Vol.25, NO.01 January, 2014

ISSN 1029 - 385 X

MEDICAL FORUM MONTHLY

APNS Member | CPNE Member | ABC Certified

RECOGNIZED BY PMDC

Journal of all Specialities

"Medical Forum Monthly" Indexed by WHO Index Medicus (IMEMR) for EMRO Region Since 1997, Recognized by Pakistan Medical and Dental Council Islamabad Since 1998, Extra Med is Covered by EXCERPTA MEDICA, Netherlands Since 2000, CAB abstract and Global Health of UK, is Registered with international serials data system of France bearing ISSN No. 1029-385X. Medical Forum is also indexed in Medlip of CPSP Karachi and Pakmedinet Islamabad Pakistan.

MEDICAL FORUM MONTHLY

ISSN 1029 - 385 X

APNS Member | CPNE Member | ABC Certified

Recognized by PMDC
(Reg.No.IP/0048)

Recognized by Higher Education Commission, Isd.
Recognized by the Information Dept. Govt. of Pak. Isd.
(Press Reg. No.1212 Copr)

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15-A, Abbot Road, Behind PS, Qila Gujar Singh, Lahore – Pakistan
Ph: 92-42-36361436 Cell: 0331-6361436
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Printed By — **Syed Ajmal Hussain**
Naqvi Brothers Printing Press, Darbar Market, Lahore

Rate per Copy — **Rs.1200.00**

Subscription Rates — **Annually**

Pakistan	**Rs.9000.00**
USA & Canada	US$ 350.00
China & Japan	US$ 300.00
United Kingdom	US$ 300.00
Middle East	US$ 250.00

Med. Forum, Vol. 25, No.1 January, 2014 **ISSN 1029-385-X**

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Editorial

Mortality may be Reduced after Hip or Knee Replacement – A New Study

Mohsin Masud Jan
Editor

The risk of death from hip or knee replacement surgery as dropped substantially in recent years. Dutch researchers found that since the early 1990s, death rates have fallen by almost two-thirds among Danish adults having the procedures. The length of patients' hospital stays also dropped — from more than two weeks, on average, to about one week.

The study did not dig into the reasons for the improvements, but it's likely that changes in post-surgical care have had a big impact, said lead researcher Arief Lalmohamed, of the Utrecht Institute of Pharmaceutical Sciences in the Netherlands.

Those changes, he said, include new blood-thinning medications that help prevent patients from developing potentially dangerous blood clots after surgery. Clots can, in some cases, lead to a heart attack, stroke or pulmonary embolism. In the United States, more than 1 million people have a hip or knee replacement each year, according to the U.S. National Institutes of Health. The surgery often is prompted by severe wear and tear on the joints due to arthritis.

The findings, reported recently in the Journal Arthritis & Rheumatology, are based on data from only one country. But Lalmohamed said he would expect to see a similar pattern in other countries that made the same changes in medical care over the years. Dr. Richard Iorio, chief of adult reconstruction at NYU Langone Medical Center in New York City, agreed that the trend would be similar in the United States. Iorio, who was not involved in the study, named a number of advances that have been made over the years to make joint-replacement surgery safer and better. Changes in the procedures and anesthesia techniques have been key, Iorio said. And patients start physical rehab much faster than they did in years past. "We get people out of bed and moving on the first day after — or the day of — surgery," Lorio said. That mobility is important, he said, because it lowers patients' risk of developing blood clots. Iorio said doctors have also gotten better at managing chronic health conditions that many patients have. That, in turn, lowers the risk of complications.

For the study, Lalmohamed's team turned to Denmark's system of national health registries. The researchers found information on more than 112,000 people who had a hip or knee replacement between 1989 and 2007. Overall, the rate of death in the two months after surgery fell over time, from about 3.4 percent each year between 1989 and 1991 to 1.4 percent per year between 2003 and 2007, Lalmohamed said. Deaths from heart attack, stroke and pneumonia all dropped, despite the fact that heart and lung disease was more common in patients who had surgery in recent years.

Lalmohamed said there's still a need for similar studies in other countries. But he also said candidates for joint replacements can be reassured by his team's findings. Lorio agreed, "Clearly, patients can take heart," he said. "This operation is safer than it was 20 years ago, and it's very effective."

However, some surgeons and hospitals are better than others. In general, surgeons and centers with the most experience in hip and knee replacements have better results than those who do fewer procedures.

And, of course, each patient is different, Lorio said. An individual's overall health — rather than age alone — is vital. But, he added, it's also possible to manage some of the health issues that can increase the risks of joint-replacement surgery. Patients can quit smoking or lose excess weight, for example.

Original Article

Anatomy

Hepatoprotective Effects of Curcuma Longa against Carbon Tetrachloride Induced Liver Injury in Rats

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ABSTRACT

Objective: To investigate the protective effect of Curcuma longa (CL) against carbon tetrachloride (CCl_4) induced liver injury in adult male Wistar rat model.

Study Design: Experimental/Analytical study

Place and Duration of Study: Animal House, Isra University Hyderabad from March to December 2013.

Subjects and Methods: Forty five adult male Wistar rats were divided into three groups; Group 1. controls received 0.9% isotonic saline, Group 2. received CCl_4 orally (1.9mg/kg) mixed in olive oil, and Group 3. received the CCl_4+CL (250mg/kg) Blood samples were collected for liver biochemical assays. The animals were sacrificed, liver tissue, after fixation in 4% formaldehyde, was embedded in paraffin. Tissue sections of 5µ thickness were subjected to haematoxylin and eosin staining and were assessed by light microscopy. The data was analyzed on SPSS 21.0 using one-way ANOVA, Fischer`s LSD and Chi-square tests. A p-value of ≤ 0.05 was taken statistically significant.

Results: The liver biochemical and histological findings reveal statistically significant differences among the controls, CCl_4 and CCl_4+CL groups (p=0.0001). Liver enzymes and histology were deranged significantly in CCl_4 group compared to controls and CCl_4 +CL group (p=0.0001). The CCl_4+CL group showed less elevation of liver enzymes and derangement in liver histology compared to CCl_4 group (p=0.001). The histological findings of congestion, inflammatory cell infiltrate, vacuolar degeneration and necrosis are found prominent in CCl_4 group.

Conclusion: The Curcuma longa protects against oxidative damages caused by carbon tetrachloride induced liver injury in rat model.

Key Words: Curcuma longa, Carbon Tetrachloride, Liver injury.

INTRODUCTION

Curcuma longa (CL) is a rhizomatous perennial herb that belongs to the family Zingiberaceae, native to South Asia and is commonly known as turmeric.[1] In Sindhi, it is commonly known as “Hade”. The turmeric plant is a popular ingredient for preparing culinary dishes. In addition, it is used as herbal remedy due to the prevalent belief that the plant has medical properties. In folk medicine, the rhizome juice from C. longa is used in the treatment of many diseases such as anthelmintic, asthma, gonorrhea and urinary, and its essential oil is used in the treatment of carminative, stomachic and tonic.[2] In traditional medicine, several plants and herbs have been used experimentally to treat liver disorders, including liver cirrhosis. [3,4] C. longa possesses antioxidant[5], anti-tumor[6], antimicrobial[7], anti-inflammatory[8], wound healing[9], and gastroprotective activities.[10]

Carbon tetrachloride (CCl_4) is a hepatotoxic compound. The CCl_4 has been has been used extensively in laboratory animals for induction of liver injury, elucidate the underlying mechanism of liver injury and hepatoprotective effects of various therapeutic agents.[11]

One of the postulated mechanism of CCl_4 induced liver injury is the formation of ROS. The ROS disrupts the hepatocyte at cell membrane level through the lipid peroxidation[11, 12] causing anatomical disruption of liver architecture and physiological disturbances.[13] The hepatocyte injury causes leakage of cytoplasmic and mitochondrial enzymes in the blood streams.[14]

The cytoplasm and mitochondrial enzymes of hepatocytes are clinically used as markers of liver injury, and for monitoring and treating the liver diseases. The liver enzymes which appear in the blood as a result of liver injury include; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase (ALP) and lactate dehyderogenase (LDH) are important enzymes that are often employed in assessing liver injury.[11,15]

The previous studies have shown that the aqueous extract of C. longa has hepatoprotective activity against carbon tetrachloride toxicity[19]. The present study aims to investigate the possible hepatoprotective effects of the ethanolic extract of Curcuma longa rhizomes against Carbon tetrachloride (CCl_4)-induced hepatotoxicity in adult male Wistar rat model.

MATERIALS AND METHODS

The present original study was conducted at the animal house of Isra University from March to December 2013. Adult male Wistar rats of 250-300 grams were included while female rats and rats weighing <250 grams or >300 grams were excluded from the study protocol. The animal's house is well equipped with essential facilities like an optimal room temperature with 55-60% humidity and exposure to 12 hour light-dark cycles. The fresh alfalfa and clean water are provided freely. The rats were divided into three groups;

Group 1. Control Group (n=15) Rats received 0.9% isotonic saline orally on alternate day for three successive weeks and served as control group,

Group 2. Carbon tetrachloride Group (n=15) Rats were given CCl_4 orally mixed in olive oil on alternate day for three successive weeks and

Group 3. Experimental Group (n=15) Rats received Curcuma longa (250 mg/kg) and CCl_4 on alternate days for three successive weeks

Experimental Details: The CL was purchased from Medical store of Isra University Hospital. The Curcuma longa was administered in a dose of 250 mg/kg orally.[16] Carbon tetrachloride was purchased from scientific drug store at Hyderabad City. The CCl4 dissolved in olive oil as vehicle (1:1 Ratio) at a dose level of 1.9 ml/kg orally on alternate day for three successive weeks and sacrificed at the end of their respective period of time.[15] The animals were sacrificed using standard method as described by Nayak et al. (2006)[17] In order to examine the liver tissue, the liver of the sacrificed animals was removed promptly and preserved in formaldehyde.

Blood sampling: The blood samples were collected from tail at twenty four hours of experimental period. Sera were separated by centrifugation at 300xs for ten minutes. Serum samples were used to estimate liver enzymes.

Biochemical assay: Liver enzyme assays were determined for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase (ALP) and lactate dehyderogenase (LDH) using commercially available diagnostic kits.

Histological studies: After fixation in 4% formaldehyde, samples were embedded in paraffin. Sections of 5µ thickness were subjected to haematoxylin and eosin Hepatic morphology was assessed by light microscopy. A total of five sections for each liver tissue sample were observed under light microscope.

In H & E staining, damaged hepatocytes graded as 0= normal, += mild damage (swollen and pale cytoplasm), ++= moderate damage (vacuolated cytoplasm), +++= severe damage and ++++= very severe damage (pyknotic nucleus and eosinophil cytoplasm).[18]

The data was analyzed on SPSS version 21.0 (IBM corporation). The continous variables were presented as mean±SD using one-way ANOVA and Fischer`s LSD test. Chi-square test was used for categorical variables. A p-value of ≤ 0.5 was taken statistically significant.

RESULTS

The present study observes major differences in liver injury among groups as indicated by blood enzyme levels in animal groups. The serum LDH, ALP, and ALT, AST of Rats treated with carbon tetrachloride were found elevated compared with control group after three weeks, with a highly significant p-value (p=0.001).

The CCl_4+ Curcuma longa (CL) group shows a significant reduction in the liver enzymes compared with the CCl_4 group (p=0.001) and control group (p=0.001). The animals CCl_4+CL group shows significant reduction in the liver enzyme elevation compared to CCl_4 group alone (p=0.001). The finding shows significant hepatoprotection by the CL in CCl_4 induced injury. The liver enzyme assays among different groups are shown in table.1.

Table. 1. Biochemical liver parameters in controls, *CCl_4 and CCl_4+ Curcuma longa (CL) groups (n=45)

Groups	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	ALP (IU/L)
Group. 1 (Controls)	48.9± 3.19	91.3± 15.1	712.4± 41.7	83.6± 8.71
Group. 2 (*CCl_4)	189.6± 11.91	479.7 ± 19.9	2278.8± 117.6	165.1± 8.02
Group. 3 (*CCl_4 + CL)	87.7± 17.92	181.3± 18.3	1938.6± 141.3	135.7± 18.1

*Carbon tetrachloride

Table. 2. Histology of liver injury of controls, *CCl_4 and Curcuma longa (CL) groups (n=45)

Groups	Inflammatory cell	Congestion	Vacuolar degeneration	Necrosis
Group. 1 (Controls)	0	0	0	0
Group. 2 (*CCl_4)	++++	++++	+++	++++
Group. 3 (*CCl_4 + CL)	+++	++	++	++

*Carbon tetrachloride

Different parameters of histological score of liver injury are shown in Table. 2. The Liver sections of the control group animals show intact central venules and hepatocytes arranged in compact cords. Normal looking hepatocytes with prominent nucleus, nucleolus and well preserved cytoplasm were seen in control group. On the contrary, the CCl_4 group shows derangement of hepatocytes cords, hydropic changes with congestion of central venules and sinusoids, and abundant

inflammatory cell infiltration. The centrilobular hepatocytes show hydropic changes and necrosis, while midzonal and peripheral hepatocytes show vacuolar degeneration and fatty changes in CCl_4 group. In CCl_4+CL animals, liver tissue sections reveal less significant derangement of hepatocytes cords, hepatocytes damage and necrosis was limited compared with CCl_4 group.

DISCUSSION

The present study is an original research work, which investigates the effect of Curcuma longa (CL) on carbon tetrachloride (CCl_4) induced liver injury in adult male Wistar rats. The Null hypothesis is rejected because the study observes hepatoprotective effects of CL as evidenced by biochemical and histological marker of liver injury.

Curcumin, the most common antioxidant constituent of Curcuma longa rhizome extract, was reported to enhance apoptosis of damaged hepatocytes which might be the protective mechanism whereby curcumin down-regulated inflammatory effects and fibrogenesis of the liver. [19]

The present study shows liver damage caused by the carbon tetrachloride as indicated by serum levels of liver enzymes compared to control group in rat model. The carbon tetrachloride induced liver injury with release of liver enzymes is comparable finding to reported previously by Hurkkeri et al.[20] The Hurkkeri et al.[20] reported elevated hepatocyte enzyme of liver as a consequence of CCl_4 induced liver injury in animal model. The release of large quantities of cytoplasmic and mitochondrial enzymes of liver is a clinical indicator of hepatocyte cell membrane damage and rupture sufficient to produces change in enzyme levels in blood.[21]

The ethanolic extract of C. longa rhizomes showed a significant hepatoprotective effect when orally administrated in doses of 250 mg/kg. The main constituents of CL extract are the flavonoid curcumin and various volatile oils, including tumerone, atlantone, and zingiberene.[1] The hepatoprotective effects of turmeric and curcumin might be due to direct antioxidant and free radical scavenging mechanisms, as well as the ability to indirectly augment glutathione levels, thereby aiding in hepatic detoxification.[22] The volatile oils and curcumin of C. longa exhibit potent anti-inflammatory effects.[23]

The present study shows that the damage of liver caused by CCl_4 is evident by the rise in serum enzymes levels beside the histological changes in liver tissue. Administration of CCl_4 significantly increases the serum levels of liver enzymes; LDH, ALP, ALT and AST, which are indices of hepatocyte damage and leakage of enzymes from cells. [24,25]

The histological examination of present research study correlates in parallel to disturbance in biochemical markers of liver injury. The histology of liver tissue shows disruption of liver architecture, hepatocytes, hepatic lobules and arrangement of hepatocytes in cords. The hepatocytes show findings of cellular injury with marked cytoplasmic vacuolization. The injured hepatocytes show pyknotic nuclei with lymphocyte infiltrations. The pyknotic nuclei are a sign of severe cellular injury caused by a toxin like carbon tetrachloride. The histological and biochemical findings of present study are comparable to those mentioned previously.[26,27] The carbon tetrachloride is metabolized to free radical during its metabolism and detoxification in smooth endoplasmic reticulum by the cytochrome P450.[28] The findings of present study are highly comparable to a recent study of Salma et al.[1] The Salma et al[1] reported hepatoprotective effects of CL in thiacetamide induced liver cirrhosis in rat models. The present study concludes that the Curcuma longa decreases the carbon tetrachloride induced oxidative stress and liver damage.

CONCLUSION

The Curcuma longa protects against oxidative damages caused by carbon tetrachloride induced liver injury in rat model. The Curcuma longa may be used as an effective protector against chemical induced liver damages, however, further studies are warranted.

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Original Article

Uterovaginal Prolapse

Proportion of Urinary Symptoms in Pre and Post-Menopausal Women with Uterovaginal Prolapse

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ABSTRACT

Objective: To compare the proportion of urinary symptoms in pre and postmenopausal women with uterovaginal prolapse.

Study Design: Comparative Study.

Place and Duration of Study: The study was carried out at the Department of Obstetrics & Gynecology Unit II, Dow University of Health Sciences and Civil Hospital Karachi from July 2005 to January 2006.

Materials and Methods: Sixty consecutive patients (30 premenopausal and 30 postmenopausal) were included in the study through structured Proforma from the out patient ward or emergency. Informed consent was obtained. A detailed history and related examinations and investigations were done. These include urine DR, Urine C/S and Urodynamic like Cystometry in selected patients.

Results: In this study the difference of urinary symptoms in pre and postmenopausal women of Uterovaginal prolapse were statistically found insignificant like frequency of urine (26.7% versus 33.3%), Urgency (20% vs 26.7%), Nocturia (26.7% vs 13.3%), Dysuria (40% vs 26.7%), Voiding problems (40% vs 46.7%), Urge incontinence (40%vs 20%). Stress incontinence was slightly higher in postmenopausal group than premenopausal (53.3%vs 46.7%) but this difference was found insignificant, while parity status between these two groups had significant difference like parity 2-5 was higher in pre-menopause group than postmenopause (66.7% vs 36.7%) and parity 6-10 was higher in postmenopause group than pre-menopause (63.3% vs 26.7%).

Conclusion: Significant difference in parity was found between pre and post-menopausal women with uterovaginal prolapse but the difference of urinary symptoms in pre and postmenopausal group was found significant. Uterovaginal prolapse associated with different urinary symptoms especially incontinence and voiding problems. These urinary symptoms effect over quality of life of women. This warrants greater attention for Gynecological health needs in our country by safe family planning practices.

Key Word: Uterovaginal Prolapse, Pre–Menopausal, Post-Menopausal

INTRODUCTION

Uterovaginal prolapse is the descent of vagina and uterus due to the loss of integrity of structures that support the contents of female pelvis[1]. The exact incidence of uterovaginal prolapse is difficult to determine. It has been estimated that a 50% of parous women lose pelvic floor support, resulting in prolapse and about 10-20% of them seek medical care[2]. A WHO multicentre collaborative study carried out in Pakistan identified at least 22% women with uterovaginal prolapse who attended health care facility[3]. The chance of a woman having a prolapse increases with age. Therefore the incidence of prolapse will rise as life expectancy increases[4]. Uterovaginal prolapse is usually the result of childbirth, menopause (due to deficiency of estrogen), increase intra abdominal pressure, injury to sacral nerves S1-S4 or diabetic neuropathy. Congenital weakness of pelvic support causes prolapse in young nulliparous women, prolapse of vagina may occur after hysterectomy[5]. Prolapse is often asymptomatic. The usual presenting symptoms in patients with uterovaginal prolapse are something coming down of vagina, lump in vagina and backache which is aggravated by standing and eased by lying down[6]. Prolapse is frequently associated with urinary complains like increase frequency, urgency, nocturia, hesitancy, urinary incontinence or incomplete emptying. In severe cases of incomplete bladder emptying, retention of urine may occur. Voiding dysfunction may result in increase frequency of urinary tract infections and occasionally, overflow incontinence. Due to the kinking of the urethra, stress incontinence and even intrinsic sphincter deficiency may be over looked[7]. The world wide prevalence of urinary symptoms in association with prolapse varies ranging from 5 to 39%. In Pakistan, hospital based study on urinary incontinence reported the frequency of urinary incontinence as 20.5%. According to one study conducted by community health centre of Agha khan University Hospital (AKUH) from November 1stto30th, 2002 stress incontinence was the highest reported complaint (38.4%) followed by burning (34.4%), frequency (26%), painful micturition (20.4%), urge incontinence (18.8%), incomplete empting of bladder (14.4%) and poor stream (8.4%) [8].

Urogynecology as a subspecialty has not been introduced in Pakistan and we believe the extent of significant lower urinary tract symptoms has been hugely underestimated [9]. The severity of these symptoms is more in postmenopausal women[10]. The rationale for conducting this study is based on the hypothesis that the proportion of urinary symptoms will be significantly different in the pre and postmenopausal women with uterovaginal prolapse. The aim of our study is to compare the proportion of urinary symptoms in pre and postmenopausal women with uterovaginal prolapse.

MATERIALS AND METHODS

The study was carried out in the department of Obstetrics & Gynecology unit II, Dow University of Health Sciences and Civil Hospital Karachi, Pakistan. This was a cross sectional comparative study from July 2005 to January 2006. Sixty consecutive patients (30 premenopausal and 30 postmenopausal) were included in the study through structured Proforma by purposive sampling technique from the outpatient, ward or emergency. Informed consent was obtained. The inclusion criteria were patients with all degree of uterovaginal prolapse, both pre and postmenopausal and married and unmarried women. Exclusion criteria were women who were pregnant with uterovaginal prolapse, patients with diagnosed renal pathology, recurrent urinary tract infection, and medical disorder such as diabetes mellitus or sclerosis. A detailed history and related examination and investigations were done. These include urine D/R, Urine C/S and urodynamic like Cystometry in selected patients. Frequency was defined as the passage of urine every 2 hrs or more than seven times a day. Nocturia was defined as interruption of sleep more than once each night of need to micturate. Urgency was a strong sudden desire to void while Dysuria defined as urethral pain during micturition. Retention of urine means failure to empty the bladder totally, bladder pressure being unable to overcome urethral resistance. Voiding problems include hesitancy, a poor stream, straining to void, incomplete bladder emptying and also frequency, urgency and dysuria. Stress incontinence was defined as the involuntary loss of urine when the intravesical pressure exceeds the maximum urethral closing pressure. While urge incontinence was defined as urinary leakage associated with the sensation of urgency. Data analysis was performed through SPSS Version 10. Age was presented by Mean ∀ standard deviation and its histogram was also presented in premenopause and postmenopause group because Means were not comparable. Frequencies and percentages were computed to present all categorical variables including .menstrual status, parity status, urinary complains and symptoms. Chi-square test was applied to compare parity status, urinary symptoms including frequent urine passing, urgent urine passing, dysuria urine passing, incontinence and retention urine passing while Fisher's exact test was applied to compare nocturia urine passing, retention urine passing between two groups. Statistical significance was taken at $P<0.05$.

RESULTS

Average age of premenopause group was computed (35.57±5.04 years, range=25-40) and postmenopause group was (58.77+_9.97 years, range 40-80). There were only two patients were either nulliparous or single parous and both these patients were found in premenopause group. Parity 2-5 was significantly higher in premenopause than postmenopause group (66.7%vs36.7%), while parity 6-10 was significantly higher in postmenopause group than premenopause group (63.3%vs26.7%).This data revealed a significant difference (X2=9.09, P=0.011) of parity status between two groups. (Table1). A symptom of frequent urine passing was reported by 8 (26.7%) patients of premenopause group and 10(33.3%) patients of postmenopause group. There was insignificant difference(X2=0.317, P=0.573) of proportion of frequency of urine between two groups. Urgency in passing urine was reported by 6 (20%) patients of premenopause group and 8(26.7%) patients of postmenopause group and had insignificant difference(x2=0.373, P=0.542) between two groups (fig 1).8(26.7%) patients of pre and 4(13.3%) patients of postmenopause group complained nocturia.

Table No. 1: Comparison of Parity Status between Pre & Most Menopausal Patients

Parity Status	Pre-Menopause (N=30)	Post-Menopause (N=30)	Total
Para 0 – 1	2 (6.7%)	0 (0%)	2
Para 2 – 5	20 (66.7%)	11 (36.7%)	31
Para 6 – 10	8 (26.7%)	19 (63.3%)*	27

***Significantly high proportion (X2 = 9.09, p=0.011) at p <0.05**

Table No. 2: Comparison of Incontinence Symptom between two Groups

Incontinence	Pre-Menopause (N=30)	Post-Menopause (N=30)	Total
Urge			
Yes	12 (40%)	06 (20%)	X^2 = 2.86 P= 0.091
No	18 (60%)	24(80%)	
Stress			
Yes	14 (46.7%)	16 (53.3%)	X^2 = 0.27 P= 0.606
No	16 (53.3%)	14 (46.7%)	

An insignificant difference (P=0.333) of proportion was observed in 4 (13.3%) of pre and 6 (20%) patients of postmenopause group. Statistically insignificant difference (P=0.731) of proportion was observed

between two groups (fig 6).Greater number of patients of premenopause than postmenopause group were found with dysuria (40%vs 26.7%, P=0.273), However this difference of proportion was insignificant statistically (fig7).Urge type incontinence was reported by 12(40%) patients of pre and 6(20%) patients of postmenopause group, however this difference of proportion was insignificant (P=0.091). Stress incontinence was slightly higher in postmenopause than premenopause group (53.35vs.46.7%,P=0.606) but this difference was insignificant(table2).Proportion of voiding problem in premenopause group was 40% and postmenopause group was 46.7%, this difference of proportion was statistically insignificant(x2= 0.271,P=0.602) (Table 2).

DISCUSSION

Women with uterovaginal prolapse may present with a plethora of lower urinary tract symptoms including urine frequency, urgency, nocturia, hesitancy, incomplete emptying and incontinence. These symptoms may or may not be related to the uterovaginal prolapse, but with careful clinical and urodynamic investigations it is usually possible to determine the underlying pathophysiology[11]. There is study conducted by weber AM, Waltar MD Schova LR, Mitchinson A, they found that women with prolapse were older than those without prolapse.(mean age 58.2 vs 49.2 years respectively)[12]. In this study we used a comparison group of pre and postmenopausal women with uterovaginal prolapse to determine the frequency and proportion of associated urinary symptoms. In this study we found that the average age of premenopausal group was 35.57yrs(range 25-40yrs) while the average age of postmenopausal women was 58.77 (range 40-61yrs). A multi country collaborative study in Pakistan found that women with multiparty were more likely to have prolapse[1]. In our study only two patients in premenopause group were either nulliparous or single parous. Other patients in premenopause group had parity 2-5 which was significantly higher than postmenopausal group (66.7%vs, 36.7%), while parity 6-10 was significantly higher in postmenopause group than premenopause (63.3%vs,26.7%). So the parity status between these two groups had significant difference (x2=9.09, P=0.001). As this study was carried out on the basis of hypothesis that there is a likely to be significant difference in urinary symptoms between pre and postmenopausal group with uterovaginal prolapse, the result of our study was found against this hypothesis that differences were insignificant. In our study urge incontinence was reported by 40% patients of premenopause and 20% patients of postmenopause group, however this difference of proportion was insignificant (P=0.091) while stress incontinence was slightly higher in postmenopause group (53.3%vs.46.7%, P=0.606) but this difference was insignificant and was not support the hypothesis on which our study based. (Risk factors for urge and stress incontinence were different). The major predictors of urge incontinence were increasing age, UTI and diabetes. Other large population based studies have reported age as an important risk factor for urge incontinence[13]. According to one study the major predictors of stress urinary incontinence were white race, higher basal metabolic index and higher waist to hip ratio[14]. In our study the urinary frequency, urgency and voiding difficulties were found more in postmenopausal group as compare to premenopausal group (33.3%vs26.7%, 26.7%vs20% and 46.7%vs 40% respectively) but statistically these differences were insignificant. There is one study conducted by Bun gay et al who reported that frequency and urgency are 20% and 15% respectively and these figures increases only slightly with age[15]. In our study greater number of patients of premenopause group than postmenopause were found with dysuria (40%vs 26.7%, P=0.273) but this difference is insignificant. According to survey conducted in Chinese population in Hong Kong the prevalence of dysuria reported by 166 of 819 women (20%). According to one study conducted by Schatzl and colleagues in 2000 only 3.1% of women younger than 80 experienced nocturia greater than twice per night, where as 26.7% of those aged 60 and older did. But in our study group nocturia found to be higher in premenopause group than postmenopause (26.7%vs 13.3%). However this difference was found to be insignificant (P=0.333).Similarly retention of urine was observed higher in postmenopause group (20%) than premenopause (13.3%) but again this difference was found insignificant. Despite thorough evaluation, the source of voiding dysfunction will not be discovered in many women with lower urinary tract symptoms. Age alone correlates with its development; regardless of existing risk factor. This may be caused by a number of processes such as occult Supratentorial central nervous system lesion, neurogenic or myogenic dysfunction at the level of the bladder and changes in extracellular matrix composition at the level of the bladder and urethra[16].

CONCLUSION

Uterovaginal prolapse is associated with different urinary symptoms commonly incontinence and voiding problems. These symptoms are usually affected with age and parity. In our study significant difference in parity was found between pre and postmenopausal group but the difference of proportion of urinary symptoms was found insignificant. All urinary symptoms need to be investigated before performing any vaginal surgery to exclude coexistent pathology such as detrusor instability. These urinary symptoms effect over quality of life of women. This warrants greater attention for gynecological health needs in our

country by safe family planning practices, strengthened with health education of women for delayed age at marriage, to reduce the risk of uterovaginal prolapse and associated urinary symptoms. Further studies are required in the community to study the natural history of the development of urinary symptoms and the relationship to prolapse.

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Original Article

Postpartum Haemorrhage

A Randomized Controlled Trial on Prevention of Postpartum Haemorrhage with Sublingual Misoprostol or Oxytocin

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ABSTRACT

Introduction: Failure of the uterus to contract adequately after child birth (a tonicity) is the most common cause of postpartum hemorrhage and misoprostol produces a rapid peak concentration, and is more effective than oral administration.

Objective: We compared the postpartum blood loss with 400µg sublingual misoprostol and after standard care using 10 iu intramuscular oxytocin.

Study Design: Randomized controlled trial.

Place and Duration of Study: This study was conducted in a Department of Obstetrics and Gynecology Ghulam Muhammad Mahar Medical College Teaching Hospital Khairpur Sindh during 2011.

Materials and Methods: 60 women for each group were assigned to receive the study medications with in 1 minute of clamping and cutting the cord. Chi-square and studentt-test were used to test categorical and continuous outcomes. Mean postpartum blood loss and PPH (>500ml), >10% pre- to postpartum decline in hemoglobin and reported side effects.

Results: The mean estimated blood loss with sublingual misoprostol was 200± 125ml (n=60) and 360±136ml with oxytocin (n=60) P-value ≤ 0.001.The incidence of PPH was 3.3% with misoprostol and 6.6% with oxytocin group. No women lost >1000 ml of blood. Hemoglobin decline of > 10% observed that 11.6% and 45.0% in women after receiving misoprostol and oxytocin (P≤ 0.001). Side effects were significantly greater in the misoprostol group than in the oxytocin group.

Conclusion: In this trial we found sublingual misoprostol more effective than intramuscular oxytocinin reducing PPH, with only transient side effects.

Key Words: Hemoglobin, misoprostol, oxytocin, Postpartum hemorrhage, sublingual.

INTRODUCTION

Postpartum hemorrhage (PPH), defined as a blood loss of > 500 ml after delivery, is the most common complication of the 3rd stage of labor and is the leading cause of maternal death in Africa and Asia.[1]

Although most women experiencing a blood loss of 500ml do not require additional treatment,[2]PPH can also exacerbate anemia and its consequences.[3]

The usefulness of misoprostol a synthetic prostaglandin E1 analog marketed for the prevention and/or treatment of peptic ulcer, also used in the active management of third stage of labor (AMTSL) in developing countries was first reported by EL-Rafaey.[4] Because of its uterotonic effects, misoprostol has been demonstrated to be effective for both the prevention and treatment of PPH.[5] With its ease of administration and storage, there has been increasing evaluation and promotion of misoprostol in developing countries.[6]

Compared with other routes of administration, sublingual and oral misoprostol have the shortest time to reach peak concentration (20 minutes), which is approximately one-third of the time of the vaginal route. Sublingual misoprostol also has the highest peak concentration and greatest bioavailability, 400µg administered sublingually approaches nearly twice the peak concentration of oral administration.[7] The avoidance of first-pass metabolism via the liver achieves a higher peak concentration by sublingual administration than by oral administration. Sublingual misoprostol more suitable than other routes of administration for clinical applications requiring a rapid onset of action, such as that required for the prevention of PPH.[8]

Oxytocin, long considered the gold standard of uterotonics, remains an efficient uterotonic , with a slightly more rapid onset of action, but has a shorter half-life than misoprostol.[9]

A study was conducted in India showed women receiving 600 and 400µg sublingual misoprostol had lower mean blood loss (96 and 26ml) than those receiving oxytocin (126ml).[10]

Our study's aim was to compare the effectiveness of a relatively low dose, 400µg of sublingual misoprostol with the standard care of 10 iu I/M oxytocin on measured postpartum blood loss.

MATERIALS AND METHODS

Women with a singleton pregnancy at >37 weeks of gestation, with cephalic presentation, anticipating a

normal spontaneous vaginal delivery and with hemoglobin > 10 g/dl at the time of presentation, who were admitted to the labor room of Obstetric Department of Ghulam Muhammad Mahar Medical College Teaching Hospital Khairpur Sindh during year of 2011 were included in the study.

Women with postdates pregnancy, medical disorders, instrumental deliveries, multiple gestation and still births were excluded from the study.

Subjectswere assigned to treatment with 1:1 ratio using simple randomization.

During one year of study period 120 women was included in the study. Sixty women for each group. One group received 2 tablets (400µg) misoprostol sublingual and other group received 10 iu I/M oxytocin within 1 minute of clamping and cutting the cord. Clinicians continued to monitor the patients.

As part of the standard of care, controlled card traction and uterine massage were provided to both groups.

Visual assessment of blood loss was measured for 2 hours after delivery, women's vital sign and side effects (nausea, vomiting, diarrhea, abdominal pain and fever) were monitored for 6 hours after delivery. A blood sample for hemoglobin and hematocrit estimation was obtained between 12 and 48 hours after delivery.

The primary outcomes are mean blood loss and postpartum hemorrhage. Secondary outcomes include side effects and > 10% postpartum decline in hemoglobin, which is directly associated with blood loss.

SPSS version 15 was used for data analysis. Student t-test and Chi-square test were used for analysis of the study results.

RESULTS

A total of 120 women were eligible during the study period. Of these, 60 were randomly assigned to the misoprostol group and 60 were randomly for the oxytocin group.

The characteristics of the study subjects and the birth weights of the newborns were comparable (Table: 1). On average all women were aged <25 years and delivered between 38-39 weeks of gestation.

Women receiving oxytocin were slightly younger (22± 3.0 years old) and more had first pregnancy (53.3%) compared with womenreceiving misoprostol (23±3.2 years old and 55% were parity more than one). Un booked women were equally more in both groups of study population. More women in the misoprostol group received antenatal iron supplementation (41.6%) compared with those receiving oxytocin (33.3%), P=0.06. The average birth weight of newborn in both groups was nearly identical (Table: 1).

Regarding complications and blood loss in third stage of labor, estimated mean blood loss was 200±125 ml in women receiving misoprostol compared with 360ml ±136 ml in women receiving 10 iu intramuscular oxytocin (P=<0.001) shown in (Table: 2). Only 2(3.33%) of women experienced PPH in misoprostol group, compared with 5(8.33%) in women who receiving oxytocin (P=0.002). There was no blood loss of >1000ml or maternal death occurred in both study groups (Table: 2).

Table No.1: Characteristics of study population

variable	Misoprostol N=60 %	Oxytocin N=60 %
-Age	23±3.2	22±3.0
-Parity -Nuliparous -Multiparous<5	 27 45% 33 55%	 32 53.3% 28 46.6%
-Gestation at delivery	38.6 weeks	38.5 weeks
-Received antenatal care	20 33.3%	18 30%
-Un booked women	40 66.6%	42 70%
-Received antenatal iron	25 41.6%	20 33.3%
-Duration of Ist stage labor	9-10 hours	8-9 hours
-Duration of 2nd stage labor	20-45 minutes	35-50 minutes
-Episiotomy	32 53.3%	30 50.0%
-Birth weight (g)	2.6-2.9 kg	2.8-3.1 kg

Table No. 2 Blood loss, Hb changes and side effects

Variables	Misoprostol N:60	Oxytocin N:60	P-value
Mean blood loss (ml)	200±125ml	360±136ml	< 0.001
PPH	2(3.33%)	5(8.33%)	<0.002
Hb decline >10%	7(11.6%)	27(45%)	<0.001
Duration of 3rd stage	10-22 minutes	10-25 minutes	<0.05
Nausea	4(6.66%)	-	-
Vomiting	3(5.0%)	1(1.6%)	<0.02
Shivering	32(53.3%)	3(5.0%)	<0.001
Fever (>38◦c)	2(3.33%)	-	-
Additional uterotonic	1(1.6%)	3(5.0%)	<0.02
Blood transfusion	1(1.6%)	1(1.6%)	<0.98
Maternal death	-	-	-

Women receiving misoprostol and oxytocin had a similar duration of third stage of labor.

More than 10% decline of postpartum hemoglobin in women receiving misoprostol and oxytocin, 11.6% and 45% respectively.

Minor type of side effects like nausea, vomiting and fever was more seen in misoprostol group as compared to oxytocin group. Shivering was more seen in misoprostol group 32(53.3%) as compared to 3(5.6%) in oxytocin receiving women. In more cases additional utero-tonic required in oxytocin group 3(5.0%) as

compared to 1(1.6%) in misoprostol group. One woman in each group required a blood transfusion.

DISCUSSION

The WHO recommends oxytocin as the preferred treatment for managing PPH due to uterine atony but oxytocin has a slower onset of action,[11]or in situation where skilled health worker are not able to provide AMTSL,WHO recommends either oxytocin or misoprostol for prevention of PPH.[12]

In 2011, the WHO added misoprostol (600µg) orally to its Model list of essential Medicines for the prevention of PPH.

This study found 400µg sublingual misoprostol, with more rapid bioavailability than oral misoprostol, to be significantly superior to 10 iu intramuscular oxytocin in reducing mean postpartum blood loss. The Cochrane review found virtually no difference in mean blood loss in its comparisons of sublingual misoprostol and injectable uterotonics.[13]

Women receiving oxytocin required additional uterotonics than sublingual misoprostol for PPH treatment in our study, consistent with Vimalaet al.[14]

The incidence of PPH in this study is higher in those receiving oxytocin I/M (8.33%) where as those receiving sublingual misoprostol had a PPH rate (3.33%), it is correlate with study of Vimalaet al.[14]

Consistent with studies comparing 600µg oral misoprostol or 400µg sublingual misoprostol with injectable oxytocin, the incidence of side effects in women receiving 600µg oral misoprostol or 400µg sublingual misoprostol is similar and may be attributable to the group and sustained bioavailability of sublingual misoprostol.[13,14] In those studies, more women receiving misoprostol experienced side effects, although, as in this study, transient shivering has often been the side effects with substantially greater incidence than that associated with oxytocin 32(53.3%), 3(5.6%) women who experienced shivering and other side effects in misoprostol group cure with simple supportive measures, all side effects, including fever, were short lived and required no medical interventions.

CONCLUSION

The transient and self-resolving nature of the side effects associated with misoprostol and the effectiveness and ease of the administration of sublingual misoprostol particularly useful in buzzy and low resource setting labor room.

This trial found sublingual misoprostol more effective than intramuscular oxytocin in reducing PPH, with only minorand transient side effects.

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Original Article

Causes and Management Out Come of Peritonitis

Peritonitis

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ABSTRACT

Objective: This retrospective study was conducted to study the different causes of peritonitis and to determine the surgical out come.
Study Design: Retrospective study
Place and Duration of Study: This study was carried out at the Department of Surgery Peoples Medical College Hospital Nawabshah from 1st January 2001 to 31st December 2001.
Materials and Methods: In this study of 51 patients of peritonitis admitted, out of them, 16 cases of typhoid ileal perforation, 8 cases of perforated duodenal Ulcer, 7 cases of tuberculosis of those 2 cases were of jujenal perforation and 5 cases of ileal perforation, 4 cases of perforated appendix, 4 cases of ruptured liver abscess, 2 cases of perforated neoplasms of those 1case each with multiple ileal/ jujenal perforation due to lymphoma ,malignant caecal perforation, gastric perforation, jujenal perforation obstructive/strangulated, infective caecal perforation each, 2 cases of post operative peritonitis, 3 cases trauma, 2 cases of blunt abdominal trauma each developed peritonitis due to ileal and jejunal perforation, 1 case of gunshot injury causing peritonitis due to colon perforation, 2 cases of ruptured ovarian cyst associated with appendicitis.
Results: In our study, male to female ratio is 4:1. Maximum frequency was observed in 20-30 years age, whereas most of the patients ranged between 13 to 40 years.
Conclusion: Surgical outcome of the peritonitis resulted poor in those cases who came late with hugely contaminated peritoneal cavity when operated. They developed post operative complications i.e. wound infection, septicemia, fecal fistula & longer hospital stays. 22 patient's outcome was poor and out of them 8 patients expired. The mean hospital stay was 23.0±17.7 days and the range was 67(3-110 days).
Key Words: Peritonitis, Causes & Management

INTRODUCTION

Generalized peritonitis is one of the most common emergencies in general surgery. It is an inflammation of peritoneum, can be aseptic or septic, bacterial or viral, primary or secondary, can be acute or chronic[1,2].
Intestinal perforations are a common cause of generalized peritonitis. It is often severe because of fecal contamination and over whelming sepsis resulting in high morbidity and mortality[2,3,4,5,7,8,9,10] even after treatment with all conventional means[2,3]. A better survival can be achieved by early diagnosis, fluid resuscitation, appropriate parental antibiotics, and prompt surgical intervention with thorough peritoneal lavage. All are often valuable adjuncts on reducing mortality and morbidity from these common conditions[2,5,6].

MATERIALS AND METHODS

This retrospective study was carried out in one year at Department of surgery Peoples Medical College Hospital Nawabshah from 1st January 2001 to 31st December 2001, a total of 51 cases with peritonitis were treated. All the cases had clinically established generalized peritonitis confirmed by clinical examination, radiological , ultrasonography, X ray abdomen erect/supine and operative findings, in order to assess the underlying etiology, a detailed history was taken in each case inquiring especially about the mode of onset and original site of pain before becoming generalized, duration, prodromal symptoms, associated symptoms and past-history. A comprehensive general physical examination and systemic examination were performed in order to assess the association of co-existing abnormalities and complications. Emphasis was laid on symptoms and signs of abdominal pain, pyrexia, guarding, rigidity, distension, generalized and rebound abdominal tenderness, absent bowel sounds, free fluid and obliteration of hepatic dullness.Complete blood count (CBC), erythrocyte sedimentation rate, random blood glucose level, blood urea nitrogeon (BUN), serum electrolytes and urine analysis and cross match blood with grouping were performed in all cases.
X-ray chest, (P.A), view was also performed in all the patients and in those suspected of suffering from tuberculosis or chest complications suggested by history and physical examination. An electrocardiogram (ECG) was also obtained in all the cases of above 40 years of age.
On arrival, all the patients were resuscitated, nasogastric suction was commenced, urinary catheter was inserted and broad spectrum parenteral antibiotic. The anemic patients were given blood transfusion.

Patients in septicemia state were given appropriate circulatory support as required in addition to intravenous fluids and antibiotics in high doses. The antibiotics used were Metronidazole, Gentamycin, Ampicillin and third generation cephalosporins and in patients with impaired renal function, replacing gentamycin and additionally ciprofloxacin in infusion form in typhoid enteric perforation. The antibiotics were continued routinely for 7-10 days. Patients suffering from intestinal tuberculosis confirmed by AFB staining, operative findings and histopathology were registered for antituberculous therapy for 9 months orally. Patients suspected of peptic ulcer perforation on history and physical and examination, were administered H2- receptor blockers. All the operations were performed as emergency under general anaesthesia after resuscitation and clinical evaluation.

Abdominal cavity explored through a grid iron / midline incisions, the quality and quantity of intra-abdonimal exudates were measured. Pus and intestinal contents were removed. Then abdominal cavity washed A definitive diagnosis was made during the operation. Samples of pus for culture were obtained in cases of obscure etiology/surgical finding and were not routinely taken in the presence of fecal peritonitis. Biopsy specimens comprising of excised tissues/resected segments and mesenteric lymph nodes were taken for histopathology wherever applicable. Outcome of surgical management was determined by intra operative complications, post-operative complications i.e. wound infection, septicemia and fecal fistulas.

On the basis of operative findings, patients were classified into 5 etiological categories as follows (graph 2& 3):

1. **Intrinsic diseases of gastrointestinal tract:** This category was used to classify all inflammatory, ischemic and neoplastic diseases of gastrointestinal tract from stomach to rectum, leading to perforation and intrinsic contamination of peritoneal cavity.
2. **Hepatobiliary category:** Cases of peritonitis from gangrenous perforating cholecystitis and ruptured liver abscess were classified under this category.
3. **Genitourinary category:** This category was exclusively used to classify cases of peritonitis resulting from urologic or female genital origin.
4. **Post-operative category**: Cases operated for abdominal problems other than peritonitis and developing peritonitis post-operatively due to dehiscence of surgical repair/anastomosis were classified under this category.
5. **Traumatic category:** This category was used to classify all those cases developing peritonitis as a result of contamination caused by blunt and penetrating abdominal trauma.

Data collection is done by Questionnaire filled at the time of admission and postoperatively Data analysis was done on SPSS Version 11.0.

Inclusion Criteria: Patients above 12 years age of either sex. All cases proved as peritonitis based on history, examination, clinical features, X-ray abdomen, ultrasound and C.T scan (if required) appearances included in the study.

Exclusion Criteria: All other causes of acute abdominal conditions without peritonitis like Gastritis, Renal Colic, Pancreatitis, and Gastroenteritis.

RESULTS

A total of 51 cases treated ,out of them, there were 40 males and 11 females ranging from 13 years to 80 years in age (mean age = 31.2 + 13.6 years). They were classified into 5 etiological categories on the basis of operative findings (Table 10) as follows:

a. **Intrinsic Diseases of Gastrointestinal Tract:** 40 cases (32 males and 8 females) were included in this category. There were 16 cases of typhoid ileal perforation alone accounting for 31.4% of the total. Besides these, there were 4 appendicular perforations (7.8%) with generalized peritonitis. 7 cases of tuberculosis out of 7. 2 cases were of jejunal perforation and 5 (9.8%) of ileal perforation. 2 cases of perforated neoplasms 1 (2%) of those with multiple perforations in ileam and jejunam due to lymphoma other was 2% of caecal perforation due to carcinoma rectum 8 cases (15.7%) duodenal ulcer perforations, 1 case 2% of gastric ulcer perforation, 1 case 2% of jejunal perforation due to intestinal obstruction caused by strangulated incisional hernia, 1 case 2%of caecal perforation due to amoebic colitis.

b. **Hepatobilliary:** Total 4 (7.8%) cases of ruptured liver abscess leading to generalized peritonitis included in the study.

c. **Post operative:** 2 Cases 3.9% were included both were of jejunal perforation who developed peritonitis few days post operatively.

d. **Traumatic:** 3 cases (5.8%)of trauma out of those 2 cases were of blunt abdominal trauma each developed peritonitis due to ileal and jejunal perforation 1 case (2%) of gunshot injury causing peritonitis due to perforation in colon.

e. **Genitourinary:** Only 2 (3.9%) case of Ruptured ovarian cyst associated with appendicitis who developed generalized peritonitis .

Sign & Symptoms: Acute generalized pain was present in all cases. 90.19% with fever, 66.66% with vomiting. Constipation 68.62%. 15.68% complained of coffee ground vomiting (duodenal perforation), and 1 case of typhoid ileal perforation attributed to stress ulceration. 9.80% patients reported Melina and 1.96% fresh bleeding per rectum. On examination, all had generalized abdominal tenderness, while rebound

tenderness was (90.19%) of cases. Abdominal rigidity (82.35%), dehydration (86.27%), abdominal distention (84.31%), absent gut sound (66.66%). Shifting dullness was elicited in (64.70%) of cases and jaundice in (1.96%) of cases.

Management Outcome: The patients of poor outcome developed post operative complications like wound infection, septicemia and faecal fistula &longer hospital stay, 8 (15.6%) patients were expired out of 22(43.4%). Patients of good outcome got smooth recovery with hospital stay was shorter, few of them developed mild wound infection which was cured. The mean hospital was 23.0±17.7 and the range was 67(3-110 days) (Table No.3).

Investigations: In all causes of perforation like Typhoid ileal perforation, Appendicular Perforation, Perforated Neoplasm, Perforated intestinal obstruction Upright abdominal X-rays showed gas under the diaphragm. Abdominal ultrasonography revealed the presence of free fluid in the peritoneal cavity. Serum revealed electrolytes imbalance

Perforated Neoplasm: Abdominal X-rays showed pneumo-peritoneum and ground glass abdomen with dilated loops of bowel and fluid in peritoneal cavity in these cases. Histopathology reports revealed small T cell lymphoma and Carcinoma rectum. Para colic nodes were positive for malignant also.

Table No.1: Hospital Stay (four groups)

Days	Cases	Percentage
Up to 10 days	13	25.5%
Up to 11-20 days	17	33.3%
Up to 21-30 days	12	23.5%
More than 30 days	9	17.6%

Mean Stay was 23.0 ± 17.7 and Range was 107 (3-110)

Table No.2: Biopsy

	Frequency	Percent
CGI TB	7	13.7
Appendices	6	11.8
SC Lymphoma	1	2.0
Widal +	14	27.5
CA Rectum	1	2.0
Nil	21	41.2
Amoebic Perforation	1	2.0
Total	**51**	**100.0**

Table No.3: Post Operative Complications

	Frequency	Percent
WI+SEP+FF	10	19.6
WI	20	39.2
WI+SEP	5	9.8
SEP	2	3.9
Nil	14	27.5
Total	**51**	**100**

- WI- WOUND INFECTION
- FF- FAECAL FISTULA
- SEP-SEPTECEMIA

Table No.4: Outcome

Outcome	Frequency	Percentage
Good	29	56.6%
Poor	22	43.5%
Total	**51**	**100%**

Table No.5: Etiological Classification on the basis of Operative Finding

Category	Total	%age
I. Intrinsic Gastrointestinal tract		
A. Typhoid ileal perforation	16	31.4%
B. Perorated appendix	4	7.8%
C. (i) perforated ileocaecal tuberculosis	5 2	9.8% 3.9%
(ii) jejunal perforation (tuberculosis)		
D. Perforated duodenal ulcer	8	15.7%
E. Perforated intestinal obstruction	1	2%
F. Perforated neoplasm's	1	2%
(i) Caecal perforation		
(ii) Multiple perforations (ileum, jejunum)	1	2%
G. Caecal perforation (amoebic)	1	2%
H. Gastric ulcer perforation	1	2%
II. Hepatobiliary		
Ruptured liver abscess	4	7.8%
III. Post-Operative		
Jejunal Perforation	2	3.9%
IV. Traumatic		
A. ASC. Trans. Colon Perf. (Gunshot)	1	2%
B. Ilieal Perf. (Blunt ABD. Trauma)	1	2%
C. Jujenal Perforation (Blunt ABD. Trauma)	1	2%
V. Genitourinary		
1. Ruptured Ovarian Cyst + Appendicitis	2	3.9%
Total	**51**	**100%**

DISCUSSION

Acute generalized peritonitis is a challenging surgical condition that requires prompt attention and appropriate surgical treatment. Despite advances in surgical techniques, good antimicrobial therapy and intensive care support, it carries high morbidity and mortality while its management remains difficult and complex [11]. Peritonitis, if not treated promptly, can lead to multisystem organ failure and death [12,13]. Worldwide there is a predominance of males presenting with this life-threatening disease [14,15] our series also shows a similar trend, with a male to female ratio of 4:1. Maximum frequency was observed in the 20-30 years

age group (52.94%) whereas most of the patients ranged between 13 to 40 years in age (84.31%).

Many etiological factors have been implicated in the pathogenesis of this generalized intra-abdominal sepsis. The objective of this research exercise is to study the different causes of generalize peritonitis (secondary peritonitis) and to determine the surgical outcome

Abdominal pain was the most common symptom and tenderness the commonest sign observed in 100 percent cases. Rebound tenderness was noted in 90% cases attributable to a possible masking effect of altered conscious level in toxaemia. Findings of pneumo-peritoneum in 56.9 percent patients and elicitation of shifting dullness in (64.70%) is comparable with similar studies. Coffee ground vomiting in (15.68%) patients reflects the diverse nature of clinical presentation that can be observed in generalized peritonitis. These included patients with typhoid heal perforation and 8 cases with duodenal perforation and was attributed to stressful effects of toxemia on stomach in cases of typhoid leading to stress ulceration. Similarly, Melina was observed in 9.80% cases, 8 of those 9 cases had a perforated duodenal ulcer and 1 had typhoid ileal perforation.

Investigations irrespective of etiology revealed anaemia, varying degrees of electrolyte imbalance, raised blood urea nitrogen. Metabolic acidosis & predominance of polymorph nuclear leukocytes in the differential count which was not markedly elevated and even below normal in some cases. Cause of this functional neutropenia has been described to be a massive shift of leukocytes along with the inflammatory exudates into the peritoneal cavity so that their number falls in circulating blood usually observed in the reactive toxic stage of peritonitis with multiple organ failure. Delayed treatment associated with other factors such as malnourishment and impaired immunity are the major reasons for high mortality and morbidity. Kaur N et al., in their study also attribute delay seeking surgical treatment as an important cause for high morbidity [16].

Almost (31.4%) of the cases were due to typhoid ileal perforation alone and the second most common cause of peritonitis was perforated duodenal ulcer and than ileocaecal tuberculosis perforation and perforated appendicitis. Quereshi AM [17] & Dorairajan LN [11] who report majority of perforations involving distal gastrointestinal tract such as ileum. Chaterjee H too reported typhoid as the commonest cause of perforations in two separate studies [18,19].

Similarly, 4 cases of ruptured liver abscess and 2 cases of perforated neoplasms were observed. No case of colonic diverticular perforation was observed which was fairly common cause of generalized peritonitis in the West .

Incidence of post-operative peritonitis was also very low and only 2 cases developed peritonitis post-operatively. In our study perforated appendix 7.8% and peptic ulcer 15.7 %. Dandpat MC studied 340 cases of gastrointestinal perforations and found that 22(6.4%) patients developed secondary peritonitis secondary to perforated appendix [20]. Primary intestinal tuberculosis is uncommon in the west [21] but is still common in developing countries like Pakistan [22]. In our study perforated tuberculosis ileocaecal perforation was 9.8 % and tuberculosis jejunal perforation 3.9%.

Blunt abdominal trauma ignored earlier by the patients was seen to be associated with serious intra-abdominal injuries leading to peritoneal contamination and generalized peritonitis. Those patients sought medical help upon development of abdominal pain and distention 24 hours to 5 days (average 2 days) after sustaining abdominal trauma. Intestinal obstruction leading to peritonitis was observed in 2 cases. Perforation was found in both cases.2 cases of malignancy were found in our study one involved the large bowel 2%, while one showed involvement of small bowel 2% .

This etiological pattern observed reflected the effects of a poor socioeconomic background and lack of hygienic conditions. Ignorance and delay in seeking medical advice was observed leading to typhoid ileal perforation whereas incomplete and inadequate anti tuberculous treatment was seen in cases of open pulmonary tuberculosis leading to gastrointestinal complications. While long history of epigastric pain found in patients of perforated duodenal ulcer which developed due to in appropriate treatment of acid peptic disease.

The patients of poor outcome developed post operative complications & longer hospital stay, 8 (15.6%) patients were expired out of 22(43.4%). postoperative complication in secondary peritonitis reported by Jhobta RS [23] are respiratory tract infections (28%), wound infection (25%), septicaemia (18%) and electrolyte imbalance (17%). Kim et al. [24] in their study report mortality rate of 9.9%. Patients of good outcome got smooth recovery with hospital stay was shorter, few of them developed mild wound infection which was cured. The mean hospital was 23.0±17.7 and the range was 67(3-110 days)

CONCLUSION

The diagnosis of acute generalized peritonitis was largely clinical and the radiological evidence of perforation was conclusive in only 57.9 percent of the cases. Majority of cases belonged to the intrinsic diseases of gastrointestinal category with the following etiological distribution; typhoid ileal perforation 31.4% , appendicular perforation 7.8%, perforated intestinal tuberculosis 13.7%, perforated duodenal ulcer 15.7%, perforated intestinal obstruction 2%, perforated carcinoma 3.9%. Ruptured ovarian cyst 3.9%, Ruptured liver abscess 7%.Majority of cases had faecal peritonitis

and rest of the cases had supperative peritonitis. Surgical outcome of the peritonitis resulted poor in those cases who came late & they developed post operative complications with longer hospital stay. Surgical outcome of patients whose arrival was early and there was less contamination of peritoneum, outcome was good with smooth recovery and there hospital stay was shorter.

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Original Article

Permethrin in Scabies

Beneficial Effect and Safety of 5% Permethrin Cream in Scabies Patients

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ABSTRACT

Objective: To evaluate the efficacy and safety of 5% permethrin in scabies patients

Background: Scabies is a contagious, itchy ectoparasitic infection. It is a common public health problem with an estimated global prevalence of 300 to 400 million cases. They increases day by day.5%permethrin is one of the effective treatment of scabies, it is highly effective, well tolerated, poorly absorbed and rapidly metabolized by skin.

Study Design: Open label clinical trial

Place and Duration of Study: This study conducted in Basic Medical Sciences Institute with collaboration of Dermatology Department from ___.

Materials and Methods: This study sample involved 65 clinically diagnosed scabies patients one was lost from follow up, the duration of study was 90 days. They were treated with 5% permethrin cream, clinical data was collected by using questionnaire. Patients were divided into three age groups and given two applications of permethrin cream on first and 15 day. Follow up was done on Day 3, Day 15, Day 30 and Day 90

Results: It was found that efficacy and safety of permethrin cream in scabies patients was highly significant which clinically improved the symptoms of patients.

Conclusion: Scabies patients should advised 5% Permethrin cream with conventional therapy which improves symptoms significantly following proper local.

Key Words: Scabies, 5% Permethrin Cream, Efficacy And Safety.

INTRODUCTION

Scabies is a common ectoparasitic infection caused by the mite sarcoptes scabiei variety hominis, an arthrobe of order Acarina.[1]

The world wide prevalence has been estimated at about 300 million cases yearly, although it is common disease of children but occur in both sexes, in all ethnic groups and at all socioeconomic levels.[2] A scabies infestation symptom includes rash and intense pruritus that is often worse at night. The lesion begins as tiny erythematous papules.[3] The diagnostic signs of scabies are the burrows[4].

Permethrin is a synthetic pyrethroid and considered as a gold standard of topical scabiecides.[5] It acts on voltage dependent sodium channels by extended channel opening causing increased sodium current, depolarization is prolonged leading repetitive filling of nerve.[6] It is well tolerated, poorly absorbed through skin and rapidly metabolized by skin esterase. Local skin irritation, such as pruritus, burning sensation or tingling has occasionally been reported but all are short duration[7,8].

MATERIALS AND METHODS

This was open label clinical trial approved by ethical Committee of Jinnah Postgraduate Medical Centre, Karachi conducted in Basic Medical Sciences Institute with collaboration of Dermatology Department. Cases diagnosed by consultant dermatologist were enrolled to participate in trial. Detailed questionnaire was completed and inform written consent was taken from patients and their relatives. Patients willing to participate were screened by applying the inclusion and exclusion criteria. Patients were divided into 3 groups according to age. Inclusion criteria are night itching, diagnosed cases of scabies either gender, age above 5 years and below 70 years, demonstration of burrows or presence of scabies lesion at the classical sites, history of similar illness in the family. Three or more criteria mentioned above made the patient eligible to include the study. Exclusion criteria are pregnant women, lactating women, crusted scabies, patient who had received treatment during last 1 month, diabetic and patients with hepatic impairment or with dermatological, cardiovascular and neurological diseases. Eligible subjects were assigned to apply 5% permethrin cream over night (over 14 hours) and then repeated the application on day 15.The duration of study was 90 days with 5 follow up visits. Total 65 scabies patients enrolled in this trial and divided into 2 groups which were further sub-divided according to age into 3 groups. Clinical efficacy assessed by appearance of new skin lesion and pruritus which was evaluate by visual analogue scales, it has both static and dynamic component. Dynamic component was scored on a scale 0-6.

Statistical Analysis: Data were analyzed using SPSS software Ver. 11.0, mean±SEM.

RESULTS

Table No.1: Visual Analogue Scale In Scabies Treated Patients on Day-0 to Day-90 of Permethrin Group

Follow up available		**Permethrin Cream Group** (n=65) (n=64)
Visual analogue scale (VAS)		Mean ± SEM
Day – 0		6.00 ± 0.00
Day – 30		3.67 ± 0.08
Day – 15		2.33 ± 0.10
Day – 30		0.94 ± 0.15
Day – 60		0.48 ± 0.18
Day – 90		0.87 ± 0.26**
Visual analogue scale (VAS) according to age groups		
5-25 years	Day – 0	6.00 ± 0.00
	Day – 3	3.46 ± 0.15
	Day – 15	2.08 ± 0.18
	Day – 30	0.71 ± 0.24
	Day – 60	0.21 ± 0.21
	Day – 90	1.00 ± 0.47**
26-45 years	Day – 0	6.00 ± 0.00
	Day – 3	3.75 ± 0.09
	Day – 15	2.35 ± 0.15
	Day – 30	1.00 ± 0.24
	Day – 60	0.55 ± 0.34
	Day – 90	0.75 ± 0.41**
46-70 years	Day – 0	6.00 ± 0.00
	Day – 3	3.85 ± 0.15
	Day – 15	2.60 ± 0.18
	Day – 30	1.15 ± 0.30
	Day – 60	0.70 ± 0.38
	Day – 90	0.80 ± 0.44**

**=Highly significant.

Total mean VAS scoring for pruritus in all scabies patients at day 0 was 6.00±0.00, at day 3 the mean VAS scoring was decreased to 3.67±0.08, at day 15 the mean VAS scoring was improved to 2.33±0.10, at day 30 the mean VAS scoring was decreased to 0.94±0.15, at day 60 the mean VAS scoring was 0.48±0.18 and at day 90 the mean VAS scoring was increased to 0.84±0.26 this increased may be due to recurrence in patients. When day 0 compared with day 90 the outcome was highly significant. According to age groups in all ages the baseline mean VAS scoring of scabies patients compared with the outcome was highly significant, as depicted in table 1. The Total percentage of new lesion in scabies patients at day 15 was 1.6%(1), at day 30 the percentage of new lesion was 9.4%(6), at day 60 the percentage was changed to10.9% (7) and at day 90 the percentage was increased to 15.6% (10) which was non significant. In all age groups the appearance of new lesion was also non-significant. As depicted in table 2.

Table No.2: Appearance of new lesion in scabies treated patients permethrin group

		Permethrin Cream Group (n=65)	
Follow-up available		n = 64	P-value
Appearance of new lesion			
Day – 3		-	0.052
Day – 15		1 (1.6%)	
Day – 30		6 (9.4%)	
Day – 60		7 (10.9%)	
Day – 90		10 (15.6%)	
Appearance of new lesion according to age groups			
5-25 years (n=24)	Day – 3	-	0.265
	Day – 15	-	
	Day – 30	1 (1.6%)	
	Day – 60	1 (1.6%)	
	Day – 90	4 (6.2%)	
26-45 years (n=20)	Day – 3	-	0.741
	Day – 15	1 (1.6%)	
	Day – 30	3 (4.7%)	
	Day – 60	3 (4.7%)	
	Day – 90	3 (4.7%)	
46-70 years (n=20)	Day – 3	-	1.000
	Day – 15	-	
	Day – 30	2 (3.1%)	
	Day – 60	3 (4.7%)	
	Day – 90	3 (4.7%)	

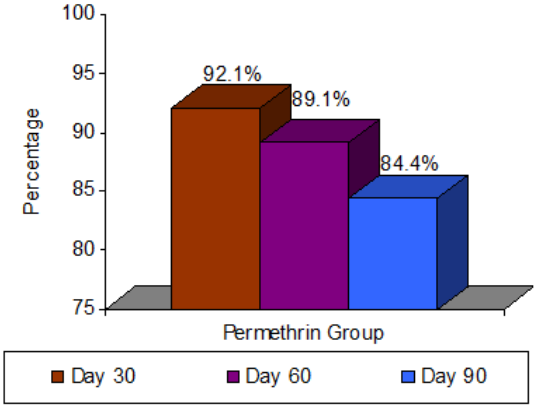

Figure No.1: Cure rate in scabies treated patients on day-30 to day-90 permethrin group

Cure rate of 64 (98.5%) Permethrin treated scabies patients at day 30 was 92.1% (59 patients), at day 60 the percentages was changed to 89.1% (57 patients) and at day 90 the percentage was decreased to 84.4% (54 patients). This reduction may be due to recurrence when day 0 compared to day 90 the cure rate was highly significant and same in all age groups. As depicted in fig 1. The safety profile clinically was

assessed by complaint of adverse effects in treated patients during the drug study period. The major adverse effects were not observed except mild burning, the percentage was 1.6 % (1), as depicted in figure 2.

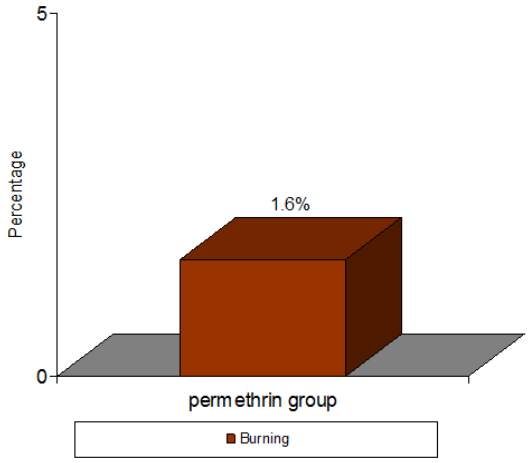

Figure No.2: Clinically safety profile assessed by adverse effects in scabies treated patients

DISCUSSION

Scabies is widely spread in our community and conventional scabiecidal therapies are difficult to instinct and hard to implant. This often results in higher failure rates because of non-compliance or reinfestation.[9] The reported incidence of scabies in Karachi is 22.7% which is more than other infection and shown alarming prevalence indicating a lack of awareness about this common skin problem.[10] Absolute confirmation can be made by the discovery of burrows and microscopical examination.[11,12] Treatment of scabies is as important as making a correct diagnosis. Patient should be properly instructed about the method of using scabiecide.[13]

The cure rate in this study was 84.4% and only one patient gave complain of mild burning. The results of our study are in accordance with other study that compared the 5% Permethrin with Ivermectin and found better results with permethrin cream.[14] In present study cure rate was assessed by the presence of new lesions. The participants who did not have any new lesion were considered as cured. Every participant was asked about pruritus which was quoted as 0/25/50/75/100% on visual analogue scale (VAS) at day 0, the pruritus was considered as 100%. Our results matched with other study who declared that permethrin act against all emerging stages of mites.[15] In the present study these participants who showed new lesions and gave complain of pruritus that had developed resistance and recurrence because of poor compliance[16]. Improper application of medicine was may be the main factor of drug resistance in study, to decrease the drug resistance of scabies patients advised the repeated prescription of scabeicidal medicines. This highly contagious nature of disease requires that all other household members be treated simultaneously whether they have no symptoms[18]. This study shows that permethrin was safe in adults, children and pregnant and lactating women[19]. For achieving good control, increased awareness and education, hygiene improvement and massive treatment campaign should be integrated[20].

CONCLUSION

5% Permethrin cream is effective and safe, but advice with conventional therapy.

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Original Article

Anatomy

Alterations in Mitochondria of Kidney Tubules by Different Doses of Diclofenac Sodium in Rabbits

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ABSTRACT

Introduction: Diclofenac sodium, a Non-Steroidal anti-inflammatory agent (NSAIDs), is being prescribed since many decades for the treatment of rheumatic diseases as well as for the relief of pain and fever. It is the inhibitor of enzyme cyclooxygenase. Unfortunately its use is often accompanied by gastro-intestinal renal and hepatic side effects. Renal dysfunction is characterized from acute renal failure to chronic injury. We herein report the damaging effect of diclofenac sodium on the ultrastructure of PCT of rabbit kidney by increasing the doses above the recommended.

Study Design: Experimental Study

Place and Duration of Study: This study was conducted at the Institute of Basic Medical Sciences (IBMS), Dow University of Health Sciences from March 2009 to March 2010.

Material and methods: In this study 88 male albino rats were selected, they were divided into 4 groups group A received normal saline 2 ml/kg, group B diclofenac sodium 2mg/kg body weight group C 4 mg/kg and group D 6 mg/kg for two weeks. At the end of experiment animals were sacrificed, dissected, kidneys were identified, fixed in 4% gluterldehyde than 1% osmium tetraoxide and passed through graded alcohols, infiltrated and embedded in resins. Semithin 3~4 µm sections were stained with toluidine, ultrathin (1µm) with uranyle acetate. Tissue sections were observed under transmission electron microscope. Tissue changes were graded as 0, +, ++ and +++, no change, mild moderate and severe changes respectively. The results were then analyzed statistically.

Results: There were non-significant changes in the cell organelles of PCT in group A and B, while significant changes were observed in group C and highly significant in group D.

Conclusion: Diclofenac sodium has damaging effect on the mitochondria of PCT cells far before the light microscopic changes. So its use should be restricted only in very painful conditions. Secondly in case of prolong treatment follow up with regular renal function test should be carried out.

Key Words: Diclofenac sodium, kidney, ultrastructure.

INTRODUCTION

NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) are very commonly prescribed drugs. During the past thirty years there has been a substantial increase in the number of NSAIDs, but their availability varies markedly between countries[1.] These drugs when grouped by generic categories they are the most widely prescribed of all medications[2]. As large population is exposed to these compounds their adverse effects are also rapidly expanding.

Diclofenac sodium, a commonly prescribed analgesic also belongs to the group non-steroidal anti-inflammatory drugs (NSAID). It is a non-selective COX inhibitor[3] and probably one of the most common non-steroidal compounds with analgesic, anti-inflammatory, anti-rheumatic and antipyretic properties with inhibitory effect on prostaglandin synthesis[4].

Its potency is substantially greater than that of indomethacin, naproxen and several other agents[5]. Chief clinical application of Diclofenac sodium is an anti-inflammatory agent in the treatment of musculoskeletal disorders such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and in dysmenorrhoea. It is also found effective for the control of postoperative pain when used preoperatively[6], pre-operative rectal diclofenac is found to delay the onset of post-operative pain and is adequate as an analgesic for early post-operative period[7].It has also been used in neonates to close the ductus arteriosus when it has remained patent[8].

The gastrointestinal toxicities are well known, while kidney is also an important site for untoward effects. In fact these agents have been known to produce a wide array of untoward renal effects that may be divided into several distinct nephrologic syndromes, including acute renal failure[9], chronic renal injury, nephrotic syndrome[10], interstitial nephritis, abnormalities of water metabolism, and abnormalities of sodium and potassium homeostasis. Renal papillary necrosis and nephritic syndrome have been reported in many patients taking Diclofenac sodium[9]. Experimental studies have also shown damage to kidney tubules at ultrastructural level[11]. Hepatotoxicity though uncommon but when occurs it is lethal.

MATERIALS AND METHODS

For this experimental study 88 male rabbits were taken from the Institute of Basic Medical Sciences (IBMS), Dow University of Health Sciences. They were about three months of age and 900-1000 gm. in weight. They were divided into four groups A, B, C, and D each comprising of 22 animals.

The animals were weighed on single pan triple beam balance on day-1 and day-14 of the experimental period. Group A served as control receiving normal saline 2ml intraperitoneally/daily, group B received diclofenac sodium (injection Voren) in the dose of 2 mg/kg body weight daily, group C received diclofenac sodium in the dose of 4mg/kg body weight daily (double the therapeutic dose), and the group D received diclofenac sodium 6 mg/kg/daily (three times the therapeutic dose), given intraperitoneally. These injections were given for 14 days. On day 15 the animals were sacrificed by a blow on head.

The kidneys were dissected out and divided into small pieces with the help of a sharp knife, and were fixed in 4% gluterldehyde for electron microscopy[12].

Tissue Processing for Electron Microscopy

All the tissue processing was done under a fume hood. Pieces of kidney were then chopped to 1 mm^3 pieces again transferred to the same fixative for further 24 hours for proper infiltration of the fixative.

a) The tissue pieces were washed with Sorensen phosphate buffer for ten minutes then post-fixed in osmium tetra oxide at 4 ºC for one hour. They were dehydrated in alcohols and infiltrated with half resin and half acetone at 37 ºC for one hour and embedded in full resin.

b) Semi-thin sections 3~4 µm were cut and stained with toluidine blue for general orientation of the tissue. After areas of interest were identified thin sections 1 µm (100 nm) stained with uranyle acetate and lead citrate were examined under transmission electron microscope (TEM Leica, Germany).

c) Tissue changes were graded as 0 no change, +, ++, and +++ as mild, moderate and sever changes respectively. The results were then analyzed statistically. P value less than 0.05 was taken as statistically significant. SPSS version 17 was used or the analysis of data.

RESULTS

The gross examination of the kidney in the group A, B, C, and D showed normal appearance. The kidney in group A appeared oval in shape, dark red in colour, soft in consistency with smooth shiny surface having delicate fibrous capsule that stripped off with ease. The kidneys of animals in groups B, C, and D were appeared normal, somewhat oval in shape with smooth looking contour, extra soft in consistency and capsule stripped with ease. The cut surface showed normal thickness of the cortex.

Electron Microscopic Observation: The kidney sections from animals of all four groups which were processed for electron microscopy and were mounted on grids; were examined under E/M. The following observations were recorded.

Group A: In group A animals the ultrastructure of kidney tubules revealed normal morphology. The plasmalemma at the microvillar brush border showed normal hazy appearance. In the cytoplasm the cell organelles appeared normal.

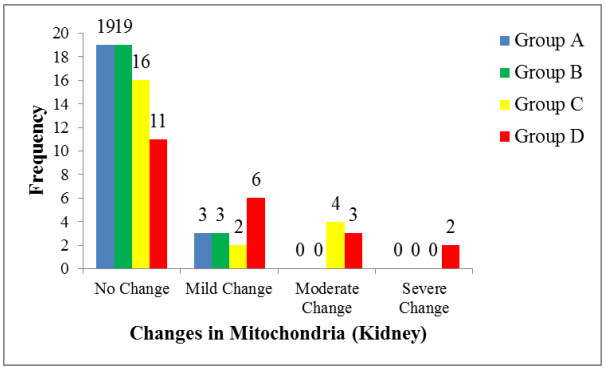
The mitochondria were elongated and rod shape, they were scattered all over the cell but gathered more near the base of the cell. Here they were arranged parallel to the long axis of cell in the basal infolding of plasmalemma. Both the inner and outer mitochondrial membranes were distinctly visible with densities in matrix; while most of the cristae were arranged transversely (Fig-1). The mean value of changes in this group was 0.14 ± 0.34 (Table-1).

Table A.1: Changes in Mitochondria of PCTs

Animal No.	Group A	Group B	Group C	Group D
1	0	0	0	1
2	0	0	0	0
3	1	0	1	2
4	0	0	0	0
5	0	0	2	3
6	0	0	0	0
7	0	1	0	2
8	0	0	0	0
9	0	0	0	1
10	0	0	1	0
11	0	0	0	0
12	1	0	0	1
13	0	0	0	0
14	0	0	2	3
15	0	1	0	1
16	0	0	0	1
17	0	0	2	0
18	1	0	0	0
19	0	0	0	0
20	0	1	0	1
21	0	0	0	2
22	0	0	2	0
Mean	0.14	0.14	0.45	0.82
Std Dev	0.34	0.34	0.78	0.98

Table A.2: Post Hoc Tests – Changes in Mitochondria of PCTs – Multiple Comparisons

	Group B	Group C	Group D
Group A	1.000	0.695	0.033
Group B		0.695	0.033
Group C			0.615



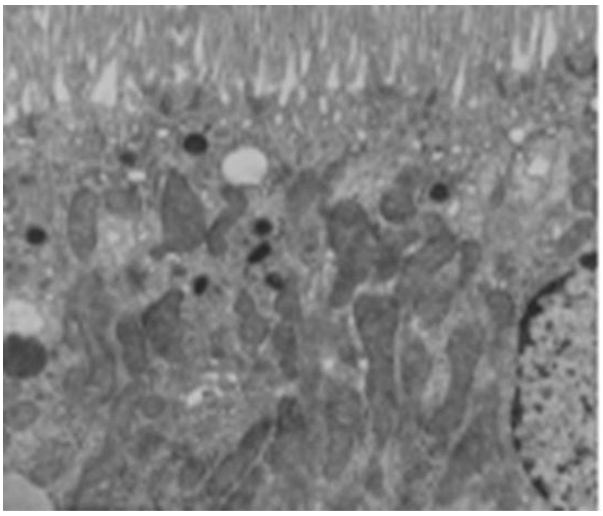


Figure 1: Electron micrograph of the rabbit kidney treated with normal saline (group A) showing a PCT cell with normal brush border, nucleus and other cell organelles x 6000

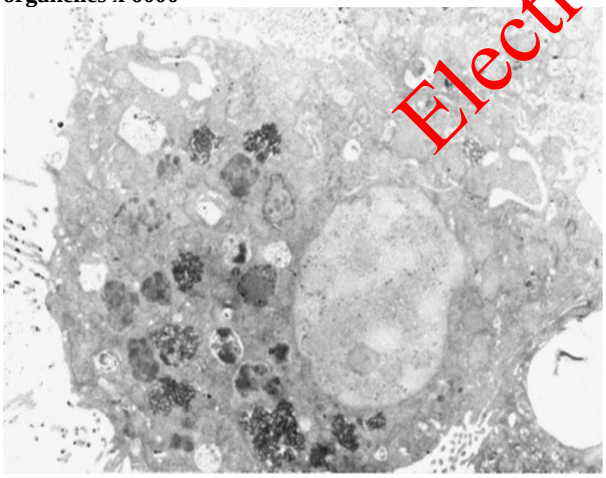


Figure 2: Electron micrograph of the rabbit kidney treated with Diclofenac sodium 6 mg/kg (group D) showing damaged mitochondria and brush border in a PCT cell x 6000

Group B: In group B animals the proximal tubular cells were almost normal under E/M. in cellular details the plasma membrane was intact and clearly visible, the cytoplasmic organelles were also studied and they showed mild to moderate change. Most of the mitochondria were elongated they were very well visualized along the basal part of the cells. They were arranged parallel within the basal infolding of plasmalemma of proximal tubular cells. Some of the mitochondria were swollen and in these mitochondria the outer membrane was not properly visualized. Mean value of the changes in group B was 0.14 ± 0.34.

Group C: The cells in proximal convoluted tubules showed many degenerative changes. Although the plasma membrane was normal bilaminar; the changes were clearly observable in other cell organelles. Mitochondria were swollen in many of the proximal tubular cells. Inner membrane was indistinct, and the cristae were short. In some of the mitochondria cristae structure was lost and it appeared as if mitochondria are filled with flocculent densities. . Mean value of the changes in group C was 0.45 ± 0.78.

Group D: The tubular lining cells showed significant ultrastructural changes. However the proximal convoluted tubules were more affected. Plasmalemma at the basal surface of the cells was almost normal while the apical surface i.e. the brush border revealed some loss.

There was marked swelling of the mitochondria in many of the proximal convoluted tubular cells. In some of the mitochondria the outer membrane was ruptured, there was loss of the cristae in inner membrane while increased densities inside the matrix were noted (Fig-2). Mean value of the changes in group D was 0.82 ± 0.98 (Table-1).

Statistically non-significant changes were observed in the mitochondria of cells in PCT in group A animals, while in groups B and C these changes were significant. Statistically highly significant changes were observed in group D ($p < 0.05$).

DISCUSSION

There are numerous reports describing various morphological and biochemical changes that occur in the kidney following administration of diclofenac sodium[12,13,14,15]. In this report an attempt has been made to study injury to cell organelle specially mitochondria of PCT under the influence of diclofenac sodium. We have tried to correlate the extent of injury with the dose of the drug. The extent of injury with the dose of the drug was also calculated statistically. An attempt has also been made to understand the mechanism of nephrotoxicity.

Rabbits treated with 2 mg/kg diclofenac sodium showed no any remarkable change in the mitochondria of cells in PCT. While those treated with higher doses i.e. 4mg/kg and 6 mg/kg body weight showed mild to severe changes. Nephrotoxicity was statistically significant in rabbits getting the diclofenac sodium 6 mg/kg.

The swelling and rupture of mitochondrial membrane is due to a phenomenon called mitochondrial permeability transition (MPT). In this phenomenon the permeability of mitochondrial membrane is increased to solutes of molecular weight less than 1500 Da. This MPT is formed at the contact sites between the inner and outer mitochondrial membrane. It results in depolarization and there is equilibrium of solutes between cytoplasm and mitochondrial matrix. This ultimately leads to the rupture of mitochondrial membranes.

Many researchers have identified the ability of diclofenac sodium to induce apoptosis and it was found that mitochondria play an important role in this mechanism[16,17]. At therapeutic dose it causes swelling of mitochondria but as the dose is increased there is rupture of mitochondrial membrane. The changes in this study are comparable with that found by Kretz Romel and Boelsterli[18]. They suggested that changes in mitochondria are dose dependent.

The E/M observations are essentially in agreement with those of Hickey EJ[19] also who proved that Diclofenac targets the mitochondria of renal proximal tubules. But he proposes the mechanism in which there is oxidative stress and massive DNA fragmentation.

The present results may have both toxicological and bioenergetic implications. First they show the potential of diclofenac sodium for toxicity in renal PCT mitochondria and secondly this drug is potential inducer of MMPT prevention. In this regard studies are necessary to evaluate the sensitivity to MMPT following diclofenac sodium exposure in vivo[20].

CONCLUSION

Diclofenac sodium has damaging effect on the mitochondria of PCT cells far before the light microscopic changes. So its use should be restricted only in very painful conditions. Secondly in case of prolong treatment follow up with regular renal function test should be carried out.

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Original Article

Dressing on Burn Wound

Efficacy of Platelet Rich Plasma Application in Comparison to Conventional Dressing Therapy in Partial Thickness Burn Wound

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ABSTRACT

Objective: To determine the efficacy of Platelet Rich Plasma application in comparison to conventional dressing therapy in partial thickness burn wound

Study Design: Comparative analytical study

Place and Duration of Study: This study was carried out at Department of Burns, Civil Hospital Karachi from March 2011 to January 2013.

Materials and Methods: A comparative analytical study was conducted to determine the efficacy of Platelet Rich Plasma application in comparison to conventional dressing therapy in partial thickness burn wound at Department of Burns, Civil Hospital Karachi. All the admitted patients of either sex having age between 20-40 years, victims of fire and scald burn, having partial thickness burn with 10-30% of TBSA involved. Patients were divided into two groups on random basis. In group "A", Platelet Rich Plasma (PRP) application was given with three day gap between two applications until full recovery of the wound. While in group "B" conventional dressing therapy was adopted till full recovery of wound.

Results: All 30 patients of group A, selected for PRP application, were recovered maximally within 18 days (6 therapies with a gap of 3 days). Whereas in other 30 cases, of group B selected for conventional dressing therapy, it took minimum 21 days or more for complete recovery. Hence recovery was found slow in conventional dressing therapy as compared to PRP and it is statistically significant at P,0.05.

Conclusion: Platelet-rich plasma application in non-healing deep partial and full thickness burn wound accelerate the wound healing as compared to conventional dressing therapy and is very effective in preparing healthy beds for grafting and provides 100% graft take.Now it is up to the Burns surgeon to select it for rapid results to save time and cost with availability of more beds in Burn Centre.

Key Words: Platelet Rich plasma, Burns, Conventional dressing, wound healing.

INTRODUCTION

Burn injuries occur as a result of fire and hot water or liquid (scald burn). It may lead to extensive damage to skin surface and also its depth, depending on the intensity of heat delivered to the skin and time of contact. As a result of damage to the skin surface, the protective function gets compromised and results in the exposure of the sub-epithelial skin layers. It depends upon the depth of injury which immediately comes in contact with the external environment specially the micro-organisms like bacteria, fungi, yeast, virus etc. that cause infection on the burnt surface, initially confined to the wound surface but as pathological events progress deeper down they may invade the circulation (bacteremia). If not stopped, further progress leads to septicemia, septic shock and finally death of the patient. In order to avoid these complications, early healing of wound and coverage of damaged skin surface in deeper injuries is necessary to decrease the chances of above mentioned life threatening consequences.

Platelet Rich Plasma has attained a significant importance in facilitating wound healing in many fields of surgery. According to many health workers platelets[1,2] and natural sources of growth factors [3,4,5] play a fundamental role in homeostasis and healing as well[6]. They also release chemotactic factors[7,8] and induce proliferation of fibroblasts and endothelial cells for neo-angiogenesis hence they have a significant effect on minimizing wound infection which is very effective in wound healing[9] in superficial burn wounds and also equally important in providing early well vascularized healthy granulation tissue for resurfacing deep wounds by grafting.

We have all the studies from abroad and no work has been done in Pakistan so far. After studying the results obtained from work done by Marquez-de-Aracena[10], Kazakook[11] and Knighton,[12] etc, we want to apply PRP on burn wounds to get a comparative analysis in partial thickness burn wound.

MATERIALS AND METHODS

This comparative analytical study was carried out from March 2011 to January 2013 at Burns Centre, Civil Hospital, Karachi. We have selected 60 patients suffering from fire and scald burn resulting in partial thickness burn. We excluded electrical, chemical and fire burn of superficial and deep thickness. We have also excluded smokers, diabetics, hypertensive and patients with other cardiac problems and also pregnant women.

We have divided these patients into two groups. Group A was treated by PRP while Group B was treated by conventional dressing therapy.

Platelet concentrate was achieved by extracting platelets from one pint of whole blood. Donor should be healthy, free of communicable blood-borne disease, ABO compatible and should have normal values of complete blood picture on lab test evaluation. Platelet is extracted by CRYOFUGE (centrifuge machine) made by JOVAN (France). The extracted platelets are suspended in 50-100ml plasma in transfusion bag having 3-5 times the concentration as compared to whole blood.

Before each application of PRP in Group A, the patient was shifted to Operation Theater. The wound was carefully examined and if any slough or dead tissue was found that was removed by desloughing or debridement. All procedures are done under strict aseptic conditions. If slough or dead tissue is not found then the wound is washed with NaCl. Before application, the platelet extract received from the blood bank in the transfusion bag is mixed with $CaCl_2$ which is present in a separate syringe provided by the blood bank. As a result of this mixing, the platelets degranulate and start releasing growth factors. The activated PRP is then applied over the wound surface and within 10 minutes the wound is covered with sterile dressing. It is left for 3 days because the activity of the platelet borne factors is supposed to be maximum during this period. So we plan next application after 3 days.

In Group B, thirty patients were selected for giving treatment with conventional dressing therapy. Dressing is done in Operation Theater under sterile conditions. The wound was examined, and washed with normal saline. The dead and devitalized tissues are removed and non-adhesive dressing is done by 1% silver sulphadiazine and then dressing is done. Initially the dressing change depends upon the soakage, the condition wound and the half life of the topical anti bacterial cream.

These procedures were adopted till the completion of wound healing. During this period, the wound was observed for the presence of infection, time of infection eradication from wound, changes in pain perception, total time of wound healing and time of hospital stay during treatment.

RESULTS

According to the analysis of the data, we have selected 60 patients suffering from fire and scald burn resulting in partial thickness burn. These patients were divided into two groups through systematic random sampling. Out of these, 29 (48.3%) patients who were suffering with 10-19%, only 5 (17.0%) required debridement, where as in 31 patients with 20-30 % of burn, 22(71.0%) required debridement which was done. Over all 27 (45.0%) patients requiring debridement (Table 1)

All 60 patients selected for this study were divided in two equal groups. Group A was selected for PRP application and its effects on wound healing were observed and Group B was treated by conventional dressing therapy. All selected patients had partial thickness burn wounds of fire and scald burns, categorized on the basis of history and clinical examination before application of PRP (Group A) and dressing (Group B).

Table No.1: Patients with % of burn requiring debridement

S. No	No. of Patients	% of Burn	Debride-ment not done	Debride-ment done	Total
1.	29	10-19	24	5	29
2.	31	20-30	9	22	31
Total:	60		33	27	60

Table No.2: Epithelization time of burn wound and no of PRP applications

No of patients	PRP applications with a 3 day gap	Total no of days of therapy
16	Three (3)	9
9	Four (4)	12
5	Six (6)	18
Total : 30	Maximum 6 therapy	Maximum 18 days for full recovery.

Table No.3: Epithelization of burn wound with conventional dressing method

No. of patients	Duration of dressing
16	21-30 days
11	31-40 days
3	>40 days
Total : 30	

PRP application was given in Group A (30 patients) at a gap of three days between two consecutive applications to achieve maximum effect of PRP which lasts for three days. 16 out of 30 (53.4%) showed epithelization after 3 applications with a 3 day gap in between every application i.e. 9-day therapy. 9 out of 30 (30%)

showed epithelization after 4 applications, 3 days apart i.e. 12 days therapy and 5 out of 30 (16.7%) showed proper epithelization after 6 applications i.e. maximum 18 days required for complete healing. (Table 2).

In conventional dressing method Group II dressing was done by 1% silver sulphadiazine and wound was covered by Gamgee dressing, 16 out of 30 (53.3%) patients took 21-30 days, 11 out of 30 (36.7%) took 31-40 days , 3 out of 30(10.0%) patients took more than 40 days to show proper epithelization (Table 3)

The results of these two groups were compared to get an idea regarding the efficacy of PRP in rapid wound healing and it was found that Platelet Rich Plasma application in comparison to conventional dressing therapy in partial thickness burn wound which is statistically significant at P<0.05 (Table 4)

Table No.4: Comparison of epithelization of burn wound treated with PRP and with conventional dressing method

No. of days	No. of patients fully recovered with PRP	No. of patients recovered with conventional dressing method
<10	16 (53.3%)	0
11-20	14 (46.7%)	0
21-30	__	16 (53.3%)
31-40	__	11 (36.7%)
>40	__	3 (10.0%)

DISCUSSION

Fire and scald burn are common in burn cases and rapid recovery is the main issue in partial thickness burn wounds. We have designed a study to compare the efficacy of PRP with conventional dressing methods in partial thickness burn wound. Partial thickness burn wound specially in fire and scald burn have an advantage of healing spontaneously nearly within 3 weeks, provided all protocols of sterilization are followed, otherwise if partial thickness burn wound gets infected, the depth of the wound may extend deep down and the partial thickness burn may turn into deep partial thickness wound where the healing takes a longer time and most of the healing occurs by formation of fibrous tissue which ends up in hypertrophic scar, keloid or contracture formation. As far as effect of PRP on burn wound is concerned, few studies are present so far, Marquez-de-Aracena et al (2007)[10] applied PRP on ten Ocular burn patients and got very good results regarding fast and excellent healing. According to him, he got very good corneal healing after application in eye suffering from chemical burn involving the cornea. He also observed minimal post burn scarring after sub-conjunctival injection of PRP.

According to Kazakos K et al (2008)[11] it is very effective in partial thickness burn wounds. No study was found on PRP application in Burn patients of higher % of body surface area involvement.

Many workers used it in deep wounds as Ganco et al[3] showed faster epithelization of chronic lower extremity ulcers where he found appropriate results. Knighton DR et al[12] also showed successful treatment of non-healing wounds after application of PRP.

In our study, the comparison clearly showed that the conventional dressing method takes twice longer time as in Group B the average healing time was 21 days to >40 days as compared to Group A where the average healing time was 9 days to maximum 18 days which definitely affects the duration of hospital stay.

The other advantage of PRP application in Group A as opposed to Group B is the decrease in pain, rapid control of infection and hence faster epithelization.

A broad majority of workers used PRP on small area of burn wounds so mostly they used autogenous PRP in their studies like Martinez Zapata[13] was in favor of autogenous PRP, but in our study we had to apply PRP on a larger % of body surface area so it was not possible to use autogenous PRP. Other factors also contributed to make it impossible such as the patients admitted in our hospital belong to very low socio-economic class and their health status is also very low at the time of admission and after admission, G.I. upset due to exposure to micro-organisms, heavy antibiotic therapy, systemic infection, intolerance to food intake, non-availability to food supplements leading to low caloric intake lead to a decreased hemoglobin making it impossible to use autogenous PRP.

The quantity of PRP required for single application for burn wound between 10-30% of body surface area needs 2 pints of blood for extraction of platelets. We have to consult blood bank and use donated blood therefore we cannot avoid chances of allergic reactions so this is a drawback of not using autogenous PRP. But its advantages over conventional dressing such as decrease in duration of healing time, decrease in pain, infection and hospital stay are more convincing for its use in partial thickness burn wounds.

CONCLUSION

According to our study results, Platelet-rich plasma application in non-healing deep partial and full thickness burn wound accelerate the wound healing as compared to conventional dressing therapy and is very effective in preparing healthy beds for grafting and provides 100% graft take. Now it is up to the Burns surgeon to select it for rapid results to save time and cost with availability of more beds in Burn Centre or to use the conventional dressing therapy according to the condition of the patient.

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Original Article

Esophageal Varices

Prediction of Large Esophageal Varices in Patients with Decompensated Cirrhosis by Child-Pugh Score, in Medical Unit-II, Chandka Medical College Hospital, Larkana

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ABSTRACT

Objective: To assess prediction of large esophageal varices in patients with decompensated cirrhosis by Child-Pugh score, in Medical Unit-II, Chandka Medical College Hospital Larkana.
Study Design: Cross sectional study
Setting and Duration of Study: This study was carried out at Medical Unit II, Chandka Medical College & Hospital, Larkana from November 2011 to November 2012.
Materials and Methods: In this study 88 consecutive cirrhotic patients with ascites (those Patients who fulfilled the inclusion criteria) were included; blood samples for Serum bilirubin, serum albumin, and INR ratio were sent to single laboratory. Then, child Pugh score were assigned to each patient on the basis of clinical and to laboratory parameter. The ultrasound of abdomen was carried out for size of liver and spleen, portal vein diameter, and quantification of ascits. Eligible patients were subjected for upper gastrointestinal endoscopy for the presence of esophageal varices and their grading. The data was analyzed using SPSS version 19.
Results: The mean age of enrolled patients was 43.19±7.1 years. Of 88 patients, 69 (78.4%) were male and 19 (21.6%) were female. Child Pugh class relation to number of patients were; 52, 20, and 16 in class A, B, and C respectively. While Child Pugh class relation to frequency of esophageal varices were; 6, 11 and 14 in class A, B, and C respectively. Frequency of grading of esophageal varices was; 4, 13, and 14 in grade one, two, and three respectively. Distribution of large esophageal varices (LEVX) in relation to child Pugh class is one in class A, two in class B and 11 in class C.
Conclusions: It is concluded from this study that as the child pugh score advances, the number and size of esophageal varices increases, and chance of absence of varices decreases.
Key Words: Esophageal varices, Child Pugh score, Grades of varices

INTRODUCTION

Cirrhosis is an end result of various hepatic diseases resulting to fibrosis with replacement of normal hepatic architecture in to nodular form leading to opening of Porto-systemic shunts. Some patients with cirrhosis are completely asymptomatic and have a reasonably normal life expectancy. Others have symptoms of end-stage liver disease and have a limited chance for survival. Chronic liver disease and cirrhosis result in about 35,000 deaths each year in the United States. Cirrhosis is the ninth leading cause of death in the United States and is responsible for 1.2% of all US deaths. Many patients die from the disease in their fifth or sixth decade of life. Cirrhosis was the 10th leading cause of death for men and the 12th for women in the United States in 2001, killing about 27,000 people each year.[1] 26% of hepatitis B and 15% of hepatitis C leads to liver cirrhosis worldwide.[2] Many patients of liver cirrhosis ultimately develop complications; amongst them portal hypertension is one of the major complication manifested as esophageal varices.[2]

The portal vein carries approximately 1500 ml/min of blood from the small and large bowel, spleen, and the stomach to the liver. Obstruction of portal venous flow, whatever the etiology, results in a rise in portal venous pressure. The response to increased venous pressure is the development of a collateral circulation diverting the obstructed blood flow to the systemic veins. These Porto - systemic collaterals form by the opening and dilatation of preexisting vascular channels connecting the portal venous system and the superior and inferior vena cava.

In Western countries, alcoholic and viral cirrhosis are the leading causes of portal hypertension and esophageal varices. Thirty percent of patients with compensated cirrhosis and 60-70% of patients with decompensate cirrhosis have gastro esophageal varices at presentation. The de novo rate of development of esophageal varices in patients with chronic liver diseases is approximately 8% per year for the first 2 years and 30% by the sixth year. The risk of bleeding from esophageal varices is 30% in the first year after identification. Bleeding from esophageal varices

accounts for approximately 10% of episodes of upper GI bleeding.

At least two thirds of cirrhotic patients develop esophageal varices during their lifetime. Severe upper gastrointestinal (UGI) bleeding as a complication of portal hypertension develops in about 30%-40% of cirrhotic, [3] the reported mortality from first episode of variceal bleeding in western studies ranges from 17% to 57% as compared to 5-10% mortality reported in our population. [4]

Upper GI endoscopy is gold standard investigation for diagnosis of esophageal varices[5]. But because of various limitations (not available commonly at primary and secondary care centers, lack of experts and cost of procedure), and not all patients of cirrhosis are developing esophageal varices, so it cannot be performed in every patients. Therefore an alternate, easy, less costly, non invasive way to identify the high risk patients for large esophageal varices has to be sorted out. So this study may help us to detect those high risk patients of liver cirrhosis.

MATERIALS AND METHODS

This Cross-sectional study was conducted in medial unit II, Chandka Medical College & Hospital Larkana, over a period of one year, from November 2011 to October 2012.

After the approval from Ethical Review Committee of Chandka medical college hospital Larkana, Patients were selected from medical unit II indoor department, those Patients who fulfilled the inclusion criteria (Cirrhotic patients with ascites, aged >12 years of either sex, without past history of upper and lower GI bleeding) were enrolled in this study. A verbal and written consent was obtained from all the patients after having fully explained the purpose and protocol of the study by researcher.

After taking history and clinical examination, blood samples for Serum bilirubin, serum albumin, and INR ratio were sent to laboratory. Then, on the basis of clinical and lab parameter child Pugh scoring were assigned. The ultrasound of abdomen was carried out for size of liver and spleen, portal vein diameter, and quantification of ascits, by an expert having at least 5 years experience.

Upper gastrointestinal endoscopy of eligible patients was performed after explaining the procedure and getting written consent from patient. After premedication with intravenous injection of 2 mg Midazolam and Xylocaine 1% spray as a topical anesthetic, procedure was done with Fiberoptic Endoscope by expert endoscopist and esophageal varices were graded on the basis of de-Franchis classification, i.e. Grade1 (small size); small straight varices, not occupying the lumen. Grade 2 (medium size); enlarged tortuous varices occupying less than one third of the lumen. Grade 3 (large size) ; large coil-shaped varices occupying more than one third of the lumen.

Following patients were excluded from our study. Patients who were taking medications for prophylaxis of variceal bleed. Patients who had previously underwent scelerotherapy or band ligation. Patients with hepatocellular carcinoma, previous portasystemic anastomosis or portal vein thrombosis. Patients with medical contraindications to upper gastrointestinal endoscopy like shock, atlanto-axial sublaxation, any coagulation disorder or not willing for endoscopy. Patients of age less than 12 years.

Data Analysis: All the data were entered and analyzed using SPSS version 19.0. Frequency and percentages was computed for categorical variables like gender, child Pugh classification and classification of esophageal varices. Mean ± S.D. was computed for quantitative variables age. Child Pugh scoring was correlated with grading of esophageal varices and their signification was assessed by chi-square test.

RESULTS

In this study 88 patients were enrolled, male were 69 (78.4%) and female were 19 (21.6%), with male to female ratio were 3.6:1.Mean age of enrolled participants was 49 + 15.538.

Out of 88 study population 52 (29.1%) patients were found in Child Pugh class A, 20 (22.7%) in class B, and 16 (18.2%) in class C. Table: 1

Table No.1: **Salient Features of Study Population**

Gender	Male. 69 (74.41%)	Female. 19 (21.59%)	Male to Female Ratio (3.6:1)
Child Pugh Class (Frequency & Percentage)			
Class A	52 (29.1%)		
Class B	20 (22.7%)		
Class C	16 (18.2%)		
Esophaeal Varices (Frequency & Percentage)			
Present	31 (35.2%)		
Absent	57 (64.8%)		
Grading Of Esophageal Varices (Frequency & Percentage)			
Grade 1 (small)	4 (4.5%)		
Grade 2 (medium)	13 (14.8%)		
Grade 3 (large)	14 (15.9%)		

Esophageal varices were present in 31(35.2%) out of 88 study population, Grade one (GI) esophageal varices were found in 4 (12.90%), Grade two (GII) in 13(41.94%) and Grade three (GIII) in 14 (45.16%) patients.

Correlation of Child Pugh class with esophageal varices and their grade shows that, in Child Pugh class

A out of 52 patients; oesophageal varices were present in 6 (11.5%) patients, and out of 6 patients 3(50%) were in grade one, 2 (33.3%) were in grade two, and 1(16.7%) was in grade three. Table: 2

In Child Pugh class B out of 20 patients; 11(55%) had esophageal varices, and out of 11 patients 1(9.1%) had grade one, 8 (72.7%) had grade two, and 2 (18.1%) had grade three esophageal varices. Table: 2

Table No. 2: Child Pugh * Grades of esophageal varices (EV) Cross tabulation

			Grades of Esophageal Varices				
			No Varices	Grade 1	Grade 2	Grade 3	Total
Child Pugh	Class A	Count	46	3	2	1	52
		% within Child Pugh	88.5%	5.8%	3.8%	1.9%	100.0%
		% within Grades	80.7%	75.0%	15.4%	7.1%	59.1%
		% of Total	52.3%	3.4%	2.3%	1.1%	59.1%
	Class B	Count	9	1	8	2	20
		% within Child Pugh	45.0%	5.0%	40.0%	10.0%	100.0%
		% within Grades	15.8%	25.0%	61.5%	14.3%	22.7%
		% of Total	10.2%	1.1%	9.1%	2.3%	22.7%
	Class C	Count	2	0	3	11	16
		% within Child Pugh	12.5%	.0%	18.8%	68.8%	100.0%
		% within Grades	3.5%	.0%	23.1%	78.6%	18.2%
		% of Total	2.3%	.0%	3.4%	12.5%	18.2%
Total		Count	57	4	13	14	88
		% within Child Pugh	64.8%	4.5%	14.8%	15.9%	100.0%
		% within Grades	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	64.8%	4.5%	14.8%	15.9%	100.0%

Chi-square = 61.273, df=6, p= < 0.05

While in Child Pugh class C out of 16; esophageal varices were found in 14 patients, among them grade one was not found in any patient, grade two in 3 (21.4%) patients, while grade three in 11(78.6%) patients. Table: 2

It means that; as the child pugh class advances (A to C) the number and size of esophageal varices increases (in Child Pugh class A 50% in grade one, in class B 72% in grade two and in class C 78% in grade three) it was statistically significant (p= < 0.05). Table: 2

DISCUSSION

Cirrhosis is the most advanced form of liver disease and variceal hemorrhage is one of its lethal complications. Over half of the patients with cirrhosis develop varices. The risk of bleeding once varices formed is 20% to 35% within 2 years,[5] and the reported mortality rate from first episode of variceal bleeding is 17% to 57%, and of those who survive the initial episode of bleeding and who do not receive active treatment, the risk of recurrent bleeding is approximately 66% and usually occurs within 6 months of the initial bleeding episode.[6,7]

Because cirrhotic patients with large esophageal varices are at a high risk for bleeding, an overt hemorrhage from the gastric mucosa occurred in 60% of patients with severe PHG. It has been documented that empiric beta-blocker therapy for the primary prophylaxis of variceal hemorrhage is a cost-effective measure, and it decreases the incidence of bleeding and an effect on bleeding related mortality. The use of screening endoscopy to guide therapy adds significant cost with only marginal increase in effectiveness. This added cost to screening endoscopy is presumably due only to the large number of unplanned endoscopies; where the endoscopic findings do not significantly alter the treatment plan, hence identification of non invasive parameters that can accurately predict esophageal varices and help to identifying patients at greatest risk is important to improve the yield and cost- effectiveness of endoscopic screening.[8]

In this study esophageal varices has been related to child-pugh categories, to identify the large esophageal varices, in order to do endoscopy in selective patients and or to start the oral beta blockers in those who are not willing or fit for endoscopy.

In this study esophageal varices observed in 31 (35.2%) patients. Of 31 cases of varices, 12.90% were of Grade I, 41.94% in Grade II and 45.16% in Grade III. While some of local and European studies suggest higher frequencies of esophageal varices observed in cirrhotic patients, like 91.7% by Said HEE et al,[9] 70% by Gill ML et al,[10] 70.6% by Prihartini J et al,[11] 70.7% by Hong WD et al,[12] 76.9% by Stojanov DB et al,[13] and 80 % by Kaji BC et al.[14]

On other hand few studies suggest little bit lower frequencies, 29% by Almani SH et al,[15] 50% by thompoulos KC et al,[16] and two studies show 51% by Carles P et al[17] and Madhotra et al, [18] 55% by schwarzenberger E et al,[19] 58.4% by Sethar GH et al,[20] and 61% by Giannini E et al.[21] In all above studies other non invasive parameters were used for the

detection of esophageal varices. In our study large esophageal varices were present in 45.16 % of cases. In comparision to other studies large oesophageal varices were observed were, 30% [10], 33%,[16] 34% [14] and 46% by Sharma SK et al,[22] 50% Barrera F et al,[23] and 65%. [13] Study by Madhotra,[18] shows distribution of esophageal variecs to child pugh category A, 35%; B, 60%; C, 69% and large varices were 29% in category A, 24 % in category B and C, while in our study frequency of large esophageal varices were, 16.66% in class A, 18.18% in class B, and 92.85% in class C, which correlates with study by Said HEE,[9] they also observed increasing frequency of esophageal varices with advancement of child-pugh class (50% in A, 93.5% in B and 100% in C). In our study frequency and size of esophageal varices is correlated rather than frequency alone.

CONCLUSION

In this study we conclude that as the Child Pugh score advances the frequency of esophageal varices increases especially the large varices. So we recommend that those patients in which upper gastrointestinal endoscopy is not feasible of any reason, and having history of upper gastrointestinal bleeding, with Child Pugh class "C" then beta blocker drugs shuld be given for prophylaxis if there are no contraindications.

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Original Article

Pharmacology

Cognition-Enhancing Effect of Oral Therapeutic Doses of Methylphenidate in Rats

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ABSTRACT

Objective: To determine the effects of oral therapeutic doses of methylphenidate, on memory. It was thought that long term use of methylphenidate possibly may lead to tolerance in the ability of the drug elicit enhancement of learning and memory. A dose-dependent effect may therefore help to extend the therapeutic use of the drug for better clinical response.

Study Design: Experimental / Analytic study

Place and Duration of Study: This study was conducted in the Department of Pharmacology, Faculty of pharmacy, University of Karachi, Karachi for a period of period of 4 weeks.

Materials and Methods: Twenty-four male Albino Wister rats (weighing 180-220g) were randomly assigned to four groups, one control and 3 test groups. The experimental protocol was designed to administer methylphenidate orally two times daily for 4 weeks. Four groups were: (i) Saline (1.0 ml/kg/ day), (ii) Methylphenidate (2mg/kg/day) (iii) Methylphenidate (5mg/kg/day) (iv) Methylphenidate (8mg/kg/day) treated groups. Behavioral activity of rats i.e. performance of rats in passive avoidance test was monitored weekly. The experiment was performed in a balanced design to avoid the order effect.

Results: In the present study methylphenidate treated rats exhibited an increased in Passive Avoidance learning as there was increased in the latency time to reach the punished compartment as compared to control rats. This memory improvement effect of methylphenidate on PA was dose dependent in 1st week as the rats treated with 8 mg/kg/day took greater time to reach the dark box than 5mg/kg/day and 2mg/kg/day, but in 2nd, 3rd and 4th week it was seen that rats treated with fore mention doses took same time but greater than 1st week to enter the punishable compartment

Conclusion: It can be concluded that high dose produce greater cognitive effect in short term treatment than low and moderate doses, however in long-term treatment all the doses produce similar improvement in memory without tolerance in cognition

Key Words: methylphenidate, oral dose, cognition, passive avoidance test.

INTRODUCTION

Cognition enhancement has received much attention in recent scientific literatures due to our aging society and the increasing prevalence of Alzheimer's disease. However, the healthy young population also engages in drug use to enhance memory. Methylphenidate a medication effective to enhance attention[1,2,3], and cognition[4] in ADHD patients, as well as in healthy subjects[5,6,7,8].This has raised concern regarding the ethical and safety aspects of potential cognition-enhancing drugs[9,10,11]. These issues aside, it is important to know if the drug does actually have cognition-enhancing effect after long-term administration.

Methylphenidate blocks the dopamine transporter and the noradrenaline transporter[12,13,14,15], thus increasing the extracellular concentrations of these catecholamines. The attention-improving characteristic of methylphenidate has been attributed to the amplification of dopamine release in the central nervous system[16]. Dopamine (DA) modulates cognitive performance in part via its regulation of the prefrontal cortex through dopamine D1 and D2 receptors[17]. Methylphenidate (MPH) increase DA signaling in the brain and are used in the treatment of attention deficit hyperactivity disorder (ADHD) and other neuropsychiatric disorders to enhance attention and cognition[18,19,20,21].

Purpose of our study was to monitor the effects of oral therapeutic doses of methylphenidate, on memory function in rats. It was thought that long term use of methylphenidate possibly may lead to tolerance in the ability of the drug elicit enhancement of learning and memory. A dose-dependent effect may therefore help to extend the therapeutic use of the drug for better clinical response.

MATERIALS AND METHODS

2.1 Test systems used (Animals):

Locally bred Albino Wister rats (weighing 180-200g) were housed individually under 12 h light and dark cycles (light on at 06:00h) and controlled room temperature (24+2℃) with free access to tap water and cubes of standard rodent diet at least 7 days before the start of experiment so that they could become familiar to the environment. They were accustomed to various handling procedures to nullify stress effects. All experiments were performed according to the protocols approved by the local animal care committee.

2.2 Behavioral assessment

Passive Avoidance Test: Passive avoidance paradigm consists of two compartments as an illuminated 'safe' and a dark 'punishable' one. Both compartments were connected with a door that enable free crossing from one compartment to another. Both compartments had a grid floor. The diameter of rods was 5 mm with 0.5 cm distance between the rods.

In the training session, rat was placed in an illuminated box. Once the rats prompted by their instinct stepped its four paws into the dark compartment, rats received 1.5 mA foot shock through the grid floor to its paws for 5 seconds. After receiving the foot shock, it immediately came back to illuminated safe compartment. During the test period (24 hour later), rats were placed in the bright compartment again for a maximum of 5minutes. The step- through latency that indicates the time elapsed before the rat entered the dark compartment was recorded in the test session as described earlier [22]. Passive avoidance test of all drug treated and control animals were performed weekly in a balance design to avoid order effect.

2.3. Drugs: Methylphenidate HCl was obtained from local medical store and prepared in 0.9% NaCl (saline). Drug was administered in a volume of 1 ml/kg of body weight by per oral route twice a day to the treated animals and control animals were treated with saline (0.9%) at the dose of 1 ml/kg per oral twice a day.

2.4. Experimental protocol: Twenty-four male Albino Wister rats (weighing 180-220g) were randomly assigned to four groups, one control and 3 test groups, each containing six animals. The experimental protocol was designed to administer methylphenidate orally two times daily for 4 weeks.

Four groups were: (i) Saline (1.0 ml/kg/day), (ii) Methylphenidate (2mg/kg/day) (iii) Methylphenidate (5mg/kg/day) (iv) Methylphenidate (8mg/kg/day) treated groups.

Behavioral activity of rats i.e. performance of rats in passive avoidance test was monitored weekly. The experiment was performed in a balanced design to avoid the order effect.

2.5. Statistical analysis: Results are represented as mean ±S.D. Statistical analysis of passive avoidance test was performed by two-way analysis of variance (ANOVA) repeated measure design. Post hoc comparison of groups was performed by Newman-Keul test. Values of $p<0.05$ and $p<0.01$ were considered as significant.

RESULTS

Dose related effect of methylphenidate on performance of rats in passive avoidance test.

Fig. shows the effects of methylphenidate doses on the performance of rats in PA test. Two-way ANOVA repeated measure revealed a significant dose ($F=200.4$, $df=3,20$, $P<0.01$), week ($F=32.9$, $df=3,20$, $P<0.01$) effect and also a significant interaction between the two factors ($F=10.97$, $df=9,60$, $P<0.01$).

Post hoc comparison by Newman-keuls test showed that retention of Passive Avoidance was improved in test rats than in controls as low dose (2 mg/kg/day), moderate dose (5 mg/kg/day) and high dose (8mg/kg/day) of methylphenidate significantly ($P<0.01$) increased latency time in all 4 weeks compared to similar week saline treated rats and in 2nd ,3rd and 4th week from 1st week values.

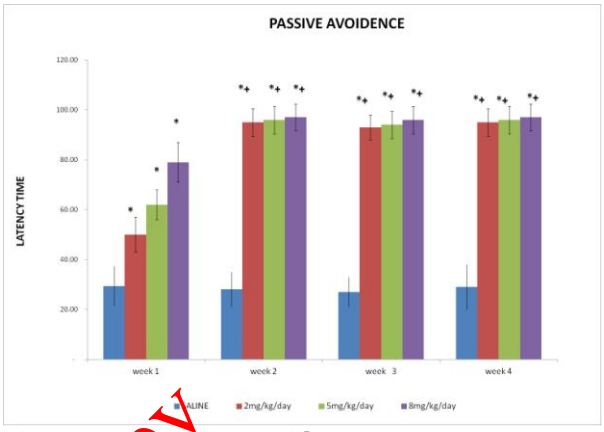

Figure No. 1: Passive Avoidance

DISCUSSION

Methylphenidate has been shown to potentiate the cognitive effect and is the main medication prescribed for attention deficit hyperactivity disorder[23] to improve memory[24] attention and concentration[25,26,27], yet there is increasing evidence that they do not promote learning[27], which can be due to tolerance to the cognitive effect of the drug.

In the current study we chose passive avoidance test to measure effects in memory function following methylphenidate administration at three different doses. Study showed methylphenidate treated rats exhibited an increased in Passive Avoidance learning as there was increased in the latency time to reach the punished compartment as compared to control rats. This memory improvement effect of methylphenidate on PA was dose dependent in 1st week as the rats treated with 8 mg/kg/day took greater time to reach the dark box than 5mg/kg/day dose and the rats treated with 5mg/kg/day day took greater time to reach the dark box than 2mg/kg/day dose, but in 2nd , 3rd and 4th week it was seen that rats treated with fore mention doses took same time to enter the dark punishable compartment which showed that high dose produce greater cognitive effect in short term treatment however in long-term treatment all the doses produce similar cognitive effect.

Central dopaminergic systems play a critical role in the regulation cognitive behavior. Memory improvement effect of methylphenidate in the present study is due to increased DA levels as there is an overwhelming evidence for a critical role of dopamine in

cognition[28,29]. Cognitive symptoms have been associated with dopamine disregulation in numerous diseases including schizophrenia[30], depression[31], drug addiction[32] and Parkinson disease[33]. Support for a role of dopamine in cognition also comes from studies of dopaminergic drug in normal subject[34]

Methylphenidate blocks the dopamine transporter[12,13,14,15] thus increasing the extracellular concentrations of these dopamine. The memory improvement effect of methylphenidate has been attributed to the amplification of dopamine release in the central nervous system[16]. Dopamine (DA) modulates cognitive performance in part via its regulation of the prefrontal cortex through dopamine D1 and D2 receptors[17]. Methylphenidate (MPH) increases DA signaling in the brain and is used in the treatment of attention deficit hyperactivity disorder (ADHD) and other neuropsychiatric disorders to enhance attention and cognition [18,19,20,21]

CONCLUSION

In summary this study provides the documentation of significant relationship between oral therapeutic doses of methylphenidate and cognation .Our results suggest that initially cognitive enhancing effect is dose dependent, but later on cognitive improvement response in low, moderate and high doses of methylphenidate became similar after long term use.

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Original Article

EPI Status

EPI Status under One Year Children and their Mothers attending Paediatric Department Sheikh Zayed Medical College & Hospital, Rahim Yar Khan

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ABSTRACT

Objective: To determine the EPI status in children <1 year of age and the status of Tetanus immunization in their mothers attending paediatric department. This will help us to determine the facts and figures in our population and to construct a plan to improve the vaccination status.

Study Design: Cross-sectional analytical study

Place and Duration of Study: This study was conducted at the Department of Paediatrics, Sheikh Zayed Medical College & Hospital, Rahim Yar khan from April 2011 to July 2011.

Materials and Methods: In the study 492 consecutive children whether admitted in the ward or visited in Paediatric OPD and their mothers were inquired about EPI status in the child and the status of Tetanus immunization in their mothers, strictly following the inclusion and exclusion criteria.

Results: Out of 492 patients, we have found three groups. Group 1 includes those children who were unvaccinated. Group 2 includes those children who were incompletely vaccinated. Group 3 include those who were completely vaccinated according to EPI schedule. Similarly three groups were found in mothers. Group 1 includes those who were unvaccinated. Group 2 includes incompletely vaccinated ladies. Group 3 include those who were completely vaccinated according to EPI schedule.

Conclusion: In our study, we found that EPI status is very poor in our low socioeconomic and illiterate population. Those people living in peripheries and remote areas are particularly not properly following the EPI programme.

Key Words: EPI (Expanded Program on Immunization), Immunization, Vaccines, Tetanus, Tetanus Toxoid(TT)

INTRODUCTION

The Expanded Program on Immunization is a World Health Organization program with the goal to make vaccines available to all children throughout the world.

For evaluation **c**ommunity-based surveys are applied using a modified cluster sampling survey method developed by the World Health Organization. Vaccine coverage is evaluated using a two-stage sampling approach in which 30 clusters and seven children within each cluster are selected. Health care workers with no or limited background in statistics and sampling are able to carry out data collection with minimal training [1]. Such a survey implementation provides a way to get information from areas where there is no reliable data source. It is also used to validate reported vaccine coverage (for example, from administrative reports) and is expected to estimate vaccine coverage within 10 percent. Surveys or questionnaires, though frequently considered inaccurate due to self-reporting, can provide more detailed information than administrative reports alone[2]. A survey in Thailand has found effective expended programme of immunization[3].

Expanded Programme on Immunization (EPI) in Pakistan: The Expanded Programme on Immunization (EPI) was launched in Pakistan in 1976 by WHO and UNICEF to protect children by immunizing them against childhood tuberculosis, poliomyelitis, diphtheria, pertussis, tetanus and measles.

The programme also vaccinates pregnant women with tetanus toxoid vaccine to protect the new born from neonatal tetanus. With support from the Government of Pakistan and development partners, the programme has added hepatitis B (Hep B) and Hemophiles influenza type B (Hib) vaccinations to its childhood immunization schedule. In 2002, the programme introduced hepatitis B vaccine with support from the Global Alliance for Vaccine and Immunization (now called GAVI). In 2006, a tetravalent combination vaccine was introduced replacing separate diphtheria, tetanus and pertussis and hepatitis B vaccines. This was later switched in 2008 to the pentavalent (DPT, Hep B, Hib) vaccine with the addition of the new Hib vaccine. Now a child needs only five visits during the first year and one visit during the second year of his/her life to complete the vaccination with four antigens against eight dreadful diseases.

Programme Development: From mid 2012 pneumococcal conjugate vaccine was added in immunization programme. This new vaccine will protect children from pneumonia and meningitis due to

pneumococcal infection. The programme also plans to introduce Rota virus vaccine, which will prevent diarrhea due to rotavirus. The new vaccines may together avert 17% of childhood mortality in Pakistan and thus help in achieving Millennium Development Goal 4 on reducing child mortality.

Safer Demmunization: The programme has adopted new technology to make immunization safer and more clients compliant. Since 2002, to prevent the risk of blood borne diseases, the programme has been using auto-disable syringes for all immunization injections and safety boxes for proper disposal of sharps waste[4].

Programme objectives: The aim is to reduce mortality and morbidity resulting from the eight EPI target diseases by immunizing children aged from 0 to 11 months and pregnant women.

The specific objectives of the programme are interruption of polio virus by 2012, elimination of neo-natal tetanus by 2015, elimination of measles by 2015, reduction of diphtheria, pertussis and childhood tuberculosis to a minimum level so that they do become a public health problem, control of other diseases by introducing new vaccines in EPI as and when they become available, using EPI as a spearhead for promoting other primary health care activities, integrating EPI into primary health care.

Current situation: Despite significant efforts by the government and its partners, Pakistan's immunization indicators have yet to reach the expected benchmarks. The key goals of polio eradication, and measles and neo-natal tetanus elimination, have not been achieved. People coming from remote areas have poor knowledge and facilities of vaccination [5,6]. A study published by Furqan Kurshid found poor vaccination coverage of mothers during pregnancy with tetanus toxoid and infant after birth [7].

Routine vaccination coverage is still suboptimal for achieving the desired goals. Poor routine coverage is also evidenced by the occurrence of numerous outbreaks of measles, pertussis and diphtheria in different parts of the country. A study conducted by Shaikh S. "Immunization status and reasons for low vaccination in children, attending O.P.D. at Liaquat University Hospital" found poor knowledge of vaccination in community [8].

MATERIALS AND METHODS

We have included the children who were below one year age. Data collected by taking interview from mothers, whether the child was admitted in Pediatric unit due to some illness or visited paediatric OPD. The Performa was filled strictly following the rules. Their mothers were asked about the EPI status of the child i.e.; BCG, polio, pentavalent (DPT, HiB, Hep B) and measles 1. Child's mother was interviewed by using a structured pre-tested questionnaire, regarding the EPI coverage of her child, her own T.T. coverage and other demographic and potential risk factors for low vaccination coverage. EPI cards were checked where ever available and if not, the subjects were inquired verbally and BCG scars were checked. We gathered information on characteristics such as basic demographics, socio-economic status, reproductive history, health services utilization, immunization coverage of mother and child and reasons for non-compliance with the EPI schedule.

The children who were below one year of age admitted in Pediatric unit due to some illness or visited paediatric OPD and their mothers were included in the study.

The children above one year of age were excluded from the study.

EPI Schedule For Children

Age	Injection or Drops
Immediate after birth	BCG , Polio (birth)
6 weeks	OPV-1, Pentavalent – Pneumococcal – 1
10 weeks	OPV-2, Pentavalent – Pneumococcal – 2
14 weeks	OPV-3, Pentavalent – Pneumococcal – 3
9 months	Measles – 1
15 months	Measles – 2

Tetanus Vaccination Schedule for Pregnant Women

Sr. No.	Schedule	Vaccination
1	During Pregnancy	TT-1
2	After one month of 1st dose of vaccination	TT-2
3	After six month of 2nd dose of vaccination	TT-3
4	After one year of 3rd dose of vaccination	TT-4
5	After one year of 4thdose of vaccination	TT-5

RESULTS

492 children of age less than one year, who were admitted in pediatric unit due to some ailment or visited Paediatric OPD and their mothers, were interviewed. The mean age of the infants was 5.4 +_3.5 months. We have found three groups. Group 1 includes those children who were unvaccinated. Group 2 includes those children who were incompletely vaccinated. Group 3 include those who were completely vaccinated according to EPI schedule.

Group 1 Unvaccinated : 92(18.69%)
Group 2 Incompletely vaccinated : 177(35.97%)
Group 3 Completely vaccinated : 223(45.32%)

Out of 492, twenty one percent had received no vaccination. Sixty two percent of mothers had received one to four doses of T.T. Seventeen percent had

received full vaccination coverage. When inquired about the total number of T.T doses received in lifetime by the mothers, we learnt that there were 102 women who had received just one dose. The rest of the women 203 had received 2-4 doses of TT.

Group 1 Unvaccinated : 103(21%)
Group 2 One to four doses of T.T : 305(62%)
Group 3 Completely vaccinated : 84(17%)

Table No.1: Demographic information of the families surveyed to determine EPI coverage

Sr. No.	Characteristic	Number of subjects (%)
1.	Fathers education	
i.	Primary	226 (46%)
ii.	Matric	147 (30%)
iii.	Inter and above	119 (24%)
2.	Mother education	
i.	Primary	290 (59%)
ii.	Matric	118 (24%)
iii.	Inter and above	84 (17%)
3.	Knowledge about vaccination	
i.	Poor	259 (53%)
ii.	Good	233 (47%)
4.	Fathers' occupation	
i.	Unskilled labor	93 (19%)
ii.	Government service	9 (1.82%)
iii.	Private service	206 (42%)
iv.	Business	73 (15%)
v.	Skilled labor	14 (3%)
vi.	Unemployed	0
vii.	Farming	44 (9%)
viii.	Landlord	19 (4%)
5.	Fathers' ethnicity	
i.	Punjabi	139(28%)
ii.	Saraiki	202(41%)
iii.	Urdu speakers	71(14%)
iv.	Balochi	32(7%)
v.	Sindhi	29(6%)
vi.	Pathan	10(2%)
vii.	Others	10(2%)
6.	Mothers' occupation	
i.	Housewives	330(67%)
ii.	Skilled labor	133(27%)
iii.	Government service	29(6%)

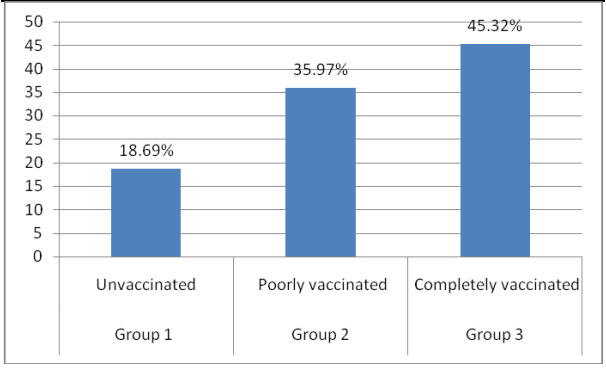

Figure No.1: Vaccination of children below one year

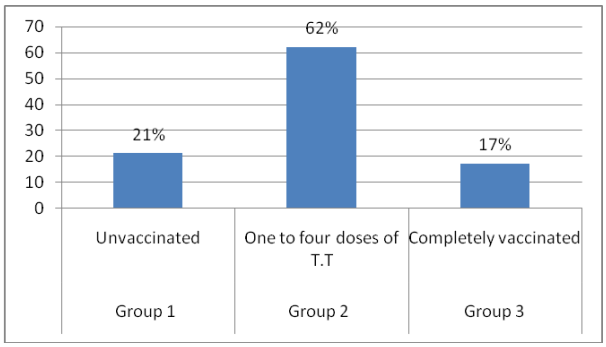

Figure No.2: Vaccination of mothers

DISCUSSION

The age-appropriate vaccination coverage for infants in our study was 54.6%. A study conducted in peri-urban Karachi To determine the age-appropriate EPI coverage of under one year old children and Tetanus Toxoid (TT) coverage of their mothers determined that 44.8% coverage [9]. In another study in Chicago, USA, among inner city pre-school children reported coverage of 47% but in this study, the age of the children was between 19 to 35 months. Another study conducted in Gondar, Ethiopia among 12-24 months old children reported 47.4% as fully immunized [10]. A baseline study done in four regions of Pakistan showed EPI coverage of 48% . In another study in the North West Frontier Province (NWFP) of Pakistan, 65% of children were fully immunized by three years of age [11]. . However a cross-sectional study conducted in Peshawar/Abottabad found poor vaccination coverage in women in reproductive age [12,13]. Fasih et al reported EPI coverage of 26.5% by age 2 years in Karachi, Pakistan[14]. Enhancing the vaccination provision may increase the coverage [15] Nisar N and colleges found poor knowledge and attitude of women regarding vaccination[16]. In our study, despite using relatively strict criteria by enrolling less than one year old children comparable or better coverage has been found

CONCLUSION

We concluded that proper knowledge of vaccination is very important. For this education of both parents plays a significant role in child's immunization coverage. Mothers' TT coverage status was significantly related with child's EPI coverage status, which reflects the health seeking behavior of a more conscious mother making good health choices for herself as well as for her child.

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Original Article

Intracapsular Hip Fractures

Functional Outcome of Cemented Versus Uncemented Hemiarthroplasty for Intracapsular Hip Fractures

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ABSTRACT

Objectives: To determine the functional outcome of cemented versus uncementedhemiarthroplasty in displaced intracapsular fractures of the hip.

Study Design: Randomized control trial.

Place and Duration of Study: This study was carried out at the Orthopaedics Department, Shaikh Zayed Hospital Lahore and Ibn e Siena Hospital & Research Institute Multan from August 2010 to August 2013.

Materials and Methods: 110 patients with hip fractures fulfilling the criteria were included,55 patients in each group were randomized. Patients in group A were having cemented hemiarthroplasty and in group B were having uncementedhemiarthroplasty respectively. After surgery all patients were mobilized as soon as they were able. All patients were reviewed at 12 weeks follow up using a pain scale of one to six and a mobility scale of zero to nine.

Results: In group A, the preoperative mean pain score was 5.91±0.29 and postoperative mean residual pain score at 12 weeks was 2.73±0.45. In group B, the preoperative mean pain score was 5.91±0.29 and postoperative mean residual pain score at 12 weeks was 3.00±0.64. P value 0f 0.000 was significant in the favor of cemented hemiarthroplasty.

In group A, the preoperative (before fracture) mean mobility score was 7.20±0.75 and postoperative mean reduction in mobility score at 12 weeks was 2.80±0.76. In group B, the preoperative (before fracture) mean mobility score was 7.20±0.75 and postoperative mean reduction in mobility score at 12 weeks was 3.20±0.76. P value 0f 0.000 was significant in the favor of cemented hemiarthroplasty.

Conclusion: The use of cemented hemiarthroplasty lead to less pain in the hip and improved return of mobility as compared to an uncemented prosthesis.

Key Words: Hip Fractures, Hip Prosthesis, Femoral Neck Fractures

INTRODUCTION

Hip fractures are relatively common injuries in elderly people. The incidence of hip fractures is increasing as the general life expectancy of the population has increased significantly during the past few decades. More than 280,000 hip fractures occur in the United States every year and this incidence is expected to double by 2050.[1] These fractures are associated with substantial morbidity and mortality.[2]

These fractures in elderly individuals occur mostly due to moderate or minimal trauma.[3,4]The goal of treating hip fractures is to return patients to their pre-fracture levels of function without long-term disability. The advantages of prosthetic replacement allow immediate weight bearing to return elderly patients to the activity and help avoid complications of recumbency and inactivity.[5,6]

Displaced intracapsular hip fractures in elderly individuals are commonly treated by hemiarthrolpasty. The two most common types of hemiarthroplasty used for treatment of a displaced intracapsular hip fracture are the uncemented Austin-Moore hemiarthroplasty and the cemented Thompson hemiarthroplasty.It is thought that cementing the prosthesis provide more secure fixation and may result in less residual pain and better functions.[7]

Most of the time uncementedhemiarthroplasty is preferred in our setup but it has been found in limited international literature that cemented hemiarthroplasty with Thompson prosthesis led to less pain, improved mobility and reduced hospital stay compared to an uncemented hemiarthroplasty with Austin-Moore prosthesis with no increase in mortality related to use of cement.[8,9,10,11,12]

Austin Moore and Thompson Prosthesis

The continued use of a mixture of uncemented and cemented prosthesis reflects uncertainty as to the relative advantages and disadvantages of using bone cement.[13,14] Keeping in view the limited evidence of improved functional outcome with cemented over uncemented prosthesis in worldwide literature, and limited or perhaps no study regarding this have been found in our setup, we have decided to conduct study on functional outcome of cemented versus uncemented hemiarthroplasty for intracapsular hip fractures.

MATERIALS AND METHODS

We undertook a randomized controlled trial of 110 patients with a displaced intracapsular fracture of the hip to determine the functional outcome of cemented versus uncemented hemiarthroplasty. The study was carried out at the Orthopaedics Department, Shaikh Zayed Hospital Lahore and Ibn e Siena Hospital & Research Institute Multan. The duration of study was 3 year from August 2010 to August 2013.

After approval from the hospital ethical committee, 110 patients with hip fractures fulfilling the criteria admitting through outpatient and emergency department were included. Written informed consent, demographic information, history and examination were taken. 55 patients in each group were randomized by the opening of a sealed opaque numbered envelope, prepared by a person independent of the study, containing detail of the procedure to be undertaken. Patients in group A were having cemented hemiarthroplasty and in group B will be having uncementedhemiarthroplasty respectively. All operations were performed or supervised by the same orthopaedic surgeon and by a standard lateral approach. Same Brand of Austin Moore and Thompson prosthesis with cement was used in all patients. Standard techniques were used for cement when femur has been prepared by reaming and saline irrigation. All patients received perioperative antibiotic prophylaxis and 14 days of low molecular weight heparin as thrombo-embolic prophylaxis. After surgery all patients were mobilized as soon as they were able, with no restriction on hip movements or weight bearing.

All patients were reviewed at four, eight and twelve weeks in outpatient department after the surgery. For the follow up assessment, pain was assessed using a pain scale of one to six. All patients' pre and postoperative mobility was assessed using a mobility scale of zero to nine. All assessments were recorded on especially designed proforma.

Inclusion Criteria:

1. Displaced intracapsular hip fracture (Garden type III and IV)
2. Patient age > 60
3. Both genders

Exclusion Criteria:

1. Patients with pathological hip fractures
2. Previous treatment to same hip for a fracture
3. Patient with significant arthritis of the hip assessed radiologically

All the collected information were entered into SPSS version 17 for the analysis of data. The quantitative variables like age, residual pain score and mobility score were presented as mean and standard deviation. The qualitative variables like gender were presented as frequency and percentage. Mobility score was calculated preoperatively (i-e before fracture) and at 12 weeks post-operatively to calculate mean reduction in mobility score.

Pain Scale (1 – 6)

No pain	1
Occasional and slight pain	2
Pain when starting walking but getting better with occasional analgesia	3
No pain at rest, pain with activities, frequent mild analgesia	4
Constant but bearable pain, stronger analgesia used occasionally	5
Constant pain with frequent strong analgesia	6

Mobility Scale (0 – 9)

Could they get about the house? Was the patient able to get out of the house? Could they do their shopping?	
Without any difficulty	3
On their own with an aid	2
Only with someone else help	1
Not at all, bed or chair bound	0

Variables of interest such as residual pain and reduction in mobility score in the two groups were compared using t-test taking p value ≤ 0.05 as significant.

RESULTS

110 patients were divided into two groups i.e. A and B. In group A cemented hemiarthroplastyandin group B uncementedhemiarthroplasty was done. The mean age of the patients in group Awas 68.44±6.73 year andin group B was 71.24±8.73 year. (Table 1)

In group A, 35 (63.6%) patients were male and 20 (36.4%) patients were female. In group B, 29 (52.7%) patients were male and 26 (47.3%) patients were female. (Table 1)

In group A, thepreoperative mean pain score was 5.91±0.29and postoperative mean residual pain score at 12 weeks of follow up was 2.73±0.45. In group B, the preoperative mean pain score was 5.91±0.29 and postoperative mean residual pain score at 12 weeks of follow up was 3.00±0.64. P value 0f 0.000 was

significant in the favor of cemented hemiarthroplasty. (Table 2 & 3)

In group A, the preoperative (before fracture) mean mobility score was 7.20±0.75 and postoperative mean reduction in mobility score at 12 weeks of follow up was 2.80±0.76.In group B, the preoperative (before fracture) mean mobility score was 7.20±0.75 and postoperative mean reduction in mobility score at 12 weeks of follow up was 3.20±0.76. P value 0f 0.000 was significant in the favor of cemented hemiarthroplasty. (Table 2 & 3).

Table No 1: Mean Age and Gender Distribution in Group A (Cemented)and Group B (Uncemented)

	Group A (Cemented)	Group B (Uncemented)
Number of Patients	55	55
Mean Age (Std. Deviation)	68.44 (6.738)	71.24 (8.737)
Male (%)	35 (63.6%)	29 (52.7%)
Female (%)	20 (36.4%)	26 (47.3%)

Table No 2. Preoperative Mean Pain Score and Mean Mobility Score in Group A(cemented) and Group B(uncemented)

Preoperative	Group A (Cemented)	Group B (Uncemented)
Mean Pain Score	5.91±0.29	5.91±0.29
Mean Mobility Score (before fracture)	7.20±0.75	7.20±0.75

Table No 3: Postoperative Mean Residual Pain Score and Mean Reduction in Mobility Score in Group A (Cemented) and Group B (Uncemented)

Postoperative Outcome (12 weeks follow up)	Group A	Group B	P-value
Mean Residual Pain Score	2.73±0.449	3.00±0.638	0.00
Mean Reduction in Mobility Score	2.80±0.755	3.20±0.755	0.00

Table No 4: Paired Samples Correlations after t-test

		N	Correlation	Sig.value after T test
Pair 1	Residual Pain Score Group A & Residual Pain Score Group B	55	0.258	0.004
Pair 2	Mobility Score Group A & Mobility Score Group B	55	-0.156	0.013

DISCUSSION

This study is the first randomised trial to date on this topic in Pakistan and confirms the results of the previous international studies of patients with an intracapsular hip fracture which found that a cemented hemiarthroplasty leads to less residual pain and a better return of mobility than an uncemented prosthesis.[15] We were able to demonstrate that the marginally increased operation time and the potential operative complications associated with cement were not detrimental. Indeed, the reverse was true, with a clear trend to fewer general medical complications, fewer re-operations and a shorter hospital stay with the cemented prosthesis. The most important outcomes measured were pain and return of function.

The first hip fracture endoprostheses were designed for cementless use, but cemented fixation has become the preferred technique with current femoral components. Numerous reports have documented improved outcomes with cemented implants. The outcome of secondary surgery, particularly revision of the implant, was not significantly different between the two groups, although there was a tendency to more revision arthroplasties in the uncemented group.

Previously published randomised trials comparing cemented and uncementedhemiarthroplasties for patients with a fracture of the hip have been identified and summarized in the Cochrane Review on this subject. Sonne-Holm, Walter and Jensen, in 1982, compared the results of a cemented and an uncemented Austin-Moore hemiarthroplasty in 112 patients.[16]There was no difference in mortality between the two groups. Better walking ability and less pain was observed in those treated with the cemented prosthesis. Similar findings were recorded in a later study of 50 patients which compared a cemented and an uncemented bipolar hemiarthroplasty.[17]There was no difference in mortality between the groups, but significantly less pain in those treated with the cemented prosthesis. Walking ability was also superior with the cemented prosthesis.

Santini et al also compared a cemented and an uncemented bipolar hemiarthroplasty in 106 patients.[18] Again, there was no difference in mortality or functional activity between the two groups. Two studies involving a total of 190 patients compared a cemented with an uncemented Thompson prosthesis. Bothreported no statistically significant difference between the groups for mortality, and significantly more residual pain in those treated with an uncemented prosthesis.[19,20]

Branfoot, Faraj and Porter also compared a cemented with an uncemented Thompson prosthesis in 91 patients and reported no significant difference in mortality.[21]The mean pain scores in the 70 surviving patients tended to be higher, indicating more pain, for the uncemented

prosthesis, although the results were not statistically significant.

We chose the two prostheses used in this study as they are currently the most commonly used in the practice. It is possible that a modern uncemented prosthesis, perhaps with hydroxyapatite coating may produce superior outcomes to the uncemented Austin-Moore prosthesis which we used, but this remains to be proved in a randomized controlled trial. The only study that has compared an uncemented Austin-Moore with a hydroxyapatite-coated Furlong prosthesis in 84 patients was too small to make any definite conclusions on any difference between the two implants.[22]

In summary, this study found that a cemented Thompson hemiarthroplasty led to less pain in the hip, improved return of mobility and a reduced hospital stay compared to an uncemented Austin-Moore prosthesis. There was no increase in complications or mortality related to the use of cement.

CONCLUSION

Our study found that a cemented Thompson hemiarthroplasty led to less pain in the hip and improved return of mobility compared to an uncemented Austin-Moore prosthesis. There was no increase in complications or mortality related to the use of cement. In conjunction with previous studies which have also reported improved outcomes for a cemented rather than an uncementedhemiarthroplasty, we suggest that when a hemiarthroplasty is used for a fracture of the hip it should be cemented in place.

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Original Article

Osteoarthritis

Efficacy of Intra Articular Injections in Different Grades of Osteoarthritis of Knee

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ABSTRACT

Objectives: To assess the efficacy of intra articular injections in different grades of osteoarthritis of knee.
Study Design: Descriptive study
Place and Duration of Study: This study was conducted at the Department of Orthopedics Railway Hospital Rawalpindi and Ibn-e-Seina Hospital & Research Institute Multan started from June 2008 to June 2012.
Materials and Methods: This study included 90 patients of either sex having mild, moderate and severe grades of osteoarthritis of knee based on radiographic findings. A study proforma was designed in which bio-statics and results of the study were recorded. Five intra-articular injections of hyaluronic acid were given on weekly basis and patients were assessed for pain and functional activity at intervals of 0, 6 and 12 weeks by KSKS, WOMAC and VAS scoring system. These patients were selected consecutively.
Results: In this study patients with mild and moderate grades of osteoarthritis of knee showed improvement but patients with severe grades of osteoarthritis either showed no response or less improvement.
Conclusion: Intra-articular injection of hyaluronic acid is an effective modality in early stages of osteoarthritis of knee.
Key Words: Osteoarthritis, Hyaluronic acid, Knee

INTRODUCTION

Osteoarthritis is the most common joint disorder in the elderly.[1] The prevalence of osteoarthritis ranges from 1% below the age of 30 years to over 50% in peoples above the age of 60.[2] In osteoarthritis of knee there is progressive cartilage destruction which results in sub-articular cysts and osteophyte formation. There is also sclerosis of the surrounding bone and capsular fibrosis. Patient with osteoarthritis of knee usually presents with pain in one or both joints, stiffness, intermittent swelling and loss of function.

Although knee arthroplasties are performed in sufficient number in Pakistan but still this group represents only a small number of patients with knee osteoarthritis. Most patients avoid surgeries due to their high cost by using a variety of non-operative treatments like oral analgesics, nonsteroidal anti-inflammatory drugs, exercise and physiotherapy, weight relieving braces or different types of intra articular injections.[3]

Hyaluronic acid is a critical component of normal synovial fluid and an important contributor to joint homeostasis.[4] In osteoarthritis, both the concentration and the molecular weight of intra-articular endogenous hyaluronic acid is decreased, which reduces the viscoelasticity of the synovial fluid.[5,6] Therefore, the original rationale for intra articular injection of hyaluronic acid was to restore the viscoelasticity of synovial fluid.[7,8] In addition, it has been found that injected hyaluronic acid augments the flow of synovial fluid, normalize the synthesis and inhibit the degradation of endogenous hyaluronic acid, and relieve joint pain.[9, 10]

Clinical trials have demonstrated the safety and efficacy of hyaluranic acid injections for the treatment of knee osteoarthritis.[11] But studies in our setup are inconclusive regarding the best responders with respect to age, grade of osteoarthritis, level of symptoms and level of physical activity. This study was undertaken to access the efficacy of intra articular hyaluronic acid in different grades of osteoarthritis of knee defined radiologically.

MATERIALS AND METHODS

This multicentered, prospective and observational study included those patients who satisfied the inclusion criteria. Patients were offered enrolment until planned number of patients were reached. Patients were enrolled between June 2008 to June 2012.

Inclusion criteria was age above 30 years, radiological evidence of symptomatic osteoarthritis of knee and failure of other non operative modalities like NSAIDS, physical therapy and knee braces.

Exclusion criteria was pregnant and lactating mothers, radiographic evidence of chondrocalcinosis or if physical examination demonstrated insufficiency of ligaments of the knee and neuropathic joints. The study was approved by the ethical committee and patients were explained about the study. Follow up of the patients was ensured.

Data including sex, age, height, weight and site of involvement was recorded. Weight bearing

anteroposterior and lateral radiographs of the involved knee were obtained. Findings on the initial radiographs were graded by the radiologist as mild, moderate and severe arthritis. Mild arthritis was defined as minimal loss of joint space and osteophyte formation. Moderate arthritis was defined as up to 75% loss of joint space, subchondral changes and more noticeable osteophyte formation. Severe arthritis was defined as total loss of joint space and more severe changes. During grading all the three compartments including medial and lateral tibiofemoral and patellofemoral were graded and worse grade was recorded.

The efficacy assessment included Knee Society Knee Score (KSKS), Western Ontario and Mcmaster Universities Osteoarthritis Index (WOMAC) score and Visual Analogue Scale (VAS) score for pain at rest and following walking and stepping activities.[12,13]

With KSKS clinical rating scale a score of < 55 point indicate a poor result, 55-64 fair result, 65-79 good result and 80-100 excellent result. WOMAC was used as self-administered questionnaire. With WOMAC a lower number represent a better result ranging from zero (Best) to 96 (Poor). Visual Analogue Scale was also administered to the patient ranging from 0-100mm with lower number representing less pain and higher number representing more pain.

All the three instruments were used to assess the patients during the time of enrollment, at 6 weeks and then at 12 weeks. One of the medical officer who was skilled to use the study instruments was appointed to assess the patients.

Hyaluronic acid injections (Hyalgan) were administered as a course of five weekly injections. All injections were given in the similar manner with the patient lying supine and the knee joint approached through superolateral pouch under the superolateral edge of patella. Ethylchloride spray was used immediately prior to the injection for the patient comfort. A 22 gauge needle was used to inject the prefilled 2ml syringe. In case of the knee effusion 18 gauge needle was used for the aspiration and then the needle was left in place for the injection. Patients was encouraged to avoid strenuous activity for a day following the intra articular injection.

The results obtained from KSKS, WOMAC, VAS were tested separately. Two-way Anova (Analysis of Variance) technique and F-Test were used.

RESULTS

This study included 100 patients having symptomatic osteoarthritis of the knee. 10 patients left the follow up. Mean age of the patients was 50.45±0.90 years with range of 35-75 years. Majority of the patients were above 40 years of age. There were 68 (75.5%) patients who were 41-60 years of age. There were 16 (17.7%) patients who were less than or equal to 40 years of age and 6 (6.6%) patients were above 60 years of age. Mean weight of the patients was 80.30±0.75 kg. (Table No.1)

There were 52 (57.8%) male and 38 (42.2%) female patients. 20 (22.2%) patients had low socioeconomic status, 55 (61.1%) had middle and 15 (16.6%) had high socioeconomic status. In 38 (42.2%) patients left side was involved while in 44 (48.9%) patients right side was involved and in 8 (8.9%) patients both sides were involved. 30 (33.3%) patients had mild, 30 (33.3%) patients had moderate and 30 (33.3%) patients had severe grade of osteoarthritis.

Table No.1: Characteristics of patients with OA Knee (n=90)

	No. of Patients (%)	No. of Patients (%)	No. of Patients (%)
Age (yrs)	≤ 40 = 16 (17.7%)	41-60 = 68 (75.5%)	≥ 60 = 6 (6.6%)
Socioeconomic Status	Low = 20 (22.2%)	Middle = 55 (61.1%)	High =15 (16.6%)
Side Involvement	Left = 38 (42.2%)	Right = 44 (48.9%)	Both = 8 (8.9%)
Arthritis Grade	Mild = 30 (33.3%)	Moderate = 30 (33.3%)	Severe = 30 (33.3%)

Table No.2: Knee Society Knee Score (KSKS) in Patients with OA Knee (n=90)

	Mild	Moderate	Severe	All
KSKS-a	N=30 Mean=62.400 S.D=4.739	N=30 Mean=54.933 S.D=2.434	N=30 Mean=37.600 S.D=4.280	N=90 Mean=51.644 S.D=11.151
KSKS-b	N=30 Mean=87.900 S.D=4.831	N=30 Mean=70.600 S.D=6.806	N=30 Mean=42.500 S.D=3.655	N=90 Mean=67.000 S.D=19.519
KSKS-c	N=30 Mean=90.900 S.D=3.872	N=30 Mean=65.567 S.D=10.516	N=30 Mean=40.267 S.D=3.483	N=90 Mean=67.444 S.D=22.006
All	N=90 Mean=80.400 S.D=13.607	N=90 Mean=65.567 S.D=10.516	N=90 Mean=40.122 S.D=4.282	N=270 Mean=62.030 S.D=19.534

Table No.3: WOMAC in Patients with OA Knee (n=90)

	Mild	Moderate	Severe	All
WAMAC-a	N=30 Mean=45.467 S.D=2.825	N=30 Mean=51.967 S.D=2.173	N=30 Mean=61.000 S.D=3.806	N=90 Mean=52.811 S.D=7.063
WAMAC-b	N=30 Mean=22.167 S.D=5.032	N=30 Mean=37.033 S.D=6.636	N=30 Mean=55.067 S.D=3.868	N=90 Mean=38.089 S.D=14.507
WAMAC-c	N=30 Mean=17.133 S.D=6.213	N=30 Mean=36.467 S.D=10.095	N=30 Mean=58.167 S.D=5.167	N=90 Mean=37.256 S.D=18.400
All	N=90 Mean=28.256 S.D=13.322	N=90 Mean=41.822 S.D=10.059	N=90 Mean=58.078 S.D=4.922	N=270 Mean=42.719 S.D=15.792

Using KSKS, mild grades showed mean score of 62.40 at the start and after 12 weeks mean score was 90.90. Moderate grades showed mean score of 54.93 at the start and after 12 week mean score was 65.56. Severe grades showed mean score of 37.60 at the start and after 12 week mean score was 40.26.

Using WOMAC, mild grades showed mean score of 45.46 at the start and after 12 weeks mean score was

17.13. Moderate grades showed mean score of 51.96 at the start and after 12 weeks mean score was 36.46. Sever grades showed mean score of 61.00 at the start and after 12 week mean score was 58.16.

Table No.4: Visual Analogue Scale (VAS) in Patients with OA Knee (n=90)

	Mild	Moderate	Severe	All
VAS-a	N=30 Mean=56.000 S.D=4.433	N=30 Mean=68.167 S.D=2.780	N=30 Mean=78.333 S.D=3.304	N=90, Mean=67.5000 S.D=9.837
VAS-b	N=30 Mean=26.500 S.D=8.610	N=30 Mean=48.000 S.D=8.367	N=30 Mean=70.687 S.D=5.231	N=90 Mean=48.456 S.D=19.493
VAS-c	N=30 Mean=20.000 S.D=8.610	N=30 Mean=45.467 S.D=15.204	N=30 Mean=74.500 S.D=6.867	N=90 Mean=46.656 S.D=24.823
All	N=30 Mean=34.167 S.D=17.178	N=90 Mean=53.878 S.D=14.317	N=90 Mean=74.567 S.D=6.102	N=270 Mean=54.204 S.D=21.233

Similarly using Visual Analogue Scale, mild grades showed mean score of 56.00 at the start and after 12 weeks mean score was 20.00. Moderate grades showed mean score of 68.16 at the start and after 12 weeks mean score was 45.46. Severe grades showed mean score of 78.33 at the start and after 12 weeks mean score was 74.50.

These results showed the improvement more in mild and moderate grades of osteoarthritis of knee than in severe grades of osteoarthritis of knee.

DISCUSSION

The present study showed the treatment effects of hyaluronic acid injection in different grades of osteoarthritis of knee. Although intra-articular injections have shown improvement in all grades but effects are more in mild and moderate grades as compared to sever grades in our population as well.

No radiological grading system has undergone interobserver and intraobserver reliability testing, this should be considered a limitation for any study that draws interference between radiological grades and outcome.

The meta-analysis by Wang CT, et al showed signification improvement in pain and functional outcome except those who had severe osteoarthritis.[14] Similarly a prospective randomized study by Petrela RJ and Petrela M showed patient satisfaction in terms of pain relief and functional improvement by hyaluronic acid.[15]

In this study 10 patients were slipped during the course of treatment as it require five weeks to complete so it is concluded that the course should be of shorter duration. As new variants of hyaluronic acid are discovered, single intra-articular injection with 6ml Hylan G-F 20 can replace routine method of once a week for five weeks.[16] The effect of hyaluronic acid at molecular level has also been established.[17,18]

CONCLUSION

Intra-articular injection of hyaluronic acid is an effective modality in early stages of osteoarthritis of knee.

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Original Article

Effect of Thiamine on Glycemic Control in Induced Diabetic Rat Model

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ABSTRACT

Objective: To evaluate the effect of high dose thiamine on postprandial blood glucose (PPBG) and hemoglobin A1C levels in induced diabetic albino rat model.

Study Design: Experimental/Analytical study

Place and Duration of Study: Animal House, Isra University Hyderabad from March to October 2013.

Materials and Methods: Forty adult albino rats were divided into four groups; Group I. Controls receiving normal diet, Group II. Controls receiving thiamine fortified diet, Group III. Diabetics receiving normal diet and Group IV. Diabetics receiving thiamine fortified diet. Diabetes mellitus was induced using Streptozocin. Thiamine was given at a dose of 1.6 g/kg body weight. Venous blood samples were drawn from animal's tail with a small bore cannula before and after 12 weeks of experimentation. The PPBG levels and Glycosylated HbA (HbA1c) were measured. The data was converted into database and analyzed on SPSS version 21.0 by ANOVA and Tukey-Kramer`s test. A p-value of ≤ 0.05 was taken statistically significant.

Results: The PPBG and HbA1c levels were found statistically significant in groups I vs. III (p=0.0001), I vs. IV (p=0.0001), II vs. III (p=0.0001), II vs. IV, (p=0.001) and III vs. IV (p=0.024) at 12th week of experimentation. The study shows significant reduction in the PPBG and HbA1c levels of rats taking thiamine compared to controls (p=0.001). Significant differences in HbA1c levels were observed between control groups I and II vs. experimental groups III and IV (p=0.024) (p=0.0001) respectively. A highly significant difference in HbA1c was observed in rats given thiamine fortified diet compared to those not given (p=0.001).

Conclusion: The thiamine improves glucose metabolism in induced diabetic rat models, hence it is concluded that the thiamine may be given along with anti- diabetic drugs to overcome defects of glucose metabolism.

Key words: Thiamine, Glycemic control, HbA1c, Diabetic rats

INTRODUCTION

The number of diabetic population older than twenty is estimated to rise from 285 million in 2010 to 439 million in 2030 as reported by International Diabetes Federation (IDF).[1] The current rise in diabetic population has put Pakistan at sixth position in the world.[2] According to an estimate, 15% of Pakistani population is diagnosed of having diabetes mellitus (DM) and millions more are unaware and undiagnosed of having DM.[3,4] According to the estimates of Pakistan National Diabetes Survey (PNDS), for each diagnosed case of DM, there are approximately two cases of undiagnosed DM and three cases of impaired glucose tolerance who remain unaware of it.[5,6] The DM is characterized by chronic hyperglycemia which in long term causes damage in the target organs like kidney, nerves, eyes, heart and blood vessels.[7,8] The DM is a multifactorial metabolic disorder, mainly characterized by abnormal glucose metabolism which is responsible for most of its symptoms and complications. But the thiamine deficiency is also linked with the pathogenesis and complicaitons of DM as has been reported in many studies.[9, 10,11] The thiamine is known as vitamin B_1. Thiamine forms an indispensable co-enzyme and participates in several steps of glucose and intermediary metabolisms. In human body it plays role in glucose metabolism by forming co-enzymes essential for functioning of enzymes of glycolysis ad citric acid cycle. The DM has been characterized by thiamine deficiency due to amplified glucose metabolism.[11] Thiamine deficiency causes disturbed glucose metabolism, and accelerates DM complications through various mechanisms.[11] The various mechanisms which have been postulated causing diabetic complications include the activation of polyol pathway, formation of advanced glycation end products (AGES), activation of protein kinase C (PKC) and increased flux through the hexosamine biosynthetic pathway (HBP).[11] These metabolic abnormalities are triggered through increased formation and elevated concentration of triose-phosphate intermediates of glycolysis.[12,13] Triose-phosphate can be suppressed by the activation of reductive pentose phosphate pathway (PPP) by high-dose thiamine therapy that would increase transketolase (TK) activity and stimulate the

conversion of glyceraldehyde-3-phosphate (GA3P) and fructose-6-phosphate (F6P) to ribose-5-phosphate (R5P), thus reducing the risk of the development of diabetic complications.[9] The present study hypothesizes that high doses of thiamine administration would be independently associated with glucose metabolism and that this association is modified by blood glucose level. The present experimental study aims to evaluate the metabolic effects of high dose thiamine on blood glucose and HbA1c in induced diabetic rat model.

MATERIALS AND METHODS

This experimental study was conducted at Animal House of Isra University Hyderabad from June 2012 to December 2013. Forty 12-week old male albino rats were selected through non-probability purposive sampling. The normal healthy rats of 150-250g body weight were included while sick rats and rats not feeding properly were excluded from study. Rats were habitated in stainless steel cages, at room temperature with 55-60% humidity. The rats were kept under standard conditions. Pelleted form of diet was given to animals throughout study period. The diet was made available freely to feed *ad-libitum*. Water was made available in separate containers. Pelleted diet was fortified with thiamine at a dose of 1.6 gms/ kg for the specified groups *as referenced*.[14] Twenty rats were separated as controls, while another twenty rats were injected streptozocin to damage β-cells to induce DM at a dose of 60mg intraperitoneally.[15,16] The rats (N=40) were divided into four groups by random selection, containing ten rats in each group. Group I (n=10) included normal healthy rats as controls receiving normal pelleted diet, Group II (n=10) included normal healthy rats as controls receiving normal diet, fortified with thiamine, Group III (n=10) diabetic rats given normal diet and Group IV (n=10) included diabetic rats given thiamine fortified diet. The study duration was twelve weeks. Venous blood samples were drawn from animal's tail with a small bore cannula, to measure random blood glucose and glycosylated HbA (HbA1c). Random blood glucose was measured by glucose oxidase method and HbA1c by enzyme calorimetric method on spectrophotometer (Hitachi, USA). Normal blood glucose level was taken as 52-105mg/dl. HbA1c range for rats was taken normal as 3-8.8%.[15] The data was converted into database onto SPSS version 21.0. The continuous variables were analysed by ANOVA and Tukey-Kramer`s test. The data was presented as mean±SD. A p-value of ≤ 0.05 was taken statistically significant.

RESULTS

The blood glucose level and HbA1c were measured before and after intervention in all groups of rats. The continous variables are presented as mean±SD in Table I for groups. The difference in blood glucose level and HbA1c in control groups I and II and experimental groups III and IV were found statistically non-significant before intervention ($p > 0.05$). However, the blood glucose level and HbA1c were found statistically significant in groups I vs. III ($p=0.0001$), I vs. IV ($p=0.0001$), II vs. III ($p=0.0001$), II vs. IV, ($p=0.001$) and III vs. IV ($p=0.024$) after intervention at twelve weeks of experimentation. The study shows significant reduction in the blood glucose level and HbA1c of rats taking thiamine compared to controls ($p=0.001$). Although the study duration was twelve weeks, still significant differences in HbA1c levels were observed between control groups I and II vs. III and IV ($p=0.024$) ($p=0.0001$) respectively. A highly significant difference in HbA1c was observed in rats taking thiamine fortified diet compared to those not taking thiamine ($p=0.001$). From the observations of present study, it is concluded that thiamine has a definitive role in regulating glucose metabolism. The thiamine shows positive effect in reducing blood glucose level through enhanced metabolism and reduces formation of HbA1c. The observations of thiamine effect on blood glucose level are of practical importance while treating diabetic subjects by clinicians.

Table No.I. Postprandial blood glucose (PPBG) level and Hemoglobin A1c in study animals (n=40)

Variable	**Group I** Control group (Thiamine $^{-ve}$ diet)		**Group II** Control group (Thiamine $^{+ve}$ diet)		**Group III** Diabetic group (Thiamine $^{-ve}$ diet)		**Group IV** Diabetic group (Thiamine $^{+ve}$ diet)	
	Before	After	Before	After	Before	After	Before	After
PPBG level (mg/dl)	79±4.7	80±3.9	80±1.5	80±3.5	78±2.5	153±15.6	80±2.7	145±13.5
HbA1c (%)	3.48±0.23	3.49±0.22	3.49±0.24	3.51±0.21	3.47±0.24	5.98±0.89	3.49±0.21	5.13±0.79

DISCUSSION

The studies regarding the effect of high dose thiamine therapy on blood glucose and HbA1c are lacking in medical literature of Pakistan. A few studies are reported on role of thiamine in diabetics.[9,15] It is reported by various studies that diabetics are thiamine deficient.[9,10,15-17] The present study shows that high doses of thiamine improves blood glucose level and results are comparable to previous studies.[9,10,15] In present study we found statistically significant

differences in blood glucose levels among in all four groups after intervention. The blood glucose levels are significantly elevated in group IV diabetics compared with group I controls and group II controls taking thiamine (p=0.0001) (Table I). Statistically significant differences in blood glucose levels are observed between group III diabetics and groups IV diabetics taking thiamine in diet. (p=0.001). The present study also observes statistically significant differences in HbA1c levels among controls, diabetics and diabetics with thiamine intake. (p=0.001). The statistically significant differences in HbA1c observed in present study are contrary to previous studies.[9,15] The differences are observed because of long duration of present study compared to previous study[15] i.e; 4 vs. 12 weeks. A previous study reported that the thiamine intake was inversely associated with blood glucose levels and this shows improved glucose metabolism.[17] The findings support our present study. Babaei-Jadidi et al.[18] conducted experimental study on diabetic rats and reported positive effects of thiamine intake on blood glucose and lipid levels. Our findings support this previous study regarding improved blood glucose levels. In that previous study, it was concluded that the hepatic expression of thiamine dependent enzymes is sensitive to decrease transketolase (TK) and pyruvate dehydrogenase (PDH) activities,[18] both of which are important in regulating intracellular glucose metabolism. The Berrone E et al.[19] conducted study on role of thiamine and benfotiamine on intracellular glucose and polyol pathway in cultured vascular cells and reported improved glucose uptake and metabolism by vascular cell cultures. The previous study concluded that high doses of thiamine and benfotiamine may prevent development of microvascular complications of diabetics.[19]

The findings are in keeping with the present study as we observed better glucose metabolism in *thiamine-fed* rats. The present study shows that the thiamine improves blood glucose levels and HbA1c in induced diabetic rat models.

CONCLUSION

The thiamine improves glucose metabolism in induced diabetic rat models as observed in present study. The present study concludes that the thiamine may be given along with antidiabetic drugs to overcome defects of glucose metabolism as it improves glycemic control.

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Original Article

Pentoxifylline on Liver

Pentoxifylline Protects against Carbon Tetrachloride Induced Liver Injury in Adult Male Wistar Rat Model

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ABSTRACT

Objective: To investigate the protective effect of Pentoxifylline (PTX) in carbon tetrachloride (CCl_4) induced liver injury in adult male Wistar rat model.

Study design: Experimental/Analytical study

Place and Duration: Animal House, Isra University Hyderabad from May to December 2012.

Subjects and Methods: Forty five adult male Wistar rats were divided into three groups; Group A. controls received 0.9% isotonic saline, Group B. received CCl_4 orally (1.9mg/kg) mixed in olive oil, and Group C. received the PTX+ CCl_4. Blood samples were collected for liver biochemical assays. The animals were sacrificed, liver tissue, after fixation in 4% formaldehyde, was embedded in paraffin. Tissue sections of 5µ thickness were subjected to haematoxylin and eosin staining and were assessed by light microscopy. The data was analyzed on SPSS 21.0 using one-way ANOVA, Tukey-Cramer and Chi-square tests. A p-value of ≤ 0.05 was taken statistically significant.

Results: The liver biochemical and histological findings reveal statistically significant differences among the controls, CCl_4 and PTX+ CCl_4 groups (p=0.0001). Liver enzymes and histology was deranged significantly in CCl_4 group compared to controls and PTX+ CCl_4 group (p=0.0001). The CCl_4+PTX group shows less elevation of liver enzymes and derangement in liver histology when compared to CCl_4 group (p=0.001). The histological findings of congestion, inflammatory cell infiltrate, vacuolar degeneration and necrosis are found prominent in CCl_4 group.

Conclusion: The Pentoxifylline protects against oxidative damages caused by carbon tetrachloride induced liver injury in rat model.

Key words: Carbon tetrachloride, Liver injury, Pentoxifylline

INTRODUCTION

Liver is the largest gland of human body. The parenchyma cells of liver are known as the hepatocyte, which performs biochemical and metabolic functions.[1]

The oxygen is a precursor of a number of free radicals which are collectively known as the reactive oxygen species (ROS). The ROS are implicated in the pathogenesis of most liver diseases both inflammatory and non-inflammatory like ischemia/reperfusion injury, chronic viral hepatitis, alcoholic hepatitis, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) and cholestasis.[2] The liver is a metabolically active organ, and protects against drug toxicity for two reasons 1). As it is interposed between porto-systemic circulation i.e. between absorption of intestinal contents containing toxins. 2). Hence preferred site of toxin metabolism and elimination through intestine. Drug induced liver injury poses body systems to possible injury if toxins access to systemic circulation.[3,4]

Carbon tetrachloride (CCl_4) is a hepatotoxic compound. The CCl_4 has been has been used extensively in laboratory animals for induction of liver injury, elucidate the underlying mechanism of liver injury and hepatoprotective effects of various therapeutic agents.[5]

One of the postulated mechanism of CCl_4 induced liver injury is the formation of ROS. The ROS disrupts the hepatocyte at cell membrane level through the lipid peroxidation[5,6] causing anatomical disruption of liver architecture and physiological disturbances.[7] The hepatocyte injury causes leakage of cytoplasmic and mitochondrial enzymes in the blood streams.[8]

The cytoplasmic and mitochondrial enzymes are clinically used as markers of liver injury, and for monitoring and treating the liver diseases also. The liver enzymes which appear in the blood as a result of liver injury include; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase (ALP) and lactate dehyderogenase (LDH) are important enzymes that are often employed in assessing liver injury.[1,5]

The Pentoxifylline (PTX) is a well known methyl xanthine. The PTX is a non-specific inhibitor of phosphodiesterase-4 type. The PTX is used clinically in peripheral arterial disease with intermittent

claudication. The mechanism underlying its therapeutic effects seems to be related to improvement of microcirculation, tissue perfusion and cell functions. The PTX is reported to possess anti-inflammatory and hepatoprotective effects against chemical-induced liver injury like alcohol.[4,9,10]

The present study aims to investigate the possible protective effects of Pentoxifylline (PTX) against Carbon tetrachloride (CCl_4)-induced hepatotoxicity in experimental animals.

MATERIALS AND METHODS

An experimental study was conducted at the animal house of Isra University on rabbit model over a period of one year, from January to December 2012. Adult male Wistar rats of 250-300 grams were included in the study. Female rats, weight <250 grams or >300 grams were excluded from the study. Animals were housed in animal house at an optimal room temperature with 55-60% humidity and exposed to 12 hour light-dark cycles. The chaw like fresh alfalfa and clean water are provided freely. The rats were divided into three groups;

Group A. Control Group (n=15) Rats received 0.9% isotonic saline orally on alternate day for three successive weeks and served as control group,

Group B. Carbon tetrachloride Group (n=15) Rats were given CCl_4 orally mixed in olive oil on alternate day for three successive weeks and

Group C. Experimental Group (n=15) Rats received PTX (200 mg/kg intraperitoneally) and CCl_4 on alternate days for three successive weeks

Experimental Details: The PTX was purchased from Medical store of Isra University Hospital. The PTX was administered in a dose of 200 mg/kg intraperitoneally.[4] Carbon tetrachloride was purchased from scientific drug store at Hyderabad City. The CCl4 dissolved in olive oil as vehicle (1:1 Ratio) at a dose level of 1.9 ml/kg orally on alternate day for three successive weeks and sacrificed at the end of their respective period of time.[1]

Sacrifice of animals: The animals were sacrificed using standard method as described by Nayak et al. (2006)[11] In order to examine the liver tissue, the liver of the sacrificed animals was removed promptly and preserved in formaldehyde.

Blood sample Processing: The blood samples were collected from peripheral veins at twenty four hours of experimental period. Sera were separated by centrifugation at 300xs for ten minutes. Serum samples were used to determine liver enzymes.

Liver enzyme assay: Liver enzyme assays were determined for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase (ALP) and lactate dehyderogenase (LDH) using commercially available diagnostic kits.

Histological studies: After fixation in 4% formaldehyde, samples were embedded in paraffin. Sections of 5µ thickness were subjected to haematoxylin and eosin Hepatic morphology was assessed by light microscopy. A total of five sections for each liver tissue sample were observed under light microscope.

In H & E staining, damaged hepatocytes graded as 0= normal, += mild damage (swollen and pale cytoplasm), ++= moderate damage (vacuolated cytoplasm), +++= severe damage and ++++= very severe damage (pyknotic nucleus and eosinophil cytoplasm).[12]

The data was analyzed on SPSS version 21.0 (IBM corporation). The continous variables were presented as mean±SD using one-way ANOVA and Tukey-Cramer test for multiple comparisons. Chi-square test was used for categorical variables. A p-value of ≤ 0.5 was taken statistically significant.

RESULTS

The present study observes major differences in liver injury between and among groups as indicated by blood enzyme levels in different animal groups. The LDH, ALP, and ALT, AST in serum of Rats treated with carbon tetrachloride were found elevated compared with control group after three weeks, with a highly significant p-value for multiple comparisons (p=0.001). The CCl_4+PTX group shows a significant reduction in the liver enzymes compared with the CCl_4 group (p=0.001) and control group (p=0.001). The animals CCl_4+PTX group shows significant reduction in the liver enzyme elevation compared to CCl_4 group alone (p=0.001). The finding shows significant hepato protection by the PTX in CCl_4 induced injury. The liver enzyme assays among different groups are shown in table.1.

Table No. 1: Liver enzyme levels in controls, *CCl_4 and CCl_4+ Pentoxifylline groups

Groups	LDH (IU/L)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Group. A (Controls)	711.5± 51.7	93.6± 8.91	48.9± 3.19	91.2± 16.81
Group. B (CCl_4)	2778.8± 139.6	167.1± 8.02	189.6± 11.91	499.7 ± 21.9
Group. C (CCl_4 + PTX)	2138.6± 153.3	136.7± 18.14	87.7± 17.92	171.3± 19.3

*Carbon tetrachloride

Different parameters of histological score of liver injury are shown in Table. 2. The Liver sections of the control group animals show intact central venules and hepatocytes arranged in compact cords. Normal looking hepatocytes with prominent nucleus, nucleolus and well preserved cytoplasm are seen in control group (Figure.1). On the contrary, the CCl_4 group shows derangement of hepatocytes cords, hydropic changes

with congestion of central venules and sinusoids, and abundant inflammatory cell infiltration (Figure.2).

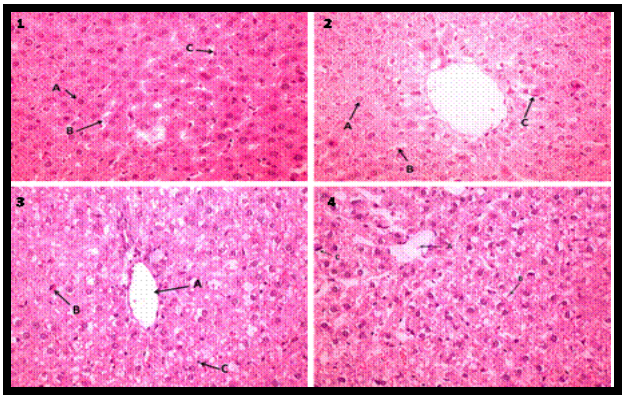

Figure No.1: Liver slide of control group shows normal looking hepatocytes arranged in cords. Central vein (CV) is shown (A) separated by sinusoids

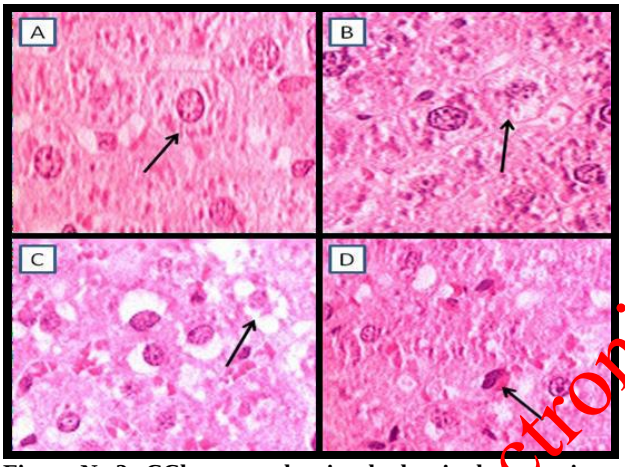

Figure No.2: CCl_4 group showing hydropic degeneration (arrow), inflammatory cell infiltrate and necrosis

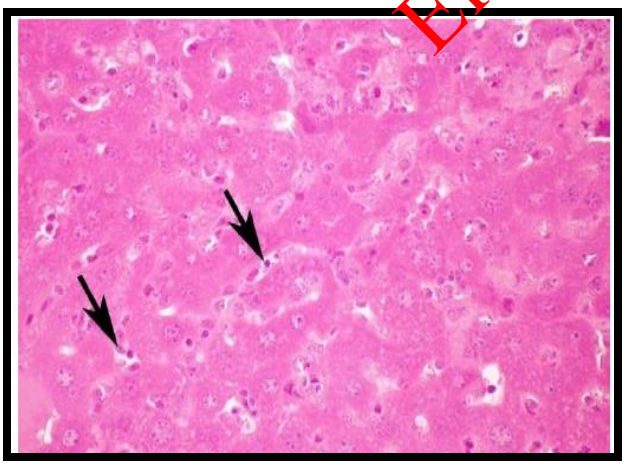

Figure No.3: CCl_4 + Pentoxifyllline group showing normal hepatocyte arranged in cords with congested sinusoids, and few lymphocytic infiltrations.

The centrilobular hepatocytes show hydropic changes and necrosis, while midzonal and peripheral hepatocytes show vacuolar degeneration and fatty changes in CCl_4 group (Figure 2). In CCl_4+PTX animals, liver tissue sections reveal less significant derangement of hepatocytes cords, hepatocytes damage and necrosis was limited compared with CCl_4 group (Figure.3).

Table No. 2: Histology of liver injury of controls, *CCl_4 and CCl_4+ Pentoxifyllline groups

Groups	Inflammatory cell infiltrate	Congestion	Vacuolar degeneration	Necrosis
Group. A (Controls)	0	0	0	0
Group. B (CCl_4)	++++	++++	+++	++++
Group. C (CCl_4 + PTX)	+++	++	++	++

*Carbon tetrachloride

DISCUSSION

The present study is an original research work, which investigates the effect of Pentoxifyllline (PTX) on carbon tetrachloride (CCl_4) induced liver injury in adult male Wistar rats. The Null hypothesis is rejected because the study observes hepatoprotective effects of PTX as evidenced by biochemical and histological marker of liver injury. The present study shows liver damage caused by the carbon tetrachloride as indicated by serum levels of liver enzymes compared to control group in rat model. The carbon tetrachloride induced liver injury with release of liver enzymes is comparable finding to reported previously by Hurkkeri et al.[13] The Hurkkeri[13] reported elevated hepatocyte enzyme of liver as a consequence of CCl_4 induced liver injury in animal model. The release of large quantities of cytoplasmic and mitochondrial enzymes of liver is a clinical indicator of hepatocyte cell membrane damage and rupture sufficient to produces change in enzyme levels in blood.[14] The effect of PTX has been studied on the hepatic encephalopathy,[15,16] hepatorenal syndrome,[17] and alcoholic hepatitis by some researchers.[18] PTX inhibits profibrogenic cytokine and procollagen I expression, [19] decreases the AST and ALT and its anti-TNF-α improves symptoms of liver tissue in patients with Non-alcoholic steatohepatitis (NASH) Non-alcoholic fatty liver disease (NALFD). [20]

The present study shows that the damage of liver caused by CCl_4 is evident by the rise in serum enzymes levels beside the histological changes in liver tissue. Administration of CCl_4 significantly increases the serum levels of liver enzymes; LDH, ALP, ALT and AST, which are indices of hepatocyte damage and leakage of enzymes from cells. [21,22]

The histological examination of present research study correlates in parallel to disturbance in biochemical markers of liver injury. The histology of liver tissue shows disruption of liver architecture, hepatocytes, hepatic lobules and arrangement of hepatocytes in

cords. The hepatocytes show findings of cellular injury with marked cytoplasmic vacuolization. The injured hepatocytes show pyknotic nuclei with lymphocyte infiltrations. The pyknotic nuclei are a sign of severe cellular injury caused by a toxin like carbon tetrachloride. The histological and biochemical findings of present study are comparable to those mentioned previously.[23-,25] The carbon tetrachloride is metabolized to free radical during its metabolism and detoxification in smooth endoplasmic reticulum by the cytochrome P450.[26] The Movassaghi, et al [4] (2013) studied hepatoprotective effects of PTX on ecstasy induced liver damage and reported effects of PTX. The PTX reduced liver injury in this previous study by reducing apoptosis and fibrosis caused by ecstasy in rat liver model. The present study concludes that the PTX decreases the carbon tetrachloride induced liver damage.

CONCLUSION

The Pentoxifylline protects against oxidative damages caused by carbon tetrachloride induced liver injury in rat model. The Pentoxifyllline may be used as an effective protector against chemical induced liver damages; however, further studies are warranted.

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Original Article

Pharmacology

Validity of Pleural Fluid Protein in Differentiating Tuberculouse from Malignant Pleural Effusion

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ABSTRACT

Objective: Validity of pleural fluid protein in differentiating tuberculouse from malignant pleural effusion keeping histopathology as gold standard.

Study Design: Cross sectional study.

Place and Duration: This study was conducted in the Pulmonology department post graduate medical institute, Lady Reading Hospital Peshawar, Khyber Pakhtunkhwa (KPK) Pakistan from March 2009 to March 2010.

Materials and Methods: One hundred and seventy nine patients having clinical suspicion of pulmonary tuberculouse and malignancy and fulfilling the inclusion criteria were subjected to Abrams needle biopsy, plural tissue was examined by histopathology. Biopsy in order to know the significant difference of pleural fluid protein level between tuberculous and malignant pleural effusion, histopathology finding and protein concentration were determined their frequency and percentage.

Results: Among total number of 179 patients one hundred and fourteen (63.69%) were male and sixty five (36.32%) were female. The age limit from 15-80 years, the result shows that 60.9% were tuberculous and 39.9% were malignant pleuraleffusion, among these malignant 20 (11.2%) showed primary and 50 (27.9%) secondary malignancy. Tuberculous PE was more common in younger age group while malignant PE in older age group, 32 number of patients falling in category A, 59 in category B, and 89 in category C.A protein level in belonging to category C, there was statistically significant difference between tuberculous and malignant PE, tuberculous PE have high concentration of protein than malignant PE, The category "A" have malignant PE.

Conclusion: Plural fluid total protein level determination and differentiating is a valuable tool in reaching to the diagnosis of suspectedtuberculouse from malignant pleural effusion provided it is used in addition to the adequate clinical scenario.

Key Words: Tuberculouse pleural effusion, malignant pleural effusion, Exudates, pleural fluid protein.

INTRODUCTION

Pleural effusion (PE) is a common clinical problem both in developed and developing countries.[1] The etiological investigation of a pleural effusion is to determine whether the effusion is a transudate or exudates. Transudates reflect the presence of systemic disease with repercussion.On the mechanism of pleural fluid production and production and reabsorption.[2]In contrast, exudates reflect, the presence of primary pleural disease and require etiological investigation.[3] So in case with trandatePE, the diagnosis is usually made without any difficulty but executive PE, require careful differential diagnosis that include tuberculouse (TB) and metastatic cancers, which are often found to be the cause in a large number of patients.[4][5] Disease in any organ can cause execute PE through a variety of mechanisms including, malignancy, immunologic response, lymphatic abnormality and non-infections inflammation.[6]Tuberculouse and malignancy are the most common causes of exudative PE in our country.[7] The gold standard for diagnosis of pleural tuberculouse is theidentification of mycobacterium tuberculouse in pleural fluid or tissue[8] however in clinical practice this identification is problematic of the low identification rate of the bacillus (less than 30% in pleural fluid and approximately 50% in the pleura) and the slow growth of mycobacterium in culture (about 60 days). [9] The diagnosis of neoplastic pleural effusion is made based on the presence of malignant cells in the pleural fluid or tissue. The positivity rate of the cytological examination ranges from 40 to 87% higher than that obtained with a needle biopsy which ranges from 35 - 65 %. [13] Several tests for the diagnosis of tuberculous in pleural effusion have been used as tuberculouse identification such as Adenosine deaminase, interferon, Lysozyme, the polymerase chain reaction. [11] and specific C antibodies.[12]However these test need specific measure and expensive equipment that are not available in most laboratories particularly in developing countries, similarly in developed countries various new parameters like pleural viscosity, C- reactive protein, carcinoembryonic antigen, interleukin, interferon, vascular endothelial growth factor, tumor necrosis factor and pleural fluid T-cells are used for the determination of tuberculous and malignancy.[13] In our

country only closed pleural biopsy and pleural fluid analysis are carried out for the diagnosis of tuberculouse and malignancy.Yetkin etal. (2007)[14] have discuss the role of viscosity in the differential diagnosis of excaudate pleural effusion. The pleural fluid protein level was > 30 g/l in excaudate in the contrast of a normal serum protein level. The Lights criteria[15]indicate the concentration of protein in exudates is ratio of pleural fluid protein/serum protein >0.5.This study was aimed to explore the role of pleural fluid protein in differentiating tuberculouse from malignant pleural effusion.

MATERIALS AND METHODS

The study was conducted at pulmonary department Post Graduate Medical Institute Lady Reading Hospital Peshawar, KPK, from March 2009 to March 2010.179 patients were selected in this study, attending to pulmonology unit OPD, Emergency department and private clinics were evaluated. Patient's unit exudate pleural effusion was subjected to Abrams needle biopsy after taken informed written consent. The specimen was sent from Histopathological examination and pleural fluid for biochemical examination. The biopsy and laboratory analysis reposts were collected and recorded in Proforma designed for this study, one standard laboratory was used for plural fluid analysis and pleural tissue was examined by well experience histopathologist to the diagnosis of malignant pleural effusion is based on the finding of neoplastic cells in pleural fluid or pleural tissue obtained by Abrams needle biopsy.Lymphocytic exudative pleural effusion, sign and symptoms consistent with Tuberculous Pleural Effusion, sign and symptom consistent with malignant pleural effusion all the patients male and female from 15-80 years of age.Transudative pleural effusion, cases of in conclusion pleural biopsy, polymorphic exudative pleural effusion/ emphyema, those patients who were uncooperative or not willing for pleural biopsy.Sample size was 179, using 9% prevalence, 95% confidence level and 4.2% margin of error, under W.H.O formula of sample size determination.In order to know the significant difference of pleural fluid level between tuberculouse and malignant pleural effusion three categories were made is category "A" having from 4-5 g/dl and category C, having protein concentration higher than 5 g/dl.Data was analyzed using statistical package for social sciences (SPSS) version 10.0. Mean ± standard deviation was calculated for age and pleural fluid protein level. Qualitative variables such as gender, pleural biopsy histopathology result were calculated in frequencies and percentages. Chi- square test was calculated for total plural fluid protein level for tuberculouse and malignant pleural effusion and P valve was significant it found < 0.05.

RESULTS

Total no of patients were 179. There were 114 (63.69%) male and 65(36.32%) were female. Age limit was from 15-80 years, age wise distribution and result of TPE & MPE are given. Table (1). Age range was as follows the no of patients with age range 15-20 years was 12 (6.7%), 20 to 40 years 72 (40.2%), 41-60 years 67 (37.4%) and the patients age range of 61-80 years old were 28 (15.6%).TPE and MPE in various age groups was analyzed as that TPE was more common in age groups of < 20 years and 20-40 years, while in age groups of 41-60 years the tuberculous pleural effusion (TPE) cases were 22(20.9%) the mean age in tuberculouse pleural effusion was 35.8+15.435D similarly in age groups of 61-80 years, the MPE was common than TPE. The mean age for malignant pleural effusion groups was 57+ 13.13 SD. TPE was common in younger age groups whole malignant, in older age group. Table (2).The pleural fluid protein were analyzed as n=32(17.9%) of patients were having PfP level of category A, n=59(33%) were in the category B, and n=88 (49.2%) of the patients were having protein level in category C, Table [3].

Table No 1: Age – wise distribution of Tuberculous and malignant pleural effusion

Age Groups in Years	Diagnosis		Total
	TPE	MPE	
< 20 Years	11 10.1%	1 1.4%	12 6.7%
20-40 Years	67 61.5%	5 7.1%	72 40.2%
41-60 Years	22 20.2%	45 64.37%	67 37.4%
61-80 Years	9 8.3%	19 27.1%	28 15.6%
Total	109 100%	70 100%	179 100%

Table No. 2: Sex-wise distribution of Tuberculous and Malignant Pleural Effusion

Sex	Biopsy Result		Total
	TPE	MPE	
Male	76 69.7%	38 56.3%	114 63.7
Female	33 30.3%	32 45.7%	65 36.3
Total	109 100%	70 100%	179 100%

Table No. 3: The Categories of Pleural Fluid Protein Sensitivity and Specificity

Category	Frequency	%age	Sensitivity	Specificity
A	32	17.9	100.00	0
B	59	33.0	97.20	41.43
C	88	49.2	73.30	90.60

The sensitivity and specificity of pleural fluid protein of various categories was analyzed that at category A, the sensitivity was reaching to 100% but the specific was having the lowest value. At category "B" the sensitivity was 97.2% and specificity was 41.43% in category "C"

the sensitivity was the lowest of three having value 73.39% while the specificity was highest reaching to 90.6%.the positive (PPV) and negative predictive (NPV) values were analyzed for the three categories of plural fluid protein as that PPV was lowest for category C having value of 2.7 while NPV was 58.57 for category B, the PPV was 26.6 while the NPV was having value of 52.87. The PPV was highest for category "A" reading to the value 73.4 similarly the PPV was also highest in this category reading to the value of 88.57.

DISCUSSION

The distinction between tuberculous and malignant pleural effusion poses a diagnostic challenge to the physician. This is mainly due to the large proportion of cases in which no confirmatory diagnosis of pleural tuberculouse is achieved by microbiological methods, and the sensitivity of cytological studies for malignancy is inadequate[16]. Tuberculouse and malignancy are the most important and commonest causes of lymphocytic exudative pleural effusion. But the ratio is different in developed and developing countries. In areas were the tuberculouse is not prevent MPE is more common than TPE. In a study, conducted in Spain (2003) on 392 patients 73% cases were MPE and 27% were TPE.[17]Our study also showed that fact that TPE was commoner than MPE, accounting for 60.9% of total classes. Rest of cases (39.1 %) was malignant PE. Age has also been an important complementary variable while deciding about tuberculous or malignant PE. In our study the TPE was commoner in younger age group than MPE, Which was more in older age group. The mean age for TPE was 35.8 + 15.43 SD while 57 + 13.13 SF was the mean age for MPE. Our findings were comparable with the international studies.Porcel JM et al (2003) reported in their study, the mean age of 30 years (range 22-40) in TPE while the mean age was 68(58-76) years in MPE cases.[18]In our study we showed that pleural fluid protein level was higher in TPE than MPE and the difference was statistically significant at > 5g/dl (category "C") at < 4g/dl(category "A"). The MPE was commoner than TPE and the difference was significant. These finding were consistent with international data.Porcel- Perez JM et al (2004) reported that 73% of TPE were having pleural fluid protein level > 5g/dl.This value was the cutoff point for considering tuberculous etiology of the lymphocytic educative PE[14]. In another Spanish study conducted on 105 patients of TPE, 57% of patients showed plural fluid protein level above 5g/dl.[19]Antonangelo et al (2007) reported higher protein level in TPE than MPE. The protein level was 5.3+0.8g/dl in tuberculous PE while 4.2+1 was the level in MPE. The difference was statically significant.[23] Along with other laboratory parameters, protein level was utilized for discrimination between tuberculous and malignant PE. The same findings were found in the study conducted by Liam et al (2000) on patients having tuberculous or malignant PE [20]. Melo et al proposed 4.5g/dl as cutoff value for diagnostic presumption of TPE [21]. Porcel JM et al (2003) reported protein level of 5.4g/dl in tuberculous while the level was 4.2g/dl in malignant pleural effusion. The difference was statistically significant. [18]The study recommended two scoring models for differentiation between tuberculous and malignant PE. It also revealed that in areas where ADA facility is unavailable, plural fluid total protein level can be used for differentiation between tuberculous and malignant PE as used in one of the scoring model lacking ADA. The pleural fluid protein showed sensitivity of 77% and specificity of 80%. These results are comparable with those of our study which showed 73.30% sensitivity and 90.60% specificity at pleural fluid protein level of > 5g/dl.

CONCLUSION

Plural fluid total protein level determination and differentiating is a valuable tool in reaching to the diagnosis of suspectedtuberculouse from malignant pleural effusion provided it is used in addition to the adequate clinical scenario.

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Original Article

Pharmacology

To Assess the In vitro Genotoxicity of Metformin and Aspartame alone & In Combination

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ABSTRACT

Objective: Metformin is a known oral antidiabetic agent belonging to the class of biguanides, widely prescribed for the treatment of type 2 Diabetes Mellitus (DM). In this study the genotoxic potential of metformin was studied alone and in combination with an artificial sweetener aspartame as most of the diabetic patients utilized this low calorie sweetener to reduce their sugar consumption per day. Many complaints regarding its potential to cause DNA damage have been submitted to FDA

Study Design: Experimental study

Place and Duration of Study: This study was carried out at the Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences Lahore from 1st Jan 2011 to Dec 2011.

Materials and Methods: Peripheral Blood Lymphocytes were exposed to various concentrations of metformin, aspartame and their combination. DNA damage was checked by comet assay. The data was analyzed by using Analysis of Variance (ANOVA) by Statistical Package of Social Sciences SPSS

Results: Exhibited dose dependant rise in comet tail lengths. Moreover the data advocates that tail lengths of lymphocytes after exposure to aspartame were high as compared to metformin. When lymphocytes were exposed to combination of aspartame and metformin and DNA damage was checked by comet assay, the results were significantly different ($p<0.05$) as compared to metformin and aspartame alone.

Conclusion: It can be concluded from the present study that aspartame is posing great genotoxic threat to the cells as compared to metformin and the combination is even more toxic to DNA, so the drug regime of diabetic patient must be closely monitored. There is further need of more studies in this regard.

Key Words: Metformin, Aspartame, Diabetes Mellitus, Comet Assay. DNA damage.

INTRODUCTION

Among the antidiabetic agents, metformin (1-(diaminomethylidene)-3,3-dimethyl-guanidine) is reported to have several properties, such as it reduces formation of Advance glycation endproducts(AGE), helps to regain the reduced levels of glutathione in DM and increases the antioxidant defense[1]. A recent study demonstrates that metformin increases AMPK activation in MIN6 cells responsible for insulin production and primary rat beta cells in dose dependant manner. Prolonged exposure of metformin (>24h) resulted in a gradual rise in apoptotic beta-cells similar to the effect which was documented for AMPK-activator AICAR[2].

AMPK is an enzyme that plays a role in cellular energy homeostasis and its persistent activation has recently been concerned with apoptosis of beta cells of pancreas and in cell death of islets of langerhans[3].

Activation of AMPK by metformin might be mediated by the mitochondria-derived RNS in vivo in C57BL6 mice[4]. There are certain studies supporting that metformin stimulate cell death in p53-deficient HCT116 cells in condition of limited glucose availability. So metformin can be beneficial to be used in patients with p53 deficient tumors which have become resistant to chemotherapy[5].

The antidiabetic drug has also been reported to cause a decline in the occurrence of mammary carcinomas in hamsters when administered in therapeutic doses. Most probably it would have damaged the genetic material of the carcinoma cells to reduce chances of cancer prevalence in mammary cells of hamster[6]. Certain reports support combination therapy of metformin and doxorubicin which kills both cancer stem cells and non–stem cancer cells in culture, and cause reduction in tumor mass and delays relapse more efficiently than metformin of doxorubicin can do alone[7].

Onaran et al. (2006) suggested that therapeutic concentrations of metformin could not check DNA damage caused by oxidative stimulus in cultured human lymphocytes regardless of its anti oxidant characteristic[8].

The use of artificial sweetener aspartame is increasing day by day by both diabetic as well as non diabetic population as a sugar substitute. Aspartame is mainly composed of phenylalanine (50%), aspartic acid (40%) and methanol (10%). In this study different doses of aspartame was administered to check the cellular effects of aspartame on brain and it was revealed that

consistent use of aspartame might cause various mental disorders due to damage to the brain cells [9].

The effect of an artificial sweetener Aspartame was studied to investigate oxidative stress and brain monoamines in normal conditions and also after administration of lipopolysaccharides intraperitoneally in mice. The results revealed that aspartame alone and in combination with any mild systemic inflammatory response enhance inflammation and oxidative stress in brain cells[10].

MATERIALS AND METHODS

Chemicals: Tested chemicals i.e., Aspartame and Metformin were provided by Popular Laboratories, Lahore Pakistan. Dimethyl Sulfoxide (DMSO) was purchased from Merck Pakistan. All other chemicals and media used were of analytical grade.

Comet Assay: Cavity slides were layered with 1% Normal melting Agarose and placed in refrigerator for 2-12 hours. Peripheral blood lymphocytes were separated by using Lymphocyte separating medium. 3ml of 1:1 ratio of peripheral blood with PBS was layered onto 4ml of Lymphocyte separating medium and after centrifuging lymphocyte suspension was made in RPMI-1640[11]. Now the lymphocytes were exposed to various concentrations of test chemicals i.e. metformin, aspartame and combination doses of aspartame and metformin. The exposed cells were mixed 1% low melting point agarose and layered onto the precoated slides. After solidification 3rd layer of Agarose 1% was layered onto the same slide and allowed to solidify. Subsequently the slides were dipped in the lysing solution (pH 10) consisting of high salt concentration and detergent for 2 hours. After that slides were exposed to alkaline buffer (pH > 13) consisting of 1mM EDTA and 300mM NAOH for DNA unwinding for 20 minutes. The cells were subjected to electrophoresis for 20 minutes at 25 volts and 300mA. Slides were washed with 0.4M TRIS buffer/ Neutralizing buffer (pH 7.5) 3 times and then stained with Ethidium bromide[12].

PBS was used as negative control and DMSO 20% was used as positive control[13].

Microscopic Analysis: The slides were visualized by using Fluorescent Microscope at 40X objective. The DNA damage was quantified by measuring tail lengths of 25 consecutive cells and quantification was done in triplicate manner. Four damage categories were established according to the method described by Valencia-Quintana and coworkers (Valencia-Quintana et al. 2012).

Class 0= Undamaged cells

Class 1= Tail length ≤Head Diameter

Class 2= Tail Length > Head Diameter BUT Tail Length ≤Double of Head Diameter

Class 3= Tail Length > Double of Head Diameter

The damage to the DNA was expressed as %age fragmentation and ultimately finding the DNA damage index of each concentration according to the following formula.

Damage Index= No. of cells in Class.1+ (2×No. of cells in Class.2) + (3×No. of cells in Class.3)

Fragmentation %= $\frac{\text{No. of cells in Class.1 + No. of cells in Class.2 + No. of cells in Class.3}}{\text{Total Number of cells under observation}} \times 100$

Statistical Analysis: The data was analyzed by using Analysis of Variance (ANOVA) by Statistical Package of Social Sciences SPSS for windows (version 16; SPSS Inc; Chicago IL; USA) and Tukey's test was applied. The value of $p<0.05$ was considered significant.

RESULTS

The results of comet assay on lymphocyte sampled from peripheral blood treated with different concentrations of metformin are presented in Table 1. Treatment with different doses of metformin induced a significant increase in DNA damage at 200, 250, 300, 350 and 400µg/ml as these values were significantly higher ($p < 0.01$) than that found in the negative control. It is noted that percentage of fragmentation of DNA and damage index increases as the concentration of metformin increases reaching a maximum value at 400µg/ml. The effect observed with 10, 20, 80, 100 and 150µg/ml do not show significant differences ($p < 0.05$) when compared with the negative control.

Lymphocytes were exposed to 10 different concentrations of aspartame presented in the Table 2. The treatment with various doses of aspartame showed same order of genotoxicity as that of metformin. Aspartame caused significant rise ($p < 0.01$) in DNA damage at 500, 1000, 2000, 4000, 8000 µg/ml as compared to the DNA damage caused by negative control. The percentage fragmentation of DNA and Damage Index becomes high when the exposed concentration of aspartame increases and the maximum DNA damage index is found at 8000 µg/ml. However the results with lower doses i.e. 12.5, 25, 50, 100 and 250µg/ml did not show any significant rise ($p < 0.05$) in the DNA damaging potential as compared to negative control.

When the cells were exposed to the combination of aspartame and metformin, the results were significantly high ($p < 0.05$) as compared to metformin and aspartame individually. Here again dose dependant rise in genotoxicity is observed in manner similar to the individual drugs. We can see a significant rise ($p < 0.01$) in DNA damage index at 500:200, 1000:250, 2000:300, 4000:350, 8000:400 µg/ml as compared to the negative control (Table 3). Percentage fragmentation and DNA damage index increases as the concentration of aspartame and metformin increases with maximum DNA damage index obtained at 8000:400 µg/ml which has highly significant difference ($p < 0.001$) with negative control.

Table No.1: DNA Damage induced by different concentration of Metformin in lymphocytes evidenced from Comet assay.

Sr. No.	Conc.	Class 0	Class 1	Class 2	Class 3	Fragmentation %	Damage Index
A	DMSO 20%	2	2	6	15	92	59
B	-ve Control	24	1	0	0	4	1
1	10µg/ml	24	1	0	0	4	1
2	20 µg/ml	23	2	0	0	8	2
3	80 µg/ml	22	3	0	0	12	3
4	100 µg/ml	22	3	0	0	12	3
5	150 µg/ml	22	2	1	0	12	4
6	200 µg/ml	20	2	3	0	28	8*
7	250 µg/ml	16	5	4	0	36	13*
8	300µg/ml	14	4	6	1	44	19*
9	350 µg/ml	13	3	7	2	48	23*
10	400 µg/ml	12	3	8	2	52	25*

***=significant difference (p<0.01) as compared to negative control analyzed by SPSS Windows Version 16 Tukey's Test.**

Table No.2: DNA Damage induced by different concentration of Aspartame in lymphocytes evidenced from Comet assay.

Sr. No.	Conc.	Class 0	Class 1	Class 2	Class 3	Fragmentation %	Damage Index
A	DMSO 20%	2	2	6	15	92	59
B	-ve Control	24	1	0	0	4	1
1	12.5 µg/ml	24	1	0	0	4	1
2	25 µg/ml	23	2	0	0	8	2
3	50 µg/ml	23	2	0	0	8	2
4	100 µg/ml	22	3	0	0	12	3
5	250 µg/ml	22	2	1	0	12	4
6	500 µg/ml	19	3	3	0	24	9*
7	1000 µg/ml	16	2	7	1	40	19*
8	2000 µg/ml	14	2	7	2	44	22*
9	4000 µg/ml	12	2	8	3	52	27*
10	8000 µg/ml	9	1	11	4	64	35*

***=significant difference (p<0.01) as compared to negative control analyzed by SPSS Windows Version 16 Tukey's Test.**

Table No.3: DNA Damage induced by different concentration of Combination of ASP: MET in lymphocytes evidenced from Comet assay.

Sr. No.	Conc.	Class 0	Class 1	Class 2	Class 3	Fragmentation %	Damage Index
A	DMSO 20%	2	2	6	15	92	59
B	-ve Control	24	1	0	0	4	1
1	12.5:10µg/ml	24	1	0	0	4	1
2	25:20 µg/ml	23	2	0	0	8	2
3	50:80 µg/ml	23	1	1	0	8	3
4	100:100 µg/ml	23	1	1	0	8	3
5	250:150 µg/ml	22	2	1	0	12	4
6	500:200 µg/ml	15	4	3	3	56	19*
7	1000:250 µg/ml	10	4	9	2	60	28*
8	2000:300µg/ml	8	3	10	4	68	35*
9	4000:350 µg/ml	6	2	12	5	76	41*
10	8000:400 µg/ml	4	2	14	5	84	45*

***=significant difference (p<0.01) as compared to negative control analyzed by SPSS Windows Version 16 Tukey's Test.**

When the all the three results were analyzed statistically for multiple comparison by applying Analysis of Variance (ANOVA) Post Hoc Test LSD by Statistical Package of Social Sciences SPSS for windows (version 16; SPSS Inc; Chicago IL; USA) it was revealed that combination doses were significantly ($p < 0.05$) highly

genotoxic as compared to metformin. However the results of combination doses were not significantly ($p < 0.05$) different as compared to aspartame individually.

DISCUSSION

The prime objective of therapy for type 2 diabetes mellitus is to obtain and sustain good glycemic control, and to reduce the risk of secondary complications associated with DM. Metformin is considered a gold standard treatment of DM along with certain interventions in lifestyle of patients. Metformin also have efficacy against polycystic ovary syndrome and various neoplasms[14].

The antidiabetic drug has been reported to cause a decline in the occurrence of mammary carcinomas in hamsters when administered in therapeutic doses. Most probably it would have damaged the genetic material of the carcinoma cells to reduce chances of cancer prevalence in mammary cells of hamster[6]. The results of comet assay on various concentrations of metformin exhibited dose dependent rise in its potential to damage DNA. Percentage fragmentation of DNA and damage index increases as the concentration of metformin increases reaching a maximum value at 400µg/ml. The results of this study are in accordance with the fact that prolonged exposure of metformin may become a potential threat of genotoxicity. A study was conducted to check the genotoxic potential of metformin in Chinese Hamster Ovary cells and DNA damage was checked by Chromosomal Aberration Assay and single cell gel alkaline electrophoresis/comet assay. Results showed significant damage to the DNA by metformin[15]. However data is available which supports that metformin is neither cytotoxic nor genotoxic when various concentrations were administered in vivo condition to diabetic and non diabetic rats[16]. Another study was conducted to evaluate the prevention of DNA damage in human lymphocytes by metformin. Cells were exposed to various concentrations of metformin and subsequently they were incubated with cumene hydroperoxide a known DNA damaging substance. The results were evaluated by comet assay revealing that metformin is unable to prevent DNA damage by any chemical causing oxidative stress to the cells[8]. Metformin kills cancer stem cells in genetically different type of breast cancer so combination therapy of metformin and doxorubicin is used to reduce tumor mass and delays relapse more efficiently that metformin and doxorubicin can do alone[7].

The underlying mechanism responsible for DNA damage by metformin may lie in the fact that metformin may inhibit mitochondrial respiration cells and ultimately there is rapid rise in the superoxide level because of inhibition of cellular respiration due to insult to the mitochondria [17]. According to Warburg theory of cancer, the key reason for development of tumor is an inadequate cellular respiration due to insult to mitochondria[18].

The use of sweetening agent by diabetic individuals is common. A survey of our diabetic clinic population showed that 65% regularly use these products[19]. It is widely used by the diabetic population for the sweet taste avoiding high calories through sugar consumption[20]. The results of comet assay revealed that aspartame exhibited significant potential of genotoxicity when different concentrations of the sweetener were exposed to the lymphocytes. Damage Index of DNA revealed that aspartame was causing more damage to the DNA of peripheral blood lymphocytes as compared to metformin when compared with the negative control. The results of comet assay are in agreement with a study exhibiting significantly high ($p < 0.01$) carcinogenic potential on prolonged use, demonstrating that these artificial sweeteners are not entirely safe although they are FDA approved[21].

Results also state that there is dose dependant rise in the DNA damaging potential of aspartame endorsing the statement that aspartame can cause chromosomal aberrations at all concentrations (500, 1000 and 2000 µg/ml) and treatment periods, in human lymphocytes, in a dose dependant manner[22]. The underlying mechanism of DNA damage caused by aspartame may lie in the fact that prolong exposure of aspartame may result in detectable amount of methanol in blood[23]. As aspartame consists of 3 components aspartic acid, phenylalanine and methanol which is most dangerous of all[24]. Various studies revealed the data that aspartame is responsible for increased frequency of lymphomas and leukemias and is also responsible in significant rise in prevalence of transitional cell carcinomas of renal pelvis at doses approximated with the ADI of aspartame for humans. These results indicate that aspartame proves to be the multipotential carcinogen greatly affecting the quality of life[25].

As far as results of the combination doses are concerned they cause severe damage to DNA when exposed to the lymphocytes and cells were quantified using comet assay. %age fragmentation and DNA damage index were significantly high ($p < 0.05$) as compared to individual results of metformin and aspartame. The data advocates the same threshold level of genotoxicity but the intensity of the genotoxic effect was relatively high in case of combination as compared to the individual drugs. The additive effect of both aspartame and metformin on DNA damage may be accredited to the fact that aspartame is responsible for increased oxidative stress to the cells[10]. Along with that metformin is associated with production of reactive nitrogen species from mitochondria[4] which ultimately hinder the cellular respiration leading to cell damage.

CONCLUSION

It can be concluded from the present study that aspartame is posing great genotoxic threat to the cells as compared to metformin. When combination of aspartame and metformin were exposed to the peripheral blood lymphocytes the results showed significantly high ($p < 0.05$) genotoxicity as compared to metformin and aspartame individually. Most of the diabetic patients utilize aspartame as an artificial sweetener along with their daily regimen of antidiabetic drug metformin. So caution must be taken while using

both these products together as it may cause significant damage to the cells of body.

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Original Article

Diabetes Mellitus

Study of Type 2 Diabetes Mellitus and its Correalation with Blood Groups

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ABSTRACT

Objectives: To ascertain any relation between T2 Diabetes Mellitus with Blood Groups of the patients coming in the OPD.

Study Design: Experimental / Analytical study

Place and Duration of Study: This study was carried out at Med Path Laboratories and Diagnostic Center Gulshan-e-Iqbal Karachi & KESC Medical center Karachi from August 2012 to December 2012.

Materials and Methods: The present study deals with the distribution of the ABO blood types in patients with diabetes mellitus. 500 samples were collected from Med Path Laborites and Diagnostic Center KESC Medical Center.

Results: The study shows blood group B was prevalent at a high percentage among patients with DM type 2. Blood group O+ were significantly higher among the male diabetics and blood group B+ among female diabetics. People with middle-aged group were seen to be more affected with type 2 diabetes mellitus.

Conclusion: It is highly recommended that this study may proceed further and the affiliation of T2DM can be screened at molecular level to find out the exact mechanism of action for susceptibility to these particular Blood Groups.

Key words: Diabetes Mellitus, Correalation, Blood Groups

INTRODUCTION

Diabetes Mellitus (DM) is a set of related diseases in which the body cannot regulate the amount of sugar (specifically, glucose) in the blood. The blood delivers glucose to provide the body with energy to perform all of a person's daily activities. The liver converts the food a person eats into glucose. In diabetes, glucose in the blood cannot move efficiently into cells, so blood glucose levels remain high. This not only starves all the cells that need the glucose for fuel, but also harms certain organs and tissues exposed to the high glucose levels.[1]

Classification of Diabetes Mellitus: Diabetes Type 1 Patients produce no insulin at all. Diabetes Type 2 – Patients don't produce enough insulin, or insulin is not working properly. Gestational Diabetes - develops diabetes just during your pregnancy.

Maturity onset diabetes of the young (Monogenic Diabetes) (MODY)-Refers to any of several hereditary forms of diabetes caused by mutations in an autosomal dominant gene disrupting insulin production.

Type 1 diabetes (T1D): The body stops producing insulin or produces too little insulin to regulate blood glucose level.

Type 1 diabetes involves about 10% of all people with diabetes. Type 1 diabetes is typically diagnosed during childhood or adolescence. It used to be referred to as juvenile-onset diabetes or insulin-dependent diabetes mellitus.[2]

Causes of Type 1 diabetes: Type 1 diabetes can occur in an older individual due to destruction of the pancreas by alcohol, disease, or removal by surgery. It also results from progressive failure of the pancreatic beta cells, the only cell type that produces significant amounts of insulin.

Type 2 diabetes (T2D): Although the pancreas still secretes insulin, the body of someone with type 2 diabetes is partially or completely unable to use this insulin. This is sometimes referred to as insulin resistance. The pancreas tries to overcome this resistance by secreting more and more insulin.

Causes of Type 2 Diabetes: Type 2 diabetes is thought to be caused by a combination of genetic and environmental factors.

Genetic causes — Many people with type 2 diabetes have a family member with either type 2 diabetes or other medical problems associated with diabetes, such as high cholesterol levels, high blood pressure, or obesity. Environmental conditions — Environmental factors such as eating habits and physical activity are, combined with genetic causes, affect the risk of developing type 2 diabetes.[3]

Gestational Diabetes: A small number (about 3 to 5 percent) of pregnant women develop diabetes during pregnancy, called "gestational diabetes."

Maturity onset diabetes of the young: MODY is a rare form of diabetes which is different from both Type 1 and Type 2 diabetes, and runs strongly in families. MODY is caused by a mutation (or change) in a single gene.

It was reported that DM type 2 is the most common type, accounting for 90-95% of all diabetic cases.(4) In 1998 it was estimated that there were almost140 million people with diabetes and the predictions by Hilary King indicate that this figure would rise up to 300 million by the year 2025.[5]

Blood Groups: Blood groups are created by molecules present on the surface of red blood cells (and often on other cells as well). The major human blood group system is ABO. The blood group of a person depends upon the presence or absence of two genes, A and B. The ABO blood groups were the first to be discovered (in 1900) by Karl Landsteiner and are the most important in assuring safe blood transfusions. The Rh blood group system is the second most significant system for blood grouping.

Rh factor refers to Rh D antigen only. Determination of Rh factor along with ABO is essential for defining the Rh +ve or Rh -ve status of the individual. Around 85% of the human population is Rh +ve while 15% is Rh -ve. [6]

The ABO & Rh systems are the most significant blood group systems from the clinical point of view.[7]

Many researchers have made attempts to determine the significance of particular ABO phenotypes for susceptibility to disease. The relationship between the ABO/rhesus (Rh) blood groups and various diseases has generated a great deal of interest[8]. Certain diseases show a strong association with the ABO/Rh blood groups, notably peptic ulcer and gastric cancer[9]. Individuals with blood group O have been found to be at a higher risk of contracting cholera than those with other blood groups. The ratio of this risk of group O to group A individuals has been reported as 1.35:1. Oral candidiasis shows a higher incidence of group O over other ABO groups[10]. Small pox virus has been found to carry an A antigen-like structure, so that individuals who possess a naturally occurring anti-A (group O and B individuals) are thought to have an increased resistance to the infection.[11]

This could be attributed to the inability of the immune system of group A individuals to recognize the A like antigen of the tumor cells as foreign and cannot destroy them, but group O and B individuals do have a naturally Occurring anti- A that are most likely to destroy tumor cells.[12]

It has been reported that group O individuals are proportionately more prone to bleeding than individuals of the other blood groups; and group an individuals with highest incidence of thrombosis than in any other group. [13]

The blood groups of diabetics have been extensively studied since McConnell's suggestion in 1955 of an increased frequency of blood group A among these patients.[14] In Copenhagen, an excess of blood group O was found in male diabetics.[15]

No diseases are known to result from the lack of expression of ABO blood group antigens, but the susceptibility to a number of diseases has been interrelated to a person's ABO phenotype.[16] Such correlations remain conflicting and include the observation that gastric cancer is more common in group A individuals, whereas gastric and duodenal ulcers occur more commonly among blood group O individuals.[17] In the present study, an attempt has been made to investigate any association with the ABO blood types and diabetes mellitus type 2.

MATERIALS AND METHODS

The present study was an epidemiological study. A total no of 500 blood samples from patients with Diabetes mellitus (Type 2) were collected from the Med Path Laboratory. Since both gender differentiations are not known to exist in the ABO blood type system, the samples collected from both males and females were pooled for the analyses.

Materials:

- BSL 2 lab
- Gloves
- Lab coat
- Blood typing tiles
- 500 diabetic patients Blood samples
- Synthetic anti-Rh (D) serum
- Synthetic anti-A serum
- Synthetic anti-B serum
- Justors (50 ul)
- Mixing sticks (blue, yellow, and white)

Method:

Blood Sample Collection: For the ABO blood types, standard serological procedures were followed using the anti-A, anti-B and anti-D antisera. A blood sample drawn from a vein in the arm or a finger-prick

Abo Test:

1. Using the justor, place a drop of the blood sample in each demo created area of the blood typing tile. Replace the tip of the jester.
2. Add a drop of synthetic anti-A (blue) to the well labeled A.
3. Add a drop of synthetic anti-B serum (yellow) to the well labeled B.
4. Add a drop of synthetic anti-Rh serum (clear) to the well labeled Rh.
5. Using a different color mixing stick for each well (blue for anti-A, yellow for anti-B, white for anti-Rh), gently stir the synthetic blood and anti-serum drops for 30 seconds. Remember to discard each mixing stick in danger box after a single use to avoid contamination of your samples.
6. Carefully examine the thin films of liquid mixture. If a film remains uniform in appearance, there is no agglutination. If the sample appears granular, agglutination has occurred. Determine the blood type

of the sample. A positive agglutination reaction indicates the blood type.
7. Record the results in the data table.
8. Thoroughly rinse the blood typing tile with a disinfectant, and then repeat steps.
9. The data were entered in Microsoft excel for analysis. Further analysis was carried out using instant graph-pad.

RESULTS

In Table 1 the distribution of the ABO blood types in patients with diabetes mellitus is shown including both male and female.

Table No.1: Correlation of blood groups

Blood Group	Frequency	Percentage
A+	124	24.80%
A-	9	1.80%
B+	150	30.00%
B-	6	1.20%
AB+	63	12.60%
AB-	2	0.40%
O+	131	26.20%
O-	15	3.00%
Total	500	100.00%

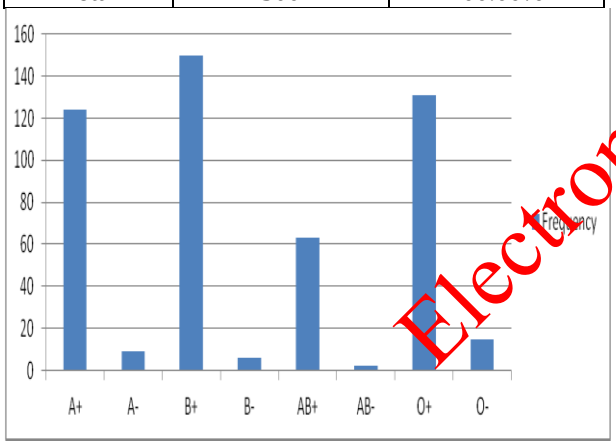

Chart: Blood group frequency

Table No.2A: Correlation of blood groups in Male

Blood Group	Frequency	Percentage
A+	71	24.83%
A-	7	2.45%
B+	66	23.08%
B-	3	1.05%
AB+	41	14.34%
AB-	2	0.70%
O+	87	30.42%
O-	9	3.15%
Total	286	100.00%

In our study, the results showed association between ABO blood groups and DM type 2. B+, O+, A+ blood group showed higher incidence of type 2 diabetes mellitus than groups which are Rh-ve signifying the higher incidence in patients which refers to Rh+ve group. However, significant association was found between DM type 2 and blood groups A (P 24.80%) and O (P 26.20%) The frequency of B blood group (30.00%) was high among patients with DM type 2. AB blood groups showed little association with DM (P 12.60%) ,which implied that AB blood group patients have less chances of DM type 2. (Table 1)

In Table 2: A and 2: B the distribution of ABO blood groups between genders with diabetes mellitus is shown.

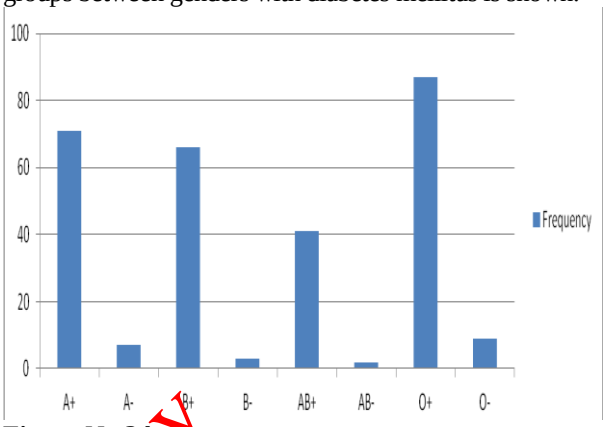

Figure No.2A: Frequency

Table No.2B: Correlation of blood groups in Female

Blood Group	Frequency	Percentage
A+	53	24.77%
A-	2	0.93%
B+	84	39.25%
B-	3	1.40%
AB+	22	10.28%
AB-	0	0.00%
O+	44	20.56%
O-	6	2.80%
Total	214	100.00%

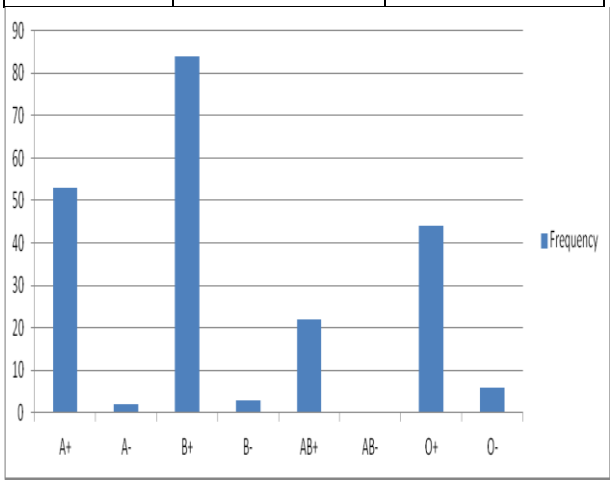

Figure No.2b: Frequency

The distribution of ABO/Rh blood groups between the genders for diabetics is shown in Table II. Blood groups O+ were more dominant in the diabetic group among men (30.42%) than women (20.56%); the type 2 DM in B+

blood groups were more common in women (39.25%) than men (23.08%). (Table 2)

Table No.3A: Correlation of blood group by Age in Male

Blood Group	20 – 40	41 - 60	61 - 80
A+	18	36	17
A-	3	4	0
B+	11	36	19
B-	1	0	2
AB+	8	21	12
AB-	0	2	0
O+	27	47	13
O-	0	4	5
Total	68	150	68

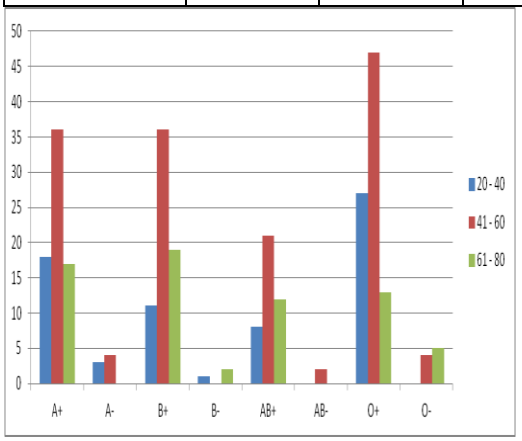

Figure No.3A:

Table No.3B: Correlation of blood group by Age in Female

Blood Group	20 – 40	41 - 60	61 - 80
A+	15	29	9
A-	0	1	1
B+	22	50	12
B-	1	2	0
AB+	7	12	3
AB-	0	0	0
O+	16	22	6
O-	1	4	1
Total	62	120	32

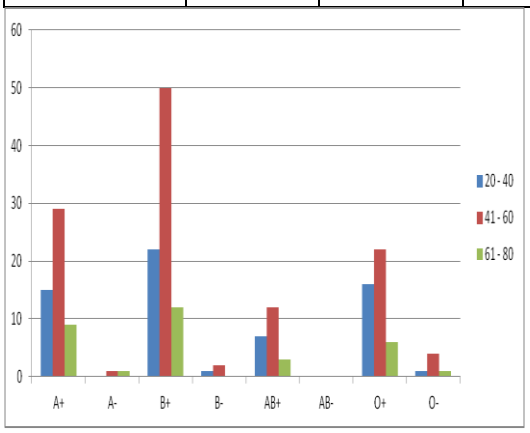

Figure No.3B:

In Table 3: A and 3: B the distribution of diabetic's blood types by age between the genders is shown.

A distinction is also drawn between diabetes in the young and in the middle-aged and in old respectively. It was observed that in middle-aged (40-60) people were more affected by DM type 2 having different blood groups. (Table 3)

DISCUSSION

It was long suggested that the ABO blood group system had evolved under a positive selection pressure in both humans and other primates.[18] This implies that certain ABO groups provide a selected vulnerability to individuals possessing a particular ABO blood group. Diabetes mellitus was observed to occur in all the ABO/Rhesus blood groups. However it was observed that the patients with type 2 diabetes mellitus were at rise in Rh+ve cases of which are A, B and O. This study clearly reveals the importance of blood group in different group of patients based on gender, age and Rh groups. This study suggests that person with Rh+ve are more prone to develop type 2 diabetes mellitus than the person with Rh -ve group.

In this study it was also found out that the following groups were more susceptible to develop type 2 diabetes mellitus in sequence of higher percentage.

Blood group	Percentage	Blood group	Percentage
B+	30.00%	B-	1.20%
O+	26.20%	O-	3.00%
A+	24.80%	A-	1.80%
AB+	12.60%	AB-	0.40%

This comparison certainly reflects that the person having B+ blood group is more likely to develop type 2 diabetes mellitus than person having blood group B.

The association of type 2 DM in male and female is somewhat equal however, in male the incidence was higher in O+ group and female shows high incidence in blood group B+.

This was also evident with regard to age which were divided in to three groups, 20-40, 41-60, 61-80 showing higher incidence in age groups of 41-60 with equal distribution of type 2 DM O+ and B+ blood groups which are more likely to develop type 2 DM in their age group.

The study of Qureshi and Bhatti demonstrated that DM type 2 and ABO blood groups are interrelated; they found that among 70 patients with DM, blood group B was more common and represented 35.71%.[19] It is interesting to note that our study did show a higher percentage of blood group B (30.00%) in the diabetic group, but this failed to achieve statistical significance, and results are in agreement with Qureshi and Bhatti. In addition, there were reports from Italy and Trinidad showing an increased frequency of blood group B among diabetics.[20]

CONCLUSION

It is concluded that the persons having these blood groups who are more susceptible to develop T2DM should take due care with reference to increased incidence of developing DM and adopt healthy life style in every respect keeping in view the danger zone in which they are living and the information they have with respect to their own blood group. It is highly recommended that this study may proceed further and the affiliation of T2DM can be screened at molecular level to find out the exact mechanism of action for susceptibility to these particular Blood Groups.

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Original Article

Pharmacology

The Effects of Monosodium Glutamate on the Histology of Fallopian Tube in Female Rats

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ABSTRACT

Objective: To investigate the effects of orally administered monosodium glutamate (MSG) on the fallopian tube histology in adult female Wistar rat model.

Study Design: Experimental/Analytical study

Place and Duration of Study: Animal House, Isra University Hyderabad from May to November 2013.

Materials and Methods: Forty adult male Wistar rats were divided into three groups; Group A. controls received 0.9% isotonic saline, Group B. received MSG orally (1.5 mg/kg), and Group C. received MSG orally (3 mg/kg). The animals were sacrificed after six weeks. Fallopian tubes were fixed in 4% formaldehyde, and were embedded in paraffin. Tissue sections of 5µ thicknesses were subjected to haematoxylin and eosin staining and were assessed by light microscopy.

Results: The fallopian tubes (FT) of the control group A showed normal histological features. The fallopian tubes of the treated groups showed some cellular hypertrophy of the columnar epithelium, distortion of the basement membrane separating the endosalpinx from the myosalpinx. There were degenerative and atrophic changes observed in some parts; these were more pronounced in those that received 3 mg/kg body weight of MSG. There were marked vacuolations and lysed red blood cells, (3 mg/kg body weight treated rats) appearing in the stroma cells

Conclusion: The monosodium glutamate may have deleterious effects on the fallopian tube histology in adult female Wistar rats particularly in high dose. Therefore caution must be taken for its frequent use in female because of possibility of female infertility.

Key words: Histology, Fallopian Tube, Monosodium Glutamate, Female Rat.

INTRODUCTION

The Fallopian tubes are paired, tubular, seromuscular organs which run medially from the cornua of the uterus toward the ovary laterally at the upper margins of the broad ligaments between the round and utero ovarian ligaments.[1] Millions of tiny hair-like cilia line the fimbria and interior of the fallopian tubes. The cilia beat in waves hundreds of times a second catching the egg at ovulation and moving it through the tube to the uterine cavity, Other cells in the tube's inner lining or endothelium nourish the egg and lubricate its path during its stay inside the fallopian tube.[2] The tubal wall consists of three layers: the internal mucosa (endosalpinx), the intermediate muscular layer (myosalpinx), and the outer serosa, which is continuous with the peritoneum of the broad ligament and uterus, the upper margin of which is the mesosalpinx. The endosalpinx is thrown into longitudinal folds, called primary folds, increasing in number toward the fimbria and lined by columnar epithelium of three types: ciliated, secretory, and peg cells. In the ampullary and infundibular sections, secondary folds of the tubal mucosa also exist, markedly increasing the surface areas of these segments of the tube. The myosalpinx actually consists of an inner circular and an outer longitudinal layer to which a third layer is added in the interstitial portion of the tube. The serosa of the tube is composed of an epithelial layer histological indistinguishable from peritoneum elsewhere in the abdominal cavity. [2,3]

Currently many studies have reported deleterious effects of Monosodium glutamate (MSG) on the histology of fallopian tubes in female rats.[3,4] The MSG is commonly known as AJINOMOTO.[5] MSG is the sodium salt of a naturally occurring amino acid; the glutamic acid. MSG is commonly marketed as a flavour enhancer and is used as a food additive particularly in West African and Asian dishes.[6,7] Generally, monosodium glutamate is accepted as a safe food additive that needs no specificied average daily intake or an upper limit intake.[8] An experimental study demonstrated that both subcutaneous injection and oral administration of MSG to immature rats and mice resulted in neuronal losses in the hypothalamus.[9] The ability of monosodium glutamate to damage nerve cells

of the hypothalamus is a pointer to the fact that it may alter the neural control of reproductive hormone secretion via the hypothalamic-pituitary-gonadal regulatory axis. The effects of such toxicants on male reproduction may be anatomical or only functional, depending on whether they produce structural changes in the reproductive system, or merely affect the functions of the reproductive organs.[10]

In the last few years, fear had increased due to the adverse reactions and toxicity of MSG, with few and limited literature regarding the histological studies of the damage in fallopian tubes of animals treated with MSG. Therefore, the present study aimed to investigate some probable histological effects of MSG on the fallopian tube histology in adult female Wistar rats.

MATERIALS AND METHODS

The present experimental study included forty young adult male Wistar rats at animal house of Isra University from May to November 2013. Adult female Wistar rats of 250-300 grams were included, while male rats weighing <250 grams or >300 grams were excluded from the study.

- **Animals:** The Animals were housed in animal house at an optimal room temperature with 55-60% humidity and exposed to 12 hour light-dark cycles. The chaw like fresh alfalfa and clean water are provided freely. The rats were divided into three groups;
  - **Group A. Control Group** (n=10) Rats received 0.9% isotonic saline orally on alternate day for three successive weeks and served as control group,
  - **Group B.** (n=10) Experimental Rats were given 1 mg/kg of monosodium glutamate orally.
  - **Group C.** (n=10) Experimental Rats were given 3mg/kg of monosodium glutamate orally.
- **Chemical:** The chemical used was monosodium glutamate (C5H9NO4-Na+). The MSG was purchased from the open market of Hyderabad under the license of Ajinomoto co.INC. Tokyo, Japan. A stock solution was prepared by dissolving 30 and 60 g of MSG crystals in 100 ml of distilled water. The dose schedule was so adjusted that the amount of MSG administration per animal was as per their respective weight. The MSG doses were given for six weeks. The applied doses were selected according to as referenced.[11]
- **Fallopian tube histology:** At the end of the experimental period, the animals were sacrificed by cervical dislocation and the abdominal cavity was opened up to expose the fallopian tube which were quickly dissected out, and fixed in 10% formal saline for routine histological techniques. The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 3–4 lm thick were obtained using a rotator microtome. The deparaffinized sections were stained routinely with hematoxylin and eosin. Sections of fallopian tube were examined by light microscope. Photomicrographs of the desired sections were obtained for microscopic details..

RESULTS

The present study was conducted to evaluate the effects of monosodium glutamate (MSG) on the histology of testis in rat model. The MSG was given in different doses in the experimental group animals as mentioned in methodology. The control group revealed normal histology. The fallopian tubes (FT) of the control group A showed normal histological features, illustrating a well defined tubal wall which consists of three layers: the internal mucosa (endosalpinx), the intermediate muscular layer (myosalpinx), and the outer serosa (Fig. 1).

The experimental groups (Groups B and C) were studied separately for the microscopic structure of fallopian tubes. The major derangements were observed in the fallopian tubes of high dose MSG treated rats (3 mg/kg body weight). The histological details of experimental rats are shown in figure 2-3.

The fallopian tubes of the treated groups showed some cellular hypertrophy of the columnar epithelium, distortion of the basement membrane separating the endosalpinx from the myosalpinx. There were degenerative and atrophic changes observed in some parts; these were more pronounced in those that received 3 mg/kg body weight of MSG. There were marked vacuolations and lysed red blood cells, (3 mg/kg body weight treated rats) appearing in the stroma cells (Figs. 2 and 3).

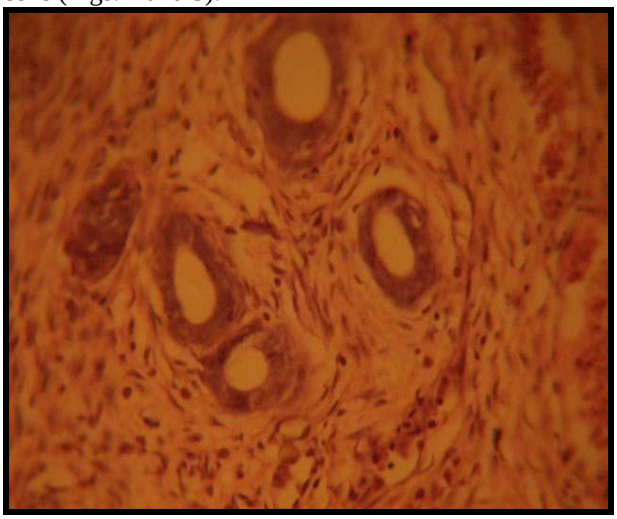

Figure No. 1: Section of testis from rats of control group (Group A) rat showing semniferous tubules (T) and interstitial spaces (N) showing normal Leydig cells (H & E stains, × 400).

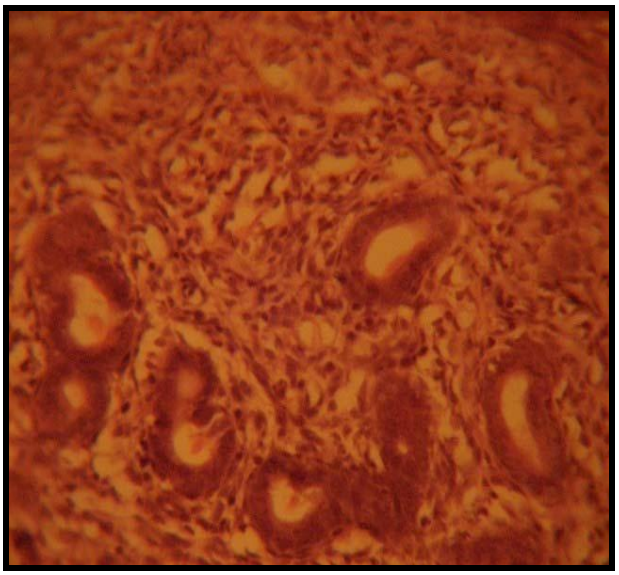

Figure No.2: Section of testis from rats (experimental group B) treated with monosodium glutamate (1mg/kg body weight) showing seminiferous tubule (T) with lots of spermatids, and oedematous interstitial space (N). (H & E stains, × 400).

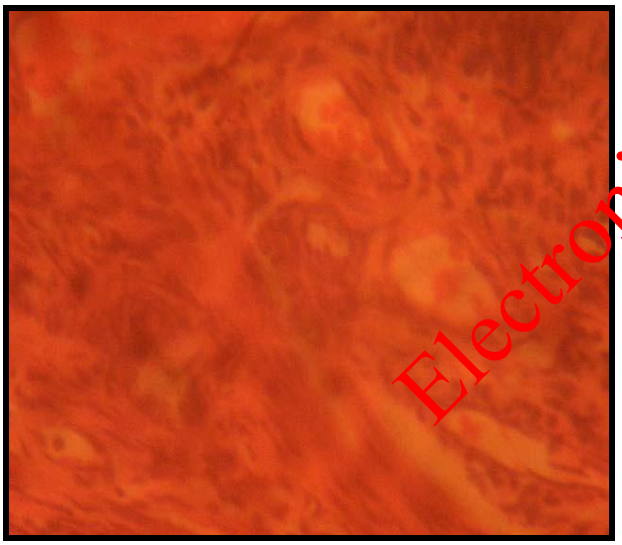

Figure No.3: Section of testis from rats (experimental group B) treated with monosodium glutamate (2mg/kg body weight) showing seminiferous tubule (T) with only few spermatids and interstitial space (N) with inflammatory exudates. H&E stains, ×400

DISCUSSION

The results of the hematoxylin and eosin staining (H & E) reactions showed some cellular hypertrophy of the columnar epithelium, distortion of the basement membrane separating the endosalpinx from the myosalpinx. Degenerative and atrophic changes were observed in some parts; these were more pronounced in those that received 3 mg/kg of MSG. There were marked vacuolations and lysed red blood cells (3 mg/kg treated rats) appearing in the stroma cells.

The increase in cellular hypertrophy of the columnar epithelium in the treatment groups as reported in this study may have been as a result of cellular proliferation caused by the improved intake of food which MSG influences.[12,13,14] The vacuolations observed in the stroma of the fallopian tubes in this experiment may be due to MSG interference. Degenerative and atrophic changes and lysed red blood cell which were observed were more pronounced in the groups treated with higher dose (3 mg/kg) of MSG.

As a result of the distortion and disruption in the lumen of the fallopian tubes, the ciliary action and other functions of the fallopian tubes may have been highly affected as a result of probable toxic effect of MSG. It may be inferred from the present results that higher dose and prolonged administration of MSG resulted in degenerative and atrophic changes observed in the fallopian tubes. The actual mechanism by which MSG induced cellular degeneration observed in this experiment needs further investigation.

Degenerative changes have been reported to result in cell death, which is of two types, namely apoptotic and necrotic cell death. These two types differ morphologically and biochemically.[15] Pathological or accidental cell death is regarded as necrotic and could result from extrinsic insults to the cell such as osmotic, thermal, toxic and traumatic effects.[16] In this experiment MSG could have acted as toxins to the epithelial cells of the fallopian tubes. The process of cellular necrosis involves disruption of membrane's structural and functional integrity which was also a landmark of this experiment.

Cell death in response to toxins occurs as a controlled event involving a genetic programme in which caspase enzymes are activated.[17].

CONCLUSION

The monosodium glutamate may have deleterious effects on the fallopian tube histology in adult female Wistar rats particularly in high dose. Therefore caution must be taken for its frequent use in female because of possibility of female infertility.

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Original Article

Malignant Lymphomas

Incidence of Malignant Lymphomas in Balochistan

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ABSTRACT

Background: Traditionally lymphomas are classified into Hodgkin's disease (HD) and Non Hodgkin's lymphoma (NHL) depending upon histo-pathological evidence on biopsy taken from an enlarged lymph node. Delayed diagnosis in lymphoma deteriorates the health eminence resulting in poor outcome.

Objectives: The aim of the study is to estimate the incidence and clinical presentation of malignant lymphomas in Balochistan.

Study Design: Prospective Study

Place and Duration of Study: This study was carried in the Department of Radiotherapy & Oncology, Bolan Medical Complex Hospital, Quetta from June, 2006 to May, 2012

Materials and Methods: A total of 263 newly diagnosed patients of both types of lymphoma from different parts of Balochistan were registered in Bolan Medical Complex Hospital in Department of Radiotherapy & Oncology. Followed by histopathology, WHO classification and Ann Arbor staging was done to assign subtype and extent of disease.

Results: It was found that the incidence of Non Hodgkin's lymphoma a (64.7%) was greater than Hodgkin's disease (35.2%) and both present bimodal distribution in age. Male patients dominate female patients in both cases (2.5:1). Lymph-adenopathy of cervical region was primary site in 44 % of cases while 27.6 % were extra nodal. Histopathology shows 57.4 % mixed cellularity variant in Hodgkin's lymphoma and 25.5% diffuse B cell pattern in Non Hodgkin's lymphoma. Ann Arbor staging reveals that 3.4% cases present with stage I and 64.5% show stage IV.

Conclusion: To conclude Non Hodgkin's lymphoma is two times more frequent than Hodgkin's lymphoma with greater male contribution. Due to delayed diagnosis resulted in late stage presentation ,health awareness is needed for physicians and general population for availability of patients at a due time for management.

Key Words: Hodgkin's lymphoma, Non Hodgkin's lymphoma, Lymph-adenopathy.

INTRODUCTION

Lymphomas are group of disorders caused by malignant lymphocytes that accumulates in lymph nodes and cause the characteristic feature of lymph-adenopaty. Occasionally, they may spill over in blood or infiltrate organs outside the lymphoid tissue [1]. Lymphatic organs play an important part in the immune system, having a considerable overlap with the lymphoid system. Lymphoid tissue is found in many organs, particularly the lymph nodes, and in the lymphoid follicles associated with the digestive system. Lymphoid tissues contain lymphocytes, but they also contain other types of cells for support [2]. The system also includes all the structures dedicated to the circulation and production of lymphocytes, which includes the spleen, thymus, bone marrow, and the lymphoid tissue associated with the digestive system[3]. The Primary lymphoid tissues generate two major types of cells, B lymphocytes and T lymphocytes. Lymphoma is a group of cancers that affect these lymphocytes. It is malignant transformation of either B cell or T cell or their subtypes, these abnormal lymphocytes may travel from one lymph node to another and some time to remote organs via lymphatic system. Care should be taken to assign the primary site of origin as it strappingly affects treatment modality. The primary site may be nodal when malignant cells originate from lymph nodes and is extra nodal when develop from the organs other than lymphatic system such as gastrointestinal tract, hypochondriam, Para-nasal sinuses, central nervous system . Lymphoma may invade the nearby organs involving extra nodal sites and vice versa [4].

Traditionally lymphomas are classified into Hodgkin's disease (HD) and Non Hodgkin's lymphoma (NHL) depending upon histo-pathological evidence on biopsy taken from an enlarged lymph node. Hodgkin's lymphoma develops from a specific abnormal B lymphocyte lineage and has characteristics Read Sternberg cells having large, abundant cytoplasm, double or multiple nuclei with its distinctive clinical features [5]. Since there are so many different types of lymphomas, its classification is very complicated [6]. Many of these subtypes look similar but they are functionally quite different and respond to different types of therapies with different probability of cure [7].

Diffuse large B cell Lymphoma is most common and is potentially curable while mantle cell lymphoma is unique subtypes B cell lymphoma and is potentially incurable [4,8,9]. Due to the varied clinical picture, many patients are misdiagnosed and treated for diseases like tuberculosis[10]. Sometimes, Benign disorders including ordinary infections, sebaceous cysts and other non-neoplastic conditions may be interpreted as malignant lymphoma and unnecessarily subjected to surgery and/or chemotherapy [11]. The first sign of lymphoma is painless enlarged lymph nodes accompanying fever for more than 3 days. Usually patient presents with low grade intermittent fever, unexplained weight loss and drenching night sweats. Confirmed diagnosis is established on the basis of histopathology findings followed by fine needle aspiration or biopsy of relevant site. Histopathology clearly marks the morphology of cells, their subtypes and resemblance to the normal cells. Clinically Non Hodgkin's lymphoma is dividing into low grade, intermediate and high grade. High grade lymphoma has cells and multiply rapidly hence aggressive in nature while low grade lymphoma cells look much similar to normal cells, multiply slowly and are indolent. Lymphomas are also categorized on the basis of tumor burden for appropriate treatment. The Ann arbor staging system is the most popular system for classifying lymphoma in different stages on the basis of number of tumor sites involved (nodal and extra nodal), location, and the presence or absence of B symptoms [5].

The aim of this study is to evaluate the incidence of malignant lymphomas and its extent at the time of presentation and to promote health awareness in population.

MATERIALS AND METHODS

In this prospective study, 263 newly diagnosed patients of both types of lymphoma from different parts of Balochistan, were registered in Bolan Medical Complex Hospital, Quetta, in Department of Radiotherapy & Oncology, from June, 2006 to May 2012. The study includes patients of all ages and sexes. Exclusion criteria from the study were the patients having chronic lymphocytic leukemia and patients with viral hepatitis. Informed consent was obtained from individual patients for collecting demographic and disease data on pre-designed questionnaire. Initial laboratory evaluation included complete blood count, erythrocyte sedimentation rate, serum electrolytes and urine analysis. Further investigation includes biochemical tests like renal function test, liver function test, total proteins and blood urea, serum creatinin, specific tests such as lactate dehydrogenate and B_2 micro globulin also performed. Final diagnosis was established by fine needle aspiration and excision biopsy of enlarged lymph node. Some cases were diagnosed by bone marrow biopsy. Histopathology confirms the specific subtype and grade of lymphoma. With the help of radiology imaging such as ultra sonogram, magnetic resonance imaging, computer tomography scan etc. staging and extent of disease assigned by using American Joint Commission on Cancer (AJCC) staging manual and Surveillance Epidemiology End Result (SEER) summary stage, respectively.

RESULTS

Two hundred and sixty nine diagnosed cases of lymphomas were included in this prospective study, out of which 169(71%) cases belonged to NHL with male to female ratio 1.5:1 while 94 (29%) cases were diagnosed having HD with male to female ratio 2:1. Demographics (Table.1) showed that in HL group 56 (60%) were males and 38 (40.4%) patients were females. The patients were divided in two groups, age<40 years consist of 62 (65.9%) patients and age ≥ 40 years consist of 32 (34%) patients. In NHL group, out of 169 (64.9%) patients ,32 (34%) patients were ≥ 40 years. In NHL group, out of 169(64.2) patients, the 123 (172.7) patients were male and 46 (27.2) patients were female. Graphical representation of frequency of age exhibited bimodal distribution, where the first peak appears between 20-29 years, both HD and NHL whereas, in HL the second larger peak stuck between 50-59 years. On general examination, lymph-adenopathy was the commonest finding and 100% patients presented with enlarged lymph node of any site. The most frequent primary sites were cervical lymph nodes (41%). It was also observed that incidence of malignant site was also very high (28%), so was the abnormal lymphocyte growth outside the lymphatic system (Fig.1). All extra nodal cases were Non Hodgkin lymphomas. Accompanied with lympha-denopathy, patient usually complained low grade fever, fatigue, weight loss and abdominal fullness. Physical and clinical findings clearly demonstrated the situation of patient at the time of presentation and it was obvious that most of the victims were suffering from B symptoms (Table.1). Fine needle aspiration cytology established the initial diagnosis and surgical resection of lymph node finally confirmed the subtype and cell surface marker by histopathology and immuno-histochemistry analysis. Among HD, rate of occurrence of mixed cellularity was highest with 77.5%, and only 18 cases of nodular sclerosis (22.5%) were indentified. In NHL, diffuse large B cell pattern was widespread with 45% cases whereas B cell Non Hodgkin's lymphoma exhibited by 23 patients (13%),seventeen patients each of follicular lymphoma and small lymphocytic lymphoma (SLL) ,7 % each were reported (Table.2). Other less common variant included anaplastic large cell lymphoma, lymphoblastic lymphoma and large cell lymphoma.

In some cases, histopathology mentioned WHO grade but most of the cases were without grading so cell nature became ambiguous and was difficult to assess whether it was aggressive or indolent. Further work up, including computerized tomography (CT) scan,

magnetic resonance imaging (MRI), bone marrow biopsy, was performed to formulate the staging and extent of disease. Eight (8.5%) cases of HL presented with stage I while 12 (12.7%) in stage II and 16 (17%) patients were in stage III and 58 (61.7%) patients were in stage IV, respectively. Stage IV had higher frequency as 61.7% cases were identified with stage IV. One the other, in NHL, 6 (3.5%) cases were stage I disease whereas 19(11.2%) and 45(26.6%) cases presented with stage II and III respectively. Stage IV was dominant in NHL with 109 (64.4%) cases. The comparison between different stages of HL and NHL showed that stage III was dominant in HL and stage IV in NHL. Stage I lied side by side in both types (Fig.2), according to SEER summary stage, stage I
and II represent local and regional disease respectively while stage III and IV symbolized for distant metastasis..

Table No. 1: Characteristics of 263 patients Presenting with(HL 94 cases and NHL 169 cases).

	HL		NHL	
Characteristics	No	(%)	No	(%)
Sex				
Male	56	60	123	72.7
Female	38	40.4	46	27.2
Age				
≤4 yr	62	65.9	61	36
≥4 yr	32	34	108	63.9
Ann Arbor Stage				
I	08	8.5	06	3.5
II	12	12.7	19	11.2
III	42	24.8	45	26.6
IV	32	34	109	64.4
Presentation				
Nodal	68	72.3	128	75.7
Extra nodal	26	27.6	41	24.2
Symptoms				
Fever	78	82.9	134	79.2
Weight loss	69	73.4	112	66.2
Lymphomatous involvement				
Spleen	72	76.5	104	61.5
Bone Marrow	31	32.9	42	24.8
Liver	48	51	71	42.0
GIT	26	27.6	36	21.3
CNS	02	2.1	03	1.7
Pleural effusion	08	8.5	11	6.5
Asities	10	10.6	16	9.4
LDH				
1. Normal	30	31.9	42	24.8
2. Abnormal	58	61.7	113	66.8
3. Not known	06	3.5	12	7.1
Extranodal involvement				
1 Site	36	21.3	45	26.6
2 or > 2 sites	51	30.1	109	64.4
Not known	7	4.1	13	7.6

Table No. 2: Different types of NHL cases

Type	WHO classification	Cases	%age
HL	Mixed callularity	54	57.4
	Nodulr sclerosis	24	25.5
	Nodular lymphocyte	10	10.6
	Predominanat Lymphocyte rich	06	6.3
NHL	Diffuse large B cell Lymphoma	85	37.8
	Small Lymphocytic Lymphoma	40	17.2
	Folicular Lymphoma	15	8.8
	Mantle cell Lymphoma	13	7.6
	Anaplastic large T-cell Lymphoma	10	5.9
	Lymhoblastic Lymphoma	8	4.7
	Peripheral T-Cell Lymphoma	5	2.9

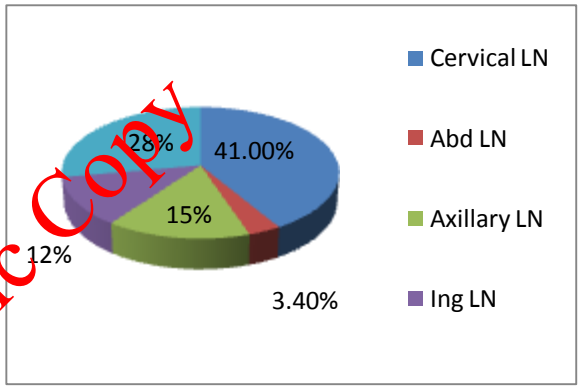

Figure No.1: Distribution of Malignant lymph nodes in NHL.

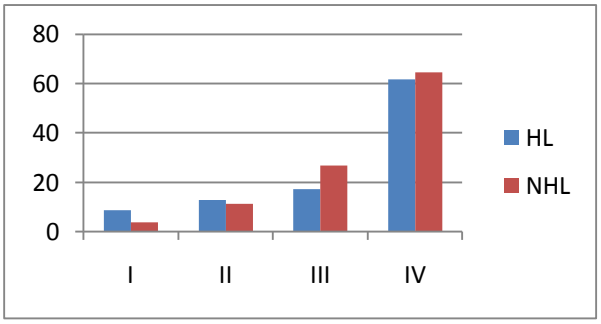

Figure No.2: Staging in HL and NHL.

DISCUSSION

Lymphoma is the most common form of hematological malignancy in the developed world. With addition of life style, malignant lymphoma is increasing in developing countries. Malignant lymphomas accounts for 5.3% and 55.6% of all blood cancers. According to the National Institute of Child Health, lymphomas account for about five percent of all cases of cancer in the United States, and Hodgkin's lymphoma in

particular accounts for less than one percent of all cases of cancer in the United States.

The patients present in the study belong to different geographic and ethnic groups and show evidence of different kinds of lymphoma. The present study shows the incidence of NHL greater then HL with approximate 2.5:1. This frequency is also affected by age as the study shows that people who develop HL are between 30-40 years of age. On the contrary, victims of NHL are young as well as aged people. Moreover, males are more susceptible then female. This pattern is validated by the previous study but no reason was yet established. Age distribution demonstrate bimodal pattern in NHL where first peak lies 30-40 years and second peak involve 41-70 years. HL shows the same pattern with one peak at 3-40 years and second at 41-49 years. These findings are consistent with previous studies [12,13]. The process of aging which contribute to health deterioration may explain this type of distribution. Lymphoma occurs when genes associated with programmed cell death (apoptosis) are irregular and the lymphocytes apoptosis response is interrupted. Consequently, the lymphocytes do not die but rather continue to proliferate and circulate causing disease and possible death. The clinical presentation of NHL and HL is also very typical. Lymph-adenopathy is a common sign of beginning of malignant disorders [14]. All HL patients present with single chain of cervical lymph node with no disseminate involvement of other lymph nodes but rather directly engross bone marrow. On the contrary, NHL cases present with cervical, intra abdominal and extra nodal sites as well. Most of the cases present with more than one nodal site diffuse extra nodal association and in 20% cases extra nodal sites are primary sites involving lymph nodes as secondary sites. Ascites, pleural effusion and focal defect in spleen and GIT involvement are also frequent. These all findings effect the staging and extent of disease. In our country the trend to go for regular check up is very low and usually self medication prevent people to go for proper to go for examination, so most of the patients are diagnosed when they develop metastasis in more than one secondary site. Hence 45% under study were diagnosed with stage III and IV in HL and NHL respectively where prognosis is very poor and rate of survival is low because in late age the immunity continues to decrease and body rapidly consequences to advance stage. As in advance stage, response to treatment is poor and chances of survival decline so it burdens health budget.

According to WHO, morphological diagnosis of NHL relies on cytological details, although the development of new technologies has helped to define several NHL tend to be sclerotic and diagnosis is possible only with excisional biopsy [15,16]. Fine needle aspiration cytology (FNAC) though minimally invasive, produces suboptimal material and reveals scanty neoplastic cells. Presence of lymphoid cell in FNAC are usually considered to be associated with the diagnosis of lymphoma However there are other types of lymphoid infiltrates that may be misleading e.g. granulomatous infiltrates like tuberculosis, lymphoid infiltrates in extra nodal site, and neoplasm containing lymphocytes [17].

Recent advances attempting at increasing the specificity of FNAC by combining it with immune flowcytometry (IFC) and immune-histochemistry (IHC), have proven unsuccessful for certain lymphomas and excisional biopsy is still generally recommended[18]. Histopathology, cytology and immuno-histochemistry analysis set the morphological variations in lymphoma. Our study reveals most common variant lymphoma Diffuse Large B Cell Lymphoma (DLBCL) (38%), since DLBCL is heterogeneous, CNS prophylaxis by adding ritoxumab in chemotherapy is promising [19,20], while mixed cellularity is dominant entity in HL rather than Nodular sclerosis. An important finding is that 12% cases of NHL and 10% cases of HL have no further specification of type and designated as not otherwise specified.

Stage III and IV are dominating in patients diagnosed with HL and NHL, respectively. This delayed diagnosis reveals unawareness of the importance of regular medical check up in general public . Patients presenting with late stage diagnosis too have poor prognosis that may have economic and social impacts by increasing the burden on health care budget and their families. Health awareness, both for physicians and general population, is required on priority basis.

CONCLUSION

To conclude Non Hodgkin's lymphoma is two times more frequent than Hodgkin's lymphoma with greater male contribution. Due to delayed diagnosis resulted in late stage presentation ,health awareness is needed for physicians and general population for availability of patients at a due time for management.

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Original Article

Glioblastoma

Adjuvant Therapy for Old Age Glioblastoma Patients

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ABSTRACT

Objective: Since the advent of Temozolomide (TMZ), optimum management for elderly patients with newly diagnosed Glioblastoma (GBM) is still elusive. The object of this study was to clarify outcomes of present management.

Study Design: Long term prospective study

Place and Duration of Study: This study was carried out on patients who were treated at the Aseer Central Hospital Abha KSA, Frontier Medical College Abbottabad, Women Medical College Abbottabad and those operated privately between August 2001 and August 2013.

Material and Methods: This is a long term study of 41 consecutive cases involving patients aged 55 years or more with newly diagnosed GBM. The patients' median age was 61 years (range 55-87 years). Twenty nine patients underwent resection and rest underwent biopsy. Patients with deep-seated lesions and multifocal lesions (12 patients= 29.26%) were preferably biopsied than gross total resection. Eighteen patients (43.90%) were treated with chemotherapy (mostly TMZ) with radiation therapy (RT) and Six (14.63%) with RT alone. Three patients (7.31%) received only palliative care after surgery.

Results: New neurological deficits developed in 5 patients (12.19%). Postoperative hemorrhage occurred in 8 patients (19.51%), all of whom underwent biopsy. Chemotherapy complications occurred in 19.51% (Advanced hematological complications in 14.63%).

The overall median values for progression-free survival and overall survival were 4.5 and 6 months respectively. Younger age, single lesion resection and adjuvant treatment were associated with better overall survival. Only adjuvant treatment was significantly associated with prolonged progression-free survival. With combined therapy containing resection, RT, and chemotherapy, the median progression-free survival and overall survival were 7.5 and 11 months, respectively.

Conclusions: The prognosis for GBM worsens with increasing age in elderly patients. When high risk factors are present, resection with adjuvant treatment are associated with prolonged survival but are with associated risks. Advanced age alone should not preclude optimal resection followed by adjuvant radio-chemotherapy.

Key Words: Glioblastoma, Old Patients, Adjuvant Therapy, Overall Survival, Progression Free Survival.

INTRODUCTION

Glioblastoma (GBM) is the second most common primary brain tumor after meningioma, accounting for 17.6% of all CNS tumors, and it is by far the most common malignant primary brain tumor.[1] The prognosis of GBM remains dismal with median survival of approximately 1 year despite advances in surgery, radiation and chemotherapy.[2] Current standard treatment consists of maximal safe resection followed by RT and concomitant and adjuvant chemotherapy with TMZ(3) (47), which results in a median overall survival of approximately 15 months or longer in appropriately selected patients.[4]

Advanced age has been reported as the most significant unfavorable prognostic factor for patients with GBM.[6] GBM peaks in incidence at 5-74 of age and affects more than 10 per 100,000 people older than 65. (23) In the Pakistani setting an age of 55 is considered to be old, relating to socio-economic standards and health awareness level. Older patients with GBM tend to be offered less aggressive treatment, such as biopsy or even palliative care, for fear of their possible intolerance of standard treatment.

However, the best treatment regimen for these patients is yet to be fully determined. Stupp et al[3] proved the efficacy of TMZ in patients with newly diagnosed GBM, but did not target individuals over the age of 70 years. Therefore, GBM therapy in this population is a difficult decision making resulting in the paucity of evidence. In an attempt to shed light on this situation, we analyzed the outcome in our series of elderly patients with newly diagnosed GBM treated at the Asir central Hospital Abha Saudi Arabia and in Pakistani settings.

MATERIALS AND METHODS

Patients aged 55 or older who underwent surgery in the Department of Neurosurgery Assir Central Hospital Abha, Saudi Arabia, Frontier Medical College Abbottabad, Women Medical College Abbottabad and those operated privately between 2001 and 2013 leading to a new diagnosis of GBM were recruited in this study. Patients with progression from previously diagnosed lower grade glioma were excluded. A total of

41 patients were studied. The patients median age was 61 years (range 56-76 years). Twenty nine patients underwent resection and 12 underwent biopsy. Patients with deep-seated lesions and multifocal lesions) were preferably biopsied than gross total resection. Eighteen patients (43.90%) were treated with chemotherapy (mostly TMZ) with radiation therapy (RT), and six (14.63%) with RT alone. Three patients (7.31%) received only palliative care after surgery.

RESULTS

Patients Data is presented in Table 1. Twenty nine patients (70.73%) presented with focal deficits including cognitive dysfunction (37%) and motor deficit (33%). Nineteen patients (46.34%) experienced seizures before surgery. Seven patients (17.07%) presented with headache and vomiting with or without decreased level of consciousness. Intra-tumoral bleeding was noted at time of presentation in 4 patients (9.75%). Associated medical conditions were common, including cancer in 6 patients (14.63%), coronary artery disease in 5 patients (12.19%), and hypertension in 14 patients(34.14%). The median maximum tumor diameter was 38 mm (range 23-76). Lesions were located most frequently in the Temporal lobe (31%). Multifocal lesions defined as multiple separate enhancing lesions with or without connection when visualized on fluid-attenuated inversion recovery sequences of MRI, were frequently seen (29%).

Surgical outcome: For most patients undergoing resection or open biopsy and for all patients undergoing frameless stereotactic biopsy, MRI or CT scan was obtained prior to surgery for stereotactic purposes. The images were transferred into Neuro-navigation system, a facility only available in Saudi Arabia, and were used during craniotomy as well as tumor resection or biopsy. For patients undergoing frame-based stereotactic biopsy, MRI or CT scan was obtained after frame placement under general anesthesia.

Table No.1:Location of the Glioblastoma in Our series

Location	No. of Patients
Frontal	12
Temporal	15
Parietal	8
Occipital	4
basal ganglia & thalamus	2
corpus callosum	2
Cerebellum	8
deep lesion	6
Bilateral Lesion	13
Multifocal Lesion	9
Eloquent Lesion	7
	23

Table No.2: Patients Data

Variable	Total (n=41)	Group	
		Resection (n=29)	Biopsy (n=12)
Age (yrs)			
Mean	64.1±5.3	64.3±5.5	65.8±5.1
Range	55-87	55-87	55-86
Sex			
Male	25	15	10
Female	16	09	07
Preop focal deficit	28	18	10
Preop Sz	33	12	07
Increased ICP Syndrome tumor size (mm)	16	07	09
Mean	44.9±17.0	46.6.1±11.5	41.1±16.4
Range	15-74	18-72	16-71

Table No.3: Patients in Treatment Groups

	Total (n-41)	Treatment Group	
		Resection (n-29)	Biopsy (n -12)
adjuvant treatment			
chemo & RT	21	18	03
RT alone	11	08	03
chemo alone	01	0	01
palliative	08	03	05

Table No.4: Complications during our Study

Type of Complication	Total (n=37)	Treatment Group	
		Resection (n = 25)	Biopsy (n = 12)
any complication	17	11	6
hematological complication	8	7	1
systemic complication†	8	6	2
dermatological complication	3	1	2
psychiatric	1	0	1

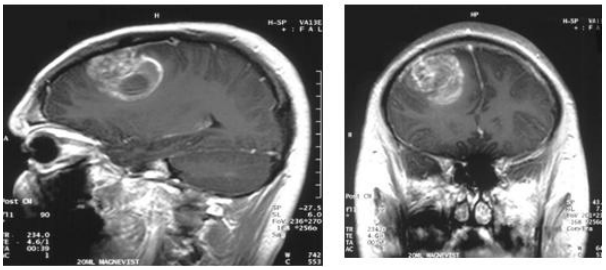

Figure No.1: Right Frontal Glioblastoma with mass effect

Target and trajectory for stereotactic biopsy were determined using the software program. The patients with pacemakers underwent CT scan instead of MRI.

Twenty nine (70.73%) patients underwent resection and 12 patients (29.27%) underwent biopsy. The resection group included 5 patients who had previously undergoing biopsy for diagnostic purposes prior to resection. There were no statistically significant differences between the biopsy and resection groups regarding age, sex, morbidities, or tumor size. Resection group presented more frequently with increased ICP and had a higher dexamethazone dose than the biopsy group.

Lesions were located more often in the temporal lobe in the resection group and less often in the parietal lobe, the basal ganglia, and the corpus callosum (Deep Lesion) in the biopsy group.

Surgical complications were divided into 3 groups as suggested by the Glioma Outcome Project . The overall complication rate was 24.8%. Neurological complications were seen in 17 patients (41.46%).

The neurological complication rate did not differ significantly between the resection group and the biopsy group. Regional complications affected 13 patients (31.70%) including hemorrhage in 6 patients (14.63%). All hemorrhagic events occurred in the biopsy group. Systemic complications occurred in 7 patients (17.07%), including thrombo-embolic events in 4 patients (9.7%).

The perioperative mortality (death within 30 days of surgery) rate was 9.7% (3 patients undergoing biopsy and one from resection group).

Outcomes for Adjuvant Treatment: Five patients (12.08%) received palliative care only without any active tumor treatment. All remaining received RT with a completion rate of 100%. The median radiation dose delivered was 51.0 Gy (range 10.0-68.0 Gy). More patients in the biopsy group received only palliative care postoperatively than the resection group of patients with hematological complications (myelosuppression), 2 required dose reduction and 3 needed to discontinue chemotherapy. One of those patients receiving TMZ required platelets. Systemic complications occurred in 6 patients Frontal ,Temporal ,corpus callosum Eloquent Lesion and included deep venous thrombosis (3 patients) and pulmonary embolism (1 patient), complications included rash, stomatitis, and depression. Among 64 patients who received RT, 8 patients (19.5%) were not able to complete the prescribed treatment. Five patients experienced clinical deterioration during RT.

Significant differences were noted between patients receiving palliative care and patients receiving adjuvant treatment Patients receiving RT alone were older than those receiving RT and chemotherapy, were more likely to have presented with increased ICP (39% vs 15%), and had a higher mean Dexamethazone dose.

Progression Free Survival (PFs) and overall survival

Median PFS, calculate from date of surgery, was 4.5 months. Factors significantly associated with poor PFS included deep lesion, multifocal lesions biopsy only, new persistent postoperative focal deficit, and palliative care (that is lack of adjuvant treatment) The median PFS for patients undergoing adjuvant treatment was 5.5 months, whereas that for patients who had palliative care only was 0.5 months. Patients receiving chemotherapy along with RT had achieved significantly longer PFS compared with those with RT alone.

The median Overall Survival differed strikingly among the age groups: 1. Months for patients younger than 70 years, but merely 4.5 months for patients aged over 70 years. Favorable survival effects of resection and adjuvant treatment in this study mainly result from relatively younger patients (< 65 years) among our elderly patient adjuvant treatment lived substantially longer if they had undergone resection compared with biopsy (median OS 11.5 months vs 6.5 months). With maximal safe resection followed by the combination of RT and chemotherapy, which is the standard treatment for unselected GBM patients, the median PFS and OS among our elderly patients with GBM were 8 months and 12.5 months, respectively. When limited to patients who were 70 years of age or older, they were 6 months and 10.0 months, respectively.

DISCUSSION

Gross total (safe) surgical resection followed by adjuvant treatment including RT and chemotherapy with TMZ has become the standard of care for patients with newly diagnosed GBM since the landmark study from Stupp and colleagues[3,21].

But patients older than 70 years were excluded from this study which is relatively common.[8,17] In clinical practice, physicians and surgeons are frequently reluctant to offer aggressive treatment to elderly patients because of concerns that it may not be tolerated due to advanced age, co-morbidities and an underlying propensity to complications. Therefore, although the age-adjusted incidence of GBM is greatest among patients older than 65 years, the best treatment strategy for these patients is not as well defined as in younger patients, the very reason this study was carried out.

Impact of Resection versus Biopsy on Survival in Elderly Patients have been performed evaluating the impact of the prospective studies9,25 have been more definitive, demonstrating an association Sanai et al,[10,14] and between resection and survival specially in younger patient impact of extent of resection (determined with or without computer-assisted volumetric assessment) on survival and concluded that the majority supported extent of resection as a prognostic factor for prolonged survival. Some authors ha reported increased extent of GBM resection and prolonged PFS in patients undergoing fluorescence-guided surgery using 5-aminolevulnic acid (5-ALA) in a prospective, randomized controlled trial"[12,20] Older patients are often believed to take longer to recover from an aggressive

surgery and carry a higher risk of peri-operative complications. Thus, biopsy is often resection for these patients.[13,1] A retrospective study from the independent predictor of survival and was associated with favorable OS among patients along with age and performance status.[14,13] However, this benefit was only evident for patients who went on to receive adjuvant treatment (median survival for resection vs biopsy: 11.5 vs 6.5 months).

Surgical Complications in Elderly Patients: High complication rates in elderly patients like new neurological deficits are associated with decreased survival, not just decreased quality of life."[15,16] Our overall complication rate of 24.8% for elderly patients compares favorably to other authors and true for patients undergoing resection in our series. The overall complication rate in these patients was 18.9%. By comparison, patients with newly diagnosed malignant glioma undergoing first craniotomy in the Glioma Outcome project had an overall complication rate of 24.2% including 8.1% permanent neurological worsening, 10% regional complications, 9.2% systemic complications, and 1.5% mortality.

Similarly, Sawaya et al reported their experience at MD Anderson Cancer Center with 400 craniotomies for intra-axial tumors (206 gliomas, 194 metastases). The overall complication rate was 32% (including 8.5% permanent neurological worsening, 7% regional complications, 7.8% systemic complications and 1.7% mortality) those undergoing resection (30.8% vs. 18.9%) although the difference did not reach statistical significance. complication rates for stereotactic biopsy in our series of elderly patients with GBM are higher than those reported in large unselected mortality rates range from 0.6% to 2.8%.[3,9,16,25]

Impact of Adjuvant Treatment on Survival in Elderly Patients: Radiation therapy has an established role in GBM therapy. RT has a significant survival benefit and appears to hold true for elderly patients.[13,14,15,17] Superiority of an abbreviated RT course (40 Gy in 15 fractions over 3 weeks) in elderly patients has been successfully proved by a prospective randomized clinical trial by Roa and colleagues.[18]

Like RT, chemotherapy has an established role in GBM treatment. For many years nitrosoureas such as BCNU or lomustine (CCNU) were the mainstay of chemotherapy for GBM patients and 2 meta-analyses consistently confirmed their efficacy for longer survival.[19,10] However, the benefits of nitrosoureas for old patients was questioned (similar to RT) especially given the higher incidence chemotherapy-related neurotoxcity and myelosuppression in this population. The changed the standard of care for patients with newly paper by Stupp et al changed the standard of care for the patients with newly diagnosed GBM by demonstrating increased survival for patients receiving RT with concurrent TMZ followed by adjuvant TMZ compared with RT alone (median survival 14.6 months vs 12.1 months), excluding 70 plus patients. Some recent papers have reported favorable survival for elderly patients with GBM receiving RT and TMZ, such as 10.6 months with the Stupp regimen for 2 patients aged 70 or older or 11 months with adjuvant RT and reduced dose of TMZ.[9,21]

Adjuvant Treatment Toxicity in Elderly Patients: Adjuvant treatment including RT and TMZ was feasible for elderly patients and was allocated with a modest complication rate.[4,5,6,9,11,12,24,25]

Most of the major complications did not seem to be directly associated with RT except for an infarct of the ipsilateral corona radiata 2 years after RT for a temporal tumor. In addition to resection and adjuvant treatment, other factors reported to favorably influence survival in unselected GBM patients include young age, good performance status, good mental status, seizure presentation, frontal lesion, superficial 3.1780.11) Younger single lesion, and the absence of postoperative complications age has consistently been one of the strongest favorable prognostic factors across many studies. It is interesting that our data suggest that this holds true even among the elderly population (age-65 years). Several hypotheses have been proposed to explain the poor clinical outcome of GBM in older patients, including the presence of comorbidities, resistance to cancer therapy, genetic aberrations, different histology, neurodegeneration and age discrimination.[2,4,19, 22,23,26]

CONCLUSION

This study demonstrates that aggressive treatment with resection and adjuvant treatment is associated with significantly longer survival for patients 65 years of age or older with newly diagnosed GBM. This finding must be interpreted with caution given that this study not randomized and patient biopsy and/or palliative care were significantly different (had tumors in deeper, more eloquent locations poorer performance status, increased dexamethasone dose) from those receiving resection and or adjuvant treatment. Nevertheless, resection and adjuvant treatment remained significant prognostic factors in a multivariate analysis and were generally well tolerated in this group of elderly patients. Although advanced age is an independent unfavorable factor, this alone should not disqualify patients from being treated with optimal tumor resection followed by.

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Original Article

Pharmacology

Comparative Study on Neem Leaf Extract and Nimolicin (NC) on Gastric Mucosa of Albino Rats

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ABSTRACT

Objective: Compare the anti ulcer effect of Methanolic Neem (Azadirachta indica, A Juss, Meliaceae) Leaf Extract (NLE) and Neem compound Nimolicin on gastric mucosa of albino rats.

Azadiradione also called Nimolicine coded as NC has been studied for its anti insect effect but anti ulcer effect has never been studied.

Study Design: Experimental study

Place and Duration of Study: This study was carried out at the Pharmacy and Physiology Department of Baqai Medical College for duration of two years.

Materials and Methods: Gastric ulcers in albino rats were induced in group-1 (check group) by a single oral dose of 1 ml 100% ethanol. After 24 hours the treatment was started. Group-2 was treated with oral administration of pea nut oil 1ml/day for 5 days (control of the treated group). Group-3 was treated with NLE (1ml/day for 5 days) and Group-4 with NC 1% (1ml/day for 5 days). The healing effects of neem were compared to oral administration of anti ulcer drugs ranitidine (50mg/kg daily for 5 days) and omeperazole (2.5 mg/kg daily for 10 days). Histopathology of the stomach was performed to confirm the presence or healing of ulcers. Ulcers were scored and indexed on the basis of histopathology.

Results: Ranitidine had the highest ulcer inhibition of 84%. NLE proved to be better than omeperazole by showing an ulcer inhibition of 82 % compared to 73% ulcer inhibition of omeperazole. NC showed least anti ulcer activity with an ulcer inhibition of only 69%. HPLC was performed to show the tissue concentration of NC, omeparazole and ranitidine showing their retention time, area and concentration compared to their controls.

Conclusion: It is concluded that NLE proved to be better anti ulcer agent as compared to NC and can be used as an anti ulcer drug after clinical trials.

Key Words: Gastric ulcer**,** neem (Azadirachta indica, A Juss, Meliaceae) leaf extract, azadiradione, nimolicin, ranitidine, omeperazole, ulcer inhibition.

INTRODUCTION

Neem (Azadirachta indica A. Juss) has been known for its medical values for the last 2000 years. Even today neem has been the centre of attraction especially for workers of medical field. All parts of neem are important particularly the leaves which is "storehouse of organic compounds"[1]. The leaves are easily available through out the year and preparation of extracts is easy. Thus they have been used extensively for medicinal applications[2].

More than 300 different compounds of neem have been isolated from different parts of tree about one third are tetranortriterpinoids (Liminoids).[3] Nimolicine coded as NC is a known neem compound. The neem fruit is divided into two parts. Fleshy outer part pericarp and mesocarp and inner part seed coat and kernel. The active ingredient of seed coat and kernel is Azadirachtin (AZ).[4] NC (Azadiradione) was isolated from fresh fruit coatings (after separation of seeds from fruits). Thus in the present study the anti ulcer effects of neem leaf extract were studied and compared with NC. The potent anti ulcer and anti secretory effect of neem leaf extract has been attributed to a glycoside.[5] Plant glycosides are known to inhibit chloride transport in gastric juice reducing gastric acidity.[6] The mechanism of action of NLE in healing of gastric ulcers is possibly because of its antioxidant effect which is independent of acid suppression.[7] This effect may be similar to the effects of omeperazole providing gastroprotection due to its antioxidant and anti apoptotic role.[5]

This study shows a comparison of anti ulcer effects of methanolic extract of neem leaves with neem compound NC showing the healing effects by histopathology.

MATERIALS AND METHODS

Wistar strain of rats were purchased from HEJ Institute of Chemistry, Karachi University and kept under optimum conditions in the animal house of Baqai Medical University. The animals were acclimatized and had free access to food and tap water ad libitum. Principals of laboratory animal care (NIH publication no.82-83, revised 1985) guidelines were followed. The animal experimentation approval was obtained from University Animal Ethical committee. NLE and NC were obtained from HEJ Institute of Chemistry, Karachi.

A total of 60 albino rats weighing 180Gm to 200Gm were divided into six groups. In each group 5 male and 5 female rats were included. Group-1 was the check group which was given 1 ml of 100% ethanol orally and sacrificed after 24 hrs to check for ulcers. Macroscopic and microscopic examination for the confirmation of ulcers was done. Once it was established that oral administration of ethanol caused gastric ulceration in rats, the next part of the study was undertaken. All the other groups were first given oral ethanol for ulcer production and then treated orally after 24 hours accordingly and then sacrificed. Group no 2 (control of the treated group) was treated with 1 ml of peanut oil daily for 5-7 days. Group-3 (test group) was treated with 1ml NLE daily for 5 days. The rats of fourth group (test group) were treated with 1% NC daily for 5 days. The fifth group (comparison group) of rats was treated with ranitidine (purity 50%) 50mg/Kg daily for 5 days. The rats of the 6th group were treated with Omeperazole (purity 83.33%) 2.5 mg/kg daily for 10 days. After completion of the respective treatment rats of Group-2 to Group-6 were sacrificed and stomach was incised. The gastric ulcer was examined in all the rats by a hand lens (power 10). The gastric tissues were then processed for histopathological examination by staining procedure of[8]. Gastric ulcers were scored on the basis of histopathology and indexed as Ulcers = 3, Heavy infiltration of polymorpho nuclear (PMN) cells = 2, Mild infiltration of (PMN) cells = 1, no ulcers = 0. The ulcer index and ulcer inhibition was determined.[9,10] The experiments were carried out in the Pharmacology Department of the Baqai Institute of Pharmaceutical Sciences and were repeated three times. The total duration of the study was three years.

High Performance Liquid Chromatography (HPLC): HPLC was performed to demonstrate the concentration of drugs in tissues. This was then compared with the standard solution prepared. One Gm of gastric tissue was homogenized in a homogenizer (OSK 9258) at 500RPM and centrifuged at 3500RPM (Labofuge-200 Heraeus). Soxhalation of the supernatant fluid was done. Sorption was done by passing the solution through Alluminium Oxide and Silica[11]. Pure methanol was used as a solvent for the mobile phase with a flow rate of 1 ml/min. A UV detector was used at a wavelength of 214 nm, pressure 200 kg/cm^2 and absorbance 0.08 with chart speed 2.5mm/min. Standard samples were prepared and run for comparison. The samples were filtered by Swiney syringe a micro filter pore diameter of 0.42 nm (Millipore Corporation Bedford MA01730). 20µL of the sample were injected by a special 25µL chromatographic syringe via the injector pore. HPLC apparatus Shimadzu (Japan) SPD-10A UV spectrophotometer with Merck reverse phase column (RP-C_{18}, 25cm X 0.46mm) was used. The chromatographic data were processed with Class LC-10 software (Shimadzu, Japan) and CBM (communication bus model) to the monitor. Peaks were recorded by software programme Real Time Analysis. The peaks were compared on the basis of retention time (RT) with the standard peaks. The area of each peak was noted to quantify the different compound residues in the samples.

RESULTS

In normal healthy rats ulcers were induced by giving 100% ethanol in the check group. This group had the highest ulcer score as shown in table-1 seen on naked eye and later confirmed on microscopic examination (Fig.2). There was no ulcer inhibition because no treatment was given to this group. The remaining 5 groups were treated with pea nut oil, neem, omeperazole or ranitidine after the induction of ulcers. The comparison of anti ulcer effects of neem with anti ulcer drugs on gastric tissue of rats have been shown in table-1 and figures 1-7.

Table No.1: Comparison of ulcer inhibition of NLE, NC, Omeperazole and Ranitidine with the control.

	Ethanol (Check Group) n=17	PNO (Control Group) n=17	NC (Test Group) n=7	NLE (Test Group) n=9	OMP (Known Antiulcer Group) n=6	Ranitidine (Known Antiulcer Group) n=7
Ulcer Score	31	31	4	3	3	2
Ulcer Index	1.82	1.82	0.57	0.33	0.5	0.29
Ulcer Inhibition	Nil	Nil	69%	82%	73%	84%

Table No.2: Shows concentration of NC, omeperazole and ranitidine in gastric tissue compared to the control by HPLC (Schimadzu)

	NC	Ranitidine	Omeprazole
Gastric tissue Conc (µg/20 µl)	3.37	3.62	7.11
Control Conc. (µg/20 µl)	7.08	14.64	9.22

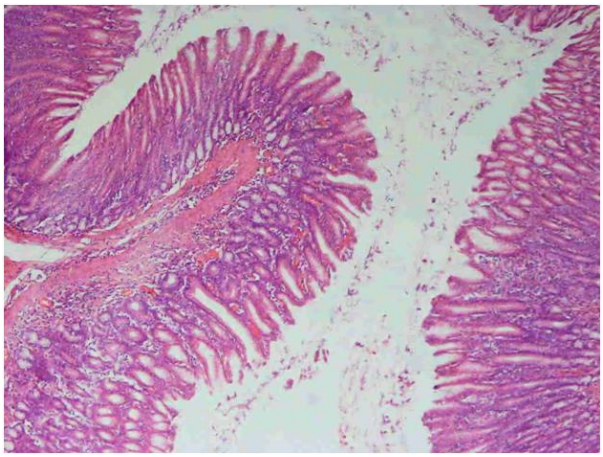

Figure No. 1: H & E stained, 5 micron thick paraffin section of stomach in an adult female rat untreated showing normal gastric mucosa. (Photomicrograph H&Ex10)

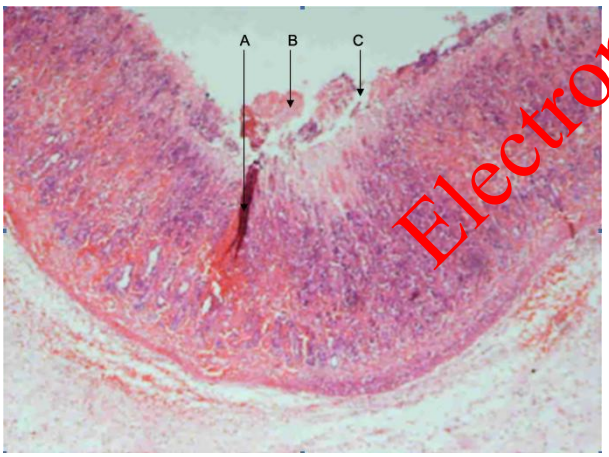

Figure No.**2:** H & E stained, 5 micron thick paraffin section of stomach in an adult female rat given oral Ethanol 100% showing massive erosions (A). The section reveals ulceration of mucous membrane with infiltration of the base with neutrophils (B & C) and dilatation and congestion of blood vessels. (Photomicrograph H&E x 10)

The normal gastric tissue Fig.1 has been compared with ulcerated gastric epithelium in Fig.2. Treated gastric epithelium has been shown in Fig.3-7. Pea nut oil used as control of treatment group has not caused a healing effect and the persistence of ulcers has been demonstrated in Fig.3. The healing effect of neem has been shown in Fig.4 & Fig.7 and this gastric tissue can be compared to the normal gastric mucosa fig.1 and healing effect of ranitidine and omeperazole Fig 6 & Fig 7. Table-1 shows that ranitidine had the highest ulcer inhibition of 84%. The ulcer inhibition of NLE was better than omeperazole by showing an ulcer inhibition of 82% compared to 73% of omeperazole. NC showed least anti ulcer activity with an ulcer inhibition of only 69% (Table-1). The ulcer score of the check group and control is the same. There was no ulcer inhibition with pea nut oil which served as the control of treated group. Tissue concentration of NC, ranitidine and omeperazole on HPLC has been shown in table -2 compared to their controls.

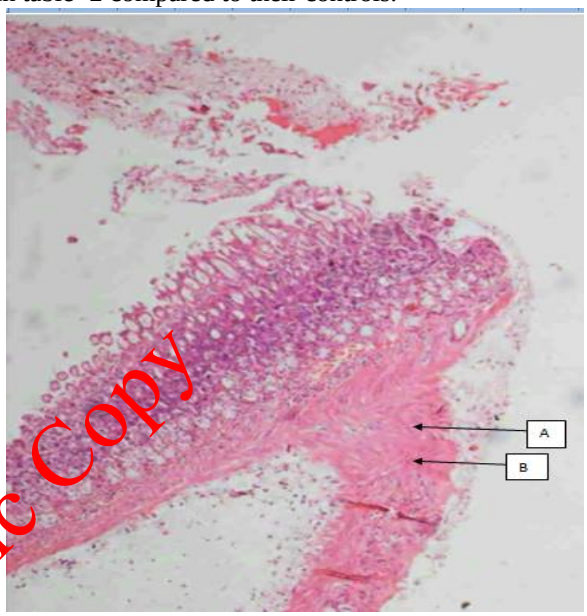

Figure No.3: H & E stained 5 micron thick paraffin section of stomach in an adult female rat treated with Pea nut oil for 5 days. The section shows the presence of chronic inflammatory cells showing a non healing effect of PNO. Ulcers are seen. A & B. (Photomicrograph H&E x 10)

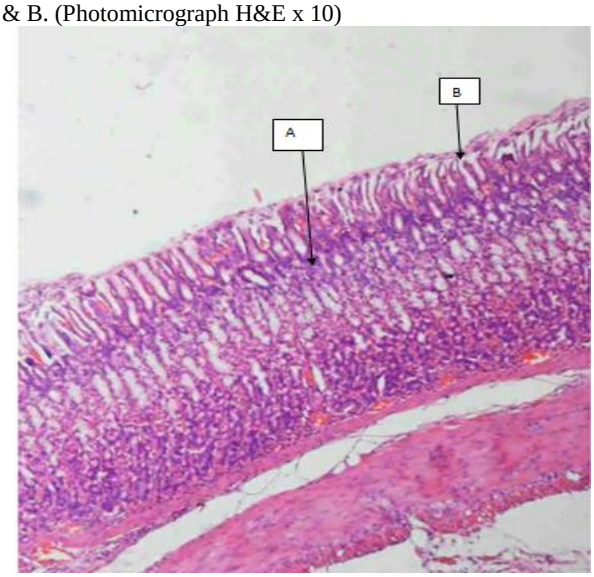

Figure No.5: Photomicrograph of H & E stained, 5 micron thick paraffin section of stomach in an adult female rat treated with neem compound nimolicin (NC). The section reveals

mild infiltration of mucosa by PMN cells, lymphocytes and occasional plasma cells. No ulcers visible. A & B. (H & E X 10).

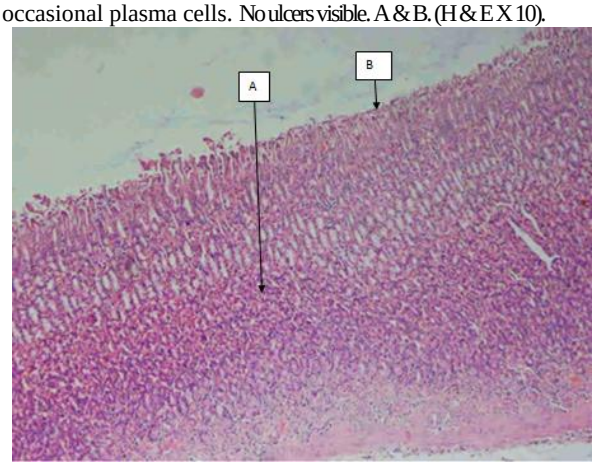

Figure No.**5:** Photomicrograph of H & E stained, 5 micron thick paraffin section of stomach in an adult female rat treated with neem compound nimolicin (NC). The section reveals mild infiltration of mucosa by PMN cells, lymphocytes and occasional plasma cells. No ulcers visible. A & B. (H & E X 10).

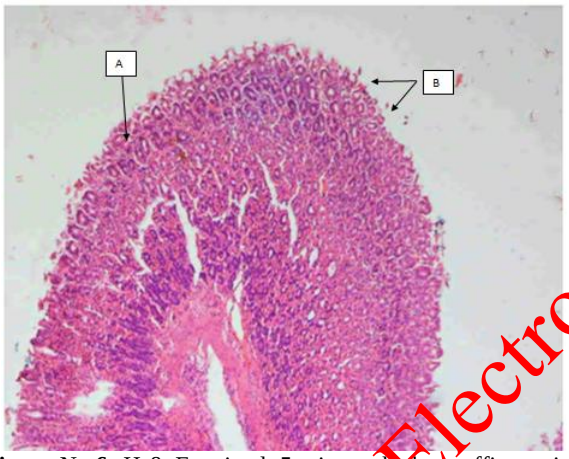

Figure No.**6:** H & E stained, 5 micron thick paraffin section of stomach in an adult female rat treated with ranitidine The section shows mild focal infiltration by chronic inflammatory cells (A). No ulcers seen (B). (Photomicrograph H & E x 10).

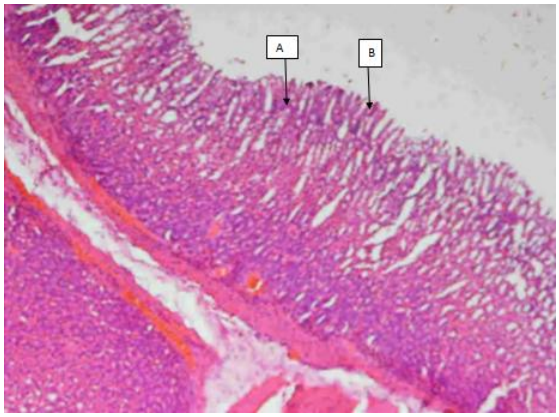

Figure No.**7:** H & E stained, 5 micron thick paraffin section of stomach from an adult female rat treated with omeperazole. The section shows mild focal infiltration by chronic inflammatory cells. No ulcers seen A & B. (Photomicrograph H & E x 10).

DISCUSSION

In the present study ulcer was induced by oral administration of ethanol which is in contrast with the findings of[12] who used mercaptomethylimidazole compound for the production of ulcer which is less potent in ulcer induction than ethanol. The anti ulcer effect of neem has been demonstrated in the present study by the induction of ulcer first, followed by treatment and confirmation by histopathology which is in contrast with the findings of[13] who studied the ulcer protective effect by pretreatment followed by ulcer induction without any histological evidence.

The anti ulcer effects of methanolic extract of NLE in the present study has been reported which has been shown to decrease ulcer index, which is similar with the findings of[14] who used aqueous extract of NLE and bark extract of neem respectively.

Hence the present study demonstrates that NLE is more effective as an anti ulcer agent compared to NC possibly because of its cumulative effect. Resistance is difficult to develop with extracts compared to isolated compounds. Clinical trials may further help in supporting our results.

CONCLUSION

It is concluded that NLE proved to be better anti ulcer agent as compared to NC and can be used as an anti ulcer drug after clinical trials.

Acknowledgements: We gratefully acknowledge the contribution of Professor Dr Beena Siddiqui of HEJ institute of Chemistry, Karachi University for providing us neem leaf extract and neem compound NC. We are also highly indebted to Madam Zehra Yaqeen, Dr Nudrat Fatima and Dr Lubna of the PCSIR laboratories Karachi for their cooperation and support during the project. We are very thankful to Dr Mirza MF Subhan, Assistant Professor, Department of Physiology, University of Kingdom of Bahrain for his expert guidance in editing this article. A special word of thanks to the department of Baqai Institute of Information Technology specially Syed Rizwan Nisar for their cooperation in computer skills in formatting and processing for publication of this article. We also are greatly indebted to Professor Dr Arshad Azmi of Karachi University for his support during HPLC. Partial financial support from HEC project no. 20-1127/R&D/2008 is gratefully acknowledged.

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