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CONTENTS

Molecular Detection and genotyping of human papilloma virus in cervical specimens among Egyptian Female Patients

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Abstract: Human Papilloma Virus (HPV) was found to be involved in a variety of malignancies; cervical cancer is the most important and prevalent. The goal of this study was to identify genital HPV: to determine its rates and possible genotypes in cervical biopsies from Egyptian female patients; to detect its association with non malignant and malignant cervical lesions and to examine the potential role of HPV in development of cancer cervix. This study was carried out on 60 Egyptian female patients with histopathological evidence of flat condyloma, CIN and cervical carcinoma in addition to 30 age matched females as control group. The molecular analysis was carried out employing MY09/11 consensus HPV L1 PCR in order to molecularly detect genital HPV. Positive PCR samples for HPV were further subjected to molecular genotyping by Southern blot using specific labeled oligonucleotide probes **(6-11-16-18)** followed by sequencing for confirmation. PCR detected HPV DNA in 76.7% of patients and in 10% of the control group. *The HPV was positive in 84.2% of patients with flat condyloma lesion of cervix; in* 71.4% of the CIN group and in **75%** of cancer cervix patients. By Southern blot genotyping, it was found that in flat condyloma HPV genotype 6 was in 62.5% followed by genotype 11 (18.8%). CIN lesions harbored high risk oncogenic HPV genotypes in 53.8%. As regards squamous cell carcinoma HPV genotype 16 was found in 90.9% while HPV 18 was the only genotype detected in adenocarcinoma. In conclusion, HPV infection was found to be common and more associated with CIN II & III lesions and invasive carcinomas. This reflects a large unscreened population so introduction of newer techniques in female screening should be a matter of intense research. HPV DNA detection and genotyping is useful for classifying oncogenic HPV thus serving as a valuable tool in picking up of high risk group.
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Keywords: HPV, CIN, Cancer cervix, Southern blot .

1. Introduction

Human papillomavirus (HPV) is a nonenveloped, double-stranded DNA virus. Most infections clear within 2 years, however, a minority persists and potentially could progress to cervical cancer (1). HPV have more than 100 genotypes (2), infection with certain genotypes, that are high-risk HPV (16&18), play an essential role in the development of uterine cervical cancer (3)**.** In contrast, other genotypes, as HPV 6 and 11, are considered as 'low risk' for the development of cervical carcinoma (4).

In malignant transformation of uterine cervical epithelia, viral DNA integrate into the host genome causing disruption of the HPV 16 E2 gene -a negative regulator of the E6/E7 promoter. Consequently, increasing expression of E6 and E7 viral oncoproteins that target the p53 and retinoblastoma (pRb) tumor suppressor proteins, respectively, thus down regulating their antitumor functions (5).

Since HPV cannot be cultured and the clinical performance of serological assays is poor, diagnosis of HPV infection is almost entirely based on molecular tools (6), including liquid hybridization as Hybrid Capture 2 (7), Southern and dot blot hybridization using HPV type-specific probes, type-specific polymerase chain reaction (PCR) (6), and general-primer PCR (8,9)

Polymerase chain reaction (PCR) primers that target the highly conserved regions of the HPV L1 open-reading frame are capable of amplifying a broad spectrum of HPV types (10) and the newly developed short-fragment PCR (11).

Accordingly, the aim of this study was to identify genital HPV; to determine its rates and possible genotypes in cervical biopsies from Egyptian female patients; to detect its association with non malignant and malignant cervical lesions and to examine the potential role of HPV in development of cancer cervix.

2. Subjects and Methods:

1-Study population:

After informed consent, 90 women attending the Early Cancer Detection Unit, Department of Obstetrics and Gynecology, Ain-Shams University Hospitals were participated in this study, in the period from June 2008 to May 2010. Their ages ranged between 28-52 years with a mean age of 39.5 ± 11.4 years. According to the histopathological results the subjects studied were classified into:

Patient group (I): comprising 60 patients.

Ia: 19 patients with flat condyloma lesion of cervix.

Ib: 21 patients with Cervical Intraepithelial Neoplasia CIN (I, II, III).

Ic: 20 patients with cancer cervix; 16 patients with squamous cell carcinoma (SCC) and 4 patients with adenocarcinoma (AD).

In addition to 30 females with normal histological findings were considered as control group (II).

Subject enrollment criteria included: no history of low or high-grade cervical squamous intraepithelial lesions within the past year (i.e., not attending clinic for referral abnormal Pap smear), not currently pregnant and no hysterectomy.

All studied groups were subjected to:

-Through detailed history laying stress on age, ethnicity, marital status, household income, education level, age at menarche, parity, smoking history, history of use of condoms, diaphragm, and birth control pills, history of partner with genital warts, history of sexually transmitted disease (STD) (chlamydia, trichomonas, gonorrhea, and herpes), and history of pelvic inflammatory disease.

-Routine gynaecological examination and cytological evaluation, colposcopic examination with directed punch biopsy and histopathological evaluation.

-HPV-DNA detection using the MY09/11 consensus HPV L1 PCR.

-Southern blot hybridization for HPV genotyping followed by HPV-DNA sequencing for positive samples for confirmation of genetic specificity.

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2. Methods:

2.1 Cytological evaluation (Pap test):

Cervical cell scrapings were collected with a cytobrush from the ecto- and endo-cervix. The cytobrush was rolled onto 2 separate glass slides which were then fixed in 95% alcohol for Pap test. The microscopic cellular changes in the cervicovaginal smear were detected according to Bethesda classification system (12). All women with abnormal Pap smears were referred to the colposcopy.The main diagnostic features of flat condyloma is Koilocytes showing a distinct perinuclear zone of cytoplasmic clearing together with peripheral zone of dense cytoplasm and atypical nucleus. Also dyskeratocytes (individual cell kertinization: parakeratotic and hyperkeratotic cells) are commonly found however not specific for HPV (13).

2.2 Colposcopic examination

Together with saline test, acetoacetic test and Schiller test for all patients with abnormal Pap smears (14). Colposcopic directed punch biopsy from the abnormal part of the cervix detected by acetoacetic acid test and Schiller test. Each biopsy was divided into 2 parts. One part was kept in formalin for histopathological examination, the other part was immediately stored at -70ºC in aluminum foil for PCR detection of HPV and Genotyping

2.3 Histopathological evaluation:

Sections of biopsies were prepared, stained with hematoxylin-eosin and examined by a single pathologist. Tissues biopsies were classified as either normal cervix (including cases of chronic inflammation), flat condyloma, CIN (I , II or III) and cancer cervix either squamous cell carcinoma, or adenocarcinoma. The pathologist had no clinical information or results of cytology, colposcopy or HPV testing (Fig1-3).

2.4 HPV detection using the MY09/11 consensus HPV L1 PCR (15):

Total DNA was extracted from approximately 25 mg of tissue biopsy using the DNeasy tissue kit (Qiagen Inc., California, USA), following the manufacturer's protocol. The polymerase chain reaction (PCR) targeting the L1 capsid protein gene was used to amplify a 458-base pair DNA fragment using consensus primers the MY09/11 that amplify a wide range of HPV genotypes. Reactions were performed in 50 ul volumes containing 0.5 mg of DNA, 200 pmol of each primer, 0.2mM of each dNTPs, 1unit thermostable Taq DNA polymerase and 1X Reaction buffer (all reagents were supplied by Promega, USA).

Cycling conditions were performed as follows: one initial denaturation step at 94 ºC for 1 min; 40 cycles of denaturation at 94º C for 1 min, annealing at 50 ºC for 1 min, and extension at 72ºC for 2 min; and one final extension step at 72 ºC for 10 min. Amplified DNA fragments were resolved by electrophoresis of 40 ul of PCR products in 2% agarose containing ethidium bromide (0.5 mg/ml) and visualized under UV transilluminator. The expected PCR products size was 458 bp (Fig4).

Each experiment was performed with separate positive and negative PCR controls. Amplification of isolated DNA was checked by β-globin PCR primers PC03 and PC04, generating a fragment of 110 bp [20].

2.5 Southern blot hybridization for HPV genotyping:

The oligonucleotides type specific probes for types 6,11,16 &18 were as follows:

Type 6: 5' CAT CCG TAA CTA CAT CTT CCA 3'
Type 11: 5'TCT GTC TCT AAA ACT GCT ACA 3'
Type 16 : 5' CAT TAC ACT CCA GCA CCC TGA 3'
Type 18: 5' GGA TGC ACC GGC TGA 3'. They were labeled using DIG oligo nucleotide 3' End Labeling Kit supplied by Boehringer Mannheim.

For typing by southern blot, electrophoresis was repeated for all positive HPV-PCR amplified products to collect them on separate membrane**.** DNA from agarose gel was then transferred by capillary blotting to the nylon membrane (Amersham, UK) using 0.4 M NaOH buffer, then the membrane was hypridized with HPV 6,11,16&18 Digoxigenin labeled oligonucleotides probes. Then the membrane was exposed to X-ray film resulting in an autoradiograph (16). To denote the genotype of HPV, the resulting bands were compared to corresponding sites of specific bands originally present in gel (Fig 5).

2.6 DNA sequencing:

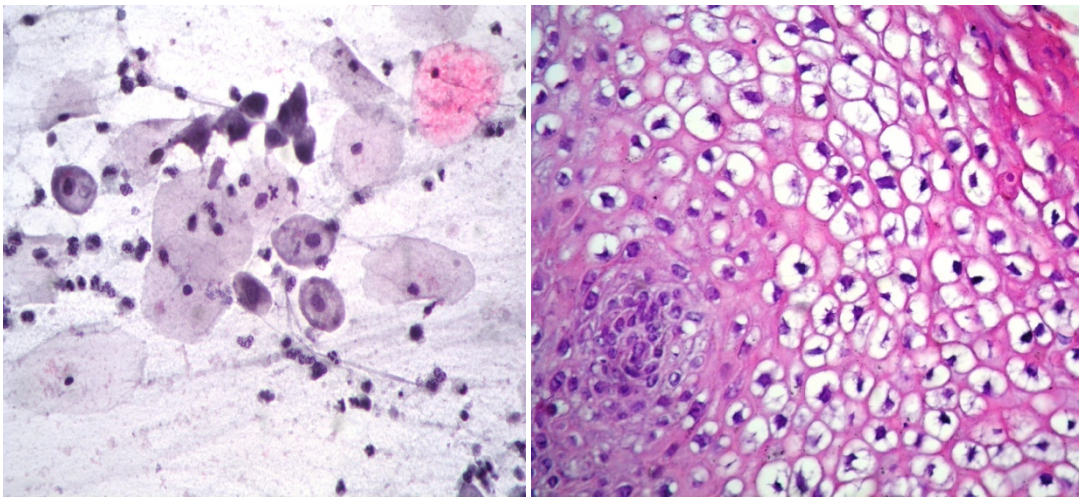
A selection of HPV DNA positive samples was sequenced for confirmation of genetic specificity. The sequencing was performed with a dye terminator cycle sequencing kit (Applied Biosystems,UK). The sequences were analyzed on an ABI PRISM 310 genetic analyzer, Perkin Elmer (Applied Biosystems, UK) according to their protocol.

The sequences were compared to other known sequences in a database search (BLAST from National Center for Biotechnology Information [NCBI]: http://www.ncbi.nih.gov/)

Statistical analysis:

The association between the detection of HPV DNA and its specific types were described by using chi-square (X^2) test. While relation to parametric data, as age

and duration of marriage, were described by Student t-test and were computed by SPSS procedures.



A B

Fig. 1: A- cervicovaginal smear; flat condyloma Pap stain X100 B- Flat condyloma of the cervix (LGSIL) H&E stain X400

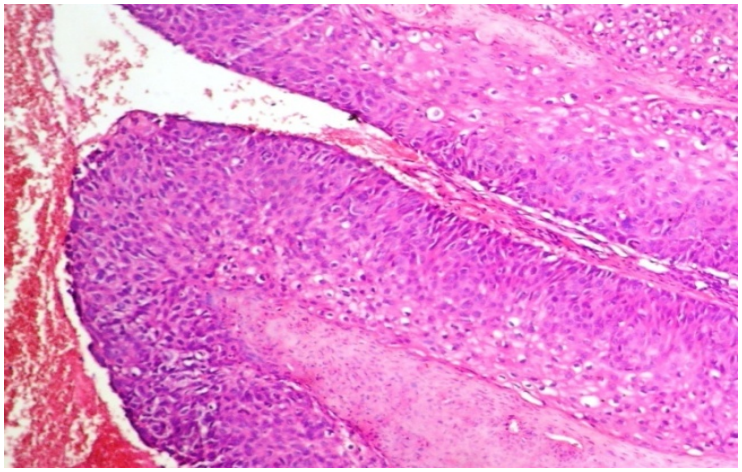


Fig. 2: Carcinoma insitu (CIN III) H&E stain X400

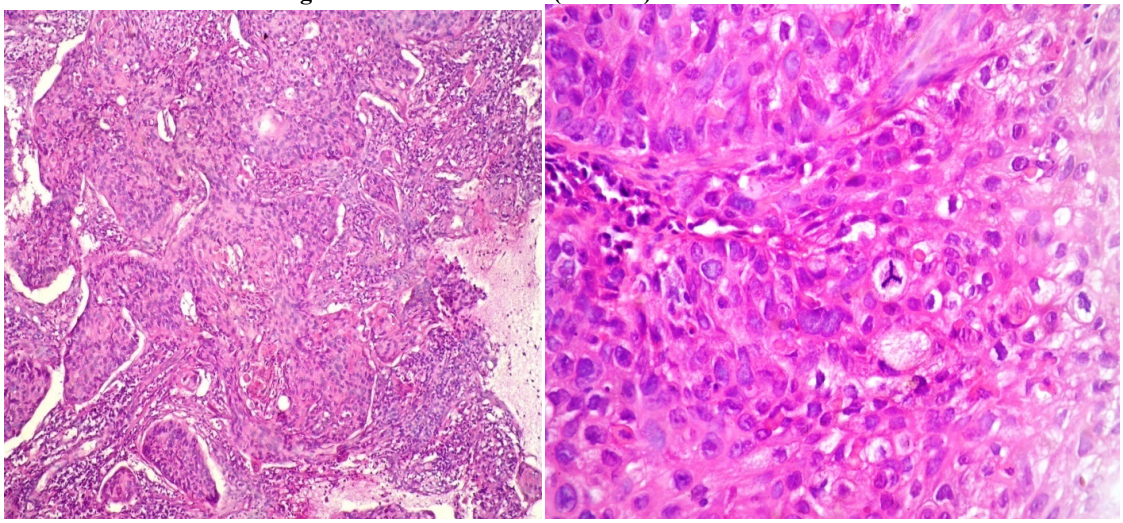


Fig. 3: squamous cell carcinoma of the cervix grade II, H&E stain X 100& X400

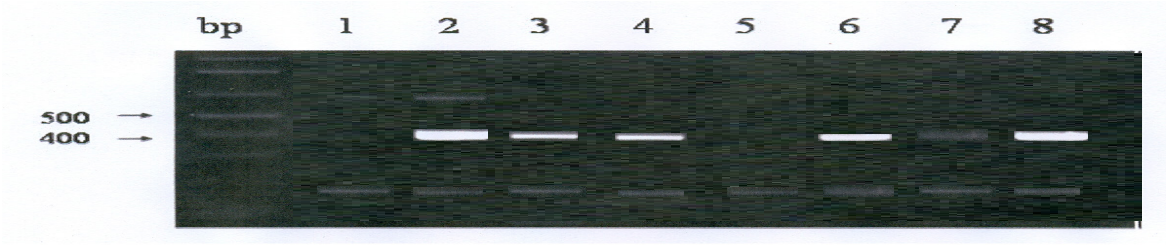
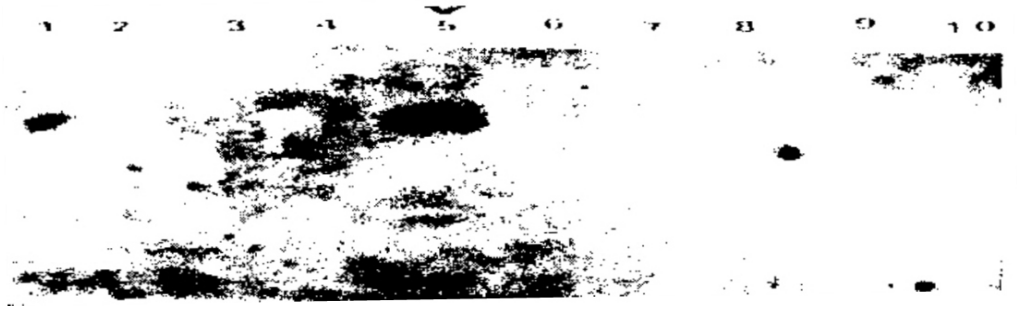


Fig. 4: Agarose gel electrophoresis of amplified DNA fragments corresponding to the L1 capsid protein gene.

Probe 6

Probe 11



Probe 16

Fig. 5: Southern blot autoradiograph showing hyperidization of HPV DNA

3. Results:

Cytological findings:

The control group (30) reveals no cellular abnormalities in all cases. In the study group (60) cytologic diagnosis was low-grade squamous intraepithelial lesions (LGSIL) in 24 and high-grade squamous intraepithelial lesions (HGSIL) in 36 of cases.

Histopathological findings:

No significant histopathological changes were seen in the control group (30). In the study group, histopathology confirmed the diagnosis of LGSIL in all 24 cases which classified as 15 flat condyloma & 9 CINI. While in HGSIL cases, histopathology proved the diagnosis in 30 out 36 case as following: 9 CINII, 1 CINIII, 20 carcinoma (16 SCC& 4 AD). The remaining 6 cases were diagnosed as LGSIL (4 flat condyloma, & 2 CINI)

HPV DNA analysis:

HPV DNA was detected in 76.7% of patients while it was detected in 10% of the control group. Age and duration of marriage showed no significant difference between HPV-DNA positive and negative cases (t=0.08&0.35 respectively; p>0.05).

HPV DNA was detected in 84.2% of patients with flat condyloma lesion of cervix. In CIN group HPV DNA was positive in 15/21 (71.4%) [CINI: 7/11 (63.6 %), CIN II: 7/ 9 (77.8%)] and the only case of CIN III was positive. Among the group with cancer cervix, HPV-DNA was detected in 15/20 (75%); squamous cell carcinoma were positive in 11/16 (68.8%), while all adenocarcinoma cases were positive for the HPV DNA (4/4) (Table 1).

HPV gynotyping

The genotype distribution among different groups is shown in table 1.

DNA sequences of high-risk HPV types by used probes (HPV16&18) were detected in 23/43 (53.5%) of patients groups while low-risk HPV types (HPV6&11) were present in 20/43 (46.5%). The remaining 3 untyped cases by the used probes were excluded (Table2).

All flat condyloma cases carried low risk types except for a case (6.2%) carrying the high risk type (HPV 16). Among the CIN group, 7/13 (53.8%) of the positive HPV DNA cases carried high risk types [CINI: 2/6 (33.3%), CINII: 4/6 (66.7%), CINIII: 1/1 (100%)]. While all cancer cervix group carried high risk genotypes.

Majority of squamous cell carcinoma (90.9%) had a single infection with HPV type 16, while adenocarcinoma cases showed infection with HPV type 18 only.

Three mixed infections were detected among 3 cases; one case of flat condyloma carrying HPV 6&11; one case with CINI showing 6&11 types and one case of SCC revealed types (16&18). The distribution of HPV genotypes (high or low-risk) among different patients groups showed a high statistical significant difference (X^2) = 24.6; p=0.0001; Table2).

Sequencing

The genotype of all positive cases for the HPV DNA were further analyzed & confirmed by sequencing. The three untyped case were excluded.

Table (1): HPV-DNA PCR and genotyping in patients and controls

Groups (n)	PCR		HPV-6 (14) n(%)	HPV-11 (7) n(%)	HPV-16 (17) n(%)	HPV-18 (5) n(%)	#Mixed (3) N (%)	•Untyped (3) n(%)
	Positive (49) n (%)	Negative (41) n (%)						
FC(19)	16(84.2)	3(15.8)	10(62.6)	3(18.8)	1(6.2)	-	1**(6.2)***	1(**6.2**)
CIN (21)								
CINI(11)	7(63.6)	4(36.4)	1(14.3)	2(28.6)	2(28.6)	-	1(14.3)*	1(14.3)
CINII(9)	7(77.8)	2(22.2)	1(14.3)	1(14.3)	4(57.1)	-		1(14.3)
CINIII(1)	1(100)	-	-		-	1(100)		-
Ca Cx (20)								
SCC(16)	11(68.7)	5(31.2)	-	-	10(90.9)	-	1(9.1)**	-
Adeno(4)	4(100)	-	-	-	-	4(100)		-
Control (30)	3(10)	27(90)	2(66.7)	1(33.3)	-	-	-	-

FC: flat condyloma; CIN: cervical intraepithelial neoplasia Ca Cx: cancer cervix; SCC: squamous cell carcinoma; Adeno: adenocarcinoma
#Mixed infection: * infection with (6 &11) genotypes ** infection with 16&18 genotypes ●untyped types by used probes were excluded

Table (2): High and low risk-HPV genotypes distribution among patients carrying HPV DNA

Groups (43)* (n)	Low Risk(20) n(%)	High Risk(23) n(%)	X^2	*P*
FC (15)	14(93.3)	1(6.7)		
CIN (13)				
CINI(6)	4(66.7)	2(33.3)		
CINII(6)	2(33.3)	4(66.7)		
CINIII(1)	-	1(100)		
Cancer Cx (15)				
SCC(11)	-	11(100)		
Adeno(4)	-	4(100)	24.6	0.0001

FC: flat condyloma; CIN: cervical intraepithelial neoplasia Ca Cx: cancer cervix; SCC: squamous cell carcinoma; Adeno: adenocarcinoma
*untyped cases by used probes were excluded

4. Discussion

Human Papilloma Virus (HPV) is becoming a menace worldwide, especially to the developing world, due to its involvement in a variety of malignancies, with cervical cancer being the most important and prevalent (17). There are many HPV genotypes; HPV 16&18 are the most carcinogenic. They can cause a variety of low or high-grade cellular abnormalities, most frequently detected in a routine Pap test. On the other hand, low-risk HPV types (LRHPV), as 6 and 11, are mostly associated with benign genital lesions and rarely progress to cancer (18).

Our results confirm the high prevalence of HPV infection among women with genital lesions showing abnormal pathology, as the HPV-DNA was detected in 76.7% of study group. It was positive in 84.2% of flat condyloma; in 63.6 % of CINI; 77.8% of CINII. While, it was detected in the case with CINIII and 75% cancer cervix. This results indicate that the prevalence of HPV infection increased with the severity of cervical lesions (2).This is in agreement with Kroupis & Vourlidis and Castle et.al(6,19) who consider HPV to be the main etiological factor for the development of cervical intraepithelial neoplasia (CIN) and cervical cancer.

HPV-DNA negative cases in this work were 25% of cervical cancers, 28.6% of CIN cases and 15.8% of flat condyloma. These results represent true negative samples for HPV-DNA as the success of amplification process with an internal control β-globin support the integrity of DNA and confirm absence of PCR inhibitors (7).

This study showed that age and duration of marriage have no significant association with HPV infection. On the contrary, other studies showed that age and sexual behavior are key risk factors for HPV infection (10, 17). This discrepancy may be explained by the difference in the study population and cultural behavior.

In the current study, HPV-DNA was detected in 10% of the control. Their molecular genotyping showed low risk types 6&11. These results strongly suggest that these cases represent subclinical or latent infection. This is in agreement with a previous studies who stated different rates of HPV detection in normal cervical samples (14,20,21). In cases with normal cervical cytology, HPVDNA was detected in a wide range from 3 to 34.3% (6,10) . This wide range is explained by the different nature of participating populations in such studies, and by technical evolutions in the diagnostic tests used (11). Others added that these women should be considered as having a real risk for progression to abnormal cytological findings (22).

HPV genotypes are classified according to their neoplastic ability as high-risk and low-risk types. High-risk HPV types 16,18,31and 33 are associated with cervical cancer or advanced precancerous stages CIN II and III, which are cytologically characterized as high-grade squamous intraepithelial lesions (HGSIL) (14). On the other hand, low-risk HPV types 6&11 are mostly associated with benign genital lesions such as flat condyloma and CINI, characterized cytologically as low-grade squamous intraepithelial lesions (LGSIL) which rarely progress to cancer (4).

Similarly in this study, the molecular genotyping to HPV-DNA in flat condyloma revealed low risk HPV types in 93.3% of patients while only one case carries high risk type . These results are similar to those reported that the majority of the flat condyloma are associated with HPV-6 and 11 (7). While the case carrying the high risk genotype should be considered as having a real risk for progression to cervical neoplasia (21). Thus, these women should be prospectively followed up by their gynecologist and submitted to appropriate testing cytology, colposcopy and other (23).

In the current report, molecular genotyping revealed that there is high incidence of the high risk HPV genotypes in precancerous CINII and III & cancer cervix. HPV-16 was found to be more predominant in squamous cell carcinoma while HPV-18 was the predominant type in adenocarcinoma. This is in accordance with previous results of Kjaer (24) denoting that different genotypes have different pathologic potentials.

Furthermore, our results are in agreement with a previous study showing that low- risk HPV were seldom detected in invasive cancers (2). On the other hand other reports showed the presence of low risk HPV associated to malignant tumors (25). This could be explained by geographic distribution which is an important variable for the prevalence of a particular type of HPV in CIN and cancer cervix.

In this study, single or multiple infection with high risk oncogenic types (16 or 18) were strongly associated with the diagnosis of invasive carcinoma. This is in concordance with previous publications (3, 23)

In conclusion, HPV infection was found in a large proportion of the population and was more associated with CIN II and III lesions and infiltrating carcinomas. This is indicative of a largely unscreened population thus the effect of introduction of newer techniques in population screening is a matter of intense research. HPV DNA detection and genptyping method could be useful for classifying oncogenic HPV and serve as a valuable tool in monitoring of HPV-related disease with a higher sensitivity, reliability and is relatively inexpensive.

Thus, pointing to the importance for developing preventive protocols and appropriate intervention targets. Furthermore, prophylactic vaccines for HPV 16/18 may be efficient and raise high expectations for the complete eradication of these types in the future.

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Antimicrobial Activity of Onion Juice (*Allium cepa*), Honey, And Onion-Honey Mixture on Some Sensitive and Multi-Resistant Microorganisms

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Abstract: The study conducted here to analyze the antimicrobial activity of onion juice alone which extracted from red Egyptian onion, honey alone (Langaneza honey, Black Forest) and honey-onion mixture (v/v: 1/1, 1/4, 4/1) with different concentrations 100 , 50 , 20 and 10% respectively, against 8 microbial species, *Streptococcus pyogenes* ATCC 19615, *Staphylococcus aureus*; (Methicillin- Sensitive *Staphylococcus aureus* - MSSA) ATCC 25923, (Methicillin-Resistant *Staphylococcus aureus* -MRSA) ATCC 10442, *Enterococcus faecalis*; (Vancomycin -Sensitive *Enterococci*-VSE) ATCC 29212, (Vancomycin - Resistant Enterococci-VRE) ATCC 51299, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10291 were investigated by broth dilution method. The results showed that onion juice at 100%, 50%, 20% and 10% concentration have a very strong effect on the growth of all tested species of microbes comparing with control and *Staphylococcus aureus* was the most sensitive microbe. Moreover, Honey at 100, 50, 20 and 10% concentration have a very strong effect on the growth of all species of microbes but significantly less than the effect of onion juice. When studying the effects of the onion- honey mixture with different concentrations, it became clear that the mixture (1/1) had a very noticeable effect on all species of examined microbes.. Results also showed that the honey-onion mixture was significantly more effective comparing with onion or honey alone.
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Key word: Onion, Honey, Onion- Honey mixture, Antimicrobial activity, MRSA, MSSA, VSE, VRE.

1. Introduction

The onion is one of the oldest cultivated vegetables in history. It is thought that bulbs from the onion family have been utilized as a food source for Millennia **(Azu and Onyeagba , 2007).** A number of studies have proven that onion having antibacterial and antifungal properties, and the potential use of onion against human pathogenic organisms. **(Hughes and Lawson, 1991; Augusti, 1996; Adeleye and Opiah, 2003; Amin and Kapadnis, 2005; Sohail *et al.,* 2011).** It is a rich source of flavonoids, polyphenols, organic sulfur, saponins and many other secondary metabolites, which are mainly responsible for its medicinal activities **(Sohail *et al.,* 2011).**In addition, the inhibitory effect of onion oil was demonstrated against the growth of various isolates of bacteria representing Gram-positive (four isolates), Gram-negative (four isolates) species and nine different species of dermatophytic fungi. The results showed that onion oil was highly active against all Gram-positive bacteria tested and only one isolate (*Klebsiella pneumoniae*) of Gram-negative bacteria, while all fungi inhibited at different concentrations **(Zohri *et al.,* 1995).** The antibacterial activity of onion extracts was studied on *Streptococcus mutans* and *Streptococcus sobrinus*, the results showed that the onion extracts possess an effect on all test bacterial strains and the effects were bactericidal. **(Kim, 1997). Mboto *et al*., 2009** found that a combination of medicinal plants like *G. kola* and *V. amygdalina extracts* suspended in honey inhibits the growth of some microbe's showing stronger effect than that observed by honey alone or medicinal plants alone.

2.Materials and Methods

Honey source and type

One brand of commercial honey, called Black Forest honey (Langaneza), Germany, is available in Saudi Arabia (Jeddah), and was used in current study.

Onion source and type

The onion which used in this study was Egyptian red onion (Allium cepa).

Microbial strains*:*

Six strains of standard microbes; *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus pyogenes* ATCC 19615, *Staphylococcus aureus*; (Methicillin-Sensitive *Staphylococcus aureus* - MSSA) ATCC 25923, (Methicillin-Resistant *Staphylococcus aureus*-MRSA) ATCC 10442, *Enterococcus faecalis*; (Vancomycin-Sensitive *Enterococci* - VSE) ATCC 29212, (Vancomycin - Resistant *Enterococci* - VRE) ATCC 51299 and *Candida albicans* ATCC 10291. All strains were collected from the Microbiology lab at King Khaled National Guard Hospital and King Abdulaziz University hospital in Jeddah, Saudi Arabia.

Preparation of *Allium cepa* extract

Fresh *Allium cepa* bulbs were rinsed thoroughly in distilled water and air dried; 200 grams were then blended. The resulting paste was allowed to

stand for 24 hours. Juice was then filtrated and squeezed out of it. The extract was stored bellow 4°C. **(Nelson** *et al*., 2007; Ige *et al***., 2009).**

Media used

Nutrient agar (Oxoid), Nutrient broth (Oxoid), and Blood agar (Oxoid) were used in this study.

3-Assay of antibacterial activity

The antibacterial effect of onion, honey and honey-onion mixture was determined by broth dilution method (**Al-Masaudi and Al-Bureikan, 2010**).

The broth Dilution Method

A - Five ml of different concentrations (100, 50,20 and 10%) of (onion or honey) were prepared in Nutrient broth in test tubes. All the tubes were inoculated with 0.1 ml of Over Night culture of the tested organisms 1.5×10^6 cfu/ml. The tubes were incubated at 37^o C for 24 hrs and serial dilutions were made using sterile Nutrient broth and counts were determined as cfu/ml using Nutrient agar plate count (**Steve & Dennis, 2001**).

B - Onion- honey mixture was prepared in different volumes (1:1), (1:4) and (4:1). Then, every volume prepared with different concentrations in Nutrient broth (100, 50, 20 and 10%) in test tubes.

3.Results and Discussion

Table (1) illustrates the antimicrobial effect of different concentrations of onion on the tested microorganisms. At 100% and 50% concentration of onion, the results showed no growth, while when we use 20% concentration of onion the result showed growth of some microbial strains. However, at 10% concentration of onion all microorganisms were grown. Despite the appearance of growth at a concentrations of 20% and 10% of onion extract the growth was significantly less than the control sample.The results clearly showed that Onion had an antimicrobial activity against all tested microorganisms at different concentrations, and it reduced the growth significantly comparing with control which is agree with many previous studies **(Hughes & Lawson, 1991; Augusti, 1996; Adeleye & Opiah, 2003 ;Amin & Kapadnis, 2005; N. C. Azu, *et al*., 2007; Nelson and Onyeagba, 2007;** Nelson *et al*., 2007**; Watson, 2008; Hannan *et al*., 2010; Sohail *et al*., 2011).** Onion has clear effect on Gram-positive, Gram-negative and pathogenic yeast this result was not in agreement with **Adeleye & Opiah, 2003; Azu *et al*., 2007b** who proved that gram negative bacteria affected by Onion more than gram positive. The Welsh onion ethanol extracts were tested for their inhibitory activity against the growth and aflatoxins production of *Aspergillus flavus* and *A. parasiticus* where the results showed that the extracts have inhibitory effect toward aflatoxins production than the preservatives sorbate and propionate at pH values near 6.**5 (Fan and Chen, 1999). Azu *et al*., 2007** estimated the antibacterial activity of raw and aqueous extracts of onions against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, (from high vaginal swab) that are common cause of nosocomial (hospital-acquired) and urinary tract infections investigated using the cup-plate diffusion method, the result showed that *Pseudomonas aeruginosa* was more sensitive to the extract of onion bulbs compared to *Staphylococcus aureus*.). Moreover, onion has antimicrobial effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from High Vaginal Swab **(**Nelson *et al*., 2007)**.** Also, in other study onion (*Allium cepa*) showed antibacterial effect against *B. subtilis*, *Salmonella* sp. and *E. coli* **(Winston, 2008). Hannan *et al*., 2010** investigated the antimicrobial potential of onion against thirty-three clinical isolates of *Vibrio cholera;* the results indicated that onion (*Allium cepa*) has an inhibitory effect on *V. cholerae*

Table 1: Effect of onion juice on the microbial count (cfu/mℓ) of different pathogenic organisms by dilution method.

Total plate count at different concentrations of onion.					
Organism	Con.	Different concentrations of Onion (%)			
		100	50	20	10
S.pyogenes	2.3×10^8	0	0	4.5×10^4	7.4×10^7
MSSA	7.8×10^8	0	0	0	3.2×10^6
MRSA	7.7×10^8	0	0	0	2.9×10^6
VSE	4.3×10^8	0	0	7.5×10^4	8.1×10^7
VRE	3.4×10^8	0	0	4.3×10^4	5.8×10^7
E.coli	1.5×10^9	0	0	0	6.8×10^8
P. aeruginosa	4.8×10^8	0	0	6.8×10^4	8.8×10^7
C. albicans	7.5×10^6	0	0	2.7×10^5	7.5×10^6

MSSA, (*Methicillin-Sensitive Staphylococcus aureus*); MRSA, (*Methicillin-Resistant Staphylococcus aureus*); VSE, (*vancomycin -sensitive enterococci*); VRE, (*vancomycin - resistant enterococci*)

Table (2) demonstrates the activity of different concentrations of Langaneza Black Forest honey against eight microorganisms. *Staphylococcus aureus* was the most affected microbe, while *Enterococcus faecalis* was the least affected organism. The results showed that honey has antimicrobial effect on all tested microorganisms at different concentrations which was in full agreement with the study performed by **Al-Masaud & Al-Bureikan, 2010; Manyi-Loh *et al.*, 2010b; Olawuyi *et al.*, 2010; Al-Waili *et al.*, 2011; Halawani & Shohayeb, 2011; Aurongzeb & Azim, 2011.** Moreover, results showed that honey significantly has similar antibacterial effect on sensitive and resistance microbes either MSSA or MRSA and VSA or VRE and this result confirms the results obtained by **Bilal & Alfalki, 1998; Cooper *et al.*, 2002; Al-Masaud & Al-Bureikan, 2010.** It could be also noted that *Candida albicans* was highly affected by honey and this is in accordance of **Zaghlooul *et al.*, 2001,** but not agree with **Lusby *et al.*,2005** who measured the antimicrobial effect of three kinds of honey on different organisms and found that *Candida albicans* did not affected with all kinds of honey. This may be attributed to use different method and different sources of honey. The effect of honey could be antibacterial or antifungal effect **(Nzeako and Hamdi, 2000;Taormina *et al.*, 2001; Al-Jabri *et al.*, 2003; Iurlina and Fritz, 2005; Lusby *et al.*, 2005; Manyi-Loh-*et al.*, 2011; Aurongzeb and Azim, 2011).** Honey is known to contain phenol, fatty acids, lipids, amylases, ascorbic acid, peroxidases and fructose and has high osmolarity and low pH. These elements acting alone or synergistically may contribute significantly to the antimicrobial activity of honey **(Al-jabri, 2005).** Moreover, honey has similar antibacterial effect on sensitive and resistance microbes either MSSA or MRSA and VSA or VRE **(Bilal and Alfalki, 1998; Cooper *et al.*, 2002enkins *et al.*, 2011).** In addition, **Zaghlooul *et al.*, 2001** proved that *Candida albicans* affected by honey. Honey has anti-*Helicobacter pylori* activity (**Manyi-Loh *et al* 2010b**). **AL-Masaudi and Al-Bureikan, 2010** proved that honey has antimicrobial effect against *Streptococcus pyogenes*, *Staphylococcus aureus;*results of the dilution method showed that all kinds of honey had a very clear effect on all types of microbes with different concentrations. *Candida albicans* was the least microbe affected by the different kinds of honey with different concentrations even at 100% concentration. On the other hand, *Staphylococcus aureus* and *Streptococcus pyogenes* were the most sensitive microbes. When studying the antimicrobial effect of honey against microbes at concentration of 50%, it was found that some kinds of honey had static effect or cidal effect on different species. Comparing the effects of honey on resistance and sensitive *Staphylococcus aureus* **(MSSA and MRSA), results showed that there was no** significant difference in the effect of honey on both MSSA and MRSA tested strains. The results of the diffusion method exhibited a contrast in the sensitivity of microbes. It was recommended to use the dilution method instead of diffusion method because it gave the real effect at different concentrations of honey **(Al-Masaudi and Al-Bureikan, 2010).**

Table 2: Effect of honey on the microbial count (cfu/mℓ) of different pathogenic organisms by dilution method.

Total plate count at different concentrations of honey.					
Organism	Con.	L.B.F Langaneza Black Forest honey (%)			
		100	50	20	10
S.pyogenes	2.3×10^8	0	1.04×10^4	2.3×10^5	2.4×10^7
MSSA	7.8×10^8	0	0	0	2.4×10^3
MRSA	7.7×10^8	0	0	0	4.8×10^4
VSE	4.3×10^8	0	8.2×10^5	1.83×10^6	1.17×10^8
VRE	3.4×10^8	0	2.3×10^5	1.29×10^6	2.4×10^7
E.coli	1.5×10^9	0	0	0	2.35×10^8
P.aeruginosa	4.8×10^8	0	0	1.6×10^3	4.1×10^7
C . albicans	7.5×10^6	0	0	2.3×10^6	3.3×10^6

MSSA , (*Methicillin- Sensitive Staphylococcus aureus*) ; MRSA , (*Methicillin-Resistant Staphylococcus aureus*) ; VSE, (*vancomycin -sensitive enterococci*) ; VRE , (*vancomycin - resistant enterococci*)

Tables (3- 5) illustrate that onion - honey mixture (v/v: 1:1, 4:1, 1:4) have antimicrobial activity on all tested microorganisms with different concentrations 100, 50, 20, and 10%. The results showed that onion - honey mixture (1:1) at concentrations of 100, 50 and 20% showed significantly the best antimicrobial effect on all tested organisms. Moreover, at a concentration of 10% the onion - honey mixture (1:1) exhibited significantly stronger antimicrobial activity on all tested organisms than onion alone and honey alone.

VRE and VSE were significantly affected by onion - honey mixture (1:1) more than onion alone and honey alone. However, the onion - honey mixture (1:1) significantly has the pest effect than onion - honey mixture (4:1, 1:4) on all organisms.

Table 3: Effect of onion - honey mixture (1:1) on the microbial count (cfu/mℓ) of different laboratory organisms by dilution method

Total plate count at different concentrations of onion - honey mixture (1:1).					
Organism	Con.	Different concentrations onion - honey mixture (%)			
		100	50	20	10
S.pyogenes	2.3×10^8	0	0	2.9×10^3	2.7×10^6
MSSA	7.8×10^8	0	0	0	2.6×10^3
MRSA	7.7×10^8	0	0	0	2.2×10^3
VSE	4.3×10^8	0	0	0	4.1×10^4
VRE	3.4×10^8	0	0	0	2.8×10^4
E.coli	1.5×10^9	0	0	0	4.2×10^7
P. aeruginosa	4.8×10^8	0	0	2.1×10^3	3.8×10^7
C. albicans	7.5×10^6	0	0	2.4×10^5	5.4×10^6

MSSA , (*Methicillin- Sensitive Staphylococcus aureus*) ; MRSA , (*Methicillin-Resistant Staphylococcus aureus*) ; VSE , (*vancomycin -sensitive enterococci*) ; VRE , (*vancomycin - resistant enterococci*)

Table 4: Effect of onion - honey mixture (4:1) on the microbial count (cfu/mℓ) of different laboratory organisms by dilution method

Total plate count at different concentrations of onion - honey mixture (4:1).					
Organism	Con.	Different concentrations onion - honey mixture (%)			
		100	50	20	10
S.pyogenes	2.3×10^8	0	0	5.5×10^4	8.7×10^7
MSSA	7.8×10^8	0	0	0	4.1×10^6
MRSA	7.7×10^8	0	0	0	3.4×10^6
VSE	4.3×10^8	0	0	6.2×10^3	8.9×10^7
VRE	3.4×10^8	0	0	5.4×10^3	6.8×10^7
E.coli	1.5×10^9	0	0	0	6.1×10^8
P. aeruginosa	4.8×10^8	0	0	4.2×10^4	6.3×10^7
C. albicans	7.5×10^6	0	0	3.7×10^5	8.2×10^6

MSSA , (*Methicillin- Sensitive Staphylococcus aureus*) ; MRSA , (*Methicillin-Resistant Staphylococcus aureus*) ; VSE , (*vancomycin -sensitive enterococci*) ; VRE , (*vancomycin - resistant enterococci*)

Table 5: Effect of onion - honey mixture (1:4) on the microbial count (cfu/mℓ) of different laboratory organisms by dilution method

Total plate count at different concentrations of onion - honey mixture (1:4).					
Organism	Con.	Different concentrations onion - honey mixture (%)			
		100	50	20	10
S.pyogenes	2.3×10^8	0	2.5×10^3	6.7×10^4	3.1×10^7
MSSA	7.8×10^8	0	0	0	3.9×10^3
MRSA	7.7×10^8	0	0	0	4.3×10^3
VSE	4.3×10^8	0	0	2.8×10^4	1.05×10^5
VRE	3.4×10^8	0	0	2.2×10^4	4.7×10^5
E.coli	1.5×10^9	0	0	0	8.9×10^7
P. aeruginosa	4.8×10^8	0	0	5.5×10^4	9.1×10^7
C. albicans	7.5×10^6	0	0	5.3×10^5	9.8×10^6

MSSA , (*Methicillin- Sensitive Staphylococcus aureus*) ; MRSA , (*Methicillin-Resistant Staphylococcus aureus*) ; VSE , (*vancomycin -sensitive enterococci*) ; VRE , (*vancomycin - resistant enterococci*)

Our results showed that the antimicrobial activity of onion was significantly stronger on tested microbes than honey, but when we use the onion and honey mixtures especially (v/v: 1:1) it becomes clear that the

onion and honey mixtures have stronger effect on most microbes than onion alone or honey alone. This results agree with (Osman *et al,* 2003) who used combination of honey plus some natural additives and they found superior results with honey compound in its antibacterial, antifungal, and wound-healing promotion properties compared with pure bee honey and some other topical wound agents. Also, our results agree with **Al-Jabri *et al*. (2005a)** who found that the combination of honey and bovine milk had stronger antimicrobial effect than honey alone or bovine milk alone. The obtained results are in agreement with **Al-Jabri *et al*. (2005b)** who found that combination of honey and gentamicin had stronger antimicrobial effect than honey alone or gentamicin alone. **Mboto *et al*., 2009** also found that a combination of medicinal plants like *G. kola* and *V. amygdalina extracts* suspended in honey inhibits the growth of some microbe's showing stronger effect than that observed by honey alone or medicinal plants alone.

From our experience, it became clear that the combination of onion- honey (1v/1v) has stronger effect on microorganisms than onion alone or honey alone.The conclusion of this result could be explained in two ways. The first way, as we noted previously in our results, onion has antimicrobial effect against tested microbes because it is a rich source of flavonoids, polyphenols, organic sulfur, saponins and many other secondary metabolites, which are mainly responsible for its medicinal activities. Honey, also, has antimicrobial effect against tested microbes because it is known to contain phenol, fatty acids, lipids, amylases, ascorbic acid, peroxidases and fructose and has high osmotic potential and low pH. These elements (either in onion or in honey) which can acting alone or synergistically may be contribute significantly to the antibacterial activity of the combination of honey and onion which resulting higher growth reduction, enhancing the killing activity. The second way, the antibacterial activity of honey and onion, both have excellent nutritional values and would be an additional enhancer of immunity in aid to the treatments of bacterial infections. Honey in combination with onion may prolong or improves the shelf life of each other. Apparently, with the increasing interest in the use of alternative therapies coupled with the development of antibiotic-resistant bacteria, honey may finally receive its due recognition **(Christy *et al.,* 2011).**

The combination of two or more antibacterial agents has been long accepted in the treatment of some microorganisms **(Al-jabri, 2005).** The combination of honey plus some natural additives has superior results in its antibacterial, antifungal, and wound-healing promotion properties compared with pure bee honey and some other topical wound agents alone (**Osman *et al.,* 2003**). The combination of medicinal plants like *G. kola* and *V. amygdalina extracts* suspended in honey inhibits the growth of some microbes stronger than honey alone or medicinal plants alone **(Mboto *et al*., 2009).** When honey mixed with an antibiotic it had best killing effect within half an hour of exposure to bacteria than either an antibiotic or honey used alone **(Al-Jabri *et al*., 2005a).** Synergy is known to exist between penicillin and streptomycin and between sulphamethoxazole and trimethoprim, between honey and gentamicin and between honey and milk **(Al-jabri, 2005).**

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Morphogenetic abnormalities of *Musca domestica* vicina induced by glycosidic groups from *Calotropis procera* plant

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Abstract: Latex samples were collected under cold ethanol (95%) from *Calotropis procera* plant which was obtained from border desert districts of Jeddah City, Saudi Arabia. The extraction of latex for its glycosidic groups was carried out by soaking or soxhelt extraction by using several solvents with different polarity. The contents were then separated by alumina – charcoal column chromatography with several solvent systems (chloroform, chloroform: ethylacetate (3:1, 1:1, 1:3) and ethylacetate. The pure components were tested against *Musca domestica* larvae to demonstrate its toxic effects on the morphogentic characters of the developmental stages. The present investigation revealed that the morphogentic aberrations has been induced by all the used plant extracts when applied topically on early 3rd larval instar of *M. domestica*.
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Key words: Structure – house fly – developmental stages – botanicals – toxicity – extraction – chemical components – chromatography.

1. Introduction

There is ample evidence to show that the plant kingdom is a vast store-house of chemical substances manufactured and used by plants in their own defense from attack by insects, bacteria, fungi and viruses. Many authors isolated and identified more other substances affected the growth of insect pests.

Much of the efforts to develop these nontoxic, safe and biodegradable natural products, have been concerned by their use as antifeedants that influence chemosensory behavior of insects growth regulators and growth inhibitors (**Deshmukh and Renaparkar, 1987**) that act upon the physiological processes of insects and as agents of strong fecundity reducing effects (**El-Zoghby *et al*., 1985**).

These substances may act as brain hormone action (**Kobayashi *et al*., 1962**), ovicide and larval growth inhibitor (**Nakajima and Kawazu, 1982**), growth regulators (**Reynold *et al*., 1984; Deshmukh and Renapurkar, 1987; and Coffelt and Schultz, 1988**), substances with juvenile hormone activity (**Bowers, 1968; Bowers and Nishida, 1980**), alkaloids (**El-Gayar *et al*., 1979 and Saxena *et al*., 1986**) and substance with moulting hormone activity (**El-Zoghby *et al*., 1985**).

Successful fly control is achieved when various methods are integrated in an overall program. Modern control of *Musca domestica* involve three interrelated approaches, namely source reduction, use of biological control methods, and control with insecticides. Conventional insecticides play an important role in the overall fly suppression programs. However, the intensive use of insecticides been challenged, especially by the house fly, and by the development of resistance of these chemicals, (**Abudulai *et al*., 2001**). Botanical insecticides, microbial pesticides and anti parasitic are highly effective, safe and ecologically acceptable (**Weinzbrt and Henn, 1991; Nathan *et al*. ,2005; Nathan and Kalaivani, 2005; Massoud *et al*., 2008; Khatter and Abuldahb, 2010; Gamal and Abuldahb, 2012**). So, the literature has directed our attention to the poisonous plant, *Calotropis procera*. A new steroidal hydroxyl ketone and calopenyl acetate were isolated from an ethanolic extract of *Calotropis procera* fresh flowers with evaporation and partitioning into chloroform and water. The aim of the present study is to isolate the toxic groups of the latex against the house fly *Musca domestica* developmental stages.

2. Material and Methods:

The tested plant (*Calotropis procera*)

This plant was reported to be insecticidal (**Farnsworth *et al*., 1975**). Its latex is very poison and yielded five crystalline bodies; calactin C29 H40 O9, calotoxin C29 H40 O10, calotropin C29 H40 O11, uscharidin C29 H38 O9 and uscharin C31 H41 O8 (**Pharmacological Exp., 1970**).

Extraction and separation of pure groups by soaking method:

Latex samples (300 ml) were immediately collected from the plant and soaked in ethanol 95% (300 ml) for 48 hours at 40-60°C on a thermostatic water bath, then it was filtered off (A). The latex coagulate was resoaked in ethanol 95% (400 ml) in the same manner and filtered off (B). The two filtrates (A+B) were mixed together and kept in a

freezer at 0-4°C for 48 hrs as crude latex and filtered off (C), the precipitate (In) was found to be white crystals (5.98 gm, m.p. 84-88°C range). The filtrate (C) was kept under cooling for further 48 hrs and filtered off (D), giving further portion of white crystals (If1) (1.8 gm, m.p. 82-90°C range). It was found that some crystals did not melt up to 290°C The filtrate (D) was also kept under cooling (0-4°C) for three weeks and filtrated off (E) to give pale yellow flakes precipitate II (1.12 gm, m.p. 84-90°C). However, If1, If2 and II portions are considered as solid mixture of crude latex cardenolides.

The filtrate (E) was concentrated to about one third of its volume using rotary evaporator, the concentrate (F) was kept under deep cooling for 48 hrs, giving a brownish yellow precipitate (3.77 gm) which was dissolved in acetone and the insoluble part was separated and dried to give pale yellow crystals (0.8 gm, m.p. 248-249°C). Another portion of concentrate (F) was further concentrated using rotary evaporator, producing an oily brown layer which was filtered off and clear filtrate was kept on water bath at 40-60°C for one hour and then cooled (0-4°C) . Silvery crystals (0.65 gm, m.p. 221.5°C) were produced.

Test insects

a- Sources of colony

Adult susceptible strain of house fly *M. domestica vicina* used in the present study were obtained from well established colony originated from the King Abdulaziz University, Faculty of Science for Girls- Biology Department.

b- Rearing technique

Egg masses were used to maintain a colony in the laboratory under constant conditions of temperature and humidity (27 ± 2°C and 60 ± 10% R.H.). Each egg mass was placed in a clean Petridish (10 cm diameter), previously constant technique described by (**Lewallen, 1954**). Full grown larvae were allowed to pupate in clean glass Petridishes. Following emergence, the adults were provided with a piece of cotton soaked in 10% sugar, 2% milk solution as a source of food.

Treatment of newly emerged 3rd larva instars (2-days old larvae):

Studies were conducted in a rearing chamber at 27± 2°C and 60 ± 10% R.H. Extracted groups (calactin, calotoxin and calotropin) were applied topically on the dorsal surface of newly moulted early 3rd larval instar (twenty five larvae) with different doses of 80 µg/larvae by using Hamilton serange. After application, larvae were put in small plastic cups, 7 cm in diameter, and covered with larval medium . This experiment was replicated three times. The larvae of control groups were treated with 1 µL of the solvent only and replicated two times. Mortality percentage of the treated and control larvae were calculated after 24, 48 and 72 hours and corrected by Abbott′s formula (**Abbott, 1925**). Treated and control larvae were also noticed periodically for moulting disturbances until pupation. Larval pupation and adult emergence were also recorded. All the pupae and adults obtained were collected and checked for abnormalities.

3. Results and Discussion

A. Toxicity of latex groups:

Results of latex crude constituents are shown in table (1). It can be reported that the solvent system of chloroform, methanol (9:1) developed different constituents in the different isolated groups. The groups were found to have similar constituents with RF values equal to 0.48, 0.61 and 0.97; whereas, three other constituents were closely developed near the baseline with RF values equal to 0.03, 0.05 and 0.15. This means that the polarity of the latex three constituents was not compatible with the solvent system used (chloroform: methanol, 9:1). The three fractions in the solvent system of ethyl acetate: ethanol, 96.4 appeared three similar constituents with RF values equal to 0.95, 0.96 and 0.98. The cold ethanolic fraction for 48 hrs and 3 weeks gave two similar constituents with 0.86 and 0.88 RF values in the same solvent system, whereas, the cold fraction for 96 hrs gave also another constituents with RF values equal to 0.75. There are tow similar constituents with RF values equal to 0.1 and 0.12, which indicated the polarity of ethyl acetate: ethanol (96:4) solvent system was not suitable for running them from latex fractions cooled for 48 hrs and 3 weeks, respectively. In case of ethylacetate : ethanol (97:3) solvent system, it was found that one compatible constituents with RF values equal to 0.93 in fraction with cooling for 48 hrs whereas, three constituents with RF values equal to 0.38, 0.75 and 0.97 were found in the fraction with cooling for 96 hrs. In the fraction with cooling for three weeks, two incompatible constituents were developed (RF values 0.1 and o.19) in addition to two suitable constituents with RF values equal to 0.87 and 0.97. So, the development of the different constituents from ethanolic crude isolated extract was based on the suitable solvent used and the ability of solvent for running with the constituents or the compatibility of polarity between the isolated constituents and solvents system used.

B. Morphogenetic abnormalities

The present investigation revealed that the morphogenetic aberrations induced by all the used

plant toxic groups when applied topically on early 3rd larval instars of *M. domestica*. *So,* exhibited various morphological abnormalities in response to all latex groups used. Most of treated larvae were able to form puparia. Yet abnormal ones and other regarding the inability of this early 3rd larval instar accomplish metamorphosis (from a pupae). These larvae become pigmented with block and brown pigment, Fig (1- A, B,C). Similar observation were mentioned by (**Saxena *et al.*, 1981; Schmutterer, 1985 and AL-Sharook, 1991**).

Many investigators described the nature of this phenomenon as follow, the formation of black bodies starts from the idea that the epidermal cells are going to secret the exuvial fluid. This fluid secreted as droplets contain inactivated lytic enzyme systems. These are activated before the cuticulin layer protecting the epidermal cells against digestion is deposited. Therefore not only parts of the endocuticle, but also the epidermis, are digested. The lytic material accumulated in the subcuticular region, where it forms the center of the black bodies. Haemocytes containing further lytic material cluster around developing black body and after flattening, form a coat. The content of these clusters undergo melanization (**Chaieb *et al.* , 2001; Roy *et al.* , 2002 and Mitchell *et al.* , 2004**).

Larval-pupal intermediate stages were also observed. The cuticle of this individual contains pupal parts which parts of still persisting last larval skin. In other cases, the individual completely covered with pupal exuvia, Fig (2- A, B, C).

Similar finding were recorded by (**Jagannadh and Nair, 1992**). Pigmented pupae, small pupae, constricted pupae, deformed pupae which do not produce adults, Fig (3 A, B, C). pupal-adult intermediate also were shown in which this type of deformed individual possess the external character of pupae and has a distinct adult head, (Fig 4).

Incomplete adult eclosion was the most observed frequently. This varied from partial to complete eclosion of adults with legs or wings glued to the puparium. In most cases, only head, or head and part of thorax managed to be release from the puparium. In other cases the head, thorax, part of the abdomen or entire abdomen, and some of the legs emerged, but the adults still attached to the puparium by 1 or more legs; by tarsi or by wing. Partially eclosed adults having crumpled wings were fairly common (Fig 5), (**Ahmed, 1981; Kandil A. M. , 1985 Fouad, 2000; Delco and Gallerani, 2002; Abel-Hady *et al.* , 2005; and Pineda *et al.*, 2007**).

In conclusion, plant extracts are similar to insect ecdysteroid (**Nakanishe, 1975**) and apparently act as an inhibitor of ecdysis (**Kubo and Klocke, 1982**) by influencing the quantity or quality of the "Pool" of moulting hormone (**Rembold *et al.* ,1982**). This mode of action was suggested by the general condition of the larvae deformed by extracts. Many were unable to shed their pupal cuticle completely, those that succeeded often and crumpled or twisted wings, misshapen abdomen and other gross morphological abnormalities.

Table (1): Rf Values and melting points of the crude constituents isolated from latex of *Calotropis procera*.

Isolated groups	Rf Values			m. p (C°)
	Chloroform : Methanol 9 : 1	Ethylacetate : Ethanol 96 : 4	Ethylacetate : Ethanol 97 : 3	
Calactin	0.3, 0.48, 0.61, 0.97	0.1, 0.2, 0.86, 0.95	0.1, 0.93	84 - 88nm.p
Calotoxin	0.05, 0.89	0.75, 0.96	0.38, 0.75, 0.97	82 – 90 m.p
Calotropine	0.15, 0.47, 0.55, 0.62, o.86, 0.97	o.12, 0.21, 0.88, 0.98	0.1, 0.19, 0.87, 0.95	84 – 95 m.p

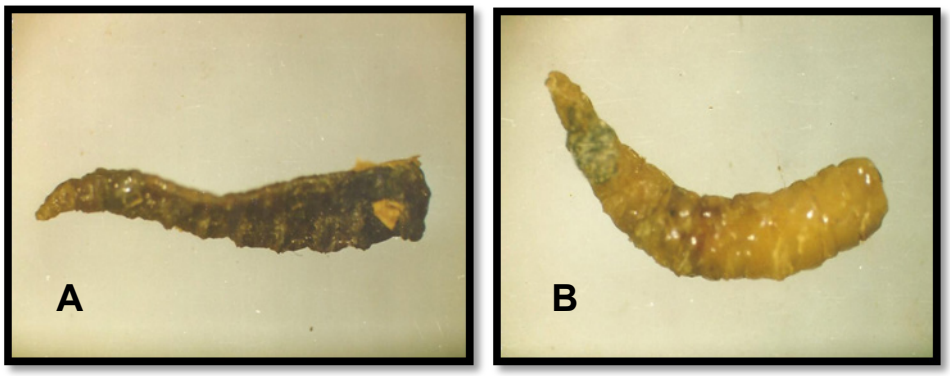

Fig (1): Deformed larvae A- Normal larva of normal size, B- larva of normal size with batches of brown pigment, C- C-shaped larvae pigmented with brown pigment, D- Burned larvae with normal size and shrankage and black pigment. (X = 25)

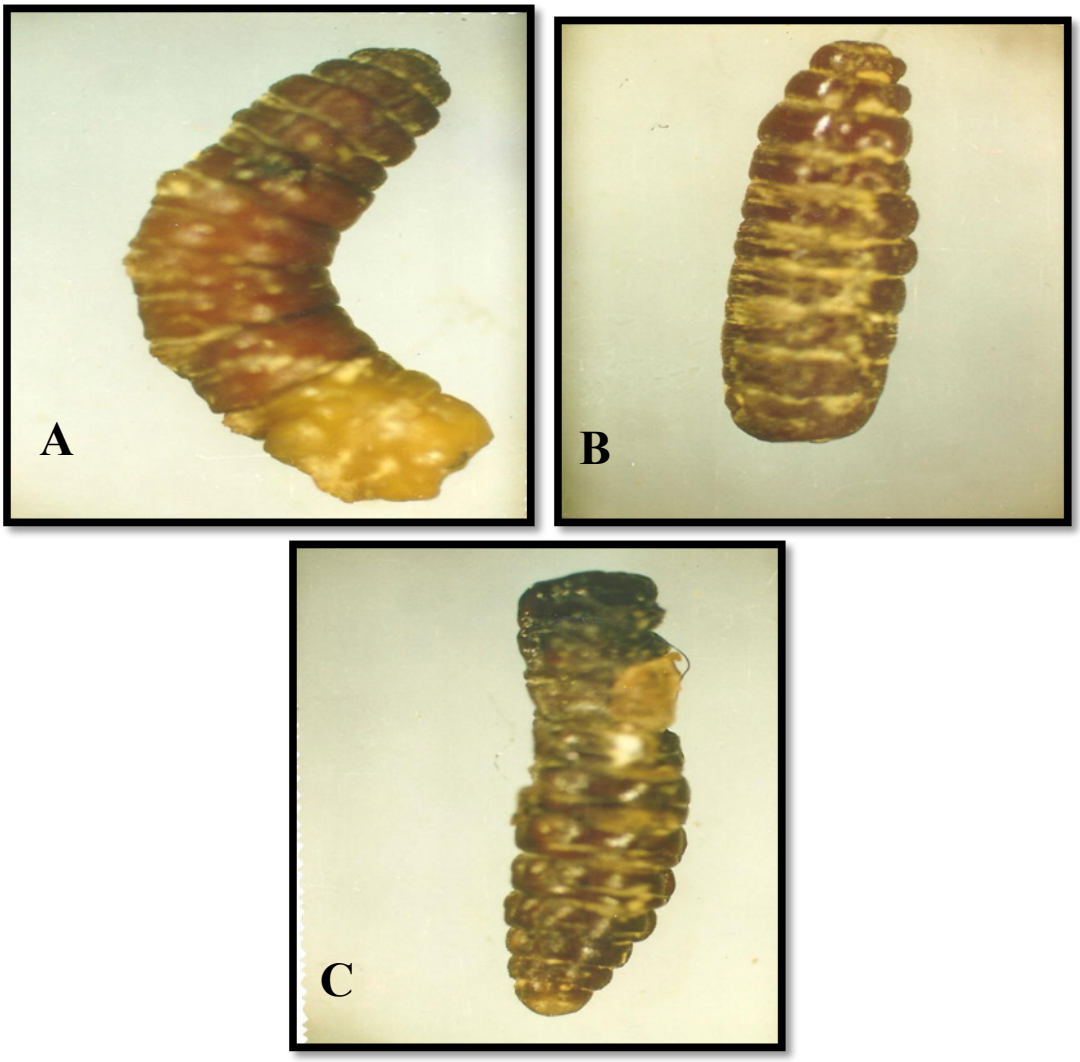

Fig (2): Larval-Pupal Intermediate.
A- C-shaped larval-pupal intermediate, the cuticle of these abnormal individuals contains part which still persisting a last larval instar skin.
B- elongated larval-pupal intermediates with normal color, it has a larviform puparia and intermediated larval cuticle.
C- Pigmented elongated larval-pupal intermediates with segmented larval cuticle (X=25).

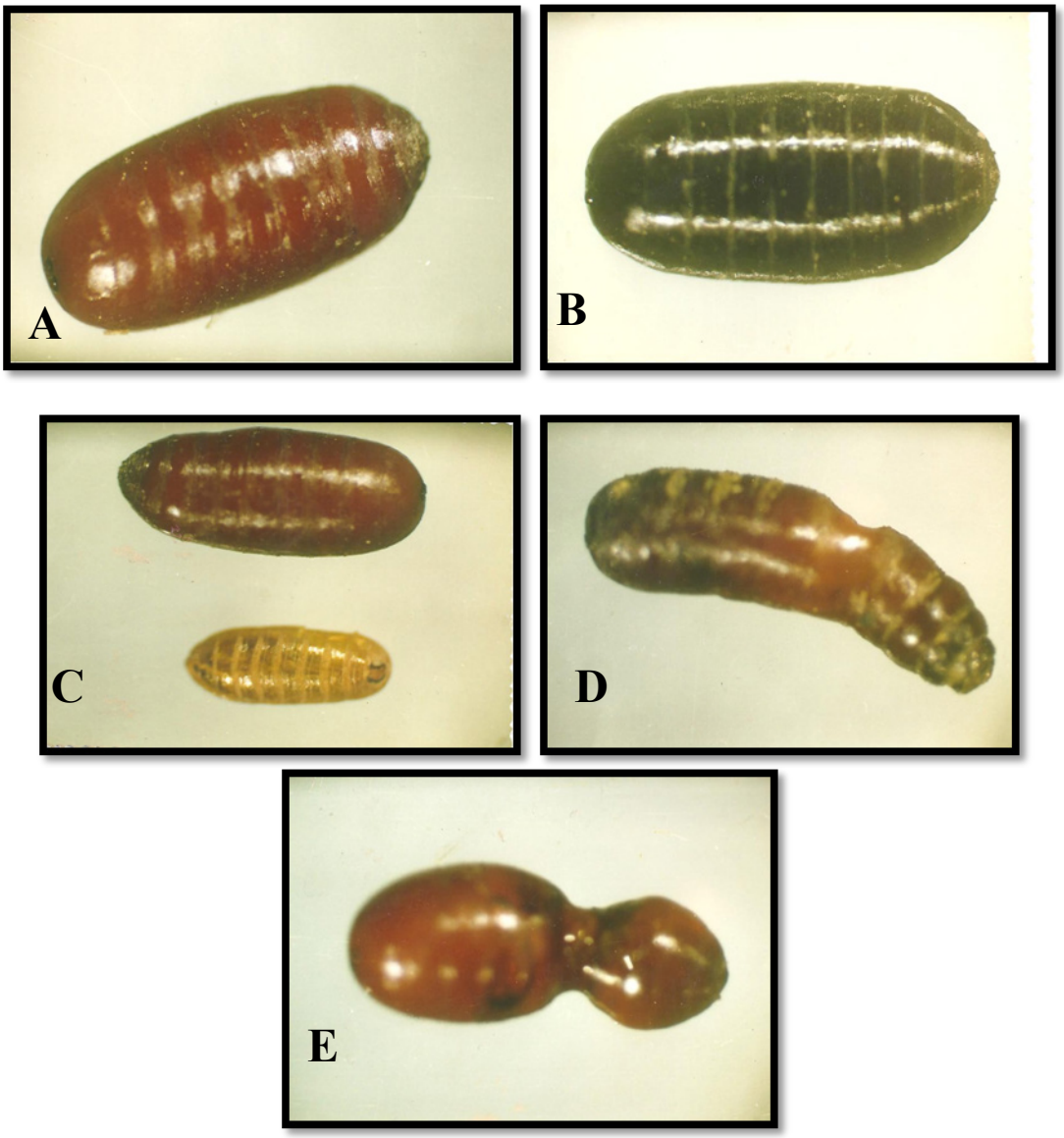

Fig (3): Deformed pupae.

A- Normal pupae.
B- B- Pupae with normal appearance, but pigmented with dark back pigment.
C- Deformed pupae. Small pupae compared with normal one, it has normal appearance but have relatively small size.
D- Fully formed pupae with conspicuous in their puparia, so that they failed to give adult.
E- Elongated melanized pupae with slightly constricted puparia. (X = 25).

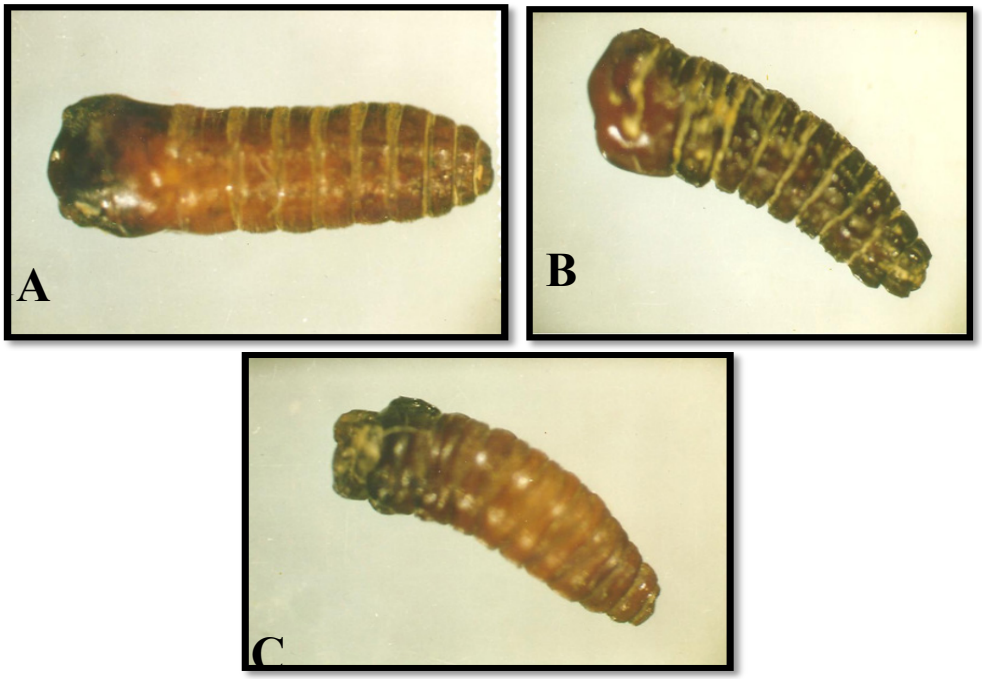

Fig (4) : Pupal – Adult Intermediates.
A- Elongated pupal – adult intermediate with distinct adult head.
B- Twisted pupal – adult intermediate with larviform puparium and distinct adult head.
C- Twisted pupal – adult intermediate with larviform puparium and distinct adult thorax. (X = 25)

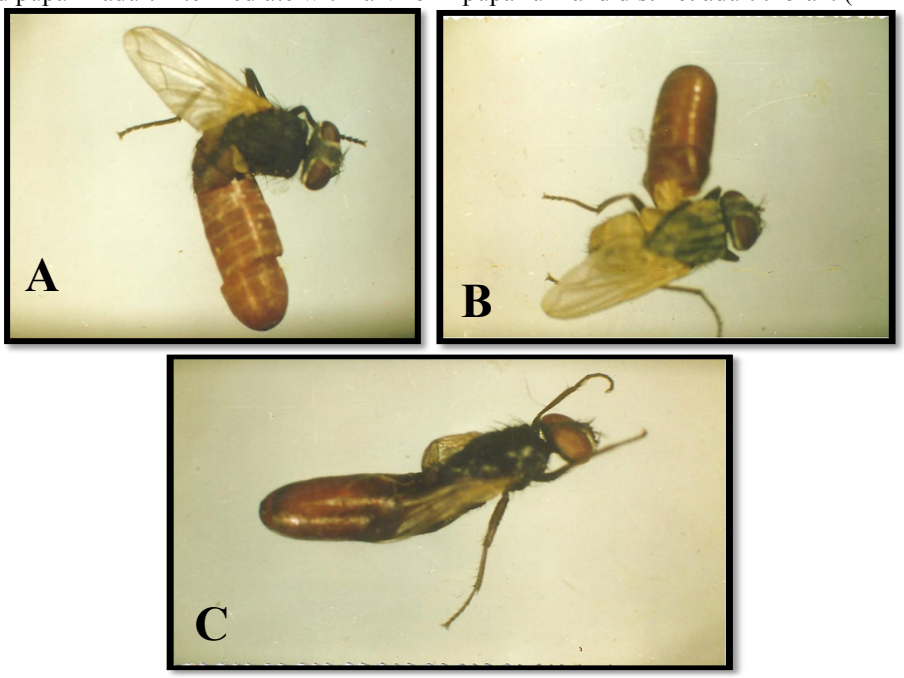

Fig (5): Deformed Adult and incompleted adult eclosion.
A- Head, thorax, one wing, legs of one side only and part of abdomen exuviated, but the remainder of the adult (abdomen, wings and legs of the another side) still retained within the pupal exuvia.
B- Pigmented thorax, crumpled wings and stiff legs are exuvated only. Abdomen and part of the wings are still retain in the pupal exuvia.
C- All the fly eclosed with undeveloped last abdominal segments. Wings and mid leg of one side failed to wirggle out the pupal exuvia. (X= 25)

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Dexmedetomidine versus Propofol for Monitored Anesthesia Care In Patients Undergoing Anterior Segment Ophthalmic Surgery Under Peribulbar Medial Canthus Anesthesia

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Abstract: Objective: Ophthalmic surgery is commonly performed under local anesthesia with propofol sedation. Dexmedetomidine, a sedative-analgesic, is devoid of respiratory depressant effects. This study compared the use of dexmedetomidine and propofol in patients undergoing anterior segment ophthalmic surgery (cataract and glaucoma). **Methods:** One hundred patients undergoing combined cataract and glaucoma surgery under peribulbar anesthesia were divided into two groups. The first group (Group D) received i.v. dexmedetomidine infusion 0.2-0.5µg/kg/min without loading. The second group (Group P) received propofol 25-75µg/kg/min i.v. infusion. Sedation was titrated using Richmond Agitation-Sedation Scale and bispectral index. Mean arterial pressure (MAP), heart rate (HR) oxygen saturation (SPO_2) and respiratory rate (RR) were recorded from the start of the infusion. Readiness for recovery room discharge (time to Aldrete score) and 7-point likert-like verbal rating scale were evaluated postoperatively. **Results**: Both groups provided a similar significant reduction in heart rate and mean arterial pressure compared with baseline. The oxygen saturation values of dexmedetomodine group were higher than those of propofol group. The respiratory rate values of the dexmedetomidine group were higher than those in the propofol group. Postoperatively, the time to achieve an Aldrete score of 10 was higher in propofol group. The patients' satisfaction was higher in the dexmedetomidine group. **Conclusion:** Compared with propofol, dexmedetomidine appears to be suitable for sedation in patients undergoing cataract surgery. While there was a slightly better subjective patient satisfaction, it was accompanied by relative cardiovascular depression and delayed recovery room discharge.

[Ashraf Darwish, Rehab Sami, Mona Raafat, Rashad Aref and Mohamed Hisham. **Dexmedetomidine versus Propofol for Monitored Anesthesia Care In Patients Undergoing Anterior Segment Ophthalmic Surgery Under Peribulbar Medial Canthus Anesthesia.** Life Sci J 2012;9(2):789-793] (ISSN:1097-8135). http://www.lifesciencesite.com. 117

Keywords: Cataract, Dexmedetomidine, Monitored anesthesia care, Propofol.

1. Introduction

Combined surgery is most frequently performed under local anesthesia with monitored anesthesia care and sedation. Several drugs have been used for sedation during this procedure including benzodiazepines[1] and opioids. However, midazolam may result in confusion, particularly when administered to elderly patients, and opioids are associated with increased risk of respiratory depression and decreased oxygen saturation. All of these untoward effects may hamper patients' co-operation during surgery, and would make these agents less than ideal for the intraoperative management of sedation. In contrast, dexmedetomidine is a selective α_2-adrenoceptor agonist with both sedative and analgesic properties and is devoid of respiratory depressant effect [2]. It has been used to premedicate and sedate patients undergoing day case surgery without adverse effects, and patients, typically, remain co-operative. These properties along with its relatively short elimination half-life of 2 hours make dexmedetomidine an attractive agent for sedation during monitored anesthesia care for ophthalmic surgery [3, 4]. Accordingly, this clinical study was undertaken to compare the effects of dexmedetomidine sedation with those of propofol sedation in patients undergoing combined cataract and glaucoma surgery under medial canthus peribulbar anesthesia guided by ultrasound.

2. Methods

After Ethics Committee approval, 100 patients participated in this clinical study. Patients were included in the study if their ages ranged between 40 - 60 years, ASA class I, II or III and were undergoing elective surgery under local anesthesia. They were excluded if they had high serum creatinine, advanced liver disease, history of chronic use of sedatives, narcotics or allergy to any of the study medications. Patients were scheduled to receive either dexmedetomidine (Group D) or propofol (Group P) for sedation during surgery**.**

Patients arrived in the operating room, without previous premedication, a 20 gauge cannula was inserted into one of the two nasal prongs of an oxygen nasal cannula, and was connected proximally to the CO_2 sampling tubing of the end-tidal CO_2 module of the patient monitor (Ohmeda-Datex) to measure patients' expired CO_2. Oxygen was administered at a rate of 2 liters/min. Other standard monitors including ECG, non-invasive arterial pressure and pulse oximeter were also applied. Group D patients received a continuous infusion of dexmedetomidine 0.2- 0.5

µg/kg/min [5], while group P patients received a continuous infusion of propofol 25-75 µg/kg/min using an infusion pump. The infusion rate was titrated every 3 min according to Richmond Agitation-Sedation Scale (Table 1) [6] and a bispectral index between 80-70 % [7].

After starting infusion of the study drug, the anesthetist applied the electrodes of bispectral index to the head. Benoxinate 0.4%, as a surface anesthetic, was applied to the eye selected for operation. Peribulbar block was then performed, under ultrasound guide, by single injection in the medial canthus of 8ml local anesthetic mixture consisting of levobupivacaine 0.25%, Lidocaine 2% (in the ratio of 1:1) and hyaluronidase 10 units/ml. Heart rate (HR), mean arterial pressure (MAP), respiratory rate (RR), oxygen saturation (SPO_2) and expired CO_2 were recorded every 5 min throughout the surgery. The infusion was stopped at the end of the surgery in both groups. In the recovery room, Aldrete score [8] was determined every 5 min until discharge and the requirement for postoperative analgesia was documented. Patients were deemed ready for discharge when they had achieved an Aldrete score of 10. Patients were asked to answer the question '*How would you rate your experience with the sedation you have received during surgery?'* using 7-point likert like verbal rating scale [9] (Fig.1). Assessment of patients' satisfaction with sedation was performed 4 hours after the end of surgery.

Table (1) Richmond Agitation-Sedation Scale (RASS scale) [6]

Score	Term	Description	
+4	Combative	Overtly combative, violent, immediate danger to staff	
+3	Very agitated	Pulls or removes tube(s) or catheter(s); aggressive	
+2	Agitated	Frequent non-purposeful movement, fights ventilator	
+1	Restless	Anxious but movements not aggressive vigorous	
0	Alert and calm		
-1	Drowsy	Not fully alert, but has sustained awakening (eye-opening/eye contact) to *voice* **(>10 seconds)**	**Verbal Stimulation**
-2	Light sedation	Briefly awakens with eye contact to *voice* **(<10 seconds)**	
-3	Moderate sedation	Movement or eye opening to *voice* **(but no eye contact)**	
-4	Deep sedation	No response to voice, but movement or eye opening to *physical* stimulation	**Physical Stimulation**
-5	Unarousable	No response to *voice or physical* stimulation	

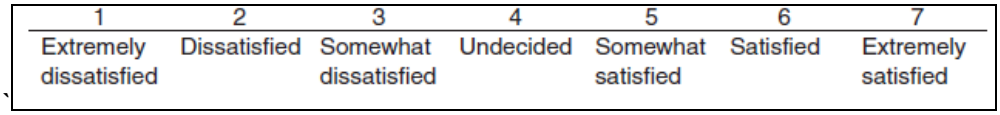

Fig. (1) A 7-point Likert-like verbal rating scale for assessment of patients' satisfaction with intraoperative sedation [9]

Statistics

The number of patients in this study was determined on the basis of the results of preliminary investigations during which the sample size was calculated to be 50 patients per group based on the reduction in heart rate in both groups during the sedation period as the primary endpoint, a population variance of (2) ^{2}, a two sided α of 0.05, and a power of 90%. Sample size calculation program version 2.1.31 was used. The statistical analysis of our results was conducted using the computer program SPSS for windows.

3. Results

A total of 100 patients were recruited in this study. The two groups were comparable with respect to the following variables: age, sex, weight, height and ASA status (Table 2). Total anesthesia time was 50.39±12.28 min in group D and 49.9± 9.566 min in group P, and operation time was 35.03±7.62 min and 37.73±6.26 min in group D and P, respectively. These were comparable between the two groups. The time required from the start of the infusion of the study drugs to achieve targeted levels of sedation was significantly longer in the dexmedetomidine group (15.36±4.66) than in the propofol group(11.96±3.27) (*P*=0.0015). Group P patients achieved an Aldrete score of 10 faster than group D, thus, ready for discharge sooner. However, there was no significant difference in the Richmond Agitation-Sedation Scale (Fig.1).

On the other hand, the 7-point Likert-like verbal rating scale for assessment of patients' satisfaction with intraoperative sedation in Group D was 6.53±0.63 compared with 5.39±0.98 in Group P (Table 2).

Changes of hemodynamic and respiratory variables are presented in figures in both groups. There was a similar significant reduction in HR and MAP compared with baseline in both groups (Figs. 3, 4) respectively.

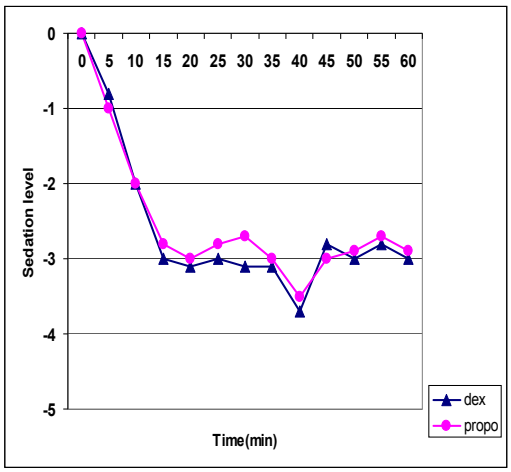

Fig.(2): Richmond Agitation-Sedation Scale during intraoperative period

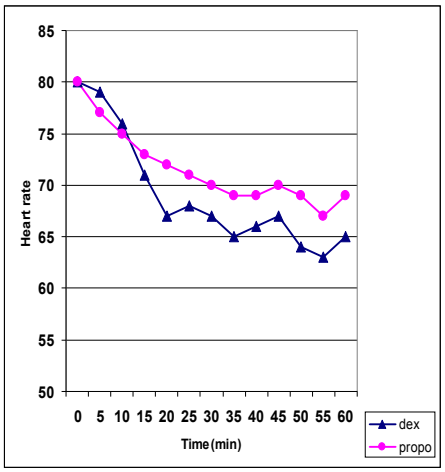

Fig.(3): Heart rate (HR) changes during intraoperative period

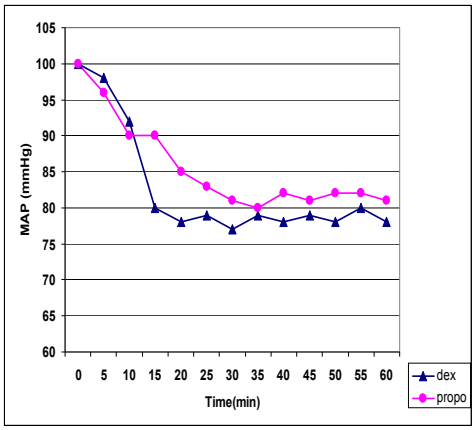

Fig.(4): Mean arterial blood pressure (MAP) changes during intraoperative period

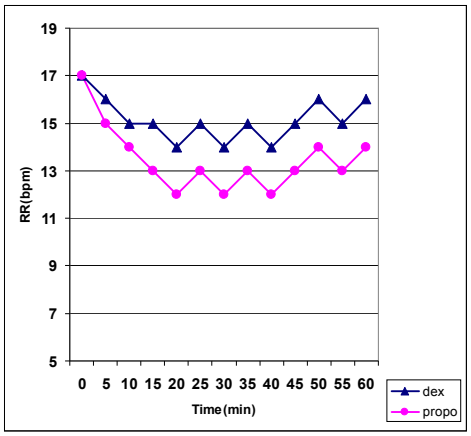

Fig.(5): Respiratory rate (RR) changes during intraoperative period

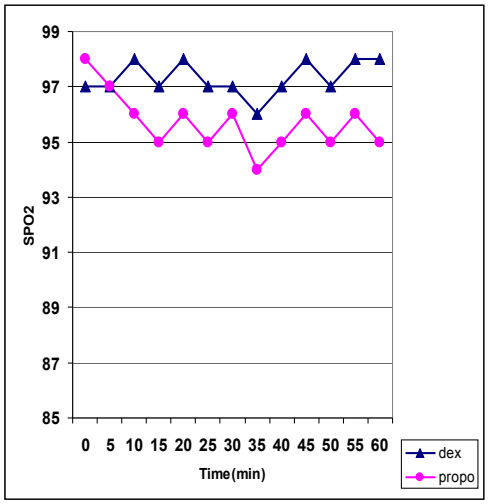

Fig. (6) Oxygen saturation (SPO_2) changes during intraoperative period

Table (2) Demographic Data

	Group D (n=50)	*Group P (n=50)*	*P value*
Age (yr)	50.26±9.44	51.9±10.02	NS
Weight (kg)	69.00±5.634	70.46±6.64	NS
Sex (M/F)	30/20	26/24	NS
Height(cm)	162.7±4.5	163.7±3.6	NS
ASA class I/II/III (n)	14/32/4	13/30/ 7	NS

Table (3) clinical data of the study groups

	Group D	*Group P*	*P-value*
Duration of surgery (min)	35.03±7.62	37.73±6.29	NS
Time to achieve adequate sedation level	15.36±4.66	11.96±3.27*	0.0015
Time to achieve an Aldrete score of 10 (min)	40.53 ±6.51	37.60±6.42	NS
Degree of patient's satisfaction (a 7-point likert-like verbal rating scale)	6.53±0.63	5.39 ±0.98*	0.026

Data presented as mean ± standard deviation * Statistically significant compared to group D
NS Statistically insignificant

RR values in the dexmedetomidine group were significantly higher than those in the propofol group during the sedation period (P<0.05) (Fig.5). The SPO_2 values in the dexmedetomidine group showed less change from baseline values, while there was significant reduction in the SPO_2 in the propofol group (P<0.05) compared with the baseline values (Fig.5). SPO2 values in the dexmedetomidine group were significantly higher than those in the propofol group during the sedation period (P<0.05) (Fig.6). The expired CO_2 values were similar in both groups. In the immediate postoperative period, all the cardiorespiratory measures returned back to the normal preoperative values within 17 minutes.

4. Discussion

In this study, our results suggest that dexmedetomidine is a good and safe drug for monitored anesthesia care (MAC) in outpatients undergoing combined cataract and glaucoma surgery. Dexmedetomidine has been used in short or long term sedation in the intensive care unit, being unique in that it does not cause respiratory depression because its mechanism of action is not mediated by the γ-aminobutyric acid system. This has been proved in critically ill patients given dexmedetomidine during surgery as well as those given the drug for short term. In addition to this singular property of dexmedetomidine, no use of rescue sedative or analgesic drugs might also contribute to less respiratory depression.

This study demonstrated that sedation with dexmedetomidine was equally effective to that of propofol in patients undergoing combined cataract surgery under local anesthesia. This was evident from the facts that none of the patients in either group required rescue sedation, and that surgeons were equally satisfied with both sedative regimens [(10)]. .These results are correlating with those reported by **Virkkila and colleagues** [(11)], who have demonstrated that a single dose of i.m. dexmedetomidine administered 45 min before operation provides sedation. Use of loading dose of dexmedetomidine is still controversial because of the development of cardiovascular depression. Dexmedetomidine at a rate of 0.25-2 µg/kg resulted in a reduction of arterial pressure and cardiac output. Although large doses (1 or 2 µg/kg) of dexmedetomidine produced the initial increase of arterial pressure temporarily, presumably due to peripheral vasoconstriction [(3)], in this current study, loading dose of dexmedetomidine was omitted. There were results reporting that appropriate sedation and stable hemodynamics were achieved in the absence of loading dose of dexmedetomidine [(12)] and the incidence of hypotension was decreased in ICU sedation without the loading dose [(13)]. For various procedures however, its efficacy outside the critical care environment has also been documented.

This study demonstrated that both drugs were effective in providing adequate intraoperative sedation, the dexmedetomidine group (Group D) patients were more satisfied with their sedation than those of the propofol group (Group P). This could be explained, at least in a way, by the additional analgesic property of dexmedetomidine that could have contributed to improved patients' perception of this form of sedation, and in another way, by potential differences in the quality of sedation of the two drugs [(14)]. The lower mean arterial pressure (MAP) and heart rate (HR) observed in the dexmedetomidine group (Group D) could be explained by the decreased sympathetic outflow and circulating levels of catecholamines that are caused by dexmedetomidine. Similar hemodynamic changes have been reported by Arain and Ebert, who compared the sedative effect of dexmedetomidine and propofol during surgery under regional anesthesia [(15)].

Moreover, intraoperative respiratory rate (RR) and oxygen saturation (SPO_2) of the dexmedetomidine group (group D) were somewhat superior to those of the propofol group (group P).

In summary; compared with propofol, dexmedetomidine does appear to be suitable for sedation in patients undergoing combined cataract and glaucoma surgery. While there was a slightly better subjective patient satisfaction, it was accompanied by relative cardiovascular depression and delayed recovery room discharge. In addition, most of the patients were outpatients and elderly, thus dexmedetomidine might have more advantages over other commonly used sedatives.

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5/5/2012

A Comparative Study between Different Phases of Menstrual Cycle Regarding Hemodynamic Response to Laryngeal Mask Airway and Intraocular Pressure Changes in Elective Ophthalmic Surgery

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Abstract: Objective: The aim of the study was to determine the effect of the different phases of the menstrual cycle on hemodynamic response to laryngeal mask airway and intraocular pressure changes in elective ophthalmic surgeries. **Methods:** 80 ASA I & II female patients were equally allocated in one of two groups: group 1 (follicular) and group 2 (luteal) according to the phases of the menstrual cycle. Patients received propofol and rocuronium and LMA was introduced. Hemodynamic variables and intraocular pressure were recorded before administration of I.V anesthetic and after LMA introduction. Rate pressure products were calculated as well as IOP measurements. **Results:** Rate pressure products values were significantly increased at the first minute after LMA introduction in group 2 (luteal) compared to group 1 (follicular) ($P < 0.001$). There was no significant statistical difference between the two groups regarding the intraocular pressure measurements. **Conclusion:** The phase of the menstrual cycle affects the hemodynamic response to LMA introduction being higher in the luteal phase than in the follicular phase, whereas the different phases have no significant effect on IOP measurements.
[Mona Raafat Fahim, Ashraf Darwish, Rehab Sami, Hala Bahy and Rashad Aref. **A Comparative Study between Different Phases of Menstrual Cycle Regarding Hemodynamic Response to Laryngeal Mask Airway and Intraocular Pressure Changes in Elective Ophthalmic Surgery.** Life Sci J 2012;9(2):794-797] (ISSN:1097-8135). http://www.lifesciencesite.com. 118

Key words: laryngeal mask airway, ophthalmic surgery, intraocular pressure, haemodynamic changes.

1. Introduction

The normal menstrual cycle is divided into two phases; the follicular phase and the luteal phase[1]. Hormonal, physical and psychological fluctuations occur during the menstrual cycle.

The hormonal changes during the different phases affect the anesthetic, analgesic and antiemetic requirements according to the phase[1,2]. Pain perception can also differ during the different phases of the cycle[3]. Laryngoscopy and tracheal intubation stimulates somatic and visceral nociceptive afferents in the airway and can significantly increase arterial blood pressure and catecholamine levels.[4] Previous studies studied the effects of the phases of the menstrual cycle on the hemodynamic response to tracheal intubation[5].

In this study, we evaluated the effects of the follicular and luteal phases of the menstrual cycle on the hemodynamic response to laryngeal make airway, also their effects, if any, on the intraocular pressure in elective ophthalmic surgery.

2. Patients and Methods

After obtaining the approval of the Ethical Committee of the Research Institute of Ophthalmology and written informed consent, 80 female patients ranging from 18 to 42 years old, ASA physical status I & II, were scheduled to receive general anesthesia with laryngeal mask airway introduction for elective ophthalmic surgeries; (cataract extraction and intraocular lens (IOL) implantation) and enrolled in this study. Exclusion criteria were: the presence of neurological or psychiatric diseases, communication problems, history of combined oral contraceptive use, irregular menstrual cycle, amenorrhea, total abdominal hysterectomy, pregnancy, any medications (including analgesics) received 24 hours prior to surgery effecting either arterial blood pressure or heart rate, patients with diagnosed glaucoma or with a history of increased intraocular pressure (even if not symptomatizing) and lengthy ophthalmic procedures.

The patients were equally assigned in two groups, 40 each, according to the phase of their menstrual cycle. The patients who were on the 1st to 12th days after the 1st day of their last menstruation were considered to be in the follicular phase of the cycle and assigned to group 1 (follicular group). Those on the 20th to 24th days after the 1st day of their last menstruation were considered to be in the luteal phase of the cycle and assigned to group 2 (luteal group).

Laboratory measurement of estrogen and progesterone levels were not done, we only abided to the above mentioned calculation. To be more precise in determining the two phases distinctively, patients in the 13th to the 19th days of their last menstruation cycles were excluded (as luteinizing hormone peaks on the 13th day and progesterone starts to increase on the 18th day of the menstrual cycle). Also, patients on

the 24th or more day of their cycle were excluded as progesterone level starts to decrease on the 24th day of the cycle. Menstrual cycle duration was recorded[5].

On arrival to the operating theatre, patients were cannulated and received premedication with 0.03 mg/kg IV midazolam 15 minutes prior to induction of anesthesia. All patients in the two groups were monitored with continuous electro-cardiography, non-invasive arterial blood pressure manometer and pulse oximetry. Intraocular pressure (IOP) was measured by an ophthalmologist blinded to the study immediately before induction, immediately after induction, immediately after laryngeal mask airway (LMA) insertion and at 5 and 10 minutes after LMA insertion.

Preoxygenation was done for 2 minutes with 5L/min fresh gas flow of 100% oxygen. After oxygenation, all patients were administered 3 mg/kg propofol over 30 seconds. After loss of eyelash reflex to touch, mask ventilation was started. Rocuronium 0.7 mg/kg was given and after around one and a half minutes, LMA was inserted and patients were instantly connected to be ventilator. Tidal volume, I:E (inspiration to expiration) ratio and RR (respiratory rate) were all calculated for each patient and were preset before patients were on the ventilator. Patients' systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), heart rate (HR) and SpO_2 (arterial oxygen saturation) were recorded at the following times: before administration of I.V. anesthetic and muscle relaxant, after administration of I.V. anesthetic and muscle relaxant, immediately after LMA insertion and at 1,2,3,4,5, and 10 minutes after LMA insertion before start of surgery.

Rate pressure product (RPP), which is calculated by the formula RPP = HR X systolic pressure was calculated for each time point and recorded.

After LMA insertion, anesthesia was maintained with 100% oxygen and 1.5% isoflurane. Ventilation was adjusted to maintain end-tidal Co_2 ($ETCo_2$) between 30 and 35 mm Hg. The surgery commenced after the data collection period (10 minutes after induction and LMA insertion). Atropine at a dose of 0.5 mg was given for bradycardia [HR < 50 beats per minute (bpm)]. 5 mg ephedrine was given if MAP decreased by 30% from the control value for a minimum of 1 minute and 1 μg/kg fentanyl was given if MAP increased by 30% from the control value for a minimum of 1 minute.

All these drugs were recorded if given. Complications related to LMA insertion such as laryngospasm, coughing or bronchospasm were also recorded. Primary outcome measures were the RPP changes in both groups while IOP changes were recorded as secondary outcome measures.

Statistical analysis

Numerical data were given as mean ± SD and were analyzed using the student's t-test. A p value < 0.05 was considered statistically significant.

3. Results

The 2 groups were comparable regarding the demographic data ($p > 0.05$) as shown in table (1).

Intraocular pressure recordings were comparable with no statistical significant difference at all time recordings (Table 2).

Table (1): Demographic Data

	Group 1 (follicular phase) (n = 40)	Group 2 (luteal phase) (n = 40)	*p* value
Age (years)	29.6±7.3	28.4±7.9	0.31
Weight (kg)	68.4±12.3	67.2±12.1	0.79
Height (cm)	165.5±4.8	163.9±4.2	0.88
Duration (days)	26.2±0.94	26.3±1.1	0.21

Table (2): Intraocular pressure measurements in the two groups at different time points

IOP (mmHg)	Group 1 (follicular phase) (n = 40)	Group 2 (luteal phase) (n = 40)	*p* value
Immediately before induction	13.2±1.1	13.4±1.3	0.33
Immediately after induction	13.4±1.5	13.7±1.2	0.41
Immediately after LMA insertion	14.1±1.6	14.3±1.5	0.37
5 min after LMA insertion	13.9±1.3	14.0±1.4	0.39
10 min after LMA insertion	13.5±1.2	13.8±1.3	0.38

Hemodynamic variables were comparable in the two groups before administration of I.V. anesthetic (propofol) ($P > 0.05$). RPP values at the first minute after LMA insertion were significantly higher in group 2 (Luteal phase) than in group 1 (follicular phase) (12.006±2063 mmHg. bpm, 9.801±1923 mmHg. bpm, respectively) ($p < 0.001$) (Tables 3 & 4).

SpO_2 and $ETCO_2$ values showed no statistical significant difference ($P > 0.05$) in the two groups. Only one patient received atropine in group 1 and two patients required fentanyl; one in group 1 and one in group 2. No patients required ephedrine. No patients developed any complications during LMA insertion.

Table (3): Systolic blood pressure (SBP), diastolic Blood pressure (DBP), heart rate (HR recordings in both groups at different time points

	SBP		DBP		HR	
	Group 1 (follicular)	**Group 2 (luteal)**	**Group 1 (follicular)**	**Group 2 (luteal)**	**Group 1 (follicular)**	**Group 2 (luteal)**
Baseline	110±5	115±3	70±3	74±1	75±3	80±1
After propofol	105±4	113±3	65±2	72±1	76±2	79±1
After muscle relaxant	98±2	106±2	63±2	71±1	75±3	78±1
1 min after LMA insertion	121±6	138±2	71±3	80±2	81±2	87±1
2 min after LMA insertion	110±3	121±2	67±3	77±1	79±1	83±1
3 min after LMA insertion	107±3	115±1	65±2	76±1	77±1	82±1
4 min after LMA insertion	106±2	113±1	64±2	75±1	75±1	81±1
5 min after LMA insertion	109±2	112±1	63±1	75±1	74±1	80±1
10 min after LMA insertion	109±1	112±2	62±2	74±1	74±1	80±1

Values are mean ± SD HR values are in beats per minute (bpm) SBP, DBP value are in mmHg

Table (4): Rate pressure product (RPP) changes in both groups at different time points

Rate pressure product (RPP)	**Group 1 (follicular phase) (n = 40)**	**Group 2 (luteal phase) (n = 40)**
Baseline	8.25±970	9.200±953
After propofol	7.980±789	8.927±972
After muscle relaxant	7.350±771	8.268±883
1 min after LMA insertion	*9.801±998	*12.006±1001
2 min after LMA insertion	8.690±926	10.043±999
3 min after LMA insertion	8.239±877	9.430±901
4 min after LMA insertion	7.950±783	9.153±993
5 min after LMA insertion	8.066±853	8.960±981
10 min after LMA insertion	8.066±853	8.960±981

RPP values are in mmHg bpm. * $p < 0.001$

4. Discussion

In previous studies, it was found that in healthy women, plasma norepinephrine levels and sympathetic activity were significantly higher in the luteal phase than in the follicular phase[6-8]. Other studies revealed that venipuncture and propofol injection pain was higher in the luteal phase compared to the follicular phase[9-11]. This finding was referred to the increased progesterone and decreased oestrogen levels[6,10]. A previous study done by **Volkan *et al.*** studied the effects of the phases of menstrual cycle on the hemodynamic response to laryngoscopy and tracheal intubation. They found that the RPP at 1 minute after tracheal intubation significantly increased in the luteal phase compared to the follicular phase (14.686 ± 2278 mmHg. bpm and 11.167±2069 mmHg. bpm, respectively)[5].

In our study, our primary hypothesis was to detect if there was a different outcome on using a laryngeal mask airway instead. Our results showed the same significant effect, being higher in the luteal phase compared to the follicular phase, but our results were still lower compared to Volkan et al. study. In our study, RPP values at 1 minute after LMA introduction were 12.006 ± 2063 mmHg. bpm in the luteal phase and 9.801 ± 1923 mmHg bpm in

the follicular phase. The explanation for the lower RPP in our study compared to Volkan's is that the hemodynamic response is lower with LMA introduction compared to laryngoscopy and tracheal intubation. Still yet, there was a significant difference between the 2 groups, hence related to the same causes mentioned by the previous authors.

As the study was performed in ophthalmic surgeries, an IOP measurement was done to reveal if the different phases of the menstrual cycle affected the IOP as well. But, there was no significant difference between the two groups.

In conclusion, we suggest that in elective ophthalmic surgeries, it is advisable to perform the operation in the follicular phase rather than the luteal phase where there is significant increased RPP response to LMA introduction. Also, using an LMA is more advisable than laryngoscopy and tracheal intubation as RPP response is still lower as compared to previous studies. Therefore, further studies comparing the two techniques could be considered to reach the best anesthetic regimen.

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Synthesis Locally Extreme pressure additives via residual sulfur of crude oil

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Abstract: The objective of this work aimed at synthesis extreme pressure additive via residual sulfur extracted from crude oil. Residual sulfur was used in Sulforization process of plant oil (i.e.: Jatropha & Linseed oils). Sulforization process for plant oils was carried out according to certain conditions. Product obtained from Sulforization of jatropha oil, was additive A, while product obtained from Sulforization linseed oil was additive B. Comparative evaluation study between our local products A&B and two imported additives were carried out through bench and performance tests. From comparative study, it was found local additives A & B give the same efficiency at the same dose of imported once, as extreme pressure functions. Also, Additives A & B were found had antioxidant efficiency than imported once. Additive A & B by this way saving environmental from pollution of residual sulfur, beside highly economic value.
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Keywords: Synthesis; pressure; additive; sulfur; crude oil

1. Introduction

Lubricant additives are chemical compounds add to a lubricant to enhance performance and improve its operating characteristics [1- 4]. It can be defined as a material which imparts a new and desirable property not originally presented in the oil [5]. Also provide the necessary protection to oil for application at high temperature, high speed and high pressure [5, 6]. Lubricating oil additives are used to reduce the oxidative or thermal degradation of an oil, to lessen the deposition of harmful deposits on lubricated parts, to minimize rust and corrosion, to control frictional properties, to reduce wear, and to prevent destructive metal to metal contact. They are also employed to alter purely physical properties of oil such as viscosity, viscosity-temperature relationship (viscosity index or VI) and tendency to form stable foam [5, 7].

Extreme pressure additives were designed to prevent wear and reduce friction in power transmission via reducers [4]. Extreme pressure additives are organic compounds that contain one or more elements are functions such as sulfur, halogen (principally chlorine) phosphorus, carboxylate salt which can react chemically with the metal surface under condition of boundary lubrication [5].

Under high temperature and high pressure or extreme boundary condition there is severe metal to metal contact, which leads to welding followed by tearing away of large pieces of metal. Also, the additives react with the metal surfaces to form compounds that have lower shear strength than that of metal. The reaction is initiated by increased temperature caused by pressure between asperities on wearing surfaces. The reaction creates a protective coating at the specific points where protection is required. This coating works as dry lubricants and reduces friction, wear, scoring seizure, and galling of wear surfaces [3, 5-8].

The residual of these additives produce hazard materials, today the worldwide production going to produce environmental friend additives by using natural product such as plant oil or organo metallic compounds.

2. Experimental

Material specifications

Materials were used through this work are: jatropha curcas oil, linseed oil, hexyl amine, imported E.P additives (X) and (Y) and extracted sulfur from crude oil. Analysis figures of these used materials were listed in Tables (1-4).

Sulforization processes

Sulforization of jatropha and linseed oils, were carried out in a three-necked spherical flask equipped with stirrer, thermometer and a reflux condenser. Adjust conditions to obtain high concentration of non-corrosive sulfur by reaction between sulfur and double bond in fatty oil.

Sulforization steps were carried out according to the following:

- Weighed (100 gm) of jatropha or linseed oil and transfer to the three neck flask
- Increase degree of temperature up to (100˚C) with stirring rate (150 rpm).
- Weighed (25gm) of extracted sulfur and dissolved in (20 gm) hexyl amine, then added drop wise to the oil.

- Increase the degree of temperature gradually up to 155 – 165 ˚C to complete reaction through 2 hrs.
- After finish the reaction, leave system to cool up to 60˚C.
- Add 10 gm isopropyl alcohol as polar solvent with stirring to prevent precipitate. Then increase temperature without condenser up to 155˚C to evaporate any excess of alcohol and amine.
- Leave system to cool at room temperature.
- Both products A (sulfurized jatropha oil) & B (sulfurized linseed oil) are dark brown colour and more viscous than original oil of jatropha or linseed oil.
- Specification of base oil blended with additives A, B, X and y was listed in table (5).

Table (1). Physical and chemical properties of Jatropha curcas and Linseed oils.

Specifications	Method	Jatropha curcas Value	Linseed oil Value
Density @ 15/4 C°	IP 235/82	0.9182	0.920
Colour	ASTM D1500/82	2.5	3.0
Kinematic Viscosity @ C°,CSt	IP 71/84	46.8	41.2
Flash point, C°	IP 35/86	188	218
Pour point, C°	IP 15/81	6+	- 9
Conradson carbon, wt %	IP 13/82	0. 60	0.037
Acid value, mg KoH/g	IP 1/81	2.87	1.42
Saponification number, mg KoH/g	ASTM D 94	193.8	193.8
Iodine value	IP 84/81	107.83	170
Sulfur, %wt	ASTM D 6443	0.15	Nill

Table (2). Specifications of imported extreme pressure additives X and Y

Specifications	Method	additive X	Additive Y
Flash point, C°, PMCC	IP 35/86	80	82
Pour point, C°	IP 15/81	- 18	- 40
Kinematic viscosity @ 100, C°,CSt	IP 71/84	5	2.5
Kinematic viscosity @ 40, C°,CSt	IP 71/84	8.5	8.5
Nitrogen, %wt	ASTM D 5291	1.17	0.76
Phosphorus, %wt	ASTM D 4951	1.93	1.32
Sulfur, %wt	ASTM D 6443	19.1	30.10

Table (3). Specifications of extracted sulfur from crude oil.

Specifications	Value
M.Wt	32.06
Melting range, C°	111 – 119
Ignition temperature, C°	235
Free acid, mg KoH/g	> 0.25%(H2SO4)
Sulphated ash, wt %	> 0.1

Table (4). Specifications of cyclo hexyl amine

Specifications	Value
Physical appearance	Clear to yellow liquid with fishy odour
Assay %	99.18
Specific gravity	0.865
Chemical formula	C6H11NH2
Flash point, C°	28.4
Refractive index at 20, C°	1.4565

Table (5) Specification of paraffin base oil SAE 90 blended with additives A, B, X &Y.

Specifications	Method	Value
Density at, 15/4 C°	IP 235/82	0.9021
Appearance	----------	Clear
Colour	ASTM D1500/82	3.5
K.V at 100 C° , cSt	IP 71/84	16.7
K.V at 40 C° , cSt	IP 71/84	121
VI	IP 266/84	92
Flash point (PMCC), C°	IP 35/86	210
Pour point, C°	IP 15/81	- 3
Total acidity, mg KoH/g	IP 1/81	0.037
Conradson carbon, %wt.	IP 13/82	0.76

To evaluate active and non active sulfur several trials were carried out by blending base oil with products A and B. Blend percentages were listed in table (6). Products A & B were blended up to 10%. Standard method to detect active sulfur was applied as cupper strip ASTM D – 130.

Table (6): Ten formulation of products A & B with paraffin base oil SAE 90

Formula No.	paraffin base oil SAE90	Product A	Product B
1	99 %	1 %	……
2	97 %	3 %	……
3	95 %	5 %	……
4	93 %	7 %	……
5	90 %	10 %	……
6	99 %	……	1 %
7	97 %	……	3 %
8	95 %	……	5 %
9	93 %	……	7 %
10	90 %	……	10 %

To evaluate the efficiency of synthesis products as extreme pressure additives another four blends were carried out between base oil and products A, B and additive X by percentage (1.75%) respectively (recommended dose), to produce gear oil SAE 90 – GL3, while additive y blended with base oil by 2.4% wt (recommended dose), to give gear oil SAE90 – GL5 were listed in table (7).

Table (7) formulations of blends from synthesized products (products A&B) and two imported additives with paraffin base oil SAE 90.

Formulation No	Product A Wt %	Product B Wt %	Imported additive x Wt %	Imported additive y Wt %	Base oil SAE 90 Wt %	Performance level
Blank	……	……	……..	……..	100	……..
11	1.75	……	……..	……..	98.25	GL – 3
12	……	1.75	……..	……..	98.25	GL – 3
13	……	……	1.75	……..	98.25	GL – 3
14	……	……..	……..	2.4	97.6	GL – 5

Four ball wear standard test (IP 239) were carried out on the four samples listed in table (7).

Also standard oxidation stability tests (IP 229) were applied on the four blends beside base oil as blank as listed in table (7).

Product A is produced by reaction between jatropha oil and extracted sulfur.

Reaction was carried out by destroy double bonds of fatty oil and enter sulfur atoms in molecules of fatty oil [9]. Physical properties of products A & B were listed in table (8).

3. Results and Discussion

Table (8): Analysis figures of products A & B.

Test	Standard test method	Product A	Product B
Density at ,15/4 C°	IP 235/82	0.9437	0.9241
Appearance	----------	Clear	Clear
Color	ASTM D1500/82	Dark – Reddish – Brown	Dark – Reddish – Brown
K.V at 100 C° ,cSt	IP 71/84	11.7	9.3
K.V at 40 C° , cSt	IP 71/84	135	94
Flash point (PMCC), C°	IP 35/86	187 C°	180 C°
Sulfur, %wt	X – ray	20.39	22.18
Pour point, C°	IP 15/81	+ 6 C°	- 3 C°
Conradson carbon, %wt	IP 13/82	0.038	0.0.041
Ash, %wt	IP 3/81	Nil	nil

FTIR spectrums of jatropha oil before and after Sulforization were illustrated in figs (1, 2) while linseed oil before and after Sulforization were illustrated in figs (3, 4).

IR spectra

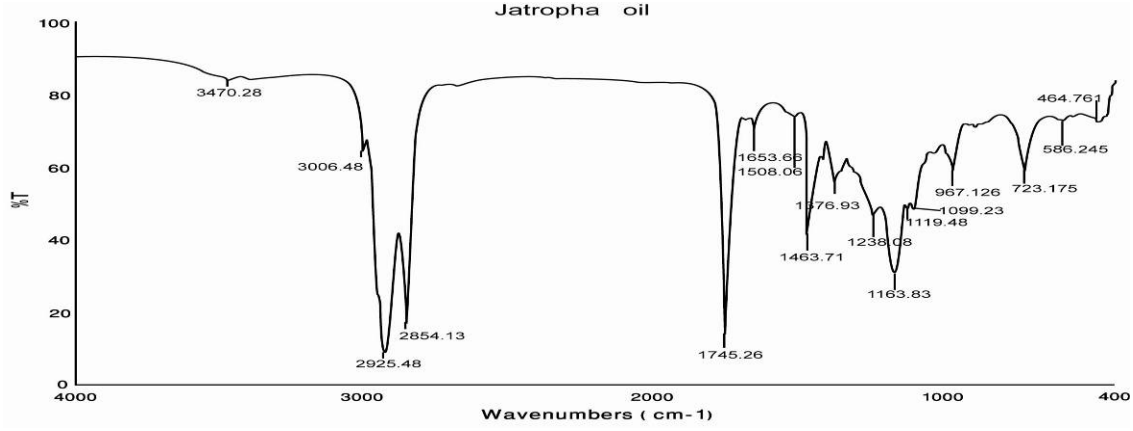

Fig. (1) IR Spectrum of jatropha oil

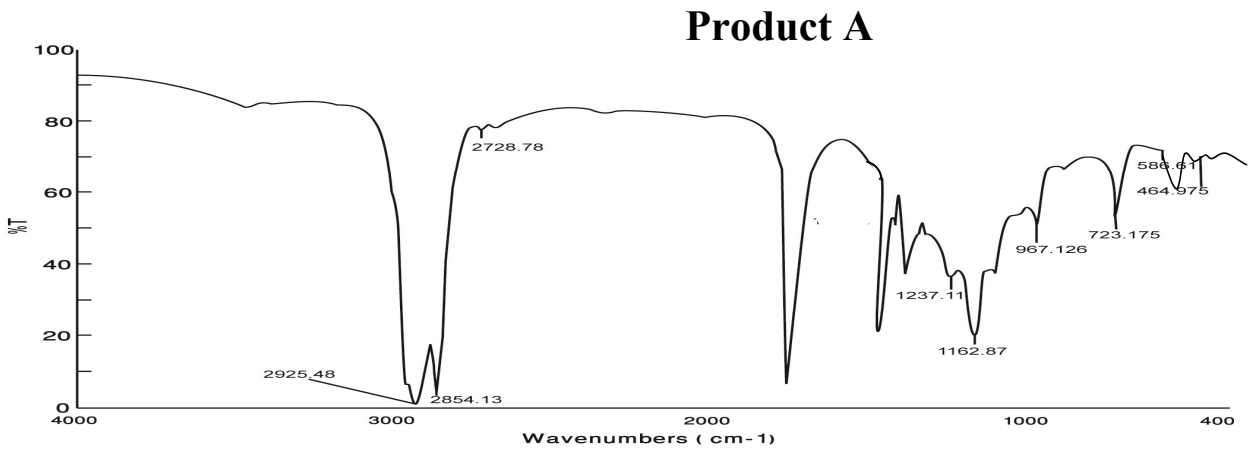

Fig. (2) IR Spectrum of product A (sulfurized jatropha oil)

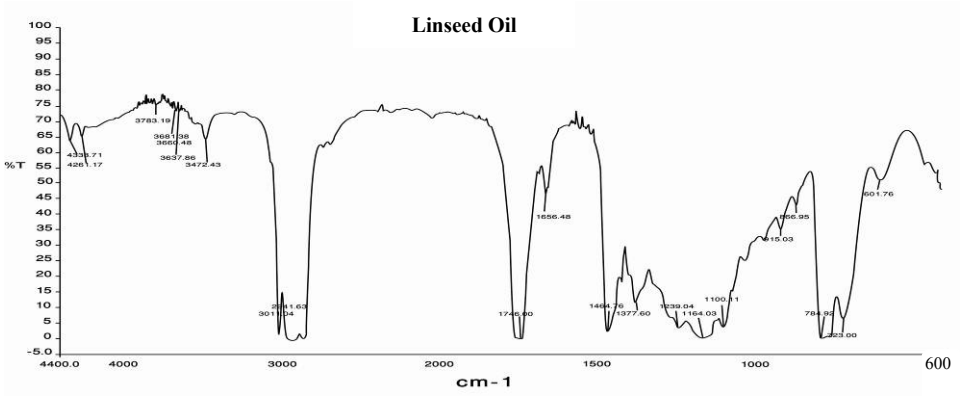

Fig. (3) IR Spectrum of Linseed oil

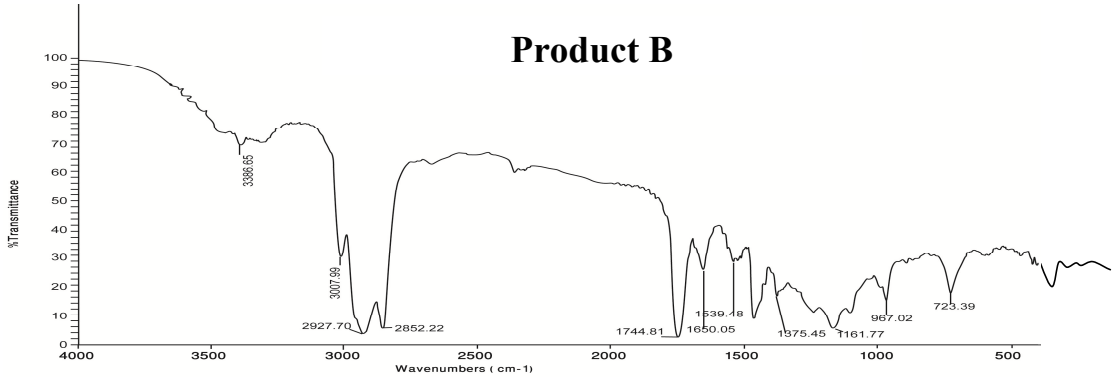

Fig. (4) IR Spectrum of product B (sulfurized linseed oil)

Evaluation study of analysis figure for product A are listed in table (8), while figs (1, 2) show FTIR spectrums before and after Sulforization.

Results obtained shows that product A contains 20.39 %wt sulfur content. Also FTIR spectrum illustrate disappearing double bonds at 1610 cm^{-1}, 907 cm^{-1}, while forming υ C – S bond at 586, 577 cm^{-1}[8,9]

This proves forming sulfurized fatty material. High percentage of sulfur contents in fatty material work as extreme pressure additives.

Also analysis figure of product B are listed in table (8). Figs (3, 4) show FTIR spectrum, before and after Sulforization.

Results obtained shows that product B contains 22.12 %wt sulfur contents, while FTIR spectrum, illustrate disappearing double bonds at 1610 cm^{-1} & 790 cm^{-1} and forming bond of υ C – S at 500 – 400 cm^{-1}. This proves forming sulfurized fatty material. High percentage of sulfur content in fatty material work as extreme pressure additive.

Results obtained of standard test ASTM – D130 for blends of products A & B with base were listed in table (9).

Evaluation study of copper strip results for standard ASTM D130 method illustrates all blends has class (1A).

Table (9): Result of cupper corrossion test for different percentage formulation from products (A & B) (ASTM D130).

Formula No.	Results (A)	Results (B)
1	1 a	……
2	1 a	……
3	1 a	……
4	1 a	……
5	1 a	……
6	……	1 a
7	……	1 a
8	……	1 a
9	……	1 a
10	……	1 a

These excellent results prove that all sulfur reacts with double bond of fatty oil (inactive sulfur), while dissolved sulfur (active sulfur) give class B or C or D.

High percentage of inactive sulfur (non corrosive) in fatty material work as extreme pressure additive with saving the machine metal surfaces from corrosion, it was also worked as antiwear additive for metal to metal surfaces.

Results obtained of performance standard four ball wear test (IP 239) were listed in table (10).

Evaluation study of scar diameter and welding load results of standard four ball wear test (IP 239) show that local products A & B have in good results than or equal imported additives.

Fig (5) shows the comparative evaluation between blends 11, 12, 13 and 14 which containing percentage of different types of E.P additives. From the figure indicate that local products A & B have in good function as extreme pressure additive similar to imported additives.

Table (10): Four ball and oxidation stability results for formulation of 4 pilot blends from paraffin base oil SAE 90 and product (A&B) and two imported additive.

Formulation No	Results		
	Oxidation Stability / min	Scar diameter/mm	Welding load/Kg
Blank	28	0.60	150
11(A)	278	0.35	280
12 (B)	314	0.3	285
13 (X)	22	0.35	275
14 (Y)	155	0.25	290

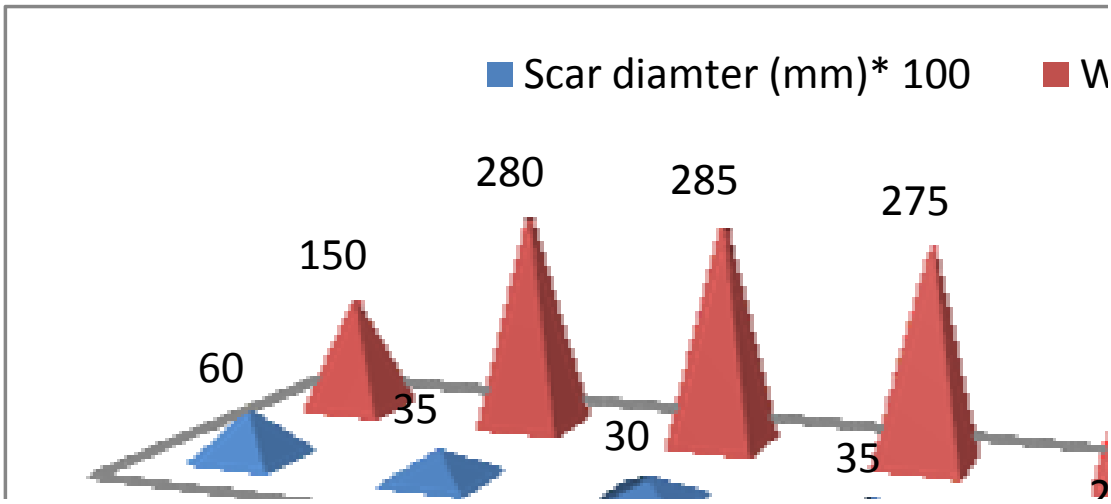

Fig (5): Shows the comparative between scar diameter and welding load of four blends and base oil.

Results obtained of oxidation stability by using standard methods IP 229 for base oil and blends 11,12,13,14 which contains 1.75%wt from product A,B and additive X with base oils as SAE 90 GL – 3 respectively, while 2.4 %wt from additive Y in blend 14 as SAE 90 GL – 5 were listed in table (10).

Evaluation study of induction periods (min) for oxidation resistance according IP (229) show blends 11 and 12 highly oxidation resistant than blends 13 & 14.

This proves that locally synthesized products A & B have in good resistant for oxidation of oils, while additives X (GL – 3 level) doesn't have any resistance and additive Y (GL – 5) have slightly resistance against oxidation.

This proves that locally products A & B have further function as antioxidants more than imported additives as shown in fig (6)

This may be due to the efficiency of natural resistant in fatty oil.

Final Conclusion

Evaluation study of this work proves that, success using extracted residual sulfur from crude oil in production useful E.P additives.

Additives produced having high efficiency as extreme pressure and antioxidant function than imported additives.

Production additives A & B by this way saving environmental from pollution of residual sulfur, beside highly economic value.

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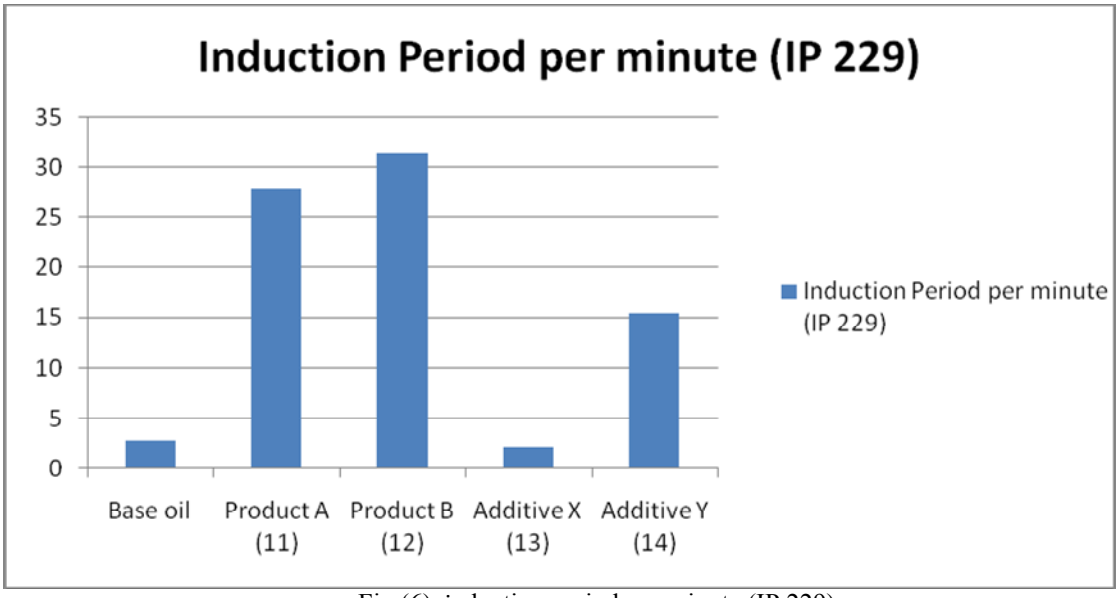

Fig (6): induction period per minute (IP 229)

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4/22/2012

The Curial Effect of Vinegar in Reducing the Serious Damages of Lead on the Histological Structure of Some Organs of Embryos and Mature Female Albino Mice

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Abstract: This research aims to study the effectiveness of apple cider vinegar to reduce the harmful impact of lead achieve this female adult mice were divided into three groups and given the first group lead acetate, while the second group was given lead and vineger, the third control group was also treated in the same way. Examined infants of (7-14-21 days). And demonstrated by histological examination over the harmful effects of lead on spleentissue and thymus in embryos. Sharp in all components of the tissue with low density of lymphocytes. The appearance of hemorrhage tissue. Proliferation in epithelial vertical glands of the uterus was observed. Also an imbalance in the number of uteringlands, lead has a devastating impact on the fabric of the ovary. This led to the lack of mature vesicles, with the appearance of hemorrhage and fibrosis in the tissue. The absence of corpus luteum while the addition of vinegar to lead led to improved tissue. In neonatal or maternal tissues and the return to almost normal structure.

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Key words: embryo- lead acetate – abnormalities –histopathology- maturity- toxicity

1. Introduction

Roiha., (1974) mentioned that an addition of 50 g of vinegar mixed with drinking water of pregnant cow twice daily led to delivering of normal calf with normal weight, heavy hair and strong legs enable them to stand just after their delivery by five minutes. Vinegar was also used to elevate their sexual ability. It is well known that toxic effects of a xenobiotic can be modified by other substances (Skoczyńska and Smolik, 1994; Brus *et al*., 1999; Institoris *et al*., 1999; Gupta and Gill, 2000). As simultaneous exposure to two or more xenobiotics can take place in the environment and/or under occupational conditions, the investigation of interactions between toxic substances is an important problem in modern toxicology. The interaction between lead can be a good example. Exposure of certain human populations to lead is often rather high (World Health Organization, 1992; Schrey*et al*., 2000) consumption continues to rise worldwide (Samson and Harris, 1992; Meyer *et al*., 2000); Some publications provide data on lead - vinger interactions (Sharma *et al*., 1991, 1992; Brus *et al*., 1995) but many aspects are still not fully recognized. According to our earlier results short- and long-term vinger administration affects lead turnover in rats, and also modifies changes in the metabolism of some essential elements by this heavy metal, (Moniuszko-Jakoniuk *et al*., 1999, 2001; Brzóska *et al*., 2000, 2002). As the uterus is an important target organ of viger (Bunout, 1999; Thurman *et al*., 1999), and the ovary of lead toxicity (Kjellström, 1986; World Health Organization, 1992; Nordberg *et al*., 1994) we have also assessed spleen, ovary and uterus histology.

Honsho *et al*., (2005) suggest that previously described hypotensive action of the beverage may be induced by the inhibition of angiotensin – converting enzyme, Fushimi and Sato (2005) conclude that a diet containing acetic acid may enhance glycogen repletion but not induce super compensation, a large increase in the glycogen level that is beneficial in improving performance ,in liver and skeletal muscle by transitory inhibition of glycolysis. Further, they indicate the possibility of transient enhancement of fatty acid oxidation in liver by acetic acid, Wang *et al*., (2006) conclude that ethyl acetate can decrease the level of serum markers of hepatic fibrosis and the expression of TGF-beta 1, Fushimi *et al*., (2006)examined the effect of dietary acetic acid the main component of vinegar for prevent hyperlipidaemia in rats and they found that dietary acetic acid reduced serum total cholesterol and triacylglycerol: first due to the inhibition of lipogenesis in liver; second due to the increment in faecal bile acid excretion in rats fed a diet containing cholesterol, Zardi et al., (2007) result show that mortality in the acetic acid -treated group was greater than in ethanol- treated group presumably due to greater acetic acid systemic diffusion and its metabolic side effect and this could be the reason why some human studies have concluded similar or even better safety and efficacy with PAI compared to PEI, Panovska et al., (2007) histomorphological. So this study substantiates the potential activity of the acetate extract lead induced spleen damage. Moon and Cha, (2008) suggest that supplementation of persimmon-

vinegar prevents metabolic disorders induced by chronic administration of alcohol. Luster et al., (1978) suggest that the T-lymphocyte rather than the B-lymphocyte is affected by lead exposure, Klein & Koch (1981) found that lead accumulated in the hepatic tissue of infants after 11-20 days of delivery. Overmann *et al* .,(1981)found that wet weight of spleen and thymus were not altered at any post natal lead treatment, Regarding lead, researches indicated that the embryo exposed to significant level of heavy metals via the blood of mother and through placenta. The lactating embryo was also affected when mother exposed to these metals during lactation. Corpas *et al.,* (2002) added that exposed mothers during gestation and lactation to the lead toxicity led to changes in the hepatic architecture in the newly born and lactating infants .Hsu and Guo (2002) suggest that lead exposure causes generation of reactive oxygen species and alteration of antioxidant defense ,the mechanisms for lead include the effect of lead on membrane, DNA, and antioxidant defense system of cells, while Death et al., (2002) suggest that the action of lead in decreasing circulating growth factor-1 contributes to the delayed puberty, the detrimental effect occurred regardless of the developmental time of exposure, although gestational exposure appeared more sensitive to the effect of lead ,the study of Gorbel et al., (2002) shows that chronic exposure to lead causes a double sexual disorder in rats: first , disorder deals with the hormonal function ,which is affected at the early stages of poisoning ,but is rapidly corrected ;second, disorder deals with the genital tract ,affecting the testis and ovary, resulting in a reduced fertility in females, in spite of presence of a normal oestrus. **Peixoto *et al* (2004)** also mentioned that exposing to heavy metals as lead caused toxicity and the level of toxicity depended on the stage of formation and the treated tissue, where the younger rats were more sensitive to the heavy metals exposure than mature ones. **ZhuZw *et al* (2005)** found the lead level of blood of infants of 14 days age was lower than that of infants of 7 days age and declared that this change depended on both the concentration and duration of exposure**. Pillet et al., (2005)** conclude that neonatal exposures to cadmium through maternal milk lead to both transitory and persistent immunotoxic effect, Nampoothiri and Gupta (2006) demonstrated that lead and cadmium are known reproductive toxicants,which accumulate in granulose cells of the ovary**, Karaca and Simsek (2007) Jihen et. al., (2011),** results indicate that lead induced increase mast cells in the ovary during the oestrous cycle of rats .

2. Materials and Methods:

Mature female mice were divided into three groups: the first group is the control group, the second group was provided with a daily dose of 0.5 ml/kg of lead acetate for a month and the third group was provided with a daily dose of lead with equivalent dose of apple vinegar **(Roiha, 1974**). Female mice, during lactation were divided into three groups from the first day of delivery to the 21st day. The first group was provided with distilled water and this is the control group. the second group was provided with a daily dose of 0.5 ml/kg of lead acetate for a month. The third group was provided with equivalent dose of apple vinegar. Female mice were dissected and specimens of the uterus and ovary were taken. Specimens of spleen and lymphatic gland were taken from dissected embryos. All organ specimens were fixed in neutral formalin 10% and the standard procedure of dehydration and clearing in xylol was followed. Specimens were sectioned and stained by eosin and heamtoxyline.

3. Results:

Lymphatic gland

At newly born age, the gland composed of the cortex only without the formation of the lobe or the lobules. It surrounded by a capsule of fiberous tissue with small lymphatic cells spreaded throughout the cortex. It was also observed that the lymphocytes number was low and presence of decaying in the lymphatic tissue (fig 1).

At two weeks after delivery, It was observed the beginning of the lobules formation but they were not complete. Despite the increasing size of the gland, the lymphocytes number was reduced. Odeama and decaying in the lymphatic tissue were also observed and the lobe did not obviously formed at this age.While,

the complete structure of the gland could be nearly recognized at this age with lobulation and differentiation of the gland to the cortex containing the lymphocytes and lobe containing lymphocytes, reticularocytes and Hasal bodies, these bodies were aggregation of flattened cells. Hasal bodies and lymphocytes were less in number especially in the cortex with decaying regions in the tissue.

2-The histological investigation of the reproductive system of treated females

A -Uterus:

Secretory columnar epithelium were proliferated that caused structural disturbance in the uterine gland. This proliferation also led to a disorder in the nuclei position shifting them away from the basement membrane and pressing of cells due to the increasing in their number. There were not disordering in the number of the uterine gland despite presence of loosen lining cells and some of these cells were observed with decayed nuclei. It was also observed that arteries were convoluted in the deep layers of the uterus lining, while the uterus stroma was composed of fiberous

connective tissues. The reticular connective tissues was less dense than that of the control group. The basal layer of the uterus was represented by muscular layer; circular and longitudinal smooth muscles separated by fine barriers of connective tissue. It could be recognized that the size of the muscular layer was reduced with expanded blood vessels and filled with blood corpuscles(fig2).

Ovary:

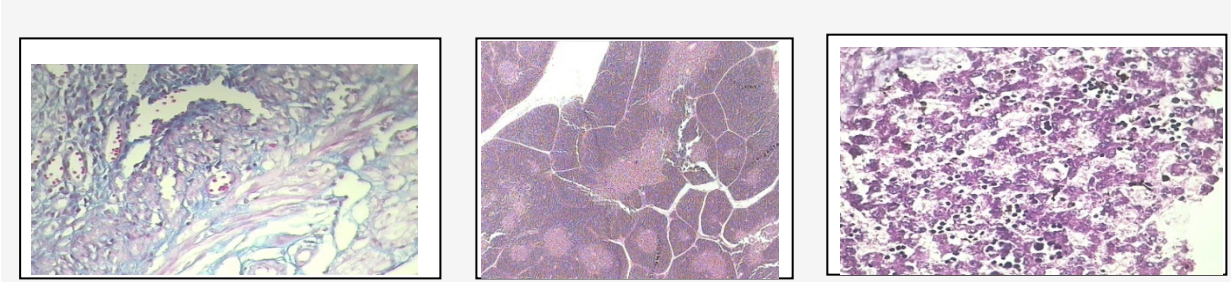
Number, decayed ova in the follicles, smaller outer wall of the follicles due to reduction in the follicular cells and presence of spaces between them with appearing some of them as compact cellular masses. A limited occurrence of primary and secondary follicles was recognized. Also spaces between follicular cells were observed indicating their decaying with decayed ovum in some due to disappearing of the radiating regions that formed by cells and supplied ovum with nutrients. Therefore, there was not any nutrient supply to the ova causing ova decaying, odeama and fibrosis of the tissue. Also the corpus luteum did not appear indicating absence of ovulation. The drug also caused a severe decrease in the ovary stroma and obvious fibrosis in all regions of the tissue.

It was observed that the ovarian columnar secretory cells and the uterine glands resumed their normal structure in the treated group. The muscular layer separated by fine barriers of reticular connective tissue. The rate of blood corpuscles filtration decreased in the blood vessels. For ovary, where all follicles stages, primary, secondary and mature were observed with ova surrounded by discus proligerus and the corpus luteum was appeared in different stages. The ovary stroma resumed its normal size and structure (fig3).

Spleen

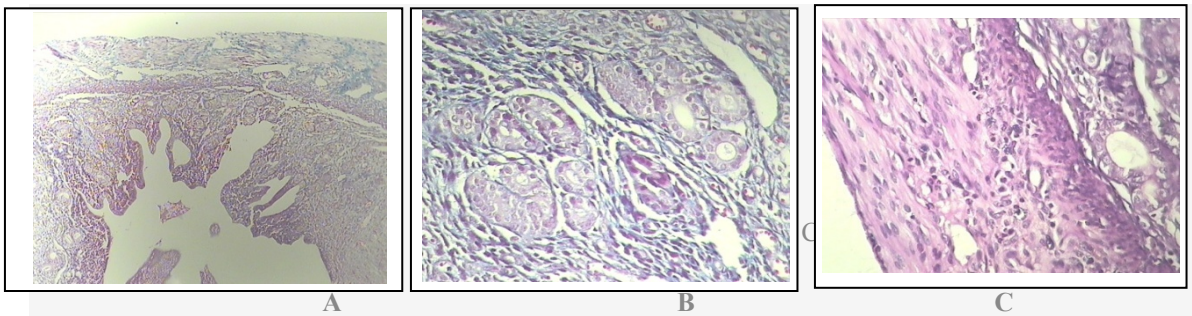
In newly born the histological investigation showed a severe decaying in all components of the tissue including veins and arteries with their lining cells. A serious decaying in the strands of the spleen, sinusoids and nodules was also observed with little presence of lymphatic cells and disappearing of the main barriers of the connective tissue. This led to a decreasing in the number of the veins and arteries and undifferentiation of white and red lobes due to the spreeding of the lymphatic cells in the whole tissue despite of their reducing number.

In one week born infant it was observed that the spleen strands decayed seriously in some regions and disarranged in others with reducing number of lymphatic cells . The white lobe was obvious while the red one was pale stained indicating less spreading of lymphatic cells and accumulation of fats between cells with more density in the white lobe. It was also observed that arteries and veins were enlarged and expanded with blood stasis in some regions and their rupture and decaying in others. Phagocytotic, mononucleated and polynucleated cells were obviously observed while the germinal center was not clear in the splenic nodules while it would be recognized clearly in the control group. In two week individuals, section lost its ability for staining with the continuity of the disassociation and decaying of the spleenic strands. This changes led to widening of the spleenic sinusoids with rupture of the rectangular lining cells and less number of lymphatic cells especially the darker ones and absence of the lymphatic nodules. Spleen was obviously composed of the red lobe indicating the increase in the number of the lymphatic cells B and decrease in the number of T-cells. It was also observed that there was an odeama in the tissue, enlarged arteries and veins, proliferation of phagocytic cells and polymorphic nuclear cells. These histological changes were continued till the weaning age, three weeks (fig4).



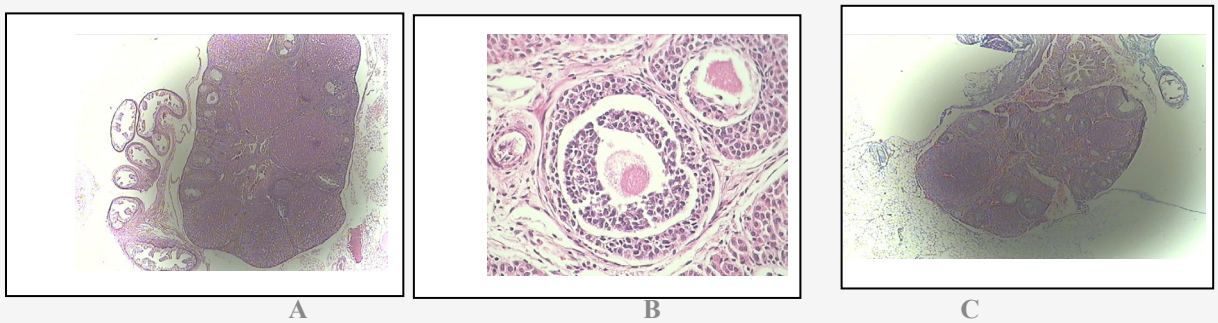
A **B** **C**

Fig. (1): Optical micrograph of the sector in the rat lymphatic gland at the age of three weeks after birth to mothers in the group shot and the vinegar treatment for the duration of breastfeeding (0-21) describes the approach to the installation of natural fabric ((40 ×-H α E E), A-normal B- lead+ vinegar C- lead.



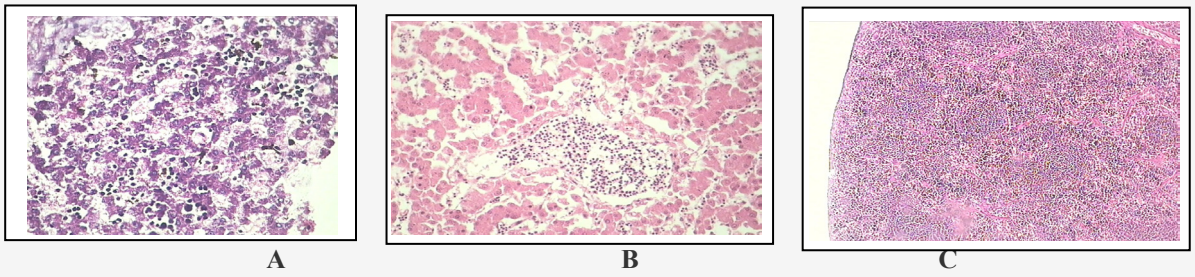
A B C

Fig. (2): Optical micrograph of the sector in the uterus of female mouse in the group shot and the vinegar treatment describes the regularity of epithelial secretory glands and increase the thickness of the uterine muscle layer (10 ×-H α E) A-normal B- lead+ vinegar C- lead. A-normal B- lead+ vinegar C- lead.



A B C

Fig. (3): Optical micrograph of the sector in the female mouse ovary in the treatment group shot shows disruption of installation are: mature follicles (A) and developing B)) and the corpus luteum ((C(10×-H α) A-normal B- lead+ vinegar C- lead.



A B C

Fig. (4) : Optical micrograph of the sector in the rat spleen at the age of three weeks after birth to mothers in the group shot and the vinegar treatment for the duration of breastfeeding (0-21) describes the approach to the installation of natural fabric ((40 ×-H α E E). A-normal B- lead+ vinegar C- lead.

4. Discussion:

The present study showed a sever decaying in all components of the spleen tissue of the newly born infants after exposure and provided daily with doses of lead acetate. the decaying represents by disappearing of the main barriers of the connective tissue and decreasing in number of veins and arteries and spreading of the lymphatic cells. These histopathological alterations extends to three weeks age after delivery and till the weaning age in which the number of the lymphatic cells B increase and decrease in the number of T cells and an odeama was also observed in the tissue.

The histological investigation of the thymus gland of newly born infants till three weeks age after delivery from females exposed to lead toxicity during gestation and lactation showed that the lobules formation were not complete after two weeks and the lymphocytes number was reduced with decaying regions in the tissue of three weeks age after delivery, these finding agree with studies of **Hsu and Guo., (2002**) who suggest that exposure causes generation of reactive oxygen species and alteration of antioxidant defense and illustrated that the mechanisms for lead include membranes ,DNA and antioxidant defense system of cells that is the reason for decaying tissue.

The spleen and thymus gland appeared structurally normal in the rope which treated with Lead and vinegar**, Wang et al,(2007)** conclude that a cupuncture of 'Zusanli'(ST 36) can suppress alcohol and vinegar induced decrease of serum gastrin and cortisol ,which may contribute to its effect in the treatment of spleen deficiency syndrome in the rats**, Teriele and Bodensteiner (2006)** investigate the mechanism of dibromo-acetic acid during pregnant and lactating female ,reproductive parameters of female neonatal rats exposed to DBA were examined maternal weight ,size and major organs weights (spleen, liver, kidneys ,uterus and ovary) and there was no different from control, and thus exposed to DBA during the period of follicular formation did not affect follicular populations in neonatal rats.

As concerning the effect of lead on the reproductive system of the treated females ,The examination of uterus showed disturbance in the uterine gland and the uterus stroma was composed of fibrous connective tissue and The reticular connective tissue was less dense than that of the control group and the lead toxicity led to reduced the size of the muscular layer, while the observation of ovaries shows a serious damage of the ovarian tissues odeama and fibrosis of the ovarian tissues which led to absence of ovulation , these finding agree with studies of **Death et al.,(2002) and Gorbel et al.,(2002)**who suggest the action of lead in decreasing circulating growth factor which led to delayed puberty and causes aduble sexual disorder deals with the hormonal function resulting in a reduced fertility.

The present study was undertaken to evaluate the function of admenstrating vinger,to protect organs against lead they affect these organs in different ways (**Kjellström, 1986; World Health Organization, 1992; Nordberg *et al*., 1994; Epstein, 1997; Sakurama, 1998; Bunout, 1999; Thurman *et al*., 1999).** Long-term EtOH consumption damages mainly the liver **(Bunout, 1999; Thurman *et al*., 1999)**, whereas chronic exposure to Cd results, first of all, in tubular dysfunction **(Kjellström, 1986; World Health Organization, 1992; Nordberg *et al*., 1994)**. Unfortunately, no data are available on the function and structure of both organs in conditions of co-exposure to Cd and EtOH.

The level of lead treatment used in this study corresponds to rats occupational exposure to this heavy metal, or environmental exposure in heavily contaminated areas **(World Health Organization, 1992**). The level of intoxication with EtOH may be tantamount to its misuse in man (**Wis′niewska-Knypl and Wrońska-Nofer, 1994).**

Since the relative weights did not change in the co-exposed rats, the decrease in their weights reflects a retardation in body weight gain, which is a consequence of reduced food **(Brzóska *et al*., 2002**) and water intake, and of Cd–EtOH interaction. Other authors also reported the unfavourable effect of co-exposure to Cd and EtOH on body weight gain **(Tandon and Tewari, 1987;Gupta and Gill, 2000**).

Lead accumulation in the thymus gland of rats exposed to this metal alone as well as in combination with vinger resulted in serious changes in the histology and function of this organs. Similar or more advanced changes in spleen and ovary histology and function under lead influence, have been reported by others **(Aughey *et al*., 1984; Kjellström, 1986; Mitsumori *et al*., 1998). Aughey *et al*. (1984)** noted early pathological changes in rat kidney already after 6 weeks of administration of 50 mg Cd/l in drinking water. After 12 weeks, they revealed signs of tubular necrosis, interstitial fibrosis and glomerular epithelial cell hypertrophy in small areas of the kidney cortex. Pathological changes in kidney ultrastructure (were observed when leadconcentration in this organ exceeded 10 μg/g and they became more pronounced as concentration increased. At a Cd level of about 30 μg/g, necrotic changes were observed **(Aughey *et al*., 1984).**

The results of this study and of other investigations **(Aughey *et al*., 1984**) show that the critical Cd concentration in the kidney cortex is lower than 200 μg/g (the kidney cortex/whole kidney ratio of Cd concentration is about 1.25). Such high Cd concentrations in the kidney cortex were measured in rats fed with diet containing 200 mg Cd/kg for 2–4 months **(Mitsumori *et al*., 1998).**

Morphological observations, together with functional tests, show that lead and vingar, administered separately and especially in combination, lead to spleen and uterus injury, thus posing a serious risk for health. The changes observed in these organs of co-exposed rats can be a result of an independent effect of lead and vingar and also of their interaction. Since vingar alone also had affected the spleen and uterus, on the basis of this study it is difficult to make any definite assessment as to whether vingar influenced lead toxicity, and if so, to what extent. However, such an effect of vingar is very likely, and can be linked to changes in lead body burden. In this work, we measured the We have noted that in the vinger group the whole lead pool in the internal organs was at the same level as in those receiving lead alone, in spite of its lower intake. In the absence of the modifying effect of viger, the concentrations and content of lead in the co-exposed animals should be lower, compared to the lead-only exposed ones.

Due to the different intakes of lead and vigar during their co-administration, than after their separate dosages, we cannot correctly interpret the interactive effects of the two substances on the liver and kidney. Nevertheless, our findings allow us to conclude that viger increases lead nephrotoxicity, although the

present results give no clear evidence of enhanced lead toxicity. However, it seems likely that, if the consumption of lead and vinger were the same in co-exposed and separately exposed animals, the disturbances in liver and kidney function as well as histology, would be more serious in the co-exposed ones. On the basis of the present and previous studies **(Brzóska *et al*., 2000, 2002),** we hypothesize that subjects exposed simultaneously to lead andvinger are more vulnerable to lead accumulation and thus its deleterious health effects, including kidney damage. Further studies are needed to explain lead- vinger interactions in conditions of long-term co-exposure and their consequences for health.However, the activity of of heavy metals is related to their physiological interaction with biological receptors depending on their concentrations. **Jang et al., (2011)** concluded that low level of lead inducephosphatidylserine exposure and erythrophagocytosis associated with anemia. Also **Jihen et. al., (2011**), explain the interrelationship between cadmium, zinc and antioxidants inliver of the rat exposed orally to relativily high doses of cadmium and zinc. Heavy metals affected polycystic ovary syndromewhile garlic treatment reduce some heavy metal accumulation in liver of wistar rats.

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Dynamic Stresses and Displacements around Cylindrical Cavities in an Infinite Elastic Medium under Moving Step Loads on the Cavity's Surface

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Abstract: Potential functions and Fourier series method in the cylindrical coordinate system are employed to solve the problem of moving loads on the surface of a cylindrical bore in an infinite elastic medium. The steady-state dynamic equations of medium are uncoupled into Helmholtz equations, via given potentials. It is used that because of the superseismic nature of the problem, two mach cones are formed and opened toward the rear of the front in the medium. The stresses and displacements are obtained by using integral equations with certain boundary conditions. Finally, the dynamic stresses and displacements for step loads with axisymmetric and nonaxisymmetric cases are obtained and discussed in details via a numerical example. Moreover, effects of Mach numbers and poisson's ratio of medium on the values of stresses are discussed.

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1. Introduction

1.1 General Remarks

Moving loads on the surfaces have been investigated by many researchers. Investigation on dynamic stresses in solids is very significant in the study of dynamic strength of materials and in the design of underground structures subject to ground blasting waves. A related but considerably simpler problem has been treated by Biot (1952), who considered space- harmonic axisymmetric standing waves and obtained a closed form solution. Another related problem was treated by Cole and Huth (1958), who considered a line load progressing with a velocity V on the surface of an elastic half- space. Because of the simpler geometry, they were able to obtain a solution in closed form. Adrianus (2002) investigated the moving point load problem in soil dynamics with a view to determine the ground motion generated by a high-speed train traveling on a poorly consolidated soil with low shear wave speed. M.C.M. Bakker (1999) revisited the nonaxisymmetrical boundary value problem of a point load of normal traction traveling over an elastic half-space. M.Rahman (2001) considered the problem of a line load moving at a constant transonic speed across the surface of an elastic half-space and derived solution of the problem by using the method of Fourier transform. Iavorskaia (1964) also studied diffraction of a plane longitudinal wave on circular cylinder. One basic method has been used for the solution of these problems, the solution is obtained by using an integral transform of the displacement potentials. The resulting transformed equations are then solved in terms of Hankel functions, and finally the stresses and displacements are found by inversion of the transformed quantities. In this paper the coefficients of the stresses and displacements are found by solving sets of coupled integral equations.

The waves are expanded into Fourier series in terms of the angle, θ , around the opening. The stress field of the wave is written in terms of potential functions which satisfy the equations of motion. These equations decoupled via introducing the potential functions and reduced to Helmholtz equations that the potentials satisfy.

These potential functions are in integral form with unknown functions in the integrands. Therefore the Fourier series coefficients of the stresses and displacements are also in integral form with unknown integrands. The applied boundary tractions (the step loads) are expanded into a Fourier series in θ and expressions for the stress and displacement components at points in the medium are derived for each term of the Fourier series as functions of the radial distance r from the cavity axis and the distance z behind the wave front.

The following three cases of step loads are considered: normal to the surface, tangential to the surface in the direction of the axis of the bore, and tangential to the circle of load application. These results can be used, by superposition, to determine the effects of other load patterns moving with the velocity V in the direction of the axis of the bore.

Numerical solution of these equations gives the values of the unknown functions. These values can

then be used to find the stresses and displacements on the boundary and also anywhere in the medium.

1.2 Problem Description

The object of this work is to obtain stresses and displacements in an elastic medium in the vicinity of a cylindrical cavity which is engulfed by a plane stress wave of dilatational travelling parallel to the axis of the cylinder, as shown in Figure 1.

The step load has an arbitrary distribution $P(\theta)$ along the circumference of the circle and moves with a velocity $V > C_1 > C_2$; therefore, the speed is superseismic with respect to both the dilatational and shear waves in the medium. Consequently, the disturbances which were initiated far behind the front on the boundary of the cavity cannot reach the vicinity of the wave front for some time after the incident wave passes.

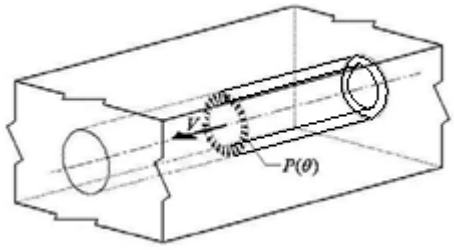

Figure 1. Moving step load

Moreover, because of the super seismic nature of the problem, it should be expected that two mach cones will be formed in the medium, as shown in Figure 2. These cones should open toward the rear of the front. Furthermore, there can be no stresses or displacements ahead of the leading front.

If a coordinate system is assumed to move along the cylinder with the wave front, it is seen that the state of stress at points close behind the wave front depends only on relative position of them with respect to the front. Thus, in the vicinity of the wave front, provided that the end of the cavity is far away, the problem may be treated as a steady-state case. In other words, in the moving coordinate system, the state of stress and displacement is independent of time.

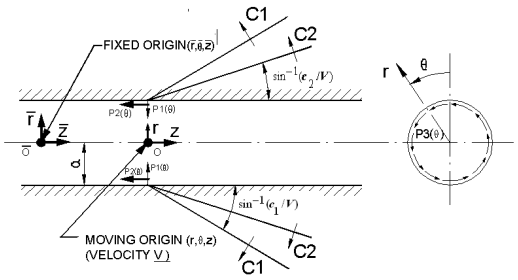

Figure 2. Geometry of the problem and the coordinate systems

2. Governing equations and general solutions

Consider a cylindrical cavity of radius r = a in a linearly elastic, homogeneous, and isotropic medium referred to a fixed coordinate system $(\bar{r},\bar{\theta},\bar{z})$ whose origin lies on the axis of the cavity.

A step load along the circle at $\bar{z}$ = -vt progresses along the interior of the cavity with a velocity V such that the stresses on the boundary r=a are:

$$\sigma_{rr}\Big|_{r=a} = \sigma_1(\bar{\theta})U(\bar{z}+vt) \quad (1)$$

$$\sigma_{r\theta}\Big|_{r=a} = \sigma_2(\bar{\theta})U(\bar{z}+vt) \quad (2)$$

$$\sigma_{rz}\Big|_{r=a} = \sigma_3(\bar{\theta})U(\bar{z}+vt) \quad (3)$$

Where the functions $\sigma_k(\theta\theta)$ define the distribution of the applied load. To determine the steady state solution, a moving coordinate system (r , θ ,z) is introduced such that:

$$r=\bar{r}, \theta=\bar{\theta}, z=\bar{z}+Vt \quad (4)$$

The following treatment is restricted to the case where the velocity V is greater than C_1 and C_2 , the respective propagation velocities of dilatational and equivoluminal waves in the medium. Hence

$$M_1=\frac{V}{C_1}>1, \quad M_2=\frac{V}{C_2}>1 \quad (5)$$

Where

$$C_1=\sqrt{\frac{\lambda+2\mu}{\rho}}, \quad C_2=\sqrt{\frac{\mu}{\rho}} \quad (6)$$

The equations of motion in cylindrical coordinates, r , θ, z, for an elastic medium, may be expressed in the following form:

$$\nabla^2 u_r - \frac{1}{r^2}(u_r + 2\frac{\partial u_\theta}{\partial\theta}) + (\frac{\lambda}{\mu}+1)\frac{\partial\Delta}{\partial r} = \frac{\rho}{\mu}\frac{\partial^2 u_r}{\partial t^2}$$

$$\nabla^2 u_\theta - \frac{1}{r^2}(u_\theta - 2\frac{\partial u_r}{\partial\theta}) + (\frac{\lambda}{\mu}+1)\frac{1}{r}\frac{\partial\Delta}{\partial\theta} = \frac{\rho}{\mu}\frac{\partial^2 u_\theta}{\partial t^2}$$

$$\nabla^2 u_{\bar{z}} + (\frac{\lambda}{\mu}+1)\frac{\partial\Delta}{\partial\bar{z}} = \frac{\rho}{\mu}\frac{\partial^2 u_{\bar{z}}}{\partial t^2} \quad (7)$$

Where the dilatation , Δ , and the laplacian operator, ∇^2 , are given by:

$$\Delta = \frac{\partial u_r}{\partial r} + \frac{u_r}{r} + \frac{1}{r}\frac{\partial u_\theta}{\partial\theta} + \frac{\partial u_{\bar{z}}}{\partial\bar{z}}$$

$$\nabla^2 = \frac{\partial^2}{\partial r^2} + \frac{1}{r}\frac{\partial}{\partial r} + \frac{1}{r^2}\frac{\partial^2}{\partial\theta^2} + \frac{\partial^2}{\partial\bar{z}^2} \quad (8)$$

As mentioned earlier, the assumption of the existence of a steady-state case and trans-formation form r , θ , $\bar{z}$ coordinates to r , θ, z results in elimination of the time variable, t , from the equations of motion.

This transformation is performed by the following relations, as given in relations (4):

$$z = \bar{z} + Vt$$

$$\frac{\partial}{\partial \bar{z}} = \frac{\partial}{\partial z}, \quad \frac{\partial}{\partial t} = V\frac{\partial}{\partial z}$$

Therefore equations (7) may be expressed as follows:

$$\nabla^2 u_r - \frac{1}{r^2}(u_r + 2\frac{\partial u_\theta}{\partial \theta}) + (\frac{\lambda}{\mu}+1)\frac{\partial \Delta}{\partial r} = \frac{\rho}{\mu}V^2\frac{\partial^2 u_r}{\partial z^2}$$

$$\nabla^2 u_\theta - \frac{1}{r^2}(u_\theta - 2\frac{\partial u_r}{\partial \theta}) + (\frac{\lambda}{\mu}+1)\frac{1}{r}\frac{\partial \Delta}{\partial \theta} = \frac{\rho}{\mu}V^2\frac{\partial^2 u_\theta}{\partial z^2}$$

$$\nabla^2 u_z + (\frac{\lambda}{\mu}+1)\frac{\partial \Delta}{\partial_z} = \frac{\rho}{\mu}V^2\frac{\partial^2 u_z}{\partial z^2}$$

(9)

Stress components are given by

$$\sigma_{rr} = \lambda\Delta + 2\mu\frac{\partial u_r}{\partial r}$$

$$\sigma_{\theta\theta} = \lambda\Delta + 2\mu(\frac{u_r}{r} + \frac{1}{r}\frac{\partial u_\theta}{\partial \theta})$$

$$\sigma_{zz} = \lambda\Delta + 2\mu\frac{\partial u_z}{\partial z} \qquad (10)$$

$$\sigma_{r\theta} = \mu(\frac{1}{r}\frac{\partial u_r}{\partial \theta} - \frac{u_\theta}{r} + \frac{\partial u_\theta}{\partial r})$$

$$\sigma_{rz} = \mu(\frac{\partial u_r}{\partial z} + \frac{\partial u_z}{\partial r})$$

$$\sigma_{\theta z} = \mu(\frac{\partial u_\theta}{\partial z} + \frac{1}{r}\frac{\partial u_z}{\partial \theta})$$

Displacement components u_r, u_θ and u_z may be expressed in Fourier series:

$$u_r(r,\theta,z) = \sum_{n=0}^{\infty} u_{n,r}(r,z)\cos n\theta$$

$$u_\theta(r,\theta,z) = \sum_{n=1}^{\infty} u_{n,\theta}(r,z)\sin n\theta \qquad (11)$$

$$u_z(r,\theta,z) = \sum_{n=0}^{\infty} u_{n,z}(r,z)\cos n\theta$$

Three potential functions are now introduced,

$$\varphi(r,\theta,z) = \sum_{n=0}^{\infty} \varphi_n(r,z)\cos n\theta$$

$$\psi(r,\theta,z) = \sum_{n=0}^{\infty} \psi_n(r,z)\cos n\theta \qquad (12)$$

$$\chi(r,\theta,z) = \sum_{n=1}^{\infty} \chi_n(r,z)\sin n\theta$$

The displacement components $u_{n,r}, u_{n,\theta}$ and $u_{n,z}$ are defined as follows:

$$u_{n,r} = \frac{\partial \varphi_n}{\partial r} + \frac{\partial^2 \psi_n}{\partial r \partial z} + \frac{n}{r}\chi_n$$

$$u_{n,\theta} = -\frac{n}{r}\varphi_n - \frac{n}{r}\frac{\partial \psi_n}{\partial z} - \frac{\partial \chi_n}{\partial r} \qquad (13)$$

$$u_{n,z} = \frac{\partial \varphi_n}{\partial z} - \left(\frac{\partial^2}{\partial r^2} + \frac{1}{r}\frac{\partial}{\partial r} - \frac{n^2}{r^2}\right)\psi_n$$

These equations may be obtained from the vector equation

$$\bar{u} = \text{grad}\,\varphi + \text{curl}\,\bar{\omega}$$

Where $\bar{\omega}$ is the sum of two independent vectors as follows:

$$\bar{\omega} = \bar{\chi} + \text{curl}\,\bar{\psi}$$

The vectors $\bar{\chi}$ and $\bar{\psi}$ have only one non- zero component which is in the z- direction in both cases.

$$\chi_r = 0 \qquad \psi_r = 0$$
$$\chi_\theta = 0 \qquad \psi_\theta = 0$$
$$\chi_z = \chi \qquad \psi_z = \psi$$

By substitution of the values given in equations (13) into the equations (9), it can be shown that the potential functions satisfy the modified wave equations.

$$\nabla^2 \varphi_n = (\frac{V}{c_1})^2\frac{\partial^2 \varphi_n}{\partial z^2}$$

$$\nabla^2 \psi_n = (\frac{V}{c_2})^2\frac{\partial^2 \psi_n}{\partial z^2} \qquad (14)$$

$$\nabla^2 \chi_n = (\frac{V}{c_2})^2\frac{\partial^2 \chi_n}{\partial z^2}$$

Stress components are expressed in Fourier series form as follows:

$$\sigma_{rr}(r,\theta,z) = \sum_{n=0}^{\infty} \sigma_{n,rr}(r,z)\cos n\theta$$

$$\sigma_{\theta\theta}(r,\theta,z) = \sum_{n=0}^{\infty} \sigma_{n,\theta\theta}(r,z)\cos n\theta$$

$$\sigma_{zz}(r,\theta,z) = \sum_{n=0}^{\infty} \sigma_{n,zz}(r,z)\cos n\theta$$

$$\sigma_{r\theta}(r,\theta,z) = \sum_{n=1}^{\infty} \sigma_{n,r\theta}(r,z)\sin n\theta \qquad (15)$$

$$\sigma_{rz}(r,\theta,z) = \sum_{n=0}^{\infty} \sigma_{n,rz}(r,z)\cos n\theta$$

$$\sigma_{\theta z}(r,\theta,z) = \sum_{n=1}^{\infty} \sigma_{n,\theta z}(r,z)\sin n\theta$$

Equations (11) and (15) may be substituted into equations (10), and as a result stress- displacement relations may be written for each term of the series:

$$\sigma_{n,rr} = \lambda\Delta + 2\mu\frac{\partial u_{n,r}}{\partial r} \qquad (16)$$

$$\sigma_{n,\theta\theta}=\lambda\Delta+2\mu\left(\frac{u_{n,r}}{r}+n\frac{u_{n,\theta}}{r}\right)$$

$$\sigma_{n,zz}=\lambda\Delta+2\mu\frac{\partial u_{n,z}}{\partial z}$$

$$\sigma_{n,r\theta}=\mu\left(-n\frac{u_{n,r}}{r}-\frac{u_{\theta}}{r}+\frac{\partial u_{n,\theta}}{\partial r}\right)$$

$$\sigma_{n,rz}=\mu\left(\frac{\partial u_{n,r}}{\partial z}+\frac{\partial u_{n,z}}{\partial r}\right)$$

$$\sigma_{n,\theta z}=\mu\left(\frac{\partial u_{n,\theta}}{\partial z}-n\frac{u_{n,z}}{r}\right)$$

where

$$\Delta=\frac{\partial u_{n,r}}{\partial r}+\frac{u_{n,r}}{r}+n\frac{u_{n,\theta}}{r}+\frac{\partial u_{n,z}}{\partial z}$$

Substitution of equations (13) into equations (16) and application of the differential equations (14) result in the following equations for stress components:

$$\frac{a^2\sigma_{n,rr}}{\mu}=\left(M_2^2-2M_1^2\right)\varphi_{n,ZZ}+2\varphi_{n,RR}+2\psi_{n,RRZ}+$$
$$+\frac{2n}{R}\left(\chi_{n,R}-\frac{1}{R}\chi_n\right)$$

$$\frac{a^2\sigma_{n,\theta\theta}}{\mu}=\left(M_2^2-2\right)\varphi_{n,ZZ}-2\varphi_{n,RR}+$$
$$\frac{2}{R}\left(\psi_{n,RZ}-\frac{n^2}{R}\psi_{n,Z}\right)-\frac{2n}{R}\left(\chi_{n,R}-\frac{1}{R}\chi_n\right)$$

$$\frac{a^2\sigma_{n,zz}}{\mu}=\left(M_2^2-2M_1^2+2\right)\varphi_{n,ZZ}-2\left(M_2^2-1\right)\psi_{n,ZZZ}$$

$$\frac{a^2\sigma_{n,r\theta}}{\mu}=-\frac{2n}{R}\left(\varphi_{n,R}-\frac{1}{R}\varphi_n\right)-\frac{2n}{R}\left(\psi_{n,RZ}-\frac{1}{R}\psi_{n,Z}\right)+ \quad (17)$$
$$+\left(M_2^2-1\right)\chi_{n,ZZ}-2\chi_{n,RR}$$

$$\frac{a^2\sigma_{n,rz}}{\mu}=2\varphi_{n,RZ}-\left(M_2^2-2\right)\psi_{n,RZZ}+\frac{n}{R}\chi_{n,Z}$$

$$\frac{a^2\sigma_{n,\theta z}}{\mu}=-\frac{2n}{R}\varphi_{n,Z}+\left(M_2^2-2\right)\frac{n}{R}\psi_{n,ZZ}-\chi_{n,RZ}$$

Where the second set of subscripts of φ_n, ψ_n and χ_n represent the partial derivatives of these functions. R and Z are the dimensionless variables form:

$$R=\frac{r}{a},\ Z=\frac{z}{a} \quad (18)$$

The values M_1 and M_2 are defined as follows:

$$M_1=\frac{V}{C_1},\ M_2=\frac{V}{C_2} \quad (19)$$

Let

$$\beta_1^2=M_1^2-1 \quad , \quad \beta_2^2=M_2^2-1$$

The differential equations (14) may be written in the following form:

$$\frac{\partial^2\varphi_n}{\partial R^2}+\frac{1}{R}\frac{\partial\varphi_n}{\partial R}-\frac{n^2}{R^2}\varphi_n=\beta_1^2\frac{\partial^2\varphi_n}{\partial Z^2}$$

$$\frac{\partial^2\psi_n}{\partial R^2}+\frac{1}{R}\frac{\partial\psi_n}{\partial R}-\frac{n^2}{R^2}\psi_n=\beta_2^2\frac{\partial^2\psi_n}{\partial Z^2} \quad (20)$$

$$\frac{\partial^2\chi_n}{\partial R^2}+\frac{1}{R}\frac{\partial\chi_n}{\partial R}-\frac{n^2}{R^2}\chi_n=\beta_2^2\frac{\partial^2\chi_n}{\partial Z^2}$$

It is seen that these equations have the same general form as the differential equations of the cylindrical waves obtained in reference (13). Therefore solutions of equations (20) may be obtained in a manner similar to that in reference (13). These solutions are given in integral form as follows (see Appendix A for verification of the solutions):

$$\varphi_n=\int_0^{\infty}f_n(Z-R\beta_1\cosh u)\cosh nu\,du$$

$$\psi_n=\int_0^{\infty}g_n(Z-R\beta_2\cosh u)\cosh nu\,du \quad (21)$$

$$\chi_n=\int_0^{\infty}h_n(Z-R\beta_2\cosh u)\cosh nu\,du$$

From consideration of the fact that the disturbances are zero ahead of the wave front, it is seen that the functions f_n , g_n and h_n are zero for the values of their arguments less than $-R\beta_1$, $-R\beta_2$ and $-R\beta_2$,respectively.

Therefore the upper limits of the integrals may be changed from ∞ to the following values:

$$u_1=\cosh^{-1}\left(1+\frac{Z}{R\beta_1}\right) \quad \text{for } \varphi_n$$
$$u_2=\cosh^{-1}\left(1+\frac{Z}{R\beta_2}\right) \quad \text{for } \psi_n \text{ \& } \chi_n \quad (22)$$

The integrals are then written with these limits:

$$\varphi_n=\int_0^{u_1}f_n\left(Z-R\beta_1\cosh u\right)\cosh nu\,du$$

$$\psi_n=\int_0^{u_2}g_n\left(Z-R\beta_2\cosh u\right)\cosh nu\,du \quad (23)$$

$$\chi_n=\int_0^{u_2}h_n\left(Z-R\beta_2\cosh u\right)\cosh nu\,du$$

3. Expressions of stresses and displacements

Substitution of equations (23) into equations (17) gives the following expressions for stress components.

$$\frac{a^2\sigma_{n,rr}}{\mu}=\int_0^{u_1}f''(\eta_1)[M_2^2-2+$$
$$+2\beta_1^2\sinh^2u]\cosh nu\,du+ \quad (24a)$$
$$+2\beta_2^2\int_0^{u_2}g'''(\eta_2)\cosh^2u\cosh nu\,du-$$
$$-\beta_2^2\int_0^{u_2}h''(\eta_2)\sinh 2u\cosh nu\,du$$

$$\frac{a^2\sigma_{n,\theta\theta}}{\mu} = \int_0^{u_1} f''(\eta_1)[M_2^2 - 2 - 2\beta_1^2\cosh^2 u]\cosh nu\,du - 2\beta_2^2\int_0^{u_2} g'''(\eta_2)\sinh^2 u\cosh nu\,du + \beta_2^2\int_0^{u_2} h''(\eta_2)\sinh 2u\cosh nu\,du \quad (24b)$$

$$\frac{a^2\sigma_{n,zz}}{\mu} = (M_2^2 - 2M_1^2 + 2)\int_0^{u_1} f''(\eta_1)\cosh nu\,du - 2\beta_2^2\int_0^{u_2} g'''(\eta_2)\cosh nu\,du \quad (24c)$$

$$\frac{a^2\sigma_{n,r\theta}}{\mu} = \beta_1^2\int_0^{u_1} f''(\eta_1)\sinh 2u\sinh nu\,du + \beta_2^2\int_0^{u_2} g'''(\eta_2)\sinh 2u\sinh nu\,du - \beta_2^2\int_0^{u_2} h''(\eta_2)\cosh 2u\cosh nu\,du \quad (24d)$$

$$\frac{a^2\sigma_{n,rz}}{\mu} = -2\beta_1\int_0^{u_1} f''(\eta_1)\cosh u\cosh nu\,du + \beta_2(M_2^2 - 2)\int_0^{u_2} g'''(\eta_2)\cosh u\cosh nu\,du + \beta_2\int_0^{u_2} h''(\eta_2)\sinh u\sinh nu\,du \quad (24e)$$

$$\frac{a^2\sigma_{n,\theta z}}{\mu} = -2\beta_1\int_0^{u_1} f''(\eta_1)\sinh u\sinh nu\,du + \beta_2(M_2^2 - 2)\int_0^{u_2} g'''(\eta_2)\sinh u\sinh nu\,du + \beta_2\int_0^{u_2} h''(\eta_2)\cosh u\cosh nu\,du \quad (24f)$$

Substitution of equations (23) into equations (13) gives the following expressions for displacement components,

$$\frac{\mu u_{n,r}}{a} = \frac{-R\beta_1^2}{n^2-1}\int_0^{u_1} f''(\eta_1)\sinh u\,[n\sinh nu\cosh u - \sinh u\cosh nu]\,du + \frac{-R\beta_2^2}{n^2-1}\int_0^{u_2} g'''(\eta_2)\sinh u\,[n\sinh nu\cosh u - \sinh u\cosh nu]\,du + \frac{-R\beta_2^2}{n^2-1}\int_0^{u_2} h''(\eta_2)\sinh u\,[\sinh nu\cosh u - n\sinh u\cosh nu]\,du \quad (25a)$$

$$\frac{\mu u_{n,\theta}}{a} = \frac{-R\beta_1^2}{n^2-1}\int_0^{u_1} f''(\eta_1)\sinh u\,[n\sinh u\cosh nu - \sinh nu\cosh u]\,du + \frac{-R\beta_2^2}{n^2-1}\int_0^{u_2} g'''(\eta_2)\sinh u\,[n\sinh u\cosh nu - \sinh nu\cosh u]\,du + \frac{R\beta_2^2}{n^2-1}\int_0^{u_2} h''(\eta_2)\sinh u\,[n\sinh nu\cosh u - \sinh u\cosh nu]\,du \quad (25b)$$

$$\frac{\mu u_{n,z}}{a} = \frac{R\beta_1}{n}\int_0^{u_1} f''(\eta_1)\sinh u\sinh nu\,du + \frac{-R\beta_2^3}{n}\int_0^{u_2} g'''(\eta_2)\sinh u\sinh nu\,du \quad (25c)$$

Where $\eta_1 = Z - R\beta_1\cosh u$, $\eta_2 = Z - R\beta_2\cosh u$

And $f(\eta_1), g(\eta_2), h(\eta_2) = f_n(\eta_1), g_n(\eta_2), h_n(\eta_2)$ and primes represent the derivatives of the functions with respect to their arguments.

4. Boundary conditions

In order to satisfy the condition of a traction boundary at the face of the cavity, r=a , three of the stress components must satisfy the following boundary conditions:

$$\left.\sigma_{n,rr}\right]_{r=a} = \sigma_{n1}u(Z), \quad \left.\sigma_{n,rz}\right]_{r=a} = \sigma_{n2}u(Z), \quad \left.\sigma_{n,r\theta}\right]_{r=a} = \sigma_{n3}u(Z) \quad (26)$$

These equations are satisfied for each term n. The coefficients of the stress, $\sigma_{n,rr}, \sigma_{n,r\theta}$ and $\sigma_{n,rz}$ are expressed in equations (24) in integral form. These integrals include the unknown functions $f''(\eta_1), g'''(\eta_2)$ and $h''(\eta_2)$ which are to be found by solving the set of three simultaneous integral equations. These values then may be substituted back into the equations (24) and (25) to find the stress components and displacement components of the waves at any point on the boundary or in the medium behind the move front.

5. Solution of the Boundary Equations

Numerical solution of the boundary equations requires finding numerical values of the functions $f''(\eta_1), g'''(\eta_2)$ and $h''(\eta_2)$.In the following paragraph, the changes in variables are used. At the boundary, the radius R is fixed, R=1. Therefore the arguments of the functions f'', g''' and h'' are:

$$\eta_1 = Z - \beta_1\cosh u, \quad \eta_2 = Z - \beta_2\cosh u \quad (27)$$

$$\text{Welet:} \quad \xi_1 = \frac{Z}{\beta_1}, \quad \xi_2 = \frac{Z}{\beta_2} = k\xi_1 \quad (28)$$

Where $K = \beta_1/\beta_2$. Equations (27) may be written as:

$$\eta_1 = \beta_1(\xi_1 - \cosh u), \quad \eta_2 = \beta_2(k\xi_1 - \cosh u)$$

A new variable ξ is now introduced by the following relations:

$$\cosh u = 1 + \xi, \quad du = \frac{d\xi}{\sinh u} = \frac{d\xi}{\sqrt{2\xi + \xi^2}} \quad (29)$$

The limits of the integrals with this variable are as follows:

$$\text{Lower limits}, \quad u = 0, \ \xi = 0$$

$$\text{Upper limits} \begin{cases} u = u_1, & \xi = \xi_1 \\ u = u_2, & \xi = \xi_2 = k\xi_1 \end{cases} \quad (30)$$

The upper limits are linear functions of Z and ξ_1; therefore, in order to perform numerical integration, the longitudinal axis ξ_1 is divided into small steps. At every point along this axis the numerical integration is performed and at each step only one new value of the functions

$f''(\eta_1), g'''(\eta_2)$ and $h''(\eta_2)$ enters into the computations.

As an example of the procedure of the numerical integration, the component of the stress in the radial direction is given symbolically below:

$$\sigma_{n,rr} = I_{rr}^F + I_{rr}^G + I_{rr}^H \quad (31)$$

Where

$$I_{rr}^F = \int_0^{u_1} f''(\eta_1)\left[M_2^2 - 2 + 2\beta_1^2 \sinh^2 u\right]\cosh nu\, du$$
$$I_{rr}^G = 2\beta_2^2 \int_0^{u_2} g'''(\eta_2)\cosh^2 u \cosh nu\, du \quad (32)$$
$$I_{rr}^H = -\beta_2^2 \int_0^{u_2} h''(\eta_2)\sinh 2u \sinh nu\, du\, du$$

The integrals I_{rr}^F, I_{rr}^G and I_{rr}^H at the p^{th} step are expressed by using the convolution theorem in summation form as follows:

$$I_{rr}^F = \left(R_{rr}^{+F}\right)_0 (f)_p + \sum_{m=1}^{P-1}\left(R_{rr}^F\right)_m (f)_{P-m} + \left(R_{rr}^{-F}\right)_p (f)_0$$
$$I_{rr}^G = \left(R_{rr}^{+G}\right)_0 (g)_p + \sum_{m=1}^{P-1}\left(R_{rr}^G\right)_m (g)_{P-m} + \left(R_{rr}^{-G}\right)_p (g)_0 \quad (33)$$
$$I_{rr}^H = \left(R_{rr}^{+H}\right)_0 (h)_p + \sum_{m=1}^{P-1}\left(R_{rr}^H\right)_m (h)_{P-m} + \left(R_{rr}^{-H}\right)_p (h)_0$$

Where (f) , (g) and (h) are the unknown functions to be evaluated.

At this stage of integration the values $(f)_m$, $(g)_m$, $(h)_m$ are known for m=0 to p-1.The only unknowns in these expressions are $(f)_p$, $(g)_p$, and $(h)_p$. Similar expressions are written for the other components of stress, at the p^{th} step. The boundary conditions are now in the form of a set of three simultaneous linear equations. Solution of this set results in the values of $(f)_p$, $(g)_p$, and $(h)_p$. The procedure is then carried on to the $(P+1)^{th}$ step; Similar operations are performed to find the values of $(f)_{p+1}$, $(g)_{p+1}$, and $(h)_{p+1}$.

As mentioned previously, when the values f, g and h are found at each step, these values are substituted into the expressions for $u_{n,r}, u_{n,\theta}$ and $u_{n,z}$ $\sigma_{n,\theta\theta}, \sigma_{n,zz}, \sigma_{n,\theta z}$ to compute the numerical values of these stresses and displacements in the medium.

6. Numerical Results and Conclusion

For the non-axisymmetric loadings characterized by n > 0, numerical values of the stress components $\sigma_{n,\theta\theta}, \sigma_{n,zz}$ and $\sigma_{n,z\theta}$ at the cavity boundary r=a are presented in this section. These stresses are given for the cases n=1, 2 for each of the three step- traction loading indicated below:

Index Applied load

K = 1 $\left.\begin{matrix}\sigma_{rr}\\ \sigma_{rz}\end{matrix}\right]_{r=a} = \left.\begin{matrix}\sigma_{n1}\cos n\theta\, U(Z)\\ \sigma_{r\theta}\end{matrix}\right]_{r=a} = 0$

K = 2 $\left.\begin{matrix}\sigma_{rz}\\ \sigma_{rr}\end{matrix}\right]_{r=a} = \left.\begin{matrix}\sigma_{n2}\cos n\theta\, U(Z)\\ \sigma_{r\theta}\end{matrix}\right]_{r=a} = 0$

K = 3 $\left.\begin{matrix}\sigma_{r\theta}\\ \sigma_{rr}\end{matrix}\right]_{r=a} = \left.\begin{matrix}\sigma_{n3}\sin n\theta\, U(Z)\\ \sigma_{rz}\end{matrix}\right]_{r=a} = 0$

The curves are shown for two sets of prameters:

Case 1: $M_1 = \frac{V}{C_1} = 2$; $\upsilon = 0.25$

Case 2: $M_1 = \frac{V}{C_1} = 1.033$; $\upsilon = 0.25$

The values of M_1 were chosen for application of the results to problems of some practical interest. The stress components in each case approach the static plain strain solution as Z approaches infinity, indicating that mathematical model produces correct results for propagation of waves in the isotropic medium. For those cases in which the static solutions do not vanish, a typical overshoot above the value of the static (long term) solutions is observed. Moreover, a decrease in the Mach number M_1 appears to compress the stress response curve into a smaller range of Z such that the asymptotic values of the lower value M_1=1,033 are obtained for smaller values of Z. Figures 12 and 13 show the stress components $\sigma_{0,\theta\theta}$ and $\sigma_{0,zz}$ at the cavity boundary r=a for the axisymmetric loading case, n=0, for the mach numbers M_1=1.033, 1.5 and 2. As in the cases where $n \neq 0$, the stress components in each case approach the static plain solutions as Z approaches infinity. Figures 14 through 19 show the displacement components $u_{n,r}$, $u_{n,\theta}$ and $u_{n,Z}$ (n=1, 2, 3, 4) for each of the three loading cases, k=1, 2, 3. Figures 20 through 22 show the $u_{0,r}$ and $u_{0,Z}$ displacement components for the case n=0. These displacement results are shown for the M_1= 2, $\nu = 1/4$ case only.

The only property of the material in the medium which enters into computations is its poisson's ratio. Figures 23 through 26 represent the effect of this parameter on the values of stress components for the axisymmetric loading case, n=0. The following values of poisson's ratio are used in this study:

$$\upsilon = 0 \text{ , } 0.15, \text{ } 0.25 \text{ and } 0.35$$

It is noticed that the change in poisson's ratio does not have a large effect on the maximum value of longitudinal stress for the load case k=2 (Figure 26), while it affects considerably the value of longitudinal stress for the case load, k=1 (Figure 25) , and hoop stress for the two cases, k=1,2 (Figures 23 and 24) for smaller values of Z.

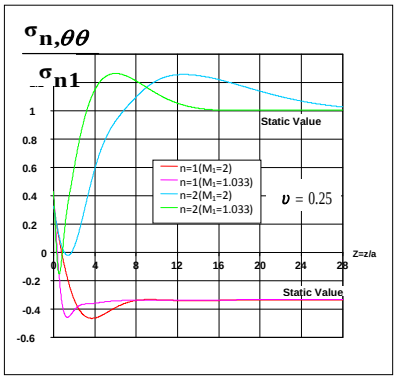

Figure 3. Stress $\sigma_{\theta\theta}$ at boundary due to step load ;

$\sigma_{rr} = \sigma_{n1}\cos n\theta u(Z); n = 1,2$

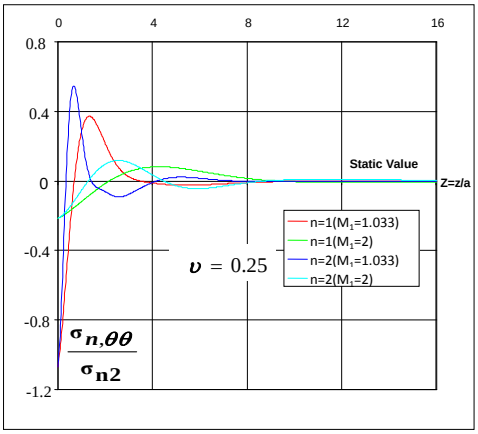

Figure 4. Stress $\sigma_{\theta\theta}$ at boundary due to step load :

$\sigma_{rz} = \sigma_{n2}\cos n\theta u(Z); n = 1,2$

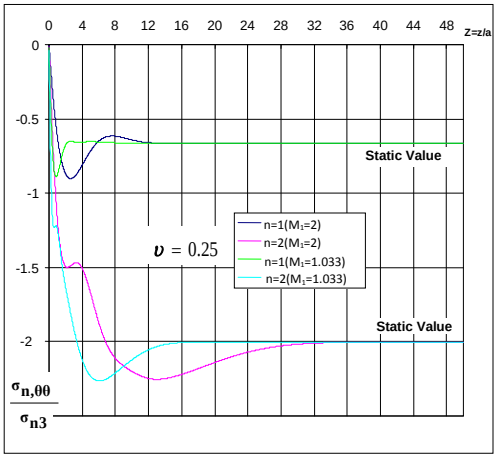

Figure 5. Stress $\sigma_{\theta\theta}$ at boundary due to step load ;

$\sigma_{r\theta} = \sigma_{n3}\sin n\theta u(Z); n = 1,2$

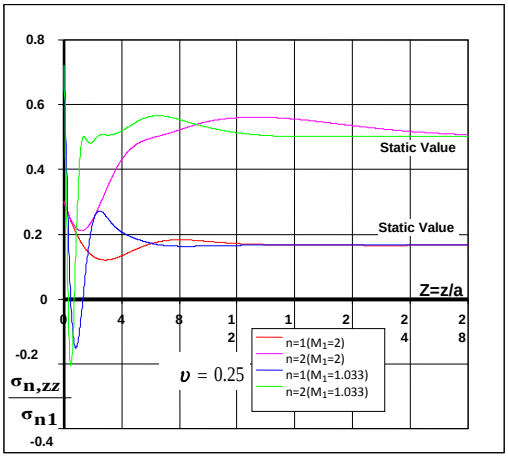

Figure 6. Stress σ_{zz} at boundary due to step load ;

$\sigma_{rr} = \sigma_{n1}\cos n\theta u(Z); n = 1,2$

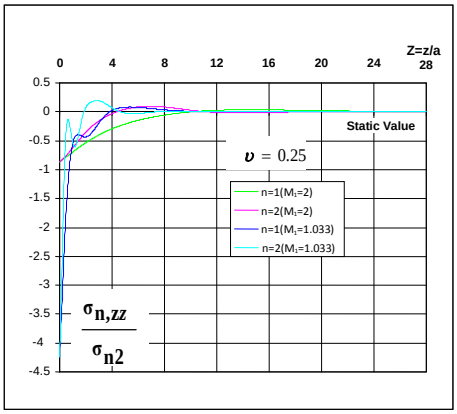

Figure 7. Stress σ_{zz} at boundary due to step load ;

$\sigma_{rz} = \sigma_{n2}\cos n\theta u(Z); n = 1,2$

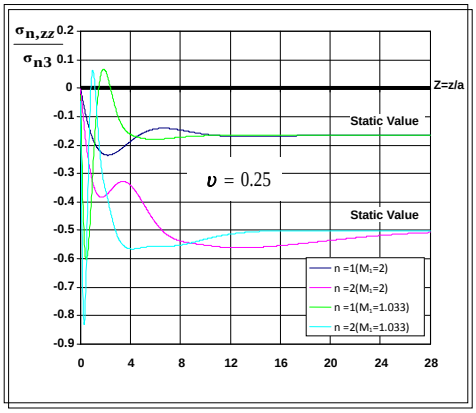

Figure 8. Stress σ_{zz} at boundary due to step load ;

$\sigma_{r\theta} = \sigma_{n3}\sin n\theta u(Z); n = 1,2$

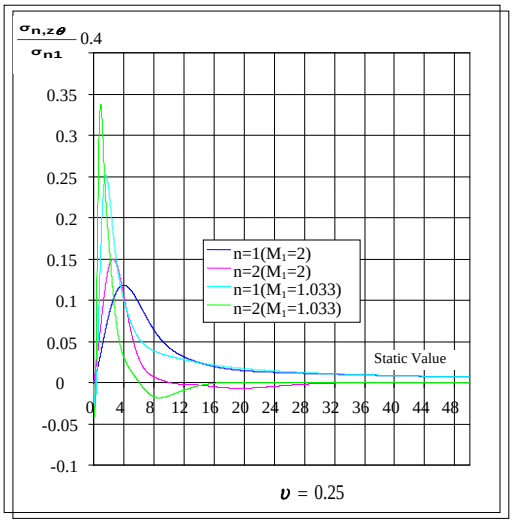

Figure 9. Stress $\sigma_{\theta z}$ at boundary due to step load ;

$\sigma_{rr} = \sigma_{n1}\cos n\theta u(Z); n = 1,2$

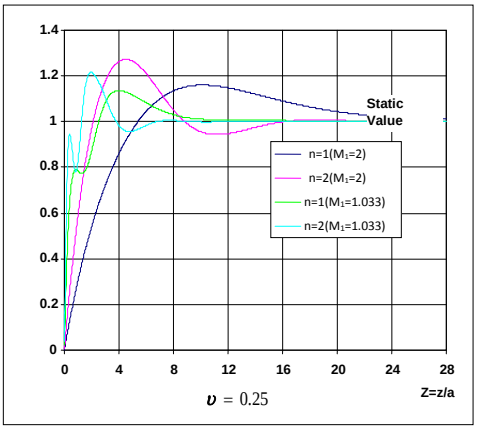

Figure 10. Stress $\sigma_{\theta z}$ at boundary due to step load ; $\sigma_{rz} = \sigma_{n2}\cos n\theta u(Z); n = 1,2$

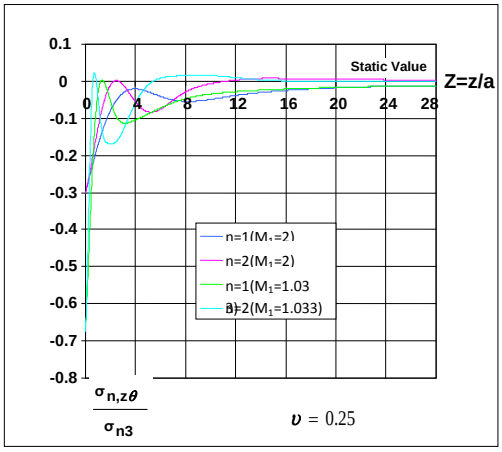

Figure 11. Stress $\sigma_{\theta z}$ at boundary due to step load ;

$\sigma_{r\theta} = \sigma_{n3}\sin n\theta u(Z); n = 1,2$

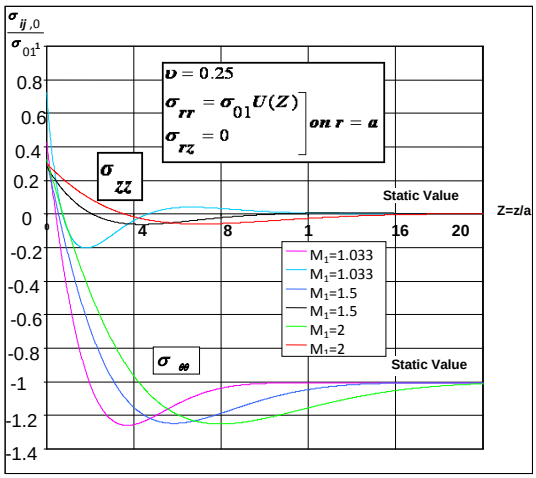

Figure 12. Stresses at boundary due to axisymmetric step load

$\sigma_{rr} = \sigma_{n1}\mathrm{u(Z)}; \mathrm{n} = 0$

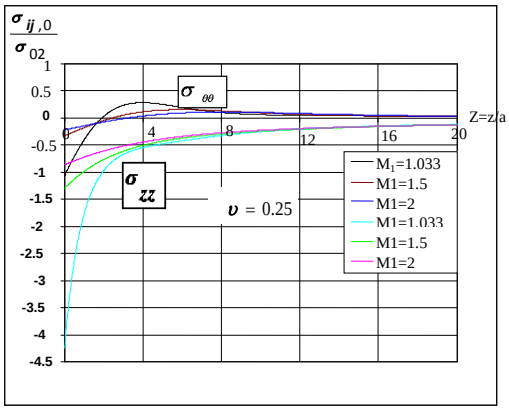

Figure 13. Stresses at boundary due to axisymmetric step load

$\sigma_{rz} = \sigma_{n2}\, u(Z); n = 0$

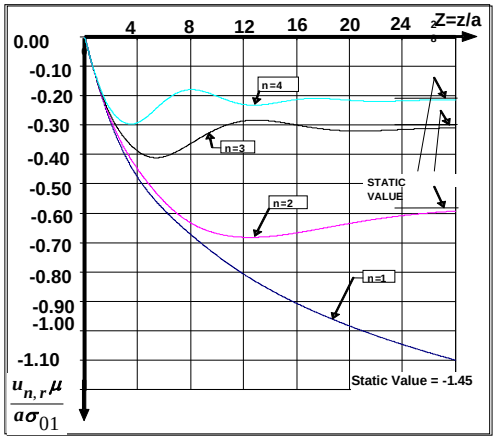

Figure 14. Boundary displacement $u_{n,r}$ due to step load $\sigma_{rr} = \sigma_{n1}\cos n\theta u(Z); n = 1,2,3,4$, $M_1 = 2$, $\upsilon = 0.25$, $\sigma_{r\theta} = \sigma_{rz} = 0$

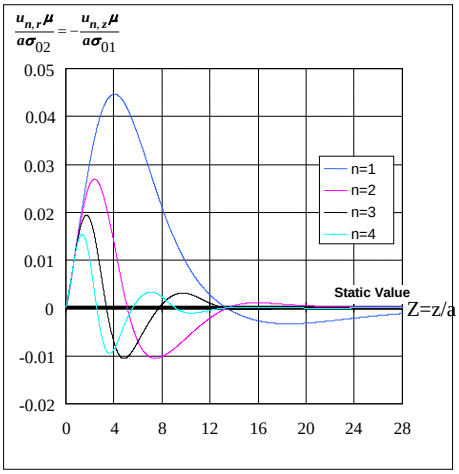

Figure 15. Boundary displacements due to step load ;n=1,2,3,4 , $M_1 = 2$, $\upsilon = 0.25$

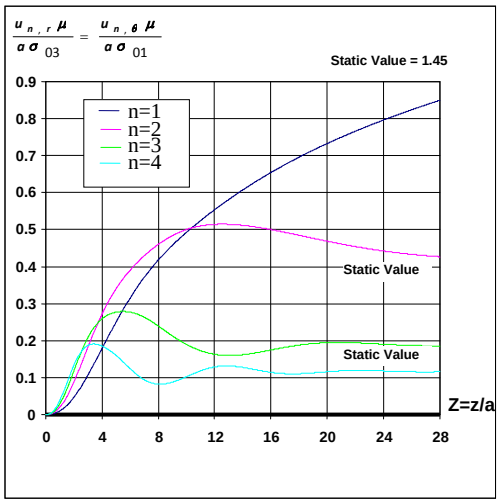

Figure 16. Boundary displacements due to step load ;n=1,2,3,4 , $M_1 = 2$, $\upsilon = 0.25$

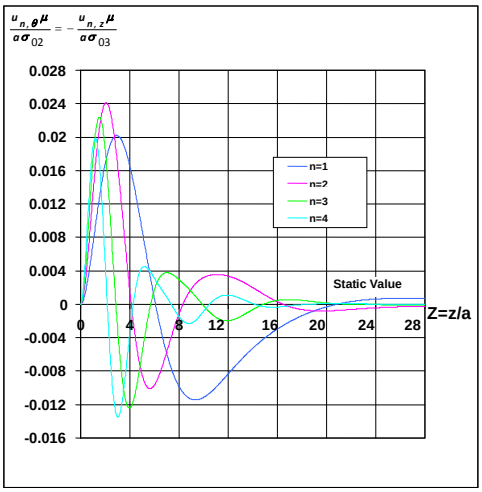

Figure 17. Boundary displacements due to step load ;n=1,2,3,4 , $M_1 = 2$, $\upsilon = 0.25$

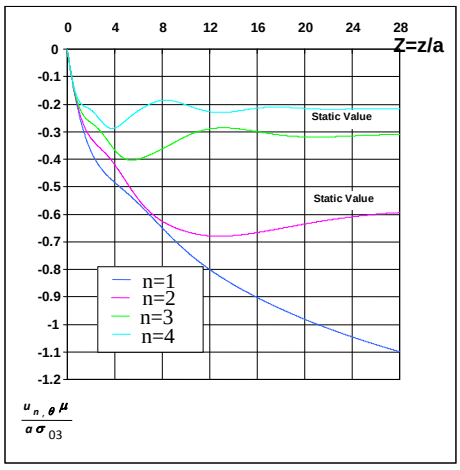

Figure 18. Boundary displacements due to step load ;n=1,2,3,4 , $M_1 = 2$, $\upsilon = 0.25$

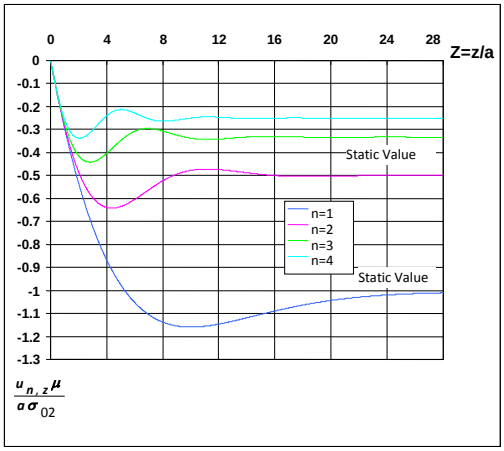

Figure 19. Boundary displacements due to step load ;n=1,2,3,4 , $M_1 = 2$, $\upsilon = 0.25$

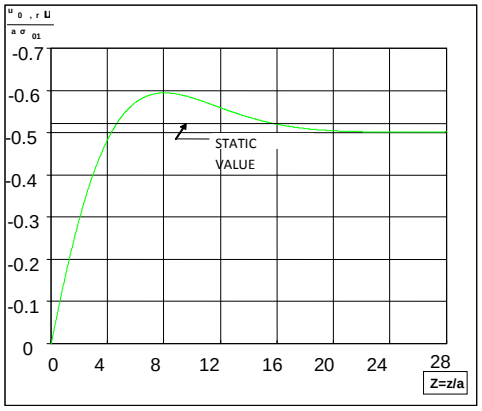

Figure 20. Boundary displacement $u_{0,r}$ due to step load σ_{01} ; n=0 , $M_1 = 2$, $\upsilon = 0.25$

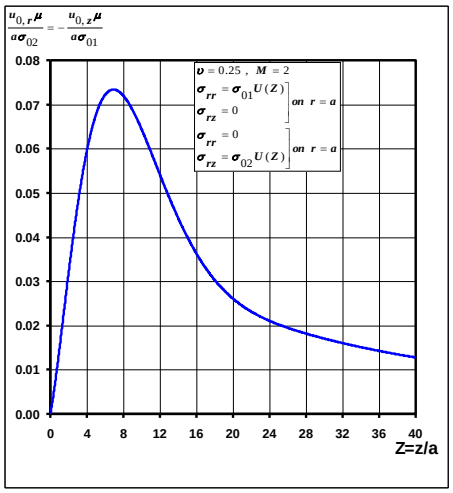

Figure 21. Boundary displacement $u_{0,r}$ due to step load σ_{02} ; n= 0 $u_{0,z}$ due to step load σ_{01} ; n= 0 , $M_1 = 2$, $\upsilon = 0.25$

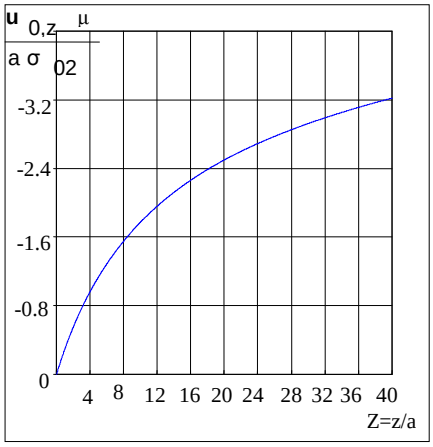

Figure 22. Boundary displacement $u_{0,z}$ due to step load σ_{02} ; n= 0

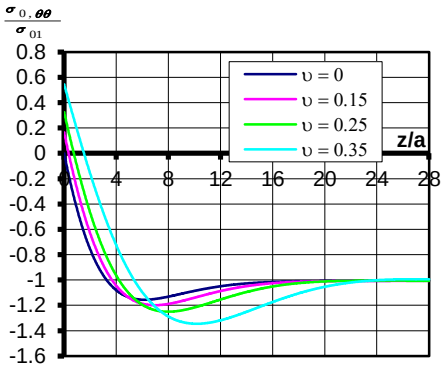

Figure 23. Comparision of Hoop stress for different values of poisson's ratio at boundary r=a due to axisymmetric step load σ_{01} ; n= 0,M_1=2

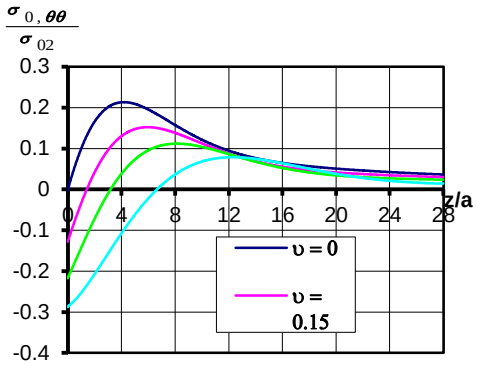

Figure 24. Comparision of Hoop stress for different values of poisson's ratio at boundary r=a due to axisymmetric step load σ_{02} ; n= 0,M_1=2

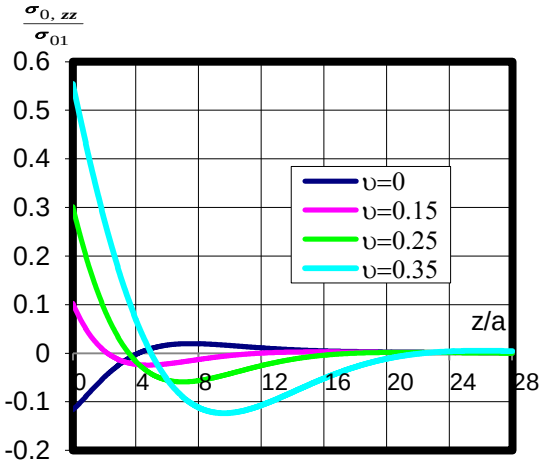

Figure 25. Comparision of Longitudinal stress for different values of poisson's ratio at boundary r=a due to axisymmetric step load σ_{01} ; n= 0, M_1=2

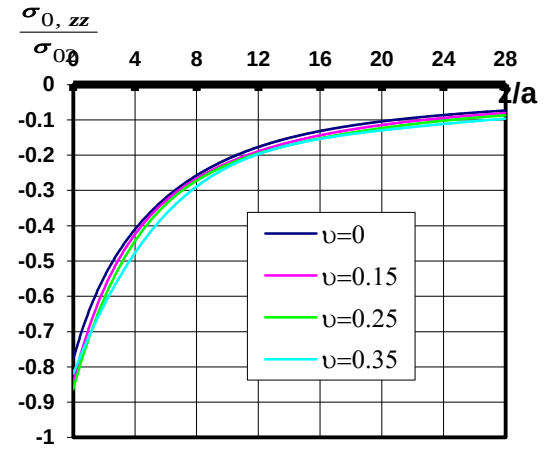

Figure 26. Comparision of Longitudinal stress for different values of poisson's ratio at boundary r=a due to axisymmetric step load σ_{02} ; n= 0, M_1=2

Appendix A

Verification of the Solution of Wave Equation

Consider the modified wave equations expressed in equations (20). A typical differential equation of this kind is expressed as:

$$\nabla^2\varphi = M^2\varphi_{ZZ}$$

Or

$$-\beta^2\varphi_{ZZ} + \varphi_{RR} + \frac{1}{R}\varphi_R - \frac{n^2}{R^2}\varphi = 0 \qquad (A.1)$$

A solution of this differential equation was represented in the following form.

$$\varphi = \int_0^{u_1} f(Z - R\beta\cosh u)\cosh nu\, du \qquad (A.2)$$

Where

$$u_1 = \cosh^{-1}(1 + \frac{Z}{R\beta})$$

In this section, the solution (A.2) is checked by substitution of φ into equation (A.1).

Partial derivatives of φ are:

$$\varphi_R = -\beta\int_0^{u_1} f'(\eta)\cosh u\cosh nu\, du$$

$$\varphi_{RR} = \beta^2\int_0^{u_1} f''(\eta)\cosh^2 u\cosh nu\, du \qquad (A.3)$$

$$\varphi_{ZZ} = \int_0^{u_1} f''(\eta)\cosh nu\, du$$

Where

$$\eta = Z - R\beta\cosh u$$

The function φ may be integrated by parts as follows:

$$\varphi = \int_0^{u_1} f(\eta)\cosh nu\, du = \frac{1}{n}f(\eta)\sinh nu\Big|_0^{u_1} + \frac{R\beta}{n}\int_0^{u_1} f'(\eta)\sinh u\sinh nu\, du \qquad (A.4)$$

The first term on the right hand side is zero, since $f(\eta)$ is zero for values of u greater than u_1. In a similar manner φ_R can be integrated by parts,

$$\varphi_R = -\beta\int_0^{u_1} f'(\eta)\cosh nu\cosh u\, du$$

$$\varphi_R = -R\beta^2\int_0^{u_1} f''(\eta)\sinh^2 u\cosh nu\, du + n\beta\int_0^{u_1} f'(\eta)\sinh u\sinh nu\, du$$

It is easily seen that substitution of the values φ, φ_R, φ_{RR} and φ_{ZZ} into equation (A.1) satisfies this equation.

Similar solutions are obtained for the functions ψ and χ.

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4/25/2012

Golgi Protein 73 (GP73) as a Novel Serum Marker for Early Detection of Hepatocellular Carcinoma in Egyptian Patients

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Abstract: Background: Serum Golgi protein 73 (sGP73) is a novel and promising biomarker for detection of hepatocellular carcinoma (HCC). However, there are few reports on the predictive values levels of GP73 in diagnosis of liver cirrhosis (LC), HCC and the relationship of this level to clinicopathologic features of patients. **Methods:** This study included 66 patients, 31 of them were proved HCC and 35 patients have LC, additionally, 20 apparently healthy subjects were included as a control group. Clinical examination, abdominal ultrasonogrphy, Triphasic C.T to patients with focal lesion. Liver function tests, complete blood cell count and serum AFP were measured. Des-γ-carboxyprothrombin (DCP) and Golgi Protein 73 (GP73) were determined by an ELISA technique. Correlations with clinical parameters were done. **Results:** The serum levels of AFP, DCP and GP73 were significantly elevated in LC and more elevated in HCC cases as compared to controls. The sensitivity and specificity of GP73 for HCC were superior to those of AFP and DCP especially in early detection of HCC, GP73 had a sensitivity of 87% and a specificity of 95% at the optimal cut-off value of 7.62 ng/ml. The area under the receiver-operating characteristic curve (AUROC) was 0.87. DCP give a sensitivity of 80.6% and specificity of 85% at a cut-off 32.64 ng/ml, while, AFP had a sensitivity of 77.4% and a specificity of 60% at a cut-off 28.51 ng/ml. However, when GP73 used in combination with AFP, they lead to an enhanced the sensitivity of HCC detection up to 90.3% and the area under receiver-operating characteristic curve (AUROC) was 0.83. A significant correlation was found between serum GP73 level and prognostic markers of LC (AST, ALT, serum albumin and child score) and more aggressive tumor characters (tumor size and vascular invasion).
Conclusion: the serum level of GP73 may be implicated in development of LC and disease progression to HCC. In combination with AFP, it had an overall performance that was better than AFP alone in early detection of HCC. Future study was needed to be confirmed in larger cohorts of patients to determine if these markers are true indicators of early HCC.

[Mona A El Shafie, Amal M Fawzy, Elhamy Abd Al Monem, Sherif Abbass, Doaa M Zakaria, Sherif El Baz. Golgi Protein 73 (GP73) as a Novel Serum Marker for Early Detection of Hepatocellular Carcinoma in Egyptian Patients. Life Sci J 2012; 9(2):823-830]. (ISSN: 1097-8135). http://www.lifesciencesite.com.122

Key words: HCC, Golgi protein 73, DCP, AFP, ELISA.

List of abbreviations:
sGP73: Serum Golgi protein 73, DCP: Des-γ-carboxyprothrombin, AFP: α-fetoprotein.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer type, and is the third leading cause of cancer mortality worldwide **[1,2].** Recent reports show that HCC is becoming more wide-spread and has dramatically increased in North America Western Europe and Japan **[3,4**].Additionally, there is an increasing incidence of the disease among younger age groups that warrants Further investigations **[5, 6**].

The progression of liver disease into liver cancer is primarily monitored by serum levels of the oncofetal glycoprotein, α-fetoprotein (AFP), or the core fucosylated glycoform of AFP (AFP- L3). However, AFP can be produced under many circumstances, including other liver diseases **[7],** and is not present in all those with HCC. Therefore, the use of AFP as a primary screen for HCC has been questioned **[8**] and more sensitive serum biomarkers for HCC are desired.

Des-γ-carboxyprothrombin (DCP), also known as PIVKA-II (protein induced by vitamin K absence or antagonist), is an abnormal, inactive prothrombin, lacking carboxylation of the 10 glutamic acid residues in the N-terminus, which is the result of an acquired post-translational defect of the prothrombin precursor in HCC cell lines. DCP was discovered in serum of patients during their anticoagulant therapy with a vitamin K antagonist. In **1984 Liebman et al. [9]** first described a higher DCP level both in patients with HCC and in cases of HCC recurrence after surgical resection, suggesting the usefulness of DCP as an HCC biomarker **[9]**. It has been proved that significant concentrations of serum DCP are present in 50%-60% of all HCC patients, but in only 15%-30% of early HCC case **[10**]. HCC is usually

diagnosed at an advanced stage resulting in limited therapeutic options and poor prognosis. The identification of prognostic biomarkers is an important issue since such markers could facilitate detection of HCC. Furthermore, such biomarkers could display potential therapeutic targets for HCC **[2]**.

In the search for serum markers of hepatocellular cancer, several investigators have recently focused on Golgi protein 73 (GP73); also known as Golgi membrane protein 1 (Golm1) or Golph2]. GP73 is a 400 amino acid, 73 kDa transmembrane glycoprotein that normally resides within the cis- Golgi complex. Its mRNA was first identified in a search for upregulated hepatic genes in a patient with syncytial giant cell hepatitis **[11]**. Subsequent studies revealed minimal GP73 expression in normal hepatocytes but marked expression in patients with acute and chronic hepatitis and liver cirrhosis **[11,12]**, regardless of the specific disease aetiology. Resolution of hepatitis is paralleled by a reduction and normalization of GP73 expression, indicating that GP73 may be triggered by the hepatic injury response **[13,14]** .In addition to hepatocytes, GP73 was consistently expressed by normal biliary epithelial cells as well as hepatic stellate cells in injured livers **[12]**. Further studies demonstrated constitutive expression in cells of the epithelial lineage, especially in the prostate, gut, breast, and thyroid, and within the central nervous system **[11]**. A circulating form of GP73 is found in the serum of patients with hepatocellular cancer (HCC**) [15].**These data indicate that serum GP73 is a promising diagnostic serum marker for liver cancer **[15,16,17].**

Aim:

The present study aimed to evaluate the serum level of GP73 as an early marker for HCC diagnosis as comparing to DCP and AFP serum levels.

2. Patient and Methods:

This study included 66 patients, selected from the hepatology department of National Liver Institute-Menoufyia University, National Cancer Institute-Cairo University, Internal Medicine-Al Zahraa University Hospital and Hepatology Centre- National Medical Centre; 31 of them were diagnosed as HCC according to clinical examination, radiological investigations including abdominal ultrasonography, triphasic C.T and laboratory investigations. All patients were newly diagnosed cases and did not receive prior chemotherapy. They were 26 males and 5 females, their age ranged from 42-71 with a mean of 59.27±9.14 years. The remaining 35 patients have post HBV or HCV liver cirrhosis, 29 males and 6 females, their age ranged from 34-

68 with a mean of 54.71±7.12 years, they were diagnosed by clinical examination, abdominal ultrasound, laboratory investigations and liver biopsy.

Additionally, 20 apparently healthy subjects (15 females and 5 males); their ages ranged from 28 to 53 years with a mean of 51.65±5.23 and all were matched for age and sex with patients were included as a control group, they had normal values of serum alanine aminotransferase (ALT) and were seronegative for hepatitis B surface markers (HBs Ag,HBeAg and HBc-Ab) and HCV antibodies. The study was approved by the local ethical committee in university hospitals and informed consent was obtained from all participated.

All patient and control groups subjected to the following:

- Full history taking, thorough clinical examination.
- Abdominal ultrasonogrphy, and ultrasound guided liver biopsy was performed by true-cut needle or liver biopsy gun for the cirrhotic patients when possible.
- Triphasic C.T. to patients with focal lesion.
- The following investigations: liver function tests including: ALT, AST, serum albumin and total bilirubin was done on Cobas Integra-400 (Roche-Germany). Prothrombin concentration was done on fibrintimer (Dade Behring-Germany). Complete blood cell counts was measured by Sysmix K-21 automatic cell counter (Japan). Serum AFP was measured using automated eleceyes (Roche- Diagnostic, Branchburg, NJ- Germany).
- Hepatitis markers (HBsAg, anti-HBc and HCV antibody) were done by EIA (COBAS- amplicore, Roche- Germany).
- HCV-RNA levels were analyzed by reverse Transcriptase polymerase chain reaction (RT-PCR) using a commercial kit (Roche Diagnostic, Branchburg, NJ) according to the manufacturer's instructions.
- Measurement of des-γ-carboxy prothrombin (DCP): It was measured using a commercially available Enzyme-linked Immunosorbent Assay (ELISA) kit (Asserachrom PIVKA-II kit, Stago, France), according to the manufacturer's instructions.
- Determination of Golgi Protein 73 (GP73): It was determined by ELISA Kit For Golgi Protein 73 (GP73) provided by Uscn, Life Science (Inc-USA), the catalogue no E91668Hu. The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of GP73 in human serum, plasma and other biological fluids. Briefly, the microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to GP73. Standards or serum samples were then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for GP73. Next, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. Then a TMB substrate solution was added to each well. Only those wells that contain GP73, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a

change in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of GP73 in the samples was then determined by comparing the O.D. of the samples to the standard curve [14].

Statistical analysis:

Data are expressed as mean ± SD. The SPSS computer program version 12.0 was used fostatistical analysis. Kruskal-Wallis test was done to compare three or more of non normally distributed variables and Tamhane test is a Post Hoc test done to variables of significant difference of more than two groups of not normally distributed data after Kruskal-Wallis test to detect the significant difference between either groups. Correlation coefficients (r) were calculated using the Pearson's correlation analysis. p value was significant at <0.05 level. Sensitivity, specificity and the area under the receiver-operating characteristic curve (AUROC) were determined.

3. Results:

Patients characteristic are shown in (Table 1). The comparison between HCC, liver cirrhosis and controls revealed that, However the age in significantly not differed, a significant increase in AST, ALT, Alkaline phosphatase, GGT, total bilirubin in both patient groups compared to control group with the higher levels in HCC group. In contrast, serum albumin level, prothrombin concentration, HB concentration and platelet counts were significantly decreased as compared in both patient groups as compared to controls (Table 2).

The serum levels of AFP, DCP and GP73 were significantly elevated in LC and HCC patient groups as compared to control group and more elevated in HCC cases than in LC cases and control group as shown in (p<0.001) for each (Table 3).

At a cut-off 28.51 ng/ml. AFP had a sensitivity of 77.4%, a specificity of 60%, positive predictive value (PPV) of 75% and negative predictive value (NPV) of 63.2% for early HCC diagnosis. DCP give a sensitivity of 80.6%, specificity of 85%, PPV of 89.3% and NPV of 73.7% at a cut-off 32.64 ng/ml. The GP73 had a sensitivity of 87%, a specificity of 95%, PPV 96.4% and NPV of 82.6% at the optimal cut-off value of 7.62 ng/ml. The area under receiver-operating characteristic curve (AUROC) was 0.87 (Figure 1). However, when GP73 used in combination with AFP for early detection of HCC, they increased sensitivity up to 90.3%, whereas, specificity was 90%, PPV was 93.3%, NPV was 85.7% and AUROC was 0.83 (Figure 2) and (Table 4).

A significant correlation was found between serum GP73 level and prognostic markers of LC (AST, ALT, serum albumin and Child score) (p<0.5) for each, and more aggressive tumor characters (tumor size and vascular invasion), (p<0.05 and p<0.01) respectively. Also, a significant correlation was detected between serum GP73 level and DCP levels (p<0.05), while AFP serum levels show no significant correlation (p>0.05) (Table 5).

Table (1) Patients demographic data

Variables	HCC (n = 31)	Cirrhosis (n = 35)
Gender (Male/Female)	**26/5**	**29/6**
Jaundice	15 (48.4%)	16 (45.7%)
Hepatomegaly	26 (83.9%)	29 (82.9%)
Splenomegaly	16 (51.6%)	21 (60.0%)
Hematemesis	19 (61.2%)	13 (37.1%)
Melena	12 (34.3%)	10 (28.6%)
Lower limb oedema	14 (45.2%)	11 (31.4%)
Ascites	17 (54.8%)	14 (40.0%)
Child Score:		
A	3 (9.7%)	9 (25.7%)
B	11 (35.5%)	12 (34.3%)
C	17 (54.8%)	14 (40.0%)
Tumor Characters:		
Single	14 (45.2%)	------
<3 cm	10 (32.3%)	
Encapsulated	18 (58.1%)	
Blood vessel invasion	4 (12.9%)	

Table (2): Comparison between HCC, cirrhotic and control groups as regards biochemical data.

Variables	HCC (n = 31)	Cirrhosis (n = 35)	Controls (n = 20)	P value
Age (years)	59.27±9.14	54.71±7.12	51.65±5.23	>0.05
AST (U/L)	121.3 ± 85.2	76.5±39.4	15.9±4.7	<0.01
ALT (U/L)	126.2 ± 89.8	57.1±29.7	17.2±5.6	<0.01
ALP (U/L)	127.5 ± 65.3	90.4±27.8	40.5±10.7	<0.01
GGT (U/L)	79.5±34.6	34.1±12.1	18.2±5.3	<0.001
TB (mg/dl)	6.2 ± 3.7	4.5±2.6	0.7±0.13	<0.001
Albumin (g/dl)	2.76±0.54	2.85±0.61	4.61±0.53	<0.001
Proth. Conc %	58.6±19.1	61.2± 7.5	95.4±4.8	<0.001
HB (g/dL)	11.3±2.4	11.06±1.6	13.5±0.49	<0.01
Platelets (/mm)	156.8± 61.7	109.3±45.2	261.4±36.2	<0.01

P<0.05 is statistically significant, p>0.05 is not statistically significant.

Table (3): Comparison between HCC, cirrhotic and control groups as regards Tumor markers

Variables	HCC (n = 31)	Cirrhosis (n = 35)	Controls (n = 20)	P value
AFP (ng/ml)	508 ±314	16.08±7.15	1.95±0.23	<0.001
DCP (ng/ml)	27.36±5.35	12.42±6.24	4.27±1.25	<0.001
GP73 (ng/ml)	10.32±2.46	3.79±2.18	1.65±0.79	<0.001

P<0.05 is statistically significant, p>0.05 is not statistically significant.

Table (4): Sensitivity, specificity, positive and negative predictive value of tumor markers for early diagnosis of HCC (n=31).

Variables	Cut-Off	Sensitivity	Specificity	PPV	NPV
AFP (ng/ml)	28.51	77.4%	60 %	75%	63.2%
DCP (ng/ml)	32.64	80.6 %	85 %	89.3%	73.7%
GP73 (ng/ml)	7.62	87%	95%	96.4%	82.6%
Combined AFP & GP73	AFP= 28.51 ng/ml & GP73= 7.62 ng/ml	90.3%	90%	93.3%	85.7%

PPV=Positive Predictive Value NPV=Negative Predictive Value

Table (5): Correlation between GP73 serum levels and prognostic parameters in HCC group (n=31)

Parameters	GP73 serum levels	
	r-value	p-value
ALT	0.48	<0.05
AST	0.52	<0.05
Serum albumin	0.49	<0.05
AFP levels	0.23	>0.05
DCP levels	0.51	<0.05
Child score	0.46	<0.05
Tumor size	0.56	<0.05
Tumor number	0.24	>0.05
Blood vessel invasion	0.67	<0.01

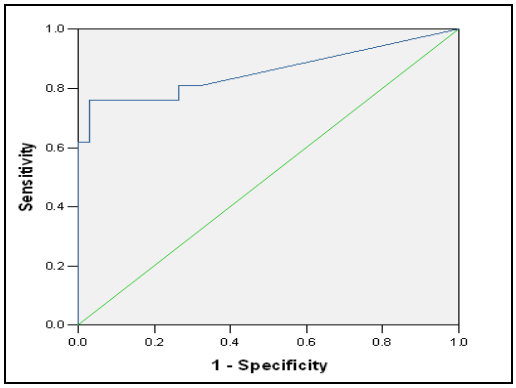

Figure (1) shows the ROC curve of GP73 in early HCC diagnosis

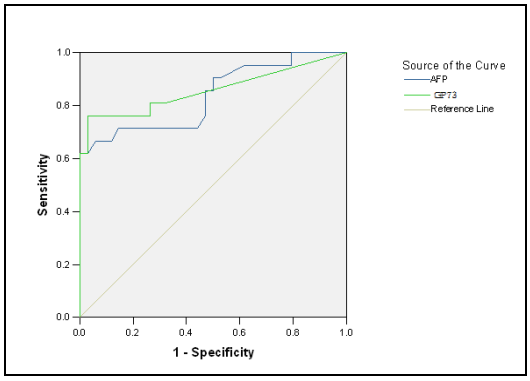

Figure (2) shows the ROC curve of combined GP73 and AFP in early HCC diagnosis

4. Discussion:

Since HCC is among the cancers with the worst prognosis, early diagnosis and treatment are the keys for effective treatment of patients with HCC. The use of serological markers in patients at the highest risk for developing HCC may thus decrease HCC mortality and reduce medical costs **[18].**

AFP has been used as a serum marker for HCC for many years, but it lack of high sensitivity and Specificity **[19]**. However, **Li and his colleagues [20]** tried to improve the detection rate using the ultrasonography. Several biomarkers such as DCP, AFP-L3, human hepatocyte growth factor, and insulin like growth factor-1 as well as AFP are promising, but none of these markers has been validated enough for clinical use. Thus, there is an urgent need for new biomarker for the detection of early HCC. DCP is a well recognized tumor marker for its high sensitivity and specificity in the screening and diagnosis of HCC **[21]**. Although some studies have identified serum GP73 as a potential biomarker for HCC **[21,22]**, the GP73 functions and the mechanisms of regulation in normal and neoplastic tissues are still unclear.

We determined the serum level of GP73 in 31 patients of HCC and 35 patients have liver cirrhosis without HCC to find its sensitivity and specificity in early detection of HCC comparing with conventional markers as DCP and AFP serum levels.

In the current study, the serum levels of AFP, DCP are significantly elevated in LC and more elevated in HCC cases. As well as the serum level of GP73 was significantly higher in HCC cases. These findings are in agreement with **Tian et al**. **[23]** who reported that, serum GP73 in LC was higher than in HCC and In all two groups were higher than those in healthy individuals. In addition, the most profound elevation of serum levels of GP73 was detected in patients who had developed an HCC on the background of HCV infection [**24**]. **Gu et al. [14]** reported that serum level of GP73 in patients with liver disease was significantly higher than in healthy individuals and in patients with other diseases. In a subsequent study by **Marrero et al. [21**] sGP73 levels were significantly increased in patients with HCV–related HCC in comparison with cirrhotic controls

Similar results were reported in a Chinese study on patients with predominantly hepatitis B virus–related liver cancer. In response to these encouraging reports, GP73 was added to a group of emerging candidate HCC serum markers **[24].** **Mao et al. [18**] have found that the elevation of serum GP73 is mildest in virus carriers, moderate in patients with cirrhosis and dramatic in patients with HCC. Therefore, serum GP73 can be used to monitor disease progression from HBV infection to cirrhosis to HCC. Moreover, they found that both liver benign tumours and non-HCC liver malignant lesions had elevated serum GP73, although the magnitude is much smaller than that in HCC. Serum GP73 can therefore be a useful tool in determining the nature (benign vs. HCC) of hepatic tumours. Furthermore, in patients with non-liver cancers also had moderate elevation of serum GP73, none of which, however, reached the level identified for HCC cases. Serum levels of GP73 diagnostic for HCC thus seemed not to be a pan-cancer marker.

Additionally, **Mao et al**. **[18]** study demonstrated that surgical resection of the tumour results in diminished serum GP73 levels and that tumour recurrence correlates with the recurrence of elevated GP73 in the blood. Reappearance of serum

GP73 indicates the existence of tumour lesions and thus may serve as an indicator for the recurrence of HCC.

The mechanism by which sGP73 reaches the circulation was worked out by **Bachert et al. [13]** in cell culture studies. Despite its steady-state localization within the cis-Golgi complex, GP73 cycles through the distal secretary apparatus and transiently reaches the apical cell membrane, from which it returns to the Golgi complex via an endosomal retrieval pathway **[25].** This secreted form is generated by N-terminal cleavage of the molecule by the proprotein convertase furin after amino acid 55, resulting in the release of the large C-terminal ectodomain into the extracellular space **[13].** Using N-terminal sequencing, **Gu et al. [14]** confirmed that the serum form of sGP73 is identical to the furin cleavage product identified in cell culture supernatants. This finding provides a mechanistic explanation for the appearance of sGP73 in serum.

The need for closer monitoring of patients with chronic hepatitis who have a high risk of developing HCC during the course of the disease has long been stated. In these patients, AFP has been a particularly unsatisfactory screening tool for early detection of HCC [**27**]. **Riener et al. [24]** concluded that GP73 is not a general HCC serum tumor marker but could rather be a valuable complementary tool in the surveillance of at-risk patients. The data presented in **Riener et al. [24**] study provides further evidence that GP73 protein is strongly expressed in HCC and bile duct carcinoma tissues and is secreted into the blood. Possibly, it is either involved in posttranslational protein modification, transport of secretory proteins, cell signalling regulation, or simply maintenance of Golgi apparatus function. GP73 has several potential glycosylation sites and up to 75% of GP73 secreted from hepatocytes is fucosylated **[28].** Endosomal trafficking of the normally membrane-bound GP73 leads to secretion into the blood, making it a potential serum biomarker for HCC **[13].**

The expression levels in benign liver lesions—focal nodular hypertrophy and hepatic adenoma were not significantly different from those of the surrounding areas.

These findings provide evidence that the increased sGP73 in HCC patients originates from cancerous hepatocytes, an important requirement for the validation of tumor biomarkers **[25].** Another novel finding is the marked up-regulation of GP73 expression in cancers of biliary origin **[29].**

In HCC diagnosis, previous studies have shown a better sensitivity of GP73 than AFP in diagnosis of HCC **[21**]. In this study, AFP had a sensitivity of 77.4% and a specificity of 60% at a cut-off 28.51 ng/ml. Parallel to these results of AFP in prediction of HCC, **Hakamada et al., [30]** reported a sensitivity of 69.3%, specificity 60%. Another two studies by **Trevisani et al. [31]** and **Gambarin-Gelwan et al**.**[32],** AFP specificity varies from about 76% to 96% and increases with elevated cut-off value.

In our study, DCP give a sensitivity of 80.6% and specificity of 85% in early HCC diagnosis at a cut-off 32.64 ng/ml. This agreed with the finding of **Durazo et al. [33],** who reported a sensitivity of 87.2 %, and specificity of 85.0% at similar cut-off. Also, in an older study by **Nakagawa et al**. **[34],** the sensitivity of DCP is 48%-62% and the specificity is 81%-98%, because they used a higher cut-off for DCP. However, AFP and DCP

are not correlated, so the combination of these two markers significantly improves HCC detection **[35, 36].**

Furthermore, whether GP73 is a better serum biomarker than AFP is controversial. The sensitivity and specificity of GP73 for HCC were superior to those of AFP, especially in early HCC, in our study; GP73 had a sensitivity of 87% and a specificity of 95% at the optimal cut-off value of 7.62 ng/ml. The area under receiver-operating characteristic curve (AUROC) was 0.87. However, when used in combination with AFP, they lead to an enhanced the sensitivity of detection of HCC up to 90.3% and AUROC was 0.83.

Previous studies by **Marrero & Lok [17]** and **Gomaa et al. [37]** postulated that, GP73 is up-regulated in HCC and measurement of serum GP73 revealed a sensitivity and specificity of 69% and 75%, respectively. In a more recent study by **Tian et al. [24],** AFP/GP73 had a sensitivity of 75.8% and specificity of 79.7% with an AUROC of 0.844. vs. 0.812 for AFP with a sensitivity of 95.2% and specificity of 47.1%; in detecting early HCC, AUROC of AFP/GP73 was 0. 804 vs. 0.766 for AFP alone. Also, **Wang et al**. **[38**] and **Mao et al. [18],** the combined measurement of GP73 and AFP can further increase the sensitivity for the detection of HCC.

These results were disappointing with two previous studies, in the first study, sGP73 was found to be elevated in patients with liver disease but did not distinguish between HCC, cirrhosis, and chronic hepatitis **[14]**. In the second study, which was reported in an abstract form, sGP73 was surprisingly found to be decreased in HCC patients **[39]**. The results of **Riener et al. [24]** study cast doubt on the diagnostic utility of sG73 as a serum marker of HCC. However, a few methodological questions will need to be addressed before the authors' interpretation is endorsed. First, the sGP73 serum levels reported by **Riener et al. [24]** were approximately 80-fold higher than those reported by **Gu et al. [14]** with a median serum concentration in normal subjects of 4 ug/mL, which is well within the range of many classical plasma proteins **[40].**

The correlation study, revealed that, a significant correlation was found between serum GP73 level and prognostic markers of LC (AST, ALT, serum albumin and child score) .This in agreement with the finding of **Tian et al. [23]**, who reported that, serum GP73 in LC patients with Child-Pugh class A was lower than in class B and C and GP73 correlated with AST, AST/ALT, albumin, A/G and alkaline phosphatase in liver cirrhosis.

Other, interesting findings in our study is the level of GP73 correlated with more aggressive tumor characters (tumor size and vascular invasion). These are similar to **Sun et al. [41**], who reported that, a significant overexpression of GP73 at both protein and mRNA levels along with overexpression of GP73 protein is associated with aggressive behavior of HCC. **Fimmel & Wright** [**29]** recorded that, the degree of GP73 expression correlated with the tumor grade. In contrast, serum levels of GP73 in patients with HCC were not consistently affected by the tumour sizes and the status of tumour differentiation [**18]**.

Conclusion:

The serum levels of GP73 concentration in patients with LC and HCC was significantly higher than in healthy individuals and it had a high sensitivity and specificity for early prediction of HCC cases. Level correlated with disease progression to HCC. In combination, measurement of AFP and GP73 has the promise to further improve the detection and treatment of HCC. Further research is needed to determine the potential of GP73 as a therapeutic target.

Conflicts of Interest:

There were no Conflicts of Interest in this study.

Authors' contributions:

All authors' contributed equally to this study.

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Influence of Mechanical properties of Cotton Fabrics on Seam Quality

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Abstract: The purpose of this article is to study the effect of mechanical properties on seam quality of cotton fabrics. Twenty cotton fabrics are used for experiment. Mechanical properties of the fabrics on warp and weft directions were tested by FAST instrument. Seam efficiency, seam puckering and seam boldness were tested to evaluate seam quality. Curve regressions were used to analyze the influence of mechanical properties on seam quality. The results showed that the shear rigidity and extensibility were closely correlated with the seam efficiency and seam puckering rate, whereas the thickness, weight and shear rigidity were affecting on the seam boldness of the cotton fabrics. The regression equations of seam quality rate were obtained**.**
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Keywords: Seam quality, seam efficiency, seam puckering, seam boldness, fabric mechanical properties.

1. Introduction

In cut and sewn apparel products, seams are formed when two or more pieces of fabrics are held together by stitches. As the seam is one of the basic requirements in the construction of apparel, seam quality has great significance in apparel products.

There are several functional and aesthetic requirements for good quality seam. Seam quality is evaluated based on various dimensions: seam efficiency, seam elongation, seam bending, seam stiffness, seam abrasion resistance, seam density, seam slippage, seam puckering, seam tightness, seam boldness and seam damage[1].Seam efficiency, seam elongation, seam density, seam slippage, seam bending stiffness and seam abrasion resistance are the dimensions for functional performance of the seam. In contrast, seam puckering, seam tightness; seam boldness and seam damage are mainly evaluated for better aesthetic performance of the seam. In order to simplify the analysis of seam quality, it is essential to choose only most important evaluating dimensions, which can well represent the overall seam quality. A subjective ranking given by different researchers to evaluate the most important dimension for the seam quality evaluation. The final ranking cleared that seam puckering; seam efficiency and seam boldness are the three critical dimensions for the evaluation of seam quality based on experts' viewpoints. The seam efficiency is used to evaluate the functional performance of seam in terms of durability [2,3]. Seam puckering and seam boldness are the two dimensions for the evaluation of seam aesthetic performance. Seam puckering used to measure the appearance along the seam line [4].Seam boldness is the dimension for evaluating the design prominence of the seam [5].Fabric properties affect the seam quality, are discussed by many previous researchers [6-11].

2. Experimental

The investigation of fabric seam quality was carried out on twenty cotton fabrics sample for production of women's outerwear. Basic properties of experimental fabrics were as follow: warp density 24-160 Ends/inch, weft density 18-78 Picks/inch. The tensile strength of the fabric was tested on an Instron Tensile Tester; model 4411.The ASTM D 5034 test method was used to measure the strength of the fabric by using the grab test procedure to measure the breaking strength of textile fabrics. The cloth cover factor was calculated using the following formula [12].

$$\text{Cloth cover factor } K_C = K_1 + K2 - \frac{K_1 + K2}{28}$$

[where k_1 = warp cover factor, k_2 = weft cover factor].Fabric mechanical properties were tested and calculated by FAST instrument under standard conditions[13]. Contents of the fabric properties were described in Table 1.All the samples were in plain weave and produced from100% cotton type of yarns. The three important dimensions for seam quality evaluation (seam efficiency, seam puckering and seam boldness) were inferred as critical dimensions for seam quality evaluation. Each selected fabric was sewn by different sewing thread size at various stitch density. Each critical dimensions measured by their standard evaluation method. Seam efficiency measures the durability along the seam line[14-16].Many studies[3,17-19] measured the seam efficiency from strength tester according to the ASTM 1683-04 standard method. In this method, seam efficiency was measured by using the following equation:

$$\text{Seam efficiency (\%)} = \frac{\text{Seam Tensile Strength}}{\text{Fabric Tensile Strength}} \times 100$$

Seam puckering appears a long the seam line of garment when the sewing parameters and sewn materials properties are not properly selected. Puckering can occur due to excess fabric and not enough thread in the seam [20-23].After analyzing the puckering behavior of various seamed fabrics, it has been found that seam puckering depends mainly on the thickness properties of the fabric[2,16].As a result, seam puckering calculated by measuring the difference in fabric and seam thickness under constant compressive load. Seam puckering calculated by using the following formula [2,3, 16, 24]:-

$$\text{Seam puckering (\%)} = \frac{t_s - 2t}{2t} \times 100$$

[Where, ts = seam thickness, t= fabric thickness]

Seam boldness is used to measure the design prominence over seam. Generally, high degree of boldness is required for better ornamentation of apparel [25].The evaluation is performed by using a standard lighting and viewing the specimens in five ratings in comparison with the appropriate reference benchmark. Seams are rated in five classes in which class 5 = highest prominence and class 1 = no prominence. Contents of the critical dimensions for seam quality evaluation (seam efficiency, seam puckering and seam boldness) were described in Table 1.

3. Results and Discussion

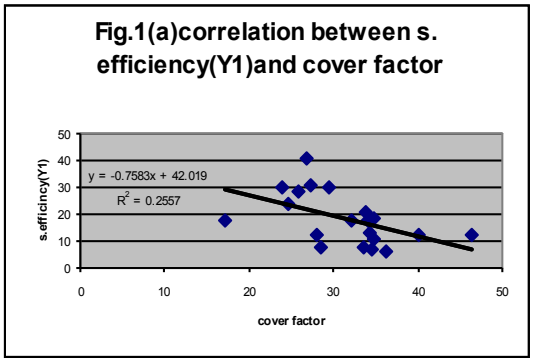
The tensile strength was measured and fabric cover factor calculated for the tested samples. Then the FAST mechanical properties were obtained for each sample. The results of fabric properties are shown in Table 1.

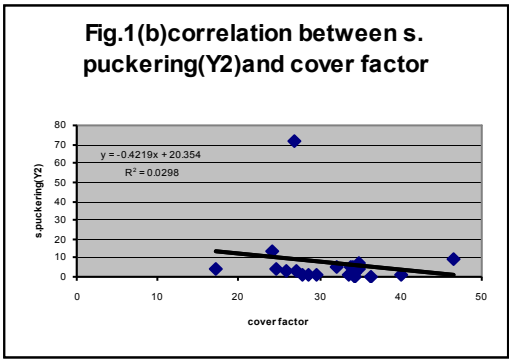
Table 1. Tested Properties of 100% Cotton Fabrics

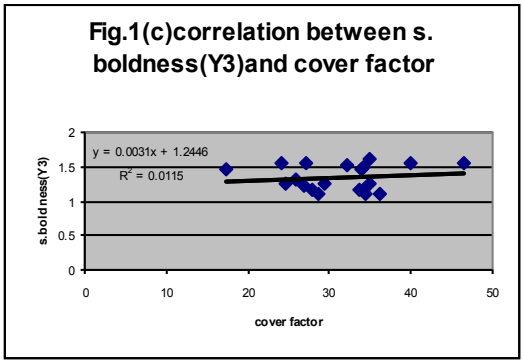
Fabric no.	Seam Effici. Y1	Seam Pucker Y2	Seam Bold Y3	Cover Factor X1	Strength (N) X2	Mean E (%) X3	Mean B(μ-Nm) X4	G (N-m) X5	T_2 (mm) X6	W g/cm^2 X7
1	6.2	0	1	36.23	803.2	3.12	5.84	13.25	0.8	380
2	7.36	0.73	1	28.59	410.7	2.93	1.98	4.63	0.85	480.2
3	7,13	0	1	34.44	426.6	2.64	1.5	9.03	0.81	344.3
4	12.06	0.78	1.15	27.92	204.4	4.49	1.05	7.7	0.83	376.2
5	8.01	0.93	1.15	33.63	479.5	5.86	0.28	3	0.57	432
6	13.36	2	1.15	34.23	510.3	7.37	0.15	2.58	0.52	273.6
7	41.09	71	1.2	26.75	440.2	8.42	0.12	1.36	0.59	214.4
8	29.6	1.02	1.25	29.47	170.5	9.22	0.06	0.96	0.42	153.2
9	30.48	3	1.55	27.18	520.4	7.61	0.05	0.72	0.43	254
10	29.71	13.9	1.55	24.01	498.3	8.86	0.04	0.74	0.47	308
11	10.82	6.95	1.5	34.83	475.7	3.39	0.13	2.38	0.49	151
12	28.17	3.55	1.2	25.88	363.8	2.85	0.24	1.36	0.5	167
13	17.89	5	1.4	34.07	532.9	5.47	0.14	0.96	0.35	150
14	20.62	5.25	1.45	33.86	663.9	7.05	0.11	0.72	0.52	181
15	12.19	8.84	1.55	46.42	492.9	6	0.1	0.74	0.46	136
16	18.61	4	1.25	34.84	803.2	6.17	0.12	2.38	0.45	151
17	12.21	1.48	1.55	39.97	410.7	6.66	0.1	4.69	0.55	184
18	18.02	4.81	1.5	32.04	426.6	8.66	0.08	1.7	0.38	131
19	23.69	4	1.25	24.53	204.4	8.17	1.03	1.79	0.42	102
20	17.87	3.98	1.45	17.24	479.5	3.15	0.12	1.62	0.44	167

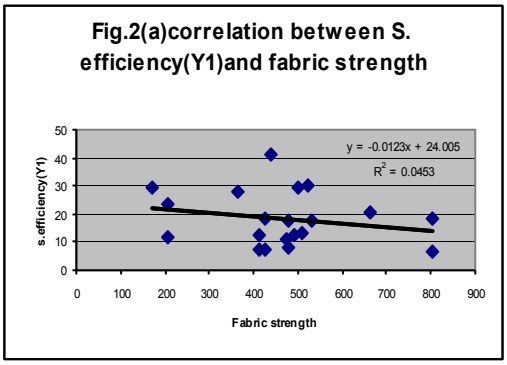
It has been found that there are different factors affecting the seam quality. They are considered as input variables of seam quality. The input variables are: cover factor (X1), tensile strength (X2), extensibility (X3), bending rigidity (X4), shear rigidity (X5), thickness(X6) and fabric weight(X7). These input variables are measured in the laboratory for seam quality evaluation of cotton fabrics. The previous input variables are given in Table 1. According to the evaluation of the different researchers, it has been found that there are three critical dimensions for seam quality evaluation. These critical dimensions considered as output variables of modeling. The output variables are: seam efficiency (Y1), seam puckering (Y2) and seam boldness (Y3). The values of output variables (Y1, Y2 and Y3) obtained from the experiments are given in Table1. It can be seen that seam efficiency is ranges from 6.2 to 41.09%. It is noticeable, that sample no. (7) obtains significantly higher seam efficiency in relation to all other fabrics This sample has higher extensibility but low bending stiffness, shear rigidity, thickness, cover factor and tensile strength (compared to other samples) and, low

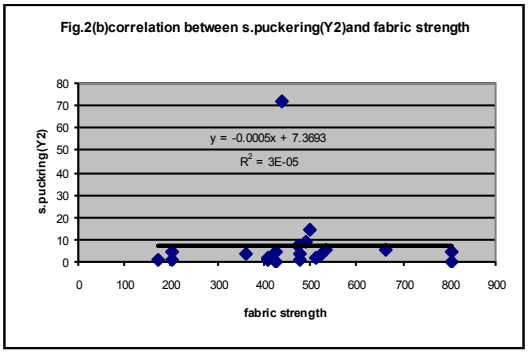
fabric weight. Also seam puckering is ranges from 0 to 71%. It is noticeable, that sample no. (7) obtains significantly higher seam puckering in relation to all other fabrics This sample has higher extensibility, cover factor, bending stiffness and thickness but low tensile strength and shear rigidity (compared to other samples) and low fabric weight. The lasting output variables seam boldness is ranges from 1 to 1.55%. It is noticeable, that samples (9,10,15,17) obtain significantly higher seam boldness in relation to all other fabrics The samples have higher extensibility but low bending stiffness, shear rigidity, thickness, cover factor and tensile strength (compared to other samples) and low fabric weight. To get idea about fabric structure parameter that gives best indication of fabric seam quality, the correlation between seam quality variables and various fabric structure parameters is investigated. There is a correlation between fabric shear rigidity (R2=0.3722), extensibility (R2=0.3704), cover factor (R2=0.2557), thickness (R2=0.2545), bending rigidity (R2=0.2042), weight (R2=0.1612) and tensile strength (R2=0.0453), with seam efficiency. Also, there is a correlation between fabric extensibility (R2=0.1067), shear rigidity (R2=0.0601), bending rigidity (R2=0.0344), cover factor (R2=0.0298), weight (R2=0.0126) and thickness (R2=0.003), with seam puckering. Finally, there is a correlation between fabric thickness (R2=0.4669), weight (R2=0.347), shear rigidity (R2=0.3357), bending rigidity (R2=0.2768), extensibility (R2=0.0807), cover factor (R2=0.0115), tensile strength (R2=0.0068),with seam boldness .The correlations between various fabric parameters and the critical dimensions for seam quality are represented in Figures from 1(a, b, c) to 7(a, b, c) respectively.

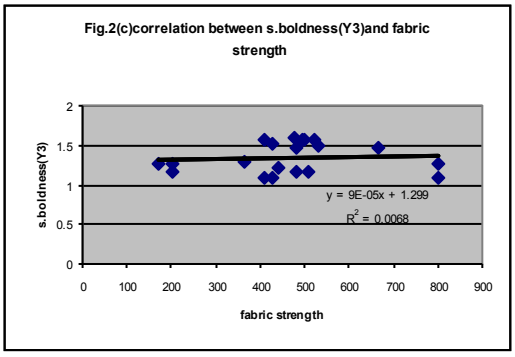












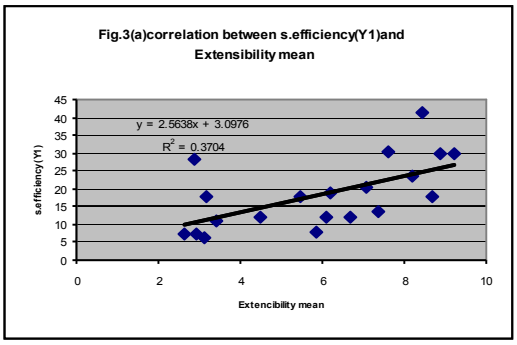


Fig.3(a)correlation between s.efficiency(Y1)and Extensibility mean
y = 2.5638x + 3.0976
R2 = 0.3704
s.efficiency(Y1)
Extencibility mean

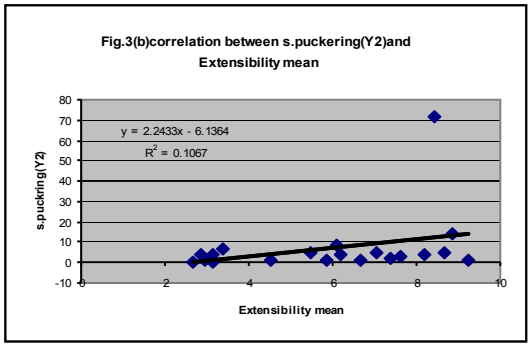


Fig.3(b)correlation between s.puckering(Y2)and Extensibility mean
y = 2.2433x - 6.1364
R2 = 0.1067
s.puckring(Y2)
Extensibility mean

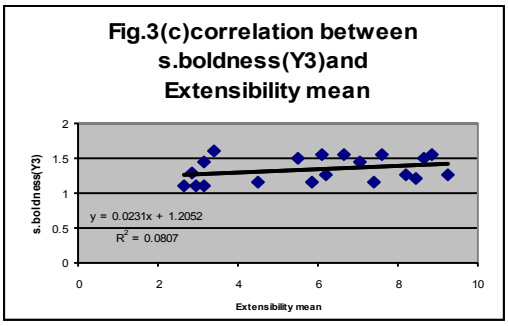


Fig.3(c)correlation between s.boldness(Y3)and Extensibility mean
y = 0.0231x + 1.2052
R2 = 0.0807
s.boldness(Y3)
Extensibility mean

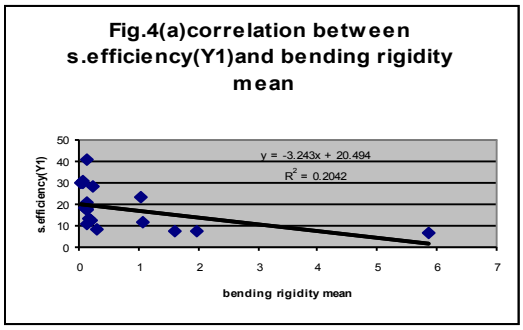


Fig.4(a)correlation between s.efficiency(Y1)and bending rigidity mean
y = -3.243x + 20.494
R2 = 0.2042
s.efficiency(Y1)
bending rigidity mean

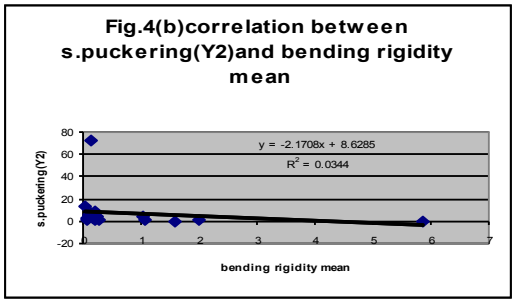


Fig.4(b)correlation between s.puckering(Y2)and bending rigidity mean
y = -2.1708x + 8.6285
R2 = 0.0344
s.puckering(Y2)
bending rigidity mean

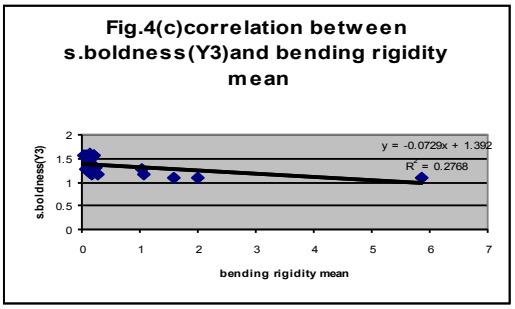


Fig.4(c)correlation between s.boldness(Y3)and bending rigidity mean
y = -0.0729x + 1.392
R2 = 0.2768
s.boldness(Y3)
bending rigidity mean

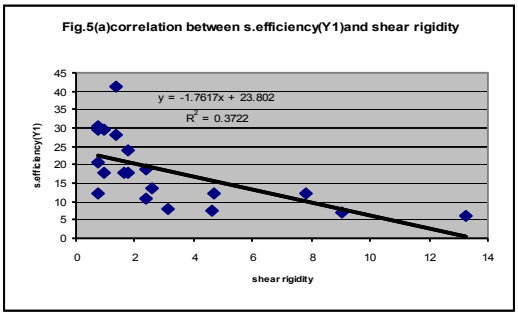


Fig.5(a)correlation between s.efficiency(Y1)and shear rigidity
y = -1.7617x + 23.802
R2 = 0.3722
s.efficiency(Y1)
shear rigidity

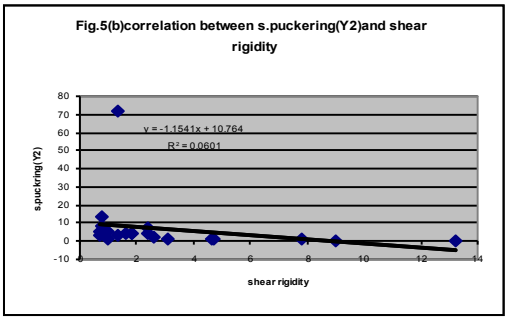


Fig.5(b)correlation between s.puckering(Y2)and shear rigidity
y = -1.1541x + 10.764
R2 = 0.0601
s.puckring(Y2)
shear rigidity

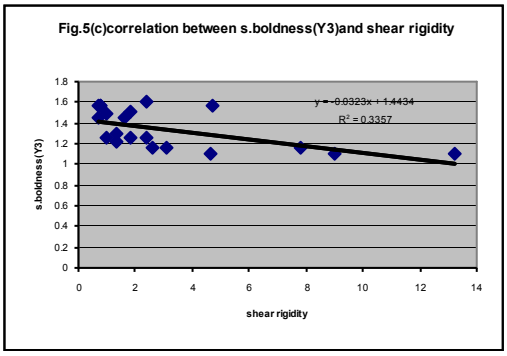


Fig.5(c)correlation between s.boldness(Y3)and shear rigidity
y = -0.0323x + 1.4434
R2 = 0.3357
s.boldness(Y3)
shear rigidity

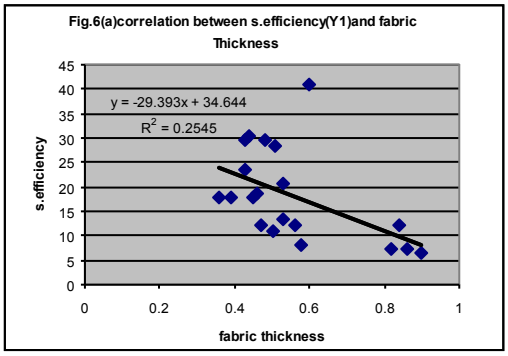
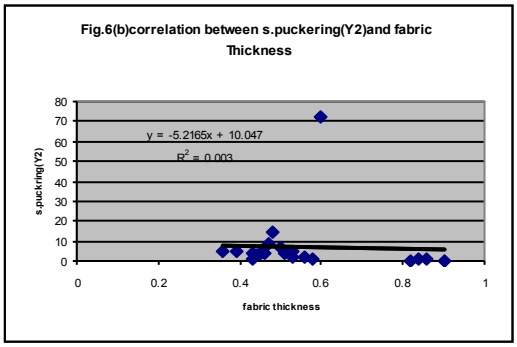
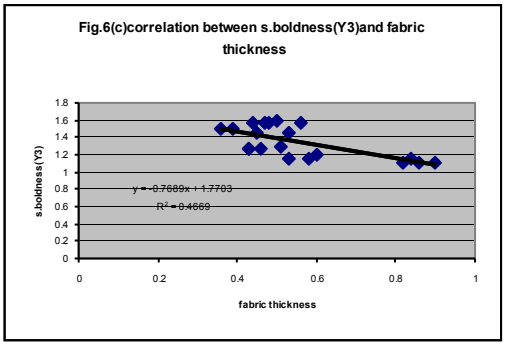
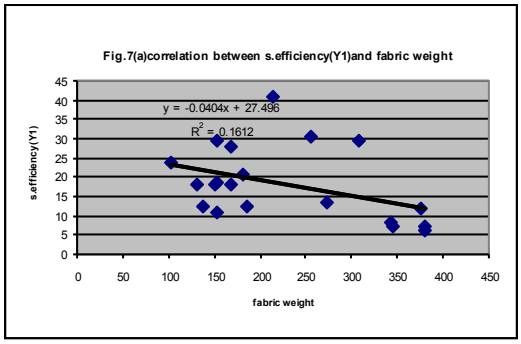
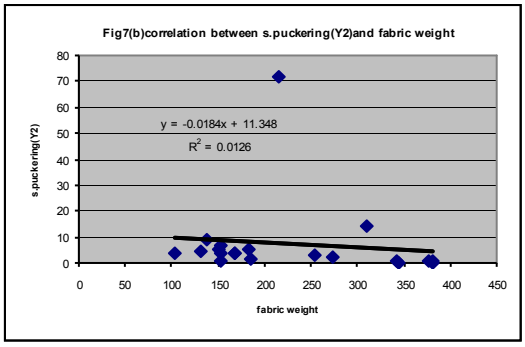


Fig.6(a)correlation between s.efficiency(Y1)and fabric Thickness
y = -29.393x + 34.644
R2 = 0.2545
s.efficiency
fabric thickness









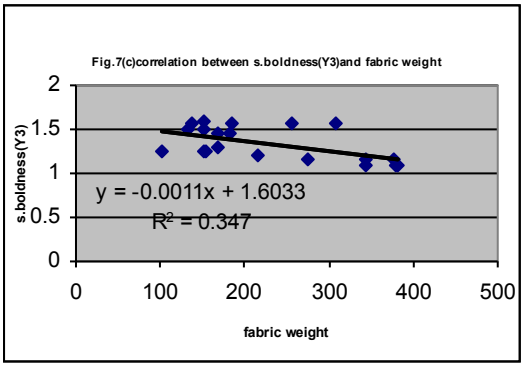


Table 2. Regression coefficient of the input variables for Y_1, Y_2 and Y_3 of cotton fabrics

Input variables	Regression Coefficients (R^2)		
	Seam Efficiency (Y_1)	Seam Puckering (Y_2)	Seam Boldness (Y_3)
Cover Factor (X_1)	0.2557	0.0298	0.0115
Tensile Strength (X_2)	0.0453	3E-05	0.0068
Extensibility (X_3)	0.3704	0.1067	0.0807
Bending Rigidity(X_4)	0.2042	0.0344	0.2768
Shear Rigidity (X_5)	0.3722	0.0601	0.3357
Thickness (X_6)	0.2545	0.003	0.4669
Weight (X_7)	0.1612	0.0126	0.347

Regression coefficients of 7 input variables (X_1-X_7) for the seam efficiency (Y_1), seam puckering (Y_2) and seam boldness (Y_3) for cotton fabric are shown in Table 2.

From Table 2, it is clear that the absolute values of regression coefficient of extensibility (X_3) and shear rigidity(X_4) are higher than the rest of input variables for the seam efficiency (Y_1) and seam puckering (Y_2) whereas, the absolute values of regression coefficient of thickness (X_6) and weight (X_7) are higher than the rest of input variables for seam boldness.

Conclusion

Based on the discussion, the following conclusion can be drawn. There are various factors for seam quality. Generally, all the fabric properties such as,weight, cover factor, thickness, tensile strength, extensibility, bending rigidity and shear rigidity have considerable effect on the seam quality of apparel products. The investigation of the seam quality of 100%cotton fabrics has shown correlation between various fabric structure parameters and critical dimension variables. The sample that has much greater

seam efficiency and seam puckering values have also much higher extensibility and lower shear rigidity,wereas the sample that has much greater seam boldness values has also much lower thickness, weight and shear rigidity. Seam efficiency has a positive value on the overall seam quality, as a high percentage of seam efficiency always represents good seam efficiency. In contrast, seam puckering always has a negative value on the overall seam quality because a greater seam puckering always leads to poor seam quality. Seam boldness may have a positive or negative value on the overall seam quality depending on the apparel. From the provided solution for evaluating the overall seam quality, the understanding of the requirements of critical dimension for seam quality of the apparel. This understanding will help in planning and control of the quality of apparel products at the time of sewing.

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Application of ordinal cumulative odds logistic regression model to analyze the influencing factors of quality of life in patients with epilepsy in rural Henan Province, China

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Abstract: Purpose: To investigate and analyze the various factors influencing the quality of life in patients with epilepsy. Methods: QOLIE-31, CES-D, SAS were used to measure multiple indexes of the study population, and multivariate ordinal cumulative logistic regression model was used to conduct univariate and multivariate analysis on the quality of life and its influencing factors among patients with epilepsy. Results: Among the 874 patients with epilepsy, the median score of the 4 different levels of QOL was 28, 44, 67, and 78 respectively. The major influencing factors of QOL among patients with epilepsy were seizure frequency, awareness rate of knowledge on epilepsy, anxiety, depression, types of medication intake and compliance. Among these, high seizure frequency, concomitant anxiety and depression were risk factors of QOL, and high awareness rate of knowledge on epilepsy, single-medication intake and good compliance were protective factors of QOL. Conclusions: The findings highlight the necessity to lay stress on intervening the modifiable influencing factors in special patients with epilepsy. Carrying out targeted health promotion and psychological intervention therapy, along with single medication intake are of vital importance to improve the QOL of patients with epilepsy. This cumulative odds logistic model is of scientific effectiveness.

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Epilepsy is a common disease that seriously harms people's health and increases the incidence of organic and mental diseases. It not only brings great suffering to the patients, but also increases the disease burden of the patients' families, the individual and the society. Awareness rate of the knowledge on epilepsy was transformed by the score of the related questionnaires. Scores of the QOL were transformed from numerical variables to the categorical variables (binary,0 and 1; polytomous: 1,2,3,…k) because they could not meet the tests of homogeneity of variance and normality, replying that different levels of QOL have orders and grades.

Ordinal cumulative odds logistic regression (Armstrong and Sloan, 1989; Brant, 1990) was a useful tool when the variables were polytomous and ordinal. In order to understand the QOL and its influencing factors in patients with epilepsy, in August 2011, we used multi phase stratified random sampling method to collect related research data in 9 exemplary sites, and conducted questionnaires survey among 900 patients with epilepsy. Multivariate cumulative odds logistic regression model was used to analyze the QOL and its influencing factors in patients with epilepsy, in order to provide basic data for targeted health promotion, epilepsy prevention and treatment.

1. Data and Methods

1.1 Study population: Since 2005, in 9 exemplary sites located in Mengzhou City and Xiuwu County of Jiaozuo City, Yancheng District, Huiyuan District, Wuyang County, Linying County, Zhaoling District of Luohe City, Xinye County and Fangcheng County of Nanyang City, 4.26 million rural people were screened and reexamined for generalized tonic-clonic seizure. After reexamination and diagnosis by well-trained neurologists, the eligible patients were enrolled in the project management and were administered standard therapy, individualized health education, psychological intervention therapy and follow-up. Comprehensive intervention group members were sampled randomly in the 5218 patients in the phenobarbital comprehensive management group, and the non-intervention control group members were sampled in the confirmed epilepsy patients who were not enrolled in the comprehensive group (diagnosed after reexamination and enrolled in the phenobarbital group). There was no statistical significance in the difference of age, gender,

profession and economic status among these two groups. Inclusion and exclusion criteria were in accordance with the Implementation scheme of epilepsy prevention, treatment and management project in rural China (Guiding group of epilepsy prevention, treatment and management project in rural area, Buerau of Disease Control, Ministry of Health. 2010; Office of the national epilepsy project 2011).

1.2 Questionnaires: 1. The QOLIE-31 scale (Cramer and etc., 1998; Liu Xueqin and etc., 2003) consists of 7 items: (1) Seizure worry, SW; (2) Overall Quality of life, Overall QOL; (3) Emotional well-being, EW; (4) Energy/fatigue, EF; (5) Cognitive functioning, CF; (6) Medication effects, ME; (7) Social functioning, SF. Scoring rules: Numeric values for responses to QOLIE-31 scales are devised so that higher and lower scores reflect better HRQOL and worse HRQOL and use ranges of values from 1 to 100. To account for these differences, the scoring system requires conversion from raw, precoded numeric values to scores of 0-100 points, with higher converted scores always reflecting better HRQOL. Converted scores for items in each scale that were answered to determine the Scale Score (range 0-100 points). The total score is not a simple sum or mean of the seven subscales. An overall score can be calculated by weighting and summing the product of QOLIE-31 scale scores times its weight and summing over all scales using an empirically derived coefficient to weight and sum scores. 2. The Center for Epidemiological Studies Depression Scale (CES-D) (Roberts and Vernon, 1983; Zhang Mingyuan, 1998) , it consists of 20 items, and it is evaluated by the frequency of related events and feelings in the previous week, the score ranges from 0-3, with higher score indicating more severe depression symptoms. 3. The Self-rating Anxiety Scale (SAS)(Zhang Mingyuan, 1998; Zung WW, 1971), it consists of 20 items, the score ranges from 1 to 4, with higher score indicating more severe anxiety symptoms. 4. General social demographic and other disease related questionnaires.

1.3 Methods of investigation: A combination of centralized survey and household survey was adopted. Investigators were acted by rigorously trained and qualified professional technical personnel. After informed consent was obtained, the investigators presented the questions to the interviewees and filled in the questionnaires according to their answers, and each visit lasted about 20 minutes. If the interviewee could not answer or could not correctly answer some questions (due to illiteracy, mental retardation, or aphasia, etc.), it was assessed according to the observation from his/her family members, guardians, nurses and other insiders. After completion, the questionnaires were carefully reviewed by the inspectors.

1.4 Statistical analysis: Epidata 3.0 software was used on the dual- independent data input, and R 2.10.0 was used for data processing and analysis, the statistical significance level of test was set at $\alpha = 0.05$. Cumulative odds logistic regression model was used to conduct univariate and multivariate analysis on the influencing factors (inclusion criterion was set at 0.05, and exclusion criterion was set at 0.10). Quality of Life (QOL) was divided into 4 levels (level 1, level 2, level 3 and level 4) according to the cut-off points: low (p25), intermediate (p50) and high (p75).

2. Results

2.1 Questionnaires survey: A total of 1642 patients received the survey, after exclusion of incomplete or ineligible questionnaires, 900 questionnaires were retrieved, and 847 questionnaires were enrolled finally. The qualifying rate was 97.1%.

Reliability test of QOLIE-31 scale: the internal consistency of Cronbach's α among all of the factors(items) in the scale was 0.9846. The internal consistency of Cronbach's α among the 7 items were respectively 0.9572、0.9615、0.9693、0.9781、0.9804、0.9597 and 0.9580.

Validity test of QOLIE -31 scale: 1.Construct validity: the KMO of the sampling data constituted by the 31 items was 0.934>0.7, this indicated that factors analysis was suitable. In Bartlett's test of sphericity, $\chi 2 = 12158.534$, $P < 0.001$, this indicated that there were common factors in the correlation matrix between the various factors, and factors analysis was suitable; 2.Content validity: analysis showed that the Pearson correlation coefficient > 0.5($P < 0.05$)in various fields and aspects of the scale; 3.Discrimination validity: differences of the scores of QOL in all kinds of fields were statistically significant.

The internal consistency of Cronbach's α among all of the items in SAS scale was 0.9498; The internal consistency of Cronbach's α among all of the items in the CES-D scale was 0.9613. KMO value of SAS scale and CES-D scale was respectively 0.885,0.939; The χ^2 value of Bartlett's test of sphericity was respectively 30116.570,28648.566（$P<0.001$）.

2.2 General demographic characteristics

Among the 847 patients with epilepsy in this study area, there were 502 male patients (57.44%) and 372 female patients (42.56%), the number of male patients was larger than the female. The age ranged between 17～82 year old, and the median age was 44 years old. There were 364 peasants,

accounting for 73.83% of the total population of epileptic patients.

Among the 847 patients with epilepsy, the minimum value of QOL was 1, the maximum value was 100, the mean value was 55.37, and the median value was 56. The 4 quartile values of QOL was respectively 28, 44, 67 and 78. The general demographic characteristics of the patients with epilepsy with different levels of QOL were seen at Table 1.

Table 1. General demographic characteristics of the patients with different levels of QOL N (%)

Demographic characteristics		Level 1	Level 2	Level 3	Level 4
Age group	17 ~ 30	3(1.41)	50(22.62)	41(20.3)	79(33.19)
	30 ~ 45	40(18.78)	85(38.46)	47(23.27)	96(40.34)
	45 ~ 59	89(41.78)	51(23.08)	68(33.66)	53(22.27)
	59 ~ 82	81(38.03)	35(15.84)	46(22.77)	10(4.2)
Gender	male	114(53.52)	133(60.18)	123(60.89)	132(55.46)
	female	99(46.48)	88(39.82)	79(39.11)	106(44.54)
Marital Status	Married	178(83.57)	152(68.78)	139(68.81)	147(61.76)
	Divorced/ separated	16(7.51)	18(8.14)	30(14.85)	22(9.24)
	widow	13(6.1)	6(2.71)	4(1.98)	2(0.84)
	unmarried	6(2.82)	45(20.36)	29(14.36)	67(28.15)
Education Level	illiteracy/semi-illiteracy	77(36.15)	41(18.55)	45(22.28)	44(18.49)
	Primary school	86(40.38)	103(46.61)	76(37.62)	106(44.54)
	Junior high school	42(19.72)	64(28.96)	60(29.7)	67(28.15)
	High/Technical school	6(2.82)	12(5.43)	20(9.9)	18(7.56)
	College and above	2(0.94)	1(0.45)	1(0.5)	3(1.26)
Occupation	“employed”	20(9.39)	33(14.93)	30(14.85)	29(12.18)
	“unemployed”	35(16.43)	38(17.19)	28(13.86)	43(18.07)
	peasant	158(74.18)	145(65.61)	139(68.81)	156(65.55)
	student	0(0)	5(2.26)	5(2.48)	10(4.2)
Economic Status	80 ~	17(7.98)	32(14.48)	61(30.2)	74(31.09)
	400 ~	57(26.76)	61(27.6)	63(31.19)	73(30.67)
	700 ~	71(33.33)	49(22.17)	19(9.41)	33(13.87)
	900 ~	43(20.19)	47(21.27)	40(19.8)	43(18.07)
	1400 ~ 2700	25(11.74)	32(14.48)	19(9.41)	15(6.3)

2.3 Univariate cumulative odds logistic regression analysis

Not take grouping into consideration, the lower, middle and upper quartile of QOL score in patients with epilepsy were 37, 56 and 74 respectively in this study. Possible factors that may influence the QOL level (Y=level 4 was set as 1, level 3 was set as 2, level 2 was set as 3, level 1 was set as 4) of patients

with epilepsy, such as demographic variables (gender, age, occupation, culture, etc) , disease related variables (duration, seizure frequency, etc) and psychological variables (depression, anxiety) were taken into cumulative odds logistic model univariate analysis, the results revealed that excluding gender, inheritance, economic status, disease duration of “20 to 30 " and " 30 to 37 ", profession (excluding students) and " mild " anxiety, QOL scores differences were statistically significant (P <0.05) in patients with different ages, marital status, ages of onset, seizure frequencies, types of medication intake, compliances, anxiety and depression.

Table 2. The results of univariate cumulative logistic regression analysis of different factors.

Influencing factors	OR(95%CI)	*SE*	*Z*	*P*
1. General demographics				
Age				
(control= 17 ~)				
30 ~ 45	1.83(1.29 ~ 2.59)	0.32	3.41	0.001*
45 ~ 59	3.67(2.58 ~ 5.24)	0.66	7.19	<0.001*
59 ~ 82	7.30(4.91 ~ 10.85)	1.48	9.82	<0.001*
Gender				
(control =male)				
female	1.04(0.82 ~ 1.33)	0.12	0.35	0.724
Marriage				
(control =married)				
Divorced / separated	0.67(0.45 ~ 0.99)	0.30	-2.02	0.043*
Widow	2.86(1.34 ~ 6.09)	0.13	2.72	0.006*
Unmarried	0.35(0.25 ~ 0.49)	0.48	-6.20	<0.001*
Education Level				
(control = Illiterate)				
Primary school	0.63(0.46 ~ 0.86)	0.10	-2.91	0.004*
Junior high school	0.53(0.38 ~ 0.75)	0.09	-3.61	<0.001*
High school /secondary school	0.38(0.23 ~ 0.64)	0.10	-3.66	<0.001*
College and above	0.45(0.10 ~ 1.98)	0.10	-2.91	0.004*
Occupation				
(control =“In-service”)				
“Unemployed”	1.09(0.70 ~ 1.68)	0.21	0.37	0.708
Peasant	1.20(0.84 ~ 1.70)	0.15	0.99	0.320
Student	0.37(0.16 ~ 0.88)	1.19	-2.26	0.024*
Economic Status				
(control=80 ~)				
400 ~	2.01(1.43 ~ 2.82)	0.09	4.00	0.358
700 ~	4.93(3.34 ~ 7.27)	0.04	8.03	0.011
900 ~	2.44(1.68 ~ 3.54)	0.08	4.67	0.395
1400 ~ 2700	3.40(2.17 ~ 5.32)	0.07	5.36	0.401

2. Clinically relevant data				
Duration of the disease				
(control =0 ~ 10)				
10 ~ 20	0.60(0.43 ~ 0.85)	0.29	-2.89	0.004*
20 ~ 30	0.76(0.54 ~ 1.06)	0.23	-1.60	0.110
30 ~ 37	0.97(0.66 ~ 1.42)	0.20	-0.17	0.864
>37	2.31(1.45 ~ 3.68)	0.10	3.52	<0.001*
Age of onset				
(control =0 ~ 11)				
11 ~ 20	1.81(1.29 ~ 2.55)	0.10	3.40	0.001*
20 ~ 34	2.04(1.44 ~ 2.87)	0.09	4.06	<0.001*
34 ~ 50	2.62(1.77 ~ 3.88)	0.08	4.81	<0.001*
≥50	5.88(3.70 ~ 9.34)	0.04	7.5	<0.001*
Seizure frequency (control=controlled)				
Mild	7.14(5.12 ~ 9.95)	0.02	11.59	<0.001*
Moderate	13.12(8.98 ~ 19.15)	0.01	13.33	<0.001*
Severe	8.71(5.77 ~ 13.15)	0.02	10.29	<0.001*
Types of Medication intake (control = Multi-medications)				
Single-medication	0.11(0.08 ~ 0.14)	0.02	-14.91	<0.001*
Familial Inheritance (control =no)				
Yes	0.73(0.49 ~ 1.08)	0.14	-1.60	0.111
Epilepsy knowledge awareness rate (control =high)				
Low	14.27(10.64 ~ 19.13)	2.13	17.78	<0.001*
Compliance (control=good)				
Bad	29.64(20.87 ~ 42.08)	5.30	18.95	<0.001*
3. Psychological data				
Depression (control=no)				
Yes	4.80(3.68 ~ 6.27)	0.65	11.53	<0.001*
Anxiety (control =no)				
Mild	1.15(0.73 ~ 1.81)	0.27	0.59	0.557
Moderate	9.51(6.02 ~ 15.03)	2.22	9.65	<0.001*
Severe	32.12(20.36 ~ 50.66)	7.47	14.92	<0.001*

*P < 0.05 , and the difference was statistically significant.

2.4 Multivariate ordinal cumulative logistic regression analysis

To further understand the different factors influencing patients' quality of life, variables which were statistically significant in univariate ordinal logistic regression were taken into multivariate ordinal cumulative logistic regression analysis to analyze the different levels of QOL（Y=1, 2, 3, 4）in patients with epilepsy(Categorical variables were taken into the equation in the form of dummy variables, and the first dummy variable for each variables was set as a reference) .

The applicability analysis of the model revealed that the χ^2 value of proportional odds assumption was 8.46 (P=0.379), in accordance with the basic assumptions of cumulative odds logistic models test(Roberts and Vernon, 1983) . The results revealed：The regression equation was statistically significant（χ^2 = 948.59 , P<0.0001）, CraggUhler(Nagelkerke) R^2= 0.707, ML (Cox-Snell) R^2= 0.662. AIC= 1.716. The frequency of seizures, awareness rate of knowledge about epilepsy, anxiety, depression, types of medication intake and compliance were the main factors influencing QOL of patients with epilepsy. Among them, high seizure frequency along with anxiety and depression were risk factors of QOL; high epilepsy knowledge awareness rate, single medication intake, and good compliance were protective factors. The details were seen in Table 3.

Table 3. Results of multivariate categorical cumulative odds logistic regression analysis

Influencing factors	OR	*β(95%CI)*	SE	*z*	*P*
Types of medication intake(control = Multi-medications)					
Single-medication	0.14	-1.96(-2.37 ~ -1.54)	0.21	-9.32	<0.001*
Compliance (control=good)					
Bad	7.00	1.95(1.39 ~ 2.50)	0.28	6.92	<0.001*
Seizure frequency(control=controlled)					
Mild	3.54	1.26(0.79 ~ 1.73)	0.24	5.29	<0.001*
Moderate	2.15	0.76(0.23 ~ 1.29)	0.27	2.82	0.005*
Epilepsy knowledge awareness rate (control=high)					
Low	4.81	1.57(1.08 ~ 2.06)	0.25	6.22	<0.001*
Depression (control=no）					
Yes	1.49	0.40(0.01 ~ 0.79)	0.20	2.03	0.043*
Anxiety (control=no）					
Mild	0.33	-1.10(-1.73 ~ -0.48)	0.32	-3.46	0.001*
Moderate	3.42	1.23(0.65 ~ 1.81)	0.30	4.16	<0.001*
Severe	13.26	2.58(2.01 ~ 3.16)	0.30	8.74	<0.001*

* P < 0.05,the difference was statistically significant.The constant terms (cut1, cut2, cut3) were not listed.

Table 4. Results of non-conditional multivariate ordinary binary logistic regression analysis.

Influencing factors	OR(95%CI)	SE	*z*	*P*
Types of medication intake (control = Multi-medications)				
Single-medication	0.09(0.03 ~ 0.29)	0.05	-4.10	<0.001*
Depression (control=no)				
Yes	9.39(3.32 ~ 26.56)	4.98	4.22	<0.001*
Seizure frequency (control=controlled)				
Low	3.58(1.45 ~ 8.89)	1.66	2.75	0.006*
Moderate	10.74(3.86 ~ 29.91)	5.61	4.55	<0.001*
High	26.22(3.25 ~ 211.62)	27.94	3.07	0.002*

* P < 0.05, the difference was statistically significant.

The results of ordinary binary (cut point set at P75, the 75% position in the percentile scale was set as the passing or qualifying point) non-conditional multivariate ordinal logistic regression analysis (variables which were statistically significant in univariate ordinal logistic regression were taken into the multivariate ordinal logistic regression): The regression equation was statistically significant. (χ^2 = 215.98，P<0.0001), Nagelkerke R^2= 0.506. Cox-Snell R^2= 0.379.AIC= 0.947. The results revealed that seizure frequency, types of medication intake and depression were the main factors influencing QOL of patients with epilepsy. The details are shown in table 4.

3. Discussion

Ordinal cumulative odds models is also known as proportional odds model or ordinal logit model, it is an extension of binary logistic regression model (Peter 1980; Armstrong and Sloan 1989; Brant 1990)，and is mainly used to handle the data whose response variables are ordinal categorical responses. Many studies(RenXiaolin and Liu Xueqin, 2003; Geng Xiang, Wu Yiwen et al., 2007; Tong Xiaoyan, Yan Junjie et al., 2010; Yang Miao and Wang Kai, 2010; Liu, Han et al., 2011; Shetty, Naik et al., 2011; Zhao, Wu et al., 2011; Gao Yan, Xu Huashan et al., 2011) used linear model (such as linear regression) directly without carrying out homogeneity of variance and normality tests, thus the model applicability was in doubt and the conclusion was not reliable. If such data is converted to binary variables to conduct ordinary logistic regression analysis, the cut point is of vital importance. For example, quality of life score in epilepsy was set as response variable, we converted the score to a 100-point scale and artificially set 60 points (split point) as the "passing" or "qualifying" point, then converted response variables to the binary variables 0 and 1, the “qualifying” point could also be set at 75 or 80 points, etc. Therefore, in addition to meaningful professional division (For example, a percentile scale of psychology set more than 80 points as positive for depression), the choice of the cut-off point often has not a certain base or standard. If there are more than 1 cut-off point, the form of the response variables is polytomous and can be divided into polytomous unordinal data and polytomous ordinal data based on whether the response variables are ordinal or not. So for the ordinal data which neither meet the requirements of the linear model nor have the professional cut-off point, it can’t be converted to the binary variables to conduct ordinary logistic regression analysis. At this time the binary is the merger of the cut points (grades) and it will inevitably lead to loss of data and reduce the reliability of conclusion.

In the study, the applicability analysis of the cumulative odds model revealed that χ^2 value of proportional odds assumption was 8.46 (P=0.379), meaning that the regression lines of different cumulative odds were parallel to each other. That means the regression coefficient of the independent variable had no correlation with the cut-off point, only intercept parameters varied, the data met the basic condition assumptions(Wang Jichuan and Guo Zhigang, 2001) of cumulative odds logistic model test. Some studies (Likang, Guo Zuchao et al., 1993; Chen Peizhen and Chen Feng, 2001) revealed that cumulative odds model was not sensitive to this "condition", however, other studies found that the test efficiency of the model would be reduced and might

lead to misleading conclusions when it did not meet this assumption condition. In this study, the cumulative odds regression results revealed that: Nagelkerke R^2=0.707,ML R^2= 0.662, the predictive accuracy(Wang Jichuan and Guo Zhigang, 2001) of the regression model was better if the value of R^2 (close to 1) was greater. AIC=1.716, AIC was the index that reflected goodness of fit of the regression model, whose value should be as small as possible. The result of ordinary binary non-conditional multivariate ordinal logistic regression analysis for the same data revealed (see table 4): Nagelkerke R^2= 0.506,Cox-Snell R^2= 0.379,the prediction accuracy of accumulative odds model was higher than that of ordinary binary logistic regression. The AIC of ordinary binary logistic regression was 0.947, this suggested that the accuracy of the ordinary binary logistic model was good, meeting the principle that the number of independent variables should be "fewer but better" (Wang Jichuan and Guo Zhigang, 2001). However, this simple model missed some important and modifiable influencing factors such as compliance, anxiety and epilepsy knowledge awareness rate. More importantly was that the corresponding results were inconsistent if the cut-off point is replaced by P60 or P70 (more than 60 or 70 percentile as "qualifying" for an ordinary binary logistic regression), this suggested that the results of the cumulative odds logistic model were more stable and reliable for the data which did not meet the requirements of the linear model.

Our research revealed that the mean QOL in patients with epilepsy without intervention was 37.89(the median level was 37), lower than that in other domestic studies, such as 55.2 by Yang Miao(Yang Miao and Wang Kai, 2010) and 58.9 by Zhu Suiqiang(Zhu Suiqiang et al., 2010). The results of different studies varied, the influencing factors analysis(Yang Miao and Wang Kai, 2010; Geng Xiang et al., 2007; Tong Xiaoyan et al, 2010; Kubota H. et al, 2010; Guekht A. B. et al, 2007)in the conclusions also varied, there may be 2 possible reasons to explain this: the first one is that the diagnostic criteria and QOL scales used in these studies were not consistent, the second one is that the statistical methods used in these studies were not consistent, and in many studies the statistical methods were wrongly used. For example, in some studies, also the data did not meet the requirements of linear model, multivariate logistic regression and variance analysis were used. More importantly, samples in these studies(Yang Miao and Wang Kai, 2010; Ren Xiaolin and Liu Xueqin, 2003; Geng Xiang et al., 2007; Gao Yan et al, 2011; Tong Xiaoyan, 2010)are clinically opportunistic collection, the size of the sample was small, and no random sampling was conducted, thus there were various types of biases. Conclusions under these kinds of circumstances were not representative and could not reflect the real conditions. In our study, the sample was collected by multi-stage stratified random sampling among patients with epilepsy who were enrolled in and not enrolled in the project management in 9 project sites in our province, the sample size was large enough to have a good representation.

By multivariate cumulative odds logistic analysis, our research revealed that seizure frequency, anxiety, depression, types of medication intake and compliance were major influencing factors of QOL among patients of epilepsy. Among the various conclusions in previous studies (Yang Miao and Wang Kai, 2010; Geng Xiang et al, 2007; Tong Xiaoyan, 2010; Gao Yan et al, 2011; Guekht A. B. et al., 2007) conducted in domestic areas and overseas, "seizure frequency is the most important influencing factor on QOL" was a widely accepted conclusion, good control of seizures indicated high QOL score, this suggests that it is necessary to carry out targeted health promotion and standardized therapy among poorly controlled patients to improve their QOL. Some research11 , 13-15,23,27 (Yang Miao and Wang Kai, 2010; Geng Xiang et al., 2007; GaoYAN et al., 2011;Tong Xiaoyan et al., 2010; Zhu Suiqiang et al., 2003; Xu Hong et al., 2008) suggested that the correlation between the compliance, anxiety, awareness rate of knowledge on epilepsy and QOL was not statistically significant, this was similar with the results of ordinary binary non-conditional multivariate analysis. This results suggested that also ordinary binary logistic regression analysis meet the "less but better" rule(Wang Jichuan and Guo Zhigang, 2001) in the choose of independent variables, it couldn't cover all the key modifiable factors, it was with "mathematical" value but couldn't be applied in the practice of targeted health promotion and epilepsy prevention and treatment project.

The results of our research revealed that patients who took single medication had higher QOL and less worry about the side effects of the medication, cumulative odds logistic regression analysis also revealed that single medication intake was a protective factor of QOL, this was in consistent with previous literatures(Baker, Jacoby et al., 1997; Zhu Dantong, Xiao Bo et al., 2002; Lin Juanxia, Sun Meizhen et al., 2009). The higher awareness rate of knowledge on epilepsy indicated higher QOL score, this suggests that it is necessary to carry out targeted health promotion and education measures among low QOL score patients to improve their compliance and awareness rate of the knowledge on epilepsy. In addition, we must pay attention to the effect of

concomitant anxiety and depression on knowledge of epilepsy, this was in consistent with previous studied conducted by Choi-Kwon(Choi-Kwon, Chung C et al., 2003) etc in South Korean population and Tong Xiaoyan15(Tong Xiaoyan, Yan Junjie et al., 2006), Xu Hong(Xu Hong, Long Faqing et al. 2006), Zhao Xiuhe(Zhao Xiuhe, Chi Zhaofu et al. 2006) in Chinese population. Our research also revealed that patients with concomitant depression had evidently lower overall health level, more worry about attacks, impaired emotional health and damaged vigour. Thus, it is necessary to conduct supportive psychological therapy among these patients to help them increase the ability to cope with difficulties and bad stimulus, and walk out the psychological dilemma.

To sum up, the QOL of patients with epilepsy in this region was relatively lower than that in other areas, this suggests that it is necessary to lay stress on intervening the modifiable factors of target patients, carrying out pertinent health promotion and health education to improve the awareness rate of knowledge on epilepsy, and strengthening standardized treatment, follow-up management and psychological intervention therapy to improve the QOL of patients and reducing the disease burden due to epilepsy. It is also necessary to conduct various broadcast and education activities with the help of the media, in aid to increase the public's insight about epilepsy, win the society's support, and change people's discriminative attitude towards epilepsy.

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Bioactive Compounds & Medicinal Properties of *Valeriana jatamansi* Jones - a review

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Abstract: *Valeriana jatamansi* Jones commonly known as "Indian Valerian" is a perennial medicinal herb, gynodioecious in nature belonging to family Valerianaceae. Today the species is highly valued medicinal plant with many pharmacopeial monographs. It occurs at an altitude of 1200-3000 m asl. Rhizomes and roots of the herb yields essential oil. The therapeutic properties of Valerian are attributed to a group of compounds known as Valmane and Valepotriates. The Valepotriates are a group of monoterpenoids of irridoid type having epoxy group and beta-acetoxy isovaleric acid. Three novel sesquiterpenoids, valerianananoids of each, forty constituents of essential oils and eleven jatamanins including a new lignin isovaleroxylariciresinol have been extracted from this valuable herb. Underground parts are used in mental disorders, scanting hair, epilepsy, leprosy, as an insecticide and as potential anti-tumour agent. Present paper highlights the information based on available published literature so as to describe the active constituents and medicinal profile of this threatened species which will serve as a valuable platform for further research and conservation of this medicinally important species.
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Key Words: Bioactive compounds; Valeportriates; *Valeriana jatamansi*

1. Introduction

Valeriana jatamansi Jones syn. *V. wallichi* popularly known as Indian Valerian (Mushkibala in Hindi/Kashmiri, Suganthdhawal or Tagara in Sanskrit) is distributed in all temperate regions of the World except Australia (Jain,1968; Polunin and Stainton,1987). Several species of genus *Valeriana* have also been reported from Chile, Brazil, South Africa and Sub-tropical Asia and among these twelve species occur in India (Polunin and Stainton,1987; Rao *et al*., 1997). *V. jatamansi* is a small herbaceous species of family Valerianaceae and is a perennial dwarf, hairy, rhizomatous herb forming a group of thick roots covered with fibers (Bagchi and Wooper, 2011). The herb mostly grows randomly in steep areas, moist, rocky, disturbed grassy slopes, on stones with coarse sandy loam soil. The herb occurs in an altitudinal gradient of 1200-3000 m asl.

Studies conducted on this species have revealed that the species falls in endangered category (Kaul and Handa, 2000) and is at the verge of becoming extinct (Nayar and Sastri, 1998) and due to its over-exploitation for its rhizomes having immense medicinal importance. Habitat degradation (Airi *et al*., 2000; Nautiyal *et al*., 2003) and other biotic interferences in its distribution ranges are the other reasons for depletion of this herb from its natural habitat (Chauhan and Nautiyal, 2005; Chauhan, 1999). The herbarium section of the Department of Botany, University of Kashmir represents 5 species of the genus *Valeriana* from different localities of Kashmir valley including Shajnar, Dara, Harwan, Gulmarg, Yusmarg, Dacksun, Ferozpur, and Sonamarg (Naqashi and Dar,1982-86). Species is gynodieocious (Raina and Srivastava, 1992) and possess pubescent stem and radical leaves with several long petioled, cordate-ovate, cauline few or much smaller entire or pinnate with hairy fruits or nearly glabrous. Flowers are white tinged with pink. Root stock thick and horizontal, aromatic and modular (Hooker, 1881).

2. Active compounds and medicinal properties

V. jatamansi Jones an important medicinal wild herb is being exploited for its roots and rhizomes which contain valepotriates (Chopra, 1956; Sood 1992) highly effective against leprosy (Kour *et al*.1999) and curing Lewybody dementia (Bagchi and Wooper, 2011) . Commercially produced Valerian (Anonymous, 1976) is obtained from roots which have antibacterial and antiprozoal activity (Theis, 1966; Von and Rehman, 1969).The isolation of valepotriates and determination of its medicinal properties resulted in wide spread use of this compound as a sedative in West Germany under the trade name "valmane" which comprises of standardized mixture of valepotriates containing valtrate (15%), didrovaltrate (80%) and acevaltrate

(5%) (Fig. 1) (Anonymous,1976; Theis, 1966; Von and Rehman, 1969). Although some clinical testing of these alkaloids were carried out earlier also, but the first report on its medicinal properties was published in 1969. The structure of three novel sesquiterpenoids, valerianananoids (Ming *et al*., 1997), forty chemical (Dongsheng and Jixian,1994) constituents of essential oils and eleven *jatamanins* including a new lignin, isovaleroxylariciresinol are extracted from *Valerian species* (Lin *et al*., 2010; Dongsheng and Jixian, 1994).

The herb is widely used in making perfumed powder and cardiac preparations. It is regarded as an aphrodisiac, antispasmodic, tranquilizer, antiseptic, expectorant, febrifuge, nerve tonic, ophthalmic, sedative supporative and tonic useful in hysteria, cholera, snakebite, scorpion sting, asthma and neurosis (Wagner *et al*., 1980; Grusla, *et al*., 1986; Gupta, et al., 1996; Nahrstedt., 1984; Diapher and Hindwarch, 2004). Cytotoxity of valeoptriates have been reported for potential anti-tumour properties which reduces the size of tumor after 24 hours application (Diapher and Hindwarch, 2004). Dried rhizomes are also employed in hair oil (Kirtikar and Basu, 1975; Kumar, 2003; Prakash and Mahrotra, 1994). Roots are acrid and bitter which are used as carminative, laxative, and hypnotic. These are also used for curing blood diseases, burning sensation, cholera, skin disease, throat troubles and ulcers (Pande *et al*., 1994). Further roots increase the lusture of eyes, promote growth and blackness of hair and are also useful in the treatment of cough, chest pain and kidney troubles.

On the basis of collection of ethnic information the tribes of Tehri- Garhwal Uttarakhand regard *Valeriana* as a sacred plant and are used in the preparation of *urtan* (a cosmetic) in marriage and religious ceremonies and also as an insect repellent (Pande *et al*., 1994). Ethno-botanical studies conducted on Gaddi tribes of Bharmour area in Himachal Pradesh has reported the use of its leaves and roots for performing *havan* (Religious ceremonies) and is locally known as *Nak Nahani* (Uniyal and Issar, 1967).

3. Conservation status

About 350 species of the genus *Valeriana* and family Valerianaceae have been over exploited due to their use in curing of wide range of diseases nationally and internationally (Gupta, 2010; Wyatt, 1981). The ever increasing demand has enforced indiscriminate collection of rhizomes by various agencies, researchers and locals causing drastic threat to its existing wild population.

The population assessment of *Valeriana* species has revealed that on an average there is a decrease of about 30 - 40 plants per 100 m^2 and is increasing with passage of time. Species of genus *Valeriana* are totally wiped out from some previously recorded localities (Wyatt, 1981; Verma *et al*., 2004), comparatively at lower altitudes while their distribution in the inaccessible terrain has dissected and shrunken. Profound impact indicates that species is exploited for its rhizome, which is the source of active principle - valeportritates, for which plant is sought after and has been depleting from its natural habitats at a fast pace, hence, it is of immediate concern that different conservation measures and strategies be adopted so as to stop its further depletion from its natural habitats**.**

4.Conclusion

Valeriana jatamansi Jones is a natural tetraploid species distributed from Afghanistan to Southwest China and Burma. Underground parts contain epoxy-iridoid esters called Valepotriates which are found in variable amounts having bioactive compounds which confer anxiolytic properties. It is cultivated in some community forest on commercial basis, but on the private land it is planted as demonstration plots not on a commercial basis. Harvesting is done during September - November. The whole plant is dug out and only rhizomes are collected. For commercial cultivation 2500 Kg (Manondar, 1976) of dry rhizome can be collected from one hectare of land. First good harvest can be done after two years of plantation. Drug obtained from rhizomes is used in perfumed powder and cardiac preparations.

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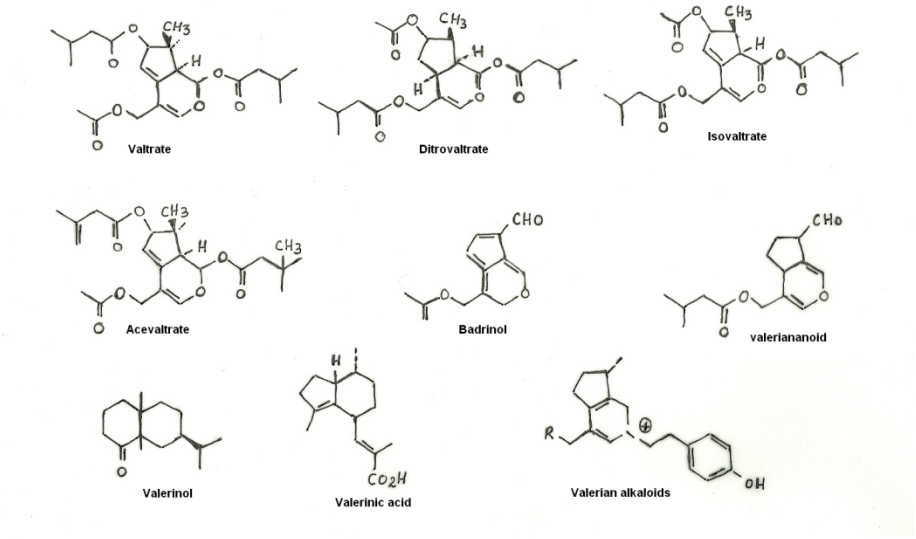
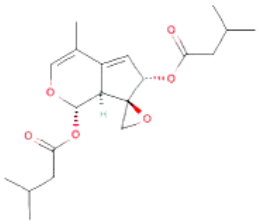
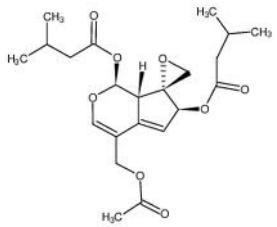


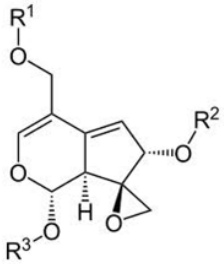
Structure of Valeportriates



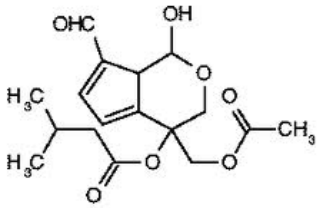
Valtrate (Baldrisedon)



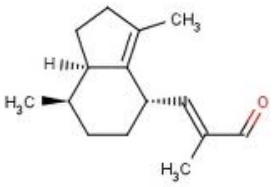
Chemical structure of valtrate



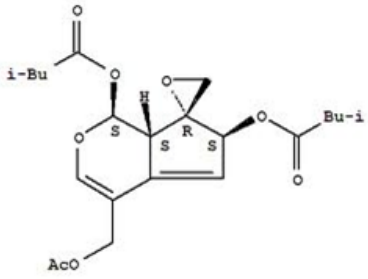
Valeportriate



Valtroxal



Valerinol



Valtrate

Fig. 1: Molecular and Chemical structures of different chemical constituents obtained from *Valeriana jatamansi*

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Influence of Methyl *tert*-Butyl Ether (MTBE) on white *Corn (Zea mays* L.) Plant Growth

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Abstract: This work was designed to investigate the phyototoxicity of methyl *tert*-butyl Ether (MTBE) to metabolic activity of white corn (*Zea mays* L.) plants. The four-week-old potted plants were subjected to four weekly doses (50 ml of MTBE) at different concentrations (0.5, 1.0, 5.0, 10 and 15 %). Growth parameters indicated significant ($p \leq 0.05$) inhibition only at the high concentrations (0.5 and 1.0 %) of MTBE. The leaf area and chlorophyll-A contents decreased as the concentration of MTBE increased. A marked increase of lipid peroxidase activity was recorded at the different MTBE concentration, while a slight decrease of catalase activity was recorded at the same MTBE concentration. The impaired growth and anabolic activities in white corn plant resulted from the oxidative stress of MTBE.

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Key word: Methyl-tert butyl ether (MTBE) , Zea Mays L., growth, leaf area, photosynthetic pigments , catalase, lipid peroxidase.

1. Introduction

Since 1992, methyl *tert*-butyl ether (MTBE) has been added to gasoline, instead of lead, worldwide as oxygenate in order to enhance combustion efficiency to lower carbon monoxide emission to reduce air pollution . It is the most commonly used as fuel oxygenate because of its low cost , high octane level and case of blending with gasoline (**Johnson, 2000**). The U.S. EPA has classified MTBE as possible human carcinogen (**Squillace, 1996**) .

Methyl *tert*-butyl ether (MTBE) is a chemical compound with molecular formula $C_5H_{12}O_5$, it is the second high produced industrial chemical in the USA and frequent ground water pollutant . MTBE is quit persistent to biotic and abiotic decomposition.

The cause of MTBE leaking into the environment is mainly attributed to gasoline spills and leaks from pipelines, underground and above ground storage tanks, and transport accidents (**An, 2002**). As a result, increased soil contamination by MTBE can lead to inhibition of soil microflora which alter soil health and fertility (**Mehler, 2001**). Toxicity of MTBE to aquatic plants has been intensively studied (**Werner, 2001**). Yet, few works has been conducted on the toxicity of MTBE to terrestrial plants (**Nellessen, 1993**). Therefore, the main objective of this study was to assess its effect on seed germination, plant growth, photosynthesis and some enzyme activity in white corn (*Zea mays* L. cv. 310) plants.

2. Material and Methods

Plant material

The experimental plant used in the current study was pure strain of *white corn (Zea mays* L. cv 310). Certified seeds were provided by the Agricultural Research Center in Giza , Egypt .

Methyl tert-butyl ether (MTBE)

The high grade chemical MTBE was obtained from ARAMCO Jeddah, Saudi Arabia.

Growth experiment

The experiment was conducted in the Botanical garden of the Faculty of Science, Taif University, Saudi Arabia in the Spring of 2011. For plantation, 30 plastic pots were filled with homogenous pre-sieved garden soil (Sandy loam). Seeds were soaked in the pot soil about 3 cm deep. All pots were watered up to saturation, then kept in the open garden and irrigated regularly to field capacity until MTBE treatments.

MTBE treatments

After two weeks from soaking, the planted pots were randomly subdivided into six equal groups (3 pots each). One group was treated with pure water and sampled as control. The other five groups were subjected to four weekly doses (50 ml of MTBE) At different concentrations (0.5, 1.0, 5.0, 10 and 15 %) added to the soil with 50 ml of ½ Hoagland nutrient solution .

Sampling and measurements

Growth vigor

After four weeks from soaking , vegetative growth parameters (shoot and root lengths, their fresh and dry weights and the area of third foliage leaf) were recorded **.**

Leaf area: The area of the third foliage leaf was determined using the formula:

A = L × W × 0.75;

where L = the leaf length , W = the leaf width , 0.75 is the factor of recalculation for maize leaf (**Montgomery, 1970; Whigham *et al*., 1974; Pearce *et al*., 1975; Aliu *et al*., 2008**).

Chemical analysis

Photosynthetic pigments

By the end of the sixth week, chlorophyll-A, Chlorophyll-B, and carotenoids were estimated (ugml^{-1}) in the third fresh foliage leaf. One gram of fresh leaf tissue was extracted by grinding in 10 ml of 80% acetone. The mixture was then centrifuged for 5 min. at 3000 rpm. The supernatant was used for spectrophotometric determination according to the method of **Lichtenlhaler and Wellborn (1983)**.

Antioxidant and oxidative enzymes

Catalase Enzyme

Catalase activity was measured according to the method of **Chen and Moely (1992)**. Extracts were prepared 4 weeks after imposing the MTBE in the nutrient solution. Plant tissues were homogenized with 0.1 M sodium phosphate buffer (pH = 6.8) in a chilled mortar and pestle. The homogenate was centrifuged at 14000 rpm for 20 min. The obtained supernatant was used for the determination of enzyme activity. The whole extraction procedure was carried out at 4° C.

Lipid peroxidase enzyme

The thiobarbituric acid reactive substance (TBARS) levels as index of malondi-aldehyde (MDA) production were measured by the method described by **Ohkawa *et al.* (1979)**. MDA, an end product of lipid peroxidation reacts with TBA-TCA complex to form a colored complex at high temperature exhibiting an absorption maximum at 535 nm. Plant tissue (0.5g) was homogenized using a potter–Elvejham homogenizer with 3 ml of 0.1 M of ice-cold phosphate buffer (pH = 7.4) . The homogenate was centrifuged for 10 min at 12000 rpm. A volume of 100 µl from the supernatant was mixed with 100 µl of normal saline and 400 µl of TBA-TCA mixture, all were incubated in a boiling water bath for 10 min., then cooled to room temperature. After centrifugation at 1200 rpm for 10 min, 100 µl from the supernatant was mixed with 100 µl of 0.7 % TBA in a cuvette and the absorbance was read at 535 nm. The concentration of MDA was calculated using a standard curve from 1,1,3,3,-tetraethoxypropane and expressed as µmolg^{-1}.

Statistical analysis

Growth parameters were statistically analyzed using multiple comparison procedure at $p \leq 0.05$ using t-test and mean separation by least significant difference (LSD) (**Steel and Torrie, 1980**).

3. Results

In response to the investigated MTBE concentrations, the results of growth parameters of white corn (Table 1) indicated non-significant ($p \leq 0.05$) effects on all growth parameters at low concentrations (0.5 and 1.0 %) of MTBE; while, the high concentrations (5.0, 10, and 15%) significantly inhibited the stem and root lengths of the plant, the stem and root fresh weights, stem and root dry weights. The severity of inhibition increased as the concentration of MTBE increased above 5%.

The leaf contents of chlorophyll a and total chlorophyll (Table 2 & Figure 1) were reduced by all the applied MTBE concentrations; while, the contents of chlorophyll b and carotenoids as well as the ratio of chl. a/chl. b were almost unchanged. The area of the third foliage leaf (Figure 2) were reduced as the concentration of MTBE increased. The activity of catalase enzyme in the tissues of white corn seedling (Figure 3) was inhibited in response to the highest concentration (15%) of MTBE. However, the activity of lipid peroxidase enzyme showed slight increases in response to the low concentration (0.5, 1.0 and 5%) of MTBE and remarkable enzyme activity in response to the high concentrations (10 and 15%) of MTBE.

4. Discussion

Among the responses of vascular plants to organic chemicals are growth parameters (**Nellssen and Fletche, 1993**). In the current investigation, a generalized inhibitory influence of MTBE on white corn plants is reported. Earlier laboratory studies reported negative growth responses of certain algae to MTBE (**Rousch and Sommerfeld, 1998**). Further studies (**Cape *et al.,* 2003**) reported on significant effects of volatile organic compounds (VOC) including MTBE on leaf water content and photosynthetic capacity of some plant species. Moreover, severe toxic symptoms were detected in weeping willow (*Salix babylonica* L.) after 120 hrs of exposure to MTBE as shown as significant reduction (35%) in transpiration. However, leaf chlorosis was not detected for the whole duration of the test (**Yu and Gu, 2006**).

Holding fairly to our results, **Youn-Joo *et al.* (2002)** reported on a reduction in seed germination, shoot and root growth of oats, sweet corn, wheat and lettuce subjected to different MTBE concentrations in the soil. Root growth, flower and pod development were found to be more sensitive to MTBE treatments,

while stem growth and photosynthetic pigment contents were more persistent to the toxicity of MTBE in common bean plants (**Beltagi, 2007**).

MTBE is quite persistent to abiotic decomposition, in other words, the natural attenuation of MTBE in aquifers is slow and, in some cases, undetectable with half-life of at least two years (**Fayolle *et al.* 2001**). Thus, MTBE is highly water soluble and is readily absorbed by plants. The obtained data of this investigation showed reductions in both shoot and root growth and reduced leaf area as well. These observations assume that MTBE might be absorbed, accumulated and transported with the plant parts from root to shoot. The decrease in the leaf area and the leaf content of chlorophyll a suggest photosynthesis as a target for the phytotoxicity of MTBE.

All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage.

A variety of environmental stresses including soil salinity, drought, extremes of temperatures, and heavy metals are known to cause oxidative damage to plants either directly or indirectly by triggering an increased production of reactive oxygen species (ROS) (**Shalini Verma and Dubey, 2003**). To combat the oxidative damage, plants have the antioxidant defense system comprising of enzymes such as catalase, peroxidase, superoxide dismutase and the non-enzymic constituents as well which remove, neutralize and scavenge the ROS. A decline in catalase activity in response to MTBE concentration was observed in our study which suggest a possible delay in the removal of H_2O_2 and toxic peroxides mediated by catalase and in turn an enhancement in the free radical mediated lipid peroxidation under MTBE toxicity. The decline in catalase activity is regarded as a general response to many stresses (**Herbinger *et al.*, 2002; Bakalova *et al.*, 2004; Jung, 2004; Pan *et al.*, 2006; Gunes *et al.*, 2008; Liu *et al.*, 2008**). The reduction in catalase activity could be attributed to the inhibition of enzyme synthesis through the change in the assembly of enzyme subunits under MTBE stressful condition. On the other hand, our results indicated an enhancement in the activity of lipid peroxidase, suggesting a significant role for this enzyme as an intrinsic defense tool to resist MTBE oxidative damage in white corn plants (**Verma and Dubey, 2003**). The enhanced antioxidative scavenging mechanism of lipid peroxidase activity might be considered as an important mechanism to cope with MTBE oxidative stress as reported by **McKersie *et al.* (1999),), Das & Csiszar *et al.* (2005) Uprety (2006) and Gunes *et al.* (2008** for peroxidase activity under drought stress.

In conclusion, MTBE causes oxidative stress in white corn plants and catalase and peroxidase enzymes appear to have a pivotal role in combating oxidative stress in white corn plants. The increased activity of the involved enzymes in removing reactive oxygen species, like lipid peroxidase, resulted from the stimulation of gene expression to alleviate the adverse effects of oxidative stress caused by MTBE.

Acknowledgments:

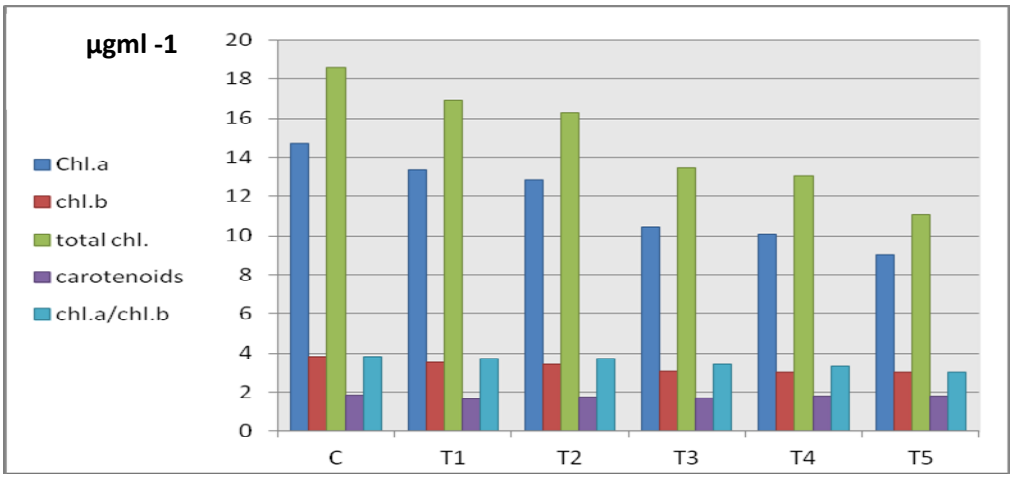
This experiment was supported by Taif University. The authors Sincerely thanks anonymous reviewers for comments on earlier drafts of this manuscript.

Table 1. Mean growth parameters of white corn (*Zea mays* L. cv. 310) plants subjected to 0.0, 0.5, 1.0, 5.0, 10 and 15 % MTBE concentrations (each value is a mean ±SD of 3 replicates).

MTBE conc. (%)	Stem fresh weight (gm)	Root fresh weight (gm)	Stem length (cm)	Root length (cm)	Stem dry weight (gm)	Root dry weight (gm)
0.0	6.08±0.005	4.18±0.045	30.81±0.76	26.32±0.080	0.47±0.015	**0.49±0.020**
0.5	5.77±0.020	3.87±0.030	29.43±0.62	23.55±0.509	0.44±0.032	**0.46±0.036**
1.0	6.47±0.040	3.60±0.064	25.20±0.06	21.17±0.282	0.42±0.020	**0.36±0.020**
5.0	2.35±0.018	1.41±0.015	18.65±0.07	20.22±0.115	0.21±0.015	**0.35±0.025**
10	2.27±0.015	0.81±0.035	18.13±0.04	19.23±0.080	0.17±0.010	**0.31±0.015**
15	1.33±0.035	0.47±0.025	15.27±0.05	15.12±0.068	0.13±0.015	**0.26±0.025**

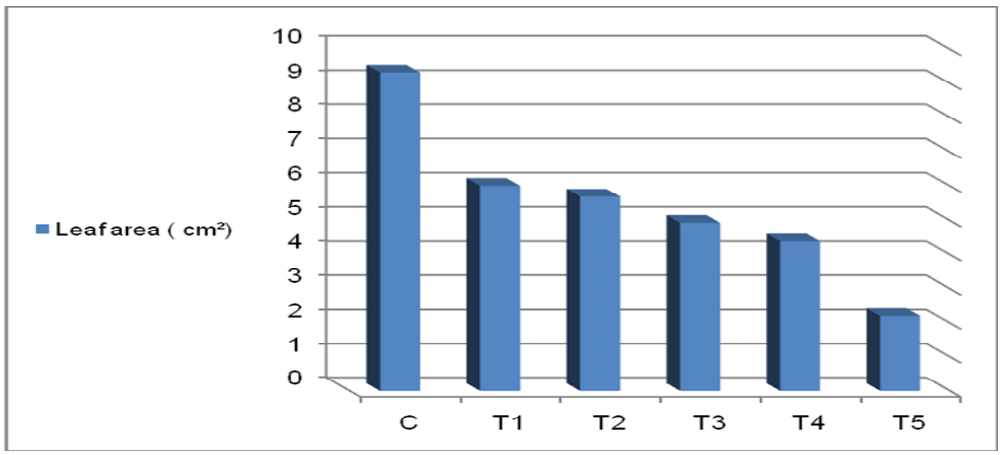
Table 2. Chlorophyll a (chl. a), chlorophyll b (chl. b), total chlorophyll (total chl.), carotenoids and chlorophyll a/chlorophyll b (chl. a/chl. b) leaf contents in white corn (*Zea mays* L. cv. 310) subjected to 0.0, 0.5, 1.0, 5.0, 10 and 15 % MTBE concentrations.

MTBE concentrations (%)	Chl.a (μgml^{-1})	Chl. b (μgml^{-1})	Total Chl. (μgml^{-1})	Carotenoids (μgml^{-1})	Chl. a/chl. B
0.0	14.73	3.85	18.58	1.83	**3.82**
0.5	13.34	3.54	16.88	1.64	**3.73**
1.0	12.83	3.45	16.26	1.73	**3.71**
5.0	10.43	3.03	13.44	1.70	**3.41**
10	10.03	3.01	13.04	1.78	**3.33**
15	09.03	3.00	11.04	1.80	**3.00**



MTBE treatments (%)

Figure 1. Chlorophyll a (chl.a), chlorophyll b (chl. b), total chlorophyll (total), carotenoids and chlorophyll a/chlorophyll b (chl.a/chl.b) leaf contents in white corn (*Zea mays* L. cv. 310) subjected to C, T1, T2, T3, T4 and T5 (0, 0. 5, 1.0, 5.0, 10 and 15 %) MTBE concentrations.



MTBE treatments (%)

Figure 2. The area of the third foliage leaf of white corn (*Zea mays* L. cv. 130) plants subjected to C, T1, T2, T3, T4 and T5 (0, 0. 5, 1.0, 5.0, 10 and 15 %) MTBE concentrations.

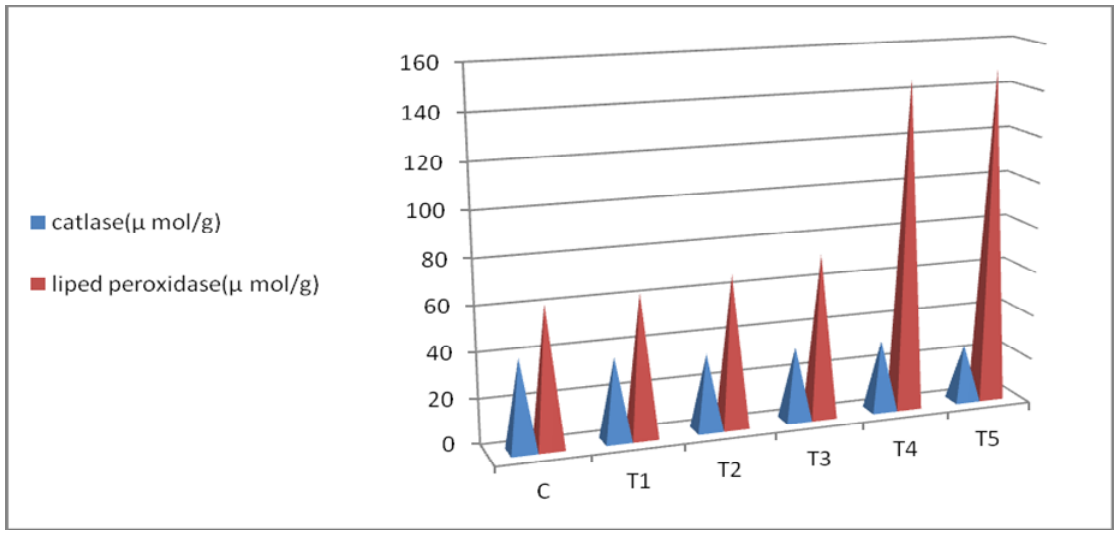


Figure 3. Activity of catalase and lipid peroxidase in leaves of white corn (*Zea mays* L. cv. 310) subjected to C, T1, T2, T3, T4 and T5 (0, 0. 5, 1.0, 5.0, 10 and 15

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4/22/2012

Direct Boundary Element Method for Calculation of Inviscid Compressible Flow past a Symmetric Aerofoil with Constant Element Approach

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Abstract: In this paper, a direct boundary element method (DBEM) is applied to calculate the inviscid compressible flow past a symmetric aerofoil whereas in our previous papers, we applied indirect boundary element method (IBEM) for this purpose. The velocity distribution for the flow over the surface of the symmetric aerofoil has been calculated using direct constant boundary element approach. The accuracy of the computed results can be increased by increasing the number of boundary elements. The validity of this method is well checked by given tables and graphs.

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Keywords: Direct boundary element method, Inviscid Compressible flow, Velocity distribution, Symmetric aerofoil, Constant element.

1. Introduction

In the present period of science and technology, the popularity of boundary element methods rises for solving fluid flow problems and modeling physics in fluid. They provide the best base for the numerical methodology to solve the fluid flow problems. As well as providing the best solution of boundary integral equation based on a discretization process. The applications of boundary element methods rose on sound footing popular with the invention of electronic computer. The boundary element methods originated within the Department of Civil Engineering at Southampton University, U.K. These methods exist under different names such as panel methods, surface singularity method, boundary integral equation method, and boundary integral equation. First of all, finite difference method, finite element method, and finite volume method etc. were being used to solve numerically the problems in computational fluid dynamics. But later on, boundary element method has received much attention from the researchers due to its various advantages over the domain type methods. One of the advantages is that with boundary elements one has to discretize only the surface of the body, whereas with domain methods it is essential to discretize the entire region of the flow field. Moreover, this method is well–suited to problems with an infinite domain. The boundary element method can be classified into two categories i.e. direct and indirect. The direct method takes the form of a statement which provides the values of the unknown variables at any field point in terms of the complete set of all the boundary data. On the other hand, the indirect method utilizes a distribution of singularities over the boundary of the body and computes this distribution as the solution of integral equation. The equation of DBEM in the past can be formulated using either as an approach based on Green's theorem or a particular case of the weighted residual method in the past by many authors. (see Lamb, 1932; Ramsey, 1942, Milne-Thomson, 1968, Kellogge, 1929 and Brebbia and Walker, 1980). The direct and Indirect methods have been used in the past for flow field calculations around bodies (Morino 1975, Hess & Smith, 1967, Kohr, 2000, Luminita, 2008, Muhammad, 2009; Mushtaq, 2008, 2009, 2010, 2011& 2012). Most of the work on fluid flow calculations using boundary element methods has been done in the field of incompressible flow. Very few attempts have been made on flow field calculations using boundary element methods in the field of compressible flow. In this paper, the DBEM has been used for the solution of inviscid compressible flows around a symmetric aerofoil.

2. Mathematical Formulation

We know that equation of motion for two – dimensional, steady, irrotational, and isentropic flow (Mushtaq, 2010, 2011 & 2012) is

$$(1 - Ma^2)\frac{\partial^2 \Phi}{\partial X^2} + \frac{\partial^2 \Phi}{\partial Y^2} = 0 \qquad (1)$$

where Ma is the Mach number and Φ is the total velocity potential of the flow. Here X and Y are the space coordinates.

Using the dimensionless variables, $x = X$, $y = \beta Y$, where $\beta = \sqrt{1 - Ma^2}$,

equation (1) becomes

$$\frac{\partial^2 \Phi}{\partial x^2} + \frac{\partial^2 \Phi}{\partial y^2} = 0$$

or $$\nabla^2 \Phi = 0 \qquad (2)$$

which is Laplace's equation.

3. Symmetric Aerofoil

The Joukowski transformation

$$z = \zeta + \frac{a^2}{\zeta} \qquad (3)$$

transforms the circle shown in figure (1) in the ζ – plane on to symmetric aerofoil in the z-plane.

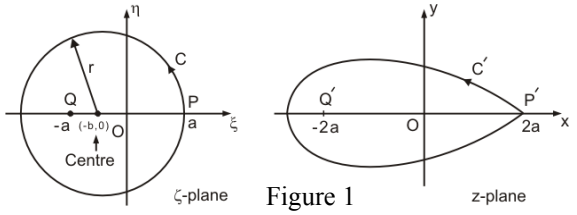


Figure 1

4. Flow Past a Symmetric Aerofoil

Consider the flow past a symmetrical aerofoil and let the onset flow be the uniform stream with velocity U in the positive direction of the x – axis as shown in figure (2) .

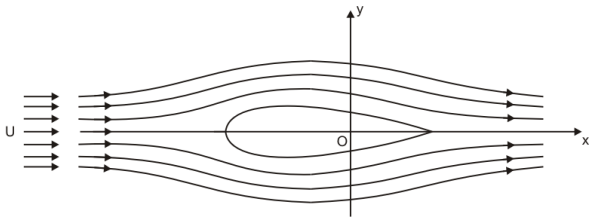


Figure 2: Flow past a symmetric aerofoil.

Exact Velocity

The magnitude of the exact velocity distribution over the boundary of a symmetric aerofoil is given by Chow [3] & Mushtaq [13, 17,18]

as $$V = U \left| \frac{1 - \left(\frac{r}{z-b}\right)^2}{1 - \left(\frac{a}{z}\right)^2} \right|$$

where r = radius of the circular cylinder,
a = Joukowski transformation constant
and b = a – r = x-coordinates of the centre of the circular cylinder

In Cartesian coordinates, we have

V = U

Error!

$$x \frac{\sqrt{[(x^2+y^2)^2 - a^2(x^2-y^2)]^2 + 4a^4x^2y^2}}{(x^2+y^2)^2 - 2a^2(x^2-y^2) + a^4}$$

Boundary Conditions

Now the condition to be satisfied on the boundary of a symmetric aerofoil is

$$\vec{V} \cdot \hat{n} = 0 \qquad (4)$$

where $\hat{n}$ is the unit normal vector to the boundary of the aerofoil .

Since the motion is irrotational

$$\vec{V} = -\nabla \Phi$$

where Φ is the total velocity potential . Thus equation (4) becomes

$$(-\nabla \Phi) \cdot \hat{n} = 0$$

or $$\frac{\partial \Phi}{\partial n} = 0 \qquad (5)$$

Now the total velocity potential Φ is the sum of the perturbation velocity potential $\phi_{s.a}$ where the subscript s . a stands for symmetric aerofoil and the velocity potential of the uniform stream $\phi_{u.s}$.(Mushtaq, 2010 & 2011)

i.e. $$\Phi = \phi_{u.s} + \phi_{s.a} \qquad (6)$$

or $$\frac{\partial \Phi}{\partial n} = \frac{\partial \phi_{u.s}}{\partial n} + \frac{\partial \phi_{s.a}}{\partial n} \qquad (7)$$

From equations (5) and (7) , we get

$$\frac{\partial \phi_{s.a}}{\partial n} + \frac{\partial \phi_{u.s}}{\partial n} = 0$$

or $$\frac{\partial \phi_{s.a}}{\partial n} = -\frac{\partial \phi_{u.s}}{\partial n} \qquad (8)$$

But the velocity potential of the uniform stream , given in Milne – Thomson [7], Shah [8], is

$$\phi_{u.s} = -U x \qquad (9)$$

then

$$\frac{\partial \phi_{u.s}}{\partial n} = -U \frac{\partial x}{\partial n}$$

$$= -U(\hat{n} \cdot \hat{i}) \qquad (10)$$

Thus from equations (8) and (10), we get

$$\frac{\partial u_{s.a}}{\partial n} = U(\hat{n} \cdot \hat{i}) \qquad (11)$$

Now from the figure (3)

$$\vec{A} = (x_2 - x_1)\hat{i} + (y_2 - y_1)\hat{j}$$

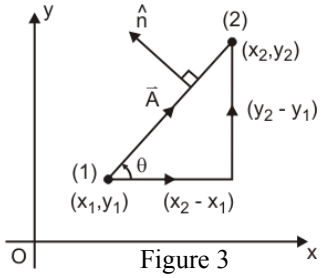


Figure 3

Therefore the unit vector in the direction of the vector $\vec{A}$ is given by

$$\vec{A} = \frac{(x_2 - x_1)\hat{i} + (y_2 - y_1)\hat{j}}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}}$$

The outward unit normal vector $\hat{n}$ to the vector $\vec{A}$ is given by

$$\hat{n} = \frac{-(y_2 - y_1)\hat{n} + (x_2 - x_1)\hat{j}}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}}$$

Thus $$\hat{n} \cdot \hat{i} = \frac{(y_1 - y_2)}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}} \quad (12)$$

From equations (11) and (12) , we get

$$\frac{\partial \phi_{s.a}}{\partial n} = U \frac{(y_1 - y_2)}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}} \quad (13)$$

Equation (13) is the boundary condition which must be satisfied over the boundary of a symmetric aerofoil.

Equation of Direct Boundary Element Method

The equation of DBEM for two–dimensional flow [Mushtaq, 2008, 2009, 2010 & 2011] is :

$$-c_i \phi_i + \frac{1}{2\pi} \int_{\Gamma - i} \phi \frac{\partial}{\partial n} \left[\log \left(\frac{1}{r} \right) \right] d\Gamma + \phi_\infty = \frac{1}{2\pi} \int_{\Gamma} \log \left(\frac{1}{r} \right) \frac{\partial \phi}{\partial n} d\Gamma \quad (14)$$

where $c_i = 0$ when i is exterior to Γ

$= 1$ when i is interior to Γ

$= \frac{1}{2}$ when i lies on Γ and Γ is smooth.

Matrix Formulation

The equation (14) for the DBEM can be written in the discretized form as

$$-c_i \phi_i + \sum_{j=1}^{m} \left[\int_{\Gamma_j - i} \frac{\partial}{\partial n} \left(\frac{1}{2\pi} \log \frac{1}{r} \right) d\Gamma \right] \phi_j + \phi_\infty = \sum_{j=1}^{m} \left[\int_{\Gamma_j} \left(\frac{1}{2\pi} \log \frac{1}{r} \right) d\Gamma \right] \frac{\partial \phi_j}{\partial n} \quad (15)$$

The integrals in equation (15) on the elements can be calculated numerically except the element on which the fixed point 'i' is located. For this element the integrals are calculated analytically. Denoting the integrals on the L.H.S. of equation (15) by $\hat{H}_{ij}$ and that on the R.H.S. by G_{ij}, then

where $$\hat{H}_{ij} = \int_{\Gamma_j - i} \frac{\partial}{\partial n} \left(\frac{1}{2\pi} \log \frac{1}{r} \right) d\Gamma \quad (16)$$

and $$G_{ij} = \int_{\Gamma_j} \frac{1}{2\pi} \log \left(\frac{1}{r} \right) d\Gamma \quad (17)$$

For the case of that element on which the fixed point 'i' is lying, these integrals have been calculated.

Thus equation (15) can be written as

$$-c_i \phi_i + \sum_{j=1}^{m} \hat{H}_{ij} \phi_j + \phi_\infty = \sum_{j=1}^{m} G_{ij} \frac{\partial \phi_j}{\partial n} \quad (18)$$

Defining

$$H_{ij} = \begin{cases} \hat{H}_{ij} & \text{when } i \neq j \\ \hat{H}_{ij} - c_i & \text{when } i = j \end{cases}$$

Equation (18) takes the form

$$\sum_{j=1}^{m} H_{ij} \phi_j + \phi_\infty = \sum_{j=1}^{m} G_{ij} \frac{\partial \phi_j}{\partial n}$$

which can be expressed in matrix form as

$$[H] \{U\} = [G] \{Q\} \quad (19)$$

Since $\frac{\partial \phi}{\partial n}$ is specified at each node of the element, the values of the perturbation velocity potential ϕ are found at each node on the boundary via equation (19). The total potential Φ is then found from equation (6) which will then be used to calculate the velocity on the symmetric aerofoil.

The velocity midway between two nodes on the boundary can then be approximated by using the formula

$$\text{Velocity } \vec{V} = \frac{\Phi_{k+1} - \Phi_k}{\text{Length from node k to k + 1}} \quad (20)$$

Process of Discretization

Now for the discretization of the boundary of the symmetric aerofoil, the coordinates of the extreme points of the boundary elements can be generated within computer programme using Fortran language as follows:

Divide the boundary of the circular cylinder into m elements in the clockwise direction by using the formula (Mushtaq 2009, 2010, 2011 & 2012).

$$\theta_k = [(m + 3) - 2k] \frac{\pi}{m}, \quad k = 1, 2, \ldots\ldots, m \quad (21)$$

Then the extreme points of these m elements of circular cylinder are found by

$$\xi_k = -b + r \cos \theta_k$$

$$\eta_k = r \sin \theta_k$$

Now by using Joukowski transformation in equation (3), the extreme points of the symmetric aerofoil are

$$x_k = \xi_k \left(1 + \frac{a^2}{\xi_k^2 + \eta_k^2} \right)$$

$$y_k = \eta_k \left(1 - \frac{a^2}{\xi_k^2 + \eta_k^2} \right)$$

where $k = 1, 2, \ldots\ldots, m$.

For constant boundary element approach there is only one node at the middle of the element and the potential ϕ and the potential derivative $\frac{\partial \phi}{\partial n}$ are constant over each element and equal to the value at the middle node of the element .

The coordinates of the middle node of each boundary element are given by

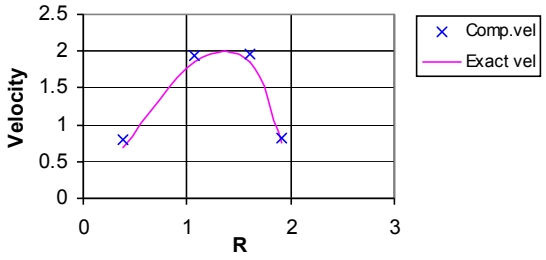
$$\left.\begin{aligned} x_m &= \frac{x_k + x_{k+1}}{2} \\ y_m &= \frac{y_k + y_{k+1}}{2} \end{aligned}\right\}$$

$$k , m = 1 , 2 , \ldots\ldots , n \qquad (22)$$

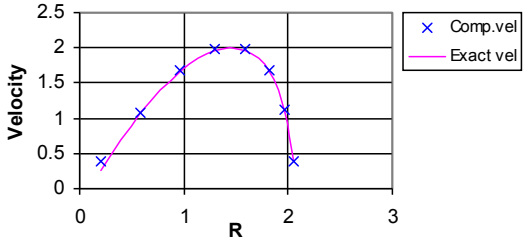
and therefore the boundary condition (13) in this case takes the form

$$\frac{\partial \phi_{s.a}}{\partial n} = U \frac{(y_1)_m - (y_2)_m}{\sqrt{[(x_2)_m - (x_1)_m]^2 + [(y_2)_m - (y_1)_m]^2}} \qquad (23)$$

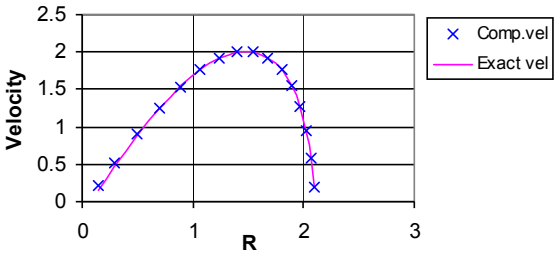
The following tables show the comparison of computed and analytical velocity distribution over the boundary of a symmetric aerofoil for 8, 16, 32, and 64 direct constant boundary elements.



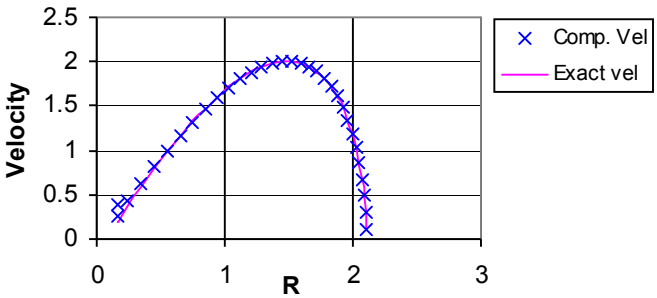
Graph 1: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 8 boundary elements with direct constant element approach for r = 1.1, a = 0.1 and Ma = 0.7.



Graph 2: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 16 boundary elements with direct constant element approach for r = 1.1, a = 0.1 and Ma = 0.7.



Graph 3: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 32 boundary elements with direct constant element approach for r = 1.1, a = 0.1 and Ma = 0.7.



Graph 4: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 64 boundary elements with direct constant element approach for r = 1.1, a = 0.1 and Ma = 0.7.

Table (1)

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-1.87	.36	1.91	.80937E+00	.75969E+00
2	-1.36	.86	1.61	.19518E+01	.18480E+01
3	-.64	.86	1.07	.19386E+01	.18561E+01
4	-.13	.35	.38	.79640E+00	.68955E+00
5	-.13	-.35	.38	.79640E+00	.68955E+00
6	-.64	-.86	1.07	.19386E+01	.18561E+01
7	-1.36	-.86	1.61	.19518E+01	.18480E+01
8	-1.87	-.36	1.91	.80937E+00	.75969E+00

Table (2)

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-2.04	.21	2.05	.39618E+00	.38702E+00
2	-1.88	.59	1.97	.11280E+01	.11044E+01
3	-1.59	.88	1.82	.16873E+01	.16594E+01
4	-1.21	1.03	1.59	.19881E+01	.19661E+01
5	-.80	1.03	1.30	.19835E+01	.19716E+01
6	-.42	.87	.96	.16713E+01	.16645E+01
7	-.12	.57	.58	.10856E+01	.10750E+01
8	.05	.19	.20	.38967E+00	.26843E+00
9	.05	-.19	.20	.38967E+00	.26843E+00
10	-.12	-.57	.58	.10856E+01	.10750E+01
11	-.42	-.87	.96	.16713E+01	.16645E+01
12	-.80	-1.03	1.30	.19835E+01	.19716E+01
13	-1.21	-1.03	1.59	.19881E+01	.19661E+01
14	-1.59	-.88	1.82	.16872E+01	.16594E+01
15	-1.88	-.59	1.97	.11280E+01	.11044E+01
16	-2.04	-.21	2.05	.39618E+00	.38702E+00

Table (3)

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-2.09	.11	2.09	.19712E+00	.19455E+00
2	-2.05	.32	2.07	.58375E+00	.57600E+00
3	-1.97	.51	2.03	.94785E+00	.93637E+00
4	-1.85	.69	1.97	.12754E+01	.12622E+01
5	-1.70	.84	1.89	.15536E+01	.15410E+01
6	-1.52	.96	1.80	.17719E+01	.17620E+01
7	-1.32	1.04	1.68	.19216E+01	.19163E+01
8	-1.11	1.08	1.55	.19969E+01	.19969E+01
9	-.90	1.08	1.40	.19947E+01	.19999E+01
10	-.69	1.04	1.24	.19149E+01	.19236E+01
11	-.49	.95	1.07	.17602E+01	.17695E+01
12	-.31	.83	.89	.15357E+01	.15417E+01
13	-.16	.68	.70	.12489E+01	.12461E+01
14	-.04	.49	.49	.90793E+00	.88934E+00
15	.06	.28	.29	.52705E+00	.47740E+00
16	.12	.09	.15	.21584E+00	.15912E+00
17	.12	-.09	.15	.21584E+00	.15912E+00
18	.06	-.28	.29	.52705E+00	.47740E+00
19	-.04	-.49	.49	.90793E+00	.88934E+00
20	-.16	-.68	.70	.12489E+01	.12461E+01
21	-.31	-.83	.89	.15357E+01	.15417E+01

22	-.49	-.95	1.07	.17602E+01	.17695E+01
23	-.69	-1.04	1.24	.19149E+01	.19236E+01
24	-.90	-1.08	1.40	.19947E+01	.19999E+01
25	-1.11	-1.08	1.55	.19969E+01	.19969E+01
26	-1.32	-1.04	1.68	.19216E+01	.19163E+01
27	-1.52	-.96	1.80	.17719E+01	.17620E+01
28	-1.70	-.84	1.89	.15536E+01	.15410E+01
29	-1.85	-.69	1.97	.12754E+01	.12622E+01
30	-1.97	-.51	2.03	.94784E+00	.93637E+00
31	-2.05	-.32	2.07	.58375E+00	.57600E+00
32	-2.09	-.11	2.09	.19712E+00	.19455E+00

Table (4)

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-2.10	.05	2.10	.98451E-01	.97672E-01
2	-2.09	.16	2.10	.29437E+00	.29110E+00
3	-2.07	.27	2.09	.48742E+00	.48207E+00
4	-2.04	.37	2.07	.67583E+00	.66862E+00
5	-2.00	.47	2.05	.85762E+00	.84901E+00
6	-1.95	.56	2.03	.10312E+01	.10216E+01
7	-1.89	.65	2.00	.11947E+01	.11847E+01
8	-1.82	.74	1.96	.13467E+01	.13367E+01
9	-1.74	.81	1.92	.14857E+01	.14763E+01
10	-1.66	.88	1.88	.16103E+01	.16021E+01
11	-1.57	.94	1.83	.17193E+01	.17127E+01
12	-1.47	.99	1.77	.18116E+01	.18072E+01
13	-1.37	1.03	1.72	.18863E+01	.18844E+01
14	-1.27	1.06	1.66	.19428E+01	.19435E+01
15	-1.17	1.08	1.59	.19804E+01	.19839E+01
16	-1.06	1.09	1.52	.19988E+01	.20051E+01
17	-.95	1.09	1.45	.19978E+01	.20065E+01
18	-.84	1.08	1.37	.19772E+01	.19882E+01
19	-.74	1.06	1.29	.19374E+01	.19501E+01
20	-.63	1.03	1.21	.18786E+01	.18922E+01
21	-.53	.98	1.12	.18014E+01	.18151E+01
22	-.44	.93	1.03	.17064E+01	.17193E+01
23	-.35	.87	.94	.15945E+01	.16052E+01
24	-.27	.80	.85	.14665E+01	.14739E+01
25	-.19	.72	.75	.13236E+01	.13260E+01
26	-.12	.64	.65	.11670E+01	.11624E+01
27	-.06	.55	.55	.99767E+00	.98398E+00
28	-.00	.45	.45	.81697E+00	.79117E+00
29	.04	.34	.34	.62619E+00	.58472E+00
30	.08	.23	.24	.42825E+00	.36984E+00
31	.12	.12	.17	.25123E+00	.18920E+00
32	.16	.03	.16	.38116E+00	.18610E+00
33	.16	-.03	.16	.38116E+00	.18610E+00
34	.12	-.12	.17	.25122E+00	.18920E+00
35	.08	-.23	.24	.42825E+00	.36984E+00
36	.04	-.34	.34	.62619E+00	.58472E+00
37	-.00	-.45	.45	.81697E+00	.79117E+00
38	-.06	-.55	.55	.99767E+00	.98398E+00
39	-.12	-.64	.65	.11670E+01	.11624E+01

40	-.19	-.72	.75	.13236E+01	.13260E+01
41	-.27	-.80	.85	.14665E+01	.14739E+01
42	-.35	-.87	.94	.15945E+01	.16052E+01
43	-.44	-.93	1.03	.17064E+01	.17193E+01
44	-.53	-.98	1.12	.18014E+01	.18151E+01
45	-.63	-1.03	1.21	.18786E+01	.18922E+01
46	-.74	-1.06	1.29	.19374E+01	.19501E+01
47	-.84	-1.08	1.37	.19772E+01	.19882E+01
48	-.95	-1.09	1.45	.19978E+01	.20065E+01
49	-1.06	-1.09	1.52	.19988E+01	.20051E+01
50	-1.17	-1.08	1.59	.19804E+01	.19839E+01
51	-1.27	-1.06	1.66	.19428E+01	.19435E+01
52	-1.37	-1.03	1.72	.18863E+01	.18844E+01
53	-1.47	-.99	1.77	.18116E+01	.18072E+01
54	-1.57	-.94	1.83	.17193E+01	.17127E+01
55	-1.66	-.88	1.88	.16103E+01	.16021E+01
56	-1.74	-.81	1.92	.14857E+01	.14763E+01
57	-1.82	-.74	1.96	.13467E+01	.13367E+01
58	-1.89	-.65	2.00	.11947E+01	.11847E+01
59	-1.95	-.56	2.03	.10312E+01	.10216E+01
60	-2.00	-.47	2.05	.85762E+00	.84901E+00
61	-2.04	-.37	2.07	.67583E+00	.66862E+00
62	-2.07	-.27	2.09	.48743E+00	.48207E+00
63	-2.09	-.16	2.10	.29438E+00	.29110E+00
64	-2.10	-.05	2.10	.98435E-01	.97670E-01

5. Conclusion

A direct boundary element method has been applied for the calculation of inviscid compressible flow past a symmetric aerofoil with constant element approach. The calculated flow velocities obtained using this method is compared with the analytical solutions for flow over the boundary of a symmetric aerofoil. It is found that from graphs 1 to 4, the computed results obtained by this method are good in agreement with the analytical ones for the body under consideration and the accuracy of the result increases due to increase of number of boundary elements.

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Serum, Urinary and Tissue Monocyte Chemoattractant Protein 1 in Patients with Lupus Nephritis (A Comparative Study)

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Abstract: **Background*:*** Lupus Nephritis (LN) is one of the most common complications and is considered a crucial determinant of poor prognosis in Systemic Lupus Erythematosus (SLE) patients. Yet it is still a challenge for scientists to establish a sensitive and specific investigations that reflect renal status and can be linked to disease outcome and most importantly easy follow up with less hassle for the patient. ***Aim of the work:*** This study was done to estimate the serum and urinary Monocyte chemoattractant protein 1 (MCP-1) levels as non invasive markers in patients with SLE with comparison to tissue MCP1 and to evaluate the role of MCP-1 as an indicator for SLE disease activity and renal involvement (lupus nephritis).***Patients and methods****:* Serum and urinary MCP-1 were determined in forty randomly selected adult SLE patients their ages in years ranged from 17-54 (27.7± 7.9 years), the control group included twenty age and sex matched volunteers. SLE Disease Activity score (SLEDAI and the Systemic Lupus International Collaborating Clinics (SLICC) Damage Index was recorded in all SLE patients. All patients were subjected to clinical and routine lab investigations. Serum and Urinary MCP1 were evaluated by ELISA technique. Renal biopsy was performed in Lupus nephritis patients for Histopathological classification, Activity and Chronicity indices and immunohistochemistry for MCP1 protein expression. ***Results:*** There was significant difference in level of urinary MCP 1 only in active than in inactive patients. In SLE with LN, serum and urinary MCP 1 showed a highly significant positive correlation with SLEDAI, proteinuria and serum creatinine and significant negative correlations with Hemoglobin. Urinary MCP1 showed highly significant difference between LN (class III&IV) and other classes of LN (p<0.001). Glomerular and tubulointerstitial MCP1 protein expression showed significant positive correlation with proteinuria (p=0.046 and 0.002 respectively).Tubulointerstitial MCP-1 protein expression showed significant difference between LN(class I, II, V) cases versus LN (class III, IV) cases (p=0.008) . Glomerular MCP1 showed highly significant positive correlation with activity index, while Tubulointerstitial MCP1 showed highly significant positive correlation with chronicity index (p <0.001). Urinary MCP1 showed positive significant correlation with both glomerular and tubulointerstitial MCP1 protein expression(p <0.001 and 0.016 respectively). Urinary MCP1 showed highly significant correlation with activity index (p <0.001), while Serum MCP1 showed no significant correlation with activity or chronicity indices**.** ***Conclusion:*** MCP1 could be a valuable marker for LN and can help in assessment of disease outcome and follow up of patients, furthermore, Urinary MCP1 in our study proved to be a sensitive , non invasive tool for assessment of LN patients that can be linked to Histopathological classes and tissue MCP1 protein expression.

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1. Introduction

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disorder that may affect multiple organ systems. Renal damage is one of the most serious complications of SLE. The majority of people with lupus have some degree of asymptomatic microscopic kidney damage. Renal involvement occurs in 40% to 70% of all patients[(1)].

While autoantibody production and complement activation are the major players in initiating the inflammatory response in lupus nephritis (LN), cellular immune mechanisms mediated through infiltrating mononuclear cells have an important role in progression of renal injury[(2)].

Chemotactic factors, such as monocyte chemoattractant protein-1 (MCP-1), appear to play a pivotal role in leucocyte entry into the kidney, enhancing endothelial and leucocyte adhesiveness and endothelial permeability in murine and human LN. This has been proven by previous immunohistochemical and *in situ* hybridization analyses of renal tissue from patients (or experimental animals) that have demonstrated local renal expression of chemotactic factors in association with inflammatory disease[(3)].

All types of renal cells (endothelial, mesangial, tubular epithelial, interstitial cells, and podocytes) can express chemokines upon stimulation. Proinflammatory stimuli, such as TNF-α, IL-1, IFN-γ

and lipopolysaccharide (LPS), within a few hours induce MCP-1, this may represent a common mechanism of injury-induced chemokine generation[4].

There is an increasing body of evidence that MCP-1 plays a major role in the pathogenesis of progression of renal disease. This is based on observations both in various animal models of renal damage and in different types of human renal disease[5].

The presence of chemokines in the urine of patients with SLE nephritis may reflect intrarenal chemokine expression[6].

This study was designed to estimate the serum and urinary MCP-1 levels as noninvasive markers in patients with SLE with comparison to tissue MCP1 protein expression and to evaluate the role of MCP-1 as an indicator for SLE disease activity and renal involvement (lupus nephritis).

2. Patients and methods

2.1 Study Subjects and Design

The current study was observational cross sectional study. Sample was estimated to be forty adult SLE patients (37 women and 3 men), collected by systematic random method. All patients were collected from Rheumatology & Rehabilitation department in Zagazig University Hospitals, in the period from October 2010 to November 2011.All investigations were done in Clinical Pathology and Pathology Departments in Zagazig University. Patients ages ranged from 17 and 54 years of mean ± SD (27.7± 7.9years), disease duration ranged between 0.5 and 14 years of mean ± SD/ (3.6± 3.0 years). Twenty (18 females and 2 males) apparently healthy volunteers were included as controls, they were age and sex matched with the patients, their ages ranged from 20 and 50 years of mean ± SD /years was (26.7 ± 8.0 years).

SLE patients fulfilled at least four of recent SLE criteria described by **Petri , [7].** SLE Disease Activity was based on the SLEDAI score amended in 2000[8]**),** patients with a score ≤ 4 were considered inactive while those with a score > 4 were considered active. The Systemic Lupus International Collaborating Clinics (SLICC) Damage Index, which has been endorsed by the American College of Rheumatology, was also used[9]**.**

Twenty out of the 40 patients were diagnosed as lupus nephritis according to SLE criteria: proteinuria ≥ 500 mg/day and/or red cell casts[7]. The diagnosis of renal involvement was confirmed in them by renal biopsy.

Lupus treatment at the time of serum sampling involved low-dose prednisolone (<0.5 mg/kg/day) in thirteen patients, high-dose prednisolone (≥ 0.5 mg/kg/ day) in twenty seven patients, intermittent intravenous cyclophosphamide in eighteen patients, oral azathioprine in twenty one patients.

Individuals with urinary tract infection were excluded by doing urine culture for all those with pyuria.

Ethical consideration: A written consent was taken from all of the participants after explaining details, benefits as well as risks to them.

2.2. Laboratory procedures

Laboratory investigations were done for all subjects including:

1- Complete blood count (CBC), Erythrocyte sedimentation rate (ESR), Complete Urine analysis together with quantitative 24 hours urinary protein excretion.
2- Complement C-3 (turbidimetric assay).
3- Antinuclear antibody (ANA) and Anti-dsDNA Ab were done by the indirect immunoflorescence technique.
4- **Monocyte chemoattractant protein 1 by ELISA technique :** Estimated in Urine and Serum.

It was done by Quantikine kit for Human MCP-1/CCL2 immunoassay (R&D Systems, Inc., USA) according to manufacturer description.

After the procedure The optical density was determined using ELISA reader (Teco-96 microplate reader, USA) set to 450 nm. To correct for optical imperfections in the plate, wavelength correction was set to 630 nm.

According to the manufacturer samples taken from healthy volunteers MCP1 in serum ranged from 200 to 722 pg/mL and in Urine from 42 to 410 pg/mL. In our study urine values were normalized for creatinine content by division of value of urine MCP-1 by pg/mL on value of urine creatinine by mg/dL.

5- Renal biopsy:

a) Histopathology: percutaneous renal biopsies taken only from lupus nephritis patients (n = 20) were evaluated according to the World Health Organization (WHO) classification of lupus nephritis[10]. The activity index (AI) and chronicity index (CI) of each biopsy specimen were scored by standard methods[11]**.** The distribution of histopathological classification of patients in this study was: class I, 1 (5%); class II, 4 (20%); class III, 7 (35%); class IV, 6 (30%); class V, 2 (10%) cases. The mean values for activity and chronicity indices of class III and IV were 6.9 ± 5.7 and 4.0 ± 3.1 respectively.

b) Immunohistochemistry:

Immunohistochemical staining was carried out using streptoavidin-biotin immunoperoxidase technique (Dako-cytomation, CA). Three to five micrometer thick sections, cut from formalin fixed

paraffin embedded blocks, were deparaffinized in Xylene and rehydrated in graded alcohol. Sections were boiled in citrate buffer (pH 6.0) for 20 min and then washed in phosphate buffer saline (pH 7.3). Then blocking of endogenous peroxidase activity by 6% H_2O_2 in methanol was attained. The slides were then incubated over night with the monoclonal anti-MCP-1 antibody (R&D systems, Oxon, UK). negative controls, obtained by substitution of primary antibodies by blocking buffer were included in the staining procedure. Incubation with secondary antibody and product visualization was performed employing (Dako Cytomation, Glostrup, Denmark) method with Diaminobenzidine (DAB) substrate chromogen. Slides were finally counterstained with Mayer's haematoxylin. Glomerular and tubular staining of biopsies were analysed using a modified histopathology score (H-score of 100–300) based on both percentage of positively stained cells and a semi-quantitative scale of immunointensity (0 = negative, 1 = mild, 2= intermediate, 3 = strong), scores ≤ 100 were considered negative[12].

Statistical methods: Data was analyzed using SPSS win statistical package version 15 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation (SD) or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test. Comparison between 3 groups was done using Kruskal-Wallis test. Spearman-rho method was used to test correlation between numerical variables. p-value < 0.05 was considered significant.

3. Results

3.1 Demographic and laboratory data of all studied subjects

Significant difference was detected comparing selected laboratory data of patient groups versus control group ($P < 0.001$). All laboratory data were significant when comparing SLE patients with LN to those without LN except serum MCP1 showed no significant difference between them (Table 1).

Table 1 Comparison between SLE patients with and without lupus nephritis

		Control (No=20)	SLE without LN (No=20)	SLE with LN (No=20)
Sex	Female n.%	18(90)	19 (95)	18 (90)
	Male n.%	2(10)	1 (5)	2 (10)
Age /years Median (Range)		29 (20-54)	26 (17-33)	27 (16-50)
SLEDAI ≤ 4	n.%	-	15*	1
Serum creatinine (mg/dl)		0.8±0.3	0.75 ±0.16	2.1 ±1.6*
Urine creatinine (mg/dl)		106.40 ±52.43	194.1 ±167.7	82.2 ±34.9*
Hemoglobin (g/dl)		12.0 ±1.3	12.4 ±1.5*	8.7 ±1.7
Proteinuria (g/24h)		0.048±0.023	0.271±0.16	2.118 ±1.35*
Serum MCP1 (pg/dl)		237.5±14.6	622±536	673±465
Urinary MCP1 (pg/mg creatinine)		1.2±0.1	2.2 ±2.4	28.5 ±25.0*

Significant when comparing SLE with LN versus SLE without LN
* Significant when $p<0.05$

3.2 Comparison between SLE patients as regard SLEDAI

SLEDAI recorded significantly higher scores in active than inactive patients. There was significant difference in level of urinary MCP 1 only in active than in inactive patients (Table 2).

Table 2. Clinical and laboratory parameters in SLE patients according to SLEDAI

	Inactive SLE (n = 16) Score ≤ 4	Active SLE (n = 24) Score >4	*P*
Serum MCP1(pg/dl)	709.75 + 460.49	871.46 + 439.44	0.255
Urine MCP1(pg/mg)	1.57 + 1.35	24.51 + 24.48*	0.001
SLEDAI	3.09 ±1.0	14.26 ±4.21*	<0.01
SLICC/ACR	4.1±1.0	4.4±0.7	>0.05

* Significant when $p<0.05$
* Significant difference comparing active versus inactive SLE patients
SLEDAI :Systemic Lupus Erythematosus Activity Index
SLICC: The Systemic Lupus International Collaborating Clinics Damage Index

3.3 Correlation between serum and urinary MCP 1 and different laboratory and clinical parameters

Urinary MCP 1 showed a significant positive correlation with proteinuria in SLE patients with and without LN but there was no correlation between serum MCP and proteinuria in both groups.

In SLE with LN, serum and urinary MCP 1 showed a highly significant positive correlation with SLEDAI, proteinuria and serum creatinine ($P < 0.001$), they had significant negative correlations with Hemoglobin ($P < 0.05$) (Tables 3 & 4).

Table 3. Correlation between serum and urinary MCP 1 and different laboratory and clinical parameters in SLE without LN

	Hemoglobin	Serum creatinine	SLEDAI	Proteinuria
Serum MCP 1	-0.092	0.104	-0.115	0.461
Urinary MCP 1	-0.013	0.547*	0.249*	0.565*

* Significant when $p<0.05$

Table 4. Correlation between serum and urinary MCP 1 and different laboratory and clinical parameters in SLE with LN

	Hemoglobin	Serum creatinine	SLEDAI	Proteinuria
Serum MCP 1	-0.441*	0.376	-0.191	0.489
Urinary MCP 1	-0.613*	0.532*	0.587*	0.666*

* Significant when $p<0.05$

3.4 Comparison between class I, II, V cases and class III, IV cases as regards (tissue, urinary and serum) MCP1 and different lab paremeters.

Glomerular MCP-1 protein expression showed near significant difference (P-value = 0.157) between class I, II, V cases versus class III, IV cases (mean value 103.1 ± 3.6 and 157.5 ± 54.9 respectively). Meanwhile tubulointerstitial MCP-1 protein expression showed significant difference (P-value = 0.008) between class I, II, V cases and class III, IV cases (mean value 102.6 ± 4.4 and 155.2 ± 43.9 respectively) (Fig. 1).

As regards urinary MCP1 and serum MCP1, only Urinary MCP1 showed highly significant difference between class III&IV and other classes of LN ($p<0.001$).

3.5 Correlation between MCP-1 protein expression in tissues and clinicopathological features in SLE patients with lupus nephritis.

Both Glomerular and tubulointerstitial MCP1 protein expression showed significant positive correlation with proteinuria(p=0.046 and 0.002 respectively). (Table 5)

Table 5. Correlation between MCP-1 protein expression in tissues and Lab parameters:

	Glomerular MCP-1 score	Tubulointerstitial MCP-1score
Creatinine (mg/dl)	0.287	0.241
Proteinuria (g/24h)	0.452*	0.656*

Values presented are correlation coefficient "r"

* Significant when $p<0.05$

**Highly significant when $p<0.001$

3.6 Correlation between MCP-1 protein expression and urinary and serum MCP-1 level in cases of SLE patients with lupus nephritis:

Urinary MCP1 showed positive significant correlation with both glomerular and tubulointerstitial MCP1 protein expression (p <0.001 and 0.016 respectively) (Table 6).

Table 6. Correlation between MCP-1 protein expression and urinary and serum MCP-1 level:

	Glomerular MCP-1 score	Tubulointerstitial MCP-1score
Urine MCP-1	0.802**	0.530*
Serum MCP-1	0.189	-0.054

* Significant when $p<0.05$

**Highly significant when $p<0.001$

3.7 Correlation between urinary MCP1 and serum MCP1 and both activity and chronicity indices in lupus nephritis patients in ISN/RPS class III and IV together.

Urinary MCP1 showed highly significant correlation with activity index (p <0.001), while it shoed no significant correlation with chronicity index. Serum MCP1 showed no significant correlation with activity or chronicity indices. Glomerular MCP1 protein expression showed highly significant positive correlation (p <0.001) with activity index , while Tubulointerstitial MCP1 immunoreactivity showed highly significant positive correlation (p <0.001) with chronicity index. (Table 7)

Table 7. Correlation between urinary MCP1 and serum MCP1 and both activity and chronicity indices in lupus nephritis patients in ISN/RPS class III and IV together.

	Urine MCP1	Serum MCP1	Glomerular MCP1	Tubulointrerstitial MCP1
Activity index	0.953**	0.536	**0.966****	0.181
Chronicity index	0.155	0.330	0.108	0.980**

* Significant when p<0.05

**Highly significant when p<0.001

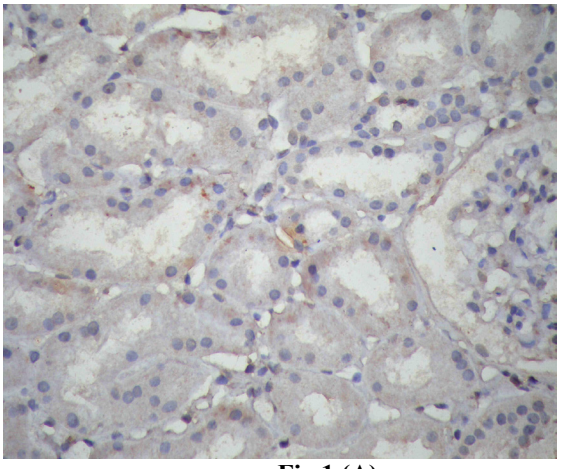

Fig.1 (A)

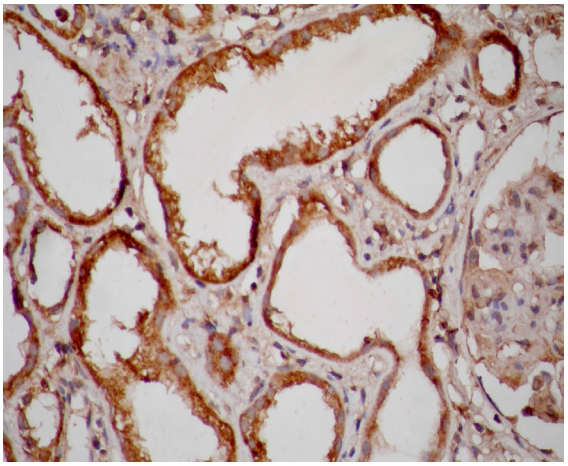

Fig.1 (B)

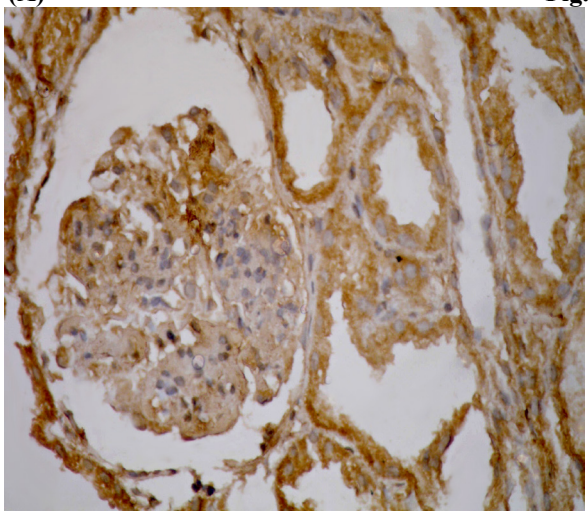

Fig.1 (C)

Fig.1: A- Faint (negative) cytoplasmic MCP-1 immunoreactivity in glomerular & tubulointerstitial tissue (original magnification x 400); **B-** moderate glomerular and strong tubulointerstitisal MCP-1 immunoreactivity (original magnification x 400); **C-** strong glomerular and moderate tubulointerstitisal MCP-1 immunoreactivity (original magnification x 400).

4. Discussion

Renal involvement is one of the main determinants of poor prognosis of SLE [13] Cellular immune mechanisms mediated through infiltrating mononuclear cells have an important role in amplification and progression of renal injury[1]. The disease course of LN is highly variable and multiple clinical, serological, histopathological and time dependent factors are responsible for its ultimate prognosis[14]. Novel biomarkers must be judged against an activity measurement that is superior to kidney biopsy[15].

In the present study, we evaluated urine and serum MCP1 as noninvasive markers and we tested them against tissue MCP1 protein expression in relation to different lab parameters, and we compared different histopathological classes as regards all types of MCP1. Moreover correlation studies were used to find the association between MCP1 and activity or chronicity indices in an attempt to find out different types of MCP1 association with disease outcome.

We found out that serum MCP-1 level was higher in SLE patients than controls. No statistically significant difference was found between SLE Patients with and without LN. This is in agreement with the results also obtained by **Li *et al*.**[16], they found that the serum MCP-1 levels were very high in many patients with SLE when compared to controls.

This was also in agreement with the results obtained by other researchers[17] who found that in SLE Patients with renal involvement the mean value of serum MCP-1 was significantly higher than healthy subjects, no statistically significant difference between SLE patients with and without renal affection.

A previous study[18] concluded that MCP-1 levels in sera of both active phase and remission phase of LN patients were markedly higher than those in controls and there no significant difference was found between the patients of active and remission phase.

We found that the mean value of urine MCP-1 in SLE patients with LN was significantly higher than those patients without LN (p<0.001) and also significantly higher than controls (p<0.001). There was no statistically significant difference between SLE patients without LN and controls (P = 0.840); this is in agreement with the results obtained by other studies[19], [20].

Li and colleagues[18] agreed with us that the MCP-1 levels in urine of active phase patients were markedly higher than those in controls, but no significant difference was found between the MCP-1 levels in urine between the remission phase patients and control.

It was found that urinary MCP-1 of patients with renal flare was significantly higher than that of healthy control subjects and higher than SLE patients with non-renal flare[21,22] and this also was similar to our study.

There is a general agreement in different literatures that the more active classes of biopsy proven LN are class III and IV, while other classes, namely class I, II, V and VI are considered less active that needs limited immunosuppressive therapy [23].

In the present study, Urinary but not serum MCP 1 levels were significantly higher in SLE patients with lupus nephritis (biopsy class III and IV together) than (biopsy class II and V together).

Tucci *et al.*[6] found that urinary levels of MCP-1 were markedly elevated in patients with LN and were correlated with the histologic class of nephritis, and they stated that elevation of urinary MCP-1 levels in patients with active LN, along with a decrease in urinary MCP-1 levels after successful treatment. This may suggest that MCP-1 overproduction is due to an inherent defect in patients with LN. Further support of this hypothesis may be the strong influence of the MCP-1 genotype on urinary MCP-1 levels, and they emphasized that ,as evidence against a constitutive up-regulation of MCP-1 in patients with LN, they found a marked decrease in urinary MCP-1 levels during treatment with cyclophosphamide.

We agreed with previous studies [24,25] that the Level of UMCP-1 was significantly correlated with total SLEDAI score however there was no correlation between serum MCP-1 and this score,

Urinary MCP 1 showed a significant positive correlation with proteinuria in SLE Patients with and without LN, but there was no correlation between serum MCP and proteinuria in both groups. This was in agreement with other researchers[4,24] who found that UMCP-1 correlated with the extent of proteinuria. Some patients had persistently elevated UMCP-1 despite improvement in proteinuria, suggesting the possibility of ongoing subclinical inflammation in the kidneys[26].

In this study there was a positive correlation between UMCP-1 and serum creatinine in both groups of SLE patients, this was similar to study of **Tucci *et al.***[6] who found that there was negative significant correlation between both serum and Urinary MCP-1 and hemoglobin in SLE patients with and without LN, also this is in agreement with the results obtained by **other researchers**[21]. This suggests that measurement of urinary chemokines may be a noninvasive method for the assessment of the severity of lupus nephritis[4].

Our study demonstrated increased urinary MCP-1 levels in patients with Lupus nephritis compared to Lupus non-nephritis patients. Urinary MCP-1 levels correlated with SLE disease activity. There were no differences in serum MCP-1 levels between those patients with and without LN. With the

measurement of urinary levels of MCP-1, it shows a positive correlation with proteinuria and thus being a useful tool for the detection and management of LN.

It was explained in previous researches[27] that The lack of significant increase of circulating levels of MCP-1 in serum of patients with nephritis is due to the possibility that locally produced MCP-1 is excreted into urine rather than circulated in the blood, and to the extremely short half-life of MCP-1 in serum .Furthermore **Li *et al.*[16]** stated that urinary MCP-1 levels reflect predominantly local production of this chemokine rather than simply filtration of MCP-1.

In our study renal biopsy was obtained from SLE patients with lupus nephritis and MCP1 protein expression in tissues was evaluated in relation to different lab parameters, urinary and serum MCP1.

No relationship was noted between serum creatinine and either glomerular or tubulointerstitial MCP-1 protein expression. **Marks and colleagues[28]** found no relationship between glomerular MCP-1 and serum creatinine.

A significant relationship was noted between the level of proteinuria and both glomerular and tubulointerstitial MCP-1 immunoreactivity.These findings were similar to those from a previous study[28] that showed a significant relationship between protein level in urine and glomerular MCP-1 . proteinuria stimulates renal tubular epithelial cells to produce cytokines such as MCP-1 that can contribute to chronic kidney damage[29]**.** However other studies reported no association between the degree of proteinuria and MCP-1 expression[30,3]**.** This discrepancy may be because all their cases had mild proteinuria < 3.5 gm/ day, however, sever proteinuria may be associated with tubulointerstitial damage.

There was a paucity of glomerular expression of MCP-1 in ISN/RPS class I, II and V. however, patients with class III and IV lupus nephritis had over-expression of glomerular MCP-1 protein with near significant difference compared to other classes (P = 0.157). This is in agreement with **Marks *et al*.[28]** who showed significant difference in MCP-1 expression between classes III & IV versus classes I, II & V

In the present study , there was a highly significant correlation between Activity index of lupus nephritis patients ISN/RPS classes III , IV and glomerular MCP-1 protein. This is in agreement with previous studies[3,28] who reported a significant association between histopathological activity of lupus nephritis and glomerular MCP-1 protein expression. These findings are consistent with previous findings showing increased MCP-1 staining in glomerular and interstitial cells in human crescentic glomerulonephritis[31]**.**

Regarding the correlation between tubulointerstitial MCP1 protein expression and Chronicity index of lupus nephritis patients ISN/RPS classes III , IV, a highly significant correlation was found in our study.

Consistent with our findings, **Chan *et al.*[3]** found significant correlation between chronic renal damage represented by histopathological chronicity index and tubulointerstitial expression of MCP-1 (P = 0.015), which is consistent with the current understanding of pathophysiology of chronic kidney disease[32]**.** Also **Dai *et al.*[30]** reported tubular of MCP-1 was strongly associated with monocyte infiltration and fibrosis in interstitium of lupus nephritis patients, suggesting that upregulation of tubular MCP-1 may initiate the process of monocyte recruitment and thus leads to interstitial fibrosis. **Wada and Furuichi[33]** suggested that MCP-1 expression contributes to interstitial fibrosis in human crescentic glomerulonephritis.

There was a highly significant positive correlation between activity index and urinary MCP1 while it showed no significant correlation with Serum MCP1. Chronicity index didn't correlate with any of them. Similarly, **Zhu *et al.*** (34) reported a positive correlation between urinary MCP1 and histopathological activity index, but not chronicity index.

A significant positive correlation was found between urinary MCP-1 and tissue MCP-1 protein expression, whether glomerular or tubulointerstitial, while another study[31] reported no association between urinary and tubulointerstitial MCP-1, but strong association between urinary MCP-1 and glomerular macrophage infiltration. No association was found between serum MCP-1 and neither glomerular nor tubulointerstitial MCP-1. This is in agreement with study done by **Dai *et al*.[30]**

Therefore, MCP-1 may have a role in the etiopathogenesis of LN and could be utilized as a potential biomarker of disease activity. therapeutic strategies with MCP-1 antagonists to ameliorate the initiation and progression of disease would be beneficial as future possible treatments. This has been demonstrated in murine (MRL lpr mice) LN [35]. In addition, anti-MCP-1 gene therapy in murine LN offers protection against renal injury due to reduced infiltration of leucocytes by significantly reducing glomerular IL-12 mRNA production and interstitium-infiltrating cell production of IL-12 and IFN-gamma mRNA [36].

Conclusion

MCP1 could be a valuable additional tool to diagnose LN and will certainly help us to suspect LN, diagnose it earlier and monitor nephritis activity in the follow up. Urinary and not serum MCP1 is a useful

non-invasive marker for the assessment of renal disease in patients with lupus nephritis.

Disclosure of Interest: None declared

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Methylenetetrahydrofolate Reductase (Mthfr C677t) Gene Polymorphism Effect on Development of Diabetic Nephropathy in Egyptien Patients with Type 2 Diabetes Mellitus

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Abstract: Introduction: Genetic predisposition has been implicated in diabetic nephropathy (DN). Methylenetetrahydrofolate reductase (MTHFR) is a regulatory enzyme of homocysteine metabolism. The C677T variant of the methylenetetrahydrofolate reductase (MTHFR) gene may play a role in the development of not only vascular disease but also diabetic microangiopathies. In this study, we examined the distribution of the MTHFR genotypes and the association between the C677T variant and diabetic nephropathy. METHODS: 50 type 2 diabetes mellitus patients classified into 2 groups according to presence or absence of nephropathy as measured by urinary albumin /creatinine ratio into 2 groups, 27 patients without nephropathy and 23 with nephropathy and 20 controls were recruited in the study. Fasting blood glucose, HbA1C and serum creatinine were measured. Plasma total homocysteine level was measured using chemilumenecent assay. MTHFR genetic C677T polymorphism was determined with PCR-restriction fragment length polymorphisms (RFLP). RESULTS: The frequency of MTHFR TT genotype and CT heterogenetic type and allele T(30.4%, 43.5%, 52%) was significantly higher in type 2 diabetes mellitus with diabetic nephropathy group than those without nephropathy (7.4%, 25.9%, 20%) or normal controls (10%, 25%, 22%). However, there was no significant difference of MTHFR genotype and allele frequency between type 2 diabetes mellitus without nephropathy and normal controls (χ^2 0.1, p value < 0.05). The presence of T allele appeared to have a stronger association with the development of diabetic nephropathy. The odds ratio was 5.7 and the 95% confidence interval was 1.7-19.3. Moreover, plasma homocysteine levels were markedly higher in patients with TT or CT genotype than those in patients with CC genotype. CONCLUSIONS: Our findings suggest that the C677T mutation in the MTHFR gene predisposes type 2 diabetes patients to the development of diabetic nephropathy. The T allele of this mutation presumably acting by elevating homocysteine levels and seems to be associated with a faster progression of nephropathy to end-stage renal failure.
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Keywords: Diabetic nephropathy, MTHFR, C677T Polymorphism, Homocysteine, Type 2 diabetes mellitus.

1. Introduction:

Diabetic nephropathy (DN) is a serious complication of type 2 diabetes (T2DM), and is the primary cause of end-stage renal failure [1,2]. The etiology of DN is multifactorial and involves both environmental and genetic factors [3,4]. Strong genetic predisposition for nephropathy in type 2 diabetes mellitus is suggested by familial clustering [5].

Homocysteine is a thiol-containing amino acid derived from methionine. It can be catabolized to cystathionine and cysteine by the action of cystathionine b-synthase, in the presence of vitamin B6. An alternative metabolic pathway consists of a remethylation process, primarily by transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine in the presence of vitamin B12, and subsequent formation of methionine [6] This pathway is regulated by the activity of 5,10-methylenetetrahydrofolate reductase (MTHFR), which, in the presence of folate, catalyzes the formation of 5-methyltetrahydrofolate [7].

Elevated homocysteine levels have been identified as a risk factor for diabetic nephropathy in type 2 diabetes [8,9]. In addition, increased plasma homocysteine is an independent risk factor for several vasculopathies including arteriosclerosis, acute myocardial infarction, cerebrovascular diseases, arterial and venous thrombosis [10–13].

The methylenetetrahydrofolate reductase (MTHFR) gene is located on chromosome 1 (1p36.3)[14] . Several mutations within MTHFR gene were reported; the best-characterized being the C677T, a valine-to-alanine substitution at amino acid 226, resulting in a thermo-labile MTHFR variant with reduced catalytic activity. [15,16]. Homozygosity for the mutation (TT genotype) predisposes to significantly elevated plasma homocysteine levels [17,18]. Elevated plasma homocysteine were shown to be associated with predisposition to developing T2DM complications, including diabetic retinopathy [19,20] and diabetic nephropathy (DN) [9-21]. **Noiri *et al.*** [9] reported an increased frequency of the CT and TT

genotypes in male haemodialysed patients with type 2 diabetes as well as a correlation between the presence of the C677T allele and the progression of renal failure. The aim of this study was to evaluate a possible role of the MTHFR C677T polymorphisms in the susceptibility to DN in T2DM patients. The allele and genotype frequencies of the C677T, together with changes in homocysteine levels, were determined for T2DM patients with DN and those without nephropathy, together with non-diabetic control subjects.

2. Subjects and Methods:

The study groups consisted of 50 T2DM patients from the Outpatient Clinic of Zagazig University Hospital diagnosed according to American Diabetes Association revised criteria [22]. These were (30 males, 20 females); 20 healthy individuals (13 males, 7 females) served as the control group. On all subjects, demographic details were recorded, which included age; gender; age of onset and duration of disease; history of hypertension, dyslipidaemia, ischaemic heart disease and other medical illness; history of chronic complications of diabetes, treatment for diabetes including date of initiation and/or discontinuation of oral agents or insulin and history of other medication. Patients with serum creatinine > 2 mg/dL, patients with blood pressure above 130/90, and those taking vitamins or folic acid containing preparation were excluded from the study. The study was approved by the institution Ethics Review Board, and written informed consent was obtained from all participants.

The patients group was classified according to presence of nephropathy into two groups 27 without DN and 23 with DN as assessed by microalbuminuria in random urine samples. Microalbuminuria is diagnosed if albumin/creatinine ratio (ACR) ranges between 30 and 300 mg albumin/g creatinine [23]. Random urine samples tested for microalbuminuria with measuring ACR. Albumin concentration in urine was measured using an immunoturbidometric assay on a Prospec nephlometry with Albumin-Urine kits (Dade Behring).

Fasting venous blood samples were collected; serum was separated for measuring fasting glucose and creatinine, another vacutainer EDTA containing tubes were used for samples collection for homocysteine and HbA1C. Urine, serum creatinine and fasting glucose concentrations were measured on ADVIA 1650 analyzer (Siemens Medical Solutions Diagnostics), glycemic control was assessed by measuring HbA1c using column chromatography (BioSystems, Middletown, CT, USA).

Homocysteine, was determined by chemiluminescent assay using commercial kits on Immulite ®, DPC US. A fasting homocysteine concentration above 15 µmol/L is the most common definition of hyperhomocysteinemia [24].

MTHFR C677T genotype:

Genomic DNA was extracted from peripheral blood leukocytes Buffy coat samples using the E.Z.N.A. ™ Blood DNA Kit (Omega – biotek. Inc). Genomic DNA samples were stored at -20°C until genotyping analysis. MTHFR C677T genotype analysis was performed by PCR-RFLP analysis using HinfI (15) digestion for C677T. The primer sequences for C677T were: forward, 5′-TGA AGG AGA AGG TGT CTG GGG GA-3′, and reverse,5′-AGG ACG GTG CGG TGA GAG TG-3′. The C677T mutation introduces a new HinfI restriction site which results in the digestion of the 198 bp amplicon into 175 and 23 bp fragments.

The PCR mixture contained 1µmol/L of each primer, two units of Taq polymerase, 25 mmol/L MgCl2, 0.2 mmol/L of each dNTP and 1 µg of DNA template in a final volume of 50 µL. The amplication was carried out in a PCR thermal cycler ,the cycling parameters were 5 min at 95°C followed by 35 cycles of 45 s at 95°C ,1 min at 55°C ,and 45 s at 72°C followed by a single 10-min extension at 72°C .

The 198-bp PCR product (10 µl) was digested with the restriction enzyme HinfI[17] at 37° C for 3–4 h in the buffer recommended by the manufacturer. HinfI can recognize the C to T substitution in the fragments. This one nucleotide substitute corresponds to a conversion of Ala-to-Val residue in the MTHFR encoding region. Twenty µL of each reaction mixture was separated on agarose gel 3% and stained with ethidium bromide and visualized under UV illumination.

The two different alleles were designated T (Val) and C (Ala). The 198-bp fragment derived from the C allele is not digested by HinfI, whereas the fragments of the same length from the T allele are digested by HinfI into 175- and 23-bp fragments. Subjects homozygous for the mutation showed two DNA fragments of 175- and 23-bp, whereas homozygous subjects without it showed a DNA fragment of 198-bp. Heterozygous subjects showed three DNA fragments of 198-, 175- and 23-bp.

Statistical analysis

Data were expressed as mean ± SD for quantitative variables, number and percentage for qualitative ones. ANOVA, t test, χ^2, and Pearson correlation were used for analysis of results .Relative risk (RR) and 95% confidence interval (CI) were performed to predict the effect of MTHFR C677T genotypes on development of DN. A *P*-value < 0.05 was considered significant. Analysis was performed

with the SPSS statistical package version 10 (SPSS Inc., Chicago, IL).

3. Results:

The demographic characteristics of the studied groups are summarized in Table 1. There was none significant differences between studied groups as regard age, sex or duration of the disease. As regard fasting blood glucose, diabetic control as measured by HbA1c and serum creatinine there was none significant difference between 2 groups of patients with or without nephropathy.

Genotype and allele frequencies were compared between diabetic patients and controls (Table 2). Although there were a higher percentage of T allele carriers among diabetic patients than control the difference was none statistically different. There was a highly significant difference of MTHFR C677T genotype between patients with or without DN (χ^2 16.8, *p* value < 0.001) (Table 3). The frequency of 677T allele was significantly higher among patients with DN, and higher frequency of C/T (RR = 4.6, CI 1.3-15.5%) and T/T (RR = 5.7, CI 1.1-30.8) genotypes.

Plasma homocysteine levels were significantly higher in patients with nephropathy than in patients without nephropathy or control subjects (Table 1). There was an association between MTHFR C677T genotype and homocysteine levels; significantly elevated homocysteine was noted in 677T/T carriers in the three groups of study subjects, as opposed to C/T or C/C genotype carriers (Table 5). The effect of MTHFR C677T polymorphism on plasma homocysteine levels was evident not only in diabetic patients but also in healthy controls (Table4).

Our results showed none significant correlation between glycemic control (HbA1C) and homocystein levels among diabetic patients (r 0.08, *P*>0.05) while there was significant correlation between plasma homocystein and serum creatinine (r 0.47 *p*< 0.01).

Table (1): Profile of T2DM patients and control subjects

Characteristics	Control (n=20)	Type 2DM without nephropathy (n=27)	Type 2DM with nephropathy (n=23)	*P* value
Age (years)	54.6±10	52.4±9	58.4±8	> 0.05
Sex male(%)	13 (65)	15 (55.6)	14(61)	> 0.05
Duration of diseases (years)	N/A	8±2.1	8.9±2	> 0.05
Fasting glucose (mg/dl)	91.2±8.4	285±78.6[a]	228.5±84.1[a]	<0.05
Hb A1C (%)	4.8±0.8	8.1±1.2 [a]	8.4±2.1 [a]	<0.05
Serum creatinine(mg/dl)	0.6±0.13	0.7±0.18	0.78±0.15	>0.05
Homocysteine (µmol/L)	10.1±2.8	12.9±4.9[ab]	19.6±5.8[ab]	<0.001

a: significant difference between T2DM patients and control subjects
b: significant difference between T2DM patients with vs. patients without nephropathy.

Table (2): Genotype distribution and allele frequency of MTHFR C677T among studied groups

MTHFR C677T Genotypes	Control subjects (n=20), No (%)	Type 2DM (n=50), No (%)	χ^2	*P* value
CC	13 (65)	24(48)	1.7	>0.05
CT	5 (25)	17(34)		
TT	2(10)	9(18)		
C allele	0.78	0.65		
T allele	0.22	0.35		

Table (3): Genotype distribution and allele frequency of MTHFR C677T among patients groups

MTHFR C677T Genotypes	Type 2DM without nephropathy (n=27), No (%)	Type 2DM with nephropathy (n=23), No (%)	χ^2	*P* value
CC	18 (66.7)	6(26.1)	9.0	<0.05
CT	7 (25.9)	10(43.5)		
TT	2(7.4)	7(30.4)		
C allele	0.80	0.48		
T allele	0.20	0.52		

Table (4): Relationships between MTHFR C677T genotypes and homocystein activity (µmol/L) in the studied groups.

MTHFR C677T Genotypes	Control (n=20)		Type 2DM patients (n=50)	
	N (%)	Homocysteine levels	N (%)	Homocysteine levels
CC	13(65)	8.7±1.8[a]	24(48)	11.4±3.4[a]
CT	5(25)	11.4±2.2[a]	17(34)	17.3±4.7[a]
TT	2(10)	15.5±0.7[a]	9(18)	24.4±4.4[a]
Test of significance	F = 13.1		F = 34.2	
***P* value**	< 0.01		< 0.001	

a: Significant difference with other genoytpes

Table (5): Relationships between MTHFR C677T genotypes and homocystein activity (µmol/L) in the patients groups.

MTHFR C677T Genotypes	Type 2DM without nephropathy		Type 2DM with nephropathy	
	N (%)	Homocysteine levels	N (%)	Homocysteine levels
CC	18(67)	11.2±3.6	6(26.1)	12.0±2.4[b]
CT	7(26)	15.2±5.3[a]	10(43.5)	18.6±4[b]
TT	2(7)	20±7[a]	7(30.4)	25.6±3[b]
Test of significance	F= 5.2		F = 20.3	
***P* value**	< 0.05		<0.001	

a: Significant difference with CC genotype
b: Significant difference with other genoytpes

4. Discussion:

Diabetic nephropathy is the leading cause of chronic kidney disease in patients starting renal replacement therapy [(25)] and is associated with increased cardiovascular mortality [(26)].

Two common polymorphisms have been described in the MTHFR gene, both single nucleotide substitutions resulting in amino acid changes C677T and A1298C [(17, 27)]. Whereas C677T unequivocally affects enzyme function and has been associated with increased plasma homocysteine concentrations and an altered balance of folate metabolites [(17,28)]. The C677T polymorphism may have a greater association with diabetic nephropathy than A1298C because of the localization of these two variants. The C677T polymorphism is in the exon 4, which is within the N-terminal catalytic domain of the enzyme, whereas the A1298C polymorphism is in the exon 7, which is within the C-terminal regulatory domain. The more dynamic effect of C677T is due to its location within the catalytic region [(29)].

The frequencies of homozygous mutated genotype and the mutated allele is higher in diabetic patients than control group. T Allele frequency was 22% in control healthy subjects while it was 37% in diabetic patients and genotype frequency was 65% for CC, 25% for CT and 10% for TT. Our results were comparable with other studies who performed screening of 114 healthy Chinese people, the allele frequency of the T mutation was 38.0%, comparable to that (33.0%) in a Hong Kong (Chinese) population. The distribution of the three genotypes was as follows: CC genotype 55.3%,; CT genotype, 27.2%; and TT genotype, 17.5% [(30)].In other study done in Tunisia T allele frequency was 22% in healthy subjects ,and more prevalent among T2DM patients, with allele frequencies of 0.36 [(31)].

Genotypes distribution was 44% for CC,38% for CT,18% for TT with none significant difference between two groups ($\chi^2 = 2.5$, $P > 0.05$).This results was similar to other study which showed that the distribution in type 2 diabetic patients in which 44.3% were CC, 34.2% were CT and 21.5% were TT. There were no significant differences in genotype distribution between type 2 diabetic patients and control group ($\chi^2 = 3.67$, $P > 0.05$)[(30)].

T Allele frequency was 20%, 59% in patients with or without nephropathy respectively. These findings indicate that the presence of the C677T polymorphism in the MTHFR gene is of pathophysiological significance, we found that patients with the 677T/677T mutation displayed a frequency of diabetic nephropathy significantly higher than the frequency displayed by those with the 677C/677C or the 677C/677T genotype. This is similar to another study by **Cui *et al.*** who found out that The 677T allele showed significant association with DN (OR = 1.97, 95% CI [1.71, 2.28], p <0.00001), but no relationship with DM (OR = 1.03, 95% CI [0.89, 1.18], p = 0.70) compared with the 677C allele in a Chinese population.[(32)]

Our results showed that risk of nephropathy increased by 6 folds among T/T genotype (RR = 5.7, CI 1.1-30.8) were in contrast to **Maeda *et al.*** [33] who found that the 677T/677T homozygote did not affect the risk for nephropathy (OR=1.17; 95% CI=0.45–3.05). These contrasts are probably due to differences in populations, number of studied cases, or gene–environment interactions.

Our results showed that hyperhomocysteinemia was frequent in T2DM patients as compared with controls, and was also higher than in patients with DN than in patients without DN, with C677T/T carriers having higher plasma homocystein concentration. The molecular mechanism of how MTHFR gene polymorphism promotes microvessel diseases has not been elucidated clearly. One suggestion is that hyperhomocysteinemia resulting from the 677T/677T mutation in the MTHFR gene may lead to the initiation and progression of microvessel diseases through induction of endothelial dysfunction followed by a wide range of pathological reactions [34-36]. Subjects who are heterozygous for the T allele have a 12% increase in homocysteine levels, whereas TT individuals have 30% higher levels, compared to CC genotypes [37].

Another explanations shown that hyperhomocysteinemia may act by inducing the expression of tissue factor (TF), an initiator of blood coagulation *in vivo* [38], by circulating monocytes, which apparently acted independent of peroxide and superoxides, since scavengers of both did not block the expression of homocystein induced TF [39]. Hyperhomocysteinemia may also act by altering endothelial cell function through upregulation of the expression and secretion of MCP-1 and IL-8, which by promoting leukocyte recruitment, may contribute to the initiation and progression of vascular disease [40].

Our results showed none significant difference between glycemic control (HbA1C) and homocystein levels among diabetic patients (r 0.08, $P>0.05$). This in contrast to other studies reported that among diabetics, homocysteine levels may be dependent on the glycaemic control [41]. There was significant correlation between plasma homocystein and serum creatinin (r 0.47 $p< 0.01$), there is a direct metabolic relationship between creatinine and homocysteine. Creatinine originates from metabolism in skeletal muscles, and the amount of released creatinine is therefore determined by muscle mass. The formation of creatine, the precursor of creatinine, depends on a methyl donation by S-adenosylmethionine, leading to the formation of homocysteine. Thus, the level of homocysteine would be expected to reflect both muscle mass and creatinine concentration [42].

We concluded that the 677T/677T mutation in the MTHFR gene could be a predictive marker of the onset of diabetic nephropathy during the initial stage of type 2 diabetes mellitus. Homocystein also has a major role so increased intake of folate and vitamins B6 and B12 can reduce plasma homocysteine levels in patients with diabetic angiopathy further studies in larger number of patients are necessary to establish a role of this interesting polymorphism in the genesis of diabetic nephropathy. It will also be important to study prospectively whether folate supplementation reduces the incidence of DN in type 2 DM in individuals who carry the C677T allele.

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Karyological Analysis of *Schizothorax labiatus* (Teleostei: Cyprinidae), a hill stream food fish of Kashmir Himalaya.

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Abstract: Karyotypic characterization of *Schizothorax labiatus* (Chush Snow trout) was carried out following Thorgaard and Disney (1990). The analysis of 80 metaphase plates revealed the chromosome number of this fish 2n=98 and a fundamental arm number (FN) =142. The diploid complement comprised 12 metacentric pairs, 10 submetacentric pairs, 1 subtelocentric pairs and 26 telocentric pairs (24m+20Sm+2St+52t). Total length of the haploid complement equalled 157.5µm with a range in the length of shortest and longest chromosome between 2-8µm. The arm ratio and the centromeric index ranged between 1-∞ and 0-50 respectively. The present study is the first to describe the chromosomal characteristics of *Schizothorax labiatus* from The Kashmir Valley.
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Key Words: Karyotype, *Schizothorax labiatus*, Kashmir, Chromosome.

1. Introduction

Kashmir Himalayas have a great array of aquatic habitats ranging from high altitude tarns, crystal clear lakes, rural and urban lakes and ponds of different trophic status as well as springs, torrential streams and mighty rivers. All these water bodies contain some kind of fish. The work on fishes of Kashmir started by J. J. Heckel (1838) who for the first time reported about sixteen species of fishes in the valley, all new to science. Since that time many others have worked on the fish and fisheries of the valley. The prominent works in the field have been those of Mukerji (1936), Hora (1936), Silas (1960), Das and Subla (1963, 1964), Qadri *et al*. (1983), Yousuf (1996), Kullander *et al*. (1999) etc.

Fish species of Kashmir belong to *Cyprinidae, Cobitidae, Siluridae, Salmonidae and Poecilidae*, with the first one dominating the aquatic systems of the valley. The fishes of this family are distributed over Africa, Asia, Europe, and North America and live almost exclusively in freshwater. Characteristics of *Cyprinidae* include presence of "Pharyngeal teeth", lack of an adipose fin and the presence of barbels in many species.

Cytogenetic analysis in fish have allowed to determine sex chromosomes (Moreira-Filho *et al*., 1993; Devlin and Nagahama, 2002; Molina and Galetti, 2007), the characterization of vertebrate models, like the zebra fish (Sola and Gornung, 2001), the evaluation of genetically modified lineages (Porto-Foresti *et al*., 2004), and to perform inferences on cytotaxonomic (Bertollo *et al*., 2000; Bertollo *et al.,* 2004) and evolutionary issues (Demirok and Unlu 2001). Karyological studies have also provided basic information on the number, size and morphology of chromosomes (Tan *et al*. 2004) which is important to undertake chromosome manipulation in fish (khan *et al*., 2000). Since 1960 karyological studies in teleost fish have made noteworthy contributions in the field of genetics, taxonomy and environmental toxicology (Cucchi and Baruffaldi, 1990). Chromosomal analysis is important for fish breeding from the view point of genetic control, the rapid production of inbred lines and evolutionary studies (Kirpichnikov, 1981). Genetic divergences of populations and their local adaptations are a potential resource for breeding programs in aquaculture and for fishery management (Phillips and Rab, 2001). The study of karyotype is also important in aquaculture in connection with the use of chromosome manipulation techniques including induction of polyploidy, gynogenesis, androgenesis and inter or intra-specific hybridization (Wu *et al*., 1986; Diter *et al*., 1993). Karyological study can be useful for addressing a variety of evolutionary and genetic questions about animals (Macgregor, 1993) and may permit detection of changes that modified an ancestral karyotype as it evolved into new lines (Winkler *et al*., 2004) and chromosomal analysis is important for genetic control, taxonomy and evolutionary studies (Macgregor and Varly, 1993; Fister *et al*., 1999; Suleyman *et al*., 2004) and is widely use in various investigations (Pisano *et al*., 2007).

Despite these advancements, only a few fishes of Kashmir have been studied for their chromosomes viz. *Schizothorax curvifrons* and *S.plagiostomus* (Farooq *et al*., 2011), *S.esocinus* (Farooq *et al.,* 2011) and *Puntius conchonius* (Ganai and Yousuf,

2011).The present study was undertaken with the aim to investigate chromosomes and karyotype of *S.labiatus* to compare it to other members of the genus and generate information that can be utilized for its management and conservation.

2. Materials and Methods.

Live fish were obtained (8 specimens, all females) from local fishermen in the River Jhelum and transported live to the Limnology and Fisheries Laboratory of Centre of Research for Development University of Kashmir and placed into 50 l fully aerated aquarium for several days. For karyological preparation the protocol of Thorgaard and Disney (1990) was followed. Fish received two doses of phytohemagglutinin (PHA) injections (4µgg^{-1} bw), in a 20-h interval at 20˚c . Fishes were pre-treated by jntraperitoneal injection of colchicine (0.05% @ 1ml/100g bw) eight hours the second dose of PHA to arrest cell division at the metaphase stage and kept alive for 2-3 hours before sacrificing. For the preparation of smears, their cephalic kidney was removed, homogenized and hypotonised simultaneously by potassium chloride 0.56% for 35 minutes at room temperature. Because of their tiny tissues, they were well mixed. Suspensions were spun at 1000 rpm for 10 minutes. Supernatant was discarded and the cells were fixed by cold fresh Carnoy (3:1 methanol and glacial acetic acid) and refrigerated for 30 minutes. This process was repeated three times and smears were prepared on cold lamellae using splash method from 1m height and air dried for 24 h, then stained with 2% Giemsa.

2.1. Chromosomal analysis

Leica DM LS2 trinoccular microscope fitted with a camera and 100x×10x oil immersion lens combination was used to scan the cells and take the photographs. Eighty well spread metaphase complements were obtained for chromosomal analysis. The chromosomes of 5 well spread metaphase complements were individually measured from photomicrographs with precision dial callipers and their centromeric indices and arm ratios were determined in order to ascribe the morphology as suggested by Levan *et al*. (1964). Using chromosomal indicators (Table II) an ideogram (Fig.2) was prepared in MS Excel 2007 software.

Table 1. Percentage frequency of the metaphases (where f % = Frequency % of chromosomes).

Species	No. of chromosomes	No. of cells	f %	modal No.
S.labiatus	94	8	10	**98**
	96	6	7.5	
	98	64	80	
	100	2	2.5	

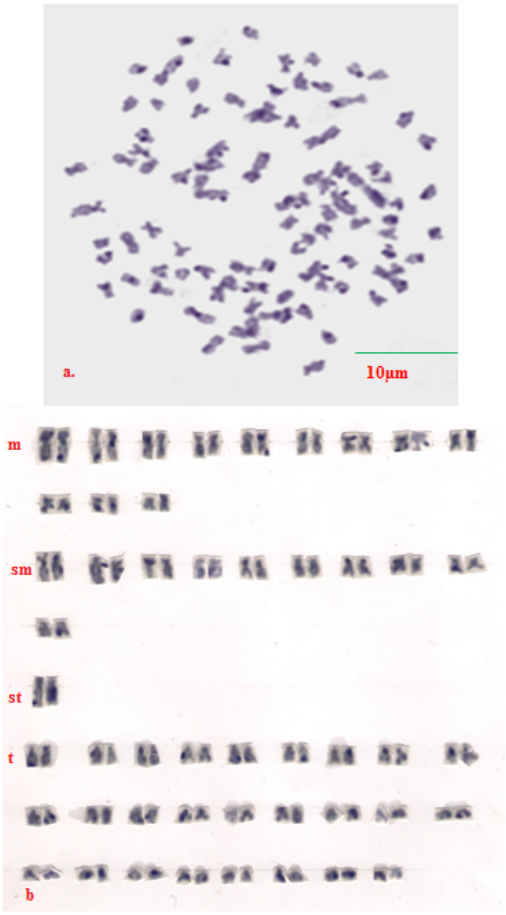

Fig. 1a-b: ***a.*** Chromosome preparation of *Schizothorax labiatus*. ***b.*** Karyotype of *S.labiatus* (m=metacentric;sm=sub-metacentric;st=subtelocentric; t=telocentric).

3. Results

A high number of small chromosomes were observed in *Schizothorax labiatus*. Eighty cells from the anterior kidney tissue were analysed in total. The overwhelming majority (80%) of the metaphase complements contained 98 chromosomes, though the count varied between 94-100 in a few cells (Table I). Cells not showing modal counts were probably caused by loss during preparation or by chromosomes being obscured by surrounding cell nuclei. The diploid complement (Fig.1a) comprised 12 metacentric pairs, 10 submetacentric pairs, 1 subtelocentric pairs and 26 telocentric pairs (Fig.1b). Total length of the haploid complement equalled 157.5µm with a range in the length of shortest and longest chromosome between 2-8µm (Table II). The arm ratio and the centromeric index ranged between 1-∞ and 0-50 respectively. The chromosomal formula can be represented as: K (2n) = 98 = 24m+20Sm+2St+52t.

Table II: Chromosome morphometry of *Schizothorax labiatus* (m= metacentric; Sm=sub-metacentric; St=sub-telocentric; t=telocentric)

Pair No.	Length of short arm (µm) 'S'	Length of long arm (µm) 'L'	Total length(µm) L+S	Arm ratio (L/S)	Centromeric index	Category
1	3	5	8	1.6	37.5	m
2	3	3	6	1	50	m
3	2	3	5	1.5	40	m
4	2	3	5	1.5	40	m
5	2.5	2.5	5	1	50	m
6	2	2.5	4.5	1.2	44.4	m
7	1.5	2	3.5	1.3	42.8	m
8	1.5	1.5	3	1	50	m
9	1.5	1.5	3	1	50	m
10	1	1	2	1	50	m
11	1	1	2	1	50	m
12	1	1	2	1	50	m
13	2	4	6	2	33.3	Sm
14	2	4	6	2	33.3	Sm
15	1	3	4	3	25	Sm
16	1	2.5	3.5	2.5	28.5	Sm
17	1	2	3	2	33.3	Sm
18	1	2	3	2	33.3	Sm
19	1	2	3	2	33.3	Sm
20	1	2	3	2	33.3	Sm
21	1	2	3	2	33.3	Sm
22	1	2	3	2	33.3	Sm
23	1	4	5	4	20	St
24	0	4	4	∞	0	t
25	0	4	4	∞	0	t
26	0	3	3	∞	0	t
27	0	3	3	∞	0	t
28	0	3	3	∞	0	t
29	0	3	3	∞	0	t
30	0	3	3	∞	0	t
31	0	3	3	∞	0	t
32	0	3	3	∞	0	t
33	0	3	3	∞	0	t
34	0	3	3	∞	0	t
35	0	3	3	∞	0	t
36	0	2	2	∞	0	t
37	0	2	2	∞	0	t
38	0	2	2	∞	0	t
39	0	2	2	∞	0	t
40	0	2	2	∞	0	t
41	0	2	2	∞	0	t
42	0	2	2	∞	0	t
43	0	2	2	∞	0	t
44	0	2	2	∞	0	t
45	0	2	2	∞	0	t
46	0	2	2	∞	0	t
47	0	2	2	∞	0	t
48	0	2	2	∞	0	t
49	0	2	2	∞	0	t

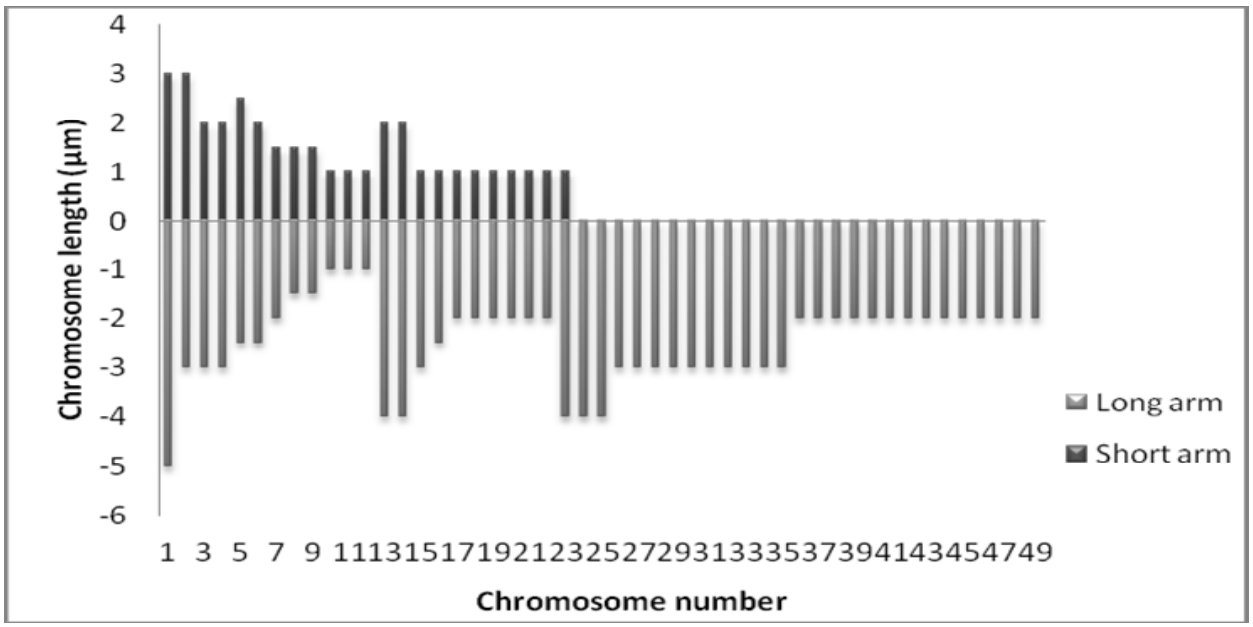

Fig. 2: Haploid ideogram of *Schizothorax labiatus.*

Table III: Showing different *Schizothorax* species worked out thus far.

S. No.	Name of the species	2n	Chromosome morphology m	Sm	St	t	NF value	Author and Year
1	*Schizothorax kumaonensis*	98	24	6	68		128	Rishi *et al*., 1998
2	*Schizothorax kumaonensis*	98	18		70	10	126	Lakara *et al*., 1997
3	*Schizothorax progastus*	98	16	20	12	50	134	Rishi *et al*., 1983
4	*Schizothorax richardsoni*	98	16		42	40	154	Lakara *et al*., 1997
5	*Schizothorax esocinus*	98	30	22	10	36	150	Farooq *et al*., 2011
5	*Schizothorax labiatus*	98	24	20	2	52	142	Present work

4. Discussion

Schizothorax labiatus analysed cytologically in the present study revealed a high number of chromosomes 2n=98. Species with high numbers are considered to have resulted through polyploidy from ancestral 2n= 48 or 50 (Rishi *et al.,* 1998). Chromosome counts in nearly all cyprinid polyploids occur in multiples or combinations of the most common karyotype (48-50) and tetraploids (96, 98 or 100) and hexaploids (148-150) have arisen through hybridisation (Dowling and Secor, 1997). This is well illustrated by a number of species of fish belonging to diverse orders. Buth *et al*., (1991) noted 52 such taxa most of which belong to cyprinidae identified through karyological analysis (Dowling and Secor, 1997) and such forms are ancestral polyploids (Ohno *et al*., 1969). Polyploidy in fishes has been associated with traits including large body size, fast growth rate, long life and ecological adaptability (Uyeno and Smith, 1972; Schultz, 1980). Since *Schizothorax* fishes are hill stream fishes, it may be that polyploidy may have resulted on account of cold temperature of their habitat. The use of thermal shocks to eggs for induction of polyploidy (Chourrout, 1988) provides support to the above assertion. The role of polyploidy in evolution and survival of fish is very important because it prevents from natural selection pressure (Oellerman and Skelton, 1990).

Interestingly *Schizothorax labiatus* showed diploid number similar to that recorded for other species inhabiting different geographical locations (Table III) e.g.,*S.esocinus*, 2n=98 (Farooq *et al*., 2011) *Schizothorax richardsonii*, 2n=98 (Sharma *et al*., 1992; Lakara *et al*., 1997), *Schizothoracichthys prograstus*, 2n= 98 (Rishi *et al*., 1983), *S. kumaonensis*, 2n=98 (Rishi *et al*., 1998; Lakara *et al*., 1997) but different fundamental arm number which may be attributed to the intra-chromosomal changes involving pericentric and paracentric inversion, suggesting origin from the same primitive ancestor. The overall similarity in the chromosome number and morphology implies that *Schizothorax* species are very closely related in that they have not been isolated as evolving entities long enough for random

chromosome changes to have taken place and become fixed, and that a particular karyotype would be selected implies an adaptive advantage for that particular configuration.

Cells lacking normal value (2n=94-100) were also encountered in the preparations and these probably resulted from losses during the preparation or addition from the neighbouring cells or hypotonic overtreatment (Nanda *et al*., 1995).

The present study is the first to describe the chromosomal characteristics of *Schizothorax labiatus* from The Kashmir Valley. The results of the study can be used for the genetic manipulation and management and conservation of the species.

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Medicinal Values of Kolanut in Nigeria: Implication for Extension Service Delivery

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Abstract: This paper reviews the medicinal values of Kolanut in Nigeria with a view of identifying the most common species in the country and discussing the problem and Prospects of Kolanut trees. Some of the values of kolanut discussed include traditional value, nutritional value, economic/industrial value and the medicinal value which is the focus of this paper. The paper recommends that retraining efforts need be focused on the forestry extension to ensure that indigenous fruit trees like Kolanut become part of the basket of livelihood options supported by extension agents.

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Key words: Kolanut, medicinal value, Nigeria, research-extension linkage

Kolanut occupies a unique place amongst West Africans where it is widely consumed by them. It is of particular importance in the social life and religious customs of people in the tropics of West Africa. In all types of traditional gatherings in these parts, kolanuts are highly esteemed channels of blessings. Kola nut belongs to the plant family S*terculiaceae*, having about 125 species of trees native to the tropical rainforests of Africa. Of these species, the most common in Nigeria are C*ola nitida* (Gbanja) with two phenotypic varieties; the white and red cultivars, C*ola acuminata* (Abata) and *Garcina cola* (Orogbo) and *Buchholzia coriacea* popularly known as wonderful kola. Nigeria accounts for about 70 percent of the total world production of kolanuts (Quarco, 1969, 1973; Jacob, 1973). About 90% of the kola produced in Nigeria is consumed within the country while 10% is exported (Quarco, 1973). The cultivation of kola in Nigeria is ecologically limited to the rain forest zones of the South and riverine areas of the Savannah region. Kola is an important economic cash crop to a significant proportion of Nigerian population who are involved in kola farming, trading and industrial utilization. It is highly valued for its perceived medicinal attributes which make it a highly desired product (Adebisi 2004). The objectives of this paper include the analysis of the problem and prospect of Kolanut trees and identifying the values of kolanut(traditional, nutritional, economic/industrial and the medicinal value of kolanut.

Prospects and Problem of Kolanut Trees

Several opportunities for improved rural development are linked to non-timber forest products, one of which is kolanut. In many areas, rural populations are traditionally dependent on local forest resources to provide additional income through collection and marketing (Arnold 1995). Where employment opportunities from traditional industries are declining, workers looking for alternative sources of income often turn to the collection of these products from the nearby forest (Adepoju & Salau 2007). In his cursory survey of people involved in the trade of Bitter kola in the J4 area of Omo Forest Reserve (south-west Nigeria), Adebisi (2004) observed that the production-to-consumption system of *Garcinia* nuts has an obvious positive impact on households of the J4 communities, its commercialization contributing to improving the standard of living of the villagers. Furthermore, the trade of kolanut is more profitable than trade in other non-timber forest products because of its high amenability to storage, both fresh and dried. The economic importance of kolanut cannot be underestimated, especially in the area of poverty alleviation among rural people. However, while the demand is rising, the production remains low because many of the trees in Nigeria are unfruitful or have very low yield due to self and cross incompatibility among trees, partial and total sterility, inefficient natural pollination, old age, field and storage pests and diseases (Odegbaro, 1973; Daramola, 1978; Jacob, 1971, 1973). Also, *kolanut* trees are currently on the decrease as a result of deforestation. Many of the farmers who have the trees on their farms indicated that they spared them during land preparation for farming, rather than planting them themselves. The swiftly decline fruit trees pose threats not only to food security, but also to wild life, environment, traditional medicine and human beings. Indigenous fruit trees which provide cheap source of proteins, vitamins, oils are also values for their medicinal properties (Ayuk et al, 1999). Various parts of the trees have been used in treating many ailments, such as skin disease (Dacryodes edulis),

black coated tongue (Chrysophylum albidum), cough and fibroid (Garcina kola) etc. (Anegheh et al, 2004).Besides, in spite of farmers' obvious interest in indigenous fruits for cash income and the range of food and medicinal products they provide, these species do not receive much attention from policy-makers, foresters or agriculturalists (Tchiegang-Megueni et al. 2001). Increasingly, there is intense competition and imperatives for vertical integration in the major markets for conventional plantation-grown tropical tree fruit and commodity/cash crops. As yet, these pressures are less evident in the expanding markets for indigenous fruit and derived products, making indigenous fruits more suitable for smallholder farmers in developing countries (Poole 2004). Furthermore, the restricted number of usually exotic species promoted by extension services cannot meet the full range of farmers' needs. The wide range of indigenous fruit trees available in many areas can enable farmers to meet their varied household needs for food, nutrition, medicines, etc. This therefore calls for urgent attention to the establishment of Kolanut plantations. Diversification of the products should be encouraged to create more market opportunities and accrued benefits.

Traditional Values of Kolanut

Kolanut is used as a masticatory stimulant by Africans and has numerous uses in social, religious, ritual and ceremonial functions by the natives in the forest region of Africa. It is used during ceremonies related to marriage, child naming, installation of Chiefs, funeral and sacrifices made to the various gods of African mythology (Nzekwu, 1961; Daramola, 1978a; Opeke, 1982). Kola nut, bitter kola and alligator pepper are traditional plants which are often eaten as snacks especially among the elderly in Nigeria. Traditionally, these nuts were chewed as a masticatory substance, to stimulate the flow of saliva (Leakey, 2001) but are now widely consumed as snack in West and Central Africa. In folk medicine, bitter kola is dried, ground and mixed with honey to make a traditional cough mixture. Traditional treatment of circumcision wounds, other wounds and chronic skin ulcers with locally prepared herbs and other natural occurring substances has been known for generations. Mboto (2000) provided evidence of accelerated healing in a combined therapy of *Garcinia kola, Vernonia amygdalina* and honey for the treatment of fresh wounds, including wounds resulting from male circumcision and chronic ulcers.

Nutritional value of Kolanut

Agro-industrial by-products and crop wastes/ residues such as wheat offals, maize offals, maize wastes, palm kernel cake, cassava peels, rice bran, cocoa pod husk, kolanut husk, kola testa, etc. have proved to be valuable in replacing a certain proportion of maize in monogastric nutrition Ogbonna and Adebowale,1993). Olubamiwa et al. (2000) reported that kolanut husk meal (KHM) shared similarity with cocoa pod husk (CPH) but had higher crude protein and lower crude fibre contents than CPH. The kola pod husk has been used in the manufacture of poultry feeds, snail feed (KOLA-T). 10 to 15 percent dietary inclusions of KPH reduced feed cost while not sacrificing bird performance (Olubamiwa *et al*. 2002). Feeding KOLA-T solely to snails was found to be better than other common snail feedstuffs.(Asogwa et al, 2006)

Economic/ industrial value of Kolanut

Buchholzia coriacea popularly known as wonderful kola possesses an invaluable but yet to be tapped potentials which, if exploited, will benefit the food industry. The fresh kola (*B. coriacea)* found to be more active on the test food borne pathogens than the hexane and methanol extracts (Ezekiel and Onyeoziri, 2008). Kolanut could be utilized in the producing countries to produce value added products such as the kola drink and thereby create and increase the income of farmers and industrialists in the country (Jayeola, 2001). It is also used in the manufacture of dyes and cola group of beverage drinks (Ajiboye and Afolayan, 2009). The kola pod husk has been used in the manufacture liquid detergent and organic fertilizer (Asogwa et al, 2006). There is also increasing demand for its usage in pharmaceutical industries and for production of soft drinks, wines and candles (Beattie, 1970; Ogutuga, 1975). Its uses have inevitably created a high demand in excess of its production (Oladokun, 1985).

Medicinal value of Kolanut

Kola nuts contain large amounts of caffeine and threobromine and are therefore used as a stimulant (Jaiyeola, 2001, Leakey, 2001; Omode et al., 1995). They produce a strong state of euphoria and well being, enhance alertness and physical energy, elevate mood, increase tactile sensitivity, suppress appetite and hunger. and are used as an aphrodisiac (http://en.wikipedia.org/wiki/kolanut; Attfield, 1865). The caffeine in the nuts also acts as a bronchodilator, expanding the bronchial air passages, hence kola nuts are often used to treat whooping cough and asthma (http://en.wikipedia.org/wiki/kolanut; Blades, 2000). Unlike other kola nuts however, bitter kola is believed to clean the digestive system, without side effects such as abdominal problems, even when a lot of nuts are eaten (Onochie and Stanfield, 1960). Atolaiye et al., (2009) observed that the extract of Eugenol, *G. kola*, Vitamin A, Vitamins A+D,

Vitamin D, *C. acuminata* (white), *C. nitida* (pink) and *C. nitida* (red) are effective as antioxidants in red cell survival and viability. Furthermore, Ibikunle et al, (2011) concluded that kola nut extracts are sufficiently trichomonacidal and therefore potentially useful as therapeutic agents in the control of trichomoniasis. The result of the experiment carried out by Esimone et al, (2007) confirmed the adaptogenic property of *G. kola* seeds (GKS). It is possible that the anti-oxidant, anti-inflammatory, and immunostimulatory properties of the flavonoids constituents of this herb are responsible for the adaptogenic effects. The findings of Okoko, (2009) show that the presence of four compounds namely garcinia biflavonoids GB1 and GB2, garcinal and garcinoic acid are partly responsible for the great antioxidant potential of *G. kola* seeds. This gives further evidence to the nutraceutical and pharmaceutical potential of *G. kola*.

Table 1: Distribution of Kolanut by traditional, nutritional, economic and medicinal Values

Traditional value of Kolanut	**Nutritional value of Kolanut**	**Economic/ industrial value of Kolanut**	**Medicinal value of Kolanut**
Ceremonies Fetish recipes Symbol of love, unity and welcome Chewing stick Believed to expel snake Local snacks Treatment of fresh wound/circumcision Masticatory substance(to stimulate the flow of saliva)	Layer mash Snail feed	Soft drinks Chocolates Dyes Kola wine Liquid detergent Organic fertilizer Candles Food industry Pharmaceutical industry	Stimulant Enhance Alertness Physical energy Elevate mood Suppress appetite and Hunger Increases tactile sensitivity Use for whooping cough Treatment of Asthma Clean digestive system Remedy against poison Treatment of fresh wound/circumcision Aphodisiac Bronchodilator Jaundice(fruit pulp) Bronchitis and throat infection Catarrh, abdominal colicky pain Anti-diabetic and antihepatotoxic activities Anti-inflammatory, antimicrobial, antiviral properties Adaptogenic property Antioxidants in red cell survival and viability Antitrichomonal activity

Conclusion

This paper has been reviewed to show the attributes of Kolanut trees on how they can improve farmers' livelihood as source of food, medicine and income. Apart from this product being a good source of foreign exchange if well managed and conserved, the food and the pharmaceutical industries have a lot of benefits to tap from this forest product. Therefore, the strategies developed by local communities to protect these species, need to be improved through application of sound scientific principles in order to help local farmers to properly manage, conserve and sustainably use this forest product well. Furthermore, retraining efforts need be focused on the forestry extension to ensure that indigenous fruit trees like Kolanut become part of the basket of livelihood options supported by extension agents.

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The Relationship Between components of information Technology and organizational effectiveness In Shiraz Regional Library of Sciences and Technology

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Abstract: Information technology (IT) has advertently or inadvertently entered our country. Therefore, the optimal use of this technology requires that all individuals commit themselves to the organization goals. IT plays a vital role in creating new concepts such as organizational effectiveness. In this line, the present study aims to investigate the relationships among information technology components and organizational effectiveness in Shiraz regional Library of Science and Technology. In this study, IT dimensions include investments, innovation, and effective tools, while IT components are selected as education, the improvement of the quality of services offered, reducing the costs and the time associated with patients' treatment, supporting managers, jobs simplification, automation, offering internet services, and using various instructions. The instruments used in the study comprised a researcher-invented IT questionnaire by Reza SabetAqadam (2007) (Coronbach Alpha = 0.8424) and organizational effectiveness questionnaire by Varmazyar (2000) developed based on Cample's effectiveness criteria (Coronbach Alpha = 0.901). The results of bivariate regression analysis indicated that there is a positive and significant relationship between variables examined in the study and organizational effectiveness, except for jobs simplification and offering internet services. A positive and significant relationship was also observed between the research hypotheses and organizational effectiveness.
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Key words: Information technology (IT), investment, innovation, cost reduction, supporting managers, jobs simplifications, automation systems, internet services, standards and instructions, effectiveness.

Introduction

An investigation of the scholars' opinions in the field of organization and management shows that there is a consensus about defining organizational environment and business methods and conditions (Sarafizadeh, 2004). Conditions that any organization may encounter were known as "calm waters", a concept that fell into disuse over the past two decades and designates a situation in which the organization withdraws from the industrial society, and steps into a world dominated by information and new ideas, which is known as "foam waters" (Robbins,2004). The requirements of the new business environment with crushing competition, on one hand, and staggering mutation in information technology (IT), on the other, have forced managers to dismiss their traditional and common trends. The important issue is the change in views concerning organizational competition, and an attempt to reduce costs and increase organizational efficacy.

It can be claimed that one of the most controversial notions among researchers in economics and management is the effective factors on organizational efficiency. Many believe that information technology does not have any effect on efficacy and efficiency of organizations or countries (Bernet&Morisson, 1995), while some believe that information technology fundamentally influences efficacy and efficiency of companies and countries. By the appropriate use of information technology, organizations can achieve sustainable competitive advantages. Today, the managers' concern is to recognize various concepts and applications of IT in carrying out organizational activities, and gaining awareness of the dimensions and multifarious effective factors in organizational efficacy to assess the role of every parameter and the degree of its effect. Unfortunately, due to the non-quantitative nature of the institutes, and numerous qualitative aims in service organizations, it is difficult to assess the rate of efficacy and efficiency. Due to the structure of governmental organizations and their special aims, it is even more complicated to measure the effects of the parameters there.

With growth in information technology and communication, and to synchronize the changes in internal and external environments and in structure and expectation level in human resources, governments have made considerable investments on different sectors. A new method for providing services in public sector, known as the "electronic government", has been formulated and has changed the fashion of providing services and carrying out activities. Over the past decade, align with

international changes; in Iran too, we have experienced extensive investments in establishing and developing electronic government, and the realizations of goals in the field. One of the important sectors playing a significant role in the perspective of organizational and institutional development includes the organizations that provide services to the public including libraries. In this study, the historical evolution and development of IT are investigated, and the aspects of effectiveness of modern information technology on enhancing efficacy is studied, and following that, the various aspects of effective parameters on efficacy are assessed as a case study in the Shiraz regional Library of Science and Technology.

1.1 Problem statement

One of the thorniest challenges in the management of organizations and manufacturing and service companies is the issue of appropriate effectiveness and optimization of human resources, skills, organizational information and communication, technology, and the opportunities for obtaining the best results. Given the increasing investment in modern scientific and industrial technologies over the past two decades, and considering the necessity for utilizing conventional information and communication technologies in the present era, it seems that the required efficacy has not been obtained, if we take into account the extent of investments and facilities (Sab\et ghadam, 2007). Also, some managers and employees believe that the computerization of an organization could eliminate the bureaucratic rules and structure and undermine the formal organizational behaviors. They also assume that proving services through computers may question human capabilities, and that special innovations like automation and simplification cannot be developed to produce efficacy.

In order to attain efficacy some strategies could be used including those based on human relation, internal processes, objectives, open systems, competing values, and satisfying the beneficiaries. In this research, the satisfaction of beneficiaries was investigated, because this approach takes into account such criteria as flexibility, morality and motivation, cooperation and assistance, making use of expert opinions and participation in decision-making. Also, if the organizations providing services fail to know their beneficiaries and their demands, they will not be able to draw up their commission statement.

In this study, the relation and role of information technology in the effectiveness process in the organization, the effective rate of organizational and technological factors on the beneficiaries' satisfaction, the individuals' role as creative agents in technical, organizational, and social structures are defined and determined. Thus, in this research, the researcher seeks to come up with an answer to the following question:

Is there a relationship between IT parameters (including investment, education, decrease in costs, enhancing quality, managers' support, simplifying jobs, automation, making use of the internet, and instructions) and organizational efficacy, as the employees of Shiraz regional Library of Science and Technology view the problem?

1.2 Research purposes

Main purpose:

To investigate the relationship between information technology parameters and organizational efficacy as the employees of Shiraz regional Library of Science and Technology view the problem.

The secondary purposes of the research are as follows:

1.2.1 To investigate the significant relationship between investment in information technology and organizational efficacy

1.2.2 To investigate the significant relationship between IT education and organizational efficacy.

1.2.3 To investigate the significant relationship between a reduction in costs due to utilizing IT and organizational efficacy.

1.2.4 To investigate the significant relationship between the high-ranking managers' support for implementing IT planes and organizational efficacy.

1.2.5 To investigate the significant relationship between the innovation arising from IT and organizational efficacy.

1.2.6 To investigate the significant relationship between the advantages of jobs simplification by using IT and organizational efficacy.

1.2.7 To investigate the significant relationship between automation of executive activities in the organization by using IT and organizational efficacy.

1.2.8 To investigate the relationship between effective tools in utilizing IT and organizational efficacy

1.2.9 To investigate the relationship between providing internet services and organizational efficacy

1.2.10 To investigate the relationship between the IT standards and instructions and efficacy.

Review of Literature

1.2.2 Domestic Research

A.Shahin and M.Jamshidian (2006) in a research entitled "Information Technology in Service Organizations", in International Journal of Information Science and Technology, found that

different type of service operations require different levels of IT. Authors have also emphasized that IT plays an important role in the service industry, especially in financial and healthcare sectors and it will continue to increase its importance as managers further appreciate the benefits that can be gained ,as IT is treated as a strategic issue and as the complexities of a large network ,demand increased capabilities in information management.

EbrahimTaghizadeh (2006), in a research entitled "Investigating IT System in Efficacy of Libraries, Museums, and Documents Centers in the AstanMoghadas Province", found that utilizing IT systems would enhance the functions of an organization. The findings also show that the aspects of IT systems (excluding two important elements of IT, namely further information storage and increased accuracy in performance) considerably affect providing services to clients. In other words, IT system users (who use such tools for providing services to clients) in the organizations, believe that the speed for doing tasks, timely retrieval of information, and the speed for accessing information have increased compared to the times when IT facilities were not utilized, and this increase in the present aspects led to organizational efficacy.

A. Divandari, M.E. Pourzarandi and S.Karimi (2010), in a research entitled "Using KPIs for Strategic Management of IT in MellatBank",in International Journal of Information Science and Management, found that Information Technology has a short precedence in Iran. Mellat Bank like other companies almost some years begins its IT plans. This is the time for all IT dependent companies for breaking their legacy metrics to monitor IT departments. The reason for migration to new metrics is the nature of IT services. Unlike other tasks, IT services have a very complicated and implicit structure that is different from other sectors of organizations. In comparison, Mellat Bank is a progressive financial company in our country. Going after technology caused some changes in IT sectors of this Bank. Finally defining KPIs for measuring IT sector came in to action in R&D part of Mellat Bank. After mentioned researches they define KPIs based on operational plan, tasks and CSFs.

Moghadasian (2008), in a research entitled "The Effect of Information Technology on Human Resources in Kowsar Economics Organization, in Accounting and Management Department of ShahidBeheshti University", measured the effects of using IT for human resources in the form of hypotheses related to reduction in human resources, specialization of human resources, self-control, and the self-alienation of the population in the study. The results showed that there is a significant relationship between using IT, self-control, specialization of human resources, and reduction in human resources. However, the relationship between using IT and self-alienation was not significant.

Vares (2001), carried out a research entitled "The Effect of Information Technology and Information Systems on Organizational Structure (Social and Physical Structure)". Considering the fact that the high-ranking manager of the organization makes use of IT as a powerful tool to study his management style in social and physical structures, he can devise a model in which, if he follows a democratic style, the IT decreases the structural complexity, formality, and concentration, leading the social structure towards an organic structure and the physical structure towards virtual administration. When a high-ranking manager follows a dictatorial task-centered management style, the complexity, formality, concentration would increase, and the social structure tends towards physical structure.

A.Azimi & F.SobhanManesh (2010) ,carried out a research entitled "A New Model to Identify and Evaluate Critical Success Factors in the IT Projects ",in International Journal of Information Science and Management ,defined that the related CSFs in the developed countries may not be directly applicable by Iranian project managers and they had to be adapted.

2.2.2 A Review over Foreign Researches

Roby (1988), establishing the relationship between IT and (non)concentrated decision-making, states that organizational structure, unlike informational processing technology, is depended upon the nature of the task environment. If the task environment is consistent, computers can contribute to concentrated decision-making, otherwise, they will cause inconsistency in the process of decision-making.

Bronel (2008) dealt with creating a strategic approach for auditory factors and organizational efficacy; in this research, he attempted to come up with a strategic model for the leaders who encourage environmental learning, which can prove to be an effective strategy. Given the fact that efficacy of leaders can effect organizational process in three analysis levels (viz. individual, team/ interact ional, and organizational), it will be discussed below that such leaders can facilitate the implementation of strategic goals, and help their organizational culture to survive, as a result of which the performance and efficacy of the employees will be enhanced.

Haung (1994), in a research entitled "The Effect of Information Technology on Organizational Efficacy in Service-Providing Organizations", concluded that the employees should frequently be

trained for using technology, and to improve technologies in organizations, the necessary investments should be made. He also points out that support systems including managers, should operate in parallel towards the technology plan, and technology systems should be capable of introducing the required innovations in the organization.

3. The research methodology:

Method of data collection in this research is a two-variable correlation. Study of the nature and purpose of this research is applicable. Generally, Independent variable in the main research hypothesis is information technology. The statistical community in the present study was examined a collection of the employees of Shiraz regional Library of Science and Technology which are in related with information technology as a data user.

Study tools are two questionnaires, including a questionnaire with 35 questions about the technology which is obtained the levels of Cronbach's alpha 8424 / 0 = ∞. The Cronbach's alpha for the third sub- hypothesis respectively were have been 5 / 75, 7 / 73, 1 / 72 percent.

The questionnaire was used by Reza SabetAghdam in 1386 in a thesis entitled "The role of information technology on organizational empowerment in terms of Fars employees of organization and management planning in Najaf Abad University.

Other instruments measure the effectiveness of a questionnaire with 12 questions was designed by Vermzyar in 1379 in a study titled "study of organizational culture and its impact on the organizational effectiveness in 'ShahidBeheshti University, and the coefficient alpha of Cronbach for this questionnaire has been reported 8852 / 0 = ∞ percent that shows high levels of reliability.

The Standard questionnaire of information technology in this study has three-dimensions (investment, innovation and effective tools) and totally it has 35 question which has been designed with Likert range. The range of five-part is such as under:

1 - Strongly Disagree 2- Disagree 3- No opinion 4- agree 5-completely agree

As shown in the following formula for Cronbach's alpha value is obtained from the above formula in which N is number of questions within the questionnaire and $\bar{r}$ is the average of internal correlation coefficient of the answer. Also, the Cronbach's alpha for the three considered sub-hypotheses are 5 / 75, 7 / 73, 1 / 72 percent, indicating an acceptable reliability for the stability of above assumptions.

$$\alpha = \frac{N \times \bar{r}}{(1+(N-1)\times \bar{r})}$$

4.1. Statistical Analysis

Descriptive characteristics

A) education

Index Variable descriptive statistics relating to education is provided In Table (4-1). According to this table, we see that the greatest number of employees have bachelor's degree and the minimum of them have masters and PHD.

B) Organizational position:

Descriptive statistics for the index of the row variables is given in Table (4-2).

According to statistics reported in the Table, Most people are in Expert post and the Manager is in the lowest.

C) Gender

It can be seen in Table (4-3) that seventy percent of the participants were female and thirty percent of them were also male. This table represents the frequency distribution of the sample and the gender variable.

4.2. The analytical assumptions of research hypothesis:

Here, the suitable statistical analysis with the hypothesis is provided. It should be noted that before doing any analysis, statistical hypothesis has been controlled.

Since the significant level is close to zero, the default significance level of test is 0 / 05.

05 / 0> 00 / 0

H0 is rejected, so the test is significant. In fact, the use of informational technology in general has an impact on the organizational effectiveness, and a direct relationship between them is established, i.e. the more use of informational technology, the more organizational effectiveness is occurred. This relationship is the number of correlation coefficient (584 / 0). It shows strongly relationship between the two variables (The absolute value is closer to one; there is strong influence and relationship between them).

Thus, linear regression equation between them can be achieved.

4.3. Sub assumptions:

Since the significant level is close to zero, the default significance level of test is 0 / 05.

05 / 0> 00 / 0

H0 is rejected, so the test is significant. In fact, the amount of investments in informational technology has an impact on the organizational

effectiveness and a direct relationship between them is established, i.e. the more amounts of investment in informational technology, the more organizational effectiveness is occurred. This relationship is the number of correlation coefficient (608 / 0). It shows strongly relationships between the two variables (The absolute value is closer to one; there is strong influence and relationship between them).

Thus, linear regression equation between them can be achieved, in which it would be the expected amount of organizational effectiveness and independent variable of investments in informational technology.

Since the significant level is close to zero, the default significance level of test is 0 / 05.

05 / 0> 00 / 0

H0 is rejected, so the test is significant. In fact, the amount of trainings of informational technology has an impact on the organizational effectiveness and a direct relationship between them is established, i.e. the more amounts of trainings of informational technology, the more organizational effectiveness is occurred. This relationship is the number of correlation coefficient (591 / 0). It shows strongly relationships between the two variables (The absolute value is closer to one; there is strong influence and relationship between them).

Thus, linear regression equation between them can be achieved, in which it would be the expected amount of organizational effectiveness and independent variable of trainings of informational technology.

Since the significant level is close to zero, the default significance level of test is 0 / 05.

05 / 0> 00 / 0

H0 is rejected, so the test is significant. In fact, the amount of quality improvement of informational technology has an impact on the organizational effectiveness and a direct relationship between them is established, i.e. the more amounts of quality improvement of informational technology, the more organizational effectiveness is occurred. This relationship is the number of correlation coefficient (524 / 0). It shows strongly relationships between the two variables (The absolute value is closer to one; there is strong influence and relationship between them).

Thus, linear regression equation between them can be achieved, in which it would be the expected amount of organizational effectiveness and independent variable of quality improvement of informational technology.

H0 is rejected, so the test is significant. In fact, the amount of cost reductions resulting from the use of informational technology has an impact on the organizational effectiveness and a direct relationship between them is established, i.e. the more amounts of cost reductions resulting from the use of informational technology, the more organizational effectiveness is occurred. This relationship is the number of correlation coefficient (652 / 0). It shows strongly relationships between the two variables (The absolute value is closer to one; there is strong influence and relationship between them).

Thus, linear regression equation between them can be achieved, in which it would be the expected amount of organizational effectiveness and independent variable of cost reductions resulting from the use of informational technology.

H0 is rejected, so the test is significant. In fact, the amount of support and awareness of manager's informational technology has an impact on the organizational effectiveness and a direct relationship between them is established, i.e. the more amounts of support and awareness of manager's informational technology, the more organizational effectiveness is occurred. This relationship is the number of correlation coefficient (346 / 0). It shows strongly relationships between the two variables (The absolute value is closer to one; there is strong influence and relationship between them).

Thus, linear regression equation between them can be achieved, in which it would be the expected amount of organizational effectiveness and independent variable of support and awareness of managers' informational technology

H0 is rejected, so the test is significant. In fact, the amount of innovation of informational technology has an impact on the organizational effectiveness and a direct relationship between them is established, i.e. the more amounts of innovation of informational technology, the more organizational effectiveness is occurred. This relationship is the number of correlation coefficient (469 / 0). It shows strongly relationships between the two variables (The absolute value is closer to one; there is strong influence and relationship between them).

Thus, linear regression equation between them can be achieved, in which it would be the expected amount of organizational effectiveness and independent variable of innovation of informational technology.

H0 is accepted, so the test is not significant. In fact, the amount of the benefits of simplified jobs of Information Technology doesn't have an impact on the organizational effectiveness, i.e. the amount of the benefits of simplified jobs of Informational Technology doesn't have relationship with organizational effectiveness. This relationship is the number of correlation coefficient (158 / 0). It shows the weak relationships between the two variables

(The absolute value is closer to zero, there is no relationship).

H0 is rejected, so the test is significant. In fact, the amount of automation of administrative activities through the use of information technology has an impact on the organizational effectiveness and a direct relationship between them is established, i.e. the more amounts of Automation of administrative activities through the use of information technology, the more organizational effectiveness is occurred. This relationship is the number of correlation coefficient (552 / 0). It shows strongly relationships between the two variables (The absolute value is closer to one; there is strong influence and relationship between them).

Thus, linear regression equation between them can be achieved, in which it would be the expected amount of organizational effectiveness and independent variable of automation of administrative activities through the use of information technology.

H0 is rejected, so the test is significant. The test level of 0 / 01 is not significant .In fact, the amount of Effective tools of IT usage has an impact on the organizational effectiveness and a direct relationship between them is established, i.e. the more amounts of Effective tools of IT usage, the more organizational effectiveness is occurred. This relationship is the number of correlation coefficient (293 / 0). It shows strongly relationships between the two variables (The absolute value is closer to one; there is strong influence and relationship between them). And it has less severe relationships than the other listed independent variables.

Thus, linear regression equation between them can be achieved, in which it would be the expected amount of organizational effectiveness and independent variable of Effective tools of IT usage.

H0 is accepted, so the test is not significant. In fact, the amount of Internet services doesn't have an impact on the organizational effectiveness, i.e. the amount of the Internet services doesn't have relationship with organizational effectiveness. This relationship is the number of correlation coefficient (095 / 0). It shows the weak relationships between the two variables (The absolute value is closer to zero, there is no relationship).

H0 is rejected, so the test is significant. The test level of 0 / 01 is not significant .In fact, the amount of standards and instructions of IT has an impact on the organizational effectiveness and a direct relationship between them is established, i.e. the more amounts of standards and instructions of IT, the more organizational effectiveness is occurred. This relationship is the number of correlation coefficient (318 / 0). It shows strongly relationships between the two variables (The absolute value is closer to one; there is strong influence and relationship between them). And it has less severe relationships than the other listed independent variables.

Demographic assumptions:

In this part, it studied informational technology and the effect of education between two groups of male and female. And finally, the effect or no effect of the people's position on the changing informational technology will be explored.

Since the significant level is more than 0/05, H0 is accepted. In fact, Informational technology has same effect on both gender and their differences are not significant.

5. Conclusion & Result:

In this study, from the perspectives of librarian employees, a significant relationship between the components of informational technology and organizational effectiveness was evaluated. The target hypothesis was that the dimensions of the IT (information technology investment, innovation and information technology tools) with the effectiveness of the organization's employees (library) have a significant relationship or not? It can be concluded that the regression model and correlation coefficient and the explained amount of variance and effectiveness by the components of informational technology is significant and these variables that can predict the effectiveness of the organization.

As described in Chapter II, Ibrahim Taqizadeh (1385) as well as research examining the effectiveness of IT systems (the Libraries, Museum and Center of Astan Quds Razavi), concluded the use of IT systems results better performance of organization. Taqizadeh worked on the other components of informational technology it had noted in the second season. Only on two exceptions (storage and the accurate increase in informational technology), others show the significant relationship between these two variables which is generally consistent with the findings of this study.

6. DISCUSSION:

The results of bivariate regression analysis indicated that there is a positive and significant relationship between variables examined in the study and organizational effectiveness, except for jobs simplification and offering internet services. A positive and significant relationship was also observed between the research hypotheses and organizational effectiveness.In general it can be concluded if the information technology (both in terms of dimensions and components of the survey) plan and implement appropriately, it will cause organizational effectiveness. These are some of the findings:

1: The Commitment and belief of Top management in the concept and effectiveness of information technology is essential. These two factors are considered as important factors for survival and growth of organizations in turbulent and highly competitive conditions of today world.

2: Application of information technology, as pervasive in the organization not just a handful of employees to access information, also contributing to the increased use of information regarding the classification of certain information.

3: Efforts to identify and eliminate barriers that increase the effectiveness of the organization and staff.

4: Having a systems thinking model in organization. Because all employees are part of a system. This thinking allows information to be transferred from one unit to other units and sections that are actually the same mechanism of organizational effectiveness.

Research Model:

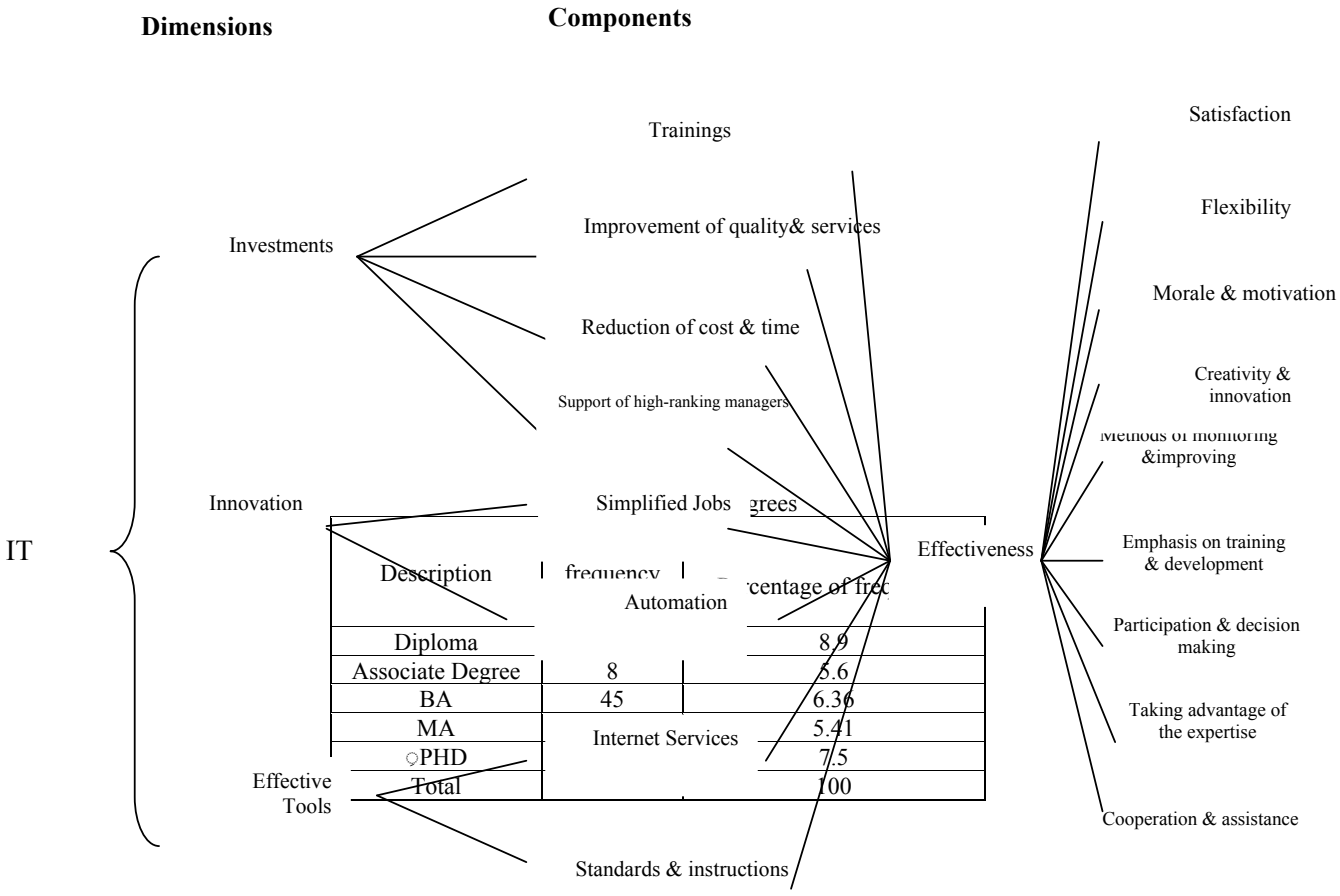

Table (4-2): Organizational position

Description	frequency	Percentage of frequency
Expert	109	6.88
Head of Unit	8	5.6
Manager	6	9.4
Total	123	100

Table (4-3): gender

Description	frequency	Percentage of frequency
Male	44	8.35
Female	79	2.64
Total	123	100

Table (4-4): The relationship between informational technology and organizational effectiveness

Variables	Correlation coefficient	Significant level
Informational Technology* Organizational Effectiveness	0/326	0/00

Table (4-5): The relationship between investment and organizational effectiveness

Variables	Correlation coefficient	Significant level
Investments in Informational Technology* Organizational Effectiveness	0/225	0/012

Table (4-6): The relationship between trainings and organizational effectiveness

Variables	Correlation coefficient	Significant level
Trainings of Informational Technology * Organizational Effectiveness	0/188	0/038

Table (4-7): The relationship between Improvement of service quality and organizational effectiveness

Variables	Correlation coefficient	Significant level
Quality Improvement of IT services * Organizational Effectiveness	0/246	0/006

Table (4-8): The relationship between cost reductions and organizational effectiveness

Variables	Correlation coefficient	Significant level
Cost reductions resulting from the use of IT * Organizational Effectiveness	0/356	0/006

Since the significant level is close to zero, the default significance level of test is 0 / 05.
05 / 0> 00 / 0

Table (4-9): The relationship between Support and awareness of managers and organizational effectiveness

Variables	Correlation coefficient	Significant level
Support and awareness of managers 'informational technology * Organizational Effectiveness	0/426	0/000

Since the significant level is close to zero, the default significance level of test is 0 / 05.
05 / 0> 00 / 0

Table (4-10): The relationship between innovations and organizational effectiveness

Variables	Correlation coefficient	Significant level
Innovation of Informational Technology *Organizational Effectiveness	0/326	0/000

Since the significant level is close to zero, the default significance level of test is 0 / 05.
05 / 0> 00 / 0

Table (4-11): The relationship between the benefits of simplified jobs and organizational effectiveness

Variables	Correlation coefficient	Significant level
The benefits of simplified jobs of Information Technology * Organizational Effectiveness	0/326	0/000

Since the significant level is 094/0, the default significance level of test is 0 / 05.
05/0 < 094/0

Table (4-12): The relationship between automation of activity and organizational effectiveness

Variables	Correlation coefficient	Significant level
Automation of administrative activities through the use of information technology * Organizational Effectiveness	0/426	0/000

Since the significant level is close to zero, the default significance level of test is 0 / 05.
05 / 0> 000 / 0

Table (4-13): The relationship between the use of effective tools and organizational effectiveness

Variables	Correlation coefficient	Significant level
Effective tools of IT usage * Organizational Effectiveness	0/438	0/000

Since the significant level is 023/0, the default significance level of test is 0 / 05.
05 / 0> 023 / 0

Table (4-14): The relationship between Internet services and organizational effectiveness

Variables	Correlation coefficient	Significant level
Internet Services * Organizational Effectiveness	0/221	0/000

Since the significant level is 094/0, the default significance level of test is 0 / 05.
05/0 < 258/0

Table (4-15): The relationship between standards and instructions, and organizational effectiveness

Variables	Correlation coefficient	Significant level
Standards and instructions of IT * Organizational Effectiveness	0/537	0/000

Since the significant level is 001/0, the default significance level of test is 0 / 05.
05 / 0> 001 / 0

Table (4-16): Usage of IT for different Genders

Variable's name	Average	T statistic	Significant level
Informational technology	02/0 -364/2 3/75 male 4/00 female		

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Employees' Intention to Use Web-based Training in South Zagros Oil and Gas Production Company, a Causal Model

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Abstract: The main purpose of this study is presenting a causal model of employees' intention to use web-based training in South Zagros Oil and Gas Production Company. The correlational study was conducted among employees of this company. The correlational study was conducted among employees of this company. The study is based upon survey approach to collect the data from 169 employees selected randomly. Path analysis and LISREL software were used to analyze the data. Results indicate that computer playfulness, Computer anxiety, and learning goal orientation have significant effects on intention directly or indirectly through perceived usefulness, perceived ease of use, perceived enjoyment, and computer self-efficacy variables. As a conclusion, Perceived usefulness had the most significant influence on intention directly, while computer playfulness had the strongest total and indirect effect on intention to use web-based training. Moreover, the final model indicated a good fitness for predicting employees' intention to use web-based training.
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Keywords- Employees, Web-based training, Intention, Path analysis

1. Introduction

Twenty-first century society of today had completely changed by the World Wide Web and the internet; in fact these technologies had changed the way of life, way of working, way of recreation and the nature of human communications (Gerg, 1386). Among this, the training had not left the ouster of these technologies. Today traditional understanding of training that is training in a physical space and certain time had changed and a new arena called e-training had been formed.

The use of web-based training as a kind of e-training which promises lower cost for training in everywhere and every time are expanding at a rapid pace around the globe. It is apparent that organizations deem this method of learning not only viable, but also prefer it in many cases to meet various training needs (Rice, 2005:37).

Web-based training can be seen as a vehicle that may increase the speed, decrease the barriers, disperse the geographical range and reduce the costs of knowledge sharing within an organization and accommodate the communication between users. Nevertheless, there are also a few inhibitors that limit its adoption and implementation by organization, such as software and hardware constraints or psychological factors (Chatzoglou et al, 2009: 878).

However, although that makes primary web-based training adoption decisions, it is the individuals within the firm who are the ultimate users and consumers of the technology (Hashim, 2008: 254), therefore, at first employees should accept this technology for their training and development.

Data about the number of employees in Iran who had actually used web-based training are not available, but it seems that this type of training in Iranian companies are still new and they use the traditional and face to face method of training for their employees. This study tries to predict employees' intention to use web-based training in South Zagros Oil and Gas Production Company by the use of TAM as one of the most common models that is used to predict user behaviour and some other factors which result from other theories and models such as learning goal orientation from Dweck's (1986) learning goal orientation theory, enjoyment from motivation theory that Davis(1992) used it in his model at first, computer self-efficacy and computer anxiety from Bandura's social cognitive theory and computer playfulness which takes its concept from Csikszentmihalyi's flow theory and Moon and Kim (2001) added it to TAM at first. Figure 1 provides the proposed model of this study.

2. Literature Review

Kim & Forsythe (2010) in their own paper, investigated the factors affecting the adoption of product virtualization technology for online shopping small consumer electronics by applying a technology acceptance model and adding two external variables (Innovativeness and Technology anxiety). Their paper results showed that perceived ease of use of PTV had a positive influence on perceived usefulness of PTV and technology anxiety also had a positive

influence on perceived ease of use and perceived usefulness. Ha and stoel (2009) predicted consumers' intention to use e-shopping by integrating e-shopping quality, enjoyment and trust into a technology acceptance model (TAM). Data collected from 208 college students of a large Midwestern university who had experience browsing and/ or purchasing products online.

Results showed that enjoyment had a positive influence on perceived usefulness. Perceived usefulness had a positive direct effect on intention to use e-shopping and perceived ease of use had a positive influence on perceived usefulness.

Aggelidis and chatzoglou (2009) in their own paper under the title of using a modified technology acceptance model in hospitals investigated personnel acceptance of information systems in Greek hospitals by adding some exogenous variables to TAM. Results indicated that perceived usefulness, perceived ease of use and self-efficacy had direct and significant influences on intention. Computer anxiety had a negative significant influence on self-efficacy. Perceived ease of use had a positive significant influence on perceived usefulness and self-efficacy had a positive significant effect on perceived ease of use. Chatzoglou et al (2009) predicted Greek employees' intention to use a web- based training process by extending the technology acceptance model and using some other related factors such as learning goal orientation, management support, enjoyment, self-efficacy, and computer anxiety. Results indicated that learning goal orientation had a significant influence on perceived ease of use, perceived usefulness, perceived enjoyment, and computer self-efficacy. Perceived enjoyment had a direct and significant influence on perceived usefulness, perceived ease of use, computer self-efficacy and intention. Computer self-efficacy had a direct and significant influence on computer anxiety. Computer anxiety had a direct and significant influence on enjoyment. Perceived ease of use and perceived usefulness had direct and significant influences on intention. Computer self- efficacy and computer anxiety had a direct and significant influence on perceived ease of use.

Peker (2010) also investigated acceptance of Hospital Management Systems in hospital in his thesis by extending the TAM. Data were collected from out of 270 Turkish government hospital personnel. The Results indicated that perceived usefulness, perceived ease of use and computer anxiety had significant influences on intention and perceived ease of use had a significant influence on perceived usefulness.

Macharia and Nyakwende (2010) also extended the TAM in his study under the title of "The Influence of E-mail on Student's learning in Higher Educations". The result from 1092 public and private Kenyan students showed that perceived usefulness had a positive and significant influence on intention. Computer self-efficacy, perceived enjoyment and computer anxiety had positive and significant influences on perceived ease of use.

Thompson (2010) in his doctoral thesis under the title of "Assessing the Determinants of Information Technology Adoption in Jamaica's Public sector using the Technology Acceptance Model" assessed the impact of the individual (computer self- efficacy) and the organizational factors (infra-structure support and technical support) on individual's intention to use IT, mediated through perceived ease of use and perceived usefulness of IT. The Results indicated that computer self- efficacy had a significant influence on perceived ease of use and perceived usefulness. Perceived ease of use had a significant influence on perceived usefulness, and perceived usefulness and perceived ease of use had significant influences on intention to use.

Al- maghrabi and Dennis (2011) identified factors that could predict continuance intentions toward e-shopping. Results indicated that perceived usefulness and enjoyment are determinants of online shopping continuance intention, but the influence of enjoyment on intention was stronger. Shin and Shin (2011) in their paper under the title of "why do people play social network games?" examined the perceived factors which contributed to a SNG user's behaviors.

The findings showed that perceived enjoyment and perceived usefulness had significant influences on intention to use, and perceived playfulness had a significant influence on perceived enjoyment.

Terzis and Economides (2011) in their paper under the title of "The acceptance and use of computer based assessment" investigated the constructs that affected student's behavioral intention to use CBA. Data were collected from 173 participants in an introductory informatics course using a questionnaire. The result showed that perceived playfulness had a positive and significant effect on intention to use. Computer self- efficacy had a positive and significant influence on perceived ease of use and perceived ease of use also had a positive and significant influence one perceived usefulness.

Al-Harbi (2011) investigated factors that were effective on acceptance of e-learning in Saudi higher education. The result showed that Internet self-efficacy had a significant influence on perceived ease of use.

Chang (2010) in his study under the title of "task-technology fit and user acceptance of online auction" also found that perceived ease of use, perceived

usefulness and perceived playfulness had significant and positive influences on intention to use and perceived ease of use also had a significant impact on perceived usefulness.

Roca and Gagne (2008) in their study under the title of "Understanding e-learning continuance intention in the workplace: A self- determination theory perspective" proposed an extended Technology Acceptance Model in the context of e-learning service. In their proposed model, perceived playfulness, perceived usefulness and perceived ease of use had significant impacts on intention to use, furthermore perceived ease of use had a significant impact on perceived usefulness.

Bertta, kristiina and Anssi (2009) in 17th European conference on information systems investigated the role of training in decreasing anxiety among experienced computer users through a longitudinal study. The results suggested that self-efficacy had a significant influence on intention to use and perceived ease of use. Perceived ease of use had a significant influence on perceived ease of use but the negative and significant impact of computer anxiety on perceived ease of use was not supported.

Celik (2008) in his study under the title of "what determiners Turkish customer's acceptance of internet banking?" investigated customers' acceptance of internet banking by extending the TAM and adding perceived risk, computer playfulness, perceived behavioral control variables to it.

Data from 161 Turkish internet users indicated that perceived usefulness had a significant impact on intention. Perceived ease of use had an indirect impact on intention through attitude and perceived usefulness variables. Computer playfulness had a significant impact on perceived ease of use but a positive and significant impact of computer playfulness on perceived usefulness was not supported.

Hackbarth, Grover and Yi (2003) supported the role of computer playfulness and anxiety as positive and negative mediators of the system experience effect on perceived ease of use. Their results indicated that computer anxiety and computer playfulness had significant influences on perceived ease of use.

3. Methodology

This paper is a descriptive correlational one based on applied goal method of data collection and analysis. Statistical group included all employees of South Zagros Oil and Gas Production Company who were familiar with web-based training. It's estimated 300 subjects in executive and engineering departments. Random sampling was conducted and sample volume was equal to 169 subjects by Cochran formula from which 144 subjects was useful in analysis. Three standard questionnaires were used to measure all variables of the study. Rating of three variables, computer playfulness, perceived usefulness and perceived ease of use were based on the seven-item Likert scale from 1= totally disagree to 7= totally agree and rating of other variables were based on the five-item Likert scale from

1= totally disagree to 5=totally agree. These questionnaires are consisting of:

perceived usefulness and perceived ease of use from Moon and Kim's questionnaire which are respectively included 8 and 9 items and rating of 1,3,5,6 items of ease of use variable are revised.

Computer playfulness from Ahn et al's questionnaire which is included 9 items.

Intention to use, learning goal orientation, computer self-efficacy, enjoyment, and computer anxiety from Chatzoglou et al's questionnaire which are respectively included 5,8,10,3,4 items.

In this study, Cronbach alpha was used to check the reliability of the questionnaire. The values listed in table 1. Path analysis and Lisrel software were used for the analysis of the results. The predicting and the mediating role of variables and direct and indirect influences between them had been calculated by using this method.

4. Results

With due attention to collected data, correlation matrix of variables is calculated. Correlation coefficients are presented in table 2. Since the purpose of this study is to consider predicting roles of variables and measuring their direct and indirect influences by path analysis, so the values of them are reported in table 3. The direct and indirect influences are standardized coefficient or regression (β) and total effect are equal to adding them up.

Computer playfulness (β=0.17, T= 2.19) had a direct and significant impact on intention to use and the research hypothesis is supported. This variable also had indirect effect on intention to use (β= 0.17) through enjoyment, usefulness and ease of use.

Computer playfulness (β=0.43, T=5.94) had a direct and significant influence on enjoyment and the research hypothesis is supported.

Computer playfulness (β=0.21, T=5.94) had a direct and significant influence on perceived ease of use and the research hypothesis is supported.

Learning goal orientation (β=0.18, T=2.36) had a direct and significant influence on perceived usefulness and the research hypothesis is supported. This variable also had indirect impact on perceived usefulness (β=0.10) through perceived enjoyment.

Learning goal orientation (β=0.25, T=3.25) had a direct and significant influence on enjoyment and the research hypothesis is supported.

Learning goal orientation (β=0.35, T=4.31) had a direct and significant influence on computer self-efficacy and the research hypothesis is supported.

Computer anxiety (β=-0.28, T=2.97) had a direct and significant influence perceived usefulness and the research hypothesis is supported. This variable also had indirect impact on perceived usefulness (β= -0.13) through computer self-efficacy and perceived ease of use variables.

Computer anxiety (β=-0.24, T=2.94) had a direct and significant influence on computer self-efficacy and the research hypothesis is supported.

Computer anxiety (β=-0.42, T=5.59) had a direct and significant influence on perceived ease of use and the research hypothesis is supported. This variable also had indirect impact on perceived ease of use (β=-0.04) through computer self-efficacy variable.

Enjoyment (β=0.43, T=5.83) had a direct and significant influence on perceived usefulness and the research hypothesis is supported.

Enjoyment (β=0.17, T=1.99) had a direct and significant influence on intention to use and the research hypothesis is supported.

Computer self-efficacy (β=0.17, T=2.29) had a direct and significant influence on perceived ease of use and the research hypothesis is supported.

Perceived ease of use (β=0.16, T=2.26) had a direct and significant effect on intention to use and the research hypothesis is supported. This variable also had indirect effect (β=0.09) on intention through perceived usefulness variable.

Perceived ease of use (β=0.30, T=3.77) had a direct and significant effect on perceived usefulness and the research hypothesis is supported.

Perceived usefulness (β=0.33, T=4.18) had a direct and significant effect on intention to use and the research hypothesis is supported.

The R^2 scores of intention to use by perceived usefulness, perceived ease of use, learning goal orientation, computer anxiety, computer playfulness, enjoyment and computer self-efficacy variables is equal to 0.38.

The R^2 scores of perceived usefulness by perceived ease of use, learning goal orientation, computer anxiety, computer playfulness, enjoyment and computer self-efficacy variables is equal to 0.36.

The R^2 scores of perceived ease of use by learning goal orientation, computer anxiety, computer playfulness, enjoyment and computer self-efficacy is equal to 0.33.

The R^2 scores of computer self-efficacy by learning goal orientation, computer anxiety, computer playfulness and enjoyment is equal to 0.24.

The R^2 score of enjoyment by computer anxiety, computer playfulness, and learning goal orientation is equal to 0.31. Figure 2 show the measurement model of study.

Model goodness of fit

In this study, chi-square, degree of freedom, Goodness of fit index, Adjusted Goodness of fit index, comparative fit index, root mean square error of approximation (RMSEA) and Normative fit index are used to measure model fit.

The values of $^{x2}/_{DF}$, GFI, AGFI, CFI, RMSEA, NFI are equal to 8, 0.97, 0.99, 0.92, 0.08 and 0.98 respectively. All index values show model good fit.

5. Conclusion

The main purpose of this study is presenting a causal model of employees' intention to use web-based training in South Zagros Oil and Gas Production Company.

In this study, the effective causes of employees' intention to use web- based training are examined by integration of Technology Acceptance Model and other related variables which come from different models and theories. Furthermore, another purpose of this paper is to analyze the roles of computer playfulness, learning goal orientation, computer anxiety, computer self-efficacy, perceived enjoyment, perceived ease of use, and perceived usefulness by path analysis causal method. The results of the path analysis showed that:

Computer playfulness had a direct and significant effect on intention to use web-based training that is consistent with the results of Roca and Gagné (2008), Change (2010) and Terzis and Econmides (2011) researches. Computer playfulness had a direct and significant effect on perceived ease of use which is consistent with the results of Celik (2008) and Hackbarth et al (2003) researches.

Computer playfulness had a direct and significant effect on perceived enjoyment which is consistent with the results of Shin and Shin (2011) and Serenko (2008) researches.

Learning goal orientation had direct and significant effects on perceived usefulness, computer self- efficacy and perceived enjoyment which are consistent with the results of chatzoglou et al (2009) research.

Computer anxiety had a direct and significant effect on perceived usefulness that is consistent with the results of Kim and Forsythe (2010) research.

Computer anxiety had a direct and significant effect on perceived ease of use which is consistent with the results of Macharia and Nyakwende (2010), Kim and Forsythe (2010), Hackbarth et al (2003), chatzoglou et al (2009) researches and is inconsistent with the result of Berta et al (2009) research.

Computer anxiety had a direct and significant effect on computer self-efficacy. It's consistent with

the results of Bertta et al (2009) and Aggelidis and Chatzoglou (2009) researches.

Enjoyment had a direct and significant effect on intention to use. It's consistent with the results of Almaghrabi and Dennis (2011) and Shin and Shin (2011) researches.

Enjoyment had a direct and significant effect on perceived usefulness which is consistent with the results of Chatzoglou et al (2009) and Ha and Stoel (2009) researches.

Computer self-efficacy had a direct and significant effect on perceived ease of use which is consistent with the results of Terzis et al (2011) , Al-Harbi (2011), Bertta et al (2009), Agglidis and Chatzoglou (2009), Chatzoglou et al (2009), Thompson (2010) and Macharia and Nyakwende (2010) researches.

Perceived ease of use had a direct and significant effect on intention to use. It's consistent with the results of Thompson (2010), Peker (2010), Chatzoglou et al (2009), Roca and Gagné (2008) and change (2010) researches.

Perceived ease of use had a direct and significant effect on perceived usefulness. It's consistent with the results of Macharia and Nyakwede (2010), Thompson (2010), Bertta et al (2009), Agglidis and Chatzoglou (2009), Roca and Gagné (2008), Terzis et al (2011) and Peker (2010) researches.

Perceived usefulness had a direct and significant effect on intention to use. It's consistent with the results of Macharia and Nyakwende (2010), Al-Maghrebi and Dennis (2011), Shin and Shin (2011), Celik (2008), Ha and Stoel (2009), Chatzoglou (2010) and Peker (2010) researches.

The R^2 score of intention (0.38) revealed the role and importance of psychological and motivational variables in predicting employees' intention to use web-based training, moreover the final model showed the good fitness for predicting intention.

On the basis of the results, Perceived usefulness has the strongest direct effect on intention, so for increasing the employees' perception of the web-based training usefulness, it proposes that the company provides conditions by which employees become familiar with web-based training more and more to perceive advantages and benefits of this training system more and better. Holding seminars and conferences, running training courses for acquaintance with web-based training and its advantages and benefits can create the belief and trust in web-based training methods and enhance employees' perception of this training system usefulness.

Given the significant relation between perceived ease of use and intention, for increasing the employees' perception of this training system ease of use, the company should provide technical infrastructure such as proper bandwidth, hardware and software for connecting to internet and the company's intranet. The required forecasting, making preparation and investing, and top management support can be effective in this way.

Based on significant effects of computer playfulness and enjoyment on intention, the company should design web-based training programs as it will be interesting, amusing, enjoyable, exciting and according to employee's taste so that it can enhance employees' perception of web- based training enjoyment and playfulness.

Given the significant effects of computer self-efficacy and computer anxiety on intention, running training course for learning skills in relation to computer, internet, software and hardware can be effective for increasing employees' computer self-efficacy and reduction of their computer anxiety.

Table 1. Cronbach alpha values of the variables

variable	value
Perceived ease of use	0.83
Perceived usefulness	0.91
Computer playfulness	0.78
Intention to use	0.81
Learning goal orientation	0.84
Computer self-efficacy	0.82
enjoyment	0.87
Computer anxiety	0.84

Table 2. Correlation matrix of model constructs

variables	1	2	3	4	5	6	7	8
Perceived ease of use	1							
Perceived usefulness	0.325**	1						
Computer playfulness	0.307**	0.484**	1					
Intention to use	0.361**	0.549**	0.463**	1				
Learning goal orientation	0.336**	0.329**	0.272**	0.419**	1			
computer self-efficacy	0.368**	0.222**	0.220**	0.243**	0.436**	1		
enjoyment	0.252**	0.508**	0.508**	0.460**	0.363**	0.150	1	
Computer anxiety	0.502**	-0.029	-0.150	-0.113	-0.386**	0.368**	-0.206*	1

P**<0.01 p*<0.05

Table 3. Direct, indirect, and total effects between variables

effects	Direct effect	Indirect effect	Total effect	T value
Computer playfulness	.....	.....	.....	.....
Intention to use	0.17	0.17	0.34	2.19
Perceived ease of use	0.21	.....	0.21	5.94
enjoyment	0.43	....	0.43	5.94
Learning goal orientation	.....	.....	.....	....
Perceived usefulness	0.18	0.10	0.28	2.36
enjoyment	0.25	....	0.25	3.25
computer self-efficacy	0.35	....	0.35	4.31
Computer anxiety	.....	.....	....	.....
Perceived usefulness	-0.28	-0.13	-0.41	2.97
Perceived ease of use	-0.42	-0.04	-0.46	5.59
computer self-efficacy	-0.24	.....	-0.24	2.94
enjoyment	.....	.....	.....	.....
Intention to use	0.17	0.14	0.31	1.99
Perceived usefulness	0.43	.....	0.43	5.83
computer self-efficacy	.....	.....	.....	.....
Perceived ease of use	0.17	.....	0.17	2.29
Perceived ease of use	.....	....	.....	.....
Intention to use	0.16	0.09	0.25	2.26
Perceived usefulness	0.30	.....	0.30	3.77
Perceived usefulness	.....	.....	.....	.....
Intention to use	0.33	....	0.33	4.18

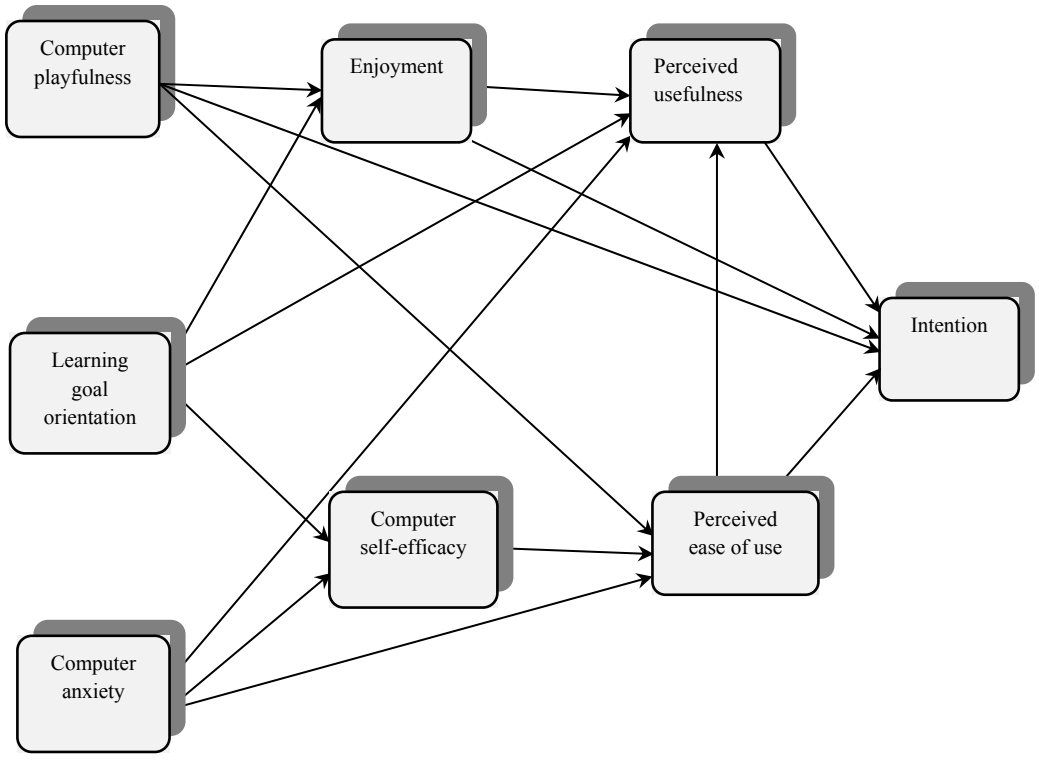

Figure 1: The proposed model of employees' intention to use web-based training

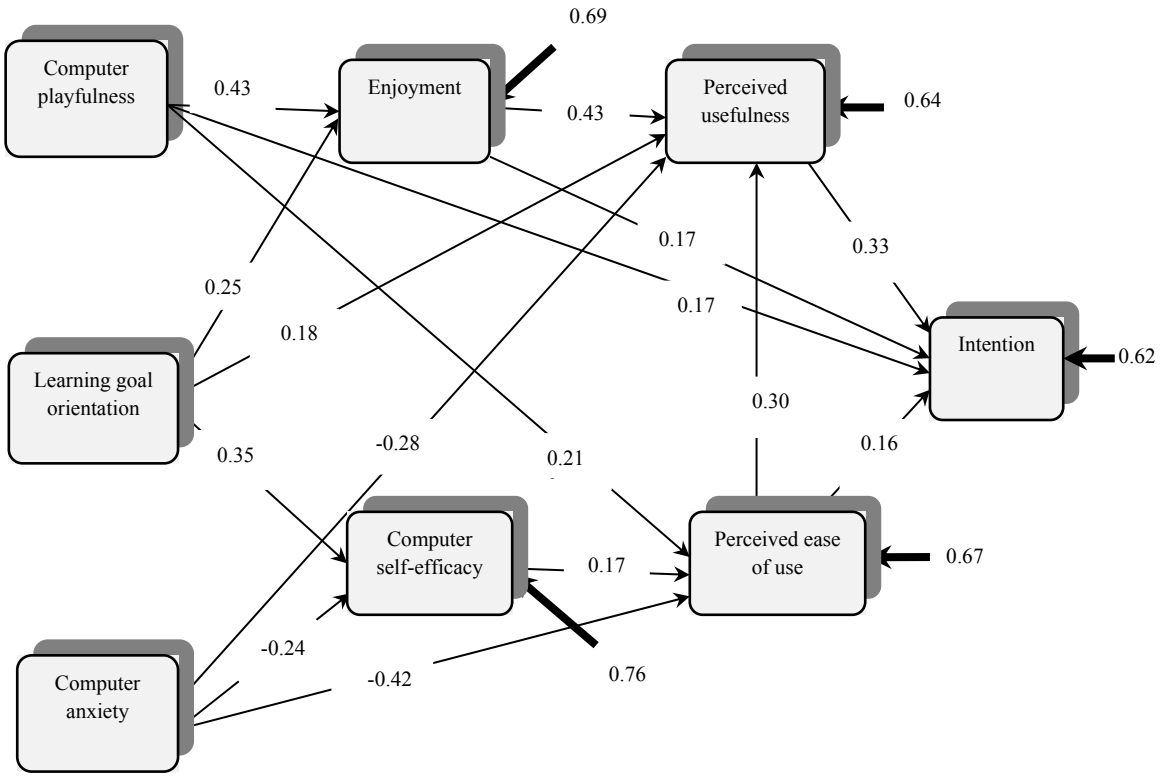

Figure 2: the measurement model of employees' intention to use web-based training

Based on significant effect of learning goal orientation on intention, the company should pay attention to individual differences of employees in order to make this training system more efficient. Finally, we propose that other effective causes of intention will be considered.

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Investigation and intervention on the psychological status of families with Hepatolenticular Degeneration children

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Abstract: Hepatolenticular Degeneration (HLD), also known as Wilson disease (WD) is an autosomal recessive copper metabolism disorder, the worldwide incidence of it is 1/100 000 to 1/30 000. In this study we will investigate the psychological status of family with Wilson's disease children, interventions to alleviate chronic sorrow of their families. And the result is that the parents of children with the observation group questionnaire sadness, anger, pain and the total degree of improvement was significantly better than the control group (all P <0.05). So we know presence of chronic sorrow the family in children with Wilson's disease, early assessment and implementation of targeted intervention, will help ease psychological burden of parents of children with epilepsy to reduce their family of chronic sorrow.

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Key words: Wilson's disease; chronic sorrow; Families of children with Wilson's disease; Adapted Burke Questionnaire (ABQ); Intervention

1. Introduction

Hepatolenticular Degeneration (HLD), also known as Wilson disease (WD) is an autosomal recessive copper metabolism disorder, the worldwide incidence of it is 1/100 000 to 1/30 000 [1]. The pathogenic gene carrier of the disease is about 1/90. It has a higher prevalence in the Chinese population mainly occurs in adolescents. The main clinical manifestations are characterized by tremors, muscle rigidity, and unclear articulation, mental disorders and cirrhosis of the liver. HLD is one of the few treatable genetic diseases of the nervous system; the key is early diagnosis, early treatment, late diagnosis or inappropriate treatment lead to disability or even death.

However, in clinical care, we found that most parents can not correctly treat the pathological behavior of the children. The lack of disease-related knowledge leads to incorrect responses, and thus can not provide a good family support to the children, impairs of treatment outcome and quality of life of the children. Olshansky [2] proposed the concept of chronic sorrow, a cycle of recurrent pain or sorrow of parents or caregivers, which can occur in different periods with children with severe or chronic disease. If the sorrow last more than 2 to 3 months, it becomes chronic sorrow. In this study, we investigated and analyzed the psychological condition of the families of HLD children and take specific care measures to improve the psychological status of the parents and caregivers.

2. Object and methods

2.1 Object 25 diagnosed HLD cases in the neurology clinic out-patient and ward of our hospital from July 2006 to July 2010, 19 males and 6 females, aged 2 to 19 years, the average age is 9.3 years. Three were brothers in the 25 cases. The first symptoms, in 13 cases (52.0%) were physical disabilities, in 6 cases (24.0%) were mental disorders, in 6 cases (24.0%) were liver damage. The age of onset was 17 years of age, duration ⩾ 1 month. Among the 25 parents, 11 were mothers, 14 were fathers, the average age of them is 35.34 years, and they all were the primary caregivers of the children. Education: junior high school and below were six (24.0%), high school were 12 (36.0%), college and above were 7 (40%). 25 parents were randomly assigned into two groups, 12 were in the observation group, and 13 were in the control group. The difference was not significant (P> 0.05) compared the proportion, age, education level and the children disease level of the two groups of parents.

2.2 Methods

2.2.1 Psychological status assess of the parents Adapted Burke, Questionnaire (ABQ) questionnaire were used [4]. The author modified some individual projects based on the specific situation of China, including eight kinds of emotional state, i.e., sadness, shock, anger, denial, pain, despair, fear and guilt. Take 0 = strongly to 3 = very strong,

four evaluation scores ranged from 0 to 24 points, the higher score means higher sad degree. Reliability Cronbach's alapha value is 0.935 [3].

2.2.2 Interventions

The control group received conventional treatment and care, and regular follow-up. The observation group received three months targeted family intervention according to the results of the assessment as follows:

2.2.2.1 Collective intervention ① HLD knowledge seminars by professionals for parents: twice a month, each time 60 ~ 90min, for three consecutive months, including the knowledge of HLD disease, medication knowledge, the importance and methods of diet care, the activities of daily living, and the role of harmonious family environment to control symptoms. After each lecture, we organized the parents to discuss and exchange the opinions and answer their questions. ②Setting up HLD hotline in the outpatient (opening every Saturday 8:30 to 11:30), neurology physicians of our hospital is responsible for answering and explaining, the parents of HLD children can ask their questions by telephone.

(1) **Psychological exchange** Family members with HLD children inevitably generate anxiety and fear. By the communication with them we found that the main reasons of anxiety and fear is the lack of understanding of the disease, the feeling of guilty for children and worried whether the brothers or sisters of HLD children is sick too when they know the HLD is a genetic disease. We explained the pathogenesis of the disease to family members of patients to inform them that the disease is curable genetic disease, and told them the treatment for the patient and the screening methods for other family members. This eased the anxiety of the families and gave them confidence to face the disease [4].

(2) **Treatment guiding** The families should develop a compliance behavior to the doctors, do not abuse their own drugs; do not change the dose and time. The drug should be stored in a cool, dry, dark, and fixed place. We explained the adverse reactions that may occur after taking the drug and told parents if the children feel sick they should come to hospital for medical treatment timely. The meals should for the HLD children should be light, easily digestible and rich in vitamins and fiber. The patients should maintain a low copper diet, do not drink the high-copper-containing water, do not eat high copper food such as seafood, nuts, mushrooms, beans and their products, do not use copper pots when cooking. Adding trace elements such as zinc, iron and calcium is recommended because they are antagonist of copper and can promote copper excretion.

(3) **Home care** Using a wet mopping for the floor, opening doors and windows regularly to ventilation. We suggested that the families purchase of air-cushion mattress, place intensity-regulate light fixture at bedside of the patients and the placed the patients in the low-rise building and low beds with shelf and air cushion mattress. We informed the potential security risks to the families to improve their security awareness, introduced the reasons early performance and dangers of pressure ulcers to the families. If the patient is bedridden, families should learn the expectoration method by chest percussion, which is an effective way to help the patients' sputum discharge.

2.2.2.2 Individual interventions Disease education and psychological counseling of the parents were specified to five specialist nurses for the specific circumstances of each family twice a month; each nurse was responsible for 3-4 families. Including: ① Adjustment of family communication, and learn to communicate with the children; to create a harmonious family environment, strengthen the exchange of feelings; to adjust expectations for children, such as the correct treatment of children with academic and daily living skills. ② The guidance for the parents of children to correctly identify adverse drug reactions, the recurrence symptoms and the corresponding approach, to improve the ability of parents to solve problems.③Answering the questions of parents of children patiently, to provide targeted guidance for children with behavioral skills and social function and rehabilitation. ④Arrangement more than 2 times family gatherings during the intervention (including children), to encourage parents to discuss their care experience and the existing problems, to establish mutual support networks. Exchange contact means between nurses and patients to communicate at any time when problem happens.

2.2.3 Methods of evaluation After three months continuous intervention ABQ questionnaire is used to evaluate the intervention effects.

2.2.4 Statistical methods SPSS software was used for statistical analysis. Repeated measures analysis of variance was used.

3. Results

ABQ score before and after the intervention of the two groups of parents (Table 1.)

Table 1. ABQ scores between two groups before and after intervention. $\bar{x} \pm s$

Group		sadness	shock	anger	denial	pain	despair	fear	guilt	Total
control (n=13)	before	2.79±1.2	2.08±1.2	2.11±0.9	2.66±1.6	2.98±1.4	2.24±1.6	2.13±1.3	2.34±1.4	2. 32±1.2
	after	2.44±1.2	1.67±0.9	2.38±1.2	2.45±1.3	2.92±1.3	2.65±1.3	2.19±1.2	2.84±0.5	2.15±1.5
Observation (n=12)	before	2.57±0.8	1.99±1.3	2.40±1.4	2.09±0.2	2.58±1.2	2.04±1.1	1.95±0.3	2.37±1.7	2.28±1.0
	after	1.64±1.1*	1.29±1.7	1.32±1.1*	1.15±1.4	1.77±0.9*	1.18±1.6	1.76±1.3	2.21±1.1	1.36±0.9*

Note: The two groups main effect, * P <0.05.

The results showed that: the improvement was significantly better than the control in the observation group in the scores on sadness, anger, pain, and the total (P <0.05).

4. Discussion

4.1 The reasons of family chronic sorrow: Chronic sorrow is a cycle, recurring pain or sorrow of parents or caregivers. It is also reported in the parents of children who suffered from mental retardation, developmental disabilities, early maturity, Down syndrome, neural tube defects, and chronic disease. Similarly, in the caregivers of adult patients with Parkinson's disease, multiple sclerosis, Alzheimer's disease and cancer it is also reported[5].In this study chronic sorrow may be related to the following factors: ① The HLD is a congenital genetic disease, parents tend to think that the pain of disease to the children is bought by themselves, so they feel pain, low self-esteem and guilt [2]. ②Knowing little about the disease: multi-system damage caused HLD, the adverse effects of the drug, and psychiatric symptoms coupled with the worry about the physical and mental development of the children, after their studies and to join the army, employment, daily living, emotional issues all these things increase the chronic sorrow in the parents of HLD children [6] ③ Economic issue is also a reason of chronic sorrow. Long-term drug application, as well as to deal with the attendant adverse effects, and periodic review of the inspection fees: all these things above give the family enormous psychological pressure and economic pressure. ④The children are very young, lack of self-expression and they can not take care of themselves, they need the care of family members for a long time. The parents have to spend much time to accompany their children so they do not have enough time with their r jobs and learning. If this last for a long time, chronic sorrow happens. All these above give the parents of HLD children many psychological problems.

4.2 Reasonable intervention to alleviate the psychological pressure of the family members of HLD patients Medical staff should give the parents of HLD children system and specification family support and health education, to help to improve the level of awareness of the disease and care of the children; should inform the parents the good prognosis of adherence treatment in this disease, so that they can see the prospect of treatment, and establish the confidence to adhere treatment, and adhere to the standardized systematized treatment; should let children get a good therapeutic effect[7], thus may improve the family atmosphere, to the ultimate improve the negative psychology of parents of children. Table1 showed that in sadness, anger, pain, as well as total scores the observed group of parents improved significantly better than the control group (P <0.05), suggesting that a reasonable intervention can effectively alleviate the chronic sorrow in the family with HLD children.

The warmth and good care of the family has a large impact on the treatment and rehabilitation of the patient member. Family not only can protect and promote the function of members of the health, but also can provide all the necessary care and support to sick members. Therefore, being a nurse, we should give mental and psychological care and support to the family members of patients, and have conversation with them by charisma and good interpersonal skills , to establish a harmonious relationship of mutual trust, so that they are willing to accept the views of nurses,

and consciously coordinate with the care and guidance.

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Nikethamide increases sodium current of inspiratory neuron via PKC pathway in mNRF of neonatal rats in vitro

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Abstract: To elucidate the roles nikethamide on sodium currents of inspiratory neurons in the medial area of nucleus retrofacialis (mNRF) and whether PKC take part in those roles, whole-cell patch were performed to record sodium current of inspiratory neuron(I neuron) and ELISA double-antibody sandwich method were used to measured concentration of PKC in mNRF. Nikethamide increases persistent and transient sodium current of I neuron. It makes steady activation curves shifted to more negative potential and steady inactivation curves shifted to more positive potential of sodium channel. Determination the concentration of PKC of neurons in mNRF by ELISA , nikethamide increases concentration of PKC of neurons in mNRF. Nikethamide makes sodium chaanel open at a lower membrane potential and close at a higher membrane potential, it increases open lasting time and open probability of sodium channel via PKC pathway.

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Keywords: Nikethamide; sodium; current; inspiratory; neuron; neonatal rats; in vitro

1. Introduction

Our researches have demonstrated that the medial area of nucleus retrofacialis (mNRF) is the site of respiratory rhythmogenesis[1,2]. Smith stated that the pre-B Ö tzinger complex (PBC) was the site of respiratory rhythmogenesis in 1991. Both mNRF and PBC are located in rostral ventrolateral medulla, although the anatomical position are different, but they are overlapped partly. Nikethamide (N, N-diethylnicotinamide) has been used widely in clinic as : first, used as a respiratory central stimulator, it can excite respiratory center selectively[3]; second, nikethamide decreases the aminopherase and jaundice levels of infant[4]; thirdly, niketnamide comprise with the polarity part and the nonpolarity part, it can be used as chaotropic agent to dissolve drugs which is difficult to dissolve in water[5]. Nikethamide increase basic rhythmic resoiratory discharge activities of medullary slice and action potential of inspiratory neuron(I neuron) of mNRF. The present study was designed and performed to to know how nikethamide increases excitability of inspiratory neuron and whether sodium current and PKC take part in those roles.

2. Materials and methods

2.1 Materials

Neonatal Sprague–Dawley rats, both males and females, 0-3 days, n=7, were supplied by Experimental Animal Center of Xinxiang Medical University. Nikethamide were bought from Sigma, others for artificial cerebral spinal fluid (ACSF) are Of analytical grade.

2.2 Medullary slice preparation.

All experiments used the transverse, rhythmic medullary slice, which can generate respiratory-related motor output. The Office for the Protection of Research Subjects, Xinxiang medical university Research Committee approved all protocols. Neonatal Sprague–Dawley rats were deeply anesthetized with ether (delivered by inhalation) and quickly decapitated at the C3-C4 spinal level. The brainstem was dissected in ice-cold ACSF containing in mM: 124 NaCl, 5 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$, 1.2 KH_2PO_4, and 30 D-glucose, equilibrated with carbogen (95% O_2 and 5% CO_2), pH 7.4. The brainstem was serially sectioned in the transverse plane until the nucleus ambiguus and inferior olive were visible. Then a rhythmic 750μm-thick slice containing the mNRF was obtained by slicing the medulla using a Vibratome (VT1000S, Leica, Germany). Slice was quickly transferred to a recording chamber and continuously perfused with oxygen-saturated ACSF at a rate of 8–10 ml/min at 27–29℃ in a 3ml recording chamber.

2.3 Electrophysiological recordings

The discharge of hypoglossal nerve (XII nerve) rootlets were recorded serving as a marker of fictive inspiration by using suction electrodes(inner diameter 180μm). Signals were amplified and band-pass filtered (100 Hz–3.3 kHz), data were sampled (5 kHz) and stored in the computer via BL-420F biological signal processing system. Whole-cell patch-clamp recordings were performed using an MultiClamp 700B amplifier (Molecular Devices) in current-clamp and voltage-clamp mode. I neuron were visualized using

infrared-enhanced differential interference contrast (IR-DIC) video microscopy(NIKON). Electrodes were pulled from borosilicate glass (outer diameter, 1.5mm;inner diameter, 0.86mm) by a horizontal puller (model P-97; Sutter Instruments, Novato, CA). The electrodes used for voltage-clamp recordings to isolate sodium currents were filled with a solution containing: 110 mM CsCl, 30 mM TEA-Cl, 1 mM CaCl2, 10 mM EGTA, 2 mM MgCl2, 4 mM Na2ATP, and 10 mM HEPES, adjusted pH to 7.2 by CsOH. The result of this pipette solution was small LJP (3mV), which was not corrected in this study.

Only I neuron active in phase with population activity were considered in this study[2,6]. The discharge pattern of each cell type was first identified in the cell-attached mode. After recording, CNQX, CPP, strychnine, and bicuculline were bath applied to isolate chemical synaptic input of neuron, the neuron which stop to burst in the absence of inspiratory population bursts is I neuron, show in Fig. 1.

2.4 Determination the concentration of PKC of neurons in mNRF by ELISA double-antibody sandwich method.

Prepare the "mNRF island" from medullary slices according Johnson SM[7], Sixteen islands were divided into two equal groups randomly: control group and nikethamide group. The control islands were incubated for 30 minutes in ACSF, islands of nikethamide group were incubated for 30 minutes in ACSF in which dissolved 0.02mol / L nikethamide. After incubated, determination the concentration of PKC by the P-PKC kit of ELISA double-antibody sandwich method(repeated three times)[8].

2.5 Statistical analysis

All values were expressed as means±SEM and statistical comparisons were evaluated using repeated measures analysis followed by Student's t-test where appropriate. A probability value less than 0.05 ($P < 0.05$) was considered as statistically significant.

3. Results

3.1 Nikethamide increased transient sodium current and persistent sodium current of I neurons.

Experiments were performed in the whole-cell patch-clamp mode with the neurons recorded in current clamp at the zero potential. Establishing the whole-cell patch-clamp configuration did not alter the firing pattern of recorded neurons. In voltage-clamp configuration, voltage steps from -80 to +20 mV and slow voltage ramps from -80 to +20 mV (90 mV/ sec) were applied to elicit transient and persistent sodium currents, respectively[9,10]. Both the persistent and transient inward currents recorded in I neurons were sensitive to TTX. The persistent sodium currents and the transient currents of I neurons were changed from 1628.33pA±276.94 pA to 1961.33±252.86 pA, 295±58 pA to 320±71 pA before and after nikethamide were purfused, show in Fig. 2($n=7$, $P < 0.05$).

3.2 Effects of nikethamide on steady-state activation curves and steady-state inactivation curves of sodium channel in I neurons.

To establish steady-state activation curves, cells were held at −70 mV, and a series of 20 ms pulse from −70 to 0 mV in 10 mV increments were followed by a 500 ms test pulse of −130 mV[9,10]. The peak currents of membrane potential were converted into conductance calculated from the equation: $G= I/(Vm -Vrev)$, where Vm is the membrane potential and Vrev is the reversal potential. The normalized conductance was fitted by a Boltzmann equation: $I/Imax=I/ \{ 1+\exp[(V-V1/2)/\kappa] \}$ where V1/2 is the membrane potential at half-activation and κ is the slope factor. The steady-state activation curves of sodium current before and after exposure to nikethamide are shown in Fig. 3A. The curve was shifted to more negative potential after application of nikethamide. The value of $V_{1/2}$ for INa activation changed from −34.49 ± 0.73 mV in the absence of nikethamide to −40.45± 2.00mV in the presence of nikethamide, with a corresponding change in slope factor κ from 5.67 ± 0.47 to 4.54± 0.32, show in Fig. 3 ($n=7$, $P < 0.05$).

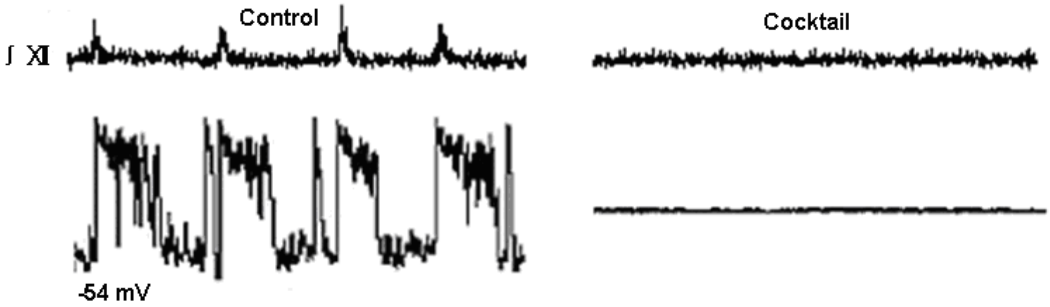

Fig. 1 Identification of inspiratory neuron

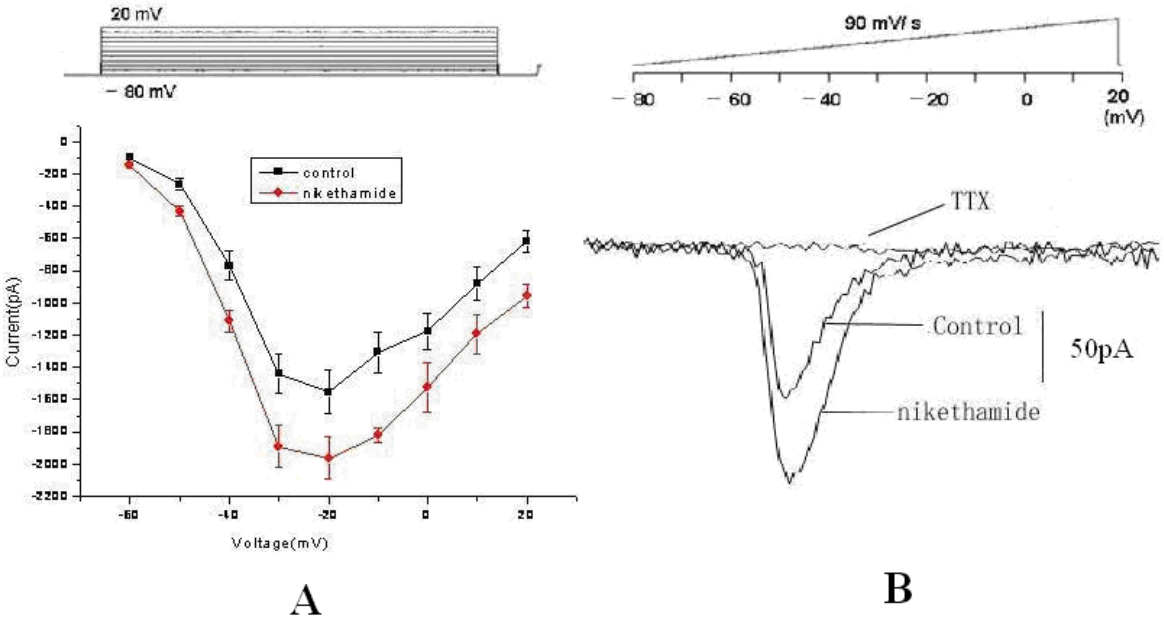

Fig. 2 Nikethamide increases transient sodium current(I-V curve, A) and persistent sodium current(B) of I neurons

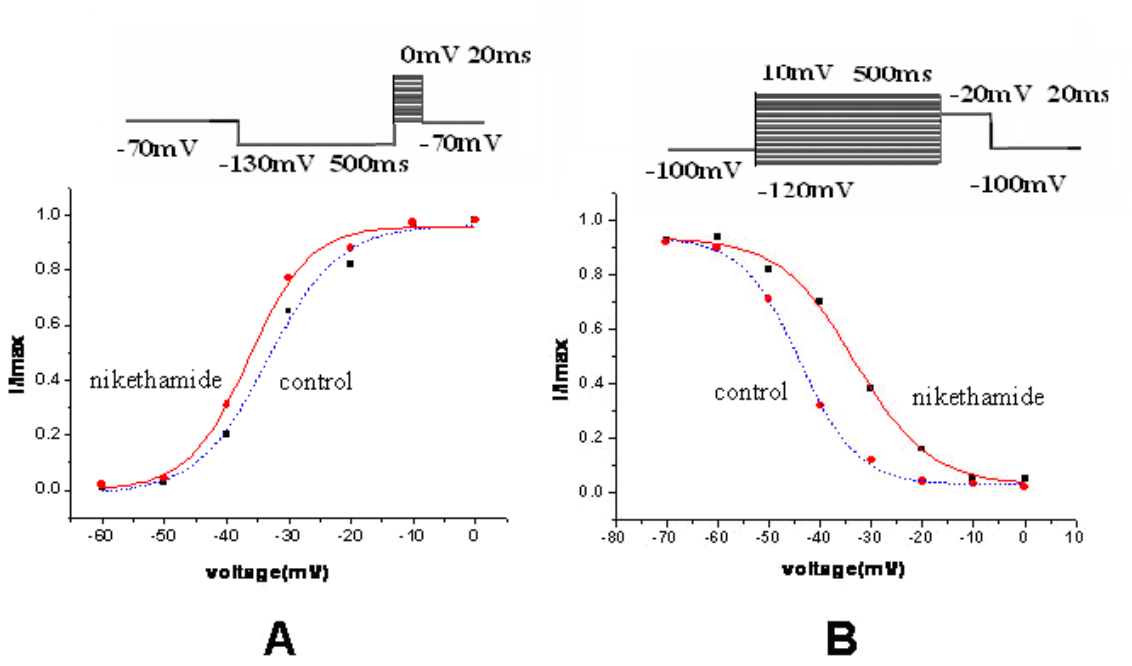

Fig. 3 Roles of nikethamide on steady-state activation curves (A) and steady-state inactivation curves (B)of sodium channel I neurons

To investigate the effects of nikethamide on steady-state inactivation properties of INa. Cells were held at −100 mV, and a series of 500 ms prepulse from −120 to 10 mV in 10 mV increments were followed by a 20 ms test pulse of −20 mV[9,10]. The inactivation curves were fitted by a Boltzmann equation:$I/I_{max}=I/\{1+\exp[(V-V_{1/2})/\kappa]\}$, where $V_{1/2}$ is the membrane potential at half-activation and κ is the slope factor. The curve was shifted to more positive potential after application of nikethamide, Fig. 3B. The value of $V_{1/2}$ was −46.85 ± 1.59 mV without nikethamide, but was changed to −35.02±1.55 mV in

the presence of nikethamide, with κ varying from 5.34 ± 0.33 to 6.67± 0.30, show in Fig. 2 (n = 7, P < 0.05).

3.3 The concentration of PKC of neurons in mNRF was 29.24% higher than control group.

After incubation with nikethamide the concentration of PKC of neurons in mNRF was 29.24% higher than control group. The concentration of PKC of niketahmide is 0.31±0.014, and the control group is 0.24±0.010.

4. Discussions

This study was performed in in-vitro brainstem slices containing the neurons critical for integration of respiratory drive. The respiratory frequency of this preparation was markedly slower than that in vivo due to the isolation of nervous system from mechanosensory afferent inputs and the removal of vagal mechanosensory afferent inputs[11]. However, the discharge patterns of respiratory motor neurons in vitro were similar to that in the intact mammal but different from gasping[12,13]. The I neurons, which appear to be fundamental components of the inspiratory pattern generation, have been proposed to be responsible for respiratory rhythm.

In this study, nikethamide has a exciting roles on sodium current, it increases transient and persistent sodium current of I neurons. Nikethamide makes sodium channel steady activation curves change towards hyperpolarization, and makes steady inactivation curves change towards depolarization. Nikethamide makes sodium chaanel open at a lower membrane potential and close at a higher membrane potential, meaning nikethamide increase open lasting time and open probability of sodium channel, increase exciting of I neuron.

After enter neuron, nikethamide transform into nicotinic amide through deacetylation, nicotinic amide transform into N-Methylnicotinamide through methylation. Nikethamide excreted through kidney in N-Methylnicotinamide. Nicotinic amide is a material for synthesizing $NADP^+$[14]. Nikethamide increase concentration of $NADP^+$, $NADP^+ \rightarrow$ superoxide $\rightarrow$ superoxide dismutase $\rightarrow$ H_2O_2, increase reactive oxygen species (ROS), and ROS activate Protein kinase C[15,16]. In our study, after being incubated with nikethamide, the concentration of PKC was higher than control. PKC increase open probability of sodium channel through phosphorylation and increase concentration of reactive oxygen species[17,18]. Reactive oxygen species can increase open probability of sodium channel too[19]. The increasing open probability of sodium channel increasing the excitability of I neurons and respiratory center.

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