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EDITORIAL

An Updated Overview on Poly Cystic Ovarian Syndrome

Saadia Sultana

BACKGROUND

Poly Cystic Ovarian Syndrome (PCOS) – Stein Leventhal syndrome is one of the most common metabolic/endocrine system disorders in women of reproductive age. Females having PCOS present with a constellation of presentations associated with the androgen excess along with menstrual dysfunction that significantly influences their quality of life. They are at augmented risk of various morbidities, including insulin resistance (leading to type-2 diabetes), obesity, cardiovascular disease (CVD), infertility, cancer, and psychological disorders.

Stein and Leventhal described it, in 1935, as a disorder in which aapproximately 10 cysts of diameter from 2 and 9 mm appear in one or both ovaries with/without increase in ovarian volume exceeding 10 ml[1]. As stated, it was considered as a disorder of women of reproductive age; however, recent evidences suggest it to be a lifelong syndrome, manifesting since prenatal age. Actually, rendering to the Rotterdam criteria, the prevalence of PCOS in youth varies between a minimum of 3% and a maximum of 26%.[2] However, its prevalence in children is still unknown.[3] PCOS costs significantly huge economic burden. Around five billion dollars are annually spent in America for screening and treatment of the disease and its complications e.g. infertility, hirsutism, diabetes mellitus etc. Women with PCOS are two times more likely to be admitted inpatient as compared to normal females.[4] Hence, early and correct diagnosis is essential not only for the prevention of future comorbidities but also to diminish financial burden on the patient and society.

PATHOPHYSIOLOGY

Numerous hypotheses developed to explain the pathophysiology of the disease. Insulin resistance might be contributing to hyperandrogenemia resulting in PCOS.[5] The best theory about the pathophysiology of PCOS explains it as a multidimensional condition involving deviant insulin signalling, wild ovarian steroidogenesis, unwarranted oxidative stress secondary to mitochondrial dysfunction, and environmental/ genetic factors. Oxidative stress can induce insulin resistance and can cause hyperandrogenism. Role of genetics in the aetiology of PCOS is supported by familial aggregation of the disease and identification of genes on PCOS-suspect loci.[6] Furthermore, a polymorphic fibrillin-3 gene associated with PCOS, has been identified in some families carrying the disease.[7] Intrinsic imperfection in theca cells can somewhat elucidate hyperandrogenemia in PCOS patients. This dysregulation disturbs granulosa cells which yield about three to four times higher levels of anti-Mullerian hormone in women with PCOS in contrast to healthy controls.

CLINICAL PRESENTATION AND DIAGNOSIS:

In adults, for the diagnosis of PCOS one can follow one of the three different guidelines as described in the following Figure.

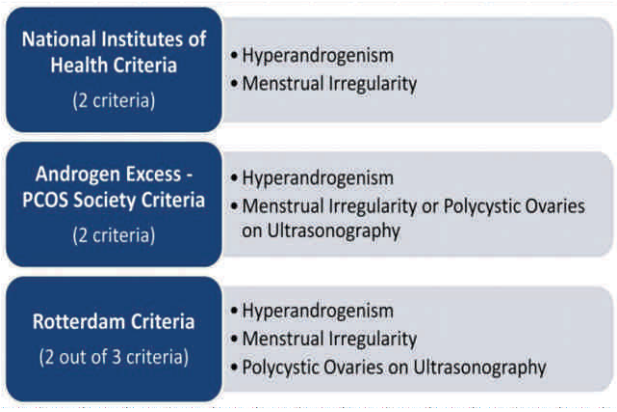

Fig 1: Guidelines for the diagnosis of PCOS.[8]

Although conditions such as obesity and insulin resistance are considered central to PCOS, none is included in the guidelines.[9] Each of the guidelines needs ruling out of any pathological situation that might describe the menstrual irregularity or hyperandrogenism. Additionally, diagnosis in

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adolescent girls is highly arguable.[10] The clinical presentation of PCOS varies with age, young women usually complaining of psychological and reproductive problems whereas older ones complaining of various metabolic symptoms. A detailed history, physical and systemic examination, and investigations should be conducted for appropriate diagnosis. Discontinuation of any hormonal medicines around a month prior to lab testing, along with planning of labs closer to the luteal phase of cycle are recommended for more precise results. Additionally, testing should include measurement of body mass index (BMI), lipid profile, and blood glucose levels. Screening for thyroid status by assessment of thyroid-stimulating hormone levels is important as these are a common cause of menstrual irregularity.

These are some key features:

- Hyperandrogenism present clinically with acne, hirsutism, alopecia, acanthosis nigricans etc. and on investigating high levels of testosterone and androstenedione are found.
- Menstrual irregularity presents as amenorrhea or oligomenorrhoea. Investigation shows high levels of luteinizing hormone.
- Polycystic ovaries on ultrasound shows twelve or more small follicles in one or both ovaries and follicle size is between 2-9 mm with an ovarian volume of 10 ml or more.

ACCOMPANYING INDISPOSITION

Insulin resistance:

Nowadays, insulin resistance is thought to be the core pathogenic factor for amplified metabolic disturbances in patients with PCOS. It explains menstrual irregularity, hyperandrogenism, and other metabolic manifestations of the disease. Hyperinsulinemia is present in 85% of women with PCOS. Increased insulin levels, along with raised luteinizing hormone, trigger the arrest of follicular growth resulting in anovulation. Hyperinsulinemia also disturbs the pulsatility of gonadotropin-releasing hormone (GnRH), decreases the sex hormone-binding globulin (SHBG) and stimulates ovarian androgen production. Lifestyle modifications and insulin-sensitizing drugs improve hyperandrogenism in women with PCOS. When insulin-sensitizing hormone, leptin is used, it decreases androgen levels and helps in regularizing menstruation in affected women. Six months of lifestyle modifications significantly reduced anovulation in affected obese women.[12] This is one of the critical aspect in the treatment of women with PCOS, which results in the contemplation of insulin-sensitizing agents as critical part of the management of the disease. These agents include metformin, thiazolidinedione and myo-inositol supplements.

Type-2 diabetes mellitus:

PCOS deliberates a significantly increased risk for type-2 diabetes mellitus and gestational diabetes. Around 1 in 5 women with PCOS will develop type-2 diabetes making glucose intolerance a common abnormality in this disease.[13]

Obesity:

An established risk factor for PCOS is Childhood obesity. Obesity is one of the most important features of PCOS. Its prevalence in patients with PCOS varies from 60 to 75%. Alternatively, women with PCOS are at a higher risk of developing obesity. Many studies reveal that females with PCOS have increased subcutaneous and visceral body fat distribution because of increased androgen production.[14] PCOS patients have an atherogenic lipid profile. Whether PCOS leads to obesity or whether obesity leads to PCOS is still debatable.[15]

Cardiovascular dysfunction:

Many studies established a burden of complex indicators of atherosclerosis (arterial stiffness, coronary artery calcification and endothelial dysfunction) with relatively early onset cardiovascular dysfunction (CVD) in patients with PCOS. In 2010, the PCOS society provided a consensus statement about increased risk of CVD in women with PCOS and developed a guideline for prevention of such complication. Uncertainty still remains concerning the increased cardiovascular morbidity and mortality in these patients.[16]

Infertility And Complications in Pregnancy:

PCOS is associated with reduced fertility because of associated gynaecologic and endocrine abnormalities that influence ovarian function and quality. A study in 2015 concluded that infertility is ten times more common in women with PCOS in contrast to healthy controls. Some studies suggested that women with PCOS who conceive after treatment usually

suffer from complications of pregnancy e.g. pregnancy induced hypertension, preeclampsia and gestational diabetes to a greater extent as compared to matched controls. Others suggest greater risk of miscarriage in women with PCOS. Women with PCOS are at a 2.5 times higher risk of giving birth to growth restricted babies in comparison to controls.[17]

Cancer:

PCOS women have a three times increased risk of developing endometrial cancer that is usually well differentiated and with good prognosis. This is explained due to the presence of many risk factors in these females which are associated with the development of endometrial cancer such as, insulin resistance, type II diabetes mellitus, obesity, and anovulation. Anovulation causes unopposed estrogen exposure to the uterine endometrium. It can subsequently trigger the endometrial hyperplasia and ultimately leading to endometrial cancer.[18]

Psychological well being:

Associated symptoms of PCOS e.g. obesity, hirsutism, acne and irregular menstrual cycles are major culprits to the psychological stress experienced by the patient due to the her body image and challenging of her female identity. (Women with PCOS are more prone to anxiety, depression, recreational drug use, abnormal eating, and psychosexual dysfunctions in contrast to healthy controls.

MANAGEMENT

Treatment of symptomatology is usually the target for the management of PCOS. The patients usually present with infertility, anovulation, acne, or hirsutism being the most common complaints. Management usually requires the involvement of an multi-disciplinary team that can include gynaecologist, family practitioner, dermatologist, paediatrician, a psychiatrist and endocrinologist.

Lifestyle modifications:

Guidelines recommend calorie-restricted diet and exercise as a central part in the management of PCOS specially the women with obesity. Lifestyle modifications and cost-effective treatment both are essential to management of PCOS.[19]

Medical treatment:

If symptomatology do not resolve by simple lifestyle changes, medical treatment must be added for the management of PCOS patients.

Metformin (Glucophage)

Metformin, a biguanide antidiabetic drug, acts by hindering hepatic glucose production and increasing the peripheral insulin sensitivity. Metformin treatment of PCOS specially of obese women with impaired glucose tolerance proved helpful in improving insulin sensitivity, increasing glucose tolerance, and in decreasing elevated androgen levels. It can also be used in combination with clomiphene citrate to treat subfertility.

Pioglitazone:

The use of pioglitazone was also studied in PCOS patients and data revealed that it results in a decrease in insulin resistance by decreasing fasting serum insulin levels.[20] Oral contraceptive pills (OCP):

Most commonly used medications for the long-term treatment of women with PCOS are the OCPs. The Task Force and the Endocrine Society and the PCOS Consensus Group have also recommended OCPs as first-line treatment for hyperandrogenism and menstrual cycle irregularities in patients with PCOS.

OCP suppress the hypothalamo-pituitary-ovarian axis, which in turn decreases LH secretions, increases sex hormone binding globulins and consequently decreases free testosterone levels. This results in hyperandrogenism-related symptoms e.g. improving acne and hirsutism, revises menstrual cycle abnormalities, and provides effective contraception.[21] A minimum of 6 months of treatment is usually required for satisfactory results in hirsutism and acne. Clomiphene citrate is considered as first line treatment of infertility (anovulatory). Laparoscopic ovarian drilling, exogenous gonadotropins and in-vitro fertilization are second line of management once clomiphene citrate with or without metformin fails.[22] D-chiro-inositol (DCI) and myo-inositol (MYO):Novel treatments of PCOS and are attaining more recognition due to minimal side effects. These are D-chiro-inositol (DCI) and myo-inositol (MYO); two isomers of inositol - insulin-sensitizing molecule. When combined with folic acid, MYO decreased hyperstimulation syndrome in PCOS females undergoing treatment. Another study showed significant improvement in symptoms in terms of

better lipid profile, more menstrual cycle regularity, and less acne, after the use of combination of MYO-DCI.[23]

SCREENING

Appropriate management of the patient needs not only to treat symptoms but also to foresee and to prevent any morbidity that might develop later in life.

These women should be routinely screened for type-II DM. Studies have shown that screening with fasting blood glucose levels alone under-diagnose type-II DM in PCOS patients, missing up to 50% of diabetics. Guidelines recommend screening such women using an oral glucose tolerance test. This screening to be done every 3–5 years in women without risk factors for type II-DM and annually in women with risk factors. Patients with PCOS should be screened routinely for CVD risk factors. Guidelines recommend body weight and BMI measurements, assessment of smoking, monitoring of blood pressure, and a complete lipid profile. Screening of PCOS patients not only for anxiety and depression but also for eating disorders, negative body image, and psychosexual dysfunctions etc. is also recommended.

While concluding, it is important to emphasize that if the physicians want to provide the finest care for these patients, future research work has to find the missing blocks in our increasing information about this syndrome/disease.

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ORIGINAL ARTICLE

Frequency of Hyperandrogenism in Females with Polycystic Ovary Syndrome

Sumera Mehnaz,[1] Rahat Akhtar,[2] Nadia Taj[3]

ABSTRACT

Objective: To find the frequency of Hyperandrogenism and Hirsutism in females with Polycystic Ovary Syndrome.

Study Design: Descriptive Case Series.

Place and Duration of Study: Department of Gynaecology and Obstetrics Nishtar Hospital Multan from 21th October 2016 to 9th April 2017.

Materials and Methods: Eighty polycystic ovary syndrome patients with in reproductive age group (15 – 49 years)[18] were included in the study through non probability consecutive sampling technique. Hirsutism was examined by FG scoring system and the assessment of Hyperandrogenism was by measurement of serum testosterone levels. SPSS 23.2 was used for data analysis. Frequency and percentage was calculated for categorical variables like Hirsutism, Hyperandrogenism mean and standard deviation was calculated for quantitative variables like age, BMI, Height , weight, serum levels of FSH, LH, And testosterone. T test was applied to check the statistical significance. Effect modifiers and confounders like age, height, weight and BMI were controlled by stratification of data. Post stratification CHI square test was used to check effect modification. P value < 0.05 was taken as significant.

Results: Among total 80 patients, the mean age of the patients was 28.93±8.24 years. Only one (0.5%) patient in our study group had normal weight, 21 (27.4%) women were overweight and remaining 58 (72.1%) patients were obese. The mean FSH, LH and testosterone levels were 6.62±5.3 IU/L, 14.01±7.65 IU/L and 65.13±23.75 ng/dl respectively. Total of 34 (43.7%) patients had Hyperandrogenism. A total of 59.5% (n=48) patients had Hirsutism (FG score >8). Among 34 patients with Hyperandrogenism, 57.8% (n=20) had Hirsutism and out of 46 patients without Hyperandrogenism 60.7% (n=28) had Hirsutism; this difference was not statistically significant; p=0.685.

Conclusion: The observations of our study concluded that a significant numbers of patients with PCOS had Hyperandrogenism and there was no difference in the number of patients with Hirsutism between hyper androgenic and non-hyper androgenic PCOS patients.

Key Words: *Ferriman Gallweyscoring, Hirsutism, Hyperandrogenism, Polycystic Ovary Syndrome.*

Introduction

Polycystic ovary syndrome (PCOS) is considered to be the one of the commonest endocrine impairment with unknown etiology in women of different reproductive ages. The condition was also explained by Stein and Leventhal in 1935.[1] The new criteria for defining PCOS is referred as ROTTERDAM criteria, which was given immediately after joint consensus between ESHRE and ASRM (European Society for Human Reproduction and Embryology and American Society for Human Reproduction) in Rotterdam in 2003.[1] Two out of following three criteria must be fulfilled for PCOS definition; Anovulation or Oligo, Hyperandrogenism (clinical or biochemical elevation of testosterone), and Polycystic ovaries assessment especially by using ultrasound (ovarian volume >10ml).[2]

Clinical sign and symptoms of PCOS include irregular menstrual cycle, obesity, Hirsutism and sub fertility or infertility.[3] Out of these clinical manifestations, Hirsutism appearance is observed obviously with Hyperandrogenism and metabolic abnormalities of PCOS. The dominating percentage of Hirsutism in females with polycystic ovary syndrome is 73.9%. But

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another study shows frequency of Hyperandrogenism in PCOS is 38%.[4]

Abnormal growth of terminal hair especially androgen dependent pattern, is seen in females that are suffering from Hirsutism.[5] The involving sites are chest, face, lower abdomen, crural areas and areola.[1] In some of the severe cases lower back, shoulder, upper arm and upper abdomen are also involved in abnormal hair growth. It is very common disorder that has a bad affect on 5-15% females of reproductive life. In 1961 Ferriman-Gallwey (FG) scoring system was given to diagnose this Hirsutism ratio in female population.[6] In conditions like, congenital adrenal hyperplasia, androgen secreting tumors and Cushing syndrome, there is always a direct relationship and association present between serum level of testosterone and Hirsutism.[7] However no sure and definite association has yet been found between serum testosterone level and Hirsutism in PCOS.[8] In such type of cases alpha reductase activity, heritability, socioeconomic, dietary and environmental features are considered to be responsible for Hirsutism.[9]

The frequency ratio of PCOS patients with Hyperandrogenism and Hirsutism have not been studied locally. The aim of this study is to calculate frequency of Hyperandrogenism and association of Hirsutism with Hyperandrogenism in females especially with PCOS. Our study will provide local reference for future research and management guidelines for patients of PCOs and Hyperandrogenism with Hirsutism.

Materials and Methods

With the ethical approval from the ethical committee of the institution this descriptive case series was conducted. The study was conducted over six months from 21th October 2016 to 9th April 2017. Sample size was calculated with WHO sample size calculator using following data level of significance = 5%, Confidence level = 95%, Sample size n = 80 patients. Patients were included using Non probability consecutive sampling technique. Other causes of Hirsutism, Congenital adrenal hyperplasia, Cushing's syndrome, Hyperprolactinemia, Androgen secreting tumors were excluded from study.

A specially designed Performa that had a complete demography of the patient to record all finding points of this study. Eighty cases of polycystic ovary syndrome who had met the inclusion criteria of our study were selected, who were also going for proper examination to the Department of Obstetrics & Gynecology, Nishtar Hospital Multan.

Informed consent was taken from all the patients one by one. The complete history and thorough clinical examination was conducted for assessment of different features of PCOS and to easily exclude other causes of Hirsutism. Biochemical work was done to record FSH, LH and serum testosterone level on the second day of regular menstrual cycle as well as on any day when the irregular menstrual cycle take place. Normal values of levels of all these hormones were also present on Performa report on which biochemical work was recorded. Assessment of Abdominopelvic ultrasound for polycystic ovary was done by ovarian volume >10ml and was used to rule out any tumor of ovary and adrenal gland. The patients who were fulfilling specific inclusion criteria of Hirsutism and PCOS were examined by FG scoring system. If total core was >8 then it was considered as diagnosis of Hirsutism.

Data was analyzed by SPSS version 23.2. Frequency and percentage was calculated for categorical variables like Hirsutism and hyperandrogenism, mean and standard deviation was calculated for quantitative variables like age, BMI, Height, weight, serum levels of FSH, LH, and testosterone, T test was applied to check the statistical significance. Effect modifiers and confounders like age, height, weight and BMI were controlled by stratification of data and post stratification CHI square test was use to check effect modification. P value < 0.05 was taken as significant.

Results

A total number of 80 female patients were included in this study. Only one (0.5%) patient in our study group had normal weight, 21 (27.4%) women were overweight and remaining 58 (72.1%) patients were obese. The mean age, BMI, height and weight of patients were 28.93±8.24 years, 32.44±4.25 BMI, 157.3 ±6.6 cm and 79.98±8.43 kg (Table I). The mean serum FSH, LH and testosterone level of patients were6.62±5.31 IU/L, 14.01±7.65 IU/L and65.13±23.75 ng/dl respectively (Table II).

The main outcome variables of this study were Hyperandrogenism and Hirsutism. Among all patients of PCOs 44% were found with

Hyperandrogenism and 56% had normal levels (Fig. 1). Total of 59.5% (n=48) patients had Hirsutism. Among 34 patients with Hyperandrogenism, 57.8% (n=20) had Hirsutism and out of 46 patients without Hyperandrogenism 60.7% (n= 28) had Hirsutism; this difference was not statistically significant; p=0.685(Table III). Hence there was no difference in the number of patients with Hirsutism between hyper androgenic and non-hyper androgenic PCOS patients.

When the patients were categorized into different age, BMI, height and weight categories, it was noted that majority of patients i.e. 56.25% (n=45) were falling in the age group 36-49 years and 43.75% (n=35) were aged from 15-35 years. 42.6 %(n=34) patients have BMI ranging from 36-40, 36.28% (n=29) patient have BMI from 25-35 and 21.26% (n=17) having BMI from 41-49. Similarly, 48.76 % (n=39) patients have height range from 140-150c, 31.27% (n= 25) patients have height range from 163-173cm and 2.05% (n=16) patients have height range from 151-162 cm. 68.78% (n=55) have weight ranging from 60-90 kg and 31.27% (n=25) have weight ranging from 91-120 kg (Table I).

Table I: Demographics Parameters of Patients

Characteristics (n=190)	Frequency	Percentage (%)
Gender		
Female	80	100.0
Total	80	100.0
Stratified Age		
15-35 years	35	43.75
36-49 years	45	56.25
Total	80	100.0
Stratified BMI		
25-35	29	36.28
36-40	34	42.6
41-49	17	21.26
Stratified Height		
140-150 cm	39	48.76
151-162 cm	16	2.05
163-173 cm	25	31.27
Stratified Weight		
60-90kg	55	68.78
91-120kg	25	31.27
Descriptive Statistics		
Age in years	28.93	8.24
BMI	32.44	4.25
Height in cm	157.3	6.6
Weight in kg	79.98	8.43

Table II: Distribution of Serum Level of Different Hormones in study Patients

Serum Hormones	Mean ±S.D	P-value
FSH	6.62±5.3IU/L	0.000
LH	14.01±7.65IU/L	0.000
Testosterone	65.13±23.75IU/L	0.000

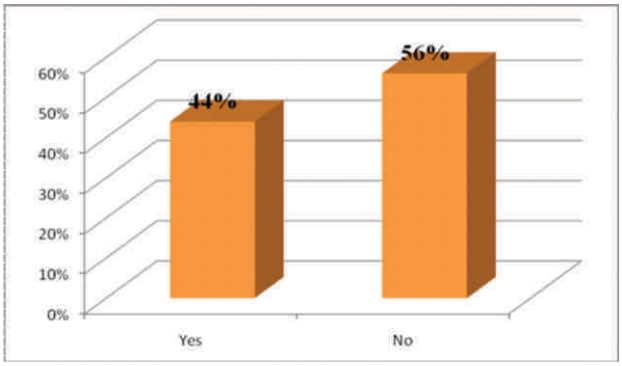

Fig 1: Hyperandrogenism *in Study Group*

Table III: Comparison of Hirsutism and Hyperandrogenism

Characteristics	Hirsutism		Total	P-value
	Yes	No		
Hyperandrogenism	57.8% (n=20)	42.1% (n=14)	43.6% (n=34)	0.685
Non Hyperandrogenism	60.7% (n=28)	39.2% (n=18)	56.3 (n=46)	
Total	60% (n=48)	40% (n=32)	100% (n=80)	

Discussion

Total and free circulating dehydroepiandrosterone sulfate (DHEAS) and testosterone levels are mostly above from normal in 50–75% women who are suffering from PCOS, the free testosterone (FT) circulating in blood is considered as the one of the very important predictive marker of PCOS in 60% PCOS patients[10] who were with increased level.In this study, we have proposed to know the, free testosterone (FT), DHEAS, and prevalence of elevated testosterone in free and combined form as well, in the large cohort of patients suffering from PCOS. By using of this information, we will be able to examine and calculate the utility of nonspecific standards in the evaluation of hyperandrogenemia especially present in PCOS and sensitivity of elevated serum hormone. From this evidence we can conclude that the Hyperandrogenism is remained as very important part of PCOS and Rotterdam criteria

includes this in major symptoms of PCOS.[11] In one of the very common endocrine disorder, nearly 7% women in their reproductive age are affected by the excess amount of androgen.

By the exclusion of specific endocrine disorder of excess androgen .i.e. non-classic adrenal hyperplasia (NCAH). Androgen-secreting neoplasm and hyper androgenic insulin- resistant acanthuses Nigerians syndrome, PCOS is mostly diagnosed in majority of females who were suffering from hyperandrogenism.[3]

By the exclusion, we have got a result that PCOS is one of those problems about which it is very difficult to enlist some standards. Both the biochemical as well as clinical manifestation of Hyperandrogenism had been observed in women suffering from PCOS.[12] One of the important clinical Evidences of Hyperandrogenism is male-pattern hair loss, Hirsutism and acne but in this study we have just explained only one manifestation that is Hirsutism of Hyperandrogenism. The elevated and accurate signs of clinical Hyperandrogenism and Hirsutism might be subjected to individual observer biases[3]. The diagnosis of PCOS could be more difficult within the ethnic variability.[13]

However, contrary to the past trends and methods to make some suitable standards for diagnosing the PCOS, in present time the hyperandrogenemia and elevated hormone levels are considered to be the standards to diagnosis the PCOS the hormonal evaluation of PCOS.[14] Most of the Past studies had normative standards based mostly on small sample size of control patients.[15]

The previous studies show Hirsutism scores and level of androgen, but these studies are unable to demonstrate the relation between severity of Hyperandrogenism and clinical Hirsutism. Coskun et al. reported that elevated hirsutism and level of androgen in the patients and control subjects from the Mediterranean region of Turkey, suggesting these people of this region mostly have much hair on body with high density. But their study is unable to find out the relationship between Hirsutism score and androgen level.

Demir et al conducted a study to find the severity of Hirsutism in the patients suffering from PCOS and its relationship with total testosterone (TT) serum levels in 87 patients suffering from polycystic ovary syndrome (PCOS) and 85 healthy were in control group belonging from the south-eastern region of Turkey. This shows that Hirsutism incidence was lower in hyper androgenic PCOS patients comparative to nonhyperandrogenic PCOS patients. Opposite to it another study designed in Europe indicates the direct correlation between Hirsutism and Hyperandrogenism in the females suffering from PCOS. However the present day study does not show any significant relationship between Hyperandrogenism and Hirsutism in females suffering from PCOS.

In our study the mean age of group was 28.93±8.24 years but in the study by Demir et al the mean age was younger i.e. 25.0 ±4.4 years. It indicates that in our study the late presentation of females suffering with PCOS may be due to difference in socioeconomic and environmental factors of both areas...72.1% women were obese with BMI > 30and 27.4 % women were overweight in our study, however in the study by Demir et al 19.5 % women were obese and 16.1% women were overweight. Similar to our study however opposite to Demir et al, in 2004 in united states another study was launched, in which 60% women suffering from PCOS were obese, at that time in common adult population about double the rate was observed.

According to the study by Demir et al the mean testosterone level was 69.5±23ng/dl but in our study the mean testosterone level was 65.13±23.75 ng/dl. A meta-analysis has been done on 3464 patients suffering from PCOS and 37% of patients have been found with elevation of serum TT level, comparing it with a previous study by Turkey in which 62% of patients had elevated serum TT level. In recent study 43.7% patients show Hyperandrogenism. It is different from Turkey study but similar to a meta-analysis. It might be possible that the socioeconomic factors as well as the ethnic heterogeneity of people suffering from Hirsutism have different levels of serum total testosterone. The very important key to find PCOS is Hyperandrogenism and it also help to determine the severity of PCOS as well as.

Among women suffering from PCOS the incidence of Hirsutism is different in different woman and it shows to depend upon two factors that is the degree of distribution of hairs on body and each woman's sensitivity to the pattern of body hair.[17] The

evaluation of Hirsutism scores and androgen level has been done by many previous studies; however a relationship between the severity of Hyperandrogenism and clinical Hirsutism has not been explained clearly. DeUgarte et al. concluded that most of the time the diagnosis of Hirsutism depends on the perception as well as mentality of patient instead of total mFG score of patients. Different studies provide different incidence of Hirsutism in patients suffering from PCOS.Some population groups have been evaluated in which rates ranging from nearly 17% and 100%. An incidence of Hirsutism of 87% has been observed by the Turkish study using the FG scoring system, forcing the authors to report that large amount of body hair is the hallmark of women living in the Mediterranean region. The present study indicates that FG scores of ≥ 8 were present in the 60% of 190 PCOS Patients. This is lesser than Coskun et al., who studied a same ethnic population with comparing social and geographical characters and might be correlated to the ethnic. Hirsutism was present almost in 15% of control group in the study from Demir et al, which is very contrasting and different to past reported rates of 4.6 – 10% in the general population. The increased rate of Hirsutism in the common population of the Demir study is according to past studies explaining much higher body density of body hair in the Mediterranean region and Asia as well.[18]

All the local studies based on local population are deficient of suitable knowledge and data. Therefore due to lack of proper statistical data regarding this in all local studies, we are unable to find the exact magnitude of PCOS present in our country. There are some barriers in our study like a single hospital is not enough for the representation of whole population and limited subjects are available. We need large multicentre case control trials to estimate Hyperandrogenism and its relationship with Hirsutism in females suffering from PCOS. To understand this important thing of association between severity of Hirsutism and serum level of testosterone must be studied.

Conclusion

The observations of our study conclude that a significant numbers of patients with PCOS had Hyperandrogenism and there was no difference in the number of patients with Hirsutism between hyper androgenic and non-hyper androgenic PCOS patients.

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ORIGINAL ARTICLE

Rehabilitation Professional Attitude Towards Persons with Disability

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ABSTRACT

Objective: The objective of this study was to determine the attitude of physical therapy and speech language pathology students toward persons with disability.

Study Design: Descriptive analytical

Place and Duration of Study: From 1st July to 31st December 2014 at Riphah College of Rehabilitation Sciences, Riphah International University Islamabad.

Materials and Methods: A sample of 246 (81 post graduate, 145 under graduate and 20 Speech language pathologists) was recruited through purposive sampling. Both gender and willing students were included in study. The standard tool for attitude toward disabled person (ATDP), A & B performa was circulated to all participations. Brief demographic information was included and finally statistical analysis was drawn through SPSS-20.

Results: The mean age of under graduate, post graduate physiotherapy students and speech language pathologists was 22.3±3.12, 25.7±2.81 and 27.6±27 years respectively. The statistical analysis showed that there was a significant difference in personal and professional attitude towards disability of under graduate and post graduate physiotherapy students ($p<0.001$). The comparison showed that there was no significant difference in personal and professional attitude towards disability of post graduate physiotherapy students and speech language pathologists ($p=0.481$, $p=0.275$) respectively.

Conclusion: The study concluded that the attitude of speech language pathologists is more positive than physiotherapy students and graduates. The attitude of under graduate physiotherapy students is more positive as compared to the post graduates. There is dire need to modify and upgrade the curriculum about the significance of disability in under grad.

Key Words: *Disabled Persons, Physiotherapy Students, Personal Attitude, Professional Attitude.*

Introduction

The disability is a condition which limits personal, physical and mental ability or situation of being disabled.[1] It can be considered as the incapacity, defect and disorder.[2] The disability is basically an interaction of person's characteristics with the environment's characteristics. In earlier decades it was considered that the disability is a consequence of biological process and the individual is responsible for disability.[3] Later they related it with some social and psychological aspects and the biopsychosocial model was introduced. Now the disability is to be considered as the human right issue by different organizations. Disability is now understood to be a human rights issue. The people are not disable by their bodies but by the society and Disability is a contextual variable with dynamic over time and circumstance.[4] The ICF model the most modern and well defined have discussed not only the single aspect of disability rather explore the multiple perspective of disability like employment, disability eligibility and social aspects.[5] The disablement process explained that disability is not an individual trait but a lack of linkage between personal disability and the environment.[6] So the main responsibility lies on the society and the environment of the persons

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with disability.[7]

The Attitudes are a complex collection of beliefs, feelings and values.[7] The attitude of society towards the disable was always negative and discriminative.[8] People thought that disability was because of their sins and the disable person is solely responsible for his/her disability.[8] This attitude created a distance and discriminative behavior among society which leads to increase the gap between disable and able persons.[8] The health care provider should be different regarding the attitude towards disability as compare to the non health care people. The health care providers have direct and long term relationship with people with disabilities. The literature showed the positive attitude of health professionals as compare to the other professionals.[8] The role of Physical Therapist in disability is also very challenging and the aim is to restore, maintain, and promote not only optimal physical function but optimal wellness and fitness.[9] The role of physical therapist is to improve quality of life of person with disabilities and play an effective role in multidisciplinary team.

In literature different studies conducted on the assessment of attitudes of health professionals towards disability. There is difference of perception of attitude in different cultural system and multi-model societies.[10] Parents and teachers have a vital role to play in shaping and modeling attitudes.[11] There are comparative studies in literature which shows the difference in attitude of different health professionals including the physiotherapy, occupational therapy, nurses, medical staff and other related persons.[12] It was supposed that the attitude of rehabilitation professionals must have positive findings as they have close relationship during the rehabilitation of person with disability.[8] The professional graduates have more positive attitude than students because they have more interaction with disability.[13] There are some confounding in the survey of rehab nurses, occupational therapy and physiotherapy and the study concluded that the occupational therapy has more positive findings than other rehab professional. The confounding includes the age, clinical setting, cultural system and the experience which have greater effect on attitude towards disability.[14]

In Pakistan, there is limited literature to explore the different perspective of disabilities. This study will contribute in enhancing the positive attitude and also creating the awareness among health professionals. The study was aimed to evaluate the attitudes of different rehabilitation professionals towards disabilities and to determine the difference of professional and personal attitude among them.

Materials and Methods

A descriptive cross sectional study design was used and a sample of (n=246) rehabilitation professionals was recruited through purposive sampling from Riphah International University, Islamabad. The study was conducted from1st July to 31st December 2014. The sample included 145 under graduate doctors of physical therapy DPT final year students, 81 post graduate students and 20 Speech and language pathologists. Both gender and willing students were included while students with disability were excluded from the sample. The demographic details were recorded and written informed consent was taken from all participants. The standard tool "Attitude towards Disabled Person" (ATDP) designed and developed by Yurker (1960) was used to evaluate the perception of attitude. This tool has been widely used for the measurement of attitude of people towards disability.[15]

There are two types of questionnaire A & B, the part of ATDP, includes the personal and professional performa. The score of scale ranges from 0 - 120 and the interpretation shows that the high score reflects positive attitude towards disability. The process of distribution of both the questionnaire was to divide the class subjects into two groups. One group was asked to fill the personal performa keeping in mind the personal attitude towards disability. Other group was asked to fill the professional with perception of professional attitude towards disability and then vice versa. After completing the process the participants were asked to fill the other performa. This process assured the random distribution and avoidance of influence of perception of students. The data was analyzed through SPSS-20. The descriptive analysis including the frequency, percentage and mean were calculated while "Independent t test" was used to compare the means between groups.

Results

The mean age of under grad, post grad

physiotherapy students and speech language pathologists were 22.3±3.12, 25.7±2.81 and 27.6±27 years respectively. The total sample has distribution of 58.94% undergrad, 32.93% post grad and 8.13% Speech and language pathologists.(Fig 1) The statistical analysis showed that there was a significant difference in personal and professional attitude towards disability of under grad and post grad physiotherapy students ($p<0.001$, $p<0.000$) respectively. (Table I) The und-grad students have positive attitude towards disability as compared to post grad. The comparison showed that there was no significant difference in personal and professional attitude towards disability of post grad physiotherapy students and speech language pathologists respectively ($p=0.481$, $p=0.275$). (Table II)

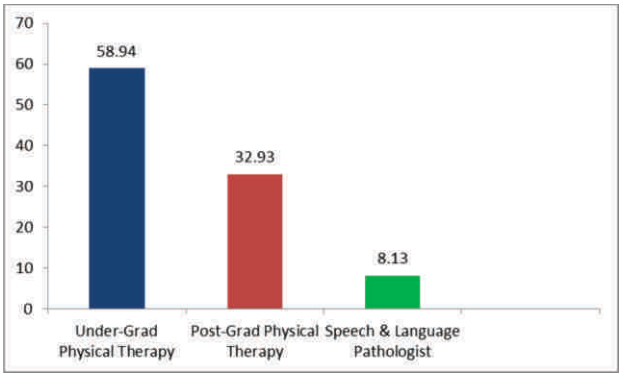

Fig 1: Shows the Sample of Different Categories

Table I: Personal and Professional Attitude of Undergrad and Post Grad PT Students

ATDP	Under grad (Mean ±Sd)	Post grad (Mean ±Sd)	p-value
Personal attitude	34.16±10.83	28.46±13.73	0.001
Professional attitude	34.16±10.77	26.90±12.56	0.000

Table II: Personal and Professional Attitude of Post Grad PT Students and Speech Language Pathologist

ATDP	Post grad (Mean ±Sd)	SLP (Mean ±Sd)	*p*-value
Personal attitude	28.46±13.73	30.90±13.94	0.481
Professional attitude	26.90±12.56	30.50 ±15.23	0.275

Discussion

The summary of results state that there is difference in attitude (both personal and professional) towards disability of under grad and post grad physiotherapy students and the under grad students have more positive attitude than post grad students.

The results also depict that there is no difference in post grad and speech language pathologists but speech language pathologists have more positive attitude towards disability. The literature shows that the occupational therapists have high and more positive score as compared to the physiotherapists The score of occupational therapy students are also above the average while physiotherapists have low score in attitude towards disability.[13] There is a difference in the attitude of final year and first year medical students towards disability, although it was not compared in this study. The personal attitude was greater than professional attitude in the final year students.[14]

The personal attitude reflects the cultural and other related factors' contribution and it is frequently more positive than professional attitude. It is difficult to modify and change the personal attitude while the professional attitude can be enhancing with the direct contact with person with disability. The focus should be to improve the professional attitude through training and other clinical oriented workshop related disability.[15,16] A study on students of occupational and physiotherapy showed that the score of physiotherapy is less than occupational therapy.[17] The medical education has significant impact on the development of attitude towards disability which leads to positive attitudes among health professionals. In one study the final year and house officers have similar attitudes towards disability.[18]

The gender has also an influence on the attitude and especially the personal attitude can be changed towards gender. But the professional attitude has not be inclined because of gender issue in attitude towards disability.[19] The curriculum of the health professional has significant outcome and plays a key role in the development of attitude and enhancing the positivity in attitude in health care professionals.[20,21]

Conclusion

The study concludes that the attitudes of speech language pathologist is more positive than physiotherapy students and graduates. The attitude of under grad physiotherapy students is more

positive as compared to the post graduates. There is a need to enhance the level of education, modify the existing curriculum and arrange workshops to improve the attitude of students towards disability. Future research should be conducted to explore the perception of students and clinicians about the significance of disability and person with disability.

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ORIGINAL ARTICLE

Does Diclofenac Sodium Provide Better Preemptive Analgesia than Ketoprofen in Impacted Third Molar Surgery? A Randomized Controlled Trial

Asma Parvez, Kamran Khan, Jawaria Bibi

ABSTRACT

Objective: To compare ketoprofen and diclofenac sodium as a preemptive analgesic in impacted third molar surgery in terms of mean pain score and mean time of first analgesic postoperatively.

Study Design: Double blinded, parallel arm Randomized Controlled Trial (RCT).

Place and Duration of Study: The study was conducted from 15th April, 2016 to 5th November, 2016 at Oral and Maxillofacial Surgery Department of Islamic International Dental Hospital, Riphah International University.

Materials and Methods: Eighty patients requiring surgical extraction of impacted mandibular third molar were selected by using randomized sampling technique. These patients were randomly assigned two groups using lottery method, resulting in sample size of 40 in each group. Preoperatively diclofenac sodium was administered intramuscularly in group A and ketoprofen in group B using double blinded technique. Pain score was measured 3 hours after surgery using visual analogue scale (VAS) and the time of first analgesic consumption post-operatively was recorded in hours. Statistical Package for Social sciences (SPSS version 16) was used for data analysis and Independent sample T test was applied to compare mean pain score and time of first rescue analgesics between two groups with 0.05 as a level of significance.

Results: Results showed that mean pain score was less in Ketoprofen group (3.42±1.08) than diclofenac sodium group (4.02±1.20) with significant p-value of 0.02. Time interval for first analgesic post-operatively was also measured in hours. Results were highly significant revealing prolonged analgesic effect in Ketoprofen group with p-value of 0.007.

Conclusion: Preemptive Ketoprofen provides better and prolonged pain control as compared to Diclofenac Sodium in impacted third molar surgery.

Key Words: *Diclofenac Sodium, Ketoprofen, Preemptive Analgesia, Pain Score.*

Introduction

Successful pain management is one of the main factors that have allowed surgery to progress to the current status.[1] It relies solely on the surgeon to attempt every possible approach to eliminate postoperative pain and discomfort in patients.[1] Surgical removal of impacted mandibular third molar is a common procedure carried out in routine dental practice which requires reflection of extensive mucoperiosteal flap and bone guttering which leads to the severe post-operative discomfort and pain.[2,3,4] Numerous studies have reported that postoperative pain reaches to its peak at 6-8 hours after surgery and require strong analgesics.[2,3,4,5] Trauma to periosteum and bone during surgery results in the initiation of nociceptive stimulus by generation of prostaglandins and leukotrienes leading to peripheral sensitization and acute pain.[6,7] Certain active mediators results in transmission of nerve impulses to brain leading to central sensitization and hyper excitability of neurons in central nervous system.[6,7]

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to relieve pain of mild to moderate intensity and have analgesic, anti-inflammatory and anti-pyretic effects.[2,4] NSAIDs are non-selective cyclooxygenase (COX) enzyme inhibitors.[2,4,7] Ketoprofen and diclofenac sodium both belong to NSAIDs and are commonly prescribed for pain control after third molar surgery.[2] Biological half-life

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of ketoprofen is 2-2.5 hours and is readily distributed into the central nervous system and crosses the blood brain barrier within 15 minutes, due to its high level of liposolubility.[2,7]Diclofenac has a biological half-life of 1.2-2 hour.[7]

Preemptive analgesia is administration of analgesics preoperatively before the initiation of nociceptive stimulus and provides better pain relief.[8,9,10,11] It blocks the nociceptive stimulus thus preventing or reducing the peripheral and central sensitization.[9,11]Idea of preemptive analgesia gained attention in 1980's.[7] Preemptive analgesia emphasizes on both the prevention and treatment of postoperative pain.[10,11]

Several studies have compared and documented the effectiveness of two different drugs as a preemptive analgesic in the literature. Velásquez et al compared ketoprofen and diclofenac sodium as a preemptive analgesic in third molar surgery, stating that ketoprofen is better than diclofenac sodium in terms of prolonging the time interval needed for the first analgesic postoperatively.[2] They also reported that ketoprofen is better than diclofenac sodium in terms of postoperative pain control[2]. Mario et al demonstrated that preoperative oral ketorolac plus submucosal tramadol results in reduced consumption of analgesics after third molar surgery than oral ketorolac alone with p-value of 0.04.[5]

There is little or no published research comparing diclofenac sodium and ketoprofen as a preemptive analgesic in third molar surgery in Pakistan. The evidence to suggest which of the two NSAIDS work better for preemptive analgesia is inconclusive. Further research in this area is warranted. This study aimsto fill this research gap bycomparing the analgesic effect of ketoprofen and diclofenac sodium in providing preemptive analgesia in third molar surgery in a tertiary care dental setup in Islamabad, Pakistan. This study will not only provide an evidence-based practice to minimize the pain intensity after surgical removal of third molar and to reduce the need of taking a large number of analgesics after surgery, but will also serve as a published literature in comparing ketoprofen and diclofenac sodium, so as to serve as a baseline for further research in Pakistan. This study is also expected to improve the patient's perception towards dental treatment which is mostly considered as a painful experience by patients by providing an evidence-based standard for treating pain and discomfort.

Materials and Methods

This was a quantitative research employing double blinded randomized control trial methodology to compare ketoprofen and diclofenac sodium as preemptive analgesics in impacted mandibular third molar surgery. This study was conducted from 15th April, 2016 to 5th November, 2016 at Oral and Maxillofacial Surgery Department of Islamic International Dental Hospital, Riphah International University. We used simple randomized sampling technique and total sample size was 80 patients with impacted mandibular third molar. All patients were between age group 18-35 years. These patients were randomly assigned to group A and B using lottery method. Double blinded technique was used while administering the drugs where neither operator nor patient knew about the medicine being received pre-operatively and all procedures were carried out by the same operator to control bias.

Ethical approval of the study was obtained from the ethical committee of IIDH (Ref.No. IIDC/IRC/2015/03/003). This was a minimal to no risk study as special care was taken to exclude all the patients who have contraindications for NSAIDs. A written informed consent was taken from all the patients before intervention. All the participants of the study were fully explained about the procedure, aim of research and all possible side effects. No participant was forced or pressurized to participate. The participants were ensured that the data will not be used for any other study without their consent. The criteria of inclusion were any patient reporting in the study setting with mesioangular class II class B mandibular impaction during the study period. The criteria of exclusion were any patient with pericoronitis, pain on the day of surgery, medical conditions in which NSAIDs are contraindicated and other oral surgical indications.

Intramuscular (IM) injection of ketoprofen 100mg was given to one group and diclofenac sodium 75 mg to other group 30 minutes prior to the surgical procedure. Then inferior alveolar nerve was blocked with 2% lignocaine and 1:80,000 epinephrine before the procedure was performed. Paracetamol 500 mg was prescribed as postoperative analgesic. Patients were advised to record the pain scores 3 hours

postoperatively with the help of visual analogue scale, ranging from 0-10(0 denotes absence of pain and 10 denotes severe pain) and the time of taking first analgesic after surgery.

We used mean pain score as a tool to measure pain that has long been used to measure pain all over the world. Thus, the tool we used to measure pain is valid optimizing the validity of our research. This study can be reproduced in any part of the world and in any community using the same methods ensuring that this is a reliable research design. The study setting is a teaching institute of Islamabad, which is the capital of country. The population of Islamabad belongs to different parts of the country and is of different socioeconomic status. So we can somewhat generalize our results to whole country.

The data was analyzed using Statistical Package for Social sciences (SPSS version 16). The independent variables are gender and age. The dependent variables are mean pain score and the time of first rescue analgesic in hours. Frequencies were measured for qualitative variable such as gender. Mean and Standard Deviation were calculated for quantitative variables such as age, pain score and time of first analgesic (hours) postoperatively. Independent sample t test was applied to compare mean pain score and time of first rescue analgesics between two groups and p-value of $\leq$0.05 was considered as significant.

Results

This double blinded randomized controlled trial aimed at comparing ketoprofen and diclofenac sodium administered through IM route 30 minutes preoperatively in patients for surgical removal of impacted third molars. Patients in group A were administered with IM Diclofenac Sodium 75mg, and in Group B with IM Ketoprofen 100mg. Group A contained 19(47.5%) male patients and 21(52.5%) female patients while group B contained 25(62.5%) male patients and 15(37.5%) female patients. Gender distribution in both groups is shown in table I.

Table I: Gender Distribution in Group A and B

Group	Male	Female	Total
Group A	19(47.5%)	21(52.5%)	40
Group B	25(62.5%)	15(37.5%)	40
Total	44(55%)	36(45%)	80

Mean age in group A was 28.98$\pm$4.00 SD and that for group B was 29.00$\pm$4.42 SD with an insignificant statistical difference (p=0.97) among both groups both groups as shown in table II.

Table II: Mean Age for Group A and B

Age	Group	N	Mean	Std. Deviation	P-value
	Group A	40	28.98	4.00	
	Group B	40	29.00	4.42	0.97

The results for independent sample T test revealed a significant difference between the mean pain scores at 3 hours among two groups that is; mean pain score at 3 hours was 4.02$\pm$1.20 in diclofenac sodium group(A) and 3.42$\pm$1.08 in ketoprofen group(B) with p-value 0.02 at 95% Confidence Interval as shown in table III.

Table III: Mean Pain Score at 3 Hours Post-operatively

Description	Group	N	Mean	Std. Deviation	P-value
Pain score at 3 hours post-operatively	Group A	40	4.02	1.20	
	Group B	40	3.42	1.08	0.02

Result clearly showed that ketoprofen provided better pain control than diclofenac sodium as a preemptive analgesic.

Results of independent sample t test for the comparison of time interval in two groups at which first analgesic was taken post operatively revealed that mean time interval for first rescue analgesic post-operatively was 2.90$\pm$1.24 hours in diclofenac group (A), and was 3.61$\pm$1.02 hours in ketoprofen group (B), with p-value of 0.007 at 95% confidence interval, so there was significant difference between both groups as shown in table IV.

Table IV: Mean time interval for 1st Rescue Analgesic (Hours)

	Group	N	Mean	Std. Deviation	P-value
Time interval for 1st rescue analgesic (hours)	Group A	40	2.90	1.24	
	Group B	40	3.61	1.02	0.007

Table V: Summary of Discussed Literature

Serial #	Authors (Reference #)	Drugs compared	Conclusion
1	Velásquez et al[2]	i)Ketoprofen ii)Diclofenac	Prolonged pain control with ketoprofen
2	Tai et al[13]	i)Ketoprofen ii)Diclofenac	Ketoprofen provided better pain control
3	Shah et al[14]	i)Ketoprofen ii)Diclofenac	Ketoprofen provided analgesic effects for longer duration
4	Niemi et al[15]	i)Ketoprofen ii)Diclofenac	Lower consumption of analgesics in diclofenac group
5	Kaczmarzyk et Al[6]	i)Ketoprofen preoperatively ii)Ketoprofen postoperatively	Postoperative administration of ketoprofen was more effective than pretreatment administration
6	Manani et al[16]	i)Ketoprofen ii)Naproxen	Ketoprofen provided better pain control
7	Lopez et al [17]	i)Diclofenac sodium ii)Methylprednisolone	Less inflammation with methylprednisolone
8	Nazar et al[18]	i)Diclofenac sodium ii)Tenoxicam	Diclofenac sodium provided better pain relief

It showed that ketoprofen provided pain control for longer duration than diclofenac sodium.

Discussion

The practice of treating pain after surgical trauma is now being replaced by preventive approaches that aim to block transmission of the painful nerve impulses before and during surgery[2]. Pre-operative administration of NSAIDs provides better and prolonged pain relief by blocking the release of prostaglandins and leukotrienes, thus blocking peripheral and central sensitization leading to reduced number of analgesics taken post-operatively and better patient compliance[2,6].

This study compares the preemptive effect of IM ketoprofen with IM diclofenac sodium and reports that ketoprofen provides better and prolonged pain control in comparison to diclofenac. The results for independent sample t test in our study clearly revealed a significant difference between the mean pain scores at 3 hours among two groups that is; mean pain score at 3 hours was 4.02±1.20 in diclofenac sodium group and 3.42±1.08 in ketoprofen group. This demonstrates that patients administered with ketoprofen pre-operatively reported significantly less pain at 3 hours post-operatively as compared to the patients administered with diclofenac. We also report the comparison of time interval in two groups at which first analgesic was taken post operatively. The results of our study revealed that mean time interval for first rescue analgesic post-operatively was significantly less in diclofenac group (2.90±1.24 hours) as compared to the ketoprofen group (3.61±1.02 hours) demonstrating that pre-operatively administered ketoprofen provided longer post-operative pain control than diclofenac sodium.

The results of our study are consistent with a similar double blinded randomized control trial carried out by Velásquez et al. They also compared analgesic effect of ketoprofen and diclofenac and report a significantly prolonged pain control with ketoprofen (p value 0 .006) supporting our results.[2] Velásquez et al, however suggest that the mean pain score at 3 hours post operatively was insignificant between two groups.[2]

The results of Tai et al also support result of our study who compared ketoprofen 200 mg and diclofenac 100 mg in multiple doses for 4 days after third molar surgery demonstrating that ketoprofen provides significantly better pain control with the p-value of 0.086.[13] Tai et al, however do not evaluate surgical difficulty level of impaction. Our study includes only mesioangular Class II and Class B impactions and excludes all the cases with surgical time above 60 mins. The results of our study are also consistent with the results of Shah et al, which state that ketoprofen induced a longer duration of analgesic effect.[14]

On the other hand, Niemi et al compared analgesic effect of intravenous (IV) diclofenac 1 mg/kg and ketoprofen 1.35 mg/kg 30 minutes before and 4 hours after surgery, and also prescribed 0.03mg/kg IV oxycodone as the rescue medication in first 24 hours. The results demonstrate a lower requirement of oxycodone in the diclofenac group in comparison with the ketoprofen with p-value of <0.01.[15] The difference of the results of this study from our results

can be explained on the basis of not considering the level of difficulty of the surgery.

In another study Kaczmarzyk T et al, compared analgesic effect of orally administered ketoprofen 100mg, 60 min preoperatively and 60 min post-operatively and reported that the mean pain score in pre-operative group was 45.7±10.53 and in post-operative group was 33.10±7.91 with p value of 0.031.[6] The study also compared mean time interval in minutes for first rescue analgesic in both groups and reported that the mean time interval for first rescue analgesic is less in pre-operative group (336.75±10.43 minutes) as compared to that in post-operative group (409.93±12.69 minutes) with p-value of 0.0013.[6] Their results showed better and prolonged pain control with post-operative ketoprofen.[6] Our study however does not evaluate the analgesic effect of post-operative administration of NSAIDs.

There is an extensive literature that compares diclofenac and ketoprofen with other medicines in terms of pain control. Manani et al, showed that ketoprofen is more effective in pain control compared with naproxen and have higher analgesic properties.[16]

In another study Lopez et al compared anti-inflammatory properties of diclofenac sodium with methylprednisolone in a third molar surgery and suggests that despite of less inflammation observed in methylprednisolone group, there is no significant difference in the reduction of trismus.[17] Nazar et al compared intramuscular (IM) diclofenac sodium with tenoxicam for the pain relief. It stated that IM diclofenac sodium produced significant pain relief compared to tenoxicam.[18] All studies discussed here are summarized in table V.

The extensive literature supports the results of our study and suggests that pre-operative administration of ketoprofen not only reduces the severity of post-operative pain but also prolongs the onset time of post-operative pain in comparison to pre-operatively administered diclofenac.

There are few limitations in the present study. Age and gender were not matched at the baseline. This may have biased the results since pain threshold may vary across gender and age variations. Another shortcoming is the selection of only mesioangular impaction with class B and type II. Therefore, the results of this study may not be used to ascertain effectiveness of both drugs for other types of mandibular 3rd molar impaction. This study includes sample from just one dental hospital in Islamabad, thus it can affect the generalizability of the results.

There is a need of further and extensive research to compare effects of different types of analgesics in Pakistan, so as to provide an evidence-based approach towards pain free experience for patients in this era of surgical advancement.

Conclusions

Based on this study we conclude that preemptive analgesia is a better option in minor oral surgical procedures. Preemptive analgesics works best in the cases of inflammation, reduces the post-operative morbidity and pain by blocking the release of prostaglandins and peripheral and central sensitization of pain. NSAIDs are more commonly used as preemptive analgesics and according to this study ketoprofen 100mg provides better analgesic effects than diclofenac sodium 75mg in terms of reducing pain intensity and pain control for longer duration.

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ORIGINAL ARTICLE

Dyslipidemia in Type 2 Diabetes Mellitus

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ABSTRACT

Objective: To determine the correlation of plasma glucose levels with lipid profile in type 2 Diabetes Mellitus (T2DM).

Study Design: Case control study.

Place & Duration of study: This study was conducted in Army Medical College Rawalpindi from 11th November 2014 to 11th November 2015.

Materials & Methods: The number of participants comprised in the study was 120. The study population was divided into two groups A & B. 60 individuals were placed in each group. Group A comprised of Type 2 diabetics and group B were healthy controls with no major illness. The patients were recruited from the Military Hospital's medical wards and Endocrinology outpatient department. The participants' blood samples were analyzed for Fasting Plasma Glucose (FPG), glycosylated hemoglobin (HbA1c) and lipid profile (Total Cholesterol, Triglycerides, LDL-Cholesterol and HDL-Cholesterol).Body mass index (BMI) was calculated by measuring the height and weight of men & women based on body fat.

Data was analyzed using SPSS version 20.

Results: FPG was (11.23±3.65 in diabetics vs 4.35±0.68 in controls), HbA1c was (6.84±0.482 vs 5.31±0.487). Serum total cholesterol was (4.68±0.96 vs 3.99±1.01 p<0.001), triglycerides (TG) were (2.42±1.22 vs 1.56±0.87 p<0.001) and LDL-Cholesterol was (2.46±0.77 vs 2.17±0.72 p<0.05). These parameters were significantly raised then the controls. Whereas HDL-Cholesterol in the diabetics were relatively lower than the controls (1.04±0.224 vs 1.21±0.222 p<0.001). The diabetics Body mass index (BMI) was also significantly more than the controls (28.57±1.97 vs 24.46±2.32 p<0.001).

Conclusion: This study shows that serum FPG, HbA1c, Total Cholesterol, TG and LDL-Cholesterol are significantly increased in T2DM while HDL-Cholesterol levels are decreased significantly which might be the reason for high coronary heart diseases incidence in T2DM.

Key Words:*Type2 Diabetes Mellitus,Dyslipidemia,Total Cholesterol,Triglycerides,LDL-Cholesterol and HDL-Cholesterol*

Introduction

"Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia caused by defective insulin secretion, ineffective insulin function or both which leads to disturbance of carbohydrate, fat and protein metabolism."[1,2] There are two major types of diabetes mellitus, T1DM previously called insulin dependent diabetes mellitus (IDDM), occurs when insulin secretion is deficient and T2DM or non-insulin dependent diabetes mellitus (NIDDM), occurring because of insulin resistance with or without insulin relative deficiency.[3]"Chronic hyperglycemia causes structural and functional damage to blood vessels and tissues leading to complications such as diabetic neuropathy, nephropathy, retinopathy, hypertension, hyperlipidemia, cerebrovascular diseases and atherosclerotic coronary heart disease."[4,5]

Diabetes mellitus is a rapidly growing epidemic. 285 million people were suffering from diabetes in year 2010. This number is going to be 439 million worldwide in year 2030.[6] Similarly there is rise in coronary mortality in diabetic patients having dyslipidemia. Expected increase in deaths from 2000

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to 2030 would be 8-11 million worldwide and most of these patients would be diabetics.

Dyslipidemia association with atherosclerosis is well established. The progression of atherosclerosis in diabetes is mainly due to the associated hyperglycemia, obesity and insulin resistance.[7,9] Excess free fatty acids (FFA) liberation from adipose tissue occurs in T2DM due to insulin resistance.[10,12] To a large extent lipoproteins hepatic metabolism is controlled by insulin. It has implications on lipid profile and increased role in coronary heart disease development.[13,14]

Atherosclerosis starts as inflammation of the blood vessels and in the presence of T2DM the process speeds up. In T2DM atherosclerosis and high blood glucose levels are related to each other.[15] All proteins glycosylation, like collagen linking arterial wall matrix proteins and collagen linking are caused by persistently raised blood glucose levels leading to endothelial cells dysfunction which further contributes to atherosclerosis. In T2DM there is 95% prevalence of dyslipidemia.[16] A large number of studies have shown a significant reduction in T2DM related complications and mortality rate due to coronary heart diseases if a normal glycemic control is maintained. Studies have shown that Langerhans beta cells apoptosis is not the only damage that high blood sugar levels causes but also leads to increased accumulation of oxidized LDL in pancreatic islets and coronary arteries thus increasing the risks of development of coronary heart disease.[17] A triad of increased levels TGs, LDL-Cholesterol and decreased levels of HDL-Cholesterol in diabetic patients is called diabetic dyslipidemia. The abnormalities of lipids in DM is mainly due to insulin resistance that affects the enzymes and pathways in lipid metabolism.[6, 9, 18, 19] Several studies have proposed that in diabetic dyslipidemia the lipid particles composition is more atherogenic than the other type of dyslipidemia.[9,20] WUL and Parhofer KG in their study showed that diabetes and increased cardiovascular risk of diabetic patients is linked mainly to the lipid changes. The increased concentration of FFA, alteration of insulin sensitive pathways and low grade inflammation being the pathophysiology, all these play an important role. The results being decreased catabolism and overproduction of intestinal and hepatic origin triglycerides rich lipoproteins. The observed changes in LDL and HDL are a sequence to this.[21] Marcello et al in their study explained that the lipoprotein abnormalities are mainly determined by increased production of very low density lipoproteins (VLDL) by the liver. The multiple mechanisms involved in this are increased FFA influx into the liver, reduction of inhibitory effects of insulin on VLDL production and enhanced de novo lipogenesis. All this leads to increased concentration of Apo-B-containing lipoproteins and abnormal function of HDL particle which may impair cholesterol removal from deposits. All these changes can lead to atherogenesis.[22]

Apart from link of diabetic dyslipidemia and atherosclerosis, uncontrolled T2DM is itself a high risk factor for atherosclerosis as Selvin et al in 2010 in their community based study done on more than 11,000 participants observed the tendency for increasing risk of stroke, heart diseases and cardiac deaths.[9,23]The current literature suggests to diagnose and treat dyslipidemia in type 2 diabetics at the earliest. It will help improve the quality of life and prevent the associated complications. The main objective of this study was to determine the correlation of plasma glucose levels with lipid profile in T2DM in our own population.

Material and Methods

This case control study was conducted in Army medical College Rawalpindi from 11th November 2014 – 11th November 2015. A total of 120 participants were included in the study. They were divided into group A & B. Group A included 60 diagnosed patients (30 males & 30 females) with T2DM. Individuals with thyroid, Liver, Kidney, Adrenal disorders were excluded from the study. Group B included 60 healthy persons (30 males & 30 females) free from any major illness.

Under aseptic conditions five ml of fasting venous sample of at least 8-12hs was obtained from left median cubital vein. The blood was centrifuged for 5 mins at 4000 rpm for serum separation. Selectra E, a fully automated chemistry analyzer was used for the FPG and lipid profile measurement. To estimate the levels of HbA1c levels Ion exchange method was used. Body mass index (BMI) was calculated by measuring the height and weight of men & women based on body fat.

Patient's selection was done from medical wards & Endocrinology Department of Military Hospital (MH) Rawalpindi using Non Probability convenient

sampling technique. Sample size was calculated using WHO calculator.

This parametric data was entered and analyzed using SPSS version 20 and results of the test were subject to appropriate statistical analysis. Quantitative data was compared using independent t-test. The data was statistically considered significant with P-value of < 0·05.

Results

120 individuals were selected for the study of which 60 were diabetics and 60 were healthy individuals who were gender & age matched. Comparison of biochemical profile of different analytes in diabetic patients (Group A) and healthy controls (Group B) are summarized in table I. FPG levels (mean ± SD) in group A was 11.23±3.65 as compared to group B 4.35±0.68, HbA1c was (6.84±0.482 group A vs 5.31±0.487 group B) and in the lipid profile, serum total cholesterol was (4.68±0.96 group A vs 3.99±1.01 group B p< 0.001), triglycerides (TG) were (2.42±1.22 group A vs 1.56±0.87 group B p< 0.001) and LDL cholesterol was (2.46±0.77 group A vs 2.17±0.72 group B p< 0.05). Whereas HDL cholesterol was (1.04±0.224 group A vs 1.21±0.222 group B p< 0.001). The BMI was raised significantly in group A than group B (28.57±1.97 vs 24.46±2.32 p< 0.001).

Table I. Comparison of Biochemical Characteristics between Cases and Controls

Variables (reference range)	Group A (Diabetic) n=60	Group B (Control) n=60	P-Value
BMI (kg/m^2) (18-24.9)	28.57±1.97	24.46±2.32	<0.001***
FBG (mmol/L) (3.3-5.6)	11.23±3.65	4.35±0.68	<0.001***
HbA1C (%) (4.0-6.0)	6.84±0.482	5.31±0.487	<0.001***
Total Cholesterol (mmol/L) (<5.2)	4.68±0.96	3.99±1.01	<0.001***
Triglycerides (mmol/L) (0.40-1.60)	2.42±1.22	1.56±0.87	<0.001***
LDL-Cholesterol (mmol/L) (<2.50)	2.46±0.77	2.17±0.72	<0.05*
HDL-Cholesterol (mmol/L) (>0.90)	1.04±0.224	1.21±0.222	<0.001***

Note: ***Significance at 0.001,
**Significance at 0.01 level, *Significance at 0.05 level

Table II. Comparison of Biochemical Characteristics between Cases and Controls in Females

Variables	Group A (Diabetic) n=30	Group B (Control) n=30	P-Value
BMI (kg/m^2)	28.68±1.90	24.84±1.91	<0.001***
FBG (mmol/L)	11.77±3.97	4.44±0.68	<0.001***
HbA1C (%)	6.91±0.58	5.41±0.45	<0.001***
Total Cholesterol (mmol/L)	4.89±0.98	4.31±0.84	<0.001***
Triglycerides (mmol/L)	2.58±1.07	1.72±1.06	<0.001***
LDL-Cholesterol (mmol/L)	2.47±0.92	2.42±0.64	0.84 NS
HDL-Cholesterol (mmol/L)	1.07±0.20	1.24±0.18	<0.001***

Note: ***Significance at 0.001,
**Significance at 0.01 level, *Significance at 0.05 level

Table II. Shows significant increase in levels of BMI, FBG, HbA1C, total cholesterol and TG in the diabetic females as compared to controls while HDL cholesterol was significantly lower in the diabetic group. In females LDL cholesterol showed no significance may be due the small sample size

Table III. Comparison Of Biochemical Characteristics between Cases and Controls In Males

Variables	Group A (Diabetic) n=30	Group B (Control) n=30	P-Value
BMI (kg/m^2)	28.46±2.08	24.08±2.64	<0.001***
FBG (mmol/L)	10.69±3.28	4.25±0.68	<0.001***
HbA1C (%)	6.76±0.34	5.22±0.51	<0.001***
Total Cholesterol (mmol/L)	4.47±0.90	3.67±1.08	<0.01**
Triglycerides (mmol/L)	2.27±1.36	1.40±0.62	<0.01**
LDL-Cholesterol (mmol/L)	2.45±0.63	1.82±0.72	0.001***
HDL-Cholesterol (mmol/L)	1.02±0.24	1.19±0.25	<0.01**

Note: ***Significance at 0.001,
**Significance at 0.01 level, *Significance at 0.05 level

Table III. Shows significant increase in levels of BMI, FBG, HbA1C, Total cholesterol, TG and LDL cholesterol in diabetic males as compared to controls while HDL cholesterol was lower in the diabetic males.

Discussion

Diabetes mellitus is a commonest systemic metabolic disorder. Prevalence of diabetes in Pakistan is very high ranging from 7.6-11% in its different regions. It is expected that number of diabetic patients may increase from 5.2 million in 2000 to 13.9 million by the year 2030. Cardiovascular disease is a major cause of morbidity and mortality in both men and women with T2DM. In patients with T2DM, risk factors like dyslipidemia and hypertension plays a major role in inducing cardiovascular disease and control of these factors is of paramount importance.

The main observation of this study is increased serum Total Cholesterol ($p<0.001$) & LDL Cholesterol ($p<0.05$) in diabetic patients. This is in agreement with study carried out by V.Siva Prabodh et al in 2012 which also showed that the frequencies of Total Cholesterol, Triglyceride and LDL-Cholesterol are higher in the diabetic group($p<0.001$).[23] However mean serum Total Cholesterol and mean serum LDL Cholesterol was not as high in our study group as compared to study carried out by Narisimhaswamy KN in 2014.[24] This is probably because of limited sample size of our study. The other observation of our study is increased serum Triglyceride levels in our patients ($p=<0.001$) as compared to the control population. This is in accordance with the prior study done by V.Siva Prabodh et al in 2012 & Narisimhaswamy KN in 2014. Another important observation of our study is decreased serum HDL Cholesterol levels in diabetic patients ($p=<0.001$) as compared to healthy controls. This is in correspondence with results obtained by Deepa Singh et al. in 2015 showing that HDL-Cholesterol was significantly lower in diabetic patients ($p< 0.005$).[25] This study also compared the BMI of the T2DM patient's vs healthy controls. BMI is significantly raised in T2DM patients ($p<0.001$). Similar results were also shown by Dr Ratna et al in 2015 quoting that obesity leads to insulin resistance which in turn causes T2DM and both together leads to dyslipidemia.[26] Hypercholesterolemia and hypertriglyceridemia are the lipid abnormalities that occur in T2DM, which is the major cause of cardiovascular diseases. Blood glucose levels and lipid profile monitoring should be done routinely. Life style modifications including weight reduction, regular exercise and use of lipid lowering drugs are recommended in these patients.

Limitations of our study was small sample size. Future work with larger sample size and of longer duration is recommended.

Conclusion

Based on the findings of current study it is concluded that serum total cholesterol, triglycerides and LDL cholesterol levels are significantly raised in T2DM whereas serum HDL cholesterol levels are significantly decreased which might be the reason for high coronary disease incidence in T2DM.

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ORIGINAL ARTICLE

Assessment of Serum Enzymatic Antioxidant Levels in Recurrent Aphthous Stomatitis Patients

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ABSTRACT

Objective: The objective of the study was to determine the serum enzymatic antioxidant levels in recurrent aphthous stomatitis patients.

Study Design: This was a case control study.

Place and Duration of Study: The study was conducted in biochemistry department of Islamic International Medical College- Trust (IIMC-T) Rawalpindi from 15 March 2016 to 14 March 2017.

Materials and Methods: The study included 160 subjects divided into two groups Aphthous group and Non-Aphthous group. Aphthous group comprised of 70 recurrent aphthous stomatitis patients with active lesion and 90 age and sex matched healthy controls were included in the non-aphthous group .From all the participants 2 ml of blood was taken. Serum glutathione peroxidase levels were measured using enzyme linked immunosorbent assay technique. This ELISA kit uses Sandwich-ELISA as the method.

Results: The comparison of mean of serum glutathione peroxidase levels between aphthous and non-aphthous group was significant (p<o.oo).This study showed that the levels of glutathione peroxidase (a biological stress marker) were lower in recurrent aphthous stomatitis patients than in control subjects.

Conclusion: Serum enzymatic antioxidant levels were lower in recurrent aphthous stomatitis patients as compared to controls, so oxidative stress plays an important role in the pathogenesis of disease.

Key words: *Glutathione peroxidase, Oxidative stress, Recurrent aphthous stomatitis*

Introduction

Recurrent aphthous stomatitis (RAS) is one of the most commonly encountered pathology of oral mucosa, affecting nearly 20% of world population.[1] Aphthous is originated from “aphtha” a Greek word meaning ulceration.[2, 3, 4, 5] Recurrent aphthous stomatitis is a painful, recurring and distressful experience for the patients because it restricts their normal oral activities.[6,7] Ulcerations can be solitary or multiple and has a shallow necrotizing center[8] and can be classified into three classes depending on their type , size , number and their healing time i-e minor, major and herpetiform.[9] Minor recurrent aphthous stomatitis are the most common type and contributes to 80% of total cases.[10] Many studies have done to find out the causes of recurrent aphthous stomatitis but the exact etiology remains unidentified .Potential factors which have been identified in the onset of disease include hereditary predisposition, local and systemic causes, hematological and micronutrient deficiencies, emotional stress and drug allergies.[11] It has been postulated that all the predisposing factors for the formation of aphthous ulcers act through a common pathway that depends on the oxidative stress by increasing free radical synthesis. Either the enhanced production of reactive oxygen species and/or decreased defense by antioxidants result in oxidative stress that is lethal as it causes tissue damage .[12]

Studies suggest that the lack of balance between reactive oxygen species and antioxidants have a pivotal role in the development and progression of inflammatory reactions, so oxidative stress may have a crucial role in development of recurrent aphthous stomatitis as it is inflammatory in nature.[13] To prevent oxidative stress, mammalian cells have developed antioxidant defense system including both enzymatic and non-enzymatic antioxidants.

Glutathione peroxidase, a selenocysteine containing

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protein is a most potent enzyme in the defense of cells against increased reactive oxygen species. It utilizes its reduced glutathione form to convert hydrogen peroxide and lipid peroxides into alcohols.[14] Gpx-1 is found in all cells, present in mitochondria, cytosole and in few cases in peroxisomes. Studies have shown that it is more efficient in getting rid of intracellular hydrogen peroxide then catalase in a number of physiological situations. Analysis of previous studies indicates that for the activity of Gpx-1, selenium (Se) which is a trace element is required which is present in the form of amino acid Sec. Hence as Gpx-1 enzymatically detoxifies non-radical hydroperoxides, it maintains cellular oxidant/antioxidant status by two ways;

a. Via direct removal of hydroperoxides
b. Secondly through oxidation of reduced form of glutathione i-e GSH [15]Regarding the insufficient studies related to this issue in Pakistan, the present study was carried to evaluate the possible relation between levels of glutathione peroxidase as a biological marker for oxidative stress and recurrent aphthous stomatitis.

Materials and Methods

This case-control study was conducted in Biochemistry department of Islamic International Medical College Trust Rawalpindi in collaboration with dental outpatient department of Pakistan Ordanance Factories Hospital, Wah Cantt from 15 March 2016 to 14 March 2017. Convenient non-probability sampling technique was used .The study population comprised of 160 subjects, 70 patients and 90 controls .Convenient non-probability sampling technique was used. The institutional review board and ethical committee of Riphah University (Islamic International Medical College) approved the study protocol. The selected patients had active lesion of aphthous ulcer and had history of recurring oral ulcer attack at least three times a year, of any age and gender. For control, healthy individuals with no history of any episode of recurrent aphthous stomatitis and any systemic disease were selected. Patients were excluded if they had any history of systemic disease or any other concurrent oral lesions. Patients were excluded if they were on any therapeutic regimen or supplement of iron, multivitamin, steroid or other immunomodulatory agents for past two months. Pregnant and lactating mothers were excluded. Smokers and alcohol users were excluded. After the informed consent of all participants in the study 2 ml of venous blood was collected using sterile disposable syringe and transferred to serum separator tubes. Serum glutathione peroxidase levels were measured by Sandwich Enzyme linked immunosorbent assay technique (Absorbance Micro plate Reader, ELX800, BioTek Instrument, Inc.USA).

The collected data was entered in statistical package for social sciences (SPSS) version 21 for analysis. Gender was expressed as percentages and frequency. Numerical variables like age, glutathione peroxidase were expressed as mean and standard deviation (±SD). Independent t –test was used to determine the significant difference of means between controls and patients.*p* values less than 0.05 were considered as significant.

Results

The Aphthous group had 45 females (64%) and 25 males (36%) while Non-Aphthous group had 54 females (60%) and 36 males (40%).The gender distribution of the both group I and group II is shown in the figure no 1.

Mean age of the Aphthous group was 28.76±9.144 and of the Non-Aphthous group was 30.53±6.404. The comparison of mean ages between the aphthous and non-aphthous group was not significant (p>0.05).

Biochemical findings:

Assessment of oxidative stress marker, Glutathione peroxidase:

With regard to oxidant/antioxidant status, mean of serum glutathione peroxidase in RAS patients was significantly lower (p <o.oo) than that of healthy controls, using t-test.

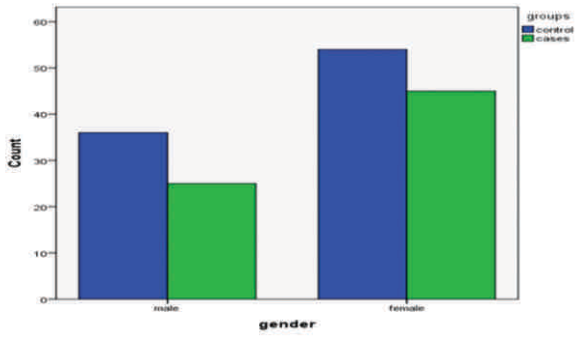

Fig 1: Gender distribution of study population

Table I: Enzymatic antioxidant levels of recurrent aphthous stomatitis patients and controls

Antioxidant parameter	RAS patients Mean±SD	Controls Mean±SD
Glutathione peroxidase -1 (Gpx-1)	733.160 ±220.500	1997.100±75.10

Discussion

Recurrent aphthous stomatitis or canker sores is an inflammatory ulcerative lesion and is one of the most common pathological disease of oral cavity.[1]

Even though extensive research regarding the predisposition of recurrent aphthous stomatitis have been done, the definitive cause of RAS remains unclear. Moreover directly or indirectly different factors involved in the pathogenesis of disease results in the loss of balance between oxidant and antioxidant system of the body. The present study was undertaken to determine serum enzymatic enzymatic antioxidant levels in patients with recurrent aphthous stomatitis and healthy controls.

Loss of balance among oxidant species and antioxidants causes variety of inflammatory oral diseases that varies from simple infections to lethal cancers. Recurrent aphthous stomatitis is also a sign of this imbalance. In several studies a possible relationship between free radical metabolism and inflammatory reaction have been demonstrated.[16] Lih-Brody et al.[17] has found that in mucosa of inflammatory bowel disease patients there is an imbalance between oxidant/antioxidant statuses. Also a study conducted by Kokcam and Naziroglu.[15] on psoriasis patients demonstrated a possible role of increased lipid peroxidation

Oxidative stress increases by the cellular damaging cytotoxic effects of reactive oxygen species. Nowadays in the maintenance of health and prevention from diseases, functional role of antioxidants has received huge attention.

Glutathione peroxidase -1 is the chief antioxidant in getting rid of hydrogen peroxide by utilizing reduced form of glutathione. Elimination of excessive hydrogen peroxide by Gpx-1 is directly related to utilization of reduced form of glutathione resulting in its decreased levels. So in recurrent aphthous stomatitis patients there is a decrease in serum levels of Gpx-1.

In the present study, serum glutathione peroxidase-1, an antioxidant marker showed significantly lower levels in recurrent aphthous stomatitis patients than the controls with a p value of zero.

This result was in agreement with the findings of Arikan et al and Cimen et al.[12] In a study conducted by Arikan, glutathione peroxidase levels were significantly lower in recurrent aphthous stomatitis patients as compared to controls .Results of our study were in contrast to the findings by Saxena who in his study found increased levels of glutathione peroxidase-1 in recurrent aphthous stomatitis patients.[19]

Selenium deficiency can occur with the decreased cellular immunity. By activating T-cell proliferation selenium supplement has an ability to markedly stimulate the immune response. Immunostimulatory role of selenium is supported by the study conducted by McKenzie.et.al who demonstrated that in the presence of oxidative stress selenium supplementation regulates the cellular response by rapidly promoting and activating the cellular antioxidant defense system.[14]

Devasagayam et al postulated that Gpx-1 deficiency causes free radical accumulation.[20]

Regarding the association of antioxidant and recurrent aphthous stomatitis, the disagreement amongst different studies may be due to different factors including different sample size, genetic variation of different population

There is a need to carry out experimental studies to find out the causative relationship between recurrent aphthous stomatitis and impaired oxidant/antioxidant status.

Further prospective studies with larger sample size are also required to identify new preventive and treatment options in recurrent aphthous stomatitis patients.

Conclusion

Glutathione peroxidase levels were significantly lower in recurrent aphthous stomatitis patients as compared to controls, which suggests there is an alteration of oxidant/antioxidant status in RAS patients.

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ORIGINAL ARTICLE

Comparing Hepatoprotective Effects of Aqueous Extract of Cassia Fistula (Amaltas) Leaves and Silymarin

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ABSTRACT

Objective: To compare the hepatoprotective effects of aqueous extract of *Cassia fistula* leaves and Silymarin on Acetaminophen induced hepatotoxicity.

Study Design: An experimental study in mice.

Place and Duration of Study: The study was conducted in the department of Pharmacology and Therapeutics, Multidisciplinary research laboratory of Islamic International Medical College, Riphah International University. The duration of study was from 16 Mar, 2016 to 16 Mar, 2017.

Materials and Methods: In present study simple random sampling was done by Balloting method and sixty adult Balb/c Albino mice were divided in four groups having 15 mice each. Mice in control group A were given normal diet and fresh water. Mice in experimental group B (positive control) were given Acetaminophenat dose of 100mg/kg/day orally for six weeks. Mice in experimental group C were treated with Acetaminophen(100mg/Kg) with concurrent 400mg/Kg body weight of *Cassia fistula* (Aqueous extract) orally for six weeks once daily while in experimental group D mice received 750 mg/kg body weight of Silymarin orally in form of aqueous suspension in 2% Cremophor1E for 6 weeks once daily concurrently treated with Acetaminophen(100mg/Kg).The cardiac puncture was done after six weeks and 2.5cc blood was collected from each mice for assessment of biochemical markers including Serum Alanine Aminotransferase (ALT) and Serum Aspartate Aminotransferase (AST).

Results: The mean value of serum ALT level was 130.4.30 ±42.90 in Group B as compared to control Group having mean value of ALT 37.30 ± 8.32. The mean value of ALT was 62.22± 19.27 and 53.90± 25.06 in Group C and in Group D respectively. In Group B mean value of serum level of AST was 134.3± 15.56 as compared to control Group A having mean value of AST31.40± 10.05.The mean value of AST in Group C and Group D were 66.40±20.50 and 44.67± 20.37 respectively.

Conclusion: The Silymarin is more beneficial than aqueous extract of *Cassia fistula* leaves in ameliorating hepatotoxic effects of Acetaminophen.

Key Words: *Acetaminophen, Cassia fistula leaves, Hepatoprotective, Silymarin.*

Introduction

Liver is a vital organ having role in metabolism of carbohydrates, proteins and lipids, bile production, maintenance of homeostasis, detoxification and vitamin storage.[1,2] Drug induced liver injury (DILI) is the leading cause of acute liver failure in most countries.[3] There are more than 1000 drugs considered toxic to liver. The incidence of DILI is 1 in 10,000 to 1 in 100,000.[4] In humans the most common cause of drug induced liver failure is acetaminophen (Paracetamol, N-acetyl-p-aminophenol,APAP) toxicity.[5] It is safe at therapeutic doses but at larger doses it can cause serious liver injury.[6,7] The cytochrome P-450 oxidation of acetaminophen overdose result in formation of reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) which initiates toxicity.[8] It depletes glutathione and binds to cellular proteins, especially in mitochondria and cause hepatotoxicity.[7,9,11] Previous studies have also identified NAPQI as important factor in acetaminophen induced hepatotoxicity.[8] Previous studies have identified some hepatoprotective agents against acetaminophen induced hepatotoxicity. Silymarin

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(SLM) is one of them having antioxidant, anti-inflammatory, antifibrotic, antiviral and immunomodulatory actions.[1,12,14] The Silymarin has hepatoprotective and antioxidant role by inhibiting free radicals.[1,15]

Herbal medicines represent one of the most important fields of traditional medicine throughout the world. *Cassia fistula* (Family Leguminosae, Sub-family Caesalpinea) is a very common plant known for its medical properties. It is commonly called Amaltas. The antioxidant property is possessed by polyphenol and flavonoids.[16]

Previous studies showed work done on methanolic and ethanolic extracts of *Cassia fistula* leaves. In present study aqueous extract was used to explore hepatoprotective effect of *Cassia fistula* leaves and its hepatoprotective effect was compared with Silymarin on Acetaminophen induced hepatotoxicity in mice and this comparison was not found in previous studies.

The aim of present study was to compare hepatoprotective effects of *Cassia fistula* leaves and Silymarin on acetaminophen induced hepatotoxicity.

Materials and Methods

An experimental study was carried out in the department of Pharmacology and Therapeutics, Multidisciplinary research laboratory of Islamic International Medical College in collaboration with National Institute of Health Sciences (NIH), Islamabad from Mar 2015 to Mar 2016. Approval of synopsis was authorized by the Ethical Review Committee of Islamic International Medical College. Mice weighing 30-50grams with normal ALT and AST levels were included in the study and mice weighing less than 30grams and more than 50grams with abnormal liver function tests were excluded. Sixty Balb/c healthy albino mice were selected randomly by balloting method. A perfect living condition indistinguishable to their class with adjusted dietary supplement was given under temperature of 24±2ºC with a 12 hour light and dark cycle.

Cassia fistula leaves were collected from Race course park, Rawalpindi. The leaves were air dried and then authenticated by herbarium department of National Agricultural Centre, Islamabad. The external dirt of freshly collected leaves was removed by washing with tap water. The leaves were shade dried and crushed coarsely by hand. About 400 gm of these leaves were boiled in distilled water for 30 minutes. These were kept for 3 days with intermittent shaking. These were filtered by Whatman No1 filter paper. The aqueous extract was obtained by concentrated the filtrate by rotary flash evaporator.[17,19]

The mice were divided into four groups randomly, each group consisting of 15 mice. Mice in Control Group A were given normal diet and fresh water orally. Experimental Group B was given Acetaminophen 100mg/Kg orally in diet for 6 weeks once daily.[20] Experimental Group C was treated with acetaminophen 100mg/Kg with concurrent 400 mg/kg body weight of *Cassia fistula*(Aqueous Extract) orally for 6 weeks once daily.[21] Experimental Group D was treated with acetaminophen 100mg/Kg orally with concurrent 750 mg/kg BW of Silymarin in form of aqueous suspension in 2% Cremophor1E for 6 weeks once daily.[22] After six weeks cardiac puncture was done and blood samples were taken for evaluation of Serum Alanine Aminotransferase (ALT) and Serum Aspartate Aminotransferase (AST) levels.

The data was analyzed by using Statistical package for social sciences (SPSS) version 22. The biochemical parameters ALT and AST were calculated by using Mean ± Standard deviation (SD). The mean difference between control and other groups was calculated by applying ANOVA. The Post Hoc Tuckey test was applied for comparison of mean difference between groups. *p*-value 0f <0.05 was considered statistically significant.

Results

The serum ALT and AST levels were significantly raised (p<0.00) in Acetaminophen treated Group B as compared to control Group A. The serum ALT and AST were reduced in *Cassia fistula* treated Group C and Silymarin treated Group D and in comparison, the results showed significant reduction in serum

Table I: Comparison of Serum ALT Level between the Groups (n=60)

Serum Levels	Group A (control group)	Group B (positive control)	Group C (Cassia Fistula)	Group D (Silymarin)
ALT (Mean± SD)	37.30± 8.32	130.4± 42.90	62.22± 19.27	53.90± 25.06
AST (Mean± SD)	31.40± 10.05	134.3± 15.56	66.40± 20.50	44.67± 20.37

P value < 0.00 *P* value <0.05 =significant

Table II: Post hoc Tukey's test for multiple comparison of ALT between the Groups

Groups	Mean Difference	Significant
Group A vs Group B	-93.13	Yes
Group A vs Group C	-24.92	No
Group A vs Group D	-16.60	No
Group B vs Group C	68.21	Yes
Group B vs Group D	76.53	Yes
Group C vs Group D	8.322	No

Table III: Post hoc Tukey's Test for Multiple Comparison of AST between the Groups

Groups	Mean Difference	Significant
Group A vs Group B	-102.9	Yes
Group A vs Group C	-35.00	Yes
Group A vs Group D	-13.27	No
Group Bvs Group C	67.93	Yes
Group B vs Group D	89.67	Yes
Group C vs Group D	21.73	Yes

levels of ALT and AST in Silymarin treated Group D as compared to *Cassia fistula* treated Group C.

Discussion

Drug induced liver injury is an unresolved health problem and Acetaminophen is known to cause hepatic damage at high doses.[23] The reactive metabolite NAPQI has been implicated to play a role in hepatic damage.[8,24] The present study is designed to compare hepatoprotective effects of aqueous extract of *Cassia fistula* leaves and Silymarin on Acetaminophen induced hepatotoxicity by measuring biochemical parameters including serum ALT and AST. In present study hepatotoxicity is induced in Group B with Acetaminophen. Group C and Group D were given aqueous extract of *Cassia fistula* leaves and Silymarin with concurrent Acetaminophen respectively to ameliorate the hepatotoxic effects of Acetaminophen on serum levels of ALT and AST.

In the present study mice in experimental group B showed abnormal increase in serum levels of ALT and AST which were given Acetaminophen. This is in accordance with findings of Amir Mohammad Kazemifar et al., who induced hepatotoxicity in male Sprague-Dawley rats with single oral dose of Acetaminophen 800mg/kg by gavage with an orogastric canula and he observed significant increase in levels of ALT and AST.[25] This is also consistent with study of Abel Felipe Freitage who found significant changes in LFTs due to hepatotoxic effect of Acetaminophen.[26]

In present study significant improvement *(p < 0.05)* in serum levels of ALT and AST in Group D is observed which was given Acetaminophen followed by Silymarin. This was also revealed by study of Amir Mohammad Kazemifar et al., who demonstrated the hepatoprotective property of Silymarin in Acetaminophen induced hepatotoxicity in male Sprague-Dawley rats.[25]

In present study a significant reduction (p < 0.05) was observed in raised serum levels of ALT and AST in Group C which was given Acetaminophen followed by *Cassia fistula* as compared to Group B which was given Acetaminophen. The hepatoprotective effect of ethanolic extract of *Cassia fistula* leaves was observed in study of Pradeep et al. at dose of 500mg/kg/day for 7 days against subacute Carbon Tetrachloride (CCl4) induced hepatic injury in rats. He found significant improvement in levels of ALT, AST.[17] Das et al. also proved the hepatoprotective effect of aqueous extract of fruit pulp of *Cassia fistula* against liver damage induced by CCl4 in Albino rats.[18] Our findings are in accordance with study of Jehangir et al. who observed significant reduction in biochemical parameters including ALT, AST, ALP and total bilirubin by ethanolic extract of *Cassia fistula* leaves against hepatotoxicity induced by Isoniazid and Rifampicin.[27] The better hepatoprotective effect of aqueous extract of *Cassia fistula* leaves could be proven with increasing duration of study. Further studies are required to see hepatoprotective effect of *Cassia fistula* leaves with higher doses and by use of different routes of administration. The molecular mechanism responsible for reducing biochemical and histopathological parameters can be explored by further studies.

Conclusion

In present study significant hepatoprotective effect was shown by aqueous extract of *Cassia fistula* leaves probably due to presence of flavonoids. Silymarin demonstrated better hepatoprotective effect than *Cassia fistula* against Acetaminophen induced hepatotoxicity.

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ORIGINAL ARTICLE

Effect of Turmeric on Mosquito Coil Induced Emphysema in Rat Lungs

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ABSTRACT

Objective: To determine the preventive effect of turmeric on mosquito coil smoke induced emphysema in rat lungs.

Study Design: Randomized control trial.

Place and Duration of Study: The study was completed in six months duration in the department of Anatomy, Islamic International Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad.

Materials & Methods: Twenty one adult male albino rats were divided into 3 equal groups. Control group X was kept in fresh air. Experimental Group M was exposed to Mosquito Coil smoke for eight weeks. Group T received oral turmeric 300mg/kg body weight and had a coil smoke exposure as well. All rats were dissected after 8 weeks and lung tissue was examined microscopically. The statistical significance of the results was calculated by applying Pearson chi square test and the obtained results were compared statistically.

Results: Marked emphysema was observed in the histological sections of rat lungs from experimental group M showing alveolar septal destruction and bullae formation in the lung tissues of all animals (100%) in group M. Emphysema was present in 1 out of 7 rats in group T thus group T showed a significant protection with only 15% of animals with emphysematous lungs.

Conclusions: Mosquito coil causes emphysema in the lung tissue and turmeric proves to be protective against this damage.

Key Words: *Turmeric, pulmonary emphysema, smoke*.

Introduction

"He who has health has hope and he who has hope has everything." But a tiny creature, named "mosquito" has always been spreading life threatening diseases like malaria and dengue fever specially in tropical and subtropical countries,[1] a major public health concern and a great hindrance to the socioeconomic development for the developing nations.[2] In the endeavor to protect himself, man has invented various means for protecting against mosquitoes. Repellency is a characteristic of the personal protection chemicals.[3] Mosquito coil repellents are widely used in domestic households to combat mosquito menace.[1,4] They are known to be an efficient mosquito repellent, are inexpensive and easy to use but produce smoke[3]. Coils being cheap are widely used in Asia. In 1996, WHO reported that the annual worldwide consumption of mosquito coils is estimated to be 29 billion pieces[4] and pollutant concentration from their emission is greater than the WHO standards.[5] They are burnt in closed rooms and are kept near the sleeping place to keep the mosquitoes at bay. Coils although effective against mosquitoes, cause indoor air pollution and users are exposed to them usually for the whole night (approximately 8 hours) and for several months every year[1], badly affecting various human organs like liver, testis and kidneys[6] specifically lungs and become a cause of debilitating respiratory disorders. Lung damage, even cancer has been shown to occur by exposure to the coil smoke. Studies have revealed that long term exposure to the coil smoke can induce asthma and persistent wheeze in children[7] and is

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teratogenic[8] most severe poisoning has been reported in infants. A chemical barrier is created by the airborne insecticide particles present inside and around the houses that prevents mosquitoes from entering. Coils that contain pyrethrins deter almost 45% to 80% mosquitoes and reduce their biting rat.[3] Burning the mosquito coil causes evaporation of insecticide and a large amount of sub micrometer particles and gaseous pollutants are released which reach the lower respiratory tract.[2] Release of particulate matter by burning 75-137 cigarettes equals that of burning one mosquito coil. Emission of formaldehyde from burning one coil is estimated to be as high as burning 51 cigarettes.[9]

Nature has always been kind and rightly labelled as a great physician and turmeric is a nature's gift for health. Curcumin(3-4%)[10] is the main bioactive component of turmeric. Turmeric holds a place of honor in traditional medicine of subcontinent for decades. It has been used as a food additive for centuries in Asia. Besides being the kitchen queen, it has been a center of attraction as a digestive aid, treatment for fever, inflammation, wounds, and infections as well as a remedy of various ailments including blood disorders. Thus it is a traditional herbal medicine.[11] In the present days, curcumin is proved to be a potent anti-inflammatory and anti-microbial agent.[12]

Certain studies have proved that curcumin has preventive effects against the tracheal responsiveness and lung pathology in asthmatic rats[13] ventilator-induced[14] and acute lung injury in rats.[15] An extensive literature search reveals that the local and international literature lacks in research with the same setup which could prove the effects of turmeric for prevention against histological alterations (damage) in lungs with allethrin based pyrethroids. Hence the study was conducted with the objective of determining the preventive effect of turmeric on mosquito coil smoke induced emphysema in rat lungs.

Material and Method

The randomized controlled experimental study was carried out in 6 months duration in the department of Anatomy, Islamic International Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, after an ethical approval from the Institutional Review Committee of Riphah International University. A total of 21 albino rats (*Sprague Dawley*), all healthy adult males weighing 250-300g were used for this study. Rats were purchased from the animal house of National Institute of Health (NIH) Islamabad. They were kept in a well ventilated room and were allowed to acclimatize for a week. The animals were kept in smoke exposure chambers at a temperature of 27±3ºC with a 12hr light/dark cycle with access to drinking water and standard laboratory diet *ad libitum.* They were exposed to mosquito coil smoke for 7 hours per day for 8 weeks. Animals were randomly divided into three groups and were kept in the same environmental conditions and received identical care. Group T was taken as control group. Experimental Group M was exposed to Mosquito Coil smoke for eight weeks. Group T received oral turmeric 300mg/kg body weight (through gastric gavage needle) and had a coil smoke exposure as well. All rats were sacrificed at the end of 8 weeks and left lungs were dissected out and preserved in the containers containing 10% formalin. Tissue processing and embedding was done in paraffin. Slides were prepared and stained with hematoxylin and eosin. Microscopic study was done first at low power, x10, and then at high power, x40 objective. Slides were studied for presence or absence of emphysema. Bullae formation and destruction of alveolar septa was taken as a criteria to label emphysema. The data obtained as presence or absence of emphysema in each rat was coded and entered in SPSS for analysis applying a Pearson Chi-Square test. P-value of <0.05 was taken as significant.

Results

The alveoli of the control group X showed normal honey comb like structure with well-formed alveolar wall. In experimental group T, which received turmeric along with inhalation of coil smoke, emphysema developed in only one rat; 6 out of 7 rats remained protected against the damaging effect of coil smoke in group T (fig 1A). Lung tissue of all the animals in group M, which received only coil smoke showed emphysema with alveolar septal destruction and bullae formation (figure 1B). Statistical analysis was performed by using SPSS version 21. Emphysema was taken as a dependent dichotomous nominal variable whereas coil smoke and curcumin were independent variables. Pearson chi square test

was applied. The data was entered by coding the presence and absence of emphysema. The statistical significance for the observed results was compared between the two experimental groups. Results showed alveolar septal destruction and emphysema with bullae formation in the lung tissues of all animals (100%) in group M (Fig 2). Emphysema was absent in 6 out of 7 rats in group T; thus group T showed a significant protection with only 15% of animals with emphysematous lungs. The difference was statistically significant with a p value of 0.000 that is <0.05. Difference of results can clearly be observed in graph (fig 2).

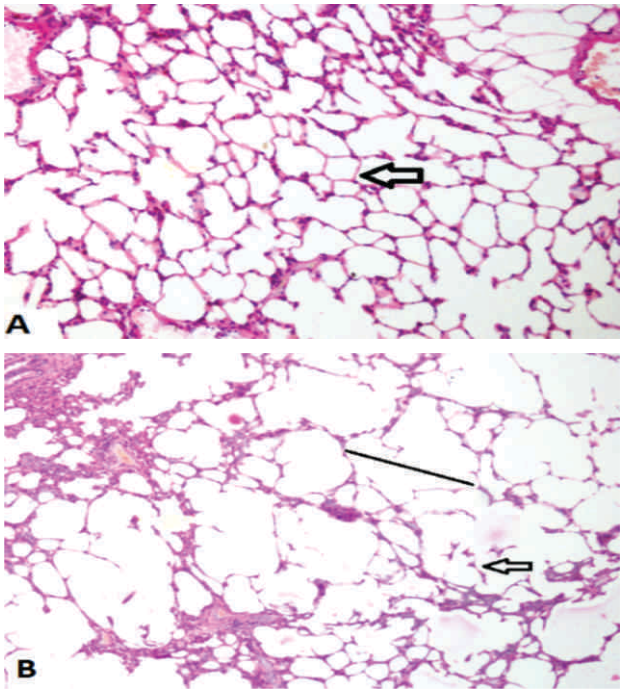

Fig 1: (A) lung of rat in Group T showing preserved alveolar structure having honey comb appearance (arrow). (B) lung of rat in group M showing destroyed alveoli (arrow) and increased alveolar space or emphysema (double arrow).

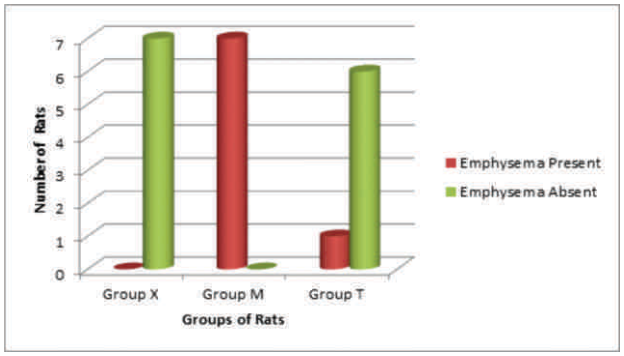

Fig 2: Graph showing frequency of emphysematous change in group X, M and T

Discussion

Emphysema is an abnormal, persistent expansion of the respiratory portion of the lung, distal to the terminal bronchioles resulting from tissue destruction.[16,17] In the lungs of all (100%) rats in group M, alveolar wall destruction and foci of collapsed alveoli with subsequent dilatation of contiguous alveolar spaces and formation of large irregular spaces (emphysematous change) was seen. Pathogenesis of emphysema involves imbalance between elastase and anti-elastases and an imbalance between oxidants (free radicals) and antioxidants. Elastase and oxidants are derived from neutrophils and macrophage[18] which were widespread in the rats of group M. The finding can be attributed to the injury of lung tissue by oxidants released by smoke exposure. Cigarette smoke is chemotactic to neutrophils and macrophages[18] elastolytic and proteolytic enzymes, released by macrophages, have destructive effects on lung airways.[19,20] Same phenomenon may be implied in mosquito coil smoke exposure because the burning of coil releases similar chemicals in even higher concentration as compared to cigarette smoke.[9] Emphysematous change has also been observed by Franks in a smoke related study.[21] After an exposure period of 16 weeks, smoke induced emphysema has also been observed in the lungs of rats by Ji-Hyun Lee in a study done in South Korea.[22] Pulmonary emphysema has also been induced by cigarette smoke in an experimental study on rats conducted in Canada to evaluate the role of neutrophils and macrophage released elastolytic enzymes in the progression of emphysema.[23] A recent study shows deranged lung function parameters in school children due to pyrethroid exposure.[24] Emphysema has also been observed in mice after an exposure to coil smoke for 120 days[25] while in our study the histological alterations have been observed in rats in a 40 days exposure period.

A recent study has proved that Curcumin has preventive effects against the tracheal responsiveness and lung pathology in asthmatic rats.[13] It has also shown its efficacy during ventilator-induced lung injury in rats by inhibiting the oxidative stress and inflammatory response.[14] A study proves that if the chemical stability of curcumin is improved than it shows incredible property of preventing the

acute lung injury in rats.[15] However, literature lacks in proving the protective effects of turmeric against the lung injury induced by allethrin based coil smoke. Group T had remarkable preservation of lung tissue in which only 15% of animals showed emphysematous change. Turmeric has shown to prevent the emphysematous changes induced by cigarette smoke.[23] Turmeric contains curcuminoids which are antioxidant and therefore can protect against the development of emphysema. As a potent immunomodulatory agent it can attenuate the activation of T cells, B cells, macrophages, neutrophils and natural killer cells.[26] and thus the tissue destructing enzymes released by these cells. It also affects the release of inflammatory cytokines.[27],therefore, can possibly protect the lung tissue from the histological alterations induced by coil smoke in the rat lungs.

Inhaling toxic chemicals daily is inevitable these days especially in the underdeveloped countries, where combustion of biomass and synthetic materials like mosquito coils are used as the main mode of prevention from the mosquitoes because of the fear of contracting lethal diseases like dengue fever and malaria. Turmeric supplementation in the diet could possibly protect lungs against environmental pollutants especially pyrethroids.

Because of the limited duration, effect of prolonged exposure of pyrethroids on lungs and the preventive effects of turmeric on other body organs could not be studied. Further human based studies of prolonged duration are recommended.

Conclusion

The present study clearly demonstrates that mosquito coil smoke adversely affects the lungs and induces histological damage causing emphysema and turmeric prevents it.

Therefore alternative ways like nets and herbal products should be used to protect oneself from mosquitoes. Exposure to the toxic repelling agents should be avoided by human population. Turmeric has protective properties and saves the lung tissue from toxic effects of pyrethroids and can be used as a dietary supplement for preventing against environmental pollutants.

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ORIGINAL ARTICLE

Endoscopic Findings in Patients with Upper Gastrointestinal Bleeding at Pakistan Railway General Hospital, Rawalpindi. A Retrospective Review of 100 Cases

Samia Kausar, Shamaila Burney, Zunera Jahanzeb, Muhammad Farooq, Asim Zulfiqar, Omar Awab

ABSTRACT

Objective: To determine the frequency of various causes of upper gastrointestinal bleeding on the basis of endoscopic findings.

Study Design: A descriptive observational study.

Place and Duration of Study: Study was done from 1st, January, 2015 to 31st December, 2017 at Pakistan Railway General Hospital Rawalpindi.

Material and Methods: The study was carried out in the Department of Medicine at Railway Hospital. The record of patients admitted with upper GI bleed through indoor/outdoor was retrieved from the Endoscopy Unit. 100 Patients fulfilling inclusion criteria were selected in the study. The relevant data and endoscopic findings were documented on Performa. The data was analyzed retrospectively.

Results: Out of 100 patients selected, 58(58%) were males and 42(42%) females. The mean age of 52.20 years ± SD 15.88.The most common cause was esophageal varices (47.90%), followed by gastritis/esophagitis 23%, peptic ulcer 14.58%. The malignant condition contributed 1.58 %. While in 9(9%) of patients, no cause of bleeding was identified.

Conclusion: Variceal hemorrhage is the most frequent cause of upper gastrointestinal bleeding in our population.

Key Words: *Endoscopy, Peptic Ulcer, UGIB, Upper Gastrointestinal Bleed, Variceal Bleed.*

Introduction

Upper gastrointestinal bleeding (UGIB) is defined as bleeding derived from a source proximal to ligament of treitz.[1] It is a common life-threatening medical emergency associated with mortality of 5-15%[2]. Its incidence is 50-150 per 100,000 people per year.[2,3] It is two times more common in males in all age groups, however mortality is same in both sexes. The patients can present with insignificant bleed to catastrophic hemorrhage. In approximately 80-85% cases, bleedings stop spontaneously while in 15-20% bleeding is continuous or recurrent. Initial evaluation of patients presenting with GI bleeding is very essential. Intensive resuscitation to achieve hemodynamic stability saves life.[4,5,6]

UGIB has been classified as variceal or non-variceal as they have different underlying mechanism, treatment algorithm and prognosis. In cirrhotics, variceal bleed is responsible for 70% of UGIBand is major cause of death.[6] Variceal bleed has higher mortality as compared to non-variceal bleed. Mortality during first episode is 15-20%.[7,8] Non-variceal causes include peptic ulcer, esophagitis, gastritis, Mallory weiss tear, and malignancies.[8,9]

The epidemiology of peptic ulcer is changing. Peptic ulcer is still common cause of UGIB[10] but incidence is decreasing. Peptic ulcer bleeding is less severe, occurs in older age group.[11]

Upper gastrointestinal endoscopy is the initially recommended procedure because of its safety.[4] The sensitivity and specificity of upper GI endoscopy for the diagnosis of UGIB is 92-98% and 30-100% respectively.[12] Timely endoscopy has vital role in modern management of UGIB.[6] Endoscopic management has shown to reduce morbidity, duration of hospital-stay, risk of re-bleeding, and overall health care cost.[5,6,13]

No large scale studies are available in Pakistan about

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prevalence of common causes of UGIB. Endoscopic services are not readily available or affordable for most patients in our setup. Patients are treated without any endoscopic evaluation to assess the etiology and response to treatment. Therefore the aim of the study was to evaluate the patients admitted with UGIB, So that future plans are made for better outcome.

Material and Methods

This was descriptive observational study. It was carried out at Department of Medicine Railway General Hospital from 1st Jan, 2015 to 31st December 2017.

Sample size was 100 patients with upper GI bleed. The sampling technique was non-probability (consecutive) sampling. Record of all patients referred with upper gastrointestinal bleeding to the Endoscopy Unit from indoor and outdoor was reviewed. Relevant data and endoscopic findings were retrieved from endoscopic register and entered in structured Performa. Those cases with incomplete record were excluded.

This study was conducted after approval by Institutional Ethical review committee

All patients had given written consent for endoscopy and had undergone hepatitis B and hepatitis C screening. In 60% of patients, endoscopy was performed within 24 hours. Biopsies were taken for suspicious lesions when required. The endoscopy was performed using endoscope GIF 130 Olympus Japan. Lignocaine gargles were used for local analgesia before endoscopy.

Endoscopic evaluation of varices was done in four grades, i.e., grade I-IV.[14] In case of peptic ulcer, FORREST classification[15] was used as follows; FI: active bleeding, FII: stigmata of recent hemorrhage, FIII: lesions without active bleeding. Los Angeles classification[16] system was used for esophagitis. Acute erosive gastritis was considered when multiple dark brown erosions were present.[17] Normal endoscopy was defined by absence of any abnormality.

Data (parametric) was analyzed by statistical software package SPSS version21. Descriptive statistics, including patient's age, gender, and admission number, were entered. Mean ± SD was calculated for age and frequency of qualitative variables was expressed as frequencies and percentages.

Results

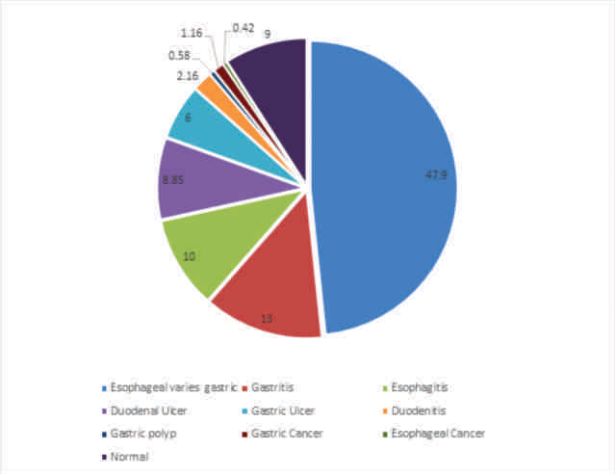
Out of 100 patients there were 58 (58%) males and 42(42%) females. The male to female ratio was 1.4:1. The mean age of patients was 52.20 ±15.58 SD years. The age range was between 18 to 80 years. The source of bleeding was endoscopically identified in 91(91%) cases and in 9 patients no source of bleeding could be identified. The commonest cause of UGIB was esophageal varices consisting of 47.90% with equal frequency in male and female. The commonest cause of non-variceal bleed was erosive mucosal disease (gastritis, esophagitis, and duodenitis) accounting for 25.16% of cases. While peptic ulcer accounted for 14.58% cases. Other less frequent causes include malignancy, gastric polyp and duodenitis. In patients with variceal bleed, anti HCV was positive in 80% and HBsAg in 10% of cases.

Table I: Distribution of patients with UGIB according to age and sex. Patient n=100

Age (years)	Number of patient (%)		
	Males	Females	Total
<30	5	6	11
31-50	18	1 5	33
51-70	27	12	39
>71	8	9	17

Table II: Distribution of endoscopic findings and its frequency with sex.

Endoscopic Finding	Males (%)	Females (%)	Total (%)
Esophageal varices	28(27.84)	20(20.16)	48(47.90)
Gastritis	9(7.54)	4(5.46)	13(13)
Esophagitis	4(5.8)	6(4.2)	10(10)
Duodenal Ulcer	5(5.22)	3(3.36)	8(8.58)
Gastric Ulcer	4(3.48)	2(2.52)	6(6)
Duodenitis	1(1.74)	1(0.42)	2(2.16)
Gastric polyp	1(0.58)	0	1(0.58)
Gastric Cancer	2(1.16)	0	2(1.16)
Esophageal Cancer	0	1(0.42)	1(0.42)
normal	4(5.22)	5(3.78)	9(9)



Upper GI bleed is a serious medical emergency associated with high morbidity and mortality. In our study esophageal varices was the most common cause followed by erosive mucosal disease and peptic ulcer. Age and sex ratio in this study was similar to other reported studies. In Pakistan there is high prevalence of hepatitis B[18] and hepatitis C[19] viruses and is the major cause of cirrhosis in our population. In variceal bleed group 80% patients were tested positive for anti HCV and 10% were positive for HBs.Ag.

Most studies conducted in Pakistan report a very high frequency of esophageal/gastric varices. A study comprising 550 patients and conducted at one of the largest tertiary care centers in Islamabad revealed that 44.4% patients reporting with UGIB had variceal bleeding while 19.7% had peptic ulcer and 6.6% had esophagitis.[20] A similar study from Multan[21] also showed high frequency of variceal bleeding (53%) with peptic ulcer being the second most frequent cause (26%). Ghouri et al. conducted a study in Jamshoro in which 54% of their patients had esophageal varices while 20% had peptic ulcer.[22]More recently, Farrukh et al. from Military Hospital Rawalpindi concluded that frequency of variceal bleeding in Pakistan has in fact risen over the years. More than two thirds of their study population (72.1%) was found to have esophageal varices.[23] Results of our study are therefore in accordance with the previous studies. Similar results are shown in a study from India.[24]

Portal hypertension secondary to hepatitis B and schistosomiasis is highly prevalent in African countries. Studies from Africa report higher frequency of esophageal varices. Jaka et al. from Tanzania reported varices in51.3% followed by peptic ulcers in 25.0% of cases.[25] While Aleema reported 40.6% cases had variceal bleed.[26]

UGIB can also result from erosive mucosal disease such as acute esophagitis, gastritis and duodenitis. Stress induced mucosal ulceration is a frequent occurrence in critically ill patients. However, clinically significant bleeding from acute stress gastritis has been reported as1.5%.[27] In the present study, erosive mucosal diseases were the second most common endoscopic finding in patients with UGIB. One in every four patients (25.16%) had esophagitis, gastritis and/or duodenitis. Results of our study closely match the findings of Shah et al. who also report erosive mucosal diseases as the second most common finding in their study after variceal bleeding. The frequency of esophageal varices in their study was 64.2% while that of erosive gastritis and peptic ulcer was 15.4% and 10.5% respectively.[28] Previously in a single center Indian study, Rathore et al. found erosive gastritis to be the most frequent cause of UGIB.[29] Similarly study by Aleema showed erosive mucosal disease second most important cause of UGIB after varices.[26]

The frequency of peptic ulcer in our study was relatively low (14.8%). While this is in accordance with results from local studies,[20,21,22,23,] although it is contrary to most data reported from the Western world. In a large scale study, Budimir et al. concluded that peptic ulcer bleeding (PUB) is the main cause of UGIB and is associated with significant re bleeding rate and mortality.[30] Peptic ulcer is strongly associated with Helicobacter pylori (H Pylori) infection. Other risk factors include alcohol abuse, chronic renal failure, non-steroidal anti-inflammatory drug (NSAID) use.

Peptic ulcer bleed and not variceal hemorrhage was the leading cause of UGIB in Pakistan in early 90's as determined in a study be Zuberi et al.[31] While this may partly be explained by the rising prevalence of chronic viral hepatitis in our country, the prevalence of peptic ulcer is overall declining.[32]This is believed to be due to an increased use of proton pump inhibitors (PPIs) and H pylori therapy. A study carried out in rural Sindh revealed that over half (51%) of patients in our setting are using PPIs with no definite indication.[33] It is therefore logical to assume that this

may very well be a contributing factor to changing trends in the frequency of PUB in recently published local data.

The endoscopy was normal in 9% of our patients. This number is much higher than the Western studies although similar to data from developing parts of the world.[26] A logical explanation of this is the fact that in the developed countries patients with UGIB undergo early endoscopy within 24 hours. In the present study, in 40% of our patients endoscopy was performed after 24 hours. Mucosal lesions are known to heal quickly, and delay in performing endoscopy can lead to normal endoscopic findings. Moreover, it is also important that detailed history must be taken to distinguish between haematemsis, haemoptysis, and gum bleed before endoscopy.

The result of our study stresses that variceal bleed is responsible for majority of cases of UGIB in our population. This requires mass efforts to stop transmission of hepatitis viruses and intensive treatment of chronic hepatitis to prevent progression to cirrhosis.

Limitation of study is that some patients had endoscopy after 24 hours of initial episode of bleeding and it is well known fact that mucosal lesion heal quickly, so bleeding source may not be identified in these patients.

Conclusion

Variceal hemorrhage is the commonest endoscopic finding in patients presenting with upper gastrointestinal hemorrhage. Amongst the non-variceal causes, erosive mucosal lesions such as esophagitis, gastritis and duodenitis are more frequently seen than peptic ulcer in our population.The authors report no conflict of interest. The authors alone are responsible for the content and writing of paper.

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ORIGINAL ARTICLE

Protective Effect of Tribulus Terrestris on Nifedipine Induced Damage to Microstructure of Testes of Rats

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ABSTRACT

Objective: To investigate the protective effect of a herb *Tribulus Terrestris* on Nifedipine induced damage on microstructure of testes.

Study Design: Animal Experimental Study.

Place and Duration of Study: The present study was undertaken for a period of 18 months with effect from October, 2012 to April, 2014 at Shifa College of Medicine, Islamabad.

Materials and Methods: Male Sprague Dawley rats (N=120) were divided into four groups, each comprising of 30 rats. Group A was given Dimethyl sulfoxide (DMSO), whereas group B, C and D were given Nifedipine only, *Tribulus terrestris* only and Nifedipine and *Tribulus terrestris* both respectively, by oral route for 56 days. At the end of experiment the diameter of seminiferous tubules and height of germinal epithelium was measured in H& E stained slides of testes in all groups and then compared. Results were analyzed on SPSS version 22.

Results: There was a significant difference in mean tubular diameter and height of germinal epithelium of testes between Nifedipine only group and treated rats which were given Tribulus extract with Nefidipine, showing its protective effect on the treated group.

Conclusion*:* Extract of *Tribulus Terrestris* helps in inhibiting the damaging effects of Nifedipine on histomorophometry of testis.

Key Words: *Infertility, Testis histomorphometry, Nefidipine, Tribulus*

Introduction

Infertility, whether primary or secondary, has always been a public health concern worldwide. As many as 15% of couples have difficulty while conceiving with male factor being responsible in up to 50% of such cases.[1] Out of a long list of risk factors, certain therapeutic drugs like cytotoxic, anti-convulsant, antimalarial and antihypertensive all have been reported to induce either permanent or temporary infertility.[2] Hypertension (HTN) is an emerging problem of modern era. It has been estimated that about 1 billion people worldwide have HTN and is expected to increase to 1.56 billion by 2025.[3] National Health Survey of Pakistan assessed that only 50% of the people with HTN were diagnosed and only half of those diagnosed were getting treatment for HTN.[4] Hypertension has to be treated vigorously as it is a stepping stone of variety of complications. Numbers of antihypertensive drugs are in use, naming a few: Diuretics, Angiotensin converting enzyme inhibitors, Beta-blockers, Calcium channel blockers (CCBs). CCBs became the best-selling antihypertensive since early 1990'still today because it is effective in controlling blood pressure at all age groups.[5]

Role of Calcium ions (Ca^{2+}) as ubiquitous second messenger system has long being recognized.[6] In male reproductive organs increase in intracellular Ca^{2+} through voltage gated calcium channels (VGCCs) induced by LH is indispensable for testosterone production.Ca^{2+} ions induce increase in activity and

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transcription of Steroidogenic acute regulatory protein (StAR) which mediates the transport of Cholesterol to the mitochondria. This step is the rate-limiting step of steroidogenesis.[7] Similarly Ca^{2+} is required for the normal spermatogenesis (spermiogenesis) and sperm motility and fertilization.[8]

Out of CCBs Nifedipine is one of the most prescribed drugs in the world. Its gaining wider acceptance for treating angina pectoris, hypertension, and congestive heart failure.[9] Belonging to Dihydropyridine(DHPs) subgroup of CCBs, it acts by blocking influx of calcium ions through the L-type VGCCs of smooth muscle cells of blood vessels thus reducing the blood pressure by decreasing peripheral vascular resistance mainly at the level of small arterioles. Though all CCBs share the same mechanism of action, DHPs differ from others induration of action, rate of onset of hypotension, predictability of response and severity of adverse effects.[10] It is known in literature that therapeutic administration of CCBs has been correlated with iatrogenic reversible male infertility.[11] The available literature has stated adverse reversible effects of Nifedipine on testicular steroidogenesis and spermatogenesis.[12, 13]Therefore, any therapeutic application of CCBs must be used with vigilance in males. Any agent that may increase testosterone production and spermatogenesis can be helpful for enhancing fertility in patients taking CCBs.

Tribulus terrestris (TT) is a natural herb (a flowering plant) used for treating many diseases. It finds important position in Ayurveda for the cure of sexual dysfunction in males.[14, 15]TT fruit particularly contains two steroidal saponins Protodioscin (PTN) and Prototribestin in its extract.[16]PTN is reported to increase the levels of testosterone, DHEA and DHT[15] hence improves libido and spermatogenesis.[17] It has been proved to be effective in stimulating spermatogenesis and activity of Sertoli cell in rats.[18]
As its a known fact that Nifedipine induces infertility through changes in calcium influx through voltage gated calcium channels and Tribulus increases the production of male sex hormone promoting spermatogenesis, its protective effect on CCB induced damage on microstructure of testes should be studied.[11,12,13] As on this protective effect of Tribulus no literature is available so far. Therefore the objective of the study was to determine the protective effect of TT on Nifedipine induced changes in microstructure of seminiferous tubules.

Materials and Methods

An animal experimental type of research was undertaken from October, 2012 to April, 2014 at the end College of Medicine, Islamabad and National Institute of Health Sciences (NIH), Islamabad. After an approval from the Institutional review committee of Shifa College of Medicine. One hundred and twenty (N=120), 90-120 days old male Sprague Dawley rats, weighing approximately 180-250g, were selected through non-probability convenience sampling from the animal house of NIH, Islamabad. Rats with any obvious physical pathology were excluded. They were divided into four groups having thirty rats each and were tagged with permanent marker on their dorsal body wall in their respective groups. Each group was kept in separate cage under standard laboratory conditions, acclimatizing for one week at a room temperature of 23-25°C with a 12 hour dark/light cycle. They were allowed to feed and drink ad *libitum* on standard pellet diet and tap water.
Nifedipine (Sigma Aldrich product no. N7634) and Dimethyl sulfoxide (Sigma Aldrich product no. D5879) was purchased from the local Distributors. Aqueous fruit extract of TT was made from fruits of TT after plant identification from herbarium of Quaid-e-Azam University. Fruit about 500 gms was powdered and soaked in 1 liter of distilled water overnight. After filtration the filtrate was dried at reduced temperature using rotary evaporator and then incubated at room temperature for 2 days. The dry mass served as an aqueous fruit extract of TT.[19]
Gavage needle was used to administer the substances orally. Group A (Control) was administered 0.5 ml distilled water and 1ml of Dimethyl sulfoxide (DMSO) per rat, once daily, orally, for 8 weeks. Group B (Nifedipine only group) was given Nifedipine at a therapeutic dose of 50 mg/kg body weight, dissolved in 1ml of DMSO, once daily, orally for 8 weeks. Group C (TT only group) was given TT aqueous extract 6mg/kg/rat, once daily, orally, for 8 weeks. Group D (Nifedipine plus TT) was given Nifedipine and TT together in the same doses, orally, once daily as were given to Group B and C

respectively for 8 weeks.

At the end of 8 weeks animals in all groups were anesthetized and sacrificed. Peritoneal cavity was opened by lower abdominal transverse incision; testes were removed, washed with saline, blotted and weighed on digital balance.[19,20]They were fixed in 10 % formalin. Transverse sections from the middle of testis were taken. Slides were prepared after cutting 5 µm thick serial sections on rotary microtome and staining with Haematoxylin and Eosin (H&E). Transversely cut seminiferous tubules were selected in the slides to measure the diameter of seminiferous tubules under light microscope at the power of 10 and height of germinal epithelium at the power of 40 with ocular micrometer. In each tubule both maximum and minimum diameters were measured and their mean was taken. Height of germinal epithelium was measured from the basement membrane to surface of the epithelium in the same tubules. Epithelial Height was measured at 4 places, equidistant from each other, in each cross-section and their mean was calculated.[21]

Mean and standard deviation (SD) was calculated for height of germinal epithelium and diameter of seminiferous tubules. The data was entered and analyzed using SPSS 22. The statistical significance of difference across the groups was determined by applying One way analysis of variance (ANOVA) followed by Tukey HSD (honestly significant difference) post hoc test to compare the mean differences among various pairs of groups. A *p*–value of <0.05 was considered as statistically significant.

Results

On macroscopic examination the testes were normal looking in all four groups. Microscopic examination of control group exhibited densely packed seminiferous tubules with little stroma in between, lodging Leydig cells. Control group, TT only group and Nifedipine plus TT group revealed evidence of active seminiferous tubules undergoing spermatogenesis and spermiogenesis(figure1,2 and 4).Different stages of spermatogenesis with spermatogonia, primary spermatocytes, round spermatids, elongated spermatids, and spermatozoa were visible in all sections of above mentioned groups. Spermiogenesis was shown by the presence of residual bodies and tails within lumen of seminiferous tubules.

In Nifedipine only group most of the tubules exhibited reduced tubular diameter and height of the germinal epithelium with depletion of few cellular layers within seminiferous epithelium. (fig3)Spermatogenesis was seen to be mostly arrested at elongating spermatid stage while Leydig cells appeared to be normal. There was no evidence of testicular degenerative changes.

Table I. Comparison of Parameters Among Four Groups By One Way ANOVA

Variables	Control group	Nifedipine only Group	TT only group	Nifedipine +TT group	*p*-value
Height of seminiferous epithelium (µm)	109.06 ± 10.54	102.36 ± 3.27	134.48 ± 13.55	129.49 ± 4.81	<0.001*
Diameter of seminiferous tubules (µm)	428.37 ± 59.15	389.79 ± 40.72	575.25 ± 39.11	547.51 ± 48.00	<0.001*

Where all values are expressed as mean ± standard deviation.

* Statistically significant values.

Table II. Group Comparison of Mean Differences in Diameter of Seminiferous Tubulesand Height of Germinal Epithelium By Post Hoc Tukey Test

Group Comparison	Diameter of seminiferous tubules (µm)		Height of germinal epithelium (µm)	
	Mean Difference	*p*-value	Mean Difference	*p*-value
Group B vs Group A (Nifedipine vs. control group)	38.58	0.011*	6.70	0.025*
Group C vs Group A (TT vs. control group)	146.88	<0.001*	25.41	<0.001*
Group D vs Group A (Nifedipine plus TT vs. control group)	119.14	<0.001*	20.43	<0.001*
Group D vs Group B (Nifedipine plus TT vs. Nifedipine group)	157.72	<0.001*	27.13	<0.001*
Group D vs Group C (Nifedipine plus TT vs. TT group)	27.74	0.112	4.98	0.150

*Statistically significant values

Discussion

Calcium Channel Blockers, most commonly used antihypertensive[22] are long been known for induction of male infertility.[23] They have been reported to induce temporary infertility either by inhibition of Leydig cell steriodogenesis, depression of spermatogenesis, destruction of the germinal cell, inhibition of sperm function or its metabolism.[24]

Our study revealed that control group, TT only group and Nifedipine plus TT group revealed evidence of active spermatogenesis but in Nifedipine only group most of the tubules exhibited reduced tubular

diameter and height of the germinal epithelium.

The main finding in Nifedipine only group showing reduction in spermatogenesis was supported by parallel studies of Latif *et al* , who had shown significant decrease in the height of seminiferous epithelium and diameter of tubules in adult male rats after giving them amplodipine for 50 days.[25] But these effects are reversible after discontinuing the drug. Similar study was reported by Adebayo A *et al* who depicted in his study the dose dependent delirious effect of amlodipine on seminiferous epithelium adult Wistar rats.[26] Lee *et al*[21] conducted study on prepubertal male mice which were given Nifedipine and Ethosuximide for 20 days. It caused a significant fall in sperm production and spermatogenic arrest mainly at the elongating spermatid stage in a dose-dependent fashion. Both T- and L-type Ca^{2+} channel blockers interfere with normal spermatogenesis and steroidogenesis firstly by blocking post meiotic germ cell maturation and/or by abolishing StAR protein expression, contributing to male infertility.[23] Sperm structure influences its motility and is also a predictor of overall health of the testes.[13] Our findings of Nifedipine only group coincided with above mentioned researches .Reduction in spermatogenesis with the use of Nifedipine may be either secondary to decrease in serum testosterone or in pituitary hormones or direct harmful effects of the Nifedipine on germ cells.[25]

There was significant increase in the height of seminiferous epithelium and diameter of tubules in TT only group because of the positive effect of aqueous extract of TT on germinal epithelium.TT has been shown to increase the height of seminiferous epithelium and diameter of seminiferous tubules as it increases the level of testosterone and LH in the body which promotes spermatogenesis.[27] Jashni *et al* reported in his study that estradiol glycosides in TT extract(PTN) cause increase in the testosterone levels by enhancing the production of androgens.[18] Histological observations made by Esfandiari *et al* after giving high dose of TT to mature and immature rats revealed increased height of germinal epithelium and diameter of seminiferous tubules in both experimental groups as compared to control groups.[28] Research conducted on rabbits by Al-Rubii et al revealed similar results.[29] Jashni *et al*[18] showed that the mean number of primary spermatocytes increased with TT dose of 10 mg/kg. In our study the histological changes became evident at 6mg/kg body weight given for same duration as above. This difference in the response might be due to geographical difference in the origin of TT plants[30] or due to aqueous extract of TT unlike alcoholic extract as used by Jashni *et al* in his study. The group Nifedipine plus TT has significant increase in the height of germinal epithelium and diameter of tubules with respect to Nifedipine only group. Nifedipine couldn't suppress spermatogenesis completely. The extract of TT is reported to increase serum testosterone. One of the suggested mechanisms is that the level of testosterone is augmented by increasing LH and the GnRH.[17] It can be direct conversion of PTN to DHEA in the body which then undergo further conversion to testosterone.[31]

TribulusTerrestris protective effects should be seen at different doses in male gonads along with changes in serum hormonal level. Cellular mechanism of action of Tribulus should be explored.

Conclusion

The current study has led us to presume that herb *Tribulus Terrestris* has protective effect on Nifedipine induced damage on microstructure of testes. Therefore temporary infertility in males induced by commonly used calcium channel blockers, Nifedipine, intake can be prevented by co-administration of *Tribulus terrestris* aqueous extract as has been confirmed by histological parameters.

Limitation of our study is non estimation of serum Testosterone and LH, which could have augmented the histological changes seen in testis

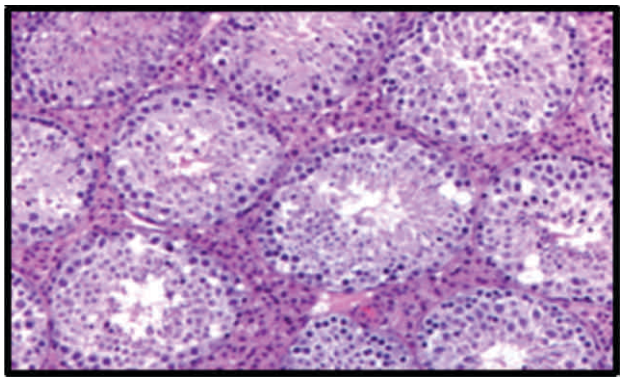

Fig 1: Photomicrograph of Testicular Tissue Showing Normal Histology in Control Group (H & E Stain x 300 approx.)

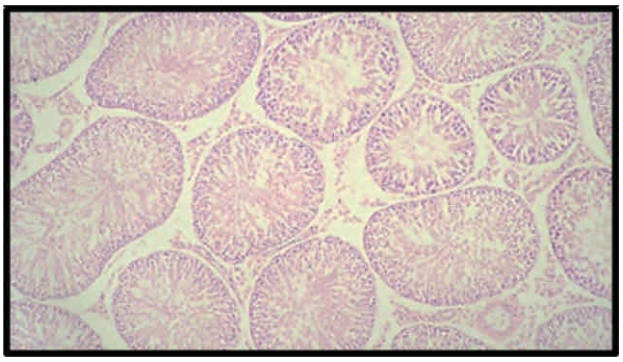

Fig 2 : Photomicrograph of Testicular Tissue Showing Testicular Histology In *Tribulusterrestris* Treated Group (H & E Stain X 300 Approx.)

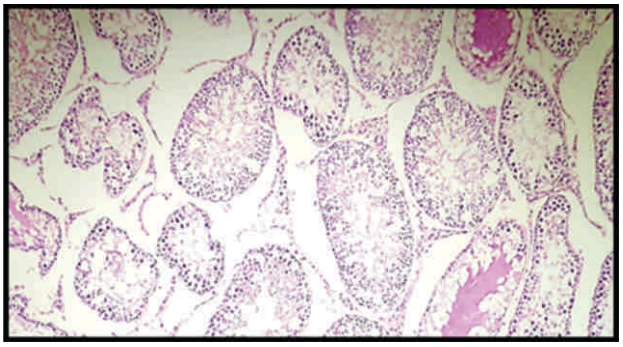

Fig 3: Photomicrograph of Testicular Tissue Showing Histological Changes In Nifedipine Treated Group (H & E Stain X 300 Approx.)

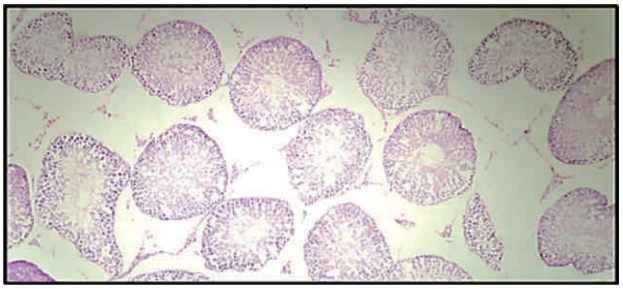

Fig 4: Photomicrograph of Testicular Tissue Showing Testicular Histology In *Tribulus Terrestris* Plus Nifedipine Treated Group (H & E Stain X 300 Approx.)

microstructure. In addition technical limitations prevented us to study the cellular mechanism of *Tribulus Terrestris* at Leydig cell level.

Recommendations

Tribulus Terrestris protective effects should be seen at different doses in female gonads along with changes in serum hormonal level.

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ORIGINAL ARTICLE

Antioxidant Effects of Spinach (Spinacia Oleracea) on Testicular and Epididymal Weight of Obese Sprague Dawley Rats

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ABSTRACT

Objective: To determine the antioxidant effects of spinach on the weight of testes and epididymis in obese Sprague Dawley rats.

Study Design: Experimental, randomized control study.

Place and Duration of Study: It was carried out from April 2016 to March 2017 in the department of physiology of Islamic International Medical College, Rawalpindi, Pakistan.

Materials and Methods: Total 40 male Sprague Dawley rats of about 8 weeks were included. They were randomly divided into Group A- Fed on standard diet and Experimental group- Fed on high fat diet to induce obesity. At the end of 6th week, after inducing obesity, experimental group was subdivided into Group B and Group C. Weight of the testes and epididymis of Group A and Group B rats was recorded. Then 5% spinach was given to Group C along with high fat diet for 4 weeks and finally weight of the testes and epididymis was measured.

Results: Weight of the testes and epididymis (g) of Group B (1.32 ± 0.53g) rats was significantly decreased (P<0.001) as compared to Group A (1.88 ± 0.92g) rats. However, weight of testes and epididymis (g) of Group C (1.92 ± 0.49g) rats was significantly increased (P<0.001) as compared to Group B (1.32 ± 0.53g) rats after spinach intake.

Conclusion: Intake of spinach supplemented diet has ameliorative effects on weight of testes and epididymis in response to deleterious effects caused by obesity-induced oxidative stress.

Key Words: *Epididymis, Obesity, Spinach, Testes.*

Introduction

Obesity, which is generally referred as anomalous and unnecessary accretion of fats in adipose tissues has become an intricate health problem.[1,2] Reported prevalence of obesity is 13% worldwide.[3] The reported prevalence of obesity in Pakistan is 10.3%.[4]

Different contributory factors are responsible for increase in weight but high fat diet and decrease physical activity are the two fundamental causes.[5] Obesity is not only the risk factor of different diseases but also accountable for harmful effects on reproductive system and increase in the incidence of infertility.[6,7]

Infertility is a global health problem, as 15% to 20% couples struggles when trying to conceive and hence look for medical guidance to increase the possibility of successful pregnancy.[8] For proper functioning of male reproductive system, testes and epididymis serves as an imperative organ as they are main site of sperm production and maturation respectively.[9]

One of the links connecting obesity with infertility is generation of reactive oxygen species.[10] In obesity, increase release of cytokines and cellular injury due to the pressure effect caused by excessive adipose tissues resulting in increased production of reactive oxygen species.[11] These reactive oxygen species are extremely unstable that gain stability by attacking the adjacent stable species, resulting in damage to surrounding cells and tissues.[12]

Thus, antioxidants utilization may be appropriate way to lessen the harmful effects of obesity.[13] These antioxidants act as scavenger against reactive oxygen species and help to overcome the unwanted consequences of obesity on male reproductive system. Currently, there is extremely curiosity

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regarding natural antioxidants found in plants as they are affordable and easily accessible and have no perilous effects.[14]

Spinach is green flourishing vegetable. It is rich in micronutrients (vitamin A, B, E, C and folic acid and oxalic acid) and minerals (calcium, phosphorus, iron, zinc, potassium and sodium).[15] It is also an established source of flavonoid and p-coumaric acid all of which have antioxidant properties.[16] So spinach can works as a shield against the damaging effect of reactive oxygen species.[14]

A number of studies on different animal models have demonstrated the antioxidant effects of spinach such as its effects on hyperlipidemia, radiation-induced biochemical changes in testes, lipopolysaccharides induced endotoxemia and slowing tumorigenic progression in prostatic carcinoma.[16,19] However, its therapeutic effects on male infertity induced by obesity are still needed to be explored and information regarding curative effect of spinach on obesity-induced changes on weight of testes and epididymis (sex organs) is still required. Thus, this will enable us to recommend this nutritional plant in the diet course of obese and overweight population to avert and alleviate these changes. As a result, the present study was intended to determine the potential effects of spinach on weight of sex organs.

Materials and Methods

The experimental randomized control study was performed in the department of Physiology and multidisciplinary research laboratory of Islamic International Medical College, Rawalpindi in association with the Animal House at National Institute of Health (NIH), Islamabad, Pakistan from April 2016 to March 2017. The study was approved by the ethical review committee and was accomplished under the guidelines, stated by the National Institute for animal experimentations.

A total of 40 male Sprague Dawley rats of about 8 weeks weighing from 160-200g were included in the study. For seven days, rats were allowed to acclimatize to NIH Animal house environment at humidity of 50-70% and at a room temperature of 24 + 2 °C, maintained at a 12 hour light and dark cycle. A standard diet in pellet form was prepared at the Animal house of NIH, Islamabad. The food and water was provided ad libitum.

Diet protocol

Spinach (Spinacia oleracea) leaves were bought from local market, and identified by taxonomist of National Herbarium department in Plant Genetic Resources Institute and National Agriculture Research Centre (NARC) Islamabad, allotting voucher number # IIMC 05.

Fresh spinach leaves were washed thoroughly with distilled water and then crushed in electric blender. The macerate was then filtered and air dried. Spinach powder was mixed with distil water and autoclaved at 121°C temperature and 15 Ibs pressure and then extract was stored.[17] Then 500 gram of this extract was added into 10kg of standard diet. Therefore, supplemented spinach diet contained standard diet along with 5% spinach hot water extract. Composition of standard and spinach supplemented diet is shown in Table I.

The composition of high fat diet constituted standard diet supplemented with 20% butter as shown in Table I.

Table I: Composition of standard diet, high fat diet and spinach supplemented diet.

Ingredients	Standard diet	High fat diet	Spinach supplemented diet
Wheat bran	2.85(kg)	2.85(kg)	2.85(kg)
Wheat flour	2.85(kg)	2.85(kg)	2.85(kg)
Dried skimmed milk powder	2.00(kg)	2.00(kg)	2.00(kg)
Fish meat	1.50(kg)	1.50(kg)	1.50(kg)
Common salt	0.05(kg)	0.05(kg)	0.05(kg)
Vitamins/minerals/amino acids	0.10(kg)	0.10(kg)	0.10(kg)
Butter		2(kg)	2(kg)
Spinach			500(gram)
Soybean oil	0.51 (Liter)	0.51(Liter)	
Total weight	10 kg	12(kg)	12.5(kg)

After acclimatization, rats were randomly divided into Group A (Control group n=13) and Experimental group (n =27). Group A rats were fed on standard diet and rats of Experimental group were fed on high fat diet to induce obesity in six weeks. Weight (g) of the rats was measured weekly by placing them on the weighing machine (TS200 electronic compact scale, Jiangyin Ditai electronic technology Co. Ltd., China).

At the end of 6th weeks, when rats of Experimental group had gained 20% weight above that of rats of

Group A (Control group), they were further subdivided into Group B (Obesity control group n =13) and Group C (Spinach treated group n=14).Then first sampling for measuring weight of testes and epididymis from Group A (Control group) and Group B (Obesity control group) Sprague Dawley rats was done by placing them in a glass jar containing cotton soaked in chloroform. The rats were kept there, until their breathing movements ceased and they were sacrificed. Then an incision was given in the abdominal wall along the midline and the right testes and epididymis were dissected out, freed from adherent tissues and weighed (g) on weighing machine (TS200 electronic compact scale, Jiangy in Ditai electronic technology Co. Ltd., China).

Then 5% spinach hot water extract was given to Group C (Spinach treated group) along with continuation of high fat diet for four weeks. At the end of 4th week, second sampling to calculate weight of testes and epididymis was done from Group C (Spinach treated group) Sprague Dawley rats similar to the method applied for the first sample collection.

Statistical analysis was performed by using Statistical package of social sciences (SPSS 21) version. All Results were expressed as Mean ± SEM. Comparisons among the groups was evaluated by using the independent sample t-test. P value of <0.05 was regarded as significant.

Results

Weight of testes and epididymis (g) of Group B rats (1.32 ± 0.53g) was significantly decreased (P<0.001) as compared to Group A rats (1.88 ± 0.92g). However, weight of testes and epididymis of Group C rats (1.92 ± 0.49g) was significantly increased (P<0.001) as compared to Group B rats (1.32 ± 0.53g) after administration of spinach hot water extract. Comparison of Mean ± SEM of weight of testes and epididymis (g) of all three Groups (A, B, C) is shown in Figure 1.

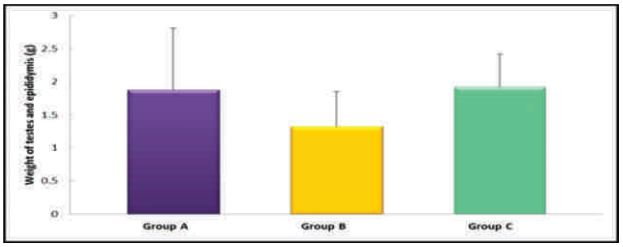

Fig 1: Comparison Of Mean ± SEM Of Weight Of Testes And Epididymis (G) Of Sprague Dawley Rats In The Following Three Groups:

Group A: Control group
Group B: Obesity control group
Group C: Spinach treated group
***=P < 0.001 is considered statistically highly significant.
***a= Group A vs B
***b= Group B vs C

Discussion

Obesity has become a great challenge for both developed and developing countries of the world.[20] Growing evidence suggests that along with other diseases, increased body fat also affect the functions of male reproductive system and cause male infertility.[21,22] Thus, our study explored the harmful effects of obesity on weight of testes and epididymis as well as the beneficent role of spinach (*Spinacia oleracea*) in restoring these changes.

In obesity, there is not only increased fat accumulation in different parts of the body but also around testes and epididymis as described by Alhashem et al. (2014).[23] These excessive adipose tissues cause cellular damage resulting in increased production of reactive oxygen species and expose the testicular microenvironment to oxidative stress. Our study also shows that obesity causes considerable decrease in the weight of testis and epididymis. Similar results have been reported by Alhashem et al., (2014) and Yan et al., (2015) that after inducing obesity, there was significant decrease in testes and epididymis weight when compared with control.[23,24] However, Ghanayem, Bai, Kissling, Travlos & Hoffler, 2010 had different observations.[25] Reason of those variations can be due to difference in genetics of animal model as well as procedure differences.

However, use of antioxidant such as spinach (*Spinacia oleracea*) could repair the oxidative damage and cause significant increase in weight of testes and epididymis in obese rats. These results are in accordance with the study carried out by Tawfeek, Ahmed & Kakel, (2006) who observed the antioxidant effect of Nigella sativa oil treatment on the sex organs in rats exposed to oxidative stress and concluded that use of antioxidant could repair the damage caused by oxidative stress.[26] Similarly, Elgazar, (2016) also showed that use of antioxidants have beneficial effect on the testicular tissue and cause significant increase in weight of sex organs

while describing the protective role of walnut seeds extract and vitamin E as an antioxidant against the oxidative stress.[27] Although we demonstrated that obesity induce injurious effects on weight of testes and epididymis, further biochemical and molecular studies are needed to clarify the effects of obesity and spinach (*Spinacia oleracea*) on the male reproductive system. Furthermore, these therapeutic effects of spinach (*Spinacia oleracea*) need to be clarify via future studies on human.

Conclusion

Thus our study shows that spinach has strong potential as an antioxidant to restore the deleterious effects on weight of testes and epididymis in response to obesity-induced oxidative stress.

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Finding source

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ORIGINAL ARTICLE

Effectiveness of Ponseti Technique in Children with Bilateral Neglected Clubfoot: A Case Report on a 7 Years Old Child

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ABSTRACT

The case is about a male 7-year-old child who was referred to our Ponseti clinic in Lady Reading Hospital for bilateral neglected clubfeet management. The patient was assessed through Pirani assessment form for club foot severity. This patient was treated through Ponseti technique and as a result the Patient went through serial casting in the LRH-MTI Club foot clinic. Tenotomy was also performed for achieving maximum desired correction. At first visit before cast the Pirani score for both feet was 6/6 and after first cast the Pirani score was reduced to 5/6 bilaterally while after 2nd cast Pirani score dropped to 4/6 for the right foot and for the left the score was 5/6. Assessment after 3rd cast revealed that Pirani score reduced to 3/6 for left side and 2.5 for right side while on 4rth cast the Pirani score was 1.5/6 for both side. After removal of the fifth cast the Pirani scoring was, 1/6 for right side and 1.5/6 for the left side. Patient was progressed to tenotomy and the Pirani score interestingly dropped to 0/6 bilaterally. Patient X was then progressed to the preventive phase.

Key Words: *Clubfeet, CTEV, Dennis brown splint neglected,Ponseti, Serial casting.*

Introduction

Clubfoot was first depicted in ancient Egyptian tomb paintings.[1] Congenital Talipes Equino Varus (CTEV) or clubfoot is a common structural congenital deformity in which affected children have abnormal bone structure in their ankle having four components equinus, hind foot varus, fore foot adduction and cavus or medial subluxation of navicular bones.[2,3] Neglected clubfoot comprises feet that had not been treated in the past.[4] Clubfoot incidence varies from about 1 in 1000 live births with approximately 50% of cases bilateral. In clubfoot male to female ratio is 2.5:1 and 24.4 % have family history of idiopathic talipes equino varus.[3] When untreated it can affect patient's mobility, productivity and cosmetic look.[5] Neglected clubfoot deformities in older children is considered a difficult surgical problem as the foot in these patients is stiff with some amount of pain and almost always had already undergone some surgical intervention.[6] Clubfoot treatment was described in India as early as 1000 B.C. The first written description of clubfoot was given to us by Hippocrates (circa 400 B.C.). He described methods for manipulative correction remarkably similar to current non-operative methods. Hippocrates treated clubfoot as soon as possible after birth. His technique involved repeated manipulations of the involved foot with his hands, followed by the application of strong bandages to maintain correction.[7] The next description of repeated stretching comes from Arcaeus, who in 1658 wrote a chapter on the treatment of clubfoot where he describes his stretching technique as well as two mechanical devices for maintaining the correction.[8] In the mid-18th century, Scarpa introduced his treatment which included forceful manipulation, not gentle stretching, and application of a complicated mechanical device, later known as Scarpa's shoe. His treatment method was never successful in other hands and for that reason was not widely accepted.[7] In the year 1806, Timothy Sheldrake published an essay entitled *Distortions of the Legs and Feet of Children.* Sheldrake used bandages like Hippocrates, and claimed that most of his patients could be cured in two to three months.[1] In 1823, Delpech performed subcutaneous tenotomy of the Achilles tendon in two patients with acquired talipes equinovarus.[9] Kite then became the leading advocate of the conservative treatment of clubfoot for many years in the early and mid 1900's.

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He continued the meticulous clubfoot cast application and molding that he had learned from Hoke. Kite corrected each component of the deformity separately instead of simultaneously. It was through his attempt to understand the pathophysiology of clubfoot, as well as his ability to learn from the mistakes of his predecessor, that Ponseti developed his current method of treatment for clubfoot. His understanding of the anatomy of the tarsus of the normal foot and of the clubfoot was greatly enhanced by the work of Farabeuf's *Precis de Manual Operatoire*, first published in 1872.[10] Since 1996, however, the non-surgical Ponseti method of correction has become increasingly popular.[10,11] Initially reserved for early correction of uncomplicated idiopathic patients, today the Ponseti method is being adapted for complex non-idiopathic patients and for patients presenting up to and beyond 2 years of age.[12] Widely implemented in high-income countries, the Ponseti method has been described as highly suitable for healthcare settings with scarce resources and is being increasingly used in low and middle income countries as well.[13]

The Ponseti technique is flexible because it gives the opportunity to recast patients who lose their corrections.[14] Successful correction of club foot is reported in 90-98% of cases treated by Ponseti method. Ponseti technique consist of intervention phase and maintenance phase, intervention phase consists of serial manipulations and casting to correct the clubfoot deformity and percutaneous tenotomy of the Achilles tendon to correct the ankle equinus. Maintenance phase consist of wearing of foot abduction braces for 2-3 years to maintain the gained correction.[15]

CASE REPORT

Patient Characteristics

Patient X was a 7-year-old child and was referred to our Ponseti clinic for club foot management. The reason behind the neglect was economic status and family support. Later, on presentation to our Ponseti clinic his family was assured that his whole treatment will be sponsored by ICRC. Detailed examination was performed to identify the type of club foot and an associated effect on the body. It was observed that the patient was walking on lateral border of the feet, and forefeet were internally rotated. It was noticed that the hip joints were in slight internal rotation, knee joints in 10 degrees genu valgum and calcaneo-cuboid joint were very prominent. Pirani scoring chart and a clubfoot detail assessment form was used as standard parameters to find the severity of the clubfoot deformity. The Pirani score was 6/6 for both feet with slight rigidity in left foot. While assessing the cavus, it was found that the arch of the foot was higher than normal. While examining for Adductus, it was observed that in both feet the forefoot was abducted towards the midline. In detailed assessment it was noted that the heel of the patient was in varus and angled towards the midline. The foot was in equinus position.

Intervention

Patient was treated through Ponseti technique serial casting with minor surgery (tenotomy). Patient had six visits, went through 5 serial casts with a minor surgery after final cast in the following order

on the head of talus for both feet. Feet were held in press position for 8 seconds to stretch of abductor hallucis longus, tibialis posterior muscles and ligament. Manipulation force was maintained, and POP cast was applied for a week.

At the second visit Pirani score was reduced to 5/6 bilaterally. Patient developed minor skin irritation on medial side of the thigh in left side while the right side was normal. Manipulation was performed again with more stretch bilaterally on the abductor hallucis longus, tibialis posterior muscles to keep feet in little supinated position and maximum abduction. Again, cast was applied but this time the medial side of the cast on thigh of left side kept a bit low from the groin to give room to the affected area of the skin.

1st Serial cast:

Pirani score for both feet was 6/6. Treatment was started with 15 repetitions of manipulation

Left foot

Varus: present

Cavus: Present

Abductus (-30 to 70): -30

Equines®(-50 to 30): -50

Pirani Scores (Hind foot):

- Posterior crease: (1)
- Empty heel: (1)
- Rigid equines: (1)

Pirani Scores(Midfoot):

- Talar head coverage: (1)
- medial crease: (1)
- Curved lateral border: (1)

Total score= 6

Right foot

Varus: present

Cavus: Present

Abductus®(-30 to 70): -30

Equines®(-50 to 30): -50

Pirani Scores(Hind foot):

- Posterior crease : (1)
- Empty heel : (1)
- Rigid equines : (1)

Pirani Scores(Midfoot):

- Talar head coverage : (1)
- medial crease: (1)
- Curved lateral border: (1)

Total score= 6

On third visit Pirani score dropped to 4/6 for the right foot and for the left the score was 5/6. A relapse was observed in the left side due to the damage to POP cast. So the position given in the second cast got recurrent and no improvement was seen in left foot. The right-side foot improved with cavus and adduction corrected.

Manipulation was applied, and more stretch was applied to the abductor hallucis longus, tibialis posterior longus, flexor digitorum longus lateralis and counter pressure was applied to the head of talus for over correction of left foot, for the right side gentle force applied and same procedure done as left side. Cast was applied to maintain correction gained through manipulation.

2nd Serial cast:

Left foot

Varus: present

Cavus: Present

Abductus(-30 to 70): -30

Equines(-50 to 30): -50

Pirani Scores(Hind foot):

- Posterior crease : (0.5)
- Empty heal : (1)
- Rigid equines : (1)

Pirani Scores(Midfoot):

- Talar head coverage : (1)
- medial crease: (0.5)
- Curved lateral border: (1)

Total score= 5

Right foot

Varus: present

Cavus: Present

Abductus(-30 to 70): -30

Equines(-50 to 30): -50

Pirani Scores(Hind foot):

- Posterior crease : (0.5)
- Empty heal : (1)
- Rigid equines : (1)

Pirani Scores(Midfoot):

- Talar head coverage : (1)
- medial crease: (0.5)
- Curved lateral border: (1)

Total score= 5

Upon fourth visit we observed that there was a bilateral mild damage to the pop cast, but the feet position was same not much disturbed, only the toes were in mild adducted position. Old cast was removed, and patient was reassessed and amazingly the Pirani score was reduced to 3/6 for left side and 2.5 for right side with only adduction in big metatarsal in both sides feet.

Manipulation was performed, and pop cast applied, dyanacast applied over the POP cast for the maintenance of the position.

On arrival for of the patient X for the fifth visit position was assessed in dyanacast was found normal. Then the old cast was removed, and patient was reassessed. This time Pirani score was 1.5/6 for both side. We observed only Equinus bilaterally and

3rd serial cast:

Left foot

Varus: present

Cavus: mild

Abductus®(-30 to 70): -10

Equines®(-50 to 30): -30

Pirani Scores(Hind foot):

- Posterior crease : (0.5)
- Empty heal : (1)
- Rigid equines : (1)

Pirani Scores(Midfoot):

- Talar head coverage : (1)
- medial crease: (0.5)
- Curved lateral border: (1)

Total score= 5

Right foot

Varus: present

Cavus: mild

Abductus®(-30 to 70): 0

Equines®(-50 to 30): -30

Pirani Scores(Hind foot):

- Posterior crease : (0.5)
- Empty heal : (1)
- Rigid equines : (1)

Pirani Scores(Midfoot):

- Talar head coverage : (0.5)
- medial crease: (0.5)
- Curved lateral border: (0.5)

Total score= 4

4th serial cast:

Left foot

Varus: present

Cavus: corrected

Abductus: (-30 to 70): 0

Equines: (-50 to 30): -20

Pirani Scores(Hind foot):

- Posterior crease : (0)
- Empty heal : (1)
- Rigid equines : (1)

Pirani Scores(Midfoot):

- Talar head coverage : (0.5)
- medial crease: (0)
- Curved lateral border: (0.5)

Total score= 3

Right foot

Varus: corrected

Cavus: corrected

Abductus: (-30 to 70): 0

Equines: (-50 to 30): -20

Pirani Scores(Hind foot):

- Posterior crease : (0)
- Empty heal : (0.5)
- Rigid equines : (1)

Pirani Scores(Midfoot):

- Talar head coverage : (0.5)
- medial crease: (0)
- Curved lateral border: (0.5)

Total score= 2.5

only mild abduction limitation in left side. On the bases of the above observation on the fifth visit we decided that left side foot will need over correction in post tenotomy POP cast. Right side was only having equinus and empty heel so normal cast applied with maximum equinus release.

Previous POP cast observed which was normal so after removal of the cast the Pirani scoring was done which was, 1/6 for right side and 1.5/6 for the left side. Finally, it was decided to perform tenotomy for the patient X to gain the maximum desired correction.

After proper positioning, knife (BP Blade-15/11 size) was placed parallel to tensed tendoachillis approximately 1 cm above the insertion at calcaneus. Then blade was turned 90 degrees, perpendicular to the tendon. A cut was applied to the tendon from medial to lateral direction. A “POP” sound was felt after the release of the tendon. An additional 10 to 15 degrees of dorsiflexion was gained after tenotomy. POP cast was applied, and more abduction was given on the left side for achieving the best possible result.

Foot Abduction Brace

After 3 weeks the pop cast was removed and Pirani scoring was performed and interestingly the score was 0/6 bilaterally. Patient X was proceeded to another phase of treatment the preventive phase in which abduction brace “Dennis brown splint” was applied. Dennis brown splint was fixed in 70-degree abduction and 20-degree dorsiflexion.

5th serial cast:

Left foot

Varus: corrected
Cavus: corrected
Abductus (-30 to 70): +20
Equines (-50 to 30): -10

Pirani Scores(Hind foot):

- Posterior crease : (0)
- Empty heal : (0.5)
- Rigid equines : (1)

Pirani Scores(Midfoot):

- Talar head coverage : (0)
- medial crease: (0)
- Curved lateral border: (0)

Total score= 1.5

Right foot

Varus: corrected
Cavus: corrected
Abductus (-30 to 70): +20
Equines (-50 to 30): -10

Pirani Scores(Hind foot):

- Posterior crease : (0)
- Empty heal : (0.5)
- Rigid equines : (1)

Pirani Scores(Midfoot):

- Talar head coverage : (0)
- medial crease: (0)
- Curved lateral border: (0)

Total score= 1.5

Tenotomy:

Left foot

Varus: corrected
Cavus: corrected
Abductus: (-30 to 70): +30
Equines: (-50 to 30): -10

Pirani Scores(Hind foot):

- Posterior crease : (0)
- Empty heal : (0.5)
- Rigid equines : (1)

Pirani Scores(Midfoot):

- Talar head coverage : (0)
- medial crease: (0)
- Curved lateral border: (0)

Total score= 1.5

Right foot

Varus: corrected
Cavus: corrected
Abductus: (-30 to 70): +30
Equines: (-50 to 30): 0

Pirani Scores(Hind foot):

- Posterior crease : (0)
- Empty heal : (0.5)
- Rigid equines : (0.5)

Pirani Scores(Midfoot):

- Talar head coverage : (0)
- medial crease: (0)
- Curved lateral border: (0)

Total score= 1

Discussion

Club foot or congenital talipes equinovarus (CTEV) is one of the most common congenital musculoskeletal deformities.[16] CTEV patients have an inward rotation of the foot, with four components: cavus, forefoot adductus, hind foot varus treatments available can be conservative (such as splinting or stretching) or surgical.[17] It is important that to have a uniform system for diagnosis, classification and follow-up to gauge the success of treatment. Pirani's classification is simpler and more recent. Pirani scoring system has proved to be useful in Ponseti management of clubfeet. Pirani score is widely used in the prediction of tenotomy and number of casts required.[18] Ponseti technique consist of two phases, an intervention phase and a maintenance phase. The intervention phase consists of serial manipulations and casting to correct the clubfoot deformity and percutaneous tenotomy of the Achilles tendon to correct the ankle equinus. Maintenance phase consist of wearing of foot abduction braces for 2-3 years to maintain the gained correction.[6]

Ponseti techniques is proved successful around the world in both developed and developing countries.[19] A study on Ponseti clubfoot techniques reported that this technique has reduced the need for extensive soft tissue release and major clubfoot surgery, and has changed clubfoot operation patterns in Nigeria.[20]

Our study reported Pirani score as (Arrival 6/6, after 1st cast 5/6 bilaterally, after second cast left 5/6 and right 4/6, after cast third left 3/6 and right 2.5/6, after fourth cast 1.5 bilaterally and after fifth cast left

Post tenotomy picture

score was 1.5/6 and right 1/6. After tenotomy Pirani scoring was performed and interestingly the score was 0/6 bilaterally.

A retrospective study which reviewed 17 children (24 feet) with congenital idiopathic club foot who presented after walking age, clinically, 16 feet (66.6%) were considered to have a good result, with a plantigrade foot and no pain, without the need for further surgery. Dynamic supination was present in four feet, but caused minimal disturbance of gait.[12]

Another prospective study by Birhanu Ayana and Peter J Klungsoyr on 22 children aged 2–10 years that the midfoot was corrected to Pirani 0 in all feet after the casts and in patients up to the age of 4 years, hyper abduction up to 60–70 degrees was achieved in the final cast. In the older children, abduction was only possible up to 30–40 degrees.[21] A study on treatment idiopathic club foot through Ponseti reported that the mean Pirani score at presentation was: hind-foot contracture score 2.5 (2 to 3), mid-foot contracture scores 2.5 (2 to 3), and a total score of 5.0 (4 to 6). The mean Pirani score after treatment for the feet which responded to treatment was: hind-foot score 0.5 (0 to 1), mid-foot score 0 and a total of 0.5 (0 to 1). Tenotomy was required in 85 (85%) of feet. A study from Brazil by Laurenco et al. (2007) has also reported successful results in 17 children having average age of 3.9 years.[12] In under developed countries, studies have shown good results of Ponseti techniques in the neglected club feet children. Sufficient deformity correction was gained in age ranging 1.5 years to 4 years children in a study conducted in Malawi.[22] A study conducted in Istanbul, TurkeY favors the results of our study, reported the efficacy of Ponseti technique using Pirani score in children aging more than 20 months.[23]

It is concluded that Ponseti method is safe and effective in managing children with neglected clubfeet. The Ponseti method markedly reduces the need for the operative treatment and the complications associated with operative treatment. In the developing world, where most neglected clubfeet occur, strategies should be formulated to reach every child with clubfeet for early intervention. Hospitals should make it mandatory to perform feet assessment after child birth and to guide the parents about clinics performing clubfeet management through Ponseti technique.

Recommendation

We recommend the use of Ponseti technique in older children with neglected clubfeet. Ponseti technique is safe and effective and it should be the treatment of choice for patients with both early presentation and neglected clubfeet.

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