT GAG ATT GCA TGT GTG GAT CCA AAA GAG TGT GAC TAC
  326  V   F   P   G   M   I   S   R   V   L   Y   P   D   E   I   A   C   V   D   P   K   E   C   D   Y
 1051 TAC TGT GGA GCC AGT GTG GGC TGC AGT AAT ATT GCT TAT CCG AAA CTA GTG GTG GAT CTG ATG CCA AAC GGT CTC
  351  Y   C   G   A   S   V   G   C   S   N   I   A   Y   P   K   L   V   V   D   L   M   P   N   G   L
 1126 AGA GGG TTG ATG TTG TCC GTC ATG CTG GCC TCT CTG ATG AGT TCA CTC ACT TCC ATC TTT AAC AGC GCT AGC ACA
  376  R   G   L   M   L   S   V   M   L   A   S   L   M   S   S   L   T   S   I   F   N   S   A   S   T
 1201 CTC TTC ACT ATG GAC ATT TAC ACC AAG ATT CGC TCC AAT GCC AAG GAA AAG GAA CTC ATG ATT GCC GGG AGG GTG
  401  L   F   T   M   D   I   Y   T   K   I   R   S   N   A   K   E   K   E   L   M   I   A   G   R   V
 1276 TTC ATG CTG TTT CTG ATC GGC GTG AGT ATC GCG TGG ATC CCC ATC GTT CAG TCG GCT CAG AGC GGG CAG CTC TTC
  426  F   M   L   F   L   I   G   V   S   I   A   W   I   P   I   V   Q   S   A   Q   S   G   Q   L   F
 1351 GAC TAC ATT CAG TCC ATC ACC AGT TAT CTG GCT CCG CCC ATC GCT GCT GTC TTC ACC CTC GCC ATT TTC TGC AAG
  451  D   Y   I   Q   S   I   T   S   Y   L   A   P   P   I   A   A   V   F   T   L   A   I   F   C   K
 1426 CGA GTC AAT GAA CCA GGT GCT TTC TAC GGG ATG TGT ATT GGT CTG TTG GTG GGT CTG GCA CGT ATG ATA ACT GAG
  476  R   V   N   E   P   G   A   F   Y   G   M   C   I   G   L   L   V   G   L   A   R   M   I   T   E
 1501 TTT GCC TAC GGC ACG GGC AGC TGC GTG AGT CCC AGT AAC TGT CCT ACG ATC ATC TGT GGA GTG CAC TAC CTC TAT
  501  F   A   Y   G   T   G   S   C   V   S   P   S   N   C   P   T   I   I   C   G   V   H   Y   L   Y
 1576 TTC GCC CTC ATC CTC TAC AGC CTG TCC TGT ATA TTG ATA CCG TGC ATC TCC CTC ATG ACT AAA CCC ATT GAC GAC
  526  F   A   L   I   L   Y   S   L   S   C   I   L   I   P   C   I   S   L   M   T   K   P   I   D   D
 1651 AAA CAT CTG TAC AGG CTC TGC TGG AGT TTG AGG AAC AGC ACT GAG GAG AGG ATT GAT CTG GAA AAA GAT GAC TGG
  551  K   H   L   Y   R   L   C   W   S   L   R   N   S   T   E   E   R   I   D   L   E   K   K   D   W
 1726 ACT GAA AAT CAG GAT TCA GAT TCT GTG CAA ACA GAA GAG GTG CGC AAG GAT CCA AGC TGT TGG AAG AAG GCC TAT
  576  T   E   N   Q   D   S   D   S   V   Q   T   E   E   V   R   K   D   P   S   C   W   K   K   A   Y
 1801 AAC TGG TTC TGC GGT TTT GAT CAA GGC AAT GCA CCT AAA CTG ACT AAA GAA GAG GAG GCA GAG TTG AAT TTG AAG
  601  N   W   F   C   G   F   D   Q   G   N   A   P   K   L   T   K   E   E   E   A   E   L   N   L   K
 1876 CTC ACA GAC ACC ACT GAG AAA CCG CTA TGG AGA AAC GTG GTC AAC GCT AAT GCT ATT ATC CTC CTC ACC GTC TGT
  626  L   T   D   T   T   E   K   P   L   W   R   N   V   V   N   A   N   A   I   I   L   L   T   V   C
 1951 GTC TTC TTC CAT GGT TTC TTT GGC TAA
  651  V   F   F   H   G   F   F   G   *
      Aaatcatcagtatcaactaactctgttaaggatattagagctgtattctgctgacagaaatagtatcttatcagtaacatgctaaacataattaatgtgattgtaag
      aagttacgaaagttcctcttgaaacacttcacacttccttataatatcctccacaaaaacgtccttactgctgcctactgttttgatttgatggcaggaagtagga
      aaaaaaaaatatttttgactttaactttaaacccaagactaaatctcataattatttccttgttttcttccttgtagtcatgtcaaatctttcttagcgtgctagtatctaat
      ttaacacatgcctgactgttttactgtacatttcacaaagtttaccgcacgttcttccttatacttttaacaaattacagagtaatagtttcaactaaaattatgaagt
      ttgagtcagctttgttgtttgttccctgtaaagtcctctgatgcattatgatcaaatcaatgcaaatacttttctgactgttttaacatgcctctcatctaccaaactc
      caaaaacatttttatatttggtcttactatttggtacgttctgattatcttgtaagtaaaaatatatattttttgtctaggacaagcaaagatattttatttaaaaacattc
      atatttttgccatggtatatgtattgtactctgtaatatattaatttaatgttttctgtctatatttaataaagattaatgaatttttagtaaaaaaaaaaaa
```

Figure 2. Nucleotide and deduced amino acid sequence of Na^+/glucose cotransporter in intestine of *Cyprinus carpio* L. The sequence contains a single open reading frame which encodes a protein with 658 amino acids. The complete 5'-untranslated region was 113 nucleotides.

Sequence analysis of *C. Carpios sglt1* gene

The deduced amino acid of carp Sglt1 using EXPASY is composed of 658 amino acids with a molecular weight of approximately 72.9 kDa and the isoelectric point of 6.35. The secondary structure of the deduced Sglt1 amino acid sequence was analyzed to seek potential transmembrane regions using TMHMM Server v. 2.0 (DTU) (Figure 3). Fourteen transmembrane domains were also putatived in this study using SOPMA (Significant improvement in protein secondary structure prediction by consensus prediction from multiple alignments) (Figure 4). The *sglt1* gene contains 47.26% of α-helix, 17.17% of extended strand, 2.74% of β-turn and 32.83% of random coil.

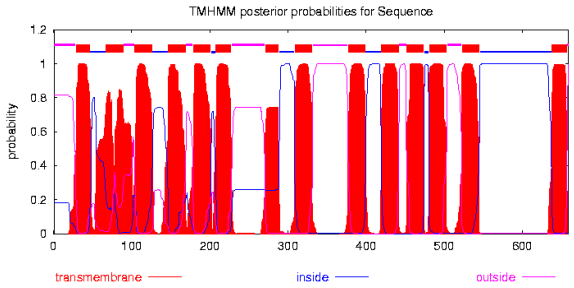


Figure 3. Secondary structure model and 14 transmembrane domains of Sglt1 Predicted by TMHMM Server v. 2.0. The C-terminal and the N-terminal of Sglt1 were out of the cytoplasm

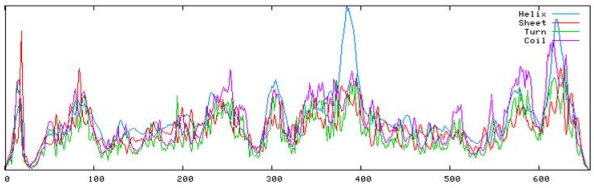


Figure 4. SOPMA result for *sglt1* from *Cyprinus carpio* L. intestine. The *sglt1* gene contains 47.26% of α-helix, 17.17% of extended strand, 2.74% of β-turn and 32.83% of random coil.

SignalP 3.0 Sercer analysis predicted a signal peptide of carp Sglt1 positioned in the amino-terminal (N-terminal) sequence (MGEEYFGFSWVRNENRKNV TIYVNNPADISVIVIYFLVVLAVGIWA) (Figure 5).

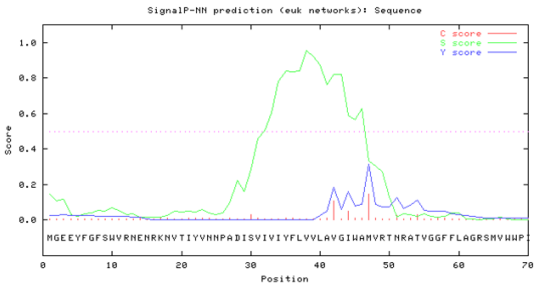


Figure 5. SignalP 3.0 Sercer analysis predicted a signal peptide of carp Sglt1 positioned in the amino-terminal (N-terminal) sequence (MGEEYFGFSWVRNENRKNVTIYVNNPADISVI VIYFLVVLAVGIWA).

Homology and phylo genetic analysis of *sglt1* genes

The *sglt1* cDNA sequence achieved in this study has been submitted to GenBank and assigned the accession No EU328389.1. The reference sequences in the analysis were downloaded from GenBank. The deduced amino acid sequence of *C. carpio sglt1* was 72.3%, 72.1%, 71.4%, 73.7%, 74.3%, 75.0%, 74.0%, 75.2% and 90.7% identical to Human (*Homo sapiens*), Horse (*Equus caballus*), Rabbit (*Oryctolagus cuniculus*), Bovine (*Bos taurus*), Bat (*Rhinolophus ferrumequinum*), Rat (*Rattus norvegicus*), Mouse(*Mus musculus*), Spiny dogfish (*Squalus acanthias*), Zebrafish (*Danio rerio*) respectively, as shown in Table 2. The homology and divergence among the sequences were calculated using the Laser-gene analysis software package (DNAMAN, USA) (Figure 6). The deduced amino acid sequence of Sglt1 in *C. carpio* was the lowest similarity with that in rabbit (71.4%) and the highest similarity with that in *Danio rerio* (90.70%)

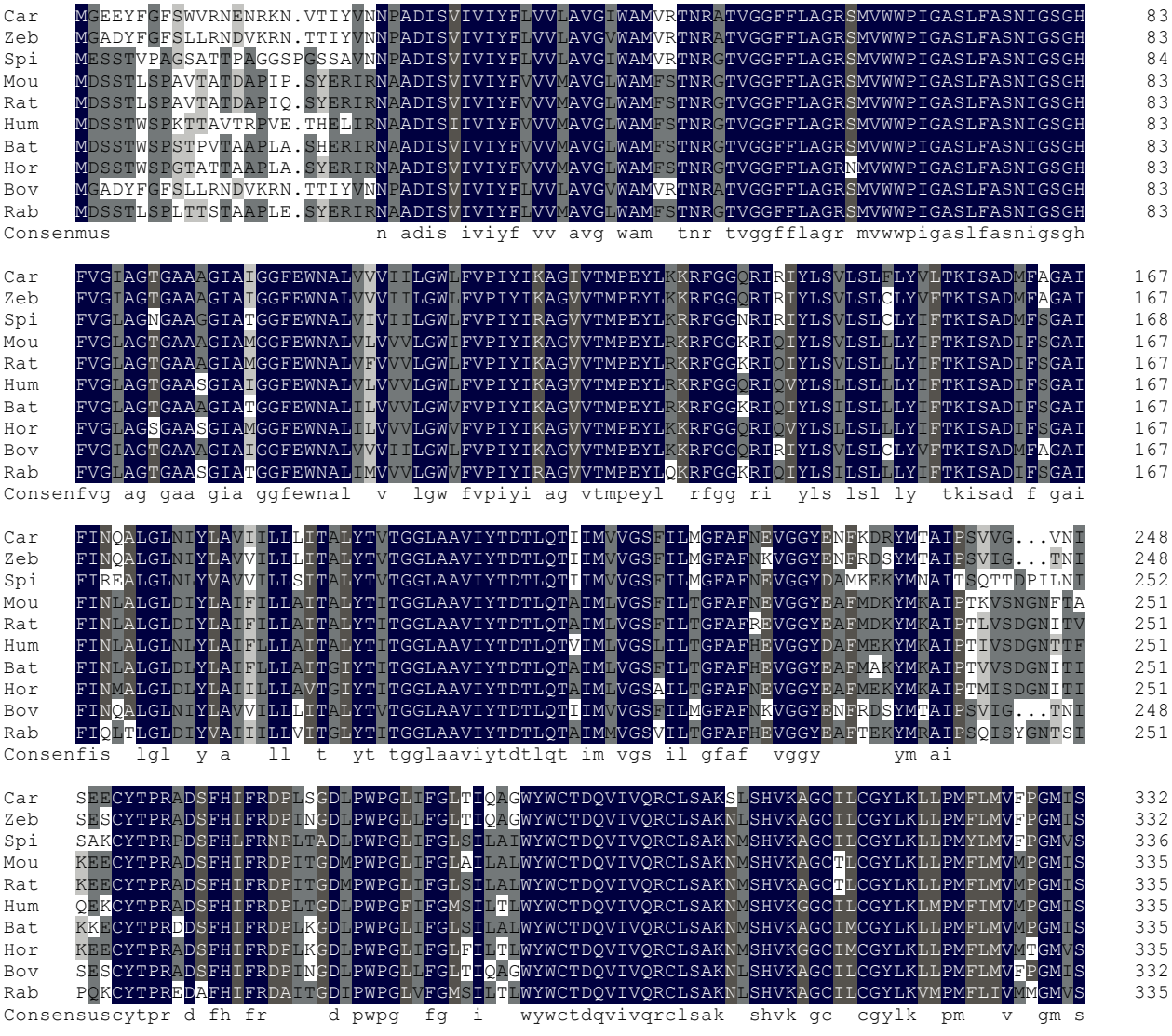
4. Discussions

The Na^+/glucose cotransporter (Sglt) are members of the expanded solute carriers SLC5A family and are predominantly expressed in the brush-border membranes of small intestine and proximal convoluted tubule of the kidney (Zhao *et al.*, 2005). Sglt1 as a member of the Na^+-glucose cotransporter family, play an important role in transporting sodium and glucose to maintain the basic physiological metabolism and nutrition requirement (Zhao *et al.*, 2005; Zhou *et al.*, 2003). Sglt1 moves 2 Na^+ ions with each glucose per cycle (D éz-Sampedro and Barcelona, 2010； Sabino-Silva *et al.*, 2010). In present study, *sglt1* was first cloned and characterized in *C. carpio*, and subsequently used to obtain new insights into the molecular mechanism of Na^+-glucose cotransporter.

Table 2. The similarity dataset of Sglt1 amino acid sequences between *C. carpio* and other species

	Car	Zeb	Spi	Mou	Rat	Bat	Bov	Hor	Hum	Rab
Car	100%									
Zeb	90.7%	100%								
Spi	75.2%	75.0%	100%							
Mou	74.0%	75.1%	72.6%	100%						
Rat	75.0%	76.0%	73.5%	95.8%	100%					
Bat	74.3%	74.0%	72.1%	89.9%	90.0%	100%				
Bov	73.7%	74.3%	72.8%	88.0%	86.9%	89.3%	100%			
Hor	72.1%	71.8%	71.5%	86.9%	86.5%	89.4%	86.6%	100%		
Hum	72.3%	72.8%	71.0%	88.0%	87.7%	88.1%	85.8%	87.2%	100%	
Rab	71.4%	72.1%	71.5%	86.0%	86.3%	86.6%	85.0%	84.6%	84.6%	100%

Note: The similarity dataset of Sglt1 amino acid sequences were from *Cyprinus carpio* L. (Car), Zebrafish (Zeb), Spiny dogfish (Spi), Human (Hum), Horse (Hor), Mouse (Mou), Rabbit (Rab), Bovine (Bov), Bat (Bat) and Rat (Rat).



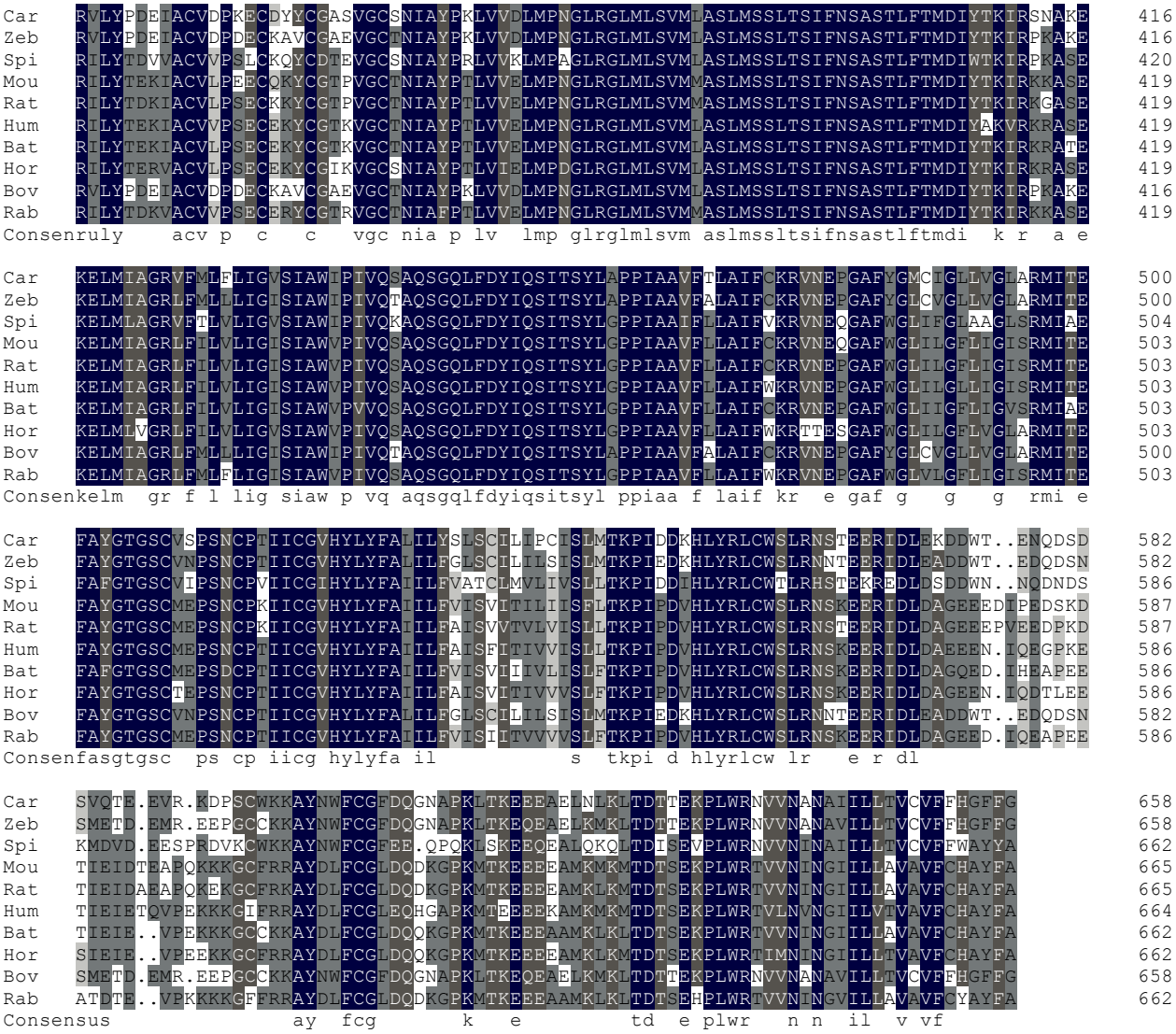


Figure 6. Alignment of deduced amino acid sequences of the Sglt1 subunit from the *Cyprinus carpio* L. (Car), Zebrafish (Zeb), Spiny dogfish (Spi), Human (Hum), Horse (Hor), Mouse (Mou), Rabbit (Rab), Bovine (Bov), Bat (Bat) and Rat (Rat). These protein sequences were aligned using the Clustal program. Identical amino acids are shown on a black background, ≥75% similar amino acids on a red background and ≥50% similar amino acids on a green background.

Totally, based on the characteristic analysis of the transmembrane and cytoplasmic domains, it was indicated to be the highest conservative between *C. carpio* and other species. For the Sglt1 subunit of the *C. carpio* it was high similar with that of Zebrafish, as well as the homologues of Spiny dogfish. On the other hand, phylogenetic trees were constructed using MEGA4.0 (Fig. 7). The nucleotide sequences of *sglt1* gene from several species were classified into two major groups. The Sglt1 subunits of other mammal were clustered into one group. The Sglt1 subunit of *C. carpio*, Zebrafish and Spiny dogfish were clustered into another group. The homology of Sglt1 was highest between *C. carpio* and Zebrafish.

The major member of Sglt1 family have been successfully cloned and sequenced (Hediger *et al.*, 1987; Pajor *et al.*, 1992; Kwon *et al.*, 1992; Kong *et al.*, 1993) in the intestine of human (Hediger *et al.*, 1989), rat (Lee *et al.*, 1994; Aoshima *et al.*, 1997), mouse (Tabatabai *et al.*, 2001), rabbit (Hediger *et al.*, 1987; Morrison *et al.*, 1991), bovine (Zhao *et al.*, 1999), and zebrafish, etc. The results of comparison analysis showed that

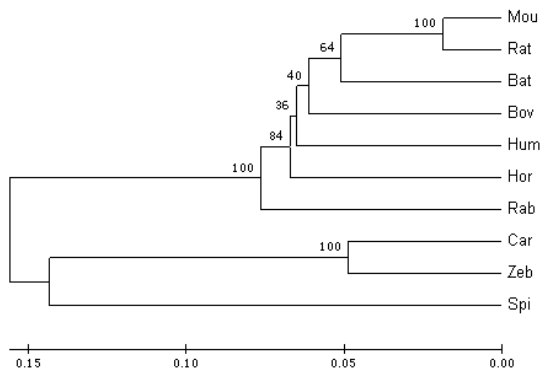
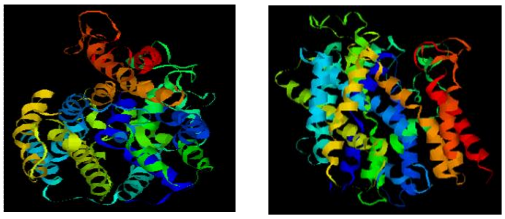


Figure 7. The Phylogenetic relationship of fish Sglt1 and its orthologues. A molecular phylogenetic tree of Sglt1 was generated based on the alignment of the amino acid sequences by MEGA4. The accession numbers for the sequences are as follows: Hum, *Homo sapiens* (AAA60320); Hor, *Equus caballus* (NP001075341); Rab, *Oryctolagus cuniculus* (CAA39040); Bov, *Bos Taurus* (AAM34274); Bat, *Rhinolophus ferrumequinum* (ACC68880), Rat, *Rattus norvegicus* (BAA03676); Mou, *Mus musculus* (AAF17249); Spi, *Squalus acanthias* (CAJ75582), Zeb, *Danio rerio* (NP956975).

the homologous sequences of other fishes were more than 90% and among different animals were more than 70% (Table II). It is showed that *sglt1* gene is the high conserved sequence.

Transporters with a C-terminal extension (e.g. hSglt1) were proposed to have an additional 14th TM (Hirsh *et al.*, 1998). Information on tertiary interactions has recently been gained by chemical cross-linking of splits of the sodium-galactose transporter in *Vibrio parahaemolyticus* (vSglt) (Reizer *et al.*, 1994). However, little is known about the tertiary status of other members of the SSSF. The 11~15 putative transmembrane domains (TMs) in a-helical conformation of SSSF proteins were the average hydropathy plot (Turk *et al.*, 1997). For PutP, it contained 13 TMs with the N-terminus located on the periplasmic side of the membrane and the C-terminus facing the cytoplasm.

To gain structural insight into the mechanistic details, the structure of vSglt were solved in the presence of Na^+ and galactose (Faham *et al.*, 2008). The 3-D protein models in this study were predicted by comparative protein modeling program SWISS-MODEL (Fig. 8). The transporter has two charged regions in common at residues 130-140 and 408-420. It was further confirmed from the similarity between sequences, when compared with the secondary structural elements such as the occurrence of a-helix in front of transmembrane regions 4. The structural core is involved in the inverted repeats of 5 TM helices (TM2-TM6 and TM7-TM11). It was suggested that glucose might be bound in the center of the core, and a Na^+-binding site might be located at the intersection of TM2 and TM9. Results of the study are in line with the result of Sodium-binding site and galactose-binding site by Faham *et al.* (Faham *et al.*, 2008).



A B

Figure 8. The predicted 3-D structure of Sglt1 in intestine of *Cyprinus carpio* L. A, Structure of Sglt1 viewed in the membrane plane. B, Structure of Sglt1 viewed from the intracellular side.

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2/3/2011

Towards Rural Women's Empowerment and Poverty Reduction in Iran

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Abstract: This paper provides an approach for rural women's empowerment to poverty reduction in Iran. Although, rural women are certainly a major contributor to poverty reduction in many rural areas in developed countries. But the result of this study found that women's empowerment is limited by the same cultural restrictions that limit their access to education and health services, and these impose serious constraints on their autonomy, mobility, and on the types of livelihoods that are available to them. The finding can assist the local and national organizations for remove this problem in face of women's participation for poverty reduction in Iran.
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Key words: Poverty reduction, women's empowerment, rural area

1. Introduction

The notion of empowerment, however, is not easy to define because of its extremely variable meaning that varies as influenced by social contexts, individual conditions and political circumstances (Quagliariello, 2009). Empowerment is a key for quality of life and human dignity, good governance, pro-poor growth, project effectiveness, and improved service delivery. Empowerment in terms of citizen inclusion and participation at the local level can help ensure that basic services reach poor people, and can lower operation and maintenance costs by comparison with centrally managed activities (World Bank, 2002).

The term 'empowerment' is a contested concept which connotes different meanings depending on different perspectives of looking at it (Asnarulkhadi & Aref, 2009). The empowerment of women means for them to have the necessary ability to undertake a number of tasks either individually or in groups, so that they have further access to and control of society resources. Empowerment is recognized as an essential strategy to strengthen the well-being of individuals, families and communities, government and non government agencies (Aref, 2010a). Empowerment has different meanings in different socio-cultural and political contexts, and does not translate easily into all languages. An exploration of local terms associated with empowerment around the world always leads to lively discussion. These terms include self-strength, control, self-power, self-reliance, own choice, life of dignity in accordance with one's values, capable of fighting for one's rights, independence, own decision making, being free, awakening, and capability—to mention only a few. These definitions are embedded in local value and belief systems (World Bank, 2002).

Since the 1990's women have been identified as key agents of rurla development and women's equality and empowerment are seen as central to a more holistic approach towards establishing new patterns and processes of development that are sustainable (Handy & Kassam, 2004a). However, in many societies around the world, women never belong wholly to themselves; they are the property of others throughout their lives. Their physical well-being – health, security and bodily integrity – is often beyond their own control. Where women have no control over money, they cannot choose to get health care for themselves or their children(Drinkwater, 2005).

In Iran there are some local organization for poverty reduction; but there are many challenges that face organizations who make it their goal to empower women (Narayan, 2002). Hence, this paper addresses the specific challenge that is faced by women's empowerment in rural area of Iran.

2. Literature Review

According to World Bank poverty is hunger. Poverty is lack of shelter. Poverty is being sick and not being able to see a doctor. Poverty is losing a child to illness brought about by unclean water. Poverty is powerlessness, lack of representation and freedom (Drinkwater, 2005). Whereas poverty is a multi-faceted phenomenon that hinders the satisfaction of basic life requirements, the tendency has been for some analysts to conceptualize it in narrow economic terms by insinuating that it is

simply the lack of money (Smith & Ross, 2006). Poverty has been defined as the "denial of opportunities and choices most basic to human development to lead a long, healthy, creative life and to enjoy a decent standard of living, freedom, dignity, self-esteem and respect from others" (Hirschowitz et al.,2000, p. 54).

Poverty can be reduced through women's empowerment. Hence this study provides an approached for development of women's empowerment for poverty reduction in Iran. Although the notion of women's empowerment has long been legitimized by international development agencies, what actually comprises empowerment, and how it is measured, is debated in the development literature. Malhotra, Schuler and Boender, (2002) provide an excellent review of this debate. They review the many ways that empowerment can be measured and suggest that researchers pay attention to the process in which empowerment occurs. The frequently used gender empowerment measure is a composite measure of gender inequality in three key areas: Political participation and decision-making, economic participation and decision-making and power over economic resources (Handy & Kassam, 2004b).

There are thousands of examples of empowerment strategies that have been initiated by poor people themselves and by governments, civil society, and the private sector. Successful efforts to empower poor people, increasing their freedom of choice and action in different contexts, often share four elements:

- Access to information
- Inclusion and participation
- Accountability
- Local organizational capacity(World Bank, 2002).

In despite of these potential, there is a number barriers for poverty reduction. For example lack of government programs and organizational capacity to respond to the opportunities provided (Jamieson & Nadkarni, 2009). Lack of formal education and skills and planning (Bushell & Eagles, 2007, p. 154). As a consequence, community facilities and services may be unacceptable for women. Hence building women's empowerment in rural communities is necessary for stakeholders involved in rural development (Bushell & Eagles, 2007).

Implementing empowerment processes needs to allow women to play more effectively their peculiar role in areas such as, for instance, food security, education, health service, agricultural development policy and natural resources management is a prerequisite for sustainable development. The essential actions leading to the strengthening of the role of women in rural area respond to some basic priorities: access to resources, support to self-determination, awareness- raising, participation in the production and right to welfare policies (Quagliariello, 2009).

Women's development priority actions in the rural areas:

1. Welfare
2. Access to Resources
3. Awareness-Raising
4. Participation
5. Self-Determination

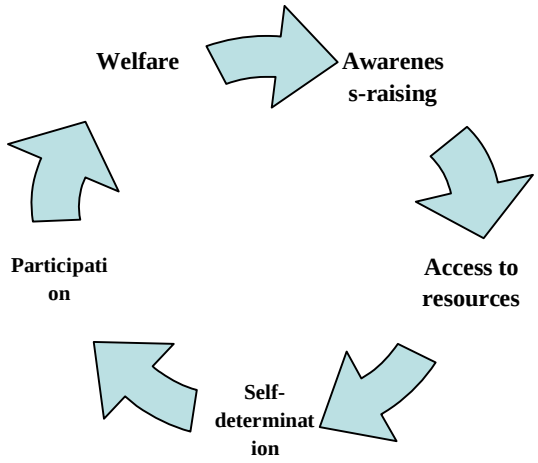

Figure 1. Women's empowerment cycle adapted from Quagliariello (2009)

3. Methods

Marvdasht in Fars Province, Iran was selected as a case study area because it provided many opportunities to develop rural development; This study is based on quantitative methodology to investigate the barriers of women's empowerment related to poverty alleviation. The participants in FGD were educated women that were engaged in education. Hence to achieve the objectives of this study, the researcher uses quantitative method. Focus group discussion (FGD) was performed to collect data from local residents. FGD conducted in a group setting and was used for obtaining a better understanding of participants' attitudes (Aref, 2010b). There is no consensus among qualitative researchers on the optimal number of participants in FGD. But the ideal number of participants in each FGD is six to ten. The respondents were educated women that participated in 10 groups. They ranged in age from 22

- 45 years. The researcher explained to them the objectives of the study and what questions would be asked. The researchers examined, categorized participants responses from each focus group of villagers that were recorded in video tapes

3. Result

Information for this study was gathered from educated women through FGD. A qualitative analysis was undertaken to determine viewed the current level of women's empowerment in poverty reduction and also barriers of women's empowerment related to poverty reduction. There were overall 55 participants with an average of 33 years old. The FGDs held on in 10 educational centers in Marvdasht, Fars, Iran. All participants were teacher in school. They were chosen because of their knowledge. The questions were asked about to contribution of women in poverty reduction and barriers of women's empowerment for poverty reduction in rural area.

In terms of women's empowerment on poverty reduction, they believe that rural women does not have important role in their villages especially on poverty reduction. The findings showed that women in rural in their villages are without any certain planning for poverty reduction. Although the FGD respondent referred to variety barriers in terms of women's empowerment for poverty reduction in their villages, the study refer to some common barriers which have been discussed in majority of FGD groups. The most barriers in terms of rural women's empowerment for poverty reduction were including:

1) Lack of suitable conditions: The majority of FGD participants believed there are no suitable conditions in their village for women's participation in social and political participation and decision making.

2) Lack of resources: The lack of financial and community resources in the villages. The most participants mentioned to this issue as main obstacles to women's empowerment for poverty reduction.

3) Lack of skill and knowledge: The participants in all groups mentioned to this issue as one barrier for poverty reduction in their villages.

5) Lack of suitable training: FGD respondents believed the lack of training; especially was behind the failure investment for poverty reduction.

6) Cultural restrictions in some families: through FGD it found the family culture is the main barriers towards women empowerment towards education and occupation.

7) *Rural traditional values*: In some case women's empowerment can limited by some values.

Overall the result indicated that in most rural area women's empowerment is limited by the same cultural restrictions that limit their access to education and health services, and these impose serious constraints on their autonomy, mobility, and on the types of livelihoods that are available to them. Their lack of access to education and resulting low-skill levels limits their opportunities for employment further.

4. Conclusion

This paper addresses the specific challenge that is faced by women's empowerment in rural communities in Marvdasht, Iran. This study has identified the barriers of women's empowerment for poverty reduction. Lack of capable organizations, lack of resources, and cultural restrictions were an important element contributing to limited rural women for poverty reduction. Overall the findings indicated that residents have negative attitude towards contribution of rural women for poverty reduction in their village. They refereed to government policy and lack of local organizational capacity as main barriers related poverty reduction. Clearly, the described barriers may not be only specific to women's empowerment strategy; some of them may also be considered as common general problems of rural in other communities in Iran. Base on the findings for women's empowerment, any project should include, include the below items:

- The integration of procedures and principles aimed at enhancing and promoting the role of women as creators of development,
-The assessment of women as a major resource for the development of a country;
-The consideration of their state of health, educational level and nutritional status as significant indicators of the degree of development of a country;
- The enhancement of the image of women as guardians of the traditional know-how so as to favor and promote their involvement in rural economic activities, and management processes.

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A Prospective and Retrospective Analysis of Patients with Post-Stroke Epilepsy Presenting at Tertiary Care Hospital

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Abstract: Stroke is one of the most common causes of disability in Saudi Arabia and when seizures complicate the stroke, the disability, cost, psychological impact and post traumatic stress on the family are tremendously increased. As for the best of author's knowledge there is no available data regarding the incidence, frequency, outcome and the risk factors or predictors of seizures after stroke in Saudi Population. Our study was conducted in King Fahd Hospital located in Eastern Province of Saudi Arabia. It is the main tertiary care Hospital in the region. We collected our sample in three consecutive years from 2007 to 2009. In the first two years data was collected from medical records system and in the third year the data of patients and controls was collected from newly admitted stroke patients. The study concluded mean incidence of post stroke epilepsy (PSE) to be about 9.6 %. A lower blood sugar and higher Rankin Disability scores were found to be significantly higher amongst patients with post-stroke epilepsy. There was no significant difference in co morbid diseases on developing PSE. The present study also showed that the occurrence of post stroke epilepsy was positively associated with increasing age and male gender.

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Keywords: Analysis; Patients; Post-Stroke; Epilepsy; Tertiary; Hospital

1. Introduction:

Post stroke Epilepsy (PSE) is one of the major causes of epilepsy in elderly population (1,2). In most studies the percentage of seizures that starts after the age of 60 years is attributed to stroke (3). The frequency of post stroke epilepsy in various populations is reported between 2.3 % to 43 %. This high variability in frequency of epilepsy is suggested to be due to different ethnic groups of studies or the definition of post stroke epilepsy or methodology and study design (4,5). In our study post stroke epilepsy was defined as a single or multiple convulsive episode/s (Fit/s) after stroke and thought to be related to cerebral damage due to stroke regardless the time of onset following the stroke.

2. Materials and Methods:

Aim of the study:

To identify, gather and study the overall risk of (post stroke epilepsy) PSE and to find the predictors for PSE both retrospectively and prospectively.

Study:

The study included all patients admitted in the hospital with stroke over a period of one year prospectively and two years retrospectively , to determine the incidence , frequency, outcome and risk factors for seizures after stroke at the King Fahd Hospital, Hofuf (KFHH).

Stroke was defined according to WHO criteria and did not include patients with subarachnoid haemorrhage, rupture of AV malformation, transient ischemic attacks.

The seizures and epilepsy were defined according to the criteria defined by International League Against Epilepsy. PSE was defined as two or more attacks of seizures after stroke.

All patients had CT scan or MRI of the brain for the diagnosis and localization of stroke. The disability after stroke was measured by Rankin Score.

Data analysis and data processing:

Data were entered and processed using SPSS (Statistical Package for Social Science) version 16.0 (SPSS Inc. Chicago IL). Both descriptive and inferential statistics were applied as appropriate. Statistical tests of significance including t-test and Mann Whiteny tests for continuous variables, Chi-square, Fisher Exact and *Z* tests for proportions were used for categorical data to detect difference between groups. Spearman Correlation coefficient was used to generate the inter-relation matrix between variables. Multivariate logistic regression model was generated

to define the association between patients characteristic and Rankin Scores (independent) to the occurrence of post stroke epilepsy (dependent), Odds ratio, 95% Confidence intervals and P value were reported for model. P value of < 0.05 was applied as a level of significance.

3. Results:

A total of 492 patients with stroke were admitted in the three years period from 2007 to 2009.

Table 1 shows the percentage of post stroke epilepsy among admitted stroke patients the three tears period the incidence of PSE ranged from 10.1 to 10.6% with an overall average of 10.4%. The incidence of PSE did not show significant relation in relation to time.

Table 2 displays basic demographic and clinical characteristics of stroke patients in relation to the occurrence of PSE. Those with post stroke epilepsy were significantly older in age and predominantly females and of rural residence. With the exception of significantly higher proportions of myocardial, coronary heart disease and reported dyslipidemia, there was no significant difference regarding the frequency and nature of the encountered co-chronic morbidities between in relation to the occurrence of PSE, although hypertension was recorded in 81.6% among those with epileptic activity compared to 70% among those without the activity.

Table 3 demonstrates the clinical characteristics of patients included in relation to the presence of PSE. It reveals that the occurrence of epileptic activity was not related to the type of stroke encountered or blood pressure measurements at admission. On the other hand, those with post stroke epilepsy recorded a significantly lower random blood sugar levels at admission and deterioration of Rankin scores on admission. The most commonly encountered type of seizures were the secondary generalized Tonic Clonic Seizures (66.7) followed by status epilepticus and simple partial types. In 78.4% of patients with PSE, seizures were controlled by using a single antiepileptic drug. Recurrence of seizures was recorded in 43.1 % of cases with mortality rate of 7.8%.

Table 4 depicts the intercorrelation matrix of potential independent variables associated with the development of post stroke epilepsy. It shows that the occurrence of post stroke epilepsy was positively associated with increasing age of the patients and female gender and negatively correlated with random blood sugar level at admission. Higher Rankin Scores were significantly positively correlated with the development of post stroke epilepsy. Neither the nature of current or previous stroke nor the multiplicity of the co-morbid disease conditions were not significantly correlated with the development of epileptic activities in the included patients.

Table 5 demonstrates the multivariate logistic regression model of predictors for the development of post stroke epilepsy. Female gender, higher Rankin score at admission and lower random blood sugar levels were significant positive predictors for the development of post stroke epilepsy among the included patients.

4. Discussion:

In our study the percentage of post stroke epilepsy among all admitted stroke patients in three consecutive years averaged 9.6 % which is almost similar in frequency as reported in western literature (6, 7, 8). A slightly higher frequency is reported from India while lower frequency of PSE has been reported from China. The variation in the frequency of PSE is most likely due to enrolment of patients in the studies and the criteria of PSE adopted by various studies. It is to be noted though that the definition of PSE influences the frequency.

Authors who included all seizures after a stroke and/or all seizures after stroke without distinguishing between acute, early, and late seizures reported a higher frequency of PSE.

Stroke incidence increases with advancing age (9). Cerebrovascular disease is the number one cause of epilepsy in the elderly population.(10) In a study of unselected population of over 2 million people in England and Wales, Wallace and colleagues found that both age specific incidence and prevalence of epilepsy are higher in older people(11). Our study also found that occurrence of post stroke epilepsy was positively associated with increasing age of the patients. This finding was not different from some studies which did not find age to be a significant predictor for PSE(6) or others in which young age to be a weak predicting factor, but the relative risk of seizures in younger patients compared with older ones did not reach statistical significance(8). The studies are at variance with regard to preponderance of PSE with regard to gender. In our study, the female were significantly at higher risk to develop post stroke epilepsy while some other studies found more common among the male gender is at higher risk (9)

Our study did not find any significant correlation regarding ischemic versus hemorrhagic stroke for onset of PSE. Some other studies has shown higher frequency of PSE among hemorrhagic stroke compared to ischemic stroke (9 -12).

Although we did not compare the size of stroke lesion in relation the occurrence of PSE, however, we found strong correlation between Rankin score and onset of PSE. Some other studies have also

found correlation between the severity of stroke and PSE. (13)

Another important finding was that PSE patients have lower random blood sugar levels compared to those without seizures. We know that hypoglycaemia can induce seizures, however, in our study none of the patient presented with hypoglycaemia. One possibility is that patients with stroke and relatively lower blood sugar compared to normals may contribute to initiation of seizures. Further studies are needed to probe this observation. We did not find any other study showing this disparity. However, we did not find any correlation between other co morbidities and onset of PSE.

In conclusion, our study shows that frequency of PSE is relatively higher compared to other studies, and that age and severity of stroke are major risk factor for developing post stroke epilepsy.

Table1. Incidence of post stroke epilepsy from 2007 to 2009 among patients admitted to King Fahd Hospital-Al Hofuf.

Year	All cases of stroke		Stroke with epileptic activity		
	No.	%	No.	%	% out of total stroke (incidence)
2007	198	40.2	20	39.2	10.1
2008	151	30.7	16	31.4	10.6
2009	143	29.1	15	29.4	10.5
Total	492	100.0	51	100.0	10.4

Chi square for trend=1.34, P= 0.368

Table 2. Basic demographic and clinical characteristics of the included stroke patients.

Variables	**Cerebral stroke (N=492)**		**P value**
	With epilepsy (N=51) No. (%)	**Without epilepsy (N=441) No. (%)**	
- Gender:			
Males	20(39.2)	387(85.7)	
Females	31(60.8)	54(14.3)	0.0001[a]
- Residence:			
Urban	25(49.0)	290(65.8)	
Rural	22(43.1)	98(22.2)	0.004[a]
Bedouins	4(7.8)	53(12.0)	
- Age in years (mean ±SD)	64.0±17.5	54.8±11.3	0.004[b]
- Occupational status:			
Working	30(58.8)	292(66.2)	
None (retired/no job/housewives)	21(41.2)	149(33.7)	0.293[c]
- Co-morbid conditions: total	49(96.1)	429(97.3)	0.647[d]
Hypertension	11(21.6)	84(19.6)	0.806 [d]
Diabetes mellitus	2(3.9)	-	--
Diabètes mellitus + Hypertension	27(52.9)	178(41.5)	0.115 [d]
Diabetes+ hypertension+ CHD	2(3.9)	52(12.1)	0.142 [d]
Others!	5(9.8)	127(29.6)	0.006 [d]
Sickle cell disease	2(3.9)	-	--
None	2(3.9)	12(2.7)	0.965 [d]
- Previous Stroke: total	51(100.0)	441(100.0)	
Hemorrhagic	3(5.9)	32(7.3)	0.941 [d]
Ischemic	45(88.2)	383(68.4)	0.952 [d]
Both hemorrhagic/ischemic	2(3.9)	-	--
Transient ischemic attack (TIA)	1(2.0)	26(5.9)	0.398 [d]

! Includes: dyslipidemia, myocardial infraction, and coronary heart disease.

[a]: = Chi-square Fisher exact [b]= t-test, [c]= Chi-square [d] = *Z* test for proportions.

Table 3. Characteristics of the included patients in relation to the presence of epilepsy and current stroke.

Variables	Cerebral stroke (N=492) With epilepsy (N=51) No. (%)	Cerebral stroke (N=492) Without epilepsy (N=441) No. (%)	P value
- Current stroke type:			
Ischemic	34(66.7)	281(63.7)	
Hemorrhagic	17(33.3)	160(36.6)	0.677[a]
- Blood pressure on admission (mmHg):			
Systolic Mean ±SD	152.1±38.2	149.4±33.7	0.723[b]
Diastolic Mean ±SD	90.2±21.6	94.6±22.4	0.364[b]
- Random blood sugar level (mg/dl):			
Mean ±SD	115.5±60.9	180.7±117.3	0.008[b]
- **Rankin Scores:** median (mean ±SD)			
Baseline	3.5(3.46±1.34)	2.0(2.10±0.93)	0.001[c]
Admission	4.0(3.73±1.26)	2.0(2.15±0.94)	0.001[c]
Discharge	4.0(3.80±1.93)	2.0(1.54±1.07)	0.001[c]
- Type of seizures:			
Simple partial	5(9.8)	-	
Complex partial	2(3.9)	-	
Secondary generalized	3(5.9)	-	
Generalized GTC	34(66.7)	-	
Status epilepiticus	6(11.8)	-	
Undefined	1(2.0)	-	
- Seizure control:			
One antiepileptic drug	40(78.4)	-	
Two anti-epileptics	10(19.6)	-	
More than two drugs	1(2.0)	-	
- **Total Rankin score**: Median (mean ±SD)	3.75(3.78±1.77)	-	
- Recurrence of seizures:	22(43.1)	-	
- Mortality:	4(7.8)	-	

SD = Standard deviation. [a]: Fisher exact, [b]= t-test, [c]= Mann Whiteny test.

Table 4. Intercorrelation matrix of variables associated with epileptic seizures among the included cases of stroke.

Variables	2	3	4	5	6	7	8	9	10
1-Age	.236*	.054	-.114	.010	.146	.293**	.212*	.186	.330**
2- Gender		.093	-.258*	-.169	.122	.495**	.141	.183	.097
3-Co-morbidity			-.071	-.226*	-.080	.043	-.027	-.101	-.166
4-Random blood sugar				-.185	.020	-.348**	-.267*	-.365**	.579**
5-Previous stroke type					-.283	-.083	.072	.078	.124
6-Current stroke type						.294	-.283	.232	.239
7-Post stroke epilepsy							.488**	.576**	.579**
8-Rankin base line								.858**	.757**
9-Rankin admission									.831**
10-Rankin discharge									

Spearman's significant correlation at * P< 0.05, ** P < 0.001

Gender (1=male, 2=female), co-morbidity (1= multiple, 2= single), previous/current stroke type (Ischemic = 1, other =2), post-stroke epilepsy (0= none, 1= present).

Table 5. Multivariate logistic regression analysis of predictors for post stroke epilepsy.

Independent variables	*B* coefficient (SE)	Odds ratio (95% confidence intervals)	P value
- Gender	.883(.642)	3.18(1.76-5.71)	0.001
- Age	-.034(.037)	0.97(0.90-1.04)	0.358
- Random blood sugar level	-.236(.652)	1.81(1.09-3.03)	0.037
- Rankin scores on admission	.670(.891)	2.43(1.22-4.73)	0.041
- Rankin scores baseline	-.236(.652)	0.79(0.22-2.83)	0.305
Constant	-4.294		
χ^2 for the model	63.241		
P value for the model	0.001		
percent predicted	90.9		

SE= standard error. Gender (1=male, 2=female).

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Assessing Employment of rural women and its effect on other empowerment

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Abstract: Women form great part of total workforce that needed for agriculture part at universe, as one of the intangible factors at agriculture economy. So, statistics that was represented in relation to extent of women's activity is very lower than real extent. Because in this statistics, mostly, seasonal jobs, part time job, no wage job and their housekeeping activities, aren't considered. rural women, have different roles and duties such as husband, mother, crops producer , participate at ranching activities, planting ,maintaining, harvesting, processing, marketing and preparing food. Rural women maybe venturing to culture cash products, while cultivating subsistence products and if they have no farm land, they have to work for others instead receiving wage. We can consider such women as agriculture propagator, production expert and even in some case as policy maker. Other than activity at agriculture field, women's participation at rural development is critical and is considered in order to supply adequate and needed food.
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Keywords: empowerment, rural women

Introduction:

Rural women constitute about half of the world's population and in the world production supply they have energetic communion and constitute a great part of agriculture workforce. They constitute% 50 of the workforce and they participate in the production of half of the foods in the agriculture section. As an example the rural women constitute about 70 to% 80 of agriculture workforce in sub-Saharan Africa, %65 in Asia, %45 in Latin American & Caribbean, %80 in Nigeria & Tunisia and %80 in India, but their role in production system is the men's supplements roles and this causes a big responsibility inside their mother & wife duties and it takes a great time and energy of them. Studies in this field show that women spend about two thirds of their time for production, management & organize of their house as the men spend only one third of their time for such things. (Varzegar & Azizi, 2001).

Although we are familiar with the rural women's role in the village and family's economic, but they direct & indirectly start a new economic relation, with finding modern jobs & financial independency. Catching loan from financial organizations has forced them to have economic schematization for loan reimbursement and to have intellectual economic behaviors. So after that rural women become active in economic activities. In rural traditional economic, women only have productive role and they don't have any role in economic planning, providence and they don't pay any attention to profits and losses. But in this new condition, for managing affairs in best way, the women have to be active in all of the affairs from production to dispense and also in others economic aspects. In other words, women will not be a productive only; they will contribute in managing of economic activities and will find various economic behaviors. . (Araghzadeh, 2002).

Importance of women issue at Iran especially rural area, at one side face with fast population growth and mass of unemployed at process of access to rural growth and development, and at other side with limitation of facilities and productive resources. Rural women at all production level of agriculture products and livestock productions work alongside men and generally, development is multidimensional process and contains different economic, social, cultural and political dimensions. Women's participation at this process is active and affective participation, and main aspect of this participation was its economic dimension for rural women. Rural women have key role as a producer at agriculture activities, rural sources and services at rural area. rural women most efficient women of society and among people who are active at productive occupations, so it is obvious that attention to rural women as a strong arm at rural development can follow positive and undeniable affects, in this purpose (Lahsaeizadeh, 2000).

Rural women are considered either directly by producing livestock and agriculture products and rural industries and either by help to agriculture part as workforce and their share at third world countries is far more than other countries. Usually statistics

about women's share at agriculture productions is less than real extent because largely, at these statistics seasonal job, part time job, no wage and housekeeping activities sere not considered. Nevertheless, they are forces for creating revolution and potential resources to progress rural economy and increase growth rate of food production (Nawab Akbar, 1997).

Women's productive activities has affective role to increase revenue, rural family welfare, and its consequents is: foods status improvement, health, preventing irregular migration, literacy enhancement and development of rural family social status. Despite clearness of affective women's role at production, economy of village and country, they don't enjoy proper social base and they were deprived of educational and welfare programs especially at rural and nomadic area(Banihashem, 1999). Thus women and their roles should be considered particularly in order that they would find that first they are important and efficient; second they have educational needs and many technical gaps; third they shouldn't forget efforts for enabling themselves. As girls and women's discussion and solving their historical lag and restoring their social right are important and necessary, it is sensitive and accurate equally, because dominant patriarchal cultures at rural societies, put women at lower status. So that at some societies, women's duties are just upbringing and reproduction and maybe they are considered as workforce, and they are deprived of decision making and opining at family and society environment. (Samadi Afshar, 2004).

Global researches show that women played critical and important role at agriculture and now at most countries, they form major workforce of this part. In spite of importance of women workforce at different systems of agriculture, they have fewer access to development resources, compare to men. although during past two decades , various programs has been performed to enable women at agriculture , but due to different problems , gained success was very fewer than required extent . One of major problem in this filed is inadequate and inappropriate access to extensional services. Low efficiency of agriculture extension systems to provide services for rural women dosen't just refer to structure and function of these organizations and systems, but refer to other issues including research and cultural barriers in this field. However, one of essential needs to extend agriculture is, determining appropriate ways and approaches to educate women at every region or country (Fami 2000). at many past decades , significant global efforts were done to provide educating how to access information , appropriate and effective technology for female farmers that led to positive effects on producing agricultural crops and consequently increasing family welfare (Janice Jiggins and et al, 2003) .

Indeed, at many development programs, women couldn't apply theories and their own basic concepts. Problems such as lack of women's access to farm, credits, suitable educational and extensional services, exist at many areas, yet. (Paknazar, 2000).

In recent years, the point was well clear that a major share of the income of rural households are obtained through the women activity, and sometimes even share of women income in the household economy is more than the share of men. For example, in 2000, about 854 million women that include 32 percent of the workforce of the world are active economically and their major activity in third world countries are in the agriculture sector and 60 percent of cultivated rice, 90 percent produce vegetables and, 50 percent cotton and oilseeds, 30 percent had affairs and gardens, 90 percent silkworm related activities and 65 percent of rearing livestock-related activities and handicrafts have the highest proportion (Emadi, 2001). This shows that the role of women as agricultural work force, not only isn't less than men but they have greater share in the process of planting, cropping, and more importantly in the sale of crops and livestock and a research specialized that 50 percent of food global production activities were owed to women.

Difference at levels of policy making, investing and receiving salary for equal activity are universal phenomena. extent of women's participation at economic activities, extent of women's activity at economic activities, and is confirmation on lack of adequate attention to women's affair and their added value, because rural women work alongside men, at all levels of producing agriculture crops and livestock products and generally all affairs, and also spend their little leisure time for handicrafts such as rugs and carpets and etc. so it is necessary to establish self acknowledgement fields, directing women's economic and social ability and programming to attract their participation at different activities. At rural area, women have more significant role on family economy and inside activities and cause economic prosperity of society. yet, women couldn't gain their real position as active citizens who have talent for participation at economic, politic, social and cultural arena at most countries, especially developing country, and still their activities in economic calculations aren't considered, and they be considered as intangible workforce. Disappointing estimation about number of active rural women and underestimate about extent of their participation at economic activities is confirmation on lack of adequate attention to women's affairs and their added

value. they are major force to create revolution and potential sources to progress rural economy and increasing extent of growth rate of producing food productions, although traditionally, farming and ranching, has been male profession, but women's role was never restricted to house and family, so they are active outside (farming, ranching, forestry and …) other than inside activity (Balali, 2005).

since , rural women take different responsibility and roles such as producers of crops , ranching and keeping poultry, children education, housekeeping, supervising family economy and managing it, collecting firewood, weaving carpet, so illiterate women who haven't possibility to utilize mass media properly too, wouldn't able to do their duties and roles and also wouldn't be affective to develop rural societies. So importance of education is very critical for rural women especially extensional educations. Approximately in most UN reports, women has been considered as greatest deprived group at human societies, while at global level, about two third of all affairs is done by women. But only one third of all recorded affairs relates to women. And also just 1% of proceeds of estates and assets of world belong to women and two third of illiterates of world are women, however they form 50% of workforce at agriculture part and they produce half of foods at all over the world. So, educating women is important because of these reasons:

1- women's historical roles at agriculture development
2- rural women as mother and manager of home
3- rural women as decision maker at home and outside activities such as agriculture and …
4- rural women as productive factor at agriculture part and rural industry
5- rural women as affective member of society for participation at rural development

Since, goal of extension base, is empowering human of society and also this issue that women have basic role at producing different agricultural productions and rural industries, it is impossible to develop rural societies without considering rural women. (Deputy of extension and system operation Ministry of Agriculture jahad, 2000).

Aside from the economic role of women that clearly has been made in the past decades, the vital role of women in social and cultural dimensions of development process in rural areas has remained hidden from the polls. They train the next generation of farmers and teach them the next generation necessary knowledge. A Chinese proverb says, "If training a man, just training a man but if you teach a woman you teach a family." Women are local knowledge and local educators themselves, in preparing and providing food, health treatments and cultural values are the next generation (Fami, 2001). Women as the first group are known to have paid agricultural work, and evidence shows that women farmers have been the first. Important factor causing women's participation in agricultural activities has been, among them we can mention the following (Fami, 2001):

A - Seasonal agricultural employment, and intensified the need for labor in certain seasons.

B - Men migrate to find better jobs and to assume responsibility for home and farm and agricultural work and its management by women:

In some countries men migrate to cities, or on bringing those to wage jobs have led to women's responsibility for 30 to 40 percent of agricultural plants and are responsible, in some areas this figure reaches 70 percent (lahsaeizadeh, 2000).

C - Effect of cultural - social conditions on women work:

Sociological experience shows that kinship networks status and community practices determine that who and in which areas women can have activity and employment. Several Kinship networks provide different economic roles for women based on age, marital status and their place in father and husband family (Movahedi, 2005).

Empowering rural women:

Empowerment is capacity that woman can obtain in cultural and social environment, for economic independency and self reliance, by controlling over emotional decision making and far from violation. Empowering means, evolution and developing activities through non governmental organizations (NGOS) that lead empowerment to improve economic dimensions. (Amiri, 2000)

Enabling is process that, during it, people of society do activities to overcome barriers of advancement that finally cause their domination to determine their own density. The term "enabling" means overcoming fundamental inequalities. So it is different from self-reliance.

Enabling, enables individual to overcome any problematic condition and consider barriers and problems as part of life and positive campaign. Finally, enabling provides energy to overcome most intellectual barriers and external problems at private life (Balali, 2005).

Thus, among all what have been said, it is possible to present suitable definition of enabling women, as follows:

"Process of explaining women about themselves (and also men about them) for instances that they must or want to do, and growth of their

willingness and courage until they reach to needed competency "(management of rural and tribal women).

it should be noted here , that major factor which should be considered about women's ability , is eliminating individual and social barriers , and finally preparing field of economic and social participation for women at all fields . purpose of women's participation , is because of their dominance on all affairs of village including decision making process , organizations , forums , enterprising posts and … that involve , participation at all social and economic dimensions (Moazami and Heidari, 2005).

Conclusion & discussion:

If rural women could provide a job for them by getting credits, loan and other financial convenience, through their income they can get self-reliance or financial independency and we will see social, cultural & economic change in village. The question here is that if these changes have positive or negative aspects in the village? It's natural that every change in social phenomenon has both positive and negative aspect, but which is Important here is that which aspect is more than the other and it depends to different condition in various societies. In our rural society there is an especial social & cultural kind that it's outcome maybe different and in some case inconsistent. With these actions rural women could be in idealistic economic condition and they could live with out dependency to their husband's income. In most of the villages in Iran there is patriarchy in the families which is not acceptable for the most of the rural people and groups. When rural women became financially independent, it's acceptable to see its cultural & social outcomes.

Giving the right that women make decision, independency to their family, increasing the cultural knowledge among them& making relation with new institutions, having independency in making decision about marriage, occupation, migration & something like this are the right that women have got it. One of the issues that government should pay attention to is rural development issue especially at undeveloped countries. in this countries due to lack of proper policy making to improve quality of people life level of these areas, villagers migration to cities has increased considerably and led to urbanization growth and emergence of problems and also psychological, social, cultural and economical abnormalities especially at agriculture and ranching part. Also method for growth and rural development growth, require research at this field which can help government in order to economic, social and cultural programming and policy making. Creating local organizations and regional institutions with affective women's attendance and villager participation to solve problems are among important and affective substances that should be considered in regional programming. at developing and changing process of developed economy system of agriculture, value of women's activity changed as form of money which previously was as no wage workforce at family , and was given to her. Other than agriculture part (i.e. industry and public services) which are main field of women's work, rural women's participation is very important. The most important issues about women's social and political participation are participating at programming, decision making, performing decisions and valuing results.

Women at most countries, have low access to economic resources at the field of economic activity. They should reinforce them at this field by supplying economic facilities. Another part that changed women's attendance at economic affairs is agriculture activities. Opportunities which they gain at this part can have important impact on economic function and related social relations.

Same discussions were presented about identifying women's role on environment changes (especially in preserving natural sources) that related to women's life and job. Women's access to agriculture credits, because increasing and improving their efficiency at agriculture. Women's membership at cooperatives, also help them to receive facilities in order to supply needed inputs of agriculture, sale productions and make some production with aim of increasing efficiency. Most of researches found that women's education is related to their agriculture efficiency. Indeed, years which women used educational programs, related to their productions meaningfully. So, by identifying their needs, demands and interests and also by determining their issues, resources and preferences, we should prepare proper extensional and educational programs for them.

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Corneal Topography and in vivo Confocal Microscopy in Different Types of Posterior Polymorphous Dystrophy

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Abstract: To observe the morphologic changes in the corneas of patients with posterior polymorphous dystrophy (PPMD), using in vivo confocal microscopy and Orbscan Ⅱ corneal topography. Four patients with clinical diagnosis of PPMD, presenting to the Henan Institute of Ophthalmology, were included in this observational case series. The eyes of the 4 patients were examined by slit-lamp biomicroscopy, Orbscan Ⅱ corneal topography, and in vivo confocal microscopy. Two patients presented with corneal steepening on topography, as well as large areas of irregular polymorphous changes of the corneal endothelium on in vivo confocal microscopy consistent with PPMD. Confocal microscopy demonstrated craters, streaks, and cracks over the corneal endothelium surface. Pleomorphism and polymegathism were present in eyes with PPMD. Guttata and clusters of abnormal endothelial cells were also identified in corneas of these PPMD patients. In vivo confocal microscopy is potentially useful for monitoring of disease progression and excluding suspected cases of subclinical PPMD. Abnormalities on the corneal topography were observed, this report brings forth the descriptions of morphologic changes on Orbscan topography.
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Keywords: posterior polymorphous dystrophy; topography; in vivo confocal microscopy

1. Introduction

Posterior polymorphous dystrophy (PPMD, OMIM#122000) is a dominantly inherited corneal disorder associated with morphologic endothelial abnormalities in which affected patients are normally asymptomatic, although corneal edema has been reported in cases associated with widespread endothelial dysfunction. In most patients with PPMD, corneal stromal edema does not develop. Instead, the diagnosis is based on the presence of characteristic bilateral endothelial bands, vesicles, and gray opacifications that do not impair visual acuity (Cibis GW,et al, 1997).

Several methods have been used to delineate the structural features of PPMD. These include clinical biomicroscopy, histopathology, electron microscopy, and specular microscopy. Herein, we present four cases of PPMD imaged by in vivo confocal microscopy and Orbscan Ⅱ slit-scanning elevation topography, demonstrating unusual features that have not previously been reported using these two techniques.

2. Material and Methods

Case 1: A 29-year-old male, who presented with a history of intermittent pain in both eyes, was noted coincidentally on slit-lamp examination to have bilateral PPMD. He had neither significant ophthalmic or medical history nor relevant family history. His ocular symptoms had fully resolved at the time of assessment. On examination, he exhibited a best spectacle-corrected visual acuity of 6/5 OU. Slit-lamp biomicroscopy revealed bilateral vesicular lesions at the level of the endothelium forming clusters. Each lesion was surrounded by a gray “halo”. Clinically, there was no associated corneal edema (Figure 1A .B). Intraocular pressures (IOP) were 23.6mmHg OD and 20.9 mmHg OS with cup-to-disc ratios of 0.6 OD and 0.3 OS. The remainder of the slit-lamp examination was unremarkable.

Case 2: A 21-year-old female presented for refractive surgery evaluation. She was a soft contact lens wearer who had no ocular history or current symptoms aside from refractive error. On examination, unaided visual acuity was 6/60 bilaterally and her best spectacle-corrected visual acuity was 6/6 OU. Slit-lamp examination of her left cornea demonstrated a curvilinear row of endothelial vesicular lesions 2 mm below the visual axis. Each vesicle was associated with a grayish halo. The remainder of the ocular examination was unremarkable (Figure 2A .B). Intraocular pressures were within normal limits at 14 mmHg OD and 16 mmHg OS.

Case 3: A 26-year-old male presented after a routine preoperative assessment of LASIK. His uncorrected visual acuity was 6/12 in the right eye

and 6/10 in the left. The best spectacle-corrected vision was 6/6 bilaterally. Aside from refractive error, he had no ocular history or current symptoms. On slit-lamp examination, an incidental finding was a "snake like" band at the level of the endothelium superiorly and inferiorly in the right paracentral cornea and "guttata-like" lesions were identified in the left corneal periphery. On retroillumination, his right cornea showed two prominent, oblique band lesions enclosing an area of abnormal endothelium with a rather guttata appearance which were thought to be cause of patient's reduced visual acuity (Figure 3A.B.C.D). Fundus examination was unremarkable.

Case 3: A 30-year-old female was examined for progressive loss of vision (6/7.5) in her both eyes. There was no improvement with refraction or pinhole in her visual acuity. Slit-lamp examination of her both eyes revealed diffuse endothelial changes with pleomorphism. Elliptical pupils were found but no iridial hole was noticed in both eyes. Broad based iridocorneal adhesion extending anteriorly to the Schwalbe's line was found by gonioscopy from 5 o'clock to 7 o'clock. On retroillumination, the posterior cornea has the appearance of beaten metal or peaud' orange. Diffuse opacities presented as irregular thickening of Descemet's membrane with grayish opacities in a swirled pattern (Figure 4A.B.C.D). There was no corneal staining with fluorescein, and intraocular pressures were 16 mmHg in both eyes. Both optic discs were healthy, with a cup-to-disc ratio of 0.3 bilaterally, and fundus examination was otherwise unremarkable.

Orbscan Ⅱ slit-scanning elevation topography (Bausch& Lomb Surgical, USA) was performed to measure corneal thickness and topography. After explanation of the procedure and obtaining informed consent, in vivo slit-scanning confocal microscopy (Confoscan3.0, NIDEK, Japan) was performed on all patients.

The patient was asked to fixate on a target, and the examination was performed with a 40 × nonapplanating, immersion lens that covers an area of approximately 0.1 mm2. A drop of Vidisic gel (0.2 % Carbomer 940, Bausch & Lomb, USA) on the objective lens served as an immersion and contact substance. For all patients, the cornea was examined using a standard setting of four passes, with a scanning range between 700 μ m and 800 μ m(throughout the z-axis) to image the full central corneal thickness and a 150 μ m scanning range to specially image the corneal endothelium and posterior stroma. The maximal light intensity was used for all examinations.

Three patients presenting with clinical signs and symptoms of PPMD were also examined using a new in vivo laser-scanning confocal microscopy, the HRT3/RCM (Heidelberg Engineering, Heidelberg, Germany). With the addition of the Rostock Cornea Module, the HRT3 is converted to an in vivo laser scanning confocal microscopy. Before examination, one drop of topical anaesthetic (oxybuprocaine chlorhydrate 1.6mg/0.4ml) and one drop of gel tear substitute (0.2 % Carbomer 940, Bausch& Lomb, USA) were instilled in the lower conjunctival fornix. The x-y position of the image and section depth were controlled manually.

Qualitative and quantitative analysis using NAVIS (Nidek Advanced Vision Information System) proprietary software was performed. Endothelial cell density was determined by choosing three representative frames in which endothelial cells were clearly visible. A manually adjusted automated cell count was performed on each frame over an area of 0.05 mm2. Mean values for endothelial cell density, cell area, and the percentage of hexagonal cells within each frame were recorded, and mean values for each cornea were calculated.

3. Results

Table1 shows the patient data. The ages of patients ranged from 21 to 30 but didn't correlate with the clinical severity of the dystrophy. Corneal changes in patient of PPMD are usually bilateral but may show marked asymmetry, and typically assume one of the three basic configurations: vesicular lesions (case 1 and 2), band lesions (case 3), and diffuse opacities (case4)

The results of endothelial analysis were showed in Table2. Endothelial densities didn't correlate with the clinical severity of the dystrophy (in terms of number of lesion seen clinically and presence of corneal edema). For example, the most severely affected case (case4) had an endothelial cell density of 1873 ± 155 cells/ mm2. Endothelial polymegathism was noted in all cases, as demonstrated by high coefficients of variation in cell area; however, endothelial pleomorphism was not a prominent feature, with all cases showing low coefficients of variation in cell shape, and high proportions of hexagonality.

Examination of the confocal images revealed that most of the abnormalities were confined to the Descemet's membrane and endothelium. Both of the in vivo slit-scanning and laser-scanning confocal microscopy revealed craters, streaks, and cracks over the corneal endothelium surface. Guttata and clusters of abnormal endothelial cells were also identified in the corneas of these PPMD patients. Interestingly, case 2,3 and 4 exhibited prominent endothelial nuclei, which were well-defined and brighter than the cytoplasm. Some endothelial cells appeared to have

more than two nuclei. In all patients with clinical PPMD, the changes were similar but varied in severity, being more marked in eyes with lower endothelial cell density.

Case1 and 2 demonstrated multiple small focal vesicular lesions (range, 20.1-93.2 μ m in diameter), which protruded into the anterior chamber. Vesicles may appear in isolation or in clusters throughout the posterior cornea. With the slit-lamp microscopy, the vesicles appeared as small blisters on Descemet's membrane. On confocal microscopy, the vesicles were surrounded by a diffuse gray halo and vesicles were composed of abnormal pleomorphic cells with indistinct borders, creating black areas in the endothelial mosaic that were surrounded by enlarged endothelial cells. Grouped vesicles may aggregate to form larger geographic lesions (Figure 5.6.9A.9B.10A.10B).

Band lesions (case 3) extended across the posterior corneal surface as two scalloped, raised ridges that run roughly parallel to each other. Like the vesicular lesions, band lesion may be present anywhere in the posterior cornea, although they are most commonly found just inferior to the central cornea (Figure7A.7B).

Diffuse lesions of PPMD were confirmed in case 4 by in vivo confocal microscopy. In vivo confocal microscopic images revealed diffuse endothelial changes with pleomorphism and polymegathism. The borders of endothelium were indistinct and the lesions were geographic shaped. The lesions contained hyperreflective areas within them. Around the lesions, we found hyperreflectivity at the level of Descemet's membrane (Figure 8A. 8B. 11A. 11B). Prominent degenerative intrastromal nerves were also noted in case 3 and 4.

Corneal topography revealed asymmetric with-the-rule astigmatism OD and oblique astigmatism OD in case 3 and 4 respectively. Prominent steepening of the posterior corneal surface can also be seen on their corneal topography (Figure 12.13).

Table1. Patient Data

Case	Eye	Age/Sex	Visual Acuity on Presentation	Slit Lamp Signs of PPMD	Confocal Featur of PPMD	Type of Lesion
1	Right	29/M	6/5	+	+	Vesicular
	Left		6/6	+	+	Vesicular
2	Right	21/F	6/60	-	-	-
	Left		6/60	+	+	Vesicular
3	Right	26/M	6/12	+	+	Band
	Left		6/10	+	+	Vesicular
4	Right	30/F	6/7.5	+	+	Diffuse
	Left		6/7.5	+	+	Diffuse

Table2. Results of Endothelial Cell Analysis

Case	Eye	Mean Endothelial Density±SD (cells/mm^2)	Age-Matched Normal Range for Endothelial Density (cells/mm^2)	Mean Endothelial Cell area±SD (μ m^2)	Coefficient of Variation(area) (%)	Coefficient of Variation(sides) (%)	Hexagonal Cells (%)
1	Right	2056±102	2056-3594	488±24	30.4	11.4	62.8
	Left	2440±319	2056-3594	417±54	41.6	15.6	48.1
2	Right	3225±72	2291-3873	310±7	36.4	13.5	50.4
	Left	2519±122	2291-3873	398±20	39.8	14.2	51.3
3	Right	1810±147	2102-3641	552±41	31.4	12.9	56.8
	Left	1377±145	2155-3696	715±53	33.0	13.5	57.9
4	Right	1873±155	2035-3572	538±44	48.1	19.3	36.8
	Left	2206±284	2035-3572	458±60	56.4	20.2	34.2

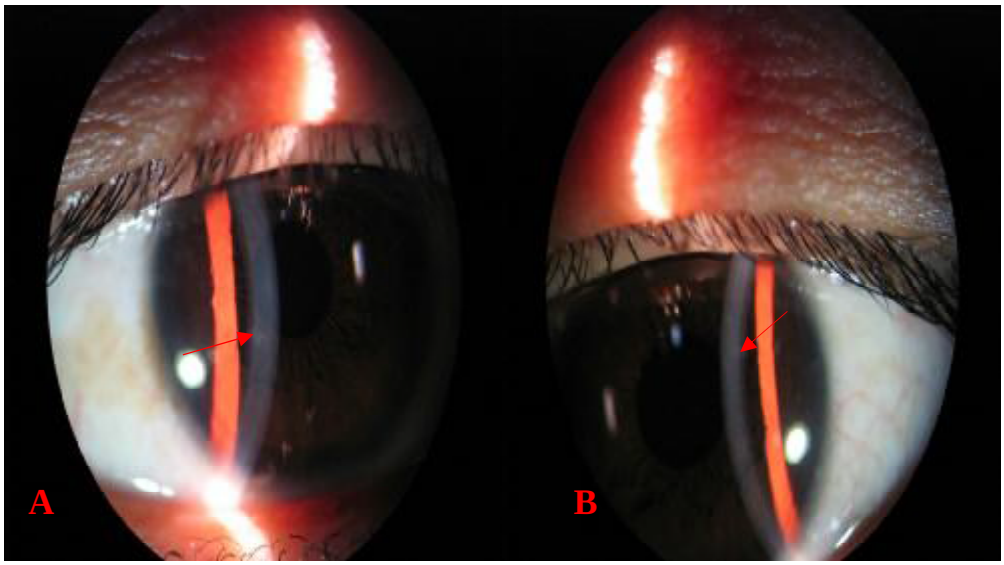
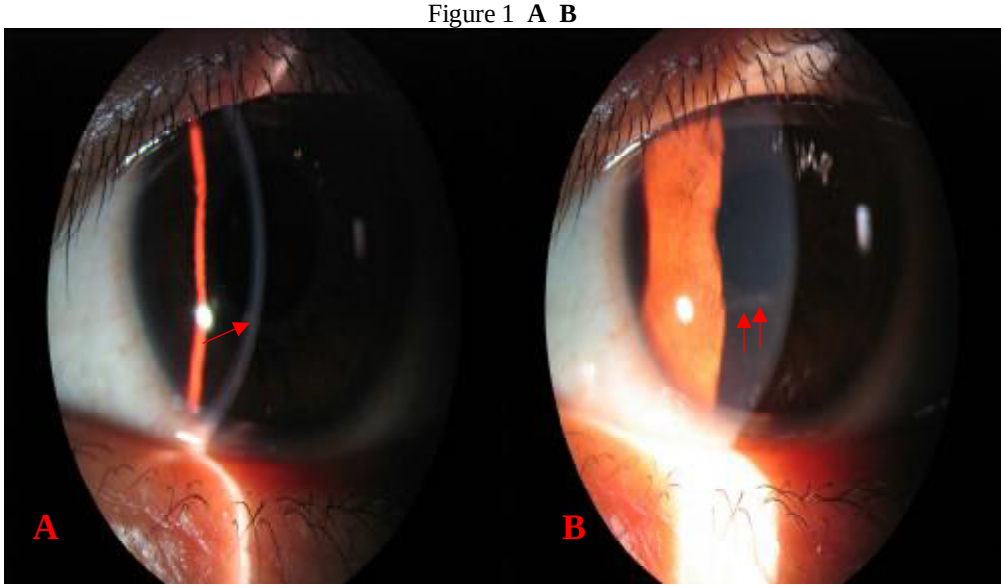


Figure 1 **A B**



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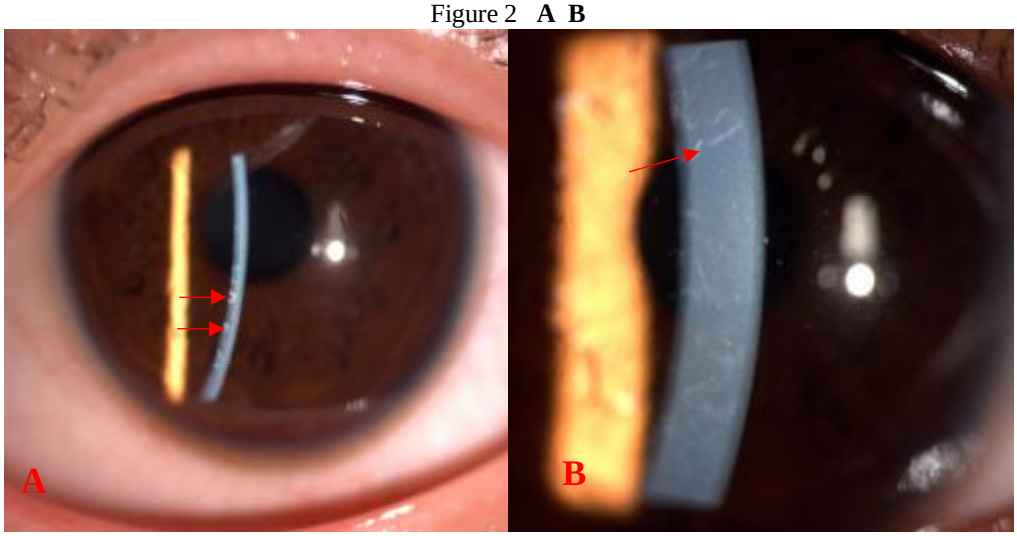


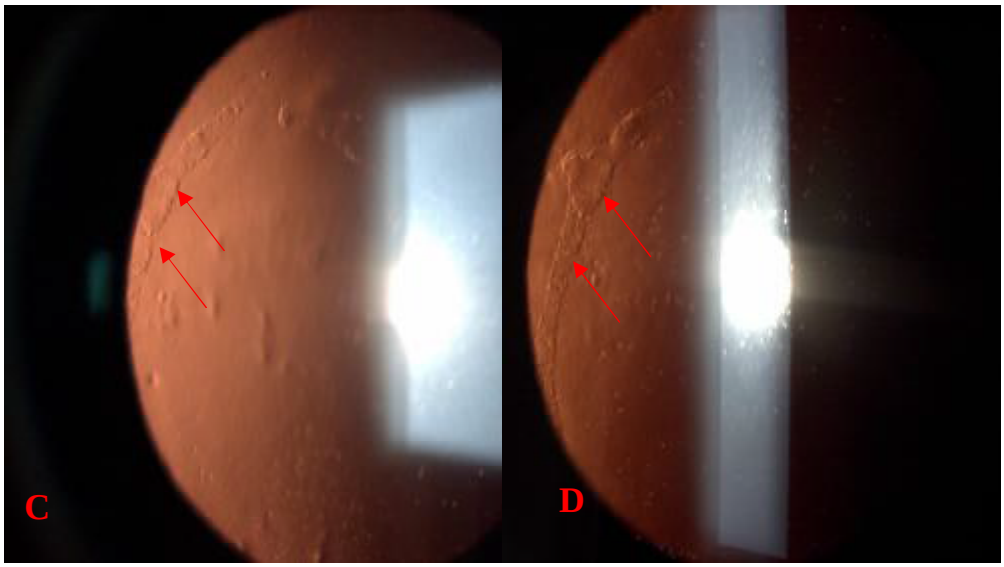


Figure 3 **A B C D**

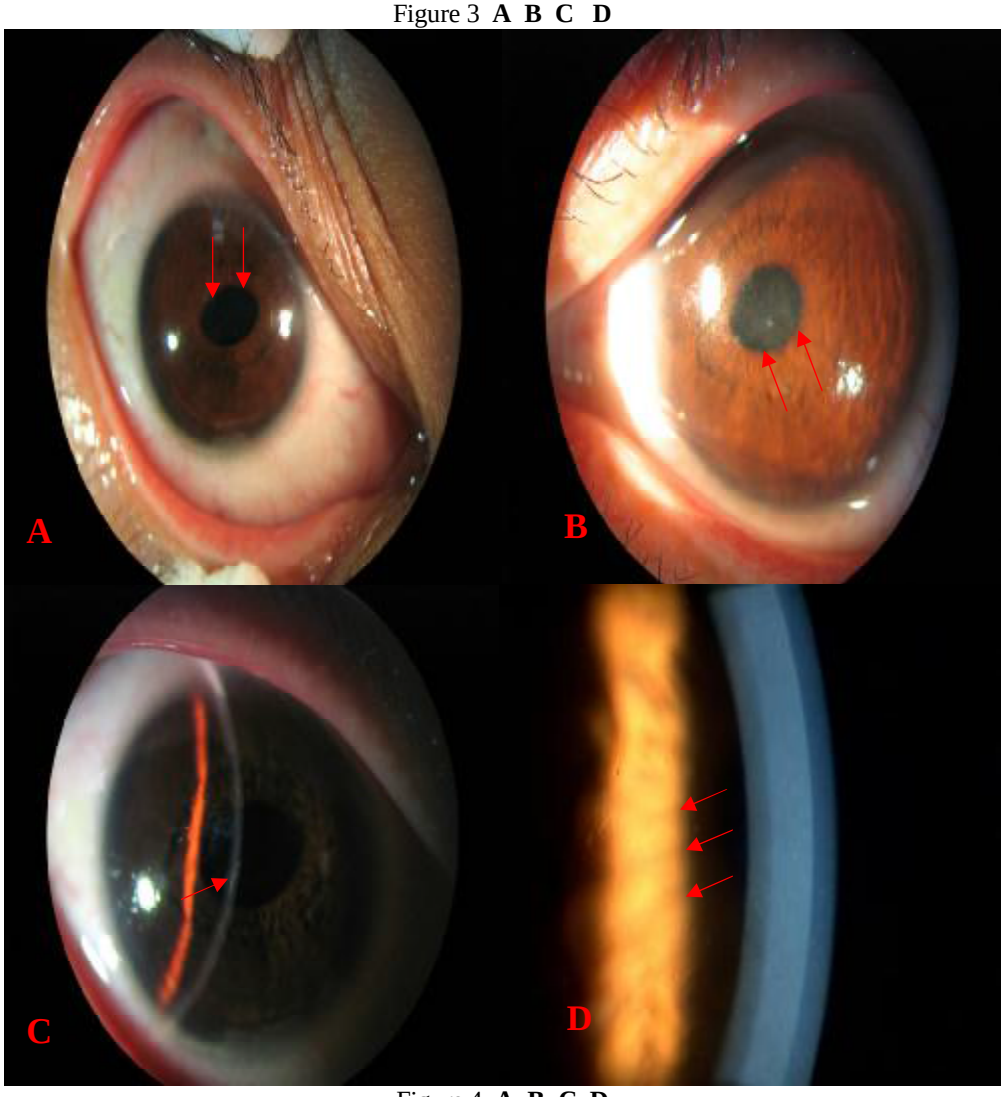


Figure 4 **A B C D**

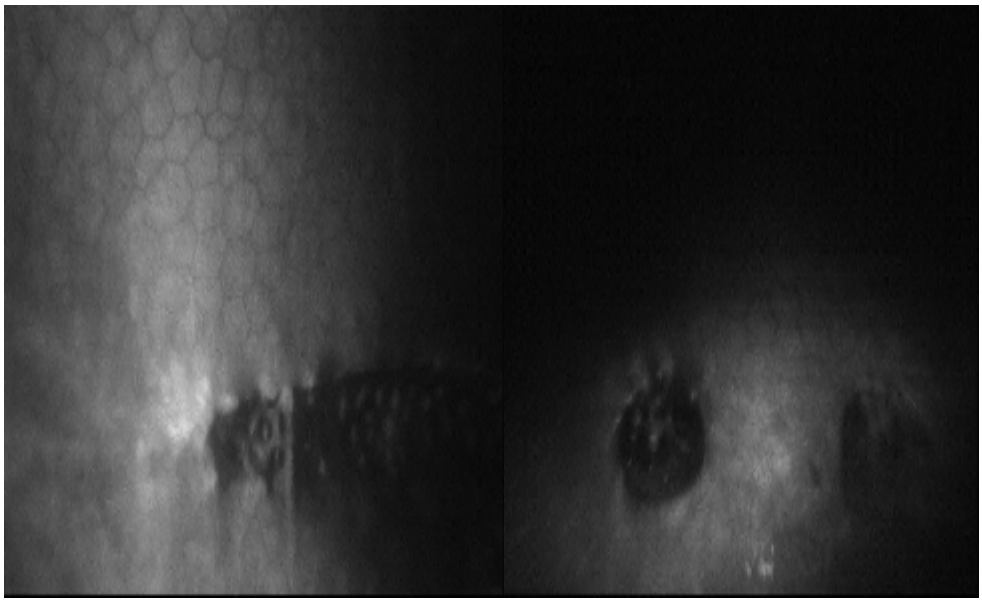
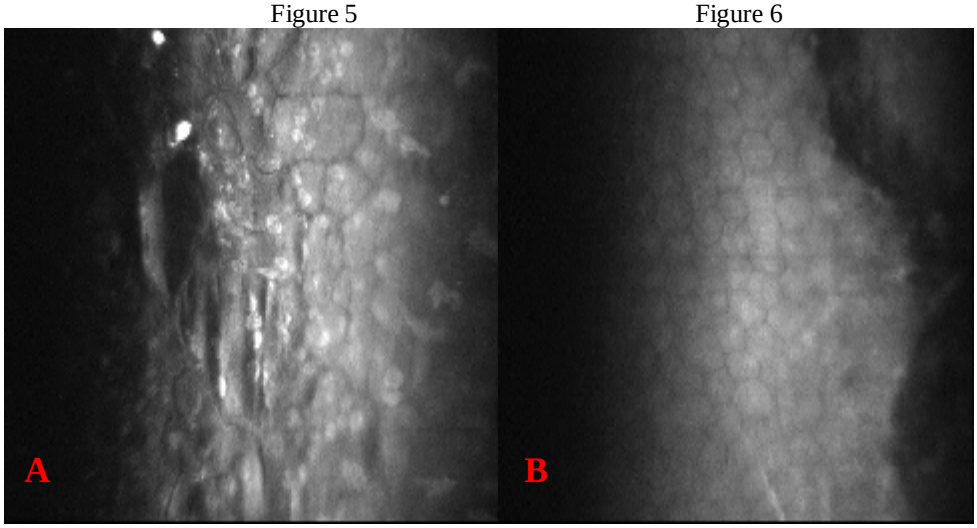


Figure 5 Figure 6



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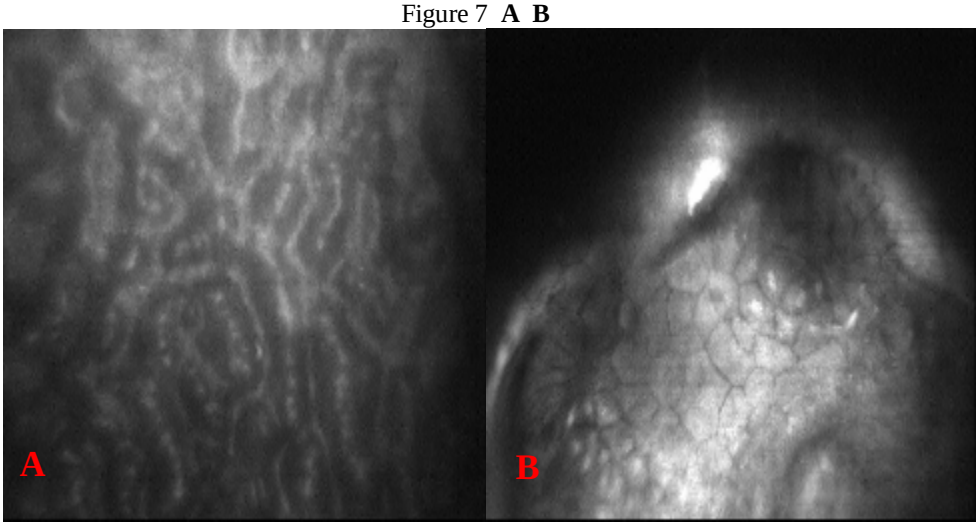

Figure 8 A B

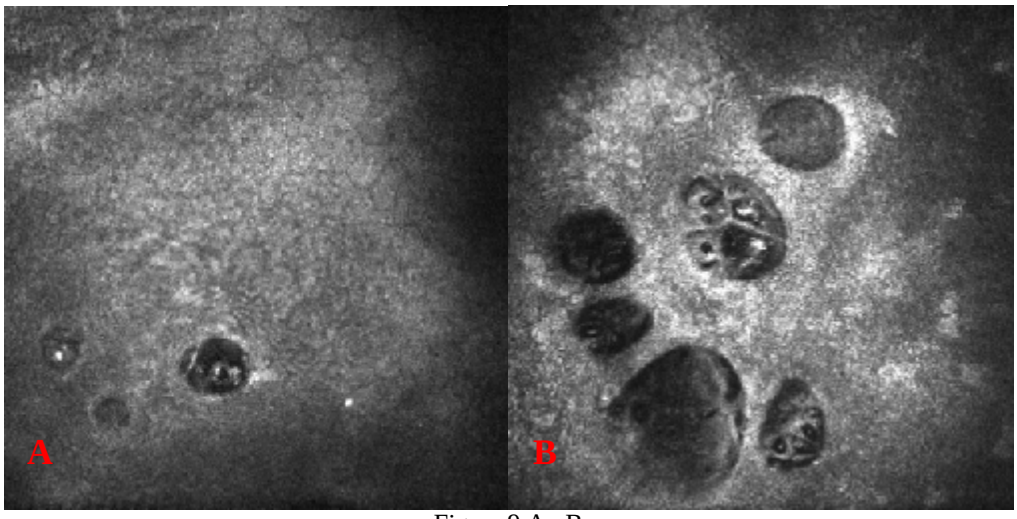

Figure 9 A B

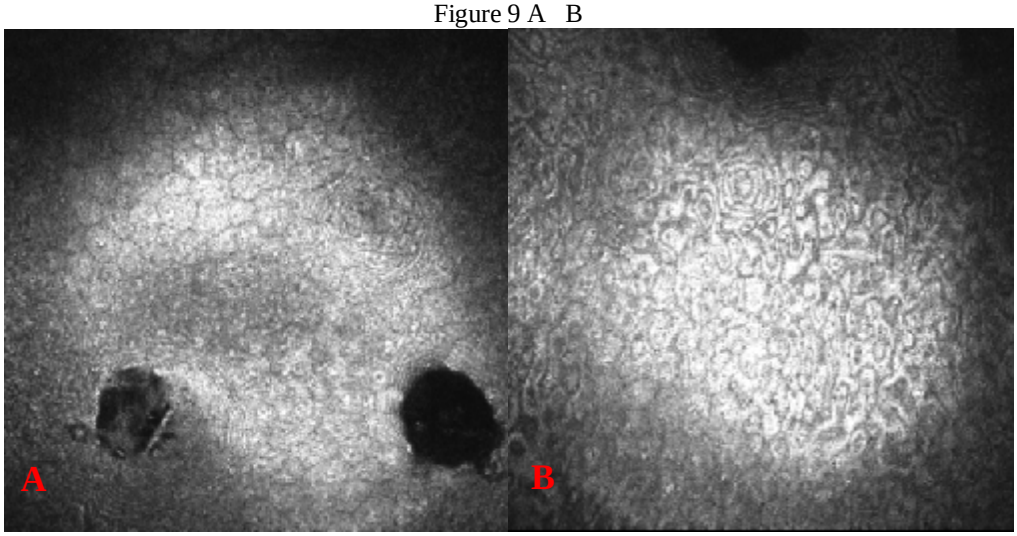

Figure 10 **A B**

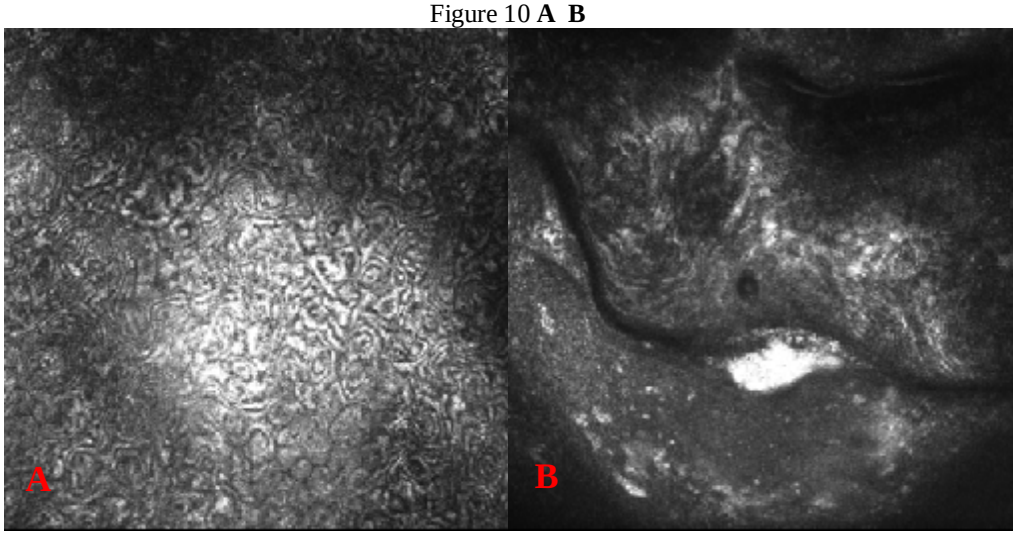

Figure 11 **A B**

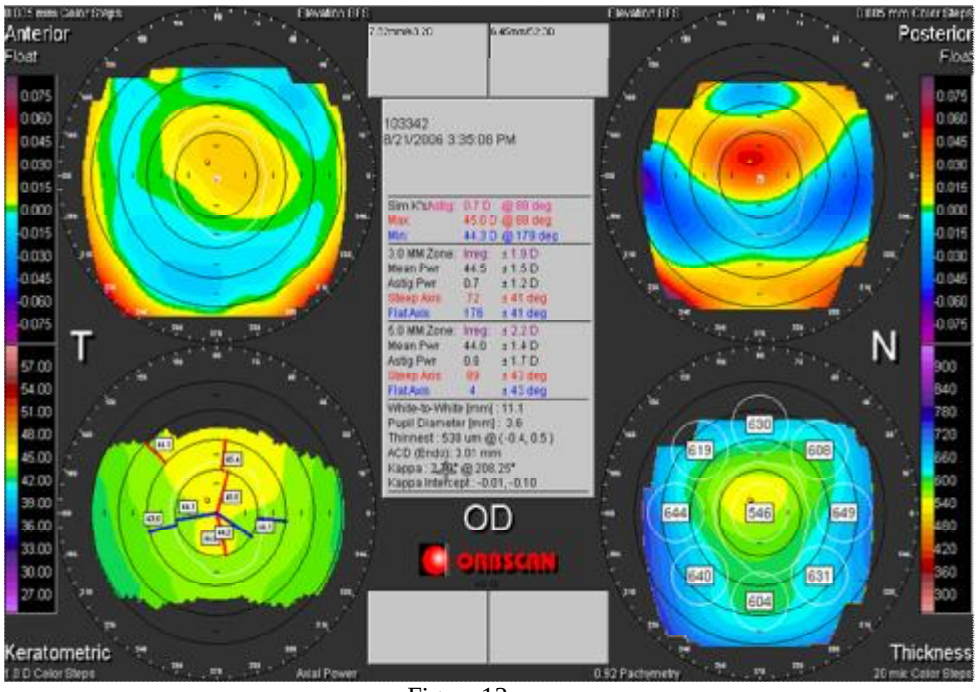

Figure 12

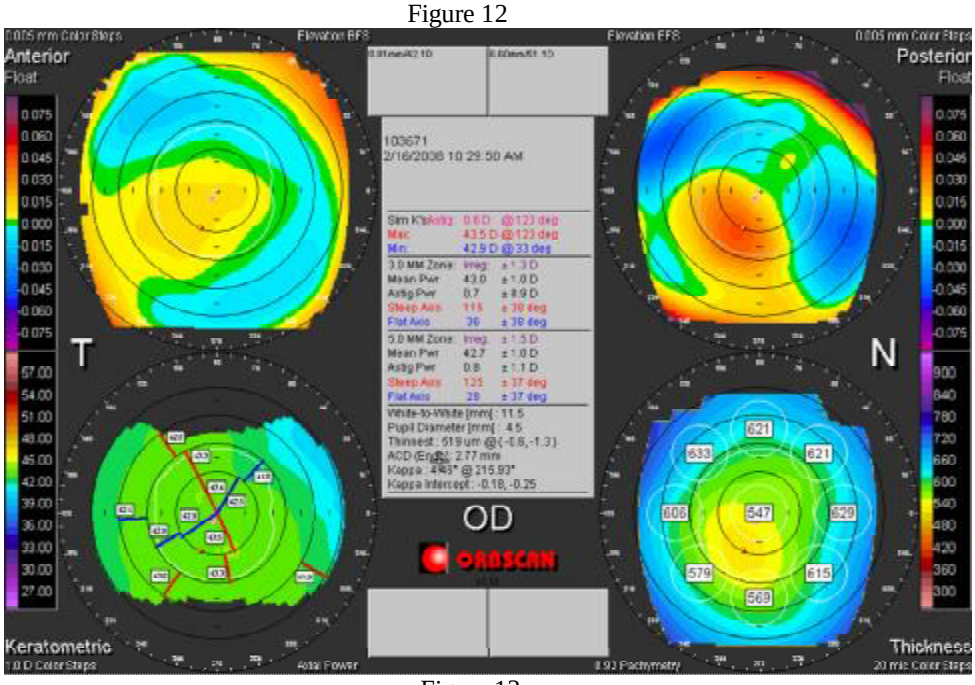

Figure 13

Figure 1 (A .B) Case 1 Slit-lamp biomicroscopy revealed bilateral vesicular lesions at the level of the endothelium forming clusters. Each lesion was surrounded by a gray "halo". Clinically, there was no associated corneal edema

Figure 2 (A .B) Case 2 Slit-lamp biomicroscopy demonstrated a curvilinear row of endothelial vesicular lesions 2 mm below the visual axis. Each vesicle was associated with a grayish halo

Figure 3 (A .B.C.D) Case 3 On slit-lamp examination, an incidental finding was a "snake like" band at the level of the endothelium superiorly and inferiorly in the right paracentral cornea and "guttata-like" lesions were identified in the left corneal periphery.

Figure 4 (A .B.C.D) Case 4 Slit-lamp examination revealed diffuse endothelial changes with pleomorphism. Elliptical pupils were found but no iridial hole was noticed in both eyes.

Figure 5 Case 1 / Figure 6 Case2 Confoscan 3.0 and Figure 9 (A .B) Case 1 / Figure 10 (A .B) Case2 HRT3/RCM Show the vesicles were surrounded by a diffuse gray halo and vesicles were composed of abnormal pleomorphic cells with indistinct borders, creating black areas in the endothelial mosaic that were surrounded by enlarged endothelial cells.

Figure 7 (A .B) Case 3 Confoscan 3.0 Show band lesions extended across the posterior corneal surface as two scalloped, raised ridges that run roughly parallel to each other. Like the vesicular lesions, band lesion may be present anywhere in the posterior cornea.

Figure 8 (A .B) Case 4 and Figure 11 (A .B) Case4 HRT3/RCM Confoscan 3.0 In vivo confocal microscopic images revealed diffuse endothelial changes with pleomorphism and polymegathism. The borders of endothelium were indistinct and the lesions were geographic shaped. The lesions contained hyperreflective areas within them. Around the lesions, we found hyperreflectivity at the level of Descemet's membrane

Figure 12 Case 3 and Figure 13 Case 4 Corneal topography revealed asymmetric with-the-rule astigmatism OD and oblique astigmatism OD respectively. Prominent steepening of the posterior corneal surface can also be seen on their corneal topography

4. Discussions

PPMD, a slowly progressive disease of the cornea, was first described by Koeppe (1916). He named the condition "keratitis bullosa interna" to describe the characteristic bullous lesions he noted in the posterior cornea.

A wide spectrum of corneal changes can be seen in PPMD, ranging from a few vesicular lesions to total opacification of the cornea with edema (Levy, et al, 1996). In PPMD, dystrophic endothelial cells exhibit epithelial features, and produce secondary abnormalities in Descemet's membrane (Sekundo, et al, 1994). Iris and anterior chamber angle structures may also be affected in some patients with PPMD (Pardos, et al, 1981; Laganowski, et al, 1991; Hirst, et al, 1983; Krachmer, 1985).

Posterior polymorphous dystrophy typically has an autosomal dominant inheritance pattern, although penetrance of the disease is low. Mutations in VSX1 homeobox (on chromosome 20q11) and COL8A2（on chromosome 1p）genes have been identified in PPMD (Heon, et al, 2002; Biswas, et al, 2001).

In addition, the clinical expression of the disease can vary considerably, even within affected families. For example, one member of the family may only be minimally affected with asymptomatic corneal lesions, whereas a sibling may have severe peripheral synechiae with glaucoma and corneal decompensation.

Clinically, PPMD is characterized by the presence of endothelial lesions, which have been classified into three basic configurations: vesicular lesions, band lesions, and diffuse opacities (Waring, et al, 1978). Vesicles appear as endothelial blisters or blebs on slit-lamp examination and these may be isolated or form clusters or curvilinear patterns (Grupcheva, et al, 2001). Band lesions are characterized by strips of guttata-like irregularities of Descemet's membrane and diffuse PPMD is seen as diffuse irregularities in Descemet's membrane , often associated with corneal edema. One study found that vesicles and bands were twice as common in women as in men, and that vesicles were mostly bilateral (94％) whereas bands were usually unilateral (85 ％) (Laganowski, et al, 1991). The significance of these findings is not known. The disease can be slowly progressive, but in many cases the findings are static. PPMD can rarely cause visual dysfunction from corneal edema, iridocorneal adhesions, corectopia, and glaucoma (Hirst, et al, 1983). The precise age of onset of PPMD is difficult to determine because most patients have no symptoms. However, a majority of patients are diagnosed in the second or third decade of life. Herein, the ages of our patients ranged from 21 to 30.

From a histological viewpoint, corneal endothelium abnormalities are the hallmark of PPMD. The primary event is believed to be "epithelialization" of the corneal endothelium (Witshcel, et al, 1980; Boruchoff, et al, 1971; Grayson, et al, 1974; Rodrigues, et al, 1980; Henriquez, et al, 1974; De Felice, et al, 1985; Matsumoto, et al, 1988; Rodrigues, et al, 1988). The stimulus for this transformation is not known. The epithelial-like endothelial cells exhibit many morphologic and growth features of the normal epithelium, including a multilaminar pattern, desmosomal junctions, microvilli, cytoplasmic keratin, sparse mitochondria, and rapid growth in tissue culture. They also stain with antisera prepared against human epidermal keratin (Rodrigues, et al, 1988). The transition between endothelial and epithelial-appearing cells can be abrupt (Henriquez, et al, 1984).

The epithelial-like cells are capable of migrating over the endothelial cells without adhering to them and can grow to cover the trabecular meshwork and iris, leading to intractable glaucoma (Rodrigues, et al, 1980). The "epithelialized" cells secrete a thick membrane that resembles an abnormal Descemet's membrane. These cells may even regenerate after corneal transplantation. Descemet's membrane is absent or markedly attenuated in the areas covered by the epithelial-like cells.

In addition to the characteristic epithelial-like cells, many other changes in the endothelium have been noted. Metaplastic fibroblast-like endothelial cells can be seen (Johnson, et al, 1981; Polack, et al, 1980; Boruchoff, et al,1990). Pleomorphism and degeneration of endothelial cells are common findings in PPMD (Polack, et al, 1980; Boruchoff, et al, 1990). Attenuated endothelial cells with disorganized organelles, large vacuoles, phagosomal inclusions, and disrupted cell membranes have been described using transmission electron microscopy. Areas of hypertrophic endothelial cells coexist with areas of focal endothelial cell loss, suggesting competing degenerative and regenerative processes (Polack, et al, 1980).

Alterations in Descemet's membrane, including deposition of abnormal collagen, guttata, and pits, are a common finding in PPMD (Rodrigues, et al, 1982). The changes are believed to be secondary to the primary alterations in the endothelium. Thickening of Descemet's membrane is the typical finding, although attenuation of this layer has occasionally been noted (Hanna, et al, 1977). The posterior collagenous layer can be interrupted by irregular excrescences, which resemble the cornea guttata observed in Fuch's endothelial dystrophy.

The pathogenesis of PPMD likely involves abnormal transformation and migration of the endothelial cells with secondary alterations in Descemet's membrane, but the stimulus for these events is unclear. The concept of metaplasia has been proposed to explain the endothelial changes in PPMD. Epithelial or fibroblastic transformation in PPMD may represent a metaplastic response of endothelial cells, although the trigger is not known (Johnson, et al, 1976). Differential staining patterns with specific epithelial and endothelial antibodies support the idea that endothelial cell transformation occurs in PPMD (Ross, et al, 1976). Some endothelial cells stain only with the endothelial antibody, some stain with both types of antibodies, and others stain only with epithelial antibody, suggesting that endothelial cells progress from a normal phenotype to a transitional phenotype, and finally to an abnormal epithelial phenotype.

Unfortunately, histologic studies have only been performed on corneal buttons after penetrating keratoplasty and therefore represent only severe cases of PPMD with corneal edema. Both specular and confocal microscopy enable imaging of the cornea in vivo and may be used to examine earlier or mild cases that represent the most common form of PPMD.

In vivo confocal microscopy is an invaluable additional tool for further investigation of PPMD. Due to a combination of high resolution and magnification, it highlights pathological findings at a cellular level in all corneal layers, not just the endothelium and Descemet's membrane. In vivo confocal microscopy provides a unique opportunity for clinicopathological assessment of corneal dystrophies such as PPMD that do not generally progress to penetrating keratoplasty.

To our knowledge, there are only a few published reports of in vivo confocal microscopy in PPMD. Chiou et al (1999) identified patchy, round hyporeflective areas at the level of Descemet's membrane in one subject and hyporeflective bands with marginal hyperreflective structures at the level of Descemet's membrane in a second subject. Unfortunately, although vesicular and band-like lesions were identified by slit-lamp microscopy, the authors didn't comment on any endothelial structures visualized by in vivo confocal microscopy. Grupcheva, et al, (2001) identified endothelial vesicular lesions composed of optically dense material, forming in deep stromal keratocyte density was noted. An interesting finding in this case was protrusion of endothelial vesicles into the anterior chamber. Confocal microscopy demonstrated craters, streaks, and cracks over the corneal endothelium surface. Pleomorphism and polymegathism were present in eyes with PPMD. Guttata and clusters of abnormal endothelial cells were also identified in corneas of our PPMD patients.

A striking feature of two of our cases presented is the abnormal changes on corneal topography. Prominent steepening of the posterior corneal surface can be seen in case 3 and 4.The lesion type of case 3 and 4 are band and diffuse respectively. This may be caused by the larger area of abnormal endothelium. PPMD has been associated with keratoconus in several reports (Gasset, et al, 1974; Weissman, et al, 1989; Bechara, et al, 1991; Blair, et al, 1992; Driver, et al, 1994). Keratoconus (OMIM#148300) is a frequent corneal dystrophy with a reported incidence that varies from 50 to 230 per 100,000 (approximately1/2000) (Rabinowitz and Keratoconus, 1998). Characteristically, the cornea assumes a conical shape as a result of progressive noninflammatory thinning of the corneal stroma. The thinning of the cornea causes irregularity in its curvature (astigmatism) and corneal protrusion resulting in a variable degree of visual impairment. Depending on the stage of the disease, every layer of the cornea may become involved in the pathological process. Although Descemet's membrane and endothelial cells may show minor changes, the major pathological defects lie in the anterior cornea, with compaction of the stroma and breaks in Bowman's membrane (Rabinowitz,1998). In case 3 and 4, the epithelium and Bowman's membrane appeared unremarkable. Subepithelial nerve plexuses with normal configuration and density were present subjacent to the Bowman layer. We also observed prominent degenerative intrastromal nerves by in vivo confocal microscopy, the relevance of which, if any, has not previously been assessed in PPMD.

Most patients with PPMD remain asymptomatic. This disease is only rarely progressive or visually impairing. Therapeutic intervention is not typically required. If epithelial edema occurs, it may be treated with hypertonic agents. Penetrating keratoplasty may be required in patients with severe disease. Overall, the prognosis for surgery is good, unless iris adhesion or glaucoma are present. Recurrence of PPMD following corneal transplantation has been reported, typically manifesting as a retrocorneal membrane or thick fibrous deposits between the epithelialized cells and Descemet's membrane (Sekundo, et al, 1994).

To the best of our knowledge, this is the first time we study the Chinese patients with PPMD by using in vivo confocal microscopy and Orbscan Ⅱ corneal topography. As there might be variations between cases, further studies, particularly about Orbscan Ⅱ corneal topography and in vivo confocal microscopy, are called for to define the distinctive features of this dystrophy.

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hTERT expression extends the life-span and maintains the cardiomyogenic potential of mesenchymal stem cells in human umbilical cord blood

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Abstract: Human umbilical cord blood-derived mesenchymal stem cells (UCBMSCs) represent a population of stem cells that are capable of differentiation into multiple lineages and are expected to serve as an excellent alternative to bone marrow-derived human mesenchymal stem cells. However, these cells exhibit senescence-associated growth arrest and phenotypic changes during long-term culture. To overcome this problem, we established UCBMSCs (hTERT-MSCs) with human telomerase reverse transcriptase (hTERT) gene. We found that the hTERT-MSCs proliferated faster than non-infected and had longer life-span. Induced hTERT-MSCs with 5-azacytidine to cardiac muscle and detected the specific marker of myocardiocyte. The hTERT-MSCs were able to form cardiomyocyte evidenced by positive staining for Connexin-43 and α-Sarcomeric actin. We concluded that the hTERT gene does not influence some type of differentiation potential of MSCs.
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Keywords: hTERT; mesenchymal stem cell; life span; cardiomyogenic potential

1. Introduction

Umbilical cord blood (UCB) is also a source of human MSCs whose cellular characteristics and multilineage differentiation capability are equivalent to those of BM-derived MSCs[Lee MW and Yang MS,2005; Lee OKand Kuo TK,2004]. UCBMSCs will be useful sources for cell transplantation, however, it is difficult to study and apply them because of their limited life span[Warren LA and Rossi DJ,2009]. Much research showed telomeres normally shorten as human cells divide. When telomeres reach a specific senescent length the cells enter senescence.

To resolve these problems, the life span of MSCs from bone marrow can be extended by retroviral transduction of hTERT gene[Basem M. Abdallah, Mandana Haack-SØensen,2005]. The researchers improved that telomerization of hMSC by hTERT over-expression maintains the stem cell phenotype of hMSC and it may be a useful tool for obtaining enough number of cells with a stable phenotype for mechanistic studies of cell differentiation and for tissue engineering protocols.

In the present study, we investigated the growth regulatory mechanism of UCBMSCs and attempted to establish UCBMSCs with hTERT (hTERT-MSCs) to overcome their limited life span. The hTERT-MSCs can serve as an alternative source of mesenchymal stem cells and may provide a unique source for cellular and gene therapy.

2. Materials and methods

2.1 Isolation and Cell Culture of UCBMSCs

Human UCB samples (>40ml/sample)obtained with the mother's consent were processed within 4h of collection. Mononuclear cells(MNCs) were isolated from UCB on a Ficoll(density,1.077/ml)gradient and were suspended in medium consisting of high glucose-DMEM, 15% fetal bovine serum, and seeded at a concentration of 1×10^7 cells/ml. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO2 with a change of culture medium every 7 days until the fibroblast-like cells at the base of the flask reached confluence. Once reaching confluence, the adherent cells were resuspended using 0.25% trypsin EDTA and reseeded at 1×10^6 cells/ml.

2.2 Flow cytometry (FACS) analysis

To detect cell generation cycle, confluent cells were detached with 0.25% trypsin-EDTA , washed with phosphate-buffered saline twice and stained for viability with propidium iodide for 10 min, then assayed for size and granularity based on forward and side scattering by FACS can flow cytometer linked with Mad fit LT for Mac 3.0 software (Becton-Dickinson).

2.3 Infection with Recombinant Retroviruses

The retroviral vector, pLNCX2-hTERT, was employed in these experiments. Amphotropic viral

supernatant containing hTERT was generated by packaging cell line PT67. One million primary UCBMSCs in a 10cm dish were exposed to viral supernatant containing retrovirus at an approximate multiplicity of infection of one to ensure single-copy integration, in the presence of 8μg/ml polybrene for 3 hours. After washing with phosphate-buffered saline, the transduced UCBMSCs were selected with 600mg/L neomycin.

2.4 RT-PCR analysis of hTERT gene transcripts expressed

Total RNA was isolated from three cell groups hTERT-MSCs, USBMSCs (negative control)and K562 cells(positive control) using the Trizon Total RNA Isolation System according to the manufacturer's instructions. The integrity and purity of total RNA was verified by gel-electrophoresis on 0.8% agarose. For reverse transcript, one hundred nanograms of total RNA was reverse transcribed and amplified with the use of the AMV First Strand cDNA Synthesis Kit. A equal volume of each sample was amplified by Polymerase Chain Reaction by using the following primers:the primers specific for retrovirally encoded hTERT were 5′TCTGGATTTGCAGGTGAACAG3′ and 5′GTAGGTGACACGGTGTCGAG′3, with a product of 310bp. The primers of β-actin were 5′GGC ATG GGT CAG AAG GAT TCC ′3 and 5′ATG TCA CGC ACG ATT TCC CGC′3, with a product of 500bp. The PCR reaction was performed at 94°C for 5 minutes, followed by amplification of 35 cycles consisting of 94°C for 30 seconds, 65°C for 50 seconds, and 72°C for 60 seconds.

2.5 Analysis of telomerase activity

hTERT-MSCs , USBMSCs (negative control)and K562 cells(positive control) were cultured in standard growth medium to 80% confluence. Telomerase activity in each sample was detected by using the Telo TAGGG Telomerase PCR ELISA kit according to the manufacturer's instruction.

2.6 proliferation rate (MTT assay)

The comparison of proliferation rate between the UCBMSCs and the hTERT-MSCs was performed by MTT assay. Cells were trypsinized and seeded in 96-well plates at a density of about 4×10^4/ml. Then cultures were maintained at 37°C in a humidified atmosphere containing 5% CO2. The measurement was done at the same time everyday. The cells were incubated with DMEM-FBS containing 0.5mg/ml MTT for 4h at 37°C. The medium was discarded and 200μl dimethyl sulfoxide was added to the wells. After 10 min of incubation at room temperature, the colored formazan salt was measured at 492nm using a spectrophotometer.

2.7 Evaluation of the cardiomyogenic potential of hTERT-MSCs

To induce cardiomyocyte differentiation. hTERT-MSCs and long-term culture, UCBMSCs were seeded at a density of 10^5 cells/ml in 6-well plates (for cytochemical staining) and grown for 24h in standard growth medium. At 80 – 90% cell confluence, the medium was supplemented with 10μmol/L 5-azacytidine and this medium was replaced after 24h. Then cultures were maintained with a change of culture medium every 3 days for at least two weeks.

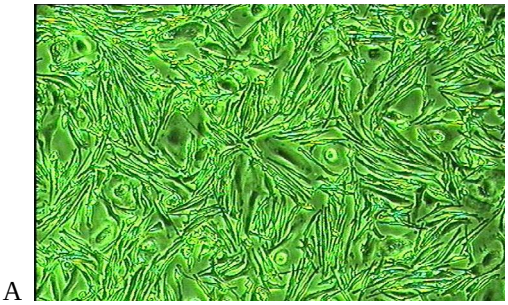
After 14 days in culture, the hTERT-MSCs and long-term culture UCBMSCs on chambered slides were fixed with 4% paraformaldehyde diluted in PBS for 20 min at 4°C and permeabilized in 0.03%TritonX-100 for 10 min , then washed with PBS and blocked in 10% sheep serum for 15min. Primary antibodies were incubated overnight at 4°C, including Anti-human α-Sarcomeric actin(mouse monoclonal antibody, 1:100) and Connexin-43(rabbit polyclonal antibody, 1:100. Secondary antibodies, anti-mouse IgG for α-Sarcomeric actin and anti-rabbit IgG for Connexin-43 were incubated on slides for 30 min at 37°C. After intensive washing with PBS, the slides were incubated in streptavidin conjugated to horseradish peroxidase in Tris-HCL buffer for 30 min. Prepared substrate-chromogen solution using DAB chromogen tablets was applied for 30s and the slides were counterstained in Meyer's hematoxylin for 2 min . Coverslips were mounted on slides with propyl gallate. Then all slides were examined under microscope.

3 Results

3.1 The proliferative ability of MSCs

Concomitant with the growing, UCBMSCs became broad and flat and ceased to proliferate. It's life span didn't exceed 40 days while hTERT-MSCs did not show the senescence phenomena (>90days).

Cell cycle analysis of UBCMSCs was performed. Results showed that during proliferation they were all normal diploid, suggesting that their karyotype remained stable during proliferation. 85.1% MSCs were in the quiescent period (G0-G1 phase), and 14.9% UBCMSCs were in the proliferating stage (S+G2+M phase), demonstrating that only a small fraction UBCMSCs are of potent proliferative ability.



(×40)

Fig.1. Photomicrographs showing hTERT-MSCs. A. Confluent hTERT-MSCs B. Cell cycle by flow cytometry analysis.

3.2 Detection of hTERT mRNA in hTERT-MSCs and UCBMSCs

We confirmed the integration and mRNA expression of the exogenous hTERT in hTERT-MSCs by RT-PCR. As shown in Fig.2, 310bp fragment was observed in the hTERT-MSCs and K562 cellsbut not in the UBCMSCs, which demonstated the integration and mRNA expression of the exogenous hTERT gene in hTERT-MSCs.

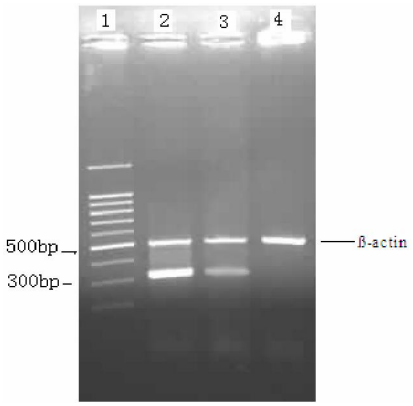


Fig.2.RT-PCR analysis of hTERT gene transcripts . 1 represents Marker, 2 represents K562 cells(as positive control) , 3 represents the hTERT-MSCs, 4 represents the UBCMSCs.

3.3 Detection of telomerase activity in transfected and untransfected MSCs

The absorbance of negative controls in which telomerase was in by heat treatment was then subtracted to remove PCR artifacts. As shown in Fig.3, As samples are regarded as telomerase-positive if the difference in absorbance(A450nm-A690nm) is >0.2 A450nm-A690nm units, we concluded that the UBCMSCs remained telomerase-negative but the hTERT-MSCs showed robust telomerase activity.

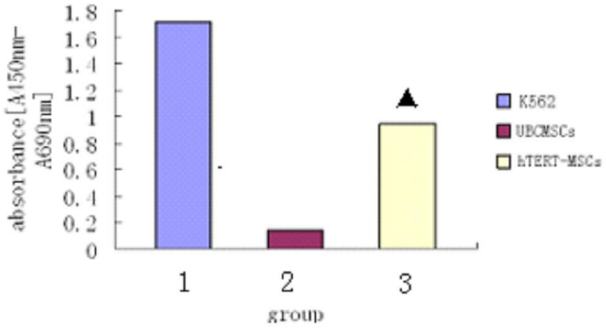


Fig. 3 the telomerase activity in transfected and untransfected MSCs, PD＝15。

3.4 Growth kinetics

Growth kinetics of hTERT-MSCs were measured by MTT assay and compared with those of UCBMSCs. hTERT-MSCs growth curves showed an initial lag phase of 1-2 days. This was followed by a log phase in which the hTERT-MSCs divided at exponential rates for 3-5 days. With the increase of passage, the hTERT-MSCs growth rates were slower and the number of cells generated by the end of 7 days in culture was reduced. UCBMSCs showed the same results. hTERT-MSCs cultures grew at faster rates as compared to UCBMSCs cultures.

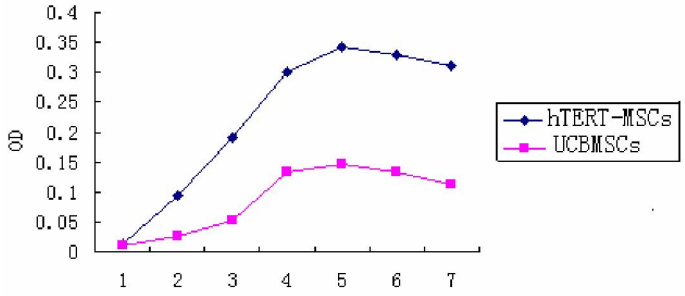


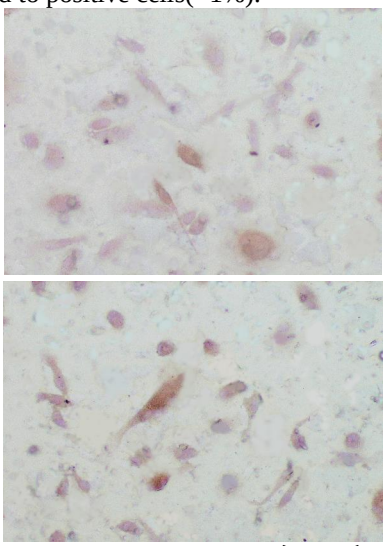
Fig.3. Growth curves of UCBMSCs and hTERT-MSCs.

3.5 hTERT-MSCs maintained differentiation capacity in vitro compared with the long-term culture of UCBMSCs

We performed studies of hTERT-MSCs (90days) and long-term (30days) culture UCBMSCs

for differentiation into the cardiomyocyte by 5-azacytidine. As shown in Fig. 4, hTERT-MSCs were able to form cardiomyocyte evidenced by positive staining for Connexin-43 (8%)and α-Sarcomeric actin(10%). But few long-term cultured UCBMSCs can be induced to positive cells(<1%).

A B



α-Sarcomeric actin（×100）
Connexin-43（×100）

Fig.4 Characteristic antibody of cardiac muscle immuncytochemistry was showed in A. Some cytoplast was stained by Connexin-43 B. Some cytoplast was stained by α-Sarcomeric actin.

4. Discussions

The use of UCBMSCs requires their in vitro expansion and the capability to preserve their differentiation potential. To achieve this goal, we attempted to prolong the life span of UCB-derived cells even to endow them with immortality. In this study, we demonstrates that hTERT-MSCs proliferated faster than non-infected and had longer life-span.

Transfection of hTERT gene was a safe and effective way to prolong cell life span. Most somatic cells from humans and other mammals lack telomerase activity and undergo senescence after only a limited number of replications. The effect of ectopic expression of hTERT on abolishing the senescence-associated growth arrest and extending the proliferative life-span has been demonstrated in several somatic cell types including fibroblasts, osteoblasts, endothelial cells, epithelial cells, and liver cells[Tsuruga Y and Kiyono T,2008]. Morales's study[Morales CP and Holt SE,1999]showed that although ectopic expression of telomerase in human fibroblasts is sufficient for immortalization, it does not result in changes typically associated with malignant transformation.

Following the proliferation of UBCMSCs, the cells will end in replicative senescence and lose their ability of differentiation. However, in addition to effects on cell proliferation, there is an increasing recognition that telomerase activity may also contribute to the biological functions of the cells. We tested the cardiomyogenic potential of the hTERT-MSCs. Approximately 10% of the hTERT-MSCs were successfully transdifferentiated into cardiomyocytes while the long-term culture UCBMSCs had only <1%. hTERT-MSCs can be a promising cellular source for cardiac stem cell-based therapy[Nishiyama N and Miyoshi S,2007].

The maintenance of the differentiation functions of cells has also been observed in other cell types. hTERT-over expression in endothelial cells led to an improved neovascularization after in vivo implantation, compared with telomerase-negative cells[Murasawa S and Llevadot J,2002]. Also, telomerized human fetal hepatocytes exhibited an extended life span and maintenance of their liver-specific characteristics[Kim YS and Yoon SJ,2008].We have found that hTERT-MSCs responded adequately to in vitro differentiation signals in contrast to the impaired responsiveness observed in senescent cells.

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2/23/2011

Phenol Toxicity Affecting Hematological Changes in Cat Fish (*Clarius lazera*)

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Abstract: Phenol and phenolic compounds are xenobiotics stressful environmental factors to which fish and animals are subjected to, and have become environmental problem due to anthropogenic impact on the environment The present study aimed to investigate the effect of phenol pollution on fish with special reference to the hematological, immunological, serum biochemical parameters, where fifty healthy *Clarius lazera* fish were divided into 3 groups. Fish of gp1 served as a control. Fish of gp. 2 & 3 were used for the determination of acute lethal concentration dose and the pathological effect of Phenol on the exposed fish. Blood samples were collected to obtain serum for biochemical studies and heparinized blood for hematological investigations. RBCs, Hb, HCt, and MCHC showed significant elevations, the serum GPT and GOT were increased significantly. L.D.H, glucose and cortisol were elevated, while serum cholesterol concentration was reduced significantly in high tem30^{o}C.
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Key words: Phenol pollution, Tilapia Zilli, Biochemical changes

1. Introduction

Fish plays an important role, not only in human food diets but also in animal and poultry rations. It is a palatabie and easily digested food which is rich in vitamins, calcium, phosphon.rs and iodine. In Egypt, fish is considered as a cheap food article if compared with other foods of animal origin. The flesh of healthy fish is considered as a marker for the natural aquatic environment (1-7).

Phenol and phenolic compounds are xenobiotics stressful environmental factors to which animals are subjected to serve animia due to phenol and have become environmental problem due to anthropogenic impact on the environment (8). They also are good research models of wide spread xenobiotics (9). Also, they are commonly present in industrial wastewaters and in non-specific pesticides, herbicides, bactericides and fungicides (10). Mukherjee *et al.* (11) reported that they are commonly found in the marine habitat and in fish tissues. Phenol induces toxic effects for fish health.

They induce genotoxic effect (12), carcinogenic effect (713), and immunotoxic effect (14).

Controversy, Stich (15) reported that phenol may act as free radical scavengers and prevent genetic damage caused by other agents. They have a high bioaccumulation rate along the food chain due to its lipophilicity. Thus phenol pollution presents a threat against natural environment and also to human health (8 and 16). When the phenol is present in the aquatic environment, fish food consumption, mean weight and fertility are significantly reduced (17). For these reasons, phenol intoxication must be taken in consideration in the fish farming systems and also in natural aquatic habitat.

Fish metabolism was adversely affected by phenol (10 and 18). The phenol and its derivatives can alter protein metabolism by altering transamination rate of amino acids by enhancing the activity of aspartate aminotransferase (ASAT, EC 2.6.1.1) and alanine aminotransferase (ALAT, EC 2.6.1.2). Also, the carbohydrate metabolism was affected by phenol by altering the activity of lactate dehydrogenase (LDH, EC 1.1.1.27) thus, affecting the interconversion of lactate into puruvate (8). Gupta *et al* (10) recorded changed ASAT and ALAT activities in different fish tissues induced by phenolic compounds. The enzymes activities (ASAT or GOT and ALAT or GPT) catalyze the interconversion of amino acids and α-keto acids by transfer of amino groups. The ASAT catalyzes the transfer of this group from aspartate to α-ketoglutarate to form glutamate and oxaloacetate, while ALAT catalyzes the transfer of the amino group from alanine to α-ketoglutarate to form glutamate and puruvate (19). The measurement of transaminase activities in serum is frequently used as a diagnostic tool in human and animals (20 and 21). Damage to the liver, kidney and gills is evident from elevated transaminase activities (20).

The present study aimed to investigate the effect of phenol toxicity on fish with special reference to the haematological, immunological, serum biochemical parameters.

2. Material and Methods:

1- Fish:

Fifty healthy fish of both sexes and 150 ± 50 gm body weight, were obtained alive and transported immediately to the laboratory. They were kept in 5 glass aquaria (100 X 30 X 50 cm) that provided daily with a tap water and continuously with filtered air. The water temperature was adjusted at 15°C along the period of experiment using thermostatic heater. The fish were fed a balanced ration daily using the formula suggested by Ahmed and Matty (22). Fish were kept under observation for 2 weeks.

Fish were divided into 3 groups (gps). Fish of gp1 (10) served as a control with no treatment. Fish of gp. 2 & 3 (20, each) were used for the determination of acute lethal concentration dose (LD_{50} /72 hr, gp2) and to investigate the pathological effect of phenol on the exposed fish (gp3).

2-Experiments:

A. Determination of acute lethal concentration dose:

To determine lethal concentration dose, fish of gp. 2 were subdivided into 5 equal subgroups. Subgroup 1 served as a control. Other 4 subgroups exposed to 35, 75, 150 and 300 mg/L of phenol; respectively. Each dose was dissolved in the distal water of each aquarium. The number of dead fish was recorded within 72 hrs post-exposure and the acute lethal concentration dose was calculated according to the formula of Brown (23).

B- Long term exposure:

Fish of gp3 were exposed to 1/100 of LD_{50} /72 hr (1.5 mg/L) of phenol for 2 weeks according to *Taylor et al* (24). The excreta were removed regularly and the water was replaced within 4 days interval. Fish were kept under observation along the 14 days of exposure.

3- Sampling:

Blood sample were collected from the caudal vein after 3, 7, 14 days of exposure, part of blood was left to clot and then centrifuged at 3000 r.p.m. to obtain serum for biochemical studies, the other part was heparinized for hematological investigations using the methods of Drabkin, 1949 (25).

4-Haematological examinations:-

The erythrocytic indices (RBCs, Hb, HCt & MCHC) were estimated according to Schalm et al (26).

5-Serum biochemical analysis:

Kits Biomericux France were used for the determination of serum glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT), lactate dehydragenase (LDH), alkaline phosphatase (AP), serum glucose, serum cholestrol, and total protein. Serum cortisol hormone was analyzed by means of a gemmacoat 125-cortisol radio-imumnassay kit (Diagnostic corporation, USA). Serum Ig M was also measured according to Fuda, et al (27).

6-Statistical analysis:

The obtained data were statistically analysed according to Snedecor and Cochran (28) by T test.

3. Results

A-Determination of acute lethal concentration dose:

Experiment 1 revealed that, the acute lethal concentration dose was 150 mg/L during the 1st 72 hrs post-exposure.

B- Long term exposure:

The effect of phenol exposure on RBC's count, Hb level, HCt and MCHC values of exposed fish were recorded in Table (1). Polycythemia was observed on the 14 day ($p<0.01$). Blood Hb, HCt, and MCHC showed a significant elevation by 14 days of experiment.

Table (2) revealed the changes of some biochemical constituents in the blood of fish due to phenol exposure. The obtained data revealed that serum GPT activity increased significantly by 14 days of exposure. A significant elevation in serum GOT activity was also observed on the 14th day ($p<0.01$). L.D.H serum activity was elevated along the whole period of experiment especially on the day 14th. Hyperglycemia was constant findings from the beginning of the experiment until the end of the experiment. Serum cholesterol concentration was increased, on the 3rd day and the 7thday and was reduced significantly, on the day 14thday. Cortisol hormone was elevated along the whole period of experiment especially on day 14th.

4. Discussion:

The aquatic environment of the River Nile subjected to many stressful factors, phenol and phenolic derivatives are one of the serious pollutants which cause serve anemia in fish. This observed hepatomegaly may partially reflect the enhancement of the liver size due to destructive changes. Barse *et al.* (21) reported elevated HSI values of *Cyprinus carpio* subjected to 4-*tert*-butylphenol.

Regarding the impact of phenol on the

hematological profile of fish polycythemia accompanied by elevated hemoglobin level, HCt value and MCHC were observed. Similar findings were reported by Mckim et al *(*29), Hilmy et al (30) and Taylor et al *(*24) recorded polycythemia in *rasy barb*. But in contrary to our finding Hb level and MCHC were reduced in *Clarias lazera* exposed to copper (31). The increased RBCs count may be due to stimulation of erythropoietin by elevated demands for O_2 or Co_2 transport as a result of increased metabolic activity or distruction of gill membranes causing faulty gaseous exchange. The increase Hb content could be explained as a process where the body tries to replace the oxidized denatured Hb (32). The increase of HCt value and MCHC may be attributed to swelling of RBCs due to increased Co_2 in blood, hypoxia or stressful procedures (33 and 34).

Exposure of fish to sublethal concentration (1.5 mg/L) of phenol for 14 days resulted in a marked increase in the activities of serum GPT, GOT, LDH and ALP. The present findings agree with our microscopic findings, which revealed a marked degeneration and necrosis of hepatocytes as the elevation in transaminases activities may be attributed to the liver injury (35).

Serum cholesterol level, in the present study, showed a significant reduction that could be due to greater level of utilization of cholesterol during corticosteroidogenesis, as it is the precursor for steroid hormones (36). In addition, they reported a rise in the blood protein resulted in a high density of lipoprotein in the serum and was suggested to be the cause of hypocholesterolemia in exposed fish.

Our results showed similar findings as that of Gill et al, (37) and *Snieszko* (38), who reported that, exposure of fish to phenol had no significant increase on blood glucose of *salmo gairnei*. The blood glucose level reflected the changes in carbohydrate metabolism under hypoxia and stress conditions. Rise of glucose level indicated the presence of stressful stimuli eliciting rapid secretion of both glucocorticoids and catecholamines from the adrenal tissue and accompined by cortisol elevation (39). Concerning serum protein level, a significant increase was noted 14 days postexposure to phenol. The elevated protein concentration may be due to the induction of protein synthesis in liver.

The serum Ig. M was determined to find out information about fish immune system which was previously investigated in different species by many authors as *Fuda et al,* (27) *O`Neill*(40), in this work, the purified Ig. M was revealed a single perception against specific polyvalent antiserum to fish Ig, similar results was obtained by Bagee et al. (41) who found that, *Coho salmon* Ig was detected by specific anti Ig 14. Our study revealed a significant decrease in Ig. M level in fish exposed to pollution if compared within control groups. *Anderson et al.* (42) found a relation between cortisol and IgM as when cortisol increased IgM decrease. The significant increase in cortisol level in fish exposed to phenol could be attributed to stress factors and the intoxication of fish (43).

We can conclude the fish exposed to phenol cause serve anemia and suppress immunity in exposed fish

Table 1: Effect of phenol on some haematological parameters in *Clarius lazera* along the period of experiment (Mean+S.E.)

Parameter	3days		7days		14days	
	Control	Exp.	Control	Exp.	Control	Exp.
RBCs $10^6/mm^3$	3.3 ± 0.22	3.3 ± 0.51	3.2 ± 0.30	4.4 ± 0.74	3.6 ± 0.68	4.7 ± 0.68*
HB g/dl	7.40 ± 0.52	8.2 ± 0.10	7.4 ± 0.26	8.7 ± 0.8	7.2 ± 0.33	8.4 ± 0.74**
H.Ct%	18.70 ± 1.30	21.9 ± 1.34	18.95 ± 0.19	26.4 ± 1.44	22.7 ± 2.64	28.7 ± 1.84**
MCHc%	32.70 ± 1.80	33.8 ± 1.30	33.52 ± 0.84	36.52 ± 1.36	32.3 ± 1.52	42.6 ± 1.27**

Exp: experimental * Significant at p<0.01. ** Non-significant

Table 2: Effect of phenol on the serum biochemical parameters *in Clarius lazera* along the period of experiment (Mean+S.E.)

Parameter	3days		7days		14days	
	Control	Exp.	Control	Exp.	Control	Exp.
SGPT (I.U/L)	28.2 ± 0.3	53.3 ± 033	34.3 ± 064	45.5 ± 1.29	35.0 ± 1.11	48.9 ± 2.68*
SGOt (I.U/L)	38.3 ± 2.2	42.7 ± 3.0	41.8 ± 1.2	48.9 ± 2.48	39.32 ± 1.0	55.40 ± 3.74**
L.D.H (I.U/L)	182 ± 4.3	192 ± 3.94	192 ± 4.3	192 ± 5.23	195 ± 3.40	199 ± 5.28*
A.L.P (U/L)	3.6 ± 2.3	3.2 ± 1.64	3.3 ± 1.84	4.7 ± 1.90	2.83 ± 1.60	5.93±2.25**
Glucose (mg / dl)	28.24 ± 2.3	33.68 ± 1.2	29.82 ± 1.2	42.20 ± 2.8	30.64 ± 1.8	62.8 ± 2.78**

Total protein (g/dl)	246 ± 0.43	2.43 ± 0.14	2.50 ± 0.64	3.1 ± 0.29	2.5 ± 0.55	4.70 ± 0.94*
Cholesterol (ng/dl)	161.4±3.4	167.6 ± 2.9	167 ± 3.0	163 ± 3.23	162.9 ± 2.4	198.4 ± 4.3*
Ig. M (ng/ml)	1.8 ± 0.13	1.60 ± 0.24	1.80 ± 0.42	1.43 ± 0.92	1.75 ± 0.12	0.3 ± 0.065*
Cortisol (ng/ml)	0.87 ± 0.24	1.53 ± 0.06	0.98 ± 1.24	1.86 ± 1.21	0.84 ± 0.73	1.95 ± 1.63*

Exp: experimental * Significant at p<0.01. ** Non-significant

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Oxdative stress on Sertoli cells of rats induced by microcystin-LR☆

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Abstract: Objective: To study the oxidative stress effects of microcystin-LR on rats Sertoli cells, and to explore the toxic mechanisms of microcystin-LR on reproductive system. **Methods:** Rat Sertoli cells were isolated and cultured. Purity of extracted Sertoli cells was identified by Feulgen staining method. Viability of primary rat Sertoli cells and the maximum dose of non-cytotoxicity of MC-LR on Sertoli cells were ascertained with MTT method after the cells were treated with the different concentrations of MC-LR for 24h. LDH, MDA, SOD, ROS in Sertoli cells were analyzed after Sertoli cells were cultured with different concentrations (0 μg/L, 0.15 μg/L, 1.5 μg/L, 15 μg/L) of MC-LR for 6, 12, 24h,. **Results:** The Sertoli cells model was obtained to study the toxic effects of MC-LR. The highest non-toxicity concentration of MC-LR was 15μg/L. The level of ROS in cells increased after exposed to the different concentrations of MC-LR, and there was a statistically significant difference when cells were exposed to 15g/L compared to control cells($P<0.05$). There was no statistically significant difference of MDA between control cells and cells exposed to MC-LR ($P>0.05$). The changes of lactate dehydrogenase (LDH) leakage amount were not significant after cells were cultured with the different concentration of MC-LR ($P>0.05$). The decreases of superoxide dismutase (SOD) were found to be dependent on the dose ($P<0.05$). **Conclusion:** MC-LR caused change of ROS in this study, but it had no effects on MDA. MC-LR had no effects on leakage rate of lactate dehydrogenase (LDH) but enhanced the activity of superoxide dismutase (SOD). The results suggested that MC-LR can induce oxidative stress in primary cultured rat Sertoli cell, but can not lead to lipid peroxidation.
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Keywords: MC-LR, Sertoli cells, oxidative stress

1. Introduction

Microcystins (MCs) are cyclic heptapeptides consisting of five common amino acids and two variable-amino acids, and their general structure is cyclo(-$_D$-Ala-L-X-erythro-X-methyl-$_D$-isoAsp-L-Y-Adda-$_D$-isoGlu-N-methylde-hydro-Ala), in which Adda(3-amino-9-methoxy-2-,6,8-trimethyl-10-phenyldeca-4, 6-dienoic acid), consisted of 20 special atomic carbons, is nearly related to the toxicity of Microcystins [1,2,3,4]

Microcystins, specific hepatotoxins produced by several cyanobacteria species in eutrophic surface waters, have received increasing worldwide concern due to their toxic potential in the past decades [1,5]. Microcystins are typically found only within the bacterial cells. However, when Microcystis cells die or are handled, Microcystins are released and can pollute drinking water sources [6,7,8]. To date, 67 kinds of Microcystin isoforms have been found [4], but Microcystin-LR (MC-LR) is the most common and the most toxic member. MC-LR has a LD_{50} value of 0.05mg/kg in mice by intraperitoneal injection [2].

MC-LR is a kind of intracellular toxin and has been documented to cause intoxication and lethalities in livestock, wildlife and even human [2]. Epidemiological investigations have indicated that Microcystins cause stomach and intestinal inflammation, disease of the spleen, and liver cancer in humans who drink water containing Microcystins. These effects correlate in part to the relative role of different organs (liver, kidney and lung) in the accumulation of Microcystins[13]. Lately, some studies have proved that Microcystins also were accumulated in the gonads, and the gonads were considered as the second target organ of Microcystins [9,10]. But so far, there have been few previous reports about the reproductive toxicity of Microcystins. For these reasons, the aim of the present study was to investigate the toxicity of MC-LR on the male rat reproductive system using primary cultured rat Sertoli cell.

2. Material and Methods

2.1. Chemicals

MC-LR was purchased from Institute of Hydrobiology (Wuhan, China). Dulbecco's modified Eagle's medium-Ham's F-12 medium(DMEM-F12 medium) were purchased from Sciencell Inc.

Penicillin-streptomycin antibody(SV30010.01)were obtained from BBI Inc. Trypsin (SH30042.01) and L-glutamine (SH30034.01) were purchased from HyClone Inc. Collagenase I were obtained from Beijing Solarbio Science & Technology Co.Ltd. Trypan blue and Tris were obtained from Sigma Inc. Dimethyl sulfoxide (DMSO) was obtained from Tianjin Damao Chemicals station. 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amresco Inc. Superoxide dismutase (SOD), Lactate dehydrogenase (LDH) and Maleic dialdehyde (MDA) test kits were purchased from Nanjing Jiancheng Bioengineering Inc(Nanjing, Jiangsu, China). Reactive oxygen species were purchased from Biyuntian Bioengineering Institutes. All other reagents were of analytical grade.

2.2. Animals

Healthy male Sprague-Dawley rats of 18-20 days weighing from 45 to 50 g were obtained from The Experimental Animal Center of Henan Province and kept in a well-ventilated room (23±1℃, 12h light/ 12h dark cycles, free access to water and standard pellet diet) for a week before used**.**

2.3. Treatment

A method was set up for obtaining a large number of viable Sertoli cells from Sprague-Dawley (SD) rats of 18-20 days. Whole testes minced from SD rats were sequentially treated with 0.25% pancreatin for 15-20 min and 0.1% collagenase for 20-25 min, and then Sertoli cells were washed and cultured in DMEM/F-12 media with 10% fetal bovine serum incubated at 37℃ in a humidified incubator with an atmosphere of 5% CO_2。After 4 days, the cells containing sperm were eliminated through lysing with a hypotonic solution of 20 mM Tris-HCL for 3 min. Feulgen staining was used to identify the purification of Sertoli cells. The viability of Sertoli cells was ascertained with MTT method after the cells were treated with the different concentrations of MC-LR for 24h.

Sertoli cells were treated with different concentrations (0μg/L, 0.15μg/L, 1.5μg/L, 15μg/L) of MC-LR. After 6, 12 and 24h, LDH, MDA, SOD, ROS of Sertoli cell line were analyzed.

2.4. Statistical analysis

The SPSS 12.0 statistical software was used for one-way ANOVA (analysis of variance, ANOVA), Bonfferoni pairwise comparison, Levene test of homogeneity of variance of data for statistical analysis (significance level $\alpha = 0.05$).

3. Results

3.1. Effect of MC-LR on the viability of Sertoli cells

Sertoli cells isolated and cultured in this study grew well, and the relative purity was more than 95%, which can be used in vitro experiments. Sertoli cells were cultured with different concentrations of MC-LR for 24h and it was found that optical density (OD) had no significant difference ($P>0.05$). That indicated MC-LR concentrations of up to 15μg/L hadn't obvious effect in cell survival rate (Table1). Based on this result, MC-LR of 0μg/L, 0.15μg/L, 1.5μg/L and 15μg/L was used in subsequent experiments.

3.2. LDH leakage rate

After treated with MC-LR (0.15, 1.5, 15μg/L) for 24h, LDH leakage rate had no statistically significant difference compared with the control group ($P>0.05$). Studying time-response relationship, LDH leakage rate of cells treated with the high dose (15μg/L) MC-LR showed a slight increase, but it was not statistically significant ($P>0.05$) (Table2).

3.3. Variation of SOD

Studying the dose-response relationship, SOD content in treated cells changed with MC-LR (0.15, 1.5, 15μg/L) after 24h. SOD activity in treated cells of 1.5μg/L MC-LR and 15μg/L MC-LR group had statistically significant difference compared with the control group($P<0.05$). When the time-response relationship was studied, SOD content in cells treated with the high-dose (15μg/L) MC-LR had no significant difference with the control group ($P> 0.05$) (Table3).

3.4. Variation of ROS

Compared with the control group, the amount of ROS in cells of each group increased. In the same group with different exposure time, the amount of ROS increased with the increase of the MC-LR concentration. In the same exposure concentration and exposure time, the amount of ROS was also increased. 6h and 24h after exposure, ROS in the treated group increased. However, only 15μg/L group had significant difference with the control group ($P <0.05$) (Table4).

3.5. MDA content in Sertoli cell

MDA content in cells with the different exposure time did not change significantly. In the different MC-LR concentration, MDA levels had no significant difference during the exposed time ($P>0.05$) (table5).

Table 1. OD value of rat Sertoli cells in different exposure concentration groups

Exposure concentration (μg/L)	n	OD value($\bar{x}\pm s$)
0	6	0.435±0.035
0.15	6	0.500±0.063
1.50	6	0.473±0.095
15.00	6	0.457±0.033

Table 2. LDH leakage rate of cells with different MC-LR exposure times and concentrations

Exposure time	LDH leakage rate(%)($\bar{x}\pm s$,n=6) with different MC-LR concentration(μg/L)			
	0	0.15	1.50	15.00
6h	56.57±1.77	56.39±0.33	57.04±1.1	58.1±0.91
12h	58.25±0.47	56.8±1.13	58.15±0.62	57.69±1.22
24h	58.35±0.35	58.52±0.79	57.92±0.17	57.54±0.64

Table 3. SOD content of cells with different MC-LR exposure times and concentrations

Exposure time	SOD content (*U/mgprot*) ($\bar{x}\pm s$,n=6)with different MC-LR concentration(μg/L)			
	0	0.15	1.50	15.00
6h	52.17±5.22	53.41±8.55	63.57±6.64	62.64±5.02
12h	61.2±10.6	68.33±10.49	67.57±18.6	67.99±17.85
24h	92.92±12	77.46±17.65	67.2±1.14 *	65.69±6.41 *

Note:*: groups exposed to MC-LR compared with control group, $p<0.05$

Table 4. OD value of ROS in cells with different MC-LR exposure times and concentrations

Exposure time	OD value of ROS($\bar{x}\pm s$,n=6) with different MC-LR concentration(μg/L)			
	0	0.15	1.50	15.00
6h	0.85±0.16	0.826±0.09	0.933±0.113	0.995±0.047
12h	1.073±0.121	1.26±0.28	1.48±0.295	1.715±0.132 *
24h	1.927±10.105	2.032±0.07	2.06±0.063	2.21±0.028 *

Note:*: groups exposed to MC-LR compared with control group,$p<0.05$

Table 5. MDA content of cells with different MC-LR exposure times and concentrations

Exposure time	MDA content (*U/mgprot*) ($\bar{x}\pm s$,n=6) with different MC-LR concentration(μg/L)			
	0	0.15	1.50	15.00
6h	0.071±0.005	0.069±0.006	0.073±0.007	0.061±0.003
12h	0.077±0.003	0.073±0.016	0.066±0.002	0.075±0.002
24h	0.089±0.003	0.073±0.006	0.075±0.001	0.075±0.005

4. Discussions

In the last decade, masses of cosmopolitan lethal animal poisonings and a plenty of cases of human illness caused by toxic cyanobacteria blooms have brought about the attention of World Health Organization and the public. Microcystins are a group of heptapeptide toxins produced by cyanobacteria in eutrophic freshwater, some of which are potent toxins, and Microcystin-LR is one of the most toxic and abundant variants in blooms. MCs form a health risk to wildlife, livestock and even humans.

The main mechanisms of Microcystins toxicity is through inhibiting the protein phosphatase of serine and threonine (PP1 and PP2A)[11] to increase the protein phosphorylation and cell toxicity of direct relevant tumor[12,13], and make the cytoskeleton proteins highly phosphorylated. Moreover, they regulate cell apoptosis by leading to important control protein phosphorylation[14]. Researches proved that exposure to low level of MC-LR can promote many cells apoptosis [15,16].

There are many kinds of statements about toxicology mechanisms of MCs. For example, some studies reported MCs could cause DNA-protein

crosslinks (DPC)[17]. But among these mechanisms, the oxidative stress is attracting more and more attention. It has been reported that MCs can cause oxidative damage in animals both in vitro[15,18]and in vivo experimental models[19,20,21]. Yan Li studied the toxic effect of MC-LR on male rats reproductive system in vitro and vivo[23]. They found MC-LR can cause oxidative stress on leydig's cell and produce cell toxicity. Besides, MC-LR played an important role in cell apoptosis, thus reducing ability of leydig's cell to secrete testosterone and produce the reproductive toxicity [22]. The damage mechanisms may be that MC-LR entered the body and caused the oxidative stress and testicular change, resulting in that testicular function was seriously damaged [23].

In this study, we have made clear that the changes of ROS, LDH, MDA and SOD were associated with MC-LR-induced reproductive system damage in vitro. Reactive oxygen species (ROS) were certain metabolites of oxygen and oxygen derivatives. Their chemical reactivity was more lively than oxygen. Under normal circumstances, ROS had very important physiological role in reproductive system. However, when the generation of ROS beyond the clearing abilities of antioxidant system, they could cause lipid peroxidation. In this experiment, we found that ROS levels of each MC-LR group were higher than that of the control group in different exposure time. This result was consistent with the result of Ding's, who reported the oxidative stress of MC-LR on liver cells [24]. This study showed that the ROS of high concentration MC-LR group had statistically significant difference with control group ($P<0.05$) after exposed for 12h and 24h. The study illustrated that the toxic effect of MC-LR on Sertoli cells was enhanced with the extension of exposure time and the increasing of exposure concentration.

LDH is a special kind of catalyzing enzymes, which is in cytoplasm and participated in glycolytic cycle. LDH leakage is generally accepted as a sign of necrosis. However, recent studies by Chong et al. [25] and Riss et al. demonstrate that LDH leakage is related cells apoptosis [26]. When cells cultured in vitro, LDH will leak out from cell to culture solution if cell membrane is damaged[27,28]. Therefore, cellular damage degree can be measured objectively by measuring LDH leakage rate. The result showed that significant difference of LDH leakage rate between each test group was not observed after Sertoli cells were treated by MC-LR for 6~24h ($P>0.05$). This illustrated that low doses of MC-LR didn't cause cell membrane damage obviously and cell permeability didn't change.

MDA is one of the final products of lipid peroxidation, and it was often used to evaluate the lipid peroxidation degree. This study found that MDA content of each MC-LR dose group hadn't significant change ($P>0.05$) compared with control group cells in different exposure time. The reason may be that the exposure dose was lesser. The results suggested that lipid peroxidation did not occur in Sertoli cells exposed to lesser dose of MC-LR.

SOD is a kind of important intracellular antioxidant enzymes. SOD can transform superoxide anion radical (O_2) into hydrogen peroxide (H_2O_2). With CAT was activated, H_2O_2 can be transformed into H_2O. Too much toxins may cause the excessive consumption of SOD activity [20]. Studies have shown that SOD is the most sensitive antioxidant enzymes after cells are exposed to the MCs. This study also found that the content of SOD in Sertoli cells was reduced with the increase of MC-LR dose. This experiment ascertained that oxidative stress strengthened along with the exposure concentration of MC-LR increasing. But SOD had no statistically significant difference between the different exposure time.

In conclusion, the results suggested that MC-LR can induce oxidative stress in primary cultured rat Sertoli cells, but it can not lead to lipid peroxidation. The present study revealed that MC-LR could exert a generally chronic toxicity on reproductive system of male rat, specially on the testes. Oxidative damage may underlie these pathological changes.

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The role of agricultural extension in Integrating indigenous knowledge and modern knowledge in rural

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Abstract: Experience shows that indigenous knowledge not only has no contradiction with formal knowledge but different indigenous knowledge features, put it as well complementary for formal knowledge. Indigenous knowledge is accessible, useful and cheap. Its perspective is holistic and its transmission is verbal. Knowledge is dynamic and time-tested, and while it has grown within local natural and social environment, so it is very sustainable with indigenous condition. Indigenous knowledge refers to both component and whole part of culture of each nation and this component and whole integration is so that stop to change traditional society of life without indigenous knowledge out of its cultural origin and therefore would lose it concept and effectiveness.
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Keywords: agricultural extension, indigenous knowledge, modern knowledge

1- Introduction:

During 1950 and 1960 decades, native (indigenous) knowledge was an inefficiency and absolute barrier for development. Nevertheless, now indigenous knowledge is recognized as a basic source. Indeed this knowledge was an answer to failure of great developmental theories by great countries and it was as a technical oriented solution for changing most peasants and farmers view in the world. (Agrawall, 2002).

The lack of indigenous knowledge about indigenous practices in many technologies in the developing countries will lead to failure. So attention to indigenous knowledge as a knowledge that is result of many thousand years experience is important in development of villages. Rural benefactors, the people who had communion in development of villages, can take efficient steps in rural development if they pay attention in the process of rural schematization for its development. Indigenous knowledge has different aspects, such as hygiene and treatment, medical plants, linguistics, livestock and agriculture, art and mystery and unprofessional things (Farrokhi and Yaghoubi, 2002).

Indigenous knowledge of each nation has enabled them to supply their needs from natural sources without reducing these sources. So, indigenous knowledge collection of world is valuable source of practices and time-tested tool that would be useful for sustainable development of all societies.

At third world countries, unconsidered triumph of world development policies has led to various social, economic, cultural and environmental issues (Agrawal, 2002).

From Robert Chambers' view, power and wealth are at industry and at cities, and poverty and deprivation are part of villager's life. Power and wealth of cities of world has absorbed experts, sources and needed research facilities for producing and disseminating knowledge. Knowledge of these modern centers is considered scientific, advanced, and valid and enjoys premium technology. He labeled this group as "first" and in contrast "last" for deprived villagers. Because, preferences and values of these two groups are different.

Their knowledge and attitudes are also different. he believes that since "first" development remedies and their attitudes have led to fault , irregular and deprivation , so deprived villager's attitudes and knowledge should be considered serious in order to reach to improve conditions for this part of human society as they need and demand(Azkia, M and Imani, 2008) .

2- Characters of indigenous knowledge :

The characters of indigenous knowledge like the definition of this knowledge are presented by experts in different ways which we will explain about them as follow:

2-1- it is based on experience:

Indigenous knowledge is the result of people's experience during many centuries.

2-2- it was tested during centuries by working on it.

2-3- it is compatible with indigenous environment and culture:

Indigenous knowledge was created through native societies and it was formed according to their needs and during time the things which were not compatible with indigenous environment were omitted, so what was remained was compatible with the environment and culture of that society (Amiri Ardekani and 2003).

2-4- it is dynamic and is changing:

Simultaneously with changing indigenous culture, the indigenous knowledge was changing too.

2-5- the knowledge of rural people was not technical:

This knowledge was consisted of rural people's wishes, values and preferences.

2-6- the rural people's knowledge is not statistic:

This knowledge was formed according to people's culture, social and economic history. The history which was written by these rural people shows that their manner and activities were efficient in changing of their conditions.

2-7- rural people's knowledge is not enough.

Maybe the rural people are knowledgeable but they like to know more and more. Because they want to be powerful in their discussions with political, economical and social forces who made these people poverty before give them technology (Zare and Yaghoubi, 2003).

2-8- rural people's knowledge has root on their political economy and is more important in political field.

The advantages that rural people can get from indigenous knowledge are the knowledge that is created and released locally and is on their authority and also depends on main factors in regional politic economy (land distribution, marketing relations, and vertical links). So improvement of their livelihoods depends on interferences which were made to pervade on these main factors.

2-9- most of the rural people are public-oriented

Mostly, they have a little information about many things which is in contrast with academic educations. Specialist people in universities have deep knowledge in little fields (of course some of these native people are specialist too) (Razavi, 1999).

2-10- indigenous knowledge systems are holist:

Local people consider the other people's problems as their problems and try to solve these problems in a whole frame with using their knowledge.

2-11- indigenous knowledge systems combine the culture and religious believes.

Religious believes as a part of indigenous knowledge are not separated from technical knowledge and these believes effect on people' do and don't.

2-12- indigenous knowledge systems prefer the less risk to most profit

Escaping of risk is important for native people, for example a native person usually keeps some goats for possible cases such as disease of his children and he and he didn't expect any incomes of these cases.

Agriculture Extension and indigenous knowledge:

Agricultural extension system as one education institute and one of the principal component of knowledge system and information of agriculture can play affective role at valuing farmer's experiences and indigenous knowledge. Extension can helps to sustaining development activities by applying more cooperative approaches, using traditional transmission channels of indigenous knowledge and try to establish mutual learning condition between components of knowledge system and information of technology, while making more adaptability of researches programs with contact's needs (Ahmed, 2000).

You can accept such functions from extension system when that agricultural extension enjoys one multidisciplinary approach as one of component of vast network of knowledge and information of agriculture. And their staffs being under education continuously in order to acquire work condition at network place.

Indigenous foundations at villages originally displays democratic system at rural societies; and from aspect of existential philosophy, principles and goals and practiced methods prepares sustainable management at villages. Indigenous foundations at villages always can be considered as popular responsible organizations, preserver of rural people's interests as an elected institution by people. Enacting every law and orders about or related to villages and also/or far from attending to indigenous foundations in villages can't be applied sustainable and never would have efficiency. Whenever establishing institutions such as cooperative organizations, rural forums, development committees and etc and consequently selecting board and CEO done by

indigenous foundations or their confirmation, so it would enjoy even more efficiency and sustainability. We can seek to most original sample of experienced NGOs about social and economic activities base on voluntary motivations and group cooperative at Iran rural area (Gigler, 2003).

Karlir (1996) emphasized that agriculture education institutes should try to identify agriculture knowledge system and also interpret their application at production systems and if possible, make current education condition compatible. Bahala represented different attitude. He says that modern technology and traditional agriculture knowledge can play important role on modernizing agriculture production systems of third world countries (Brouwer 1998).

Achieving to this purpose is dependent on integration and synthesis of these two knowledge at extension and education programs. He believes that integrating these two knowledge systems can:

Cover wide range of aimed opportunities at traditional and marginal parts, and make basic changes at traditional production systems through applying new technology.

Establish ways at extension programming and agricultural education which trough that they would enable to extend obtained interest of technology without much spending and various facilities. At developing societies such as Iran, factors such as far distance, being far from information centers or lacks of appropriate IT and trained people caused that villager being depraved from accessing to information and IT compared to large cities. According to its necessity at developing agriculture, this issue not only harmful for villagers but also is harmful for agriculture development and national development. So applying and integrating extension organization by using functions of information itself would cause extension, continuity and solidarity of process of relation between three links of extension, research and education. At one hand, accessing to clientele would be facilitated too. Briefly it can be said that applying IT at extension system leads to effectiveness of realization of extension goals and finally realization of development goals.

Most important mission of IT is distributing knowledge and information and from this viewpoint and according to direct relation of using knowledge and producing science at on society and its progress, it is possible to understand more about real position of IT in relation to multi-dimensional development (Amiri Ardakani and Shahvali, 2003) .

IT in removing barriers of access to new knowledge is among economic opportunities and social cooperative that was emphasized by extension system and plays important role. Considering that indigenous knowledge, is same issue that people living with , and base on that they should follow norm and abnormalities of their thought philosophy framework at the their different life fields. Exchange, interaction and constant refinement is considered as obvious features of indigenous knowledge. This knowledge that is based on change, revolution and changes, caused that be dynamic in contrast to its appearance. So, aforementioned knowledge has kept its structure at past and also would keep it at future but its content would be changed.

Local men are guarder of indigenous knowledge of each nation. Farmers, peasantry, rural craftsmen and ranchers, each own his specific skill. These arts and indigenous knowledge first seems simple but these simple methods have enabled villagers to supply their needs by limited sources. This isn't worthless outcome but planners' propagators of new technologies always ignore rural abilities like their needs, preferences and innovations. While, by understanding this knowledge and local acknowledge would provide stable basis for presenting technical questions at agriculture researches. Scholars and experts of Third world countries usually repeatedly deny its people's indigenous knowledge. Their prejudice partly is rooted in European social science scholar's theories and 19 century America that until now is dominated on thought of third world countries especially non-western educated People. While, many case of studies and scientific researches, has proven value and effectiveness of local knowledge. Educated people of new sciences should accept base on new framework and principle of epistemology that their knowledge at all dimensions isn't superior to indigenous people's knowledge (Appleton and Jeans, 1995).

So, at learning process they should confess to weakness of their knowledge and to strengths of indigenous knowledge in order to identify and improve local men's life condition. Nowadays, most scholars especially academic researchers want to understand certain condition of village just by one or more short visits. Obviously, these kinds of visits rarely can inform researchers of real delicate issues of villages and also about complicated economic, social, politic and cultural issues (Farrokhi, S and Yaghoubi, 2002).

Conclusion and discussion:

On the research which was done by Bozarjomhari (2004) with this title "analyzing native knowledge position on rural sustainable development". It was specified that although there are many differences between native and modern knowledge but they are not in contrast with each other, because they are each other's supplement and

we can't be success when we use them separately. According to new parameters in rural development, for solving rural problems, at the first we should use of native solutions and if it was not efficient, we can use and test external solutions.

Research findings which was done by Emadi and Amiri (2004) with this title " compilation of native and modern knowledge is necessary for reaching agriculture sustainable development" signify that The believe of educated people to native people and their knowledge " precondition for making them close" is called combination and compilation. Making evolution in modern system for attention to tentative knowledge is the main necessity for this compilation. Another necessity for this evolution is the researcher's attention to experimental accumulated wisdom and historical exploit by using qualitative and communion methods. Also applying compilation methods and making evolution among government, educational centers, farmers and peasant is the necessity and pre condition for combination of modern and native knowledge.

In order to develop agriculture extension activities, considering indigenous knowledge is critical because, sense of self-esteem and reliance on local sources would be reinforced by citing of vast application of Iranian indigenous knowledge and others ancient culture of world at sustainable development of industrial countries.

also, necessity of considering indigenous knowledge at developing extension programs is emanated from where that is considered as principal components and sustainable human development items is emanated from same sources. At sustainable human development, people are considered as “goal” of social and economic policies that their range of their selections would be extended in order to actively participate at decision making. Therefore, people’s participation is one of tools of sustainable agriculture development. But active rural people’s participation at extension programs as a form of sustainable would not be possible unless by believing role of rural people’s knowledge, vision and skills (Brouwer 1998).

Necessity and importance of indigenous knowledge and sustainable human development prepared field for establishing “united nation conference, about nature and development” at 1992.

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The detection of Chlamydia Trachomatis Antigen in cervical secretions and serum antibodies in infertile females undergoing ICSI and its impact on pregnancy success.

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Abstract: Background: Chlamydia vaginosis is a commonly reported bacterial infection, with an incidence that varies according to population. Some researchers have studied the association of such condition and infertility. In our study we aimed at evaluation of the incidence of cervical Chlamydia trachomatis infection in infertile women undergoing assisted reproductive techniques & its impact on its success to achieve pregnancy. **Methods:** A study group of 150 infertile females attending infertility centers for ICSI and a control group of 150 multiparus females attending outpatient clinics for IUD introduction have been included. Endocervical swabs from cases and controls have been examined for chlamydial antigen using immunochromatography. Also sera were examined for IgG and IgM for Chlamydia trachomatis in both groups. **Results:** 12 positive cases for serum Ig G in study group (8.0%) and 16 in controls (10.67%) has been revealed. While serum IgM was found in 4 study cases (2.7%), with no positives in the controls. Regarding chlamydia antigen detection in endocervical swab, there was 6 positive study group cases (4.2%), while no cases were positive in controls. **Conclusion:** our study reports a very low prevalence rate of *Chlamydia trachomatis* infection in Egyptian females, which minimizes its role as cause of infertility in Egyptian population and subsequently its impact on success of ICSI is not much expressed. Cultural impact on sexual life style in Egyptian population could justify our findings.

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Key words: Chalmydia, antigen , antibody

1. Introduction

Chlamydial vaginosis is a commonly reported bacterial infection specifically in developed countries. The causative agent of the condition is chlamydia trachomatis (Stamm, 1999), with an incidence of infection that ranges from 8.6% up to 40% according to population with a reported higher association with infertility (Woods, 2003). *C. trachomatis* being the causative agent of a widespread sexually transmitted disease can lead to urethritis, cervicitis pelvic inflammatory disease (PID), ectopic pregnancy and tubal factor infertility in females (Mayaud, 2001). Persistence, chronicity and subclinical presentation are the common characters of the *C. trachomatis* infection (Schachter, 1999). It has been suggested that impaired ovarian function and low ovarian response to ovulation induction are associated with *C. trachomatis* infection. (Malik et al, 2006) In females, infection usually starts in cervix and urethra with an abnormal vaginal discharge or a burning sensation in micturition. Then it usually spreads to the fallopian tubes and uterus and can progress to pelvic inflammatory disease. Still, silent infections are prevalent. (Stephen, 1999)

Earlier studies have shown that pathogenesis of the disease was not only induced by the infectious agent but also due to immune response to infected tissues. (Paavonen et al 1999).

Various diagnostic assays are available for Chlamydia infection . Previously, cell culture of cervical, vaginal swab and urine was the main tool for objective diagnosis of the case. Immunological tests by detection of antibodies (IgG, IgM) were also available. However, it is reported that serum antibody was not always consistent with infection (Brunham et al 1983). Molecular methods such as PCR are considered as reliable methods for diagnosis nevertheless, they are technically demanding and with low cost effectiveness in case of screening. Detection of chlamydia antigen in different samples have been reported to be high sensitive and specific and rapid kits are available commercially with simplicity of usage and low cost. (Machado, 2001) The aim of this study is to evaluate the incidence of cervical *Chlamydia trachomatis* infection in infertile women undergoing assisted reproductive techniques & its impact on its success to achieve pregnancy.

2. Subjects and methods.

Study group: Demales attending fertility centers in Alexandria seeking ICSI (intracytoplasmic sperm injection) for treatment of infertility.

Control group comprising multiparus

females attending the contraception clinics for IUD introduction was included for comparison. Cases included in this study have been informed and consented for this study. Criteria for selection of cases are summarized as follows:

Inclusion criteria :
1- Age range between 20 and 40 years.
2- Duration of infertility more than 3 years.

Exclusion criteria:
1- Systemic diseases such as diabetes, hypertension, renal disease & systemic lupus
2- Local disease such as fibroid, uterine anomalies as uterine septum & adenomyosis diagnosed by 3 D ultrasound or hystrosalpingography.
3- Moderate to severe endometriosis
4- Severe male factor of the male partner (count of sperm <5 millions /ml, abnormal forms of sperms more than 90% and motility less than 10%).

Cases included in this study have been subjected to:

1. Collection of endocervical swab: after cleaning of cervical canal from mucus, a sterile swab has been rubbed for 15 seconds to the cervical canal after passing squamocolumnar juction (ie: tip of the swab is not visible). Chlamydia Antigen in the collected samples was detected using rapid Immunochromatographic assay using commercially available kits according to manufacturer's instructions *(Prechekbio,Invitro diagnostics, Korea).* (Woolley et al, 1997) Results have been considered positive in case of presence of two distinct colored lines: one in control line region & another one in the test region. Negative result was considered in case of detection of only one colored line in the control region.
2. Withdrawal of 2 ml venous blood and serum was separated for detection of Chlamydia antibodies (IgM, IgG) using ELISA. The Chlamydia trachomatis antigen which is coated on the plates is comprised of elementary bodies. *(IBL International GmbH, USA).* (Herrman et al, 1991, Simms et al, 1987, Thomas et al 2000).
3. Follow up for pregnancy results of ICSI: (Intracytoplasmic sperm injection) by estimating B-hCG and ultrasound visualization of fetal heart beats.

Statistical analysis:

The Data was collected and entered into the personal computer. Statistical analysis was done using Statistical Package for Social Sciences (SPSS/version 15) software. The statistical test used as follow: Arthematic mean, standard deviation, for categorized parameters, chi square test was used while for numerical data, for two groups non parametric data U-test was used, while for two groups (parametric data), t-test was used. The level of significant was 0.05.

3. Results:

This study was carried out on 300 married females. Study group included 150 females attending fertility clinics for management of infertility, while a group comprising 150 multiparus females attending the outpatient clinic for IUD introduction for contraception were included as control. The age range in the study group was 20 – 36 years with a mean of 29.05±4.18 and in control group it was 27 – 35 years with a mean of 30.21±2.917. There was no significant difference between the two studied groups (p>0.05). No difference in the body mass index between the study and control groups was observed which ranged from 22.1 to 40 (mean 27.15±3.826) and 21.2 – 39 (mean 28.53±2.917) in the study and control groups respectively (P=0.166) .The duration of infertility in the study group was 3 – 10 years with mean of 6.07 ± 2.461. We report that there were 12 positive cases for serum Ig G in the study group (8.0%) and 16 positive cases in control group (10.67%). Further, in the study group, serum IgM was found only in 4 cases (2.7%), while no one in the control group were positive for serum IgM. There was no statistical significant difference between the two studied groups with respect to serum IgM and IgG. Chlamydia antigen was detected in 6 cases (4.2%) in the study group, while no positives were detected in the control group. There was no statistical significant difference between the study and control groups regarding chlamydial antigen positivity.

Table (1): Comparison between study and control groups regarding laboratory assays used to diagnose Chlamydial infection.

Assay	Patients "n=150"		Control "n=150"		p
	No.	%	No.	%	
Chlamydial Ag positive in endocervical swabs	6	4.2	0	0.0	0.36
Serum IgM positive	4	2.7	0	0.0	0.618
Serum Ig G Positive	12	8.0	16	20.0	0.086

There was no difference between the negative and positive cases of the study group concerning the age which ranged from 20 – 36 (mean 29.01±4.155) years in negative cases while in positive cases it ranged from 23 – 35 years (mean 30.17±5.115) years, (p>0.05). The body mass index in the negative cases ranged from 22.1 – 40.0 (mean 27.09±3.867), while in the positive it ranged from 25.0–32 (mean 28.67±2.422). There was no statistical

significant difference between the two studied groups regarding body mass index (p > 0.05). The duration of marriage in the negative cases ranged from 3-10 years (mean 6.04±2.452), while in the positive cases it ranged from 3 – 10 years (mean 6.83±2.787) with no statistical significant difference between the two studied groups (p>0.05). In order to determine whether other conditions might be associated with infertility in the study group, a wide variety of parameters were considered. It was found that the most common cause (30.7%) for infertility was polycystic ovaries (PCO), while the travelling of husband was the least (6.7%). It was also found that there were 8 cases with unexplained cause (Table 2).

Table (2): Distribution of the studied cases regarding other conditions associated with infertility.

Cause of infertility	Number	Percent
Endometriosis	16	10.7
Male factor*	38	25.3
PCO	46	30.7
Travelling husband	10	6.7
Tubal factor	32	21.3
Unexplained	8	5.3
Total	150	100.0

* Male factor 5-10 million in count, motility10-40% and abnormality 60-80%.

Correlation between chlamydial antigen results obtained by endocervical swabs and serum IgG detection, it was found that 11 cases (7.6%) of the negative for chlamydia antigen were positive for serum IgG, whereas in cases positive for chlamydia antigen only 1 case was positive for serum IgG. There was no statistical significant difference between the negative and the positive Chlamydia antigen cases in relation to serum IgG. It was also found that there were no cases that were positive for IgM in the samples that were negative for chlamydia antigen In cases positive ~~cases~~ for chlamdia antigen 4 cases were positive for ~~a~~ positive serum IgM. There was no statistical significant difference between the negative and the positive chlamydia antigen in relation to serum IgM.

Table (3): Relation between Chlamydia antigen detection and serum IgM in study group cases.

	Chlam (Ag) in endocervical swab				p
	Negative"n=144"		Positive"n=6"		
	No.	%	No.	%	
IgM Negative	144	100	2	33.3	0.152
Positive	0	0	4	66.7	
IgG Negative	133	92.4	5	83.3	0.399
Positive	11	7.6	1	16.7	

On analyzing the pregnancy outcome of the study group cases after ICSI , it was found that there was 47 cases were positive for pregnancy test (quantitative B-hCG (31.3%). It was found that 2 cases were positive (33.33%) in cases positive for chlamydia antigen. It was found that 45 cases were positive in patients negative for chlamydia antigen (31.3%), There was no statistical significance difference in B-hCG results with respect to Chlamydia antigen positivity.

Table (4): Pregnancy after ICSI in the study group

Pregnancy	Study group cases "n=150"		Positive for chlamydia Ag		Negative for chlamydia Ag	
	N	%	N=6		N=144	
Negative	103	68.7	4	66.67%	99	68.7%
Positive	47	31.3	2	33.33%	45	31.3%
p			0.415			

4. Discussion:

Underreporting is an obstacle in assessing the total burden of Chlamydia infection in the Middle East. Many factors contribute to this underreporting. Some of them include the culture of the population that judge sexually transmitted diseases as a stigma and the financial problem of high cost and technically demanding diagnostic assays for the medical condition that makes these diagnostics not available in routine labs. A previous study reported that Chlamydia trachomatis prevalence was found to be 5% in Cairo. (Sadek et al, 1991)

Various methods are available for the diagnosis of Chlamydia infection such as cell culture of cervical-vaginal swab, (Marrazzo et al 1997) serological testing by detection of specific antibodies: IgM for recent active infection, IgG for previous or chronic infection. Inspite of being useful as diagnostic tool for ocular or respiratory infection, studies reported that the production of the specific antibodies for chlamydia in case of genital tract infection is not consistent and may be absent in some cases (Simms et al 1991). Detection of Chlamydia antigen in different samples according to site of infection has been widely used in the developed countries for screening of cases for its simplicity, cheapness and rapidity. (Neuer et al, 2004, Johnson et al, 2002, Lossick et al 1990). Detection of the nucleic acid of chlamydia organism via Polymerase chain reaction (PCR) is still a gold standard for diagnosis though it is expensive and technically demanding. (Nelson et al, 2001, Rosenwaks et al, 1995)

Many researchers investigated the role of different microbial agents in infertility and the impact of their existence on outcome of assisted reproductive techniques. In this study we aimed at assessing the incidence of chlamydia trachomatis infection in

infertile females. As regards the incidence of positive serum IgG in our study, it was found that positive cases in infertile females (8.0%) where lower than in control (10.67%), however this difference was not significant.Different countries reported widely variable incidence of chlamydial genital infection. in Australia, incidence reported of Chlamydia trachomatis antibodies in patients attending infertility clinics was at minimum 30%. (Torode et al, 1994) Sharara et al. in 1997 in the United States found that the prevalence of elevated serum chlamydial IgG Ab in female patients presenting for IVF was (55.2%) which was higher than in the general population. (Sharara et al 1997). The lower incidence of positive chlamydial IgG in our study compared to studies conducted in western countries could be attributed to lower frequency of predisposing factors of chlamydial infection including early sexual life and female multiple partners.

Regarding serum IgM the comparison between the two studied groups showed that there were only 4 cases (2.7%) in the study group positive for serum chlamydial IgM, while no one in the control group, still with no significant difference. A study conducted by Annika Idahl, in Sweden, reported higher prevelance of positive serum chlamydial IgM which was about 13% (Idahl, 2009).

In the present study, the higher incidence of positive chlamydial IgG in comparaison to IgM could be attributed to IgM antibody being reflecting acute infection while infertile patients have usually chronic infection, also positive IgG result does not necessarily indicate a genital chlamydia trachomatis infection, as it could be an infection elsewhere in the body. Regarding prevelance of chlamydia (Ag) among study group cases, a low prevalence 4.2% has been revealed, while no positive cases were detected in control group. Still, due to low prevalence in study group there is no significant difference with controls.

Results obtained by Osser S et al. (study conducted in Sweeden on 121 infertile female with tubal factor (Osser et al, 1990). Other researchers reported no significant impact on ICSI outcome (Tasdemir et al, 1994). On the other hand, Witken et al. study showed decrease in embryo characterization in positive cases for chlamydia (Ag), this was justified by heat shock protein induced by inflammatory reaction in response to infection that may impair embryo implantation and/or facilitates immune rejection after uterine transfer of in vitro fertilized embryos (Witkin et al, 1994).

In the present study, the association of chlamydial Ag in endocervical swab and serum IgG, it was found that there was 11 cases had negative chlamydial antigen while their serum had positive serum IgG, whereas there was only one case had positive chlamydial (Ag) in endocervical swab show positive serum IgG. There was no significant association between prevelance of chlamydial (Ag) in cases and serum IgG. The higher rate of positive chlamydial IgG may be explained by the chronicity of infection in the patients. It was found that all cases negative for chlamydial antigen results had no positive serum IgM, whereas in the 6 positive cases for the antigen only 4 cases showed positive serum IgM that could be considered as acute chlamydial infection. Many previous studies that had assessed the effect of chlamydia trachomatis infection on the success rate of assisted reproductive techniques, reported that pregnancy outcome of IVF was not affected with previous exposure to chlamydia trachomatis (Sharara et al 1997, Tasdemir et al 1994). Moreover, Acharya, et al study reveal although strong association of bacterial vaginosis and past chlamydial infection with tubal infertility but with no impact on in vitro fertilization success rates (Acharya et al 1999).

Our study reports a very low prevalence rate of Chlamydia trachomatis infection in Egyptian females, about 4.2%. This low prevalence limits the study of its association with success of ICSI cycles. Whereas in other countries with higher prevalence, impact of chlamydial infection on infertility is much expressed. Cultural and religious impact on sexual life style in Egyptian population could justify our findings. As a consequence of our findings, Chlamydia trachomatis genital infection can't justify raising prevalence of primary infertility in Egyptian population. It could be undertaken by the clinicians in such population in management of infertility.

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3/2/2011

A study on GRP ground wave method for the variation of dune in surface soil water content in summer

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Abstract: The Changes in soil moisture in summer is one of the important factors that influence the state of germination and plant growth. The variation of dunes in the surface of the soil water content was measured using GPR ground wave method from May to July 2010. The results show that in this period, the water content of soil on top of the dunes is declining. It shown that during summer, the appearances of the high amount precipitation and the evaporation were the important factor in the distribution of soil water content.
[Khampasith, T., Jianguo, B., Mupenzi, J.P. and Bounthanome, S. A study on GRP ground wave method for the variation of dune in surface soil water content in summer. Life Science Journal. 2011;8(2):264-268] (ISSN:1097-8135). http://www.lifesciencesite.com.

Keywords: GPR ground wave method; precipitation and Evaporation; soil water content

1 .Introduction

The plant life is always linked to water content of soil is one of the determining factors for their growth. During the summer, space is always facing to the problem of a severe shortage of water resources due to high evaporation. This period weakens the plant growth because their growths depend on soil water.

Several studies conducted in this field showed that the gravimetric method performed on the basis of soil samples is the standard method and direct that should be used to measure soil water content. However, field disturbance, labor and the inability to perform repeated measurements can be major drawbacks to the success of this method (Topp et al, 1980; 2002). Other studies have considered the reflectormetry time domain (TDR) as the most accepted method for measuring soil water content and is the most widely used (Whalley, 1993; Nielsen et al, 1995)

In several parts of the world, remote sensing methods were also used for the estimation of soil water below 0.1 m depth over a large area, with a spatial resolution that each pixel contains an actual size varies a few square meters to more than 1 km^2 (Huisman et al, 2003; Galagedara et al., 2005a; 2005b)

In this study, the main objective was to analyze the mane moisture content of a dune near the surface of the soil water during the drought period under study the variation of the water content in the surface VTENA

2. Materials and methods

Study area

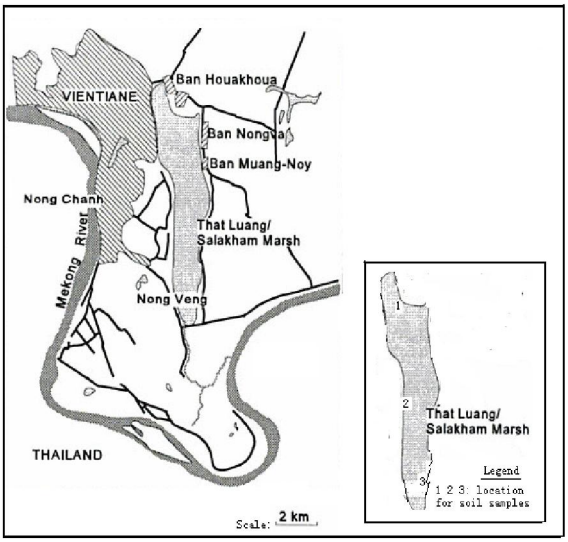

Figure 1 mapping of Vientina salakham Marsh

The Salakham marsh (That Luang marsh) is one of the largest wetlands located in peri-urban Vientiane with an area of 68 km^2. Geographically, Vientiane features a tropical wet and dry climate with a distinct monsoon season and a dry season. Vientiane's dry season spans from November through March. The average rainfall is ranging between 1360mm- 1400mm. April marks the onset of the monsoons which in Vientiane lasts about seven months. Vientiane tends to be hot and humid throughout the course of the year, though temperatures in the city tend to be somewhat cooler during the dry season than the wet season.

The main activity in this valley is agriculture with 2,000 hectares under hoe cultivation. Also, Fish

ponds are generally located along the margins of the marsh with an estimated of 15,000 people are involved with fishing-related activities on both commercial and subsistence levels (Coates, 2002).. Other activities are affecting the wetland's natural functions among them are: the construction a drainage canal through the swamp by the Vientiane municipality and the construction of a pumping station to remove water for paddy irrigation. The studies have shown that soil water content have also indicated that seepage of saline groundwater into the marsh may be occurring which would have a dramatic impact on the marsh ecosystem. Multiple measurements were taken to make comparisons on May 15, June 25 and July 8 in in year 2010; with the following indications about soil water content.

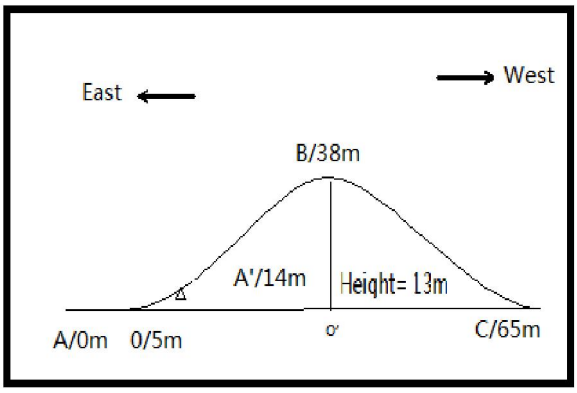

Figure 2 A schematic diagram of the chosen dune's profile

The GPR measurements

For this study, GPR instruments which include a host IDS RIS-K2 multi-channel and two radar transmitter-receiver systems were used. The speed of travel of electromagnetic waves emitted by the soil depends on complex soil dielectric properties that are fundamentally determined by its water content. The water soil samplings were conducted at a time resolution of about 0.1 ns and a spatial resolution about 0.1 m. The technique is an RPG based on the ground, noninvasive geophysical survey of soil conditions instantaneously. During experience, it is shown that the transmitter produces a high frequency electromagnetic wave transmitted by the soil. Davis and Annan (1989) revealed that any basement contrast in dielectric properties reflects part of the wave energy at the surface and the reflected wave is detected by the receiving antenna as a function of time. Note that GPR derived water contents, Time-domain reflectormetry (TDR) and gravimetric method were used for verification.

The direct ground wave travel time t_{GW} from the transmitter to receiver, the ground wave velocity V can be calculated as follows:

$$V = \frac{L}{t_{GW}}, \quad (1)$$

Where

V is the ground wave velocity; L is the separation between the transmitting and receiving antenna.

The V is converted to by using the electromagnetic wave velocity in free space:

$$\varepsilon = \left(\frac{c_0}{V}\right)^2, \quad (2)$$

Where

ε is the relative dielectric permittivity of the soil, c_0 is the electromagnetic wave velocity in free space.

$$t_{off} = t_{meas}^{air} - \frac{L}{c_0}, \quad (3)$$

where t_{meas}^{air} is the measured travel time of the airwave and L/c_0 is then correspondence to the theoretical direct airwave travel time.

$$t_{GW} = t_{meas}^{GW} - t_{off} = t_{meas}^{GW} - t_{meas}^{air} + \frac{L}{c_0}, \quad (4)$$

where t_{meas}^{GW} is the measured travel time of the ground wave and t_{GW} is the required absolute travel time for equation

The relationship between apparent permittivity ε and volumetric soil water content θ was calculated as follows:

$\theta = -5.3\times10^{-2} + 2.92\times10^{-2}\varepsilon - 5.5\times10^{-4}\varepsilon^2 + 4.3\times10^{-6}\varepsilon^3$ (Topp et al. (1980) (5)

This method about bulk permittivity of a soil-water-air system developed by Roth et al.(2006) and Friedman (1998), where ε_b, was expressed with the Complex Refractive Index Model (CRIM) as follows :

$$\varepsilon = \left[\theta\varepsilon_w^{\alpha} + (1-n)\varepsilon_s^{\alpha} + (n-\theta)\varepsilon_a^{\alpha}\right]^{\frac{1}{\alpha}}, \quad (6)$$

Where

n is the soil porosity; ε_w, ε_s and ε_a are the permittivity's of water, soil particles and air respectively; α is a factor accounting for the orientation of the electrical field .

The soil water content was obtained as follows:

$$\theta = \frac{\varepsilon^{\alpha} - (1-n)\varepsilon_s^{\alpha} - n\varepsilon_a^{\alpha}}{\varepsilon_w^{\alpha} - \varepsilon_a^{\alpha}}, \quad (7)$$

3. Results and Discussions

The results detailed in Table 1 indicate the GPR profile measurements taken in summer (May to July 2010) in in peri-urban of Vientiane

Table 1 Details for GPR profile measurements in peri-urban Vientiane

Dates	Antennas Information			Temperature(℃)		Measuring Time*	Profile Ends
	Frequencies (MHz)	weather	Separations (m)	Soil	Air		
2010/5/15	200	cloudy	0.94	0.2	1.2	13:24	O-B
2010/6/25	200	sun	0.82	0.2	1.52	16:04	O-B-C
2010/7/8	200	cloudy	1.62	6.67	6.1	13:32	O-A-B-C

From May to July, the Precipitations were irregulars according to the daily air temperature data obtained from the meteorological station located near to the Vientiane city.

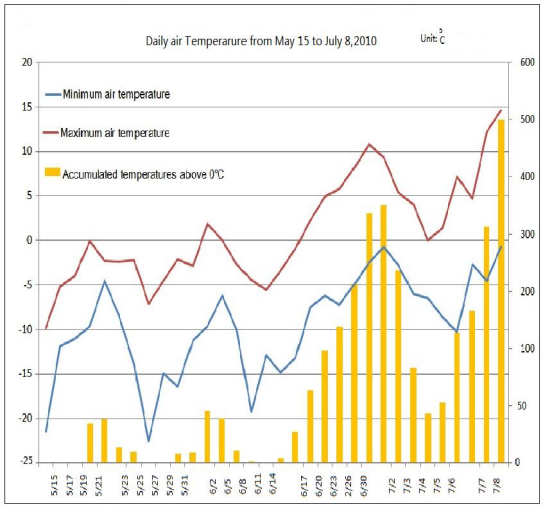

Figure 2 The daily air temperature from May 15 to July 8 obtained from the meteorological stations in Peri-urban Vientiane

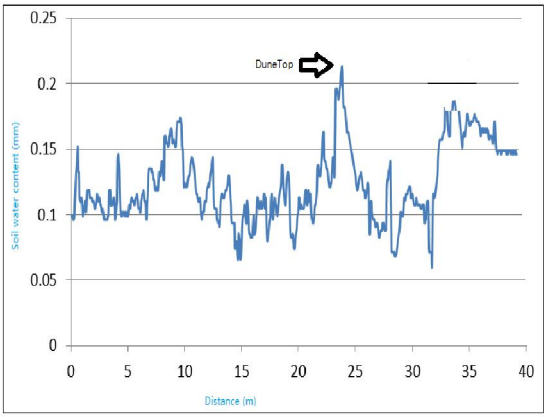

Figure 3 The surface soil water content on June 25, 2010 in peri- urban Vientiane

The upper soil water content was about 0.1~0.15, some TDR measurements were performed to get the permittivity and water content of 0-10cm depth soil, Therefore, the TDR results were very different as indicated in Tab 2.

Table 2 The TDR measurements on May 15, 2010

Description	Measuring Time	Temperature for evaluation [°C]	Averaged permittivity [As/Vm]	Averaged water content [-]
From start of profile in one meter steps towards dune top	9:00	6	5.89	0.2
	9:00	6	6.42	0.1
	9:00	6	9.24	0.126
	9:00	6	8.12	0.25
	9:00	6	5.03	0.1
	9:00	6	5.53	0.09
	9:00	6	6.11	0.063
Measurements at the 2.7 m spot over 40 minutes	15:00	6	9.32	0.17
	15:05	6	11.23	0.193
	15:40	6	8.43	0.13
	15:40	6	10.15	0.2
	15:40	6	10.25	0.16

Since the early summer from May 15 to July 15, 2010; there were changes in the soil water content in the region of Vientiane, these changes have been very clear shown through GPR image (Figure 4). Due to the length of the measure itself, the water content below included in the table 2, 3, and 4 justify the coverage of Precipitation that were almost with the same amount from May to July. The level of the evaporation was higher than that of precipitation

Tab 3 The results of TDR measurements on June 25, 2010

Description	Measuring Time	Temperature for evaluation [°C]	Averaged permittivity [As/Vm]	Averaged water content [-]
From start of profile in 1 m steps towards dune top	11:00	5	7.2	0.25
	12:00	5	6.92	0.12
	13:00	5	5.4	0.43
	14:00	5	8.97	0.25
	15:00	5	7.6	0.1
	16:00	5	5.57	0.87
	17:00	5	7.21	0.5
Measurements at the 13 m spot over 45 minutes north of the profile	16:00	7	9.02	0.16
	17:00	7	11.32	0.193
	18:00	7	9.2	0.13
	19:00	7	9.56	0.25
	20:00	7	11.12	0.1

Table 4 The TDR measurements on July, 8, 2010

Description	Measuring Time	Temperature for evaluation [°C]	Averaged permittivity [As/Vm]	Averaged water content [-]
From start of profile in 1 m steps towards dune top	10:00	9.2	7.11	0.28
	11:00	9.2	6.92	0.25
	12:00	9.2	5.4	0.1
	13:00	9.2	8.97	0.09
	14:00	9.2	7.6	0.1
	15:00	9.2	5.57	0.87
	16:00	9.2	7.21	0.5
Measurements at the 1.3 m spot over 45 minutes north of the profile	15:30	9	8.84	0.16
	16:30	9	7.394	0.193
	17:30	9	6.533	0.13
	18:30	9	9.56	0.25
	19:30	9	12.2	0.2

The results detailed in Table 2, 3and 4 indicate the soil situation at depth of 0~20 cm and 0~10 cm respectively. It showed a lower value means water content of soil layer at depth 0~10 cm lower than the soil layer at depth10~20 cm . This situation may be due to the evaporation that could me higher than precipitation.

From May to July 2010, the precipitations were estimated between 0.6-0.8 mm in this Region, that may have big impact to the soil water content ;the evaporation was high than precipitation and the decrease of soil water content was observed as indicated in table 2, 3 and 4 that describe the declining of soil water contents.

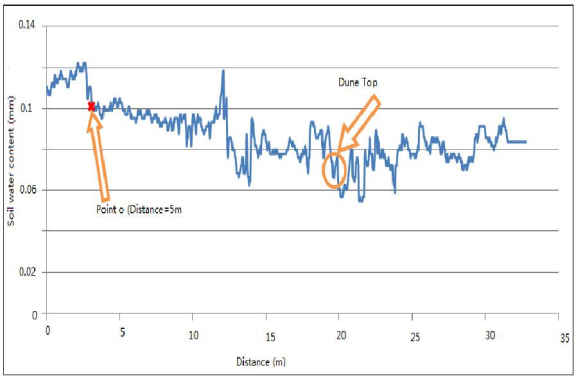

Figure 4 The soil water content calculated by Ground Wave Method on summer, 2010 at Vientiane

For a better demonstration and good understanding of the situation of subsurface conditions, one vertical soil profile was dug on the dune top and near the GPR profile on May15, and two vertical soil profiles were dug on the dune top and the flat dune base respectively on June and July. The results show that in this period, the water content of soil on top of the dunes is declining and this has big impact on soil water content in peri-urban of Vientiane.

4 Conclusions

This study was conducted with the main purpose of analyzing the water content of soil in the peri-urban of Vientiane. The results obtained using the GPR measurements showed lower content of soil water content in this area. It was shown that the soil water content decreased over time, consistently since the month of May until July. The sunshine and evaporation are the major factors that influence the amount of water content during the summer because the rapid acquisition of changes on the surface soil moisture is of great importance. This shall mean that the precipitation and evaporation are not balanced to maintain the stability of the water contained in the soil.

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Assessing Criteria of rural women empowerment

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Abstract: Enabling is process that, during it, people of society do activities to overcome barriers of advancement that finally cause their domination to determine their own density. The term "enabling" means overcoming fundamental inequalities. So it is different from self-reliance. Enabling, enables individual to overcome any problematic condition and consider barriers and problems as part of life and positive campaign. Finally, enabling provides energy to overcome most intellectual barriers and external problems at private life.
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Keywords: empowerment, rural women

Introduction:

According to women's role at family, they can be considered as base of development and progress and unfortunately according to universal tangible realities, they possess unfavorable position at international level (Changizi Ashtiani, 2003).

For example, difference at levels of policy making, investing and receiving salary for equal activity, are universal phenomena. extent of women's participation at economic activities, extent of women's activity at economic activities, is confirmation on lack of adequate attention to women's affair and their added value, because rural women work alongside men, at all levels of producing agriculture crops and livestock products and generally all affairs, and also spend their little leisure time for handicrafts such as rugs and carpets and etc. so it is necessary to establish self acknowledgement fields, directing women's economic and social ability and programming to attract their participation at different activities (Saadi and Arab Mazar, 2005). At rural area, women have more significant role on family economy and inside activities and cause economic prosperity of society. yet, women couldn't gain their real position as active citizens who have talent for participation at economic, politic, social and cultural arena at most countries, especially developing country, and still their activities in economic calculations aren't considered, and they be considered as intangible workforce. Disappointing estimation about number of active rural women and underestimate about extent of their participation at economic activities is confirmation on lack of adequate attention to women's affairs and their added value. they are major force to create revolution and potential sources to progress rural economy and increasing extent of growth rate of producing food productions, although traditionally, farming and ranching, has been male profession, but women's role was never restricted to house and family, so they are active outside (farming, ranching, forestry) other than inside activity (Balali, 2005).

Rural women are among those major groups at society who previously were considered less by planners, due to specific reasons in the past. And this problem is more observable at developing countries. While, by looking at women's history of economic and social life, we can find that this great group, continuously have played basic role in forming economic condition of country. This great group consistent with men have had active role at areas of social-economic activities and always have had major part on economic production of society. Nowadays, supporting family supervisor women is adopted by universal society, as politic, economic a social concern and nearly all countries applied related approaches, and however these efforts have resulted in failure, in so many cases (Banihashem, 1999). paying part of cost of life by government or charities, establishing forums to analyze family supervisor women's problems, supplying necessary facilities to grow and improve child's life quality and paying facilities to provide sustainable employment, are among most important approaches to support family supervisor women. Paying credit facilities to access sustainable employment with easy terms at limited time, is one of the most important approaches to support family supervisor women. Because alongside supplying their continues needs, their esteem wouldn't be marred. Currently, this approach is used

at many countries and positive results have emerged. (Ghaffari, 2000).

Increasing Suffrage, lack of relying on vast patriarchal families, increasing cultural acknowledgment, relation with newer institutions, having intellectual independence, making decision for marrying, occupation, emigration and etc are those rights that they gain. gaining aforementioned rights by women in context of cultural and social framework followed some changes that maybe lead to disfunctions and even create disorders and abnormalities at traditional, familial and kinship relations that dominated on villages (Fakhraee 2002).

What that performing credits programs, has made in recent years, was on broad outlook with purpose to access to same results as above findings.

Thus, in one inclusive outlook, it is possible to use micro-credits programs to solve those issues which involved with rural women's economic limitations, so that lead them toward social empowerment, in the context of economic growth(Rahmani andalibi, 2001).

Development along with economic growth and income increase is an important goal for most countries. Recently the growth of awareness about destructive effects of poverty has made countries believe that the best way to achieve sustainable development is to eradicate poverty; therefore most development programs have been oriented towards poverty eradication by micro-credit services.

Supporting poor to raise their life standards should be based on the belief that the poor are able to help themselves. Explicitly, this proves that among a variety of deprivation they do consider their survival.

In the new system of advanced agricultural economy, the value of women's work that previously was unpaid labor now must be paid in cash. Expect for agriculture which is rural women's main work field they have rarely participated in tow other fields of economy. The most important issue of women's social and political participation is to take part in planning, decision making, implementation of decisions, and evaluation of results. Generally they have had a little share in such processes. Although in recent years rural women have participated more in villages' management, social and cultural organizations, and cooperative institutions' management; but having a lower level of literacy, education, income and social status than urban women they still have the smaller share of administrative and official jobs. Some barriers to women's participation which can be categorized in 3 groups of personal, familial, and social include: law literacy level, large volume of work both inside and outside of home for many reasons including seasonal migration of men and the great diversity of rural women's activities (nursing, housekeeping, agriculture, handicrafts, livestock), malnutrition, law health indicator, Patriarchal structure of society, father or husbands disagreement with a woman's participation in social and economic activities for various reasons like cultural reasons or unwilling to lose the labor force at home, negative attitudes towards women's abilities, gender discrimination, family's poverty, superstitious beliefs, misleading customs like fatalism, law access of women to credit and facilities, inaccessibility of extension services, men-orientated social activities and participation plans, deficiency of professionals needed to educate rural women, problems of access to health services and social facilities, low income of rural women compared with men, lack of non-governmental organizations dealing with rural women's problems, few women managers in rural area. (Rahimi, 1380)

Empowering rural women:

Empowerment is capacity that woman can obtain in cultural and social environment, for economic independency and self reliance, by controlling over emotional decision making and far from violation. Empowering means, evolution and developing activities through non governmental organizations (NGOS) that lead empowerment to improve economic dimensions. (Amiri, 2000)

Enabling is process that, during it, people of society do activities to overcome barriers of advancement that finally cause their domination to determine their own density. The term "enabling" means overcoming fundamental inequalities. So it is different from self-reliance.

Enabling, enables individual to overcome any problematic condition and consider barriers and problems as part of life and positive campaign. Finally, enabling provides energy to overcome most intellectual barriers and external problems at private life.

Thus, among all what have been said, it is possible to present suitable definition of enabling women, as follows:

"Process of explaining women about themselves (and also men about them) for instances that they must or want to do, and growth of their willingness and courage until they reach to needed competency "(management of rural and tribal women). it should be noted here, that major factor which should be considered about women's ability, is eliminating individual and social barriers, and finally preparing field of economic and social participation for women at all fields. purpose of women's participation, is because of their dominance on all affairs of village including decision making process, organizations, forums, enterprising posts that involve,

participation at all social and economic dimensions (Moazami, M, Rahimi A. and Azam tayefe Heidari, 2005).

Criteria of empowering women:

Enabling as a theory of policy making for women, in it present five criteria:

Welfare, access, Concientisation, participation and control.

1- welfare criteria:

In this criteria, men and women as human resources of development should enjoy of desirable welfare conditions and equality.

Most of timing developmental programs, have worked on base of women's welfare. They have considered and provided some services for women who were passive recipient of these services. But these services were limited to physical needs and mostly were considered to revive their role of productivity, again. sometimes, it has been said that this approach has begun at colonial era and has considered women from poor country and intended services for them that dose not exceed from that poverty level. Agricultural and industrial projects were designed for men and social programs for women and children. Most of welfare programs were inadequate or its success was limited. Considerable point in this criteria is that men and women as human resources of development should enjoy equality and desirable welfare conditions. At this stage, women's material welfare and their enjoyment of welfare programs, compared to men (nutrition, death rate) were considered. And women's role as producer to supply their own needs isn't very important.

2- access criteria :

Lack of access or limited access for women to sources including (fields, job, capital and training) cause that their functions at production is less than men. Access to facilities, sources, designed program and projects for women and access to schools are in this part. Just whenever most of other legal, cultural and social issues being solved, men and women would equally access to sources and facilities. Concept of enabling at this stage is that women have equal right to access to sources at family and greater society.

3- Concientisation criteria

Women should know that their problems aren't due to their individual inefficiency and shortage but it has emerged by social system in which discriminations has become formal and acceptable issue. (Araghzadeh, 2002). This stage is more critical and important than other stages. Because women can participate at development activities not just be passive users. Women have real equality at development, just when be aware. Concientisation will help to increase women's ability to equality at participation at society. At this stage, women face with critical analysis with society and will find that what has been considered natural and unchangeable reality, is changeable. (Bakhshoodeh, 2005).

4- Participation criteria

One the most important items that this criteria has considered, is men and women's equal participation at decision making process of affairs of family at society. Men and women both should participate at process of assessment needs, designing, performing and evaluation of projects and development programs. In summary, this criterion means women's participation at all stages of surveying needs, detecting problems, planning, management, performing and valuation.

5- Control criteria

This criterion emphasize on this point that in addition to equal access of men and women to development sources, they must have adequate control on these sources that this issue is balance criterion, between men and women so that no one exceed other one. Women should have opportunities for decision making at workplace and home. If woman is producer, should be shared with part of her interest and wage. Women like men, should be able to choose her individual and social field and able to make decision and also development activities should be facilitator of these processes (Kar, 2000).

FAO (food and agricultural organization) addresses these three purposes as strategic goals while enabling women:

1- equality between men and women to access production sources
2- women's participation at policy and decision making
3- decreasing rural women's workload and increasing job opportunity and income for them

within theoretical framework of enabling women, having control on sources is presented as highest stage at women's participation process on development, but existing data at most developing countries, indicates that not only rural women haven't any control on financial resources of family but even they were deprived to access to sources and credits, specially through formal credits system (Farghdan, 2001).

The question that arises here is that what relation is there between enabling women and micro-credits programs? Nowadays, micro-credits are considered as effective mechanism to eradicate poverty for

women. Interests of micro-credits further increasing women's income, include:

- improving women's role in family
- Increasing women's confidence, not only through obtain financial success through business activity, but through increasing women's access to social services and communication with other women.
- Changing at social level (social class) at perspective of women's role.

Discussion and conclusion:

Supplying credits and analyzing credits approaches cause opportunity to activate poor men's working power, establishing field for sustainable production and income, prevent usurers and pre shoppers of agriculture productions to plunder poor rural men and finally empowering poor people especially women who can work but were deprived to have capital and work tools, and extension accordance to their activities such as needs assessment, identifying target group, organizing poor people, giving needed specialized and public training have important role on effectiveness and make effective activities of these credits.

Woroniukو Schalkwyk (1998) at their conducted research believe that now, micro credits, micro finance sources and small business unites are most effective mechanism to decrease poverty.

Plitt and others, conducted research as they called it "do credits programs, can empower women"? Results showed that corporation at credits programs helps empowering women.

Goetz و Sengupta (2003), presented negative image of credits effects on empowering women. They concluded that most women have minimum control on their loans. And when repayment period is short, this shortage of control has devastating effects on women welfare.

Hashemi and others (2004) found that joining to Gramin Bank, has meaningful positive affects on controlling women, and helps to family income.

In researches that conducted by Nanda (2004) became clear that women participation in credits programs had positive affects on their demand about health care.

Fiona Steele and etal (2008) in researches that conducted as called " influences of credits programs on empowering women at Bangladesh, found that women who joined to credits programs, have participated in more educational programs and have married with more educated men and also they have saved more and they had more cash.

Ellen and her colleagues (2009) used approach called it "credits and education at Bolivia, Ghana, Honduras, Mali and Thailand". This approach looks for empowering women through financial services with education. In this approach, women get familiar with importance of credits through education and extension and also familiar with ways to access it through establishing different groups.

Shahnaj and chaudhury(2009) in research as "credits and its role on empowering women " concluded that there is meaningful relation between attending in credits programs and empowering women, at economical dimensions.

Ruhal amin and others (2010) found that those who joined credit funds had more ability rather than those who didn't.

Jameela (2010) presented that credit programs has shown lot of affects on empowering women so that has increased their social, politic and economic ability.

Thus it is obvious that credits programs and its educational and empowering programs can be affective on social, humane and economic development or rural society, if it be associated with proper and gradual practices and base on reciprocal communications principles and apply opinion of local society(Bahar, 2001).

Maybe the main challenges that threaten credits associations, is lack of necessary emphasizes on social dimensions and on reinforcing their basics, that practically cause that this social foundations lose its efficiency soon and practically changed to unsuccessful institution.

In order to overcoming dominant consideration, experts believe that we should consider following in protection process of these social institutions.

- establishing and reinforcing through supporting without ant direct government involvement
- evaluating and constant modifying of financial management mechanisms
- improving organization effectiveness
- establishing constant relation and interaction with similar and equal systems.
- establishing local, regional and national networks
- establishing support and cover systems in order to decrease risk
- establishing balance and interaction with financial systems greater decision making include: capital market (local, regional, national) and governmental.

Also following suggestions have been offered:

- helping to marketing and establishing many exhibitions for member's productions, credit programs, guiding and training them in line with group and

workshop activity, can assist them on economic empowerment.

- since women have pointed to education deficiency as major barrier for empowering them, thus educating rural women at the field of exploiting different credits and channels of receiving credits, and also various educations, is so that lead to enabling them, that contain considerable importance.
- providing extension educations for men in order to believe economic role of their women, and give them chance of corporation on all economic, credits fields
- Since that base of credit association, forms base on People Corporation, so it's good chance to use these communities to expand extension-education activities. so it is better to consider special programs on different extensional filed such as agriculture, ranching, family health, housekeeping economy and other fields accordance to condition of region and rural women's needs.
- it is suggested that vast and exact programming happens at following fields:

  a- extending insurance, facilities for amenities

  b- educating women about awareness of their own individual and social rights

  c- persuading rural women about importance of participating at cooperatives and other educational institutes

  d- educating women about job management and income management

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Adiponectin in African Egyptian Obese Adolescents

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Abstract: Background: Adiponectin is the most abundant adipokine shown to have insulin-sensitizing, antiatherogenic, and anti-inflammatory properties. Adiponectin level, unlike that of other adipocytokines, is decreased in obesity and type 2 diabetes and increased after weight reduction. Recent studies suggest that adiponectin plays an important role in linking obesity with different cardiometabolic risk factors. Neverseless, a few studies have investigated this relationship in obese children. Racial differences in adiponectin level were observed, but little work has been done to determine if plasma adiponectin concentrations differ as a result of ethnicity. In few studies in African American, lack of a relationship between plasma adiponectin, obesity, and insulin sensitivity were reported despite the prevalence of obesity, diabetes, and insulin resistance in this population. However todate, there are no reports examining similar relationship of adiponectin and different cardiometabolic variables in African Egyptian. Aim of the study: To investigate the relationship between adiponectin, and different cardiometabolic and anthropometric variables in African Egyptian adolescence, in a trial to further explore, whether this relation in the African race differ from other ethnic population. Subjects and methods: A cross-sectional survey was conducted by the National Research Centre, Egypt. The survey comprised 3708 adolescents (48% boys and 52% girls), aged from 12 to 18 years (± 6 months). Of the total sample, only 340 students (9.2%) were obese (8.1% boys and 10.2% girls); with mean age 14.36±1.66 years. The included 180 obese students; who accepted to share in the laboratory tests; underwent complete physical examination, including different anthropometric measures (Height, weight, body mass index, waist circumference, hip circumference and waist/hip ratio). Blood pressure was also measured. Fasting venous blood samples were collected to detect fasting blood glucose, fasting serum insulin and adiponectin levels. Meanwhile insulin resistance was calculated. Results: Serum adiponectin level was low compared to the kit reference range. It did not show any significant correlations with the studied anthropometric parameters; both the systolic and diastolic blood pressure, the fasting plasma glucose, insulin level and insulin resistance (HOMA-IR). Conclusion: Although the present study, proved that serum adiponectin level was low in the studied African Egyptian obese adolescence, but it could not prove a direct link between adiponectin and the studied anthropometric measures, and cardiometabolic variables. This may provide additional support for the notion that what applies to other ethnic populations might not apply to the African population.

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1. Introduction:

Over the past decade, childhood obesity has increased in a dramatic fashion. With increasing number of young obese populations diagnosed with the metabolic syndrome (MS), which is characterized by such complications as hypertension, glucose intolerance, dyslipidemia and hyper-insulinemia [1,2] . The molecular basis of the pathogenesis of obesity-linked disorders has not been fully elucidated. Adipose tissue serves not only as an energy storage organ, but also as an endocrine organ. It releases many factors with autocrine, paracrine and endocrine functions. Adipokines such as leptin, resistin, tumor necrosis factor-α, interleukin-6, adipsin, visfatin, and adiponectin are biologically active molecules produced by adipose tissue. They play a role in energy homeostasis, and in glucose and lipid metabolism [3].

Adiponectin is one of the most abundant adipose tissue–specific cytokines that is closely linked to obesity in both children and adults [4,5]. Adiponectin level, unlike that of other adipocytokines, is decreased in obesity and increased after weight reduction [6,7].

The clinical relevance of adiponectin remained obscure for a number of years [8]. However, starting in 2001, several studies highlighted the potential antidiabetic, antiatherosclerotic and anti-inflammatory properties of this protein complex. Recently a growing body of evidence suggests that,

low level of adiponectin may constitute an early biomarker, identifying obese youth at high risk for the future development of diabetes and atherosclerosis [5,9].

Adiponectin levels have been shown to decrease with age in both normoweight [10] and overweight youth [11], and thus, aging and maturation may be important regulators of adiponectin metabolism and disease risk in youth [5]. Moreover the association between variants in the adiponectin gene and metabolic profiles may differ between ethnic groups [12].

Several studies involving different age groups [13,14,15] proved that adiponectin level is lower in normal-weight African-Americans compared with Caucasian peers, furthermore the prevalence of obesity,[16] diabetes,[17] and insulin resistance [18] were noticed in African-American population compared to Caucasians of similar age and total adiposity. Whether this difference is because of the racial differences in adiponectin level is currently unknown. However recent studies suggest that differences in adiponectin gene polymorphism could underlie the observed racial differences in adiponectin and its association with physical and metabolic parameters. . In fact, little is understood about the determinants influencing adiponectin levels, particularly at critical stages of development such as adolescence [19].

So this study was done on African Egyptian adolescence in a trial to investigate more about the influence of African race on the relation between adiponectin and different variables including adiposity anthropometric measures, and cardiometabolic variables (insulin level, insulin resistance, blood sugar and blood pressure).

2. Subjects and methods:

A cross-sectional survey, comprised 3708 adolescents (1779 boys (48%) and 1929 girls (52%)), aged from 12 to 18 years (±6 months) was conducted by the National Research Centre, Egypt. The studied adolescence was recruited from two Preparatory and two Secondary public Schools situated in Giza governorate, during the period of October, 2007 to April 2009. Permission to perform the study was granted by the Ministry of Education, and the directors of the school included in the research. Parents were informed about the purpose of the study and their permission in the form of written consent was obtained. Another assent was taken from the students to be involved in this research. The protocol was approved by the "Ethical Committee" of the "National Research Centre".

Of the total sample, three hundred and forty students (9.2%); who were diagnosed as having obesity, as they fulfilled the inclusion criteria which are BMI greater than the 95th percentile for age and gender based on the Egyptian Growth Reference Charts 2002 [20], were included. They were 144 boys (8.1%) and 196 girls (10.2%); with mean age of 14.36±1.66 years. Subjects were excluded if they had a prior major illness, including type 1 or 2 diabetes, received medications or had a condition known to influence body composition, insulin action or insulin secretion (e.g. glucocorticoid therapy, hypothyroidism and Cushing's disease). Informed consent and assent were obtained from 180 students and their parents who accepted to share in the laboratory data.

Each student underwent a complete physical examination, including anthropometric measures. The height and weight were measured. The height was measured to the nearest 0.5 cm using a Holtain portable anthropometer, and the weight was determined to the nearest 0.01 kg using a Seca scale Balance with the subject dressed minimum clothes and no shoes. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference **(WC)** was measured at the level of the umbilicus with the subject standing and breathing normally, hip circumference **(Hip C)** at the level of the iliac crest, using non-stretchable plastic tape to the nearest 0.1 cm. Waist/hip ratio **(WHR)** was calculated (cm/cm). Each measurement was taken as the mean of three consecutive measurements, using standardized equipments and following the recommendations of International Biological programmes [21]. Blood pressure was measured with a standard mercury sphygmomanometer after the subjects had rested at least 10 min. Systolic blood pressure was recorded at the appearance of sounds, and the diastolic blood pressure was recorded at the disappearance of sounds. Fasting venous blood samples were collected into plain tubes using standard venipuncture aseptic technique. The samples were allowed to clot and sera were separated by centrifugation and stored in aliquots at - 80º until assays. Fasting blood glucose was measured using quantitative enzymatic colorimetric commercial kit provided by Stanbio according to glucose oxidase method [22]. Fasting serum insulin and adiponectin levels where measured using commercially available enzyme linked immunosorbent (ELISA) assay kits, provided by DRG Instruments GmbH, Germany and AviBion Orgenium Laboratories, Finland respectively. **HOMA-IR** (The homeostatic model assessment for insulin resistance) was calculated:

HOMA-IR= fasting insulin concentration (µU/mL) x fasting glucose concentration (mmol/L)/22.5.

Statistical analysis

Data were expressed as mean ± S.D. BMI was expressed in terms of standard deviation score (Z-score), using the standard growth curves for Egyptian children and adolescents 2002 as reference population [20]. Student's unpaired t test was used to examine the sex differences. Pearson's correlation coefficients were used to assess the relationships between independent variables. The level of significance was set at a probability of less than 5% ($p<0.05$).All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS/Windows Version 9.05, SPSS Inc., Chicago, IL, USA).

3. Results:

The prevalence of obesity among 3708 adolescents' students (1779 boys and 1929 girls), aged from 12 to 18 years was 9.2% (8.1% in boys and 10.2% in girls); their mean age was 14.36±1.66 years. However, the prevalence of overweight (those with BMI between 85th and 95th percentile for age and gender based on the Egyptian Growth Reference Charts 2002 [20]), was 11.4%; 9.9% in boys and 12.6% in girls (table 1).

The clinical characteristics of the obese adolescents are provided in Table 2. A highly significant sex difference was observed in BMI-Z score, and significant differences in systolic and diastolic blood pressure, where the girls had higher values more than the boys. In contrast, boys were highly significant taller and had significant wider WC and higher values of fasting plasma glucose than the girls. Serum adiponectin level was low for both sexes compared to the kit reference range. However, there are insignificant sex differences in the age, weight, hip C, WHR, and the other laboratory data: fasting adiponectin, insulin level and HOMA-IR.

Correlations between adiponectin and the anthropometric data, blood pressure and laboratory data for the total sample are presented in table 3. Inspite of the finding that serum adiponectin level was low compared to the kit reference range, it showed insignificant correlations with the anthropometric parameters under study; both the systolic and diastolic blood pressure, and the other laboratory data; fasting plasma glucose, insulin level and HOMA-IR. The same findings were observed when the correlations were repeated for each sex separately.

Table 1: Prevalence of obesity and overweight in the studied adolescents

Sex	N	Obese		Overweight	
		N	%	N	%
Boys	1779	**144**	**8.1**	**177**	**9.9**
Girls	1929	**196**	**10.2**	**244**	**12.6**
Total	3708	**340**	**9.2**	**421**	**11.4**

Table (2): Comparisons between anthropometric and laboratory data of the obese adolescents by sex

	Boys (N = 82)		**Girls (N= 98)**		
	Mean	SD	Mean	SD	*P*
Age	14.19	1.23	14.27	1.87	NS
WEIGHT(Kg)	84.33	13.12	85.19	11.39	NS
Height(cm)	162.71	9.90	159.28	6.58	0.008
BMI-Z Score	2.51	0.62	3.21	0.96	0.000
WC (cm)	100.12	14.35	95.16	18.11	0.048
Hip C (cm)	110.93	9.86	110.88	18.10	NS
WHR (cm/cm)	0.90	0.12	0.88	0.27	NS
SBP(mm Hg)	114.76	12.76	119.06	13.74	0.034
DBP (mm Hg)	75.85	8.88	82.09	14.01	0.013
Lab.:					
Adiponectin (µg/L)	1.45	0.26	1.48	0.29	NS
Fasting glucose (mg/dl)	98.42	14.72	91.85	15.65	0.004
Fasting insulin(µU/ml)	10.52	8.18	10.58	9.04	NS
HOMA-IR (µU/mL)/ (mmol/L)	2.46	1.90	2.71	1.96	NS

Table (3): Correlations between adiponectin and the anthropometric data, blood pressure and laboratory data for the total sample

		Adiponectin
Age	r	- 0.007
	p	0.921
Weight	r	0.004
	p	0.959
Height	r	-0.015
	p	0.842
BMI-Z Score	r	0.004
	p	0.957
WC	r	-0.033
	p	0.661
Hip C	r	-0.069
	p	0.367
WHR	r	0.018
	p	0.808
SBP	r	0.046
	p	0.549
DBP	r	0.029
	p	0.707
Fasting glucose	r	-0.141
	p	0.058
Fasting insulin	r	0.043
	p	0.567
HOMA-IR	r	0.008
	p	0.922

4. Discussion:

The prevalence of obesity, and metabolic syndrome (MS) and its associated risk factors in children and adolescents have increased dramatically over decades [4]. Adipokines leptin and adiponectin are proposed as biomarkers in children for predicting MS, Type 2 diabetes, or cardiovascular disease [23].

In Youth, investigators could emphasize the role played by adiponectin in the development of insulin resistance, hypersinsulinemia, dyslipidemia, and metabolic syndrome, which make adiponectin an attractive therapeutic target for obesity-related conditions [24, 6]. In general serum adiponectin levels were found to be lower in obese children than in non-obese children, however, still little is known about change in adiponectin plasma levels during puberty [25].

In the light of evidence showing the important role of adiponectin and the possible ethnic difference in its level, the present cross sectional study done on 180 obese African Egyptian school adolescence, showed that adiponectin level were found to be low compared to the reference range. Other studies involving different age groups prepubertal [13], adolescents [15], and adults [14] proved that adiponectin level is lower in normal-weight African-Americans compared with Caucasian peers. The mechanism(s) responsible for the lower adiponectin levels in African population remain to be undetermined [12].

Furthermore this study gave results that could not declare correlation between adiponectin and adiposity anthropometric measures (BMI, waist circumference, hip circumference and waist/hip ratio), or the metabolic variables (fasting insulin concentrations, insulin resistance and fasting blood glucose level). This goes in concordance with that of, Lee et al. [12] who did not demonstrate any correlations between plasma adiponectin, BMI, insulin concentrations, and insulin resistance in African Americans when compared with the Caucasians peers who showed statistically significant negative correlations between adiponectin and any of the above parameters. An equally intriguing finding was that, in Indian teenagers or adults' adiponectin did not correlate directly with measures of insulin sensitivity, overweight, and other cardiometabolic variables [26]. Also in pubertal Spanish children, adiponectin was weakly related to anthropometric variables (weight, BMI, WC and HC) and was not correlated with body fat. Whether these different findings can be attributable to the ethenic differences, is unknown and warrants further investigation [27].

However the present results were at variance with several publications showing inverse relationship between plasma adiponectin concentrations and both adiposity markers and fasting insulin levels in both adults and children [7, 25]. Studies in other populations including Pima Indian, Hispanic, and Asian-American children showed positive correlation of adiponectin with glucose metabolism and negative correlation of adiponectin with fasting insulin level, insulin resistance, proinsulin, and BMI [9,28] . Also a significant inverse relationship between plasma adiponectin concentrations and BMI was noted in Taiwanese school children >10 years old [7]. Kettaneh et al., [29] noted that adiponectin in Indian teenagers correlated with waist circumference and BMI, and insulin resistance in boys but not in girls and they concluded that these correlations with adiponectin display a sex-specific picture throughout puberty.

It is interesting to note that although it is generally accepted that hypoadiponectinemia is a novel predictor of hypertension [30], the present study, could not prove that serum adiponectin levels correlate with systolic or diastolic blood pressure (SBP or DBP) in obese adolescence which is consistent with previous reports done on obese boys showing also lack of correlation between adiponectin and either SBP or DBP [31]. Lambert et al., [32] stated that although animal studies support the role of leptin

and adiponectin in controlling BP, they are not independently associated with BP in youth.

In contrary other cross-sectional studies, reported independent association between hypoadiponectinemia and hypertension [33, 34]. This was confirmed by Chow et al. [35] who demonstrated, that hypoadiponectinemia predict the development of hypertension on long-term follow-up of the normotensive subjects, independent of the effects of known risk factors of hypertension, including sex, age, and BMI. Other clinical studies showed that the association between adiponectin and hypertension is evident, and that hypoadiponectinemia is a risk factor for hypertension independent of insulin resistance and diabetes [36].

Ohashi and his colleagues [37] suggested that hypoadiponectinemia obesity-related hypertension, may be due to its direct effect, in addition to its effect via insulin resistance, and that adiponectin therapy can be potentially useful for hypertension in patients with the metabolic syndrome .

Nevertheless, very few studies address the relationship between adiponectin and hypertension at a mechanistic level. So, further studies on the relationship between adiponectin and childhood hypertension will be needed [37].

5. Conclusion:

In the studied African Egyptian obese adolescence, although serum adiponectin level was low, but there were no direct link between adiponectin and the selected anthropometric measures, fasting insulin level, insulin resistance, blood glucose concentration and blood pressure. Which may provide additional support for the notion that what applies to other ethnic populations might not apply to the African population. This finding may spark future research to establish the relationship among adiponectin and different variables in African population.

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Memantine decreases apoptosis and attenuates the activation of caspase-3 and MDA release in rats with ischemia-reperfusion injury

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Abstract: To investigate the effects of memantine on neuron apoptosis and the expression of Caspase-3 and malonaldehyde (MDA) during cerebral ischemia-reperfusion injury in rat. 135 male Wistar rats were randomly divided into 3 groups: sham operation group, cerebral ischemia-reperfusion model group and memantine intervention group. The changes of cell morphology and the expression of caspase-3 in cerebral cortex neurons at 12h, 24h, and 48h after ischemia-reperfusion were observed by Haematoxylin Eosin (HE) and immunohistochemistry staining respectively. The expression of caspase-3 activity and MDA levels at different time points were detected by spectrophotometer. Meanwhile, the apoptosis in situ in the CA1 region of hippocampus of the rats were investigated with TdT-mediated dUTP nicked labeling (TUNEL) method. Results show that in memantine intervention group, the expression levels of caspase-3 and MDA in ischemia-reperfusion injury region increased, in comparison with sham-operated group (p=0.00), while lower than that of cerebral ischemia-reperfusion model group (p =0.00). Caspase-3 activity remarkably increased in ischemia-reperfusion brain in rats in a time-depended manner. The number of TUNEL positive cells in the CA1 region of hippocampus in the memantine treated rats (7.00±2.04) and model rats (11.57±2.64) were significantly increased compared with the sham operation controls (1.57±4.72) (p =0.00), while the number of TUNEL positive cells in the memantine treated rats decreased as compared with that of the model rats (p =0.00). Suggesting that mamantine may probably have the function of neuroprotection in rats with cerebral ischemia-reperfusion injury by suppressing the expression of caspase-3 activity and MDA and inhibiting the apoptosis of pyramidal neurons in the CA1 region of hippocampus in ischemia-reperfusion rats.
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Key words: Mamantine; cerebral ischemia-reperfusion; Caspase-3; MDA; apoptosis

1. Introduction

As one of a leading cause of death in the world, stoke has severely been threatening people's health, with high incidence, prevalence and mortality rates that increased with age. Recently, scientists paid more attention to the ischemia-reperfusion damage, which increased with the application of thrombolysis and interventional techniques for the treatment of acute ischemic stroke. The pathophysiological mechanisms of transient cerebral ischemia are different from that of permanent cerebral ischemia. In the early cerebral ischemia period, the restoration of oxygen and glucose supply may aggravate cerebral injury which occurs in the ischemic penumbra area (Kuroiwa et al, 1988).

It is almost impossible to reverse the harmful outcome of the primary impact on the neural tissue by medical or surgical means. The main target of medical treatment is considered on prevention of secondary injury, which mainly concentrated on the sub-cellular level of organization additional injury death of the peripheral zone caused by the initial damage. (Özsüer et al, 2005). The aim of neuroprotection is to prevent delayed neuron apoptosis in the zone of the ischemic penumbra (Muir, 2002 and Culmsee et al, 2004).

As a nerve protectant, memantine (1-amino-3, 5-dimethyladamantane) is an uncompetive N-methyl-D-aspartate (NMDA) receptor antagonist. In most clinical practice, memantine has been frequently used for the treatment of Parkinson's disease, senile dementia, spastic diseases and viral infectious diseases without serious side effects (Dogan et al, 1999). For the treatment of ischemic stroke, plenty of studies mainly focused on inhibition of excitatory amino acids and calcium influx. While it was founded recently that cell apoptosis and lipid peroxidation may be the underlying mechanisms in ischemia-reperfusion damage (Ikeda and Long, 1990).

As the degradation product of MDA was considered to reflect the degree of lipid peroxidation, and the apoptosis of cells are closely related to caspase-3 reactivity, in this study we investigated the effect of memantine on caspase-3 activity, MDA release and the apoptosis of pyramidal neurons in the CA1 region of hippocampus in rats with cerebral ischemia-reperfusion injury to explore the possible brain protection function(s) of memantine.

2. Material and Methods

Animal treatments: All animal experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986(86/609/EEC). One hundred and thirty-five, 260-280g weights, male Wister rats were bought from the Laboratory Animal Center of Zhengzhou University. Outcome assessments were made by investigators blinded to the experimental group. All experimental procedures have been done according to the regulations for the administration of affairs concerning experimental animals, approved by laboratory animal management and ethical committee of Zhengzhou University.

Main instruments and reagents: WFZ UV-2000 UV visible spectrophotometer(Yu nika, China); micro camera system (Lecia, Germany); analytical system (Biosens Digital Imaging System v1.6, China); Caspase-3 immunohistochemical antibody (Epitomics, U.S.A.); Caspase-3, MDA activity detection kit, TUNEL detection kit (Germany); Bradford protein concentration determination kit (Beyotime, China); Memantine hydrochloride tablet (Lundbeck A/S, Bafch number: 843101, Each 10 mg H., Denmark).

Grouping and model preparation: All rats were randomly divided into three groups: sham operation group, ischemia-reperfusion model group and memantine intervention group (n=45). Middle cerebral artery (MCA) occlusion was performed on rats in ischemia-reperfusion group and memantine intervention group using an intraluminal thread embolism method as described by Zea Long et al (Longa et al, 1989). After 90 minutes of cerebral ischemia, pull out the filament slowly. The successful cerebral-reperfusion model is that animals have left Honer sign and hemiplegia on right limbs when awaked. In sham group, animals were only operated by isolating blood vessels, without thread embolism. Rats in memantine intervention group were given 20 mg/kg/d dose of amantine through a gastric tube immediately after cerebral ischemia-reperfusion and 24h later. In contrast, rats in the other two groups received physiological saline of the same volume.

HE staining and immunohistochemistry: After 12 h, 24 h and 48 h ischemia-reperfusion, 5 rats in each group were randomly selected and killed. Then perform 4% paraformaldehyde perfusion and fixation followed by ethanol dehydration, xylene and embedding, coronal slices as thin as 5μm for HE and immunohistochemistry staining.

The expression of caspase-3 in cerebral cortex of was detected by SP immunohistochemical staining, strictly in accordance with the instruction in kit. The apoptosis of hippocampal cells were investigated in situ in the CA1 region of hippocampus of the rats by TdT-mediated dUTP nicked labeling (TUNEL), 48 h after cerebral ischemia-reperfusion (n=5).

Image Analysis: The average integrated optical density of the positive area was analyzed by Lecia microscope camera system and Biosens Diosens Digital Imaging System v1.6 analysis system. Five visions in the same area of each slice were randomly selected and tested, then the average integrated optical density of the positive area of the target area were calculated.

MDA, Caspase-3 Levels in hemisphere cortex homogenate: 12 h, 24 h and 48 h after ischemia-reperfusion, another 5 rats in each group were randomly picked out and taken the left cerebral hemisphere cortex homogenate. The levels of MDA, Caspase-3 of cerebral tissue were assessed respectively in accordance with operating instructions in MDA, Caspase-3 detection kits and Bradford protein concentration assay kit.

Statistical Analysis: All data were expressed as the mean ± SD and were analyzed by a repeated measures analysis of variance (ANOVA) followed by contrasts in repeated measures design and Student's t test, and LSD method with a comparison of between two groups with SPSS 13.0.

3. Results

HE staining of rat cortical neurons

The neurons in rat brain tissue of sham operation group were well arranged, uniform and neat. Nuclei were round or oval, chromatin was uniform; While the size of neurons in rat brain of model group were relatively large, the nucleoli were disappeared and empty halo was found around the cytoplasm; In the memantine intervention group the normal neurons were less than that of sham group, but more than that of model groups (Figure 1).

Caspase-3 positive area integrated optical density of cortex by immunohistochemistry

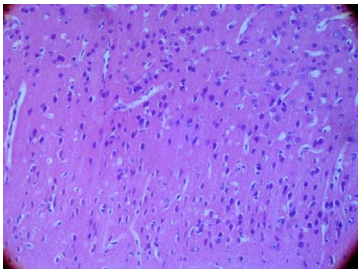
Caspase-3 positive cells appeared brown granules within the cytoplasm. The color of Caspase-3 positive cells cytoplasm in model group was most deep, brownish yellow. The density of Caspase-3 positive cells in memantine intervention group was less than that of model group, but higher than that of sham group. There were significant differences in density. At different time points the Caspase-3 positive area average integral optical density of intervention group was significantly reduced compared with that of ischemia group (p <0.05), but significantly increased than that of sham group (p <0.01) (Table 1).

MDA, Caspase-3 Levels of Cortex homogenate

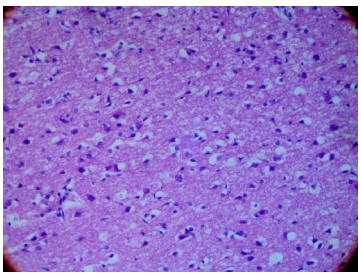
At different time points the Caspase-3 level and MDA activity unit generated content of cortex homogenate in Memantine treatment group were significantly reduced compared with that of model group respectively (P<0.05), but significantly increased

than that of sham group(P <0.01) (Table 2, Table 3).

A



B



C

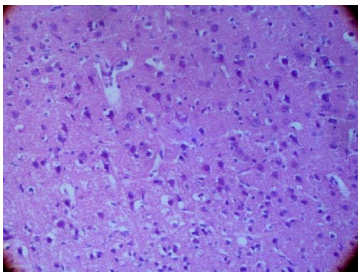


Figure 1 HE staining of rat cortical neurons(48h): A.. Sham group; B. model group; C. Intervention group

TUNEL positive cell in CA1 region of hippocampus

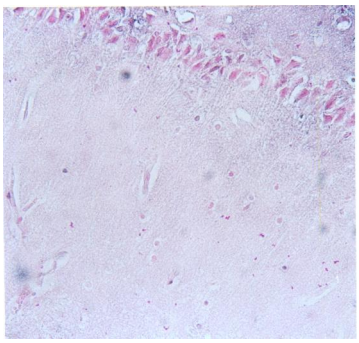
The number of TUNEL positive cells in the CA1 region of hippocampus in the memantine treated rats (7.00±2.04, figure 2C) and model rats (11.57±2.64, figure 2B)were significantly increased compared with the rats in sham operation control group (1.57±4.72, figure 2A) (P <0.01) , while the number of TUNEL positive cells in the memantine treated rats was decreased as compared with that of the model rats (P <0.05) (Figure 2).

Table 1. The caspase-3 positive area integrated optical density of cerebral cortex at different time points ($\bar{x} \pm s$)

group	Sham operation (n=10)	Memantine (n=10)	Model (n=10)
12 h	96.07±5.08	125.77±7.94※▲	134.28±10.00※
24 h	98.51±3.41	139.92±6.69※▲	147.36±5.35※
48 h	97.38±3.48	145.14±5.24※▲	151.36±4.15※

Note：compared with sham operation group $P<0.01$；compared with model group ▲$p<0.05$，△$P<0.01$

A



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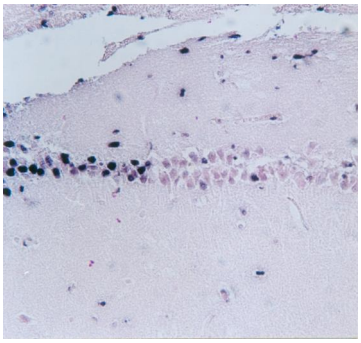

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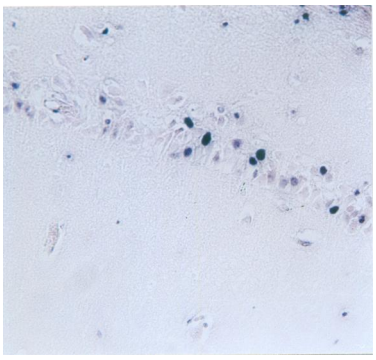

Figure 2. The apoptosis of hippocampal cells in situ in the CA1 region of the rats by TUNEL(48h): A., Sham group; B. model group; C. Intervention group

Table 2 The Caspase-3 expression enzyme activity unit of cerebral cortex at different time points (U/mg, $\bar{x} \pm s$)

group	Sham operation (n=5)	Memantine (n=5)	Model (n=5)
12 h	0.175±0.062	0.289±0.013※▲	0.397±0.0215※
24 h	0.175±0.063	0.454±0.045※▲	0.520±0.043※
48 h	0.176±0.068	0.523±0.016※▲	0.595±0.049※

Note: compared with sham operation ※$P<0.01$；compared with model ▲$P<0.05$，△$P<0.01$

Table 3 The expression of MDA of cerebral cortex at different time points ($\mu mol/mg, \bar{x} \pm s$)

group	Sham operation (n=5)	Memantine (n=5)	Model (n=5)
12 h	0.047±0.007	0.394±0.025※▲	0.440±0.033※
24 h	0.049±0.006	0.664±0.079※▲	0.850±0.080※
48 h	0.049±0.008	0.469±0.037※▲	0.591±0.033※

Note：compared with the sham operation group※ $P<0.01$；compared with model group ▲$P<0.05$，△$P<0.01$

4. Discussion

Reperfusion after a certain period of cerebral ischemia may aggravate the progression of cerebral damage. During ischemia-reperfusion injury process, oxidative stress occurs in cortical neurons, resulting in a large number of oxygen free radicals, which contribute to a large number of excitatory amino acids. It has been shown that there was a linear correlation between the severity of ischemic damage and the amounts of glutamate (Rothman et al, 1986). In addition, intracellular calcium overload leading to harmful signal transduction pathway activated, that eventually cause neuronal death and delayed apoptosis. NMDA receptor blockers are proved having the function of suppress neurotoxicity damage caused by excitatory amino acids (Blanpied et al, 2005, Davis et al, 2000 and Lees et al, 2001). One of the underlying mechanisms for the neuroprotection function of memantine lies in blocking of Ca^{++} influx through the NMDA-operated Ca channel (Dogan et al, 1999). Apoptosis develops in a certain period of ischemia while excitotoxic cell damage arises within a few minutes, and accumulated in over subsequent hours (Krivonos OV et al, 2010). Neuronal death occurs in the core area of ischemia, while apoptosis occurs in ischemia peripheral areas. For apoptosis cells can be saved, rescuing apoptotic cells as therapeutic targets may become a reality. The aim of neuroprotection is to prevent delayed apoptosis in the zone of the ischemic penumbra (Dávalos et al, 2006 and Suslina et al, 2000). Ischemia-reperfusion brain injury induced neuron apoptosis. As a key enzyme involved in apoptosis, the activity of caspases can be inhibited to hinder apoptosis process. Among the protease family related to apoptosis, Caspase-3 plays a crucial role in the cascade reaction. The damage of ischemia reperfusion cell is also closely related to the degree of lipid peroxidation. MDA is a degradation product of lipid peroxidation, which can reflect the degree of neuronal lipid peroxidation. Thus, it is feasible to explore the potential protective effect of memantine on neurons by detecting caspase-3 activity and MDA release in cerebral ischemia-reperfusion injury rats.

In this study, we still use memantine at a dose of 20 mg/kg after ischemia-reperfusion injury, which has been previously showed it is the necessary dose to exhibit the significant protective of memantine after permanent focal cerebral ischemia (Krieglstein et al, 1997). NMDA receptors are widely distributed in the brain, especially densely distributed in hippocampus and cerebral cortex (Monaghan et al, 1985) that makes it possible for mematine to protect the cortical neurons. Our results showed that, during ischemia-reperfusion injury process, in memantine intervention group, the increase of expression level of caspase-3 in cortex neuron and MDA release in cortex homogenate were inhibited significantly on 12 h, 24 h, and 48 h time points, compared to that of rats in ischemia-reperfusion model group. Such results indicate that memantine can alleviate ischemia-reperfusion injury and has neuron protective function, which is supported by the recent studies that memantine can attenuate staurosporine-induced or isoflurane-induced neuronal apoptosis by inhibiting the expression of high activity of caspase-3 in mouse (Zhang et al, 2008 and Jantas-Skotniczna et al, 2006). In ischemia-reperfusion injury process, the main excitatory neurotransmitter in central nervous system, glutamate as well as aspartate, releases in large amounts, that resulting in excessive activation of NMDA receptors. Glutamate or aspartate binding to receptors induce excitation of postsynaptic neurons, which leading to followed excitatory postsynaptic potential and develop cell damage eventually. Excess excitatory neurotransmitters lead to over activation of NMDA receptors and the influx of calcium ions, and then activate the lipase and protease, that cause cell damage (Bormann et al, 1989 and Dogan et al, 1999). Memantine, as an uncompetive NMDA receptor antagonist, can inhibit the toxic effects of excitatory neurotransmitters (Matsumoto et al, 1996) and reduce lipid peroxidation levels after closed head trauma in rats (Özsüer et al, 2005). In our study, memantine can inhibit the caspase-3 activity expression and MDA release amounts, suggesting a new mechanism of neural protection effect of memantine to prevent delayed apoptosis.

In conclusion, memainte may suppress the Caspase-3 expression level and MDA release, thus inhibit the neuron apoptosis and lipid peroxidation in ischemia-reperfusion injury, as well as resist intracellular calcium overload and relieve neurotoxicity caused by excitatory neurotransmitter to play neural protection role in ischemia-reperfusion injury. Our study drives the further development of clinical application of uncompetive NMDA open channel blockers in the treatment of ischemia-reperfusion injury in brain. Further study is needed to explore such neural protective mechanisms in-depth.

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3/1/2011

Two Different Methods of Endovascular Treatment for Ruptured Intracranial Aneurysm Associated with Moyamoya Disease and Review of the Literature

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Abstract: The purpose of this study was to evaluate efficacy and feasibility of two different embolization methods for the treatment of intracranial aneurysm with moyamoya disease. Two intracranial aneurysms with moyamoya disease treated with coils embolization and glue embolization respectively between September 2006 and December 2010 were analyzed and the related literatures were reviewed as well. The two intracranial aneurysms were successfully embolized and no complication of endovascular therapy occurred. We think that endovascular treatment may be a safe and efficacious method for the intracranial aneurysm with moyamoya disease, if coil embolization is difficult for some aneurysm, glue emboliation may be a choice.
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Keywords: Endovascular treatment; intracranial aneurysm; moyamoya disease

1. Introduction

The incidence of intracranial aneurysm (ICA) in adult patients with moyamoya disease has been estimated at 3%–14% (Borota et al, 1996). In contrast to the general population, ruptured ICA in moyamoya disease patients has a poorer prognosis (Kwak et al, 1984). Therefore, prevention of rebleeding from ruptured moyamoya related-ICA is of importance. There are two distinct options of treatment for ruptured ICA associated with moyamoya disease: direct surgical clip and endovascular embolization. The former is a standard approach, however, which is often difficult due to complicated moyamoya-like vessels in the operative field (Michael et al, 2009), moreover, moyamoya- like vessels are fragile and easy to rupture (Nishlo et al, 2004). The latter can avoid damaging brain tissue and such vessels (Suzuki et al, 2006). We report our successful experience in the treatment of ruptured ICA associated with moyamoya disease using two endovascular methods (detachable coil embolization and "glue" embolization). To the best of our knowledge, this is the first case report introducing two different endovascular methods treating ruptured ICA associated with moyamoya disease simultaneously.

2. Material and Methods

Case 1

A 52-year-old man presented with severe headache and vomiting for eight hours. On admission, he was fully conscious without focal signs. His condition was categorized as Hunt and Hess grade 1. Head CT showed subarachnoid hemorrhage in suprasellar cistern and cisterna ambiens (Fig 1-A). Cerebral angiography performed on the same day demonstrated occlusion in the terminal portion of bilateral internal carotid artery with moyamoya vessels and a 3.5-mm saccular ICA located on the left P2 segment of posterior cerebral artery (Fig 1-B, C,D,G,H). Following induction of general anesthesia, a 6F sheath was inserted in the right femoral artery and a 6F guiding catheter (Envoy; Cordis USA) was placed in the right vertebral artery. The patient was heparinized to an activated clotting time of 250 to 300 seconds. An Excelsior 10 microcatheter (Target/Boston Scientific) was navigated into aneurysm lumen. The aneurysm was then embolized with GDC-10 (3mm×40mm, 2mm×20mm, 2mm×10mm Target/Boston Scientific USA). Angiography after coil embolization demonstrated occlusion of the body and dome of the aneurysm with excellent blood flow through left posterior cerebral artery. (Fig 1-E, F). No change in the patient's neurological status was noted after the procedure and recovered in good condition.

Case 2

A 20-year-old man who presented with complaints of sudden, severe headache, nausea, and vomiting was transferred to our hospital after a diagnosis of lateral cerebral ventricle hemorrhage at an outside emergency department. He was fully conscious with left hemiparesis and was categorized as Hunt and

Hess grade 2. Head CT revealed right lateral cerebral ventricle hemorrhage (Fig 2-A). cerebral angiography revealed severe stenosis of bilateral internal carotid artery with moyamoya vessels and a 3-mm aneurysm located in a branch of right lateral posterior choroidal artery (LPChA) (Fig 2-B, C). On the day after the occurrence of bleeding, endovascular treatment was administered while the patient was under general anesthesia. Using the above method, an Excelsior 10 microcatheter was placed in the right lateral posterior choroidal artery over a 0.010-inch Transcend EX microguidewire (Target/Boston Scientific USA); however, the microcatheter could not catheterize the parent branching vessel. Then this vessel was catheterized by using a 1.3 F floating microcatheter (Marathon, ev3 USA). Superselective angiogram confirmed the ICA (Fig 2-D). 0.5 mL of a 7:1 mixture (at concentration of 12.5%) of iodized oil (Cordis Neurovascular) and cyanoacrylate glue (Glubran 2, GEM Italy) was injected through the microcatheter for 5 seconds under roadmap. Filling both the aneurysm and the adjacent parent artery with the cyanoacrylate glue, the microcatheter was then aspirated and quickly withdrawn. A control left vertebral artery angiogram demonstrated complete embolization of the aneurysm and the parent vessel (Fig 2-E). The patient remained neurologically unchanged and recovered in good condition.

3. Discussions

About half of adult patients with moyamoya disease develop intracranial bleeding, due to rupture of moyamoya-like vessels or ICA (Satoshi et al, 2008). Currently, the formation of a moyamoya-related ICA is generally believed to be result of the increased wall stress due to high flow imposed by collateral circulation or anomalous arteriovenous shunt (Dietrichs et al, 1992).

In the general population, almost 5–10% ICA is located in the posterior cerebral circulation, whereas in moyamoya disease patients, this proportion is 43.3% (Murakami et al, 2004). In moyamoya disease, occlusive or serious stenosis of distal segment of internal carotid artery and proximal segment of middle cerebral artery may decrease the flow dynamics across anterior cerebral circulation which increase flow through posterior cerebral circulation and originate turbulence that increase possibility of ICA formation and rupture. In this report, both ICAs located in the posterior cerebral circulation.

There are two treatment options available to moyamoya-related ICA: craniotomy with clip ligation (clipping) and endovascular embolization with detachable coils (coiling) or liquid embolic agent (glue) (Murakami et al, 2004) (Kuroda et al, 2001). Clipping is often difficult due to complicated and fragile moyamoya-like vessels in the operative field, moreover, surgical treatment in posterior cranial fossa are relatively complicated and dangerous (Kuroda et al, 2001). Detachable coils are now widely used to treat ICA which is a minimally invasive therapeutic approach, avoiding patients some of the hazards associated with craniotomy and surgical clipping. Therefore, endovascular treatment may be particularly suitable for moyamoya related ICA (Burns et al, 2009).

However, peripheral artery aneurysms commonly distribute in deep brain and the parent arteries are diffusely narrowed. For this reason, microcatheter is usually unable to reach aneurismal lumen; this means that the aneurysm could not been embolized with coils. Under the circumstance, liquid embolic agent embolization may be a worthy choice.

Systematic review of the literature was performed via PubMed search (key words: Moyamoya disease, aneurysm, glue), with careful manual review of the references from relevant articles. As expected articles retrieved represented only six intracranial aneurysms of six patients with Moyamoya disease treated by using liquid embolic agent embolization (Weigele et al, 2002) (kim et al, 2009) (Murakami et al, 2004) (Yu et al, 2010). The above aneurysms were all peripheral artery aneurysms. The embolic materials were NBCA or glubran 2 which belong to adhesive glue. NBCA (N-butyl-Cyanoacrylate) is a monomer acrylic glue which polymerizes on contact with blood, and subsequently causes a permanent occlusion. Glubran 2 is an acrylic glue authorized for use in surgical and endovascular procedures. The glue needs to be mixed with lipiodol before use to enable its fluoroscopic visualization. The co-monomer of Glubran 2 is comprised of a monomer of NBCA and a monomer of MS (owned by GEM). MS allows the monomer of NBCA to polymerize with a smaller exothermic reaction (45℃) and a slightly longer polymerization time. Compared with the monomer NBCA, Glubran 2 is associated with a lower risk of adherence of the catheter to the tissue, allowing a greater ease of use (Leonardi, et al, 2002) (Yakes et al, 1997). Therefore, the aneurysm can be embolized with glue (glubran 2) at appropriate concentration. Before glue embolization, deep penetration of microcatheter is needed. In the second case, we not only accessed the microcatheter near the neck of aneurysm, but also injected low concentration of glue to make the glue to reach the aneurysm avoiding occlusion of the parent vessel only. Maekawa et al reported a ruptured ICA on lateral posterior choroidal artery associated with moyamoya disease embolized with NBCA, but the patient emerged a large hemispheric infarct that extended well beyond the typical LPChA territory, the authors found that the reason of complication was the parent artery provided collateral hemispheric blood

flow (Maekawa et al, 1999). Weigele et al using NBCA embolized two aneurysms on LPChA of two patients without moyamoya disease, both patients got optimal outcome (Weigele et al, 2002).

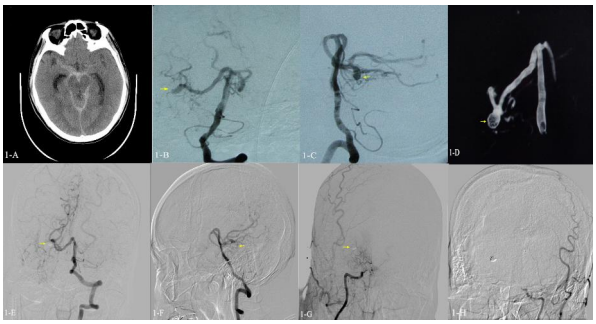

Fig 1-A: CT scan shows subarachnoid hemorrhage in suprasellar cistern and cisterna ambiens, 1-B, C, D: Right vertebral angiograms shows a 3-mm saccular aneurysm at left P2 segment of posterior cerebral artery (1-B: Frontal view, 1-C: Lateral view, 1-D: 3D reconstruction view); 1-E, F: Right vertebral angiogram shows occlusion of aneurysm with coils (1-E: Frontal view, 1-F: Lateral view), 1-G,H: carotid angiograms shows obstruction at the terminal portion of internal carotid artery and moyamoya vessels. (Arrow point to aneurysm).

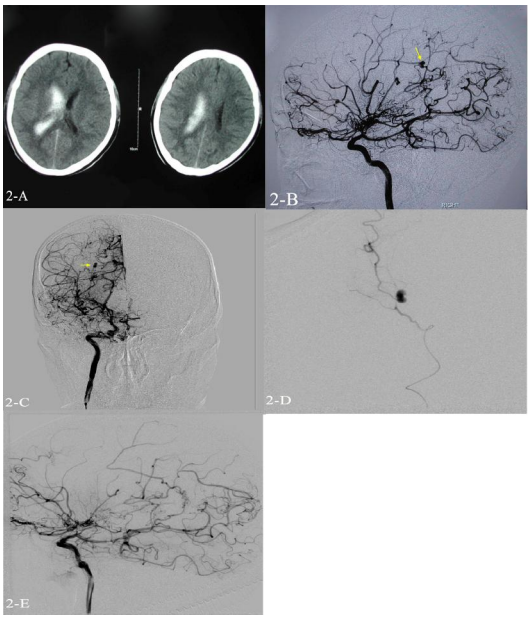

Fig 2-A: CT scan shows right intraventricular hemorrhage; 2-B, C: Left vertebral angiograms shows a 3-mm aneurysm arising from the distal portion of right lateral posterior choroidal artery (1-B: Lateral view, 1-C: Frontal view); 2-D: Superselective angiogram shows a 3-mm aneurysm, 2-E: Left vertebral angiograms shows complete obliteration of the aneurysm. (Arrow point to aneurysm).

We do not recommend onyx embolization for such aneurysm, which is another kind of liquid embolic agent. In comparison with NBCA and glubran 2, the diffusion ability of onyx is relatively poor. Onyx may only occlude the parent vessel and could not embolize the aneurysm when the tip of microcatheter could not be placed in the lumen and was in the parent vessel.

Conclusion

The endovascular embolization of intracranial aneurysm associated with moyamoya disease is a reasonable and effective treatment. If peripheral artery aneurysms associated with moyamoya disease can not be embolized with detachable coils, “glue” embolization may be an alternative method.

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1/14/2011

How the villagers participate in Participatory Rural Appraisal (PRA)

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Abstract: Much of the spread of participatory rural appraisal (PRA) as an emerging family of approaches and methods has been lateral, South-South, through experiential learning and changes in behavior, with different local applications. Rapid spread has made quality assurance a concern, with dangers from "instant fashion", rushing, formalism and ruts. Promising potentials include farmers' own farming systems research, alternatives to questionnaire surveys, monitoring, evaluation and lateral spread by local people, empowerment of the poorer and weaker, and policy review. Changes in personal behavior and attitudes, and in organizational cultures, are implied. PRA parallels and resonates with paradigm shifts in the social and natural sciences, business management, and development thinking, supporting decentralization, local diversity, and personal responsibility.
[Mohammad Abedi, Sharareh Khodamoradi. How the villagers participate in Participatory Rural Appraisal (PRA). Life Science Journal. 2011;8(2):290-294] (ISSN:1097-8135). http://www.lifesciencesite.com.

Keywords: Participatory Rural Appraisal (PRA), participation

Introduction:

There exist different methods of data collection and analysis, each with its own strengths and weaknesses. Through time, more appropriate and refined methods have been developed. In the context of rural development, information regarding the communities, their livelihoods, their beliefs, the physical environment in which they live, and their resource endowments need to be gathered and interpreted in a manner that identifies their priorities with a view of developing better understanding of their status and designing appropriate intervention projects directed at resolving their problems. The different ways of data collection and interpretation can be seen under two perspectives(IUCN, 2001): qualitative versus quantitative, and participatory versus top down. While the quantitative methods generate information that can be captured numerically, the qualitative methods generally do not generate specific numbers. Qualitative methods are concerned with exploring meanings, processes, reasons, and explanations(lnglis, 1992).

RRA was criticized for being extractive and highly dependent on expert interpretation. It was thus found useful to replace it with PRA which involves a process of learning from, with and by rural people about rural conditions. PRA shares much with its parent, RRA, but is distinguished from it in practice by correcting two common errors: roles of investigation are reversed; and rushing is replaced by relaxation and rapport. At the heart of all these developments was Robert Chambers, although Paulo Friere has also had strong influence especially in similar developments in education circles (Provention Concertium).

PRA techniques(Gibson, 1992**):**

The most common methods are the following:

1- Diagramming, Mapping and Modeling:
- transects
- maps (resource, social, farm)
- venn diagrams
- seasonally analysis
- historical analysis (time lines, trend lines, activity profiles)

2- Ranking and scoring
- pair wise ranking
- matrix ranking
- matrix scoring
- well-being analysis and wealth ranking
- proportional piling
- pie charts (injera charts)

3- Problem analysis
- identification and specification
- causal chaining
- prioritization

PRA has evolved and spread from beginnings in Ethiopia, India, Kenya, Sudan and elsewhere, and in early 1994 is known to be being quite widely practiced in parts of Bangladesh, Botswana, Ethiopia, francophone West Africa, India, Indonesia, Kenya, Nepal, Nigeria, Pakistan, the Philippines, Sri Lanka, Sudan, Uganda, Vietnam, and Zimbabwe, while starts have been made in at least a score of other countries in Latin America, Africa and Asia. Hundreds of nongovernment organizations

(NGOs) have adopted PRA and developed applications, as have a number of government departments. The use of PRA methods is being increasingly explored by students and faculty in universities for research, and by training institutes for fieldwork. Spread appears to be accelerating.

Five key principles that form the basis of any PRA activity:

1. **PARTICIPATION :**

PRA relies heavily on participation by the communities, as the method is designed to enable local people to be involved, not only as sources of information, but as partners with the PRA team in gathering and analyzing the information.

2. **FLEXIBILITY :**

The combination of techniques that is appropriate in a particular development context will be determined by such variables as the size and skill mix of the PRA team, the time and resources available, and the topic and location of the work(Dunn, 1991).

3. **TEAMWORK :**

Generally, a PRA is best conducted by a local team (speaking the local languages) with a few outsiders present, a significant representation of women, and a mix of sector specialists and social scientists, according to the topic.

4. **OPTIMAL IGNORANCE:**

To be efficient in terms of both time and money, PRA work intends to gather just enough information to make the necessary recommendations and decisions.

5. **SYSTEMATIC:**

As PRA-generated data is seldom conducive to statistical analysis (given its largely qualitative nature and relatively small sample size), alternative ways have been developed to ensure the validity and reliability of the findings. These include sampling based on approximate stratification of the community by geographic location or relative wealth, and cross-checking, that is using a number of techniques to investigate views on a single topic (including through a final community meeting to discuss the findings and correct inconsistencies).

PRA are good for:

• Providing basic information in situations where little in known
• Identifying and assessing problems
• Appraising, designing, implementing, monitoring, and evaluation programs and projects
• Getting a better picture of needs and organizations' ability to meet them
• Developing and transferring appropriate technologies
• Appraising emergencies
• Planning projects that are more relevant, restructuring administrations, assisting in decision-making and policy formation
• Generating hypotheses, ruling out inappropriate ones
• Providing guidelines for survey designs and assessing the applicability of their results to other places.
• Fleshing – out complementing, interpreting, or giving depth and context to information obtained through other methods.

PRA is not very useful for:

Working in situations in which the problem is not usefully addressed at the local or group level, for example, in situations where large-scale structural reorganization is necessary (but even then, local views may help to shape the change).

The objectives of the PRA are:

• to enable rural people to organize their knowledge, share experience among
themselves and gather information on resources they have
• to understand the rural environments and social as well as economic
dynamism
• to understand the trends in the rural socio economic conditions
• to enable the community identify their problems, causes of these problems and
possible solutions
• to enable the community develop a community action plan to address their
problems

In order to limit the PRA to the objectives set and to have consistency in conducting the PRA in the different villages, a PRA manual was prepared by the socio economic team. In line with the manual, emphasis was accorded to the following topics:

1) Village History. The first day of the PRA discussion begins with history of the village which enabled participants to easily and comfortably tell about the history of their village.

2) Agriculture and Livestock. Focus group discussions were made on agriculture and livestock rearing practices including the problems encountered and possible solutions.

3) Social service. The provision of social services like education and health including the associated

problems were also discussed in focus group discussions.

4) Village institutions. Institutions, both from within the village and outside, as well as formal and informal with which the rural communities interact have been addressed.

5) Trend lines. Trends in food availability, forest, population growth, wealth, rainfall and poverty are addressed in this section.

6) Wealth ranking, problem analysis, and community action plan. Finally, the participants ranked the community on the basis of its wealth, discussed the major problems and formulated action plan. The PRA is to be followed with a more quantitative and structured socioeconomic survey, which will then be followed by specialized researches in specifically selected areas; notably, poverty and coping mechanisms, microfinance, marketing, utilization and management of natural resources, and gender.

At the end of the 1980s, Participatory Rural Appraisal was developed in response to the too mechanistic and extractive implementation of RRAs. In PRAs the target group is encouraged to learn and the role of outsiders is reduced to a facilitator of the learning process. PRA aims to empower local people by encouraging them to share, enhance and analyse their knowledge of life and conditions and to plan, act, monitor and evaluate.

As with RRA it is hard to define what exactly a PRA is (some even prefer not to define it and just refer to "a family of approaches"). PRA shares the basic principles of RRA (quick, multidisciplinary, observations, etc.), yet now it is the local people who are encouraged to analyse their own situation and plan activities to improve it. The three basic pillars of PRA (and the basic differences from RRA) are:

1. the behaviour and attitude of outsiders, who facilitate rather than dominate;
2. the methods, which are open, group-oriented, visual and comparative;
3. sharing of information, food, experiences, etc. between in- and outsiders.

For the tools used, two issues stand out:

1. 'Handing over the stick': instead of outsiders trying to understand the knowledge of the local people, PRA tries to facilitate local people to develop their capabilities. They collect and analyse the data and propose actions to be undertaken.
2. Visualisation and sharing: local people convey their ideas and knowledge in a visual way. In verbal communication, outsiders dominate the dialogue more easily (via eye contact, cross-checking, etc.) than in communication via visual aids. When a map is drawn by a stick in the soil all can contribute, and local people feel more confident than when outsiders try to draw a map on a piece of paper with a pen - a typical tool of powerful outsiders. Sharing also explicitly involves the food and shelter during the PRA.

The most commonly used tools are:

- participatory mapping: a group of villagers makes a map of the community. The way they do this and what they find important provide good entry points for discussions about crucial aspects of village life;
- village transects: together with a (small) group of villagers the team walks through the village (or another relevant area) and discusses the things observed;
- ranking: people are asked to compare units (e.g. families /trees /crops) and to group them according to their own criteria. For example, via pair-wise comparing the importance of certain trees, people find out which criteria they use to assess the usefulness of these. Ranking is also used to stratify the local population, e.g. via wealth ranking. Both the results of the ranking and the criteria used provide entry points for further discussions.
- historical recalls: the lifestory of families are recalled and the main events are used as reference points in the analysis of the present situation;
- calendars: people indicate how things change over time, e.g. in which months they have to borrow money, when their children get malaria, when the rains are normally expected, etc.

Combining information obtained from all the tools provides the villagers with an explicit picture of their daily life. This not only helps them to start a discussion on their main problems and how to tackle them, it also boosts their self-esteem because they are able to make this analysis themselves.

Conclusion:

It is imperative that development activities/initiatives should not be attempted until participatory rural appraisal (PRA) or participatory action research (PAR) has been carried out and that the socio – economic and other factors affecting communities are well understood by the people confronted with the problem.

Kamla Bhasin (1999) suggests that development practitioners should constantly ask themselves: "am I increasing the confidence of the poor, their faith in themselves, and their self – reliance, or am I making them instruments of my own plans of action, imposing my own ideas on them and that of my organization and/or institution?" Social Development is a process of gradual change in which people increase their awareness of their own capabilities and common interests, and use this knowledge to analyse their needs; decide on solutions; organize themselves for cooperative

efforts; and mobilize their own human, financial and natural resources to improve, establish and maintain their own social services and institutions within the context of their own culture and their own political system. To give effect to this understanding of social development, participation of communities in their own development is important. The participatory approaches, including PRA provides first step/stage in sustainable community development.

As a result of the PRAs, the communities are expected to attain many benefits including:

• Expressing their own ideas and concerns;
• Organizing their knowledge about the past and present;
• Identifying as a community their problems, the causes of these problems and possible solutions;
• Developing a common plan to address these problems;
• Developing the ability to use their own resources more effectively and attract more resources from the outside.

The academicians/researchers involved in the PRAs are expected to get the following benefits:

• Developing better understanding of rural environments and social as well as economic dynamism taking place there;
• Appreciating the fact that communities are capable of analyzing their problems and outlining possible solutions to their problems;
• Participating in designing possible solutions to community problems;
• Utilizing the results of the PRA work as a research output for publications and presentations;
• Building their research and problem investigation capabilities;
• Supporting their classroom discussions to students with practical examples from the PRA findings.

The main objectives of the current PRA are:

1. empowerment of rural communities by assisting them to systematically utilize their local knowledge to identify problems and strengths, develop skills of analysis, and design appropriate mechanisms for intervention by themselves and/or by development agents;
2. advancement of understanding by academicians/researchers of local knowledge and acknowledgement of the capacity of communities to gather data, conduct analysis, and identify as well as prioritize problems and solutions;
3. utilization of the research questions/problems identified during the PRAs for further investigation;
4. documenting and presenting the outcomes of the PRAs to development agents (governmental and non-governmental) and other stakeholders so that they could undertake interventions in line with the findings.

PRA consists of a series of participatory exercises which help community members better assess their history, resources, and overall situation as concerns agriculture, health, marketing, credit, coping mechanisms, education, and other important areas.

During the conduct of the PRAs, rural communities in the selected villages will gather information on the resources they already possess; organize their knowledge; share experience among themselves; learn from each other; identify and prioritize local development needs; and develop action plans which respond to these needs.

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Information and communication technologies (ICT) and agricultural extension

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Abstract: Policy makers and service providers have increasingly come to view information and communication technologies (ICT), and particularly the Internet, as an important tool in providing disadvantaged groups and areas with access to information, services and markets that would otherwise be inaccessible. The concept of development of the rural, today, is not just project initiatives and governance; it is much more beyond that. This paper uncovers a whole plethora of ICT emergence as a technology of the new millennium. Against the backdrop of the ongoing ICT boom, this paper makes an attempt towards studying its applications and usage planning process and policy making for the rural communities focusing on how it helps in aligning the key factors and reduce the problems of alienation, fragmentation and dislocation of knowledge.
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Keywords: information and communication technologies (ICT), agricultural extension

Introduction:

Globalization and technological changes, the processes in the past fifteen years have been quickly lead to a new global economy have been driven with the reinforced technology and fuel (energy) that by providing information and knowledge.

The global economy requires the kind of necessity and purpose of educational institutions. Since the current trend towards reducing incomplete information and access to accurate information is growing, other schools can not control time to transfer a set of prescribed information from teacher to student during a fixed time point are, but schools must to promote Culture of "Teaching for Learning For example, acquisition of knowledge and continuous learning skills which make possible during the individual's life. According to Alvin Toffler, illiterate in 21st century, who was not read and write but those who do not know which fail to learn or remember are illiterate. (Jauhari, 2004).

In the rural context, development involves use of physical, financial and human resources for economic growth and social development of the rural economies (Burkey, 2000). The term rural development also represents improvement in quality of life of rural people in villages. As per Chambers (1983) “Rural Development is a strategy to enable a specific group of people, poor rural women and men, to gain for themselves and their children more of what they want and need.” Singh (1999) defines Rural Development as “A process leading to sustainable improvement in the quality of life of rural people, especially the poor”. The fact of the matter is that three quarters of the world’s poor, about 900 million people are in rural areas, and the Millennium poverty target set by Millennium Development Goals (MDG), cannot be met unless the world addresses rural poverty. “Sustainable Rural Development can make a powerful contribution to four critical goals of: Poverty Reduction, Wider shared growth, Household, national, and global food security and Sustainable natural resource management” (World Bank, 1997). Hence worldwide there is a growing emphasis on development of rural economy of the countries. Any improvement, in the social or economic status of rural areas would not just directly benefit rural poor but would also bring down the migration-pressures on cities and contribute by positive ripple effect in global stride towards development.

Institutions and experts accept Governance as a reflexive process, wherein policies, institutions, outcomes and analysis interact, to maximize the process of participatory development (UNDP, 1997; Ludden, 2005; Mehta, 2006).

Information and communication technologies (ICT), including radio and television and the newer digital technologies like computers and the Internet as potentially are introduced powerful tools and activators of educational reform and changes. different ICT, when properly applied can be developed to help access to education and the relationship between training and workshops to strengthen the increasingly digital, the quality of education also helped to create teaching and learning in an active process connected to real life high take. However, the experience of being raised by ICT in

the classroom and other educational sites around the world during the last few decades proves that is not automatic fully realize the potential benefits of ICT training. (Guptaand et al, 2004)

With the help of state and local funding, information technology has been purchased for schools ever since the 1980s. The state has also found many ways to support teacher training in the use of IT, and it has also allocated funds for the production of IT programs. Instruction in the use of IT has also played an important role in teacher training organized by local school authorities (Becker, 2000).

It is against this background that the need arose to find out how far we have progressed in the application of ICT in education and what impacts these significant economic investments have had. It is also time to start a value-oriented discussion of how strongly the future of the Iran society—and with it, of education and training— will be linked to the vision of an information society brimming over with technology (Mohseni, 2003).

The importance of communication in the development process has been acknowledged for many years by the development community. FAO has spent at least thirty years pioneering and promoting - both in thinking and practice - the centrality of communication in development. The most essential ingredient of good communication – putting people at the centre of the communication process - has similarly been understood and documented for many years.

agriculture extension and farmer-outreach programs face three major challenges – cost-effective outreach, solutions tailored to needs of individual farmers and an image that is farmer-friendly. The internet and mobile networks have the potential to provide agro-information services that are (i) affordable, (ii) relevant (timely and customized), (iii) searchable and (iv) up to date. Large sections of the farming community, particularly the rural folk, do not have access to the huge knowledge base acquired by agricultural universities, extension-centers and businesses. While telecenters are beginning to dot the rural landscape [1], one of the big barriers remains the lack of agro-content that (i) is in the language of the farmers (ii) is relevant to their needs and (iii) is delivered in a form that is of immediate use to them.

Information Technology, more precisely the Information and Communication Technology (ICT), has emerged world over as a technology of the new millennium. By augmenting the process of information exchange and reducing the transaction costs, this ubiquitous technology is instrumental in increasing productivity, efficiency, competitiveness and growth in all spheres of human activity. The potential benefits of, however, can be harnessed only if the technology diffuses across the different sectors of the society. Unfortunately, we are living in a world of 'digital divide' wherein half of the world population have never made a telephone call . The digital divide is not only an international problem, but for most developing nations including is also a national phenomenon. Nonetheless, it has been argued that in an era of globalization, the ability to harness this technology for the 'rural' improves the capability of the developing country.

Information technology (IT) has connected the world globally and is now changing our lifestyle and social consciousness dynamically. Of late, it has emerged as a best tool for information sharing and mutual communication. None of the walks of life have been left untouched by the IT sector be it grain threshing or global business. Agriculture has also been greatly influenced by IT in the present era though the share of IT in agriculture is only 1.3%.

Problems faced is that:

- The population of the earth is burgeoning every minute and there is sufficient evidence of impending food

crisis, especially in the developing countries even after attaining self-sufficiency.

- Even the major powers in the world are finding it difficult to balance the agricultural productivity with the

environmental requirement and meet the expectation of the millions round the world.

- The politics and economics in any country and the world trade mechanism are now dependent on the balance of supply and demand of the food.
- Inefficient recording and storage of data in spite of huge data collection.
- Lack of timely forecasting of weather and agriculture productivity.

In the current scenario, the role of IT assumes great importance and only with proper integration of IT with agriculture, the problem of food crisis can be solved and the world can move towards a sustainable production.

Integration of IT with agriculture must be done with following main objectives in mind:

- Develop multi-level decision support models for synergising the natural resource system with economic and social imperatives.
- To develop indicators of sustainability for agricultural production system.
- Based on the above scientific assessment, suggest alternatives to conserve and improve the health of natural resource system.

Two fundamental steps exist in establishing an innovation as a valuable, readily used tool: diffusion and adoption. Both diffusion and adoption must occur in order for an innovation to successfully reach its target user and be implemented (Mahajan, et al.). First, diffusion, the process by which an innovation "is communicated through certain channels over time among the members of a social system" (Rogers), must occur. In this study, the StratSoy project was a major factor in IT diffusion process in state soybean organizations. Other factors that influenced diffusion included the media, word-of-mouth, and experiences of friends, associates and family members.

In addition to individuals having access to a new technology, adoption must also occur, which means individuals accept the innovation as valuable and use it. Numerous factors could influence IT adoption and use in agricultural organizations and can be grouped into five categories: access to IT, demographic, IT training/education, trust, and time. It is possible for adoption factors to fit into more than one category.

In the case of IT, access to the technology means an individual must have access to a computer equipped with IT such as e-mail and access to the WWW. The category "access to IT" would not only include the use of a computer with IT ability, but would also include the ability to upgrade computer hardware and software to facilitate IT use. The price of needed computer equipment and the expense of Internet use are also related to access to IT. It is predicted that the higher the level of access to IT, the higher the level of IT use by an individual.

The demographic category includes adoption factors such as age, education level, gender, and income level. It is hypothesized that factors in the demographic category will not significantly influence IT adoption and use. Although previous literature suggests that IT use will be higher for younger, more educated individuals (Batte, et al.), 1997 survey results suggest that demographic factors have little influence on IT adoption and use. This may reflect that demographic factors may influence the decision to adopt a new technology, but once that decision to adopt is made, demographic factors may have little influence on use.

Another category of IT adoption factors is IT training/knowledge. This IT adoption factor can be measured with variables such as type of IT training, days of IT training, and the level of knowledge on IT use. It is hypothesized that as the quality and level of IT training increases, the use of IT will also likely increase.

An important factor influencing the adoption of any new technology is an individual's perception of that technology. It is hypothesized by this research that one of the key perception aspects influencing the adoption of IT is level of trust that potential adopter has in IT system and in those who use IT. Trust can be defined as "an individual's optimistic expectation about the outcome of an event" (Hosmer 1995). There are different aspects of trust related to IT.

An individual must first trust that information technologies will work and that IT will be beneficial in accomplishing his/her goals and in completing his/her tasks. An individual must also trust that the information they obtain via IT is accurate and the information they send via IT will not be tampered with and privacy levels will be maintained.

Trust proves to be a difficult variable to measure. Factors included in the trust category include an individual's perception of the ease of use of IT as well as the benefit of IT. In this study, trust is measured by variables such as helpfulness of IT for work-related communication, problem solving ability, and banking and shopping via the Internet. Some individuals, either due to their background or current environment, have a fear of IT and feel that it is difficult to use. It is hypothesized that an individual will use IT more if they have a positive perception or high trust level in IT.

The final IT adoption category proposed by this research is the passage of time. It is hypothesized that individuals will increase their use of IT over time, as access to IT becomes more commonplace. In this study, the same group of people were surveyed twice to evaluate their changes in IT use over time. Time was measured by establishing a dummy variable where each survey response from the 1997 survey was assigned a value of zero and each survey response from 1998 was assigned a value of one. Time-interaction variables were also created for each variable by multiplying the original variable by the time variable. For example, the "days of training" variable (tdays) was multiplied by the time variable and became the "timeinfluenced days of training" variable (tdayst).

Information Technology and its Components

Induction of IT as a strategic tool for agricultural development and welfare of rural requires that the necessary IT infrastructure is in place. The rapid changes and downward trend in prices in various components of IT makes it feasible to target at a large scale IT penetration into rural. Some of the broad factors to be noted with respect to various components of IT are listed below:

1. Input devices:

Radical improvements are witnessed with respect to the means of communication by human beings with computers such as key boards, mouse

devices, scanners. The advent of touch screen monitors that allow users to give input to computers by touching on the appropriate location of the monitor has made it possible to develop user-friendly interface for farmers which is easy, intuitive, circumvents language barrier and at the same time provides a relaxed environment to users. The present day digital cameras make it possible to capture and store good quality graphics and large video clips. The small size and low weight of these digital cameras, which are increasingly becoming affordable, open up possibilities of providing the computer based demonstration clips to educate farmers.

2. Output devices :

Monitor screens, printers & plotters, data projectors support high resolution and good quality output. The quality of these output devices have the potential of generating renewed interest in farmers in using IT based services. The light weight portable data projectors can be easily carried by agricultural extension personnel for serving larger audience. Similarly, speakers can also be attached to computers to incorporate voice based trainings for farmers.

3. Processors:

The processing speeds of computers have gone up. At present, Intel P-IV based processors @ 1.5 Ghz are available in the PC range which makes it possible to undertake substantial processing of data at the client side.

4. Storage Devices :

40GB and even higher hard disk drives have become common in PC range of computers. This makes it possible to store substantial information at the local level which facilitates faster access. Similarly, high capacity floppy disk drives, CDs make it possible to transfer large volumes of data to locations which can not be connected to networks immediately. These storage devices are also used for backup of crucial data. As a precaution, many corporates store their backups at locations away from the place of work.

5. Software :

Various operating systems are available which act as interface between the user and the machine. The graphic user interface (GUI) has become an accepted prerequisite for end users. Microsoft's 'Windows' continues to be a favourite. Application softwares which can support complex user requirements are available. Of the shelf solutions for office automation packages, groupware applications, complex database solutions, communication products, solutions based on remote sensing & geographical information systems are available. In addition, solutions based on some or all of these are also readily available. The present downward trend in the IT industry provides an opportunity get customised application for any specific task developed at an affordable price. Rapid Application Development and Deployment (RADD) is a popular model for quick development and deployment of applications. Development environment itself is simplified with tools that quicken the pace of software specialists. Project management and monitoring software are available that facilitate efficient execution of large and complex applications that are required for rural

6. Networking devices :

The capacity of modems, used to convert the data from digital to analog and vice versa, which are popularly employed to use telephone lines have increased. Internal modems are available integrated into the computer so that they are not exposed to outside environment. The capacities of other networking devices such as routers have also gone up which makes it possible to create large networks with smooth data transmission.

7. Transmission Media:

The media through which the data transfer takes place has also undergone revolutionary change. Telephone lines are still the popular source although the reliability and low bandwidth are still major issues. High capacity cables, optical fibre, radio, wireless local loops, satellite transmission and various solutions based on a combination of these are already being used in many parts of the country.

8. Other accessesories :

Uninterrupted Power Supply (UPS) devices are crucial to ensure the longetivity of the IT equipment as well as provide backup mechanisms. The potential of solar power packs to provide a feasible solution to shortage of power in the rural areas needs to be exploited.

CONCLUSION

A common strategy in higher education ministries in developing countries is public and private sector partnership in strategy or pursue rapid ICT projects is based. This partnership has different forms such as grant aid private sector interaction with public assistance, donated educational equipment and components by companies to public schools, providing technical assistance for planning, management and consolidation tools and human resources at the local level. But after financial aid, testing programs based on ICT is critical.

Many of the ICT training programs based on the charitable agencies aid have been unable to have high durability. Because the government has failed in its financial assistance in this situation none of the local communities to provide resources do not needed to continue these programs. Two strategies in here "to support government and local communities to move" are important. Since the 21st century, is century of education support about youth in Asia, to find sustainable ways to bridge the digital age in Asian countries is a real priority. And work through partnership that local leaders and guides are experts it can be lasting forever.

Several recommendations that emerged from the discussions emphasized on the need to think of ICT in education beyond computer aided learning and investigate the potential other technologies like community radio and other medium. These mediums could not only be cost effective but also has a greater outreach potential. It was also pointed out that low cost software solutions for e-learning that have scopes for innovation, should be incorporated in large scale projects. With an indication to open source solutions, the sessions recommended that such solutions should become a part of the overall policy for implementating technology supported education interventions. Sustainability and scalability of project are also issues that needed serious considerations. While moving beyond the pilot and experimental phase, projects especially those that needs a considerable financial contribution should have a viable sustainability model for up scaling. It was also recommended that implementers needs to be cautious when selecting areas for implementing ICT in education projects.

Projects should also not lose priority of the education objectives. In some cases ensuring school accountability system and teachers attendance may be more important that investing time and resources in ICT integration in schools. One fact that emerged in the sessions was that ICTs effectively computers, initiated in government department and schools were being used as decision support in education. Essentially, clear criteria, norms and standards needs to be developed for the information that was being used for decision-making.

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Myeloid and lymphoid neoplasms with FGFR1 rearrangement—one case report and lecture review

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Abstract: We report one case of myeloid and lymphoid neoplasms with FGFR1 rearrangement (EMS) here. The patient presented with generalized lymphodenopathy and fever.The bone marrow aspirates indicated CML but karyotype analysis discovered the translocation of t(8;13)(p11;q12),not t(9;22) and the BCR-ABL fusion transcript was not found.The histology was T-cell lymphoblast lymphoma(LBL) from the lymph node biopsy. Therefore the diagnosis of EMS was made.After chemotherapy,bone barrow assessment improved and most of the lymph nodes shrinked to untouchable.But the t(8;13) still remained.From the lecture review,we can see that on most occasions EMS presents as an atypical myeloproliferative disease characterized by myeloid hyperplasia, eosinophilia, translocation always involes the band 8p11 and high incidence of T-LBL.The fibroblast growth factor receptor-Ⅰ which locates at 8p11 is broken and fuse the other paterner gene to start the malignant transformation. By now, only allogeneic stem cell transplantation appears to cure.

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[Key words] Myeloproliferative disease; Eosinophilia; 8p11; Lymphoma; Fibroblast growth factor receptor-Ⅰ

Introduction

Myeloid and lymphoid neoplasms with FGFR1 rearrangement,which is originally named as 8p11 myeloproliferative syndrome(EMS),is a rarely happened hematological malignancy characterized by myloid hyperplasia,eosinophilia,high incidence of lymphoblast lymphoma(LBL).The chromosome translocation always involes the band 8p11 where is the locus of fibroblast growth factor receptor-Ⅰ (FGFR1).The fusion protein constitutionaly activates the tyrosine kinase and influence the cell growth, differentiation, apoptosis through several signal transduction pathways.So far, more than forty cases were reported but seldom in China[1,2]. Here we report one case we found with lecture review.

Case information

In June of 2007, the 7 year old boy had been found with lymphadenectasis in bilateral neck and in July of 2008, the boy got fever with the temperature 39--40℃ and the lymphadenectasis spread to many parts of the body. He was treated as lymphadenitis in local hospital with antibiotics for 10 days(name and dose of the medicine unclear) and the results were unsatisfactory. The complete blood count(CBC) in another hospital showed WBC 74.58×10^9/L , hemoglobin 139g/L, platete count 208×10^9/L and one course of oral hydroxycarbamide followed by one course of intravenous ara-C was given because chronic myelogenous leukemia(CML) was suspected from bone marrow aspirate. Because of the poor effect of the treatment, the patient came to our hospital. The physical examination showed the lymphadenectasis in the lower mandible, neck, armpit and inguen.Sternum tenderness and splenohepatomegalia were also found.The blood smear showed a prominent myeloid left shift and eosinphilia with 1% promyelocyte, 3% myelocytes, 7% metamyelocytes, 3% bands, 48% neutrophils and 10% eosinophils. Bone marrow aspirate showed a extremely hypercellular marrow with 77.2% of myeloid lineage including 4% myeloblasts, 4% promyelocytes , 20.8% myelocytes, 12% metamyelocytes, 20.4% bands and 5.2% neutrophils and 9.6% eosinophils(**Figure 1**). The score of neutrophil alkaline phosphatase was 71 with 50% positive cells. Cytogenetic analysis of the bone marrow aspirate showed the following karyotype: 46, XY, t(8;13)(p11;q12)[20] (**Figure 2**), and the BCR-ABL fusion transcript was not found. Biopsy of the lymph node demonstrated diffuse infiltrate composed of intermediate-sized mononuclear cells with scant cytoplasm and large round nuclei with fine chromatin. Immunohistochemical stains showed that these cells were positive for CD3, CD99, CD43, terminal deoxyribonucleotidyl transferase (TdT) and negative for CD20, Pax-5, consistent with a diagnosis of T lymphoblastic lymphoma(T-LBL) (Figure 3a-3f). Combining the excessive myelopoiesis of the bone marrow, diagnosis of T-LBL and the chromosome karyotype, it was concluded that the patient had the myeloid and lymphoid neoplasms with FGFR1 rearrangement[3].

The patient began his treatment in our hospital with 2 course of CHOPE(cyclophosphamide,

vincristine, etoposide, prednisone)[4], and achieved a partial remission. The bone marrow aspirate showed 0.4% myeloblast, 0.8% promyelocyte, 9.2% myelocytes, 9.6% metamyelocytes, and 7.6% eosinophils and the total myeloid lineage count 52.4%. The CBC showed WBC 4.31×10^9/L, hemoglobin 84 g/L, platelet count 236×10^9/L. The lymphadenectasis and splenohepatomegalia were eased and the fever stopped.But the karyotype of t(8;13)(p11;q12) of the bone marrow aspirate still remained.From September to November, we gave the patient 3 course of Hyper-CVAD(cyclophosphamide, vincristine, doxorubicin and dexamethasone)[5], repeat bone marrow aspiration, cytogenetic analysis, CBC and clinical condition showed no further improvement.

Discussions and lecture review

When we review the reports, we can see that in 1983, there were 2 cases of myeloproliferative disease(MPD) with the chromosome translocation of t(8;9)(p11;q33-34). They were finally diagnosed as Ph chromosome negative CML[6,7]. In 1992, Abruzzo and his collegues summarized the main characteristics of the disease in his 3 cases report: bcr/abl negative MPD usually accompanied by precursor T-LBL, eosinophilia and the chromosome translocations always involving 8p11,where is the locus of FGFR1[8]. Macdonald gave out the name of the disease, 8p11 myeloproliferative syndrome(EMS) in 1995[9]and in the WHO Classification of Tumors of Haematopoietic and Lymphoid Tissue in 2008, EMS was redefined as myeloid and lymphoid neoplasms with FGFR1 rearrangement.The abnormality of 8p11 translocation can be found present in both the myloid and lymphoid malignancies suggests that the pluripotent stem cell is the target of transformation[10]. So this disease is also called stem cell leukemia/lymphoma syndrome (SCLL). The rearrangements disrupt the FGFR1 gene at chromosome 8p11 and form fusion transcripts with different partner genes. So far, 8 partner genes have been identified in association with FGFR1 rearrangements in EMS (Table 1).

Table 1 Translocations and FGFR1 gene fusion partners in EMS

Translocation	FGFR1 gene fusion partner	References
t(8;13)(p11;q11-12)	ZNF198	Xiao et al[11]
t(6;8)(q27;p11)	FOP/FGFR1OP	Popovici C et al[13]
t(8;9)(p11;q33)	CEP110	Guasch G et al[12]
t(8;22)(p11;q22)	BCR	Fioretos T et al[14]
t(8;19)(p11;q13)	HERV-K	Guasch G et al[15]
ins(12;8)(p11;p11p21)	FGFR1OP2	Grand EK et al[16]
t(7;8)(q34;p11)	TIF1	Belloni E et al[18]
t(8;17)(p11;q23)	MYO18A	Walz C et al[17]

All the partner genes are fused to the exon 9 of FGFR1 with the cytoplasmic tyrosine kinase domain of FGFR1.So EMS and CML share a common molecular pathogenesis in that they both involve tyrosine kinase fusion genes. FGFR1 normally exists as monomeric plasma membrane proteins that dimerize upon ligand binding of fibroblast growth factors and transducer intracellular signals via phosporylated signaling intermediates. And the most common translocation associated with EMS, the t(8;13), fuses FGFR1 with the partner gene ZNF198, which locates at 13q11.It is composed of five zinc finger domains and a proline-rich domain. Both of the domains are conserved in the fusions and the proline-rich domain is essential for the oligomerization of the fusion protein, and makes the FGFR1 fusions exhibit aberrant tyrosine kinase activity. Subsequent activation of various downstream signal transduction pathways, notably STAT 5, culminates in unregulated cell proliferation and neoplastic transformation[19,20].It is similar with FOP-FGFR1 through PLC-γ 、MAPK/ERK 、PI3K/AKT/mTOR pathways and BCR-FGFR1 through STAT5、MAPK pathways in cell transformation[21,22].

Clinical phenotype

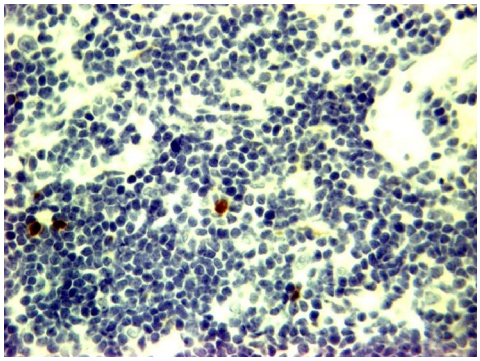
Marked leukocytosis in the peripheral blood can usually be found at presentation and the predominant cell types are neutrophils, metamyelocytes and myelocytes, similar to CML in certain aspects. Eosionphilia is a distinguishing feature of EMS and is seen in 90% patients in either bone marrow, peripheral blood or both. The bone marrow showed myeloid hyperplasia in most cases while certain variability were also found. Some cases, especially those with t(8;9) resembled chronic myelomonocytic leukemia but without major dysplasitic signs in either lineage[17,23,24].Several cases had the marrow findings of AML at the time of diagnosis of EMS while few had ALL or bilineal acute leukemia[14, 25 26,27]. One of the most striking finding of EMS is the high frequency of LBL which is seen in more than two thirds of EMS

cases and the patients with t(8;13) had a higher incidence than other translocations[28]. The histology findings showes that most cases demonstrat the feature of T-LBL while few present the findings of myeloid sarcoma[29,30]。 EMS has a natural history similar to CML in that both of them have a chronic phase.But median duration of it for EMS lasts only 6～9 months and most patients progress to AML or less commonly, B-lineage ALL without effective treatment [31].

Treatment

Up to now, only stem cell transplantation(SCT) offers the prospect of cure because the malignancy can not be eradicated by conventional chemotherapy. In the absence of SCT, disease progression was observed in the vast majority of cases and most patients died from resistant disease or early relapse within 1.5 years of diagnosis.Because of the obvious effect of imatinib to CML, much efforts are putting on the research of similar tyrosine kinase inhibitor. PKC412 (N-benzoylstaurosporine) is a small molecule tyrosine kinase inhibitor of many protein kinases and was shown to inhibit ZNF198-FGFR1 activity in cell lines. In the mouse model of EMS, the group of PKC412 had prolonged survival than control group. For a EMS patient with t(8;13), the treatment of PKC412 led to both dramatic decrease of phosphorylated tyrosine and clinical control of the disease in 6 month before SCT[32].These results raised the possibility that small molecule tyrosine kinase inhibitor that specially target FGFR1 fusion proteins may be available in the future for EMS patients.

Figure 1. Bone marrow aspirate with myloid hyperplasia and eosinophilia (Wright's-Giemsa Stain, ×1000)



Fifure 2. Chromosome translocation t(8;13)(p11;q12)

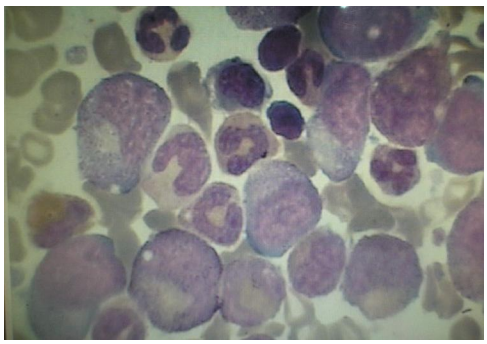


Figure 3a. Lymphoma cells negative for Pax5 (Immunohistochemical SP stain,×400)

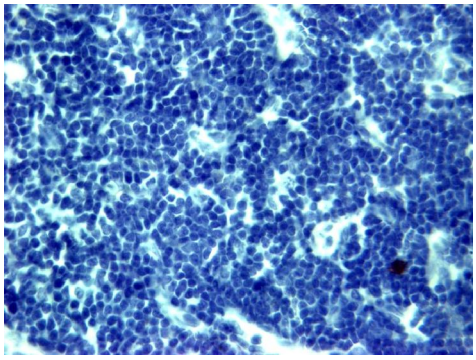


Figure 3.b Lymphoma cells negative for CD20 (Immunohistochemical SP stain,×400)

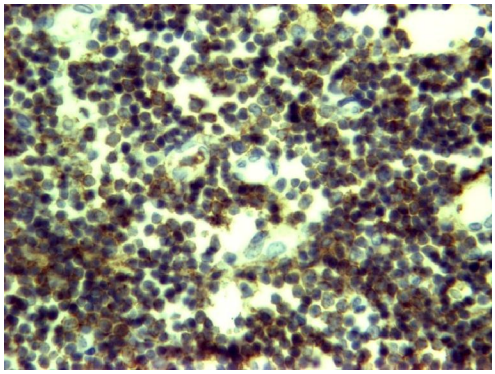


Figure 3c Lymphoma cell membrane positive for CD43 (Immunohistochemical SP stain,×400)

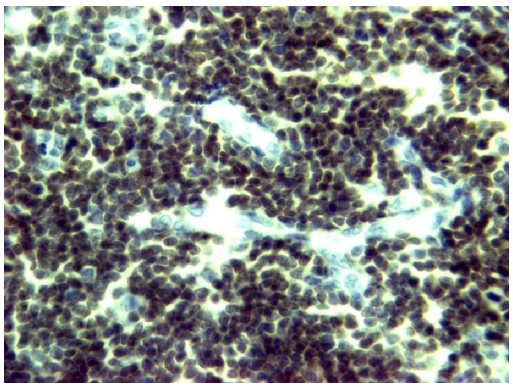


Figure 3d Lymphoma cell membrane positive for CD3 (Immunohstochemical SP stain,×400)

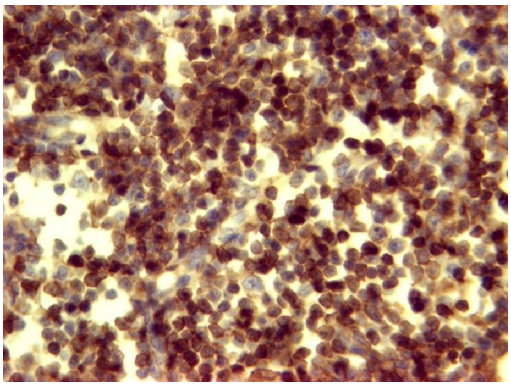


Figure 3e. Lymphoma cell membrane positive for CD99 (Immunohistochemical SP stain,×400)

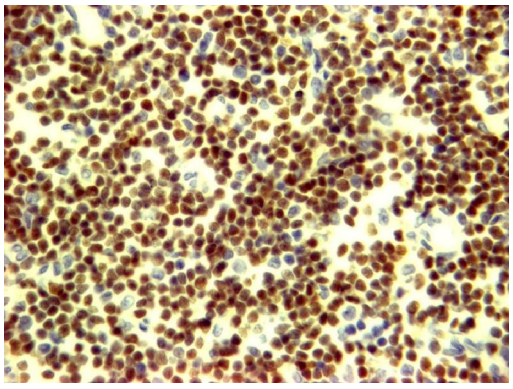


Figure 3f Lymphoma cell nuclei positive for TdT (Immunohistochemical SP stain,×400)

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3/13/2011

Effective technological pectinase and cellulase by *Saccharomyces cervisiae* utilizing food wastes for citric acid production

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Abstract: The production of a notable and highly effective pectinase and cellulase, by the commercial baker's yeast *Saccharomyces cervisiae* utilizing potato processing wastes, was achieved in 5-day solid state fermentation (SSF) cultures, at temperature 25 °C, pH range 4.0-5.0 and additive of ferric chloride. Pectinase and cellulase activities were stimulated by using potato wastes supplemented with urea, as the sole carbon and nitrogen sources, resulted in 70.20 and 98.85 % reduction of viscosity. It is concluded that citric acid production from pectinolytic and celluolytic *Saccharomyces cervisiae* optimization with *Aspergillus niger* MAF3, maximally 46.67 and 68.44 g/kilogram solid potato wastes.

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Keywords: *Saccharomyces cervisiae*, Potato wastes, Solid state fermentation (SSF), Pectinas and cellulase, Citric acid.

1. Introduction:

At the present, the fundamental exploitation of food waste, which participate in pollution, is the controlled biological degradation of the wastes by microorganisms for the production of valuable compounds such as enzymes, citric acid and others as raw materials for medical and industrial uses.

In Egypt potato is one of the most important crops grown for local consumption, export and processing. The area cultivated with potatoes about 212,000 acres producing about 2.2 million tons with an average of 10.5 tones per acre (Hegazy, 2009). In 2002, the world production of starch amounted to approximately 58 million tons (roughly 69% from corn, 10% from cassava, 9% from sweet potatoes, 6% from wheat, 6% from potatoes, and less than 1% from other sources) (Peters, 2007). Different methods created for potato wastes utilization were reported by many authors (Mahmood *et al.*, 1998; Huang *et al.*, 2003; Parawira *et al.*, 2005 and Darwish *et al.*, 2009). In addition, enzymatic hydrolysates exploited as substrate for the production of organic acids (Kuhad and Singh, 1993; Khare *et al.*, 1995; Sarangbin and Watanapokasin, 1999; Saber *et al.*, 2010).

Pectinases have widespread applications in the food and textile industries (Henriksson *et al.*, 1999), and in addition to plant tissue maceration, wastewater treatment and degumming of natural fibers (Baracat-Pereira *et al.*, 1994). Cellulases have diverse applications in environmental, food and agricultural industries (Deshpande *et al.*, 1992), also, they act synergistically (Kim *et al.*, 2003), and used to modify the surface properties of cellulosic fibers and fabric in order to achieve a desired surface effect (Kotchoni *et al.*, 2003). Moreover, citric acid is of industrial importance because, it is widely used in dairy, medicine and biochemical industries (Wang and Liu, 1996; Tongwen and Weihua, 2002). Yeasts like *Saccharomyces cerevisiae* and *Candida* sp. have been reported to produce pectinolytic enzymes (pectin lyase, polygalacturonases, and pectinesterases) (Gainvors *et al.*, 1994). Also, cellulase and pectinase were produced from some aquatic hyphomycetes by Osman *et al.* (2008).

This work aims at biodegradation of potato waste of potato processing industry in Egypt for production of pectinase and cellulase enzymes by ***a*** commercial bakery yeast and using their optimizations in prefermentation and mixed fermentation with the highly citric acid producer (*Aspergillus niger* MAF3). This methodology has two benefits, the first one is environmentally safe, the second is the utilization of low cost production of the enzymes pectinase and cellulase as well as citric acid.

2. Materials and Methods:

Materials:

Organisms and inoculums

The commercial live bakers' yeast, *Saccharomyces cerevisiae* was obtained, in the forms of active dry yeast, from the Egyptian Company for Advanced Foodstuff Industries. Active dry yeast (5g) was dispersed in 99 mL of 0.1% sterile peptone water prewarmed at 38°C for 20 min. The yeast solution contained 2×10^5 viable cells/mL and used as

inoculum for fermentation medium (Wang *et al.*, 1997).

The fungal species *A. niger* MAF3 was obtained from Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut, Egypt. *A. niger* MAF3 spores were produced in Czapek-Dox Broth (50 ml) in a 250 ml Erlenmeyer flask, incubated at 28°C for eight days. A spore suspension was prepared by adding 25 ml distilled water with Tween-80 (0.1%) and was stored at 4°C for a maximum of two weeks. It contained 10^7 spores/ml according to the method described by Vandenberghe *et al.* (2000).

Fermentation medium

Solid potato wastes were obtained from potato processing industry (chips), Assiut, Egypt. The other components of the SSF culture media were obtained from Merck and Sigma in the highest purity. Ten grams of solid potato wastes were taken in Erlenmeyer flask (250 ml), mixed with 10 ml of Czapek-Dox mineral solution, and sterilized at 121 °C for 30 min. Cultivation was carried out by adding 1.0 ml of the inoculum then the flasks were incubated at 30°C statically.

Preparation of crude enzyme extract

Culture media and cells were harvested after incubation for 7 days by filling to 100 ml of sterilized distilled water. The mixture was vigorously stirred for about 15 min and filtered through Whatman No. 1 filter paper on Büchner funnel, and centrifuged at 5000 rpm and 4 °C for 15 min. The supernatant thus obtained was used as crude enzyme preparation.

Methods:

Assay for pectinase activity

Pectinase activity was measured as reduction percentage in pectin solution viscosity using Ostwald viscometer according to White and Fabian (1953).

Assay for CM-cellulase activity.

CM-cellulase was measured viscometrically as described by (Eriksson and Hollmark, 1969; Child *et al.*, 1973, El-Sheekh *et al.*, 2009).

Pre-fermentation and mixed fermentation

This experiment was performed as the method of Khare *et al.* (1995). In this set of experiments, SPW was pre-fermented with the optimization of SSF of pectinolytic and celluolytic *Saccharomyces cervisiae*, prior to inoculation with the citric acid- producing *A. niger* MAF3. In one set pre-fermented SPW samples were sterilized after the fifth day to kill the pre-fermenting *Saccharomyces cervisiae* and then inoculated with *A. niger MAF3* (PF-1). In a second set, *A. niger* MAF3 was inoculated on the fifth day without killing *Saccharomyces cervisiae* (PF-2), and the third set was a mixed fermentation created by inoculating both the organisms simultaneously at the start (PF-3). Other fermentation conditions were kept as described above in Section 2.2, unless, the supernatant fluid thus obtained was used for citric acid estimation.

Analysis for citric acid

The concentration of citric acid in culture filtrate was measured by titration with 0.1 N NaOH as described by (Imandi *et al.* 2007; Khosravi-Darani and Zoghi, 2008). After titration, citric acid was determined spectrophotometrically at 420 nm by the acetic anhydride-pyridine method according to (Marrier and Boulet, 1958; Imandi *et al.*, 2008).

3. Results and Discussion

Several created methods for utilizing potato wastes in order to support the growth and extracellular hydrolytic enzyme production have been described (Mahmood *et al.*, 1998; Parawira *et al.*, 2005; Darwish *et al.*, 2009). In addition, (Kuhad and Singh, 1993; Khare *et al.* 1995; Sarangbin and Watanapokasin, 1999; Saber *et al.*, 2010) pointed out that the enzymatic hydrolysates exploited as substrate for the production of organic acids which strongly pronounced them for multienzyme complexes' production through microbial biodegradation. This therefore justified this study for the perfect utilization of SPW, excluded in the potato processing factories in Egypt (chips), as a substrate material suitable for a commercial *baker's* yeast strain capable of the production of pectinase and cellulase enzymes, as well as utilization of their hydrolysates for production of citric acid, essential for numerous applications.

When the commercial bakers yeast (*Saccharomyces cerevisiae*) was grown on SPW as a solid support in SSF, could produce pectinase and cellulase as high as 17.45 and 49.20 % reduction of viscosity after 5 days incubation (Fig. 1). Similarly, *Aspergillus carneus* NRC1 produced the highest yield of pectinase, cellulase and high hemicellulase after 5 days (El-Sheekh *et al.*, 2009). Also, the optimum production of pectic enzymes by *Trichoderma lignorum* attained after 5-day (Abdel-Fattah *et al.*, 1977). In addition, the maximum filter paperase (FPase) activity was 19.5 IU g^{-1} in 4 days, while, the highest CMCase activity was concurrently obtained after 5-6 days of fermentation when *A. niger* KK2 was grown on rice straw alone as a solid support in SSF (Kang *et al.*, 2004).

In relation to these results, the cellulose (CEL) culture were statistically similar to those from the 3rd and 4th days of the untreated sugar cane bagasse (SCB) using *Penicillium echinulatum* 9A02S1 culture, and the 5th and 6th days gave the greatest filter paper activity (FPA) (Camassola and Dillon, 2009). On contrast, the maximum cellulase secretion from *A. niger* using maize straw was after 3

days of incubation (Milala *et al.*, 2005), moreover, the maximum of FPase, carboxy methyl cellulase (CMCase) and xylanase activity was obtained after 7 days incubation of *Aspergillus oryzae* MTCC 1846 at 28 ± 0.5) (Chandel *et al.*, 2009).

The addition of different mono- and disaccharides in equal carbon basis in the production medium led to low or high enzyme activities. The control culture characterized by the highest levels of enzymes activity (58.50 and 65.0 % reduction of viscosity) for pectinase and cellulase, respectively. This distinctly reflects the effect of inducible substrate type of the enzymes involved by the commercial bakery yeast (Fig. 2). The inducible nature of the aforementioned fungal enzymes was previously reported (Ismail, 1996; Silva *et al.*, 2002; Bai *et al.*, 2004). In addition, the control culture using *Penicillium echinulatum* 9A02S1, gave the greatest FPA (Camassola and Dillon , 2009), while, the cellulase production from *A. niger* NRRL 2001 and *A. niger* NRRL 2007 reporting 5 and 6 U/ml of cellulase, consequently, using de-starched corn fiber as a carbon source (Dien *et al.*, 2006).

The initial pH of the basal medium ranged from 3.0 to 11.0 (Fig. 3) and that was suitable for pectinase and cellualase enzymes produced by *Saccharomyces cervisiae*. The marked effect of initial pH 4 and 5 was mainly on pectinase and cellulase enzyme productivities which reached their maximal value of 52.12 and 69.50 % reduction of viscosity, consequently. An increase in initial pH of fermentation above 4 and 5 shows a decrease in pectinase and cellulase enzymes productivity, consequently. Similarly, Foda *et al.* (1984) reported the optimum initial pH values for polygalacturonase (PG) enzyme production were 4.0–5.0 for *A. aculeatus*. Birgisson *et al.* (2003) concluded the production of PG by *Cystofilobasidium larimarini* (55,000 U/l) and *C. capitatum* (32,000 U/l) was maximum at an optimum pH of 3.2 and 3.9, respectively. CMCase showed optimal activity at pH 5.0, while xylanase, pectinase and FPase activities were optimal at pH 6.0 as reported by Parawira *et al.* (2005).

In contrast to these results, Gummadi and Kumar (2008) reported the optimum pH for pectin lyase (PL) and pectate lyase (PGL) production by *Debaryomyces nepalensis* was found to be 7.0. From one hand, while PL production was achieved at an initial pH 6.5 on the other (Manachini *et al.*, 1988). In addition, the optimum pH for pectin lyase activity was 8.0 (Solís *et al.*, 2009). Hence, in all subsequent experiments, initial pH of the medium was maintained at 4 and 5 for pectinase and cellulase enzyme productivities.

Pectinase and cellulase production was investigated in a temperature range of 15–60 °C. The highest production of pectinase and cellulase activities (66.0 and 98. 85 % Reduction of viscosity), respectively, were obtained at 25 °C and at higher temperatures their production decreased gradually (Fig. 3). The incubation temperature in previous range is favored and denoting good mesophilicity of pectinase and cellulase and their producer *Saccharomyces cervisiae*. This adds good applicable advantage to the crude *Saccharomyces cervisiae* pectinase and cellulase. Thus, *Saccharomyces cervisiae* was, a little similar to, *Aspergillus foetidus* (NRRL 341, ATCC 16878) which optimally produced pectinases at 30 °C (Hours *et al.*, 1988), and *A. niger* A-20 was optimally produced multienzyme systems of pectinases, cellulases and xylanase at 30 °C (Ismail, 1996) from one hand, and differ to, *A. carneus* NRC1 which favored pectinases production at 50 °C (El-Sheekh *et al.*, 2009). In respect to enzyme activity, the simultaneous addition of glucoamylase and yeast (*Saccharomyces cerevisiae*) was performed at 30 °C (Srichuwong *et al.* (2009), while, and the optimal activities was at 50 and 60 °C for pectinase and CMCase, respectively (Parawira *et al.*, 2005).

Study of the effect of the SPW quantity in the SSF culture medium using pectinas and cellulase enzymes, indicated that their productivities increased parallely with the added SPW till 25 and 80g which represented the most proper concentrations for obtaining the highest productivities (69.3 and 96 % reduction of viscosity), respectively (Fig. 5).

Break down of cell-wall materials causing by the hydrolytic activities of enzymes was clearly observed. According to the results of these studies, orange bagasse 50% (w/w) and wheat bran mixture was the most proper for the maximal pectin lyase productivity in SSF cultures of *Penicillium viridicatum* Rfe3 (Silva *et al.*, 2002). Taking the pectin content of orange peels and pulps (OPP) into consideration, the proper percentage 6% (w/v) equals 1.44% (w/v) pectin (El-Sheekh *et al.*, 2009) and also this was near to that concluded for apple pectin by Abdel-Fattah *et al.* (1977) for optimum production of pectic enzymes by *Trichoderma lignorum*. Moreover, the maximum cellulase secretion from *A. niger* obtained using 6% maize straw concentration (Milala *et al.*, 2005).

Table 1. Effect of pre-fermentation and mixed fermentation in the optimized medium pectinase and cellulase enzymes productivity by *Saccharomyces cervisiae* cultures for citric acid production.

Treatment	Optimized pectinase medium		Optimized cellulase medium	
	Citric acid (g/kg SPW)	Final pH	Citric acid (g/kg SPW)	Final pH
OM	15.86	2.98	2.47	5.11
PF-1	17.61	2.34	14.69	2.33
PF-2	6.17	6.52	4.61	4.53
PF-3	46.67	2.02	68.44	2.05

OM Optimized medium of each enzyme produced by *Saccharomyces cervisiae*.
PF-1 Prefermented with *Saccharomyces cervisiae* for 5 days and sterilized followed by *A. niger* MAF3.
PF-2 Prefermented with *Saccharomyces cervisiae* for 5 days followed by inoculation with *A. niger* MAF3.
PF-3 Mixed fermentation with both fungi inoculated at 0 day. Citric acid measured at 5 days after inoculation of *A. niger* MAF3.

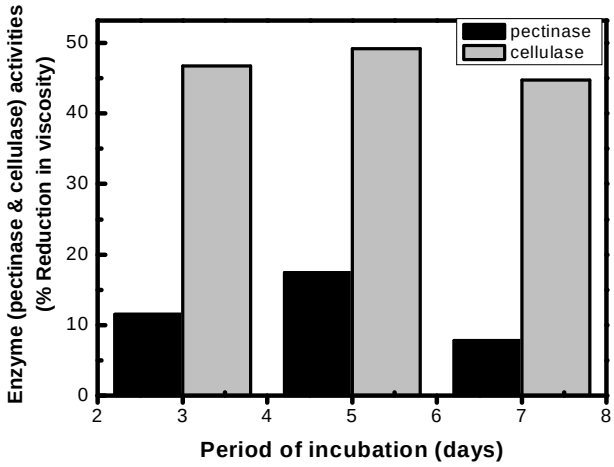

Fig. 1. The pectinase and cellulase enzymes activities (% reduction in viscosity) of the SSF culture filtrates of static *Saccharomyces cerevisiae* cultures during different periods of incubation

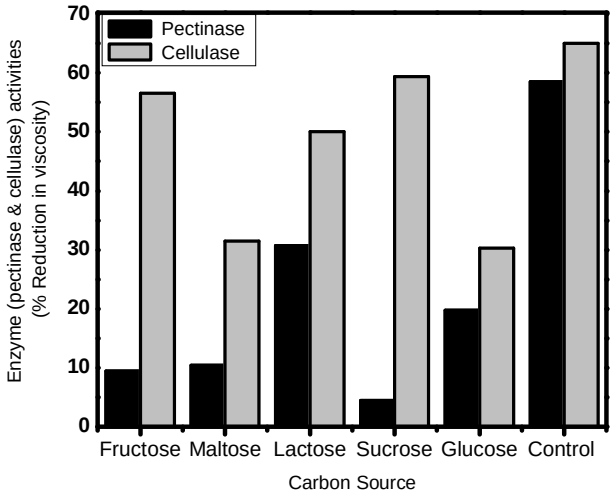

Fig. 2. Effect of different carbon sources on pectinase and cellulase enzymes productivity by *Saccharomyces cervisiae* in 5-day static cultures.

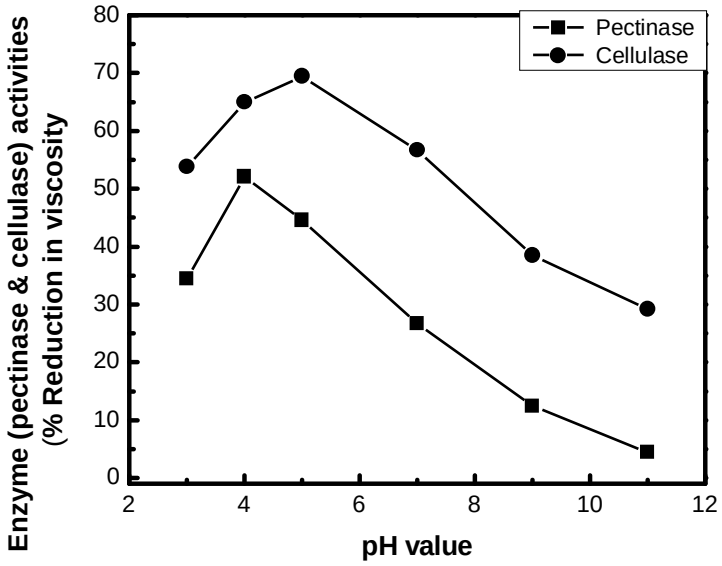

Fig. 3. Influence of initial pH value on pectinase and cellulase enzymes productivities by *S. cerevisiae* grown for 5 days in static cultures.

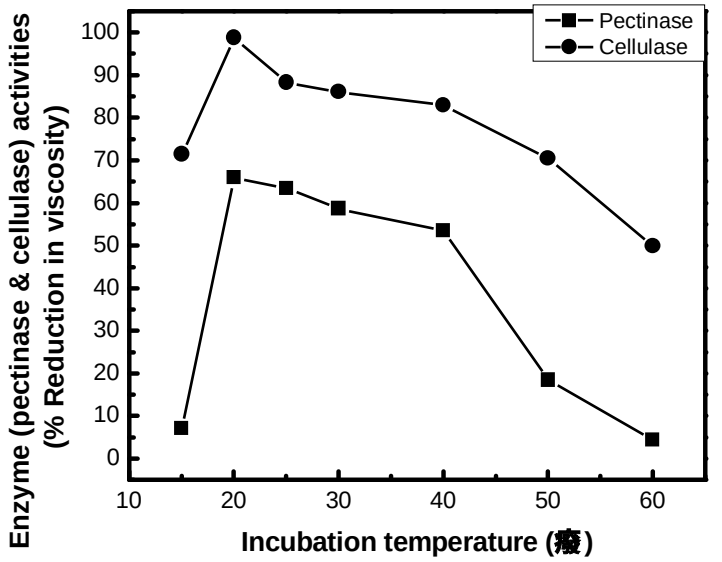

Fig. 4. Effect of incubation temperatures on pectinase and cellulase productivities by *Saccharomyces cervisiae* in 5-day SSF cultures.

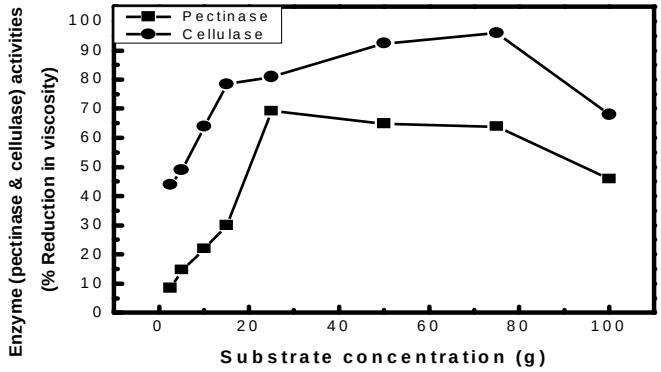

Fig. 5. Effect of solid potato waste (SPW) quantity in SSF cultures on pectinase and cellulase productivity by *Saccharomyces cervisiae* in 5-day static cultures.

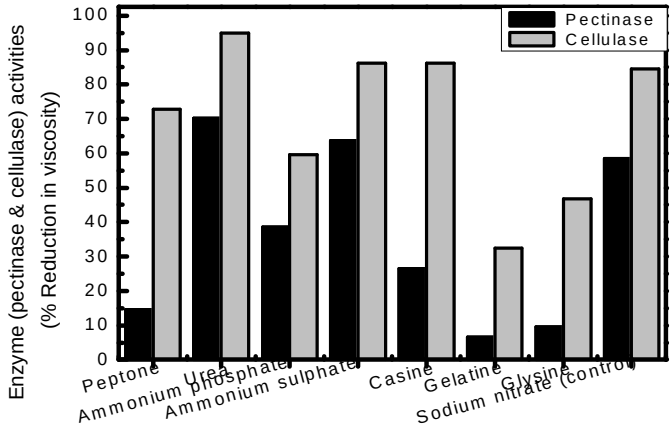

Fig. 6. Effect of different Nitrogen sources on pectinase and cellulase enzymes productivities by *Saccharomyces cervisiae* grown for 5 days in static SSF cultures.

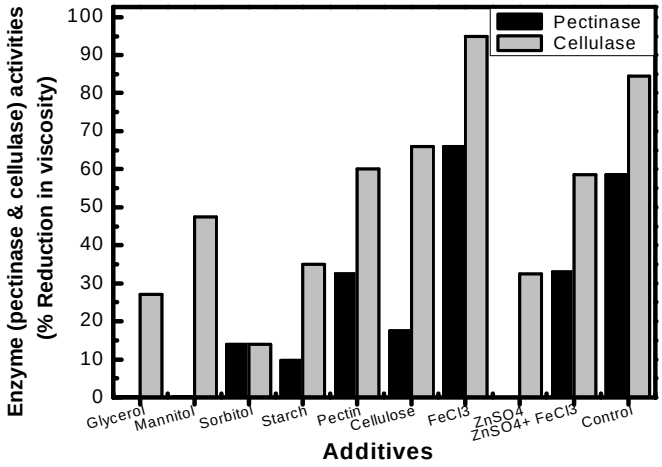

Fig. 7. Effect of different additives (1% w/w) to the optimizes medium pectinase and cellulase enzymes productivity by *Saccharomyces cervisiae*grown in 5- day static SSF cultures.

The effect of different nitrogen sources was investigated by replacement of $NaNO_3$ in equal nitrogen basis by any of N sources (peptone, urea, ammonium phosphate, ammonium sulphate, casein, gelatin or glysine) to verify their suitability for pectinase and cellulase enzymes production by *S. cerevisiae*. As shown in Fig. 6, the replacement of $NaNO_3$ in the SSF culture medium by any of the above-mentioned N sources led to many dissimilar effects on the productivity of the pectinase and cellulase enzymes. Generally, some N sources tested led to moderate or good pectinase (except peptone, gelatin and glysine show a low effect) and cellulase productivities, particularly urea by which the highest pectinase and cellulase yield was attained, and resulted in 70.20 and 98.85 (% reduction of viscosity), consequently.Yeast cells require sufficient nutrients to survive the osmotic stress and maintain their metabolic functions. Nitrogen limitation for protein synthesis and yeast growth is particularly observed in very high gravity (VHG) fermentation, which can be remedied by the addition of assimilable nitrogen sources such as yeast extract, urea and ammonium salts (D'Amore et al., 1988; Jones and Ingledew, 1994;; Blateyron and Sablayrolles, 2001).

The effect of some additives on pectinase and cellulase enzymes productivities by *Saccharomyces cervisiae*was was graphically illustrated in Fig.7.

These additives include*d*, some polyalcohols (glycerol, mannitol and sorbitol), polysaccharides (starch, pectin and cellulose), and metal ions (FeCl3, ZnSO4, and FeCl3 + ZnSO4 0.5+0.5% w/w) and added in equal weight basis (1%, w/w).

Among all the additives above-mentioned FeCl3, highly stimulated cellulase and moderately stimulated pectiase, productivity (95 and 60% reduction of viscosity), respectively, while, the others had varied stimulatory effects. Each of glycerol, manitol and Zn^{2+} had completed inhibitory effects. Fungal pectinases production media formulated by many authors and principally contained orange bagasse or peels were devoid of the metal ions Zn^{2+} and Fe^{3+} (Ismail, 1996; Silva *et al.*, 2002).

Study of the effects of the prefermentation and mixed ermentation pointed out that a maximum of a many-folds increase in citric acid yield was obtained in the case of mixed fermentation with both *Saccharomyces cervisiae* and *A. niger* MAF3 inoculated at 0 day (PF-3), and resulted in the production of 46.67 and 68.44 g citric acid/kilogram potato waste, consequently, which is quite, similar to the condition recorded by Khare *et al.*, 1995, and comparable to the yields obtained by fermentation of other agro-wastes (Panda *et al.*, 1984; E1-Abayad *et al.*, 1992; Khare *et al.*, 1995; Pramod and Lingappa, 2008).

5. Conclusion:

In conclusion, using a pectinolytic and celluolytic *Saccaromyces cerevisiae* utilizing solid potato wastes, as sole carbon source at a concentration of 15 and 85g at pH 4 and 5, supplemented with urea and FeCl3, and incubated at 25 °C for 5 days, represented the optimal conditions to attain the highest pectinase and cellulase yield (70.2 and 98. 85 % reduction of viscosity), consequently. These conditions were, used in pre and mixed fermentation with the highly citric acid producer *A. niger MAF3,* maximally produced 46.67 and 68.44 g citric acid/kilogram solid potato waste in pectinase and cellulase mediaum optimization, respectively. This offers an opportunity to recover potato chips by-products and used as substrate for producing the cheapest with the highest productivities of cellulase and pectinase, as well as citric acid to be used in food and biochemical industries.

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