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EDITORIAL

Breast Carcinoma—A Crisis in the Making

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According to the World Health Organization, breast cancer rates are increasing and its incidence is on the rise in even younger age groups[1].

An estimated 1.5 million are affected each year worldwide and of these, approximately 170,000 are of the triple negative breast cancer (TNBC) phenotype[2]. Therapeutic options for breast carcinoma vary from primary surgery to neo adjuvant/adjuvant chemotherapy, hormonal therapy or targeted radiotherapy as dictated by subtypes distinguished by expression of ER, PR and HER-2 neu proteins[3].

Although the tumour requires eight years to reach a size of 1cm, nevertheless, metastases can appear in different regions even with a lesion less than 0.5cms in size[5].

The diagnosis of breast cancer is a trauma in itself, but unfortunately, that is just the beginning of a treacherous journey. The treatment leaves deep psychological and physical scars that can take a very long time to heal. Even if the treatment is successful, the residue of emotional and physical fatigue, and the danger of its recurrence still linger.

One of the most common side effects of cancer is depression, which has its roots in many different areas ranging from physical changes to mental exhaustion. In a latest survey of 266 women who underwent breast cancer surgical treatment, the scientists found that more or less one-third of the participants had experienced psychological issues following the treatment, with problems becoming the most rigorous four months after the surgery[4].

In Pakistan, the issue of breast carcinoma is gaining attention nowadays because of its increasing prevalence. Dismally low public spending on health and a strictly traditional society collude to worsen the impact of breast cancer in the country.

Upto 90,000 breast cancer cases are reported in Pakistan every year with over 40,000 deaths annually. This is the highest rate of breast cancer in Asia, even when early diagnosis can save lives and make a difference[1]. In Pakistan, we receive patients in comparatively later stages and metastatic presentation unlike in the western countries where early screening and early diagnosis takes place[6]. As the custodians of medical fraternity, we need to reassess our objectives and strategies in tackling this situation.

I have already raised this issue in one of my research papers and concluded that the late clinical presentation of carcinoma of breast is because of lack of information, ignorance about the disease, misconceptions about the treatment and strong belief in traditional medicine resulting in delays in seeking medical attention. Poverty and the absence of healthcare services especially in the rural areas, worsen the situation. This leaves doctors unable to perform modern surgical techniques like breast conservation[7].

For early diagnosis and treatment of carcinoma breast, purpose built breast clinics and screening programmes should be started in all hospitals. Social awareness programmes are needed to find the prevalence of disease and spread knowledge in masses about early detection.

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ORIGINAL ARTICLE

Comparison of Steroidogenesis Under Acute and Chronic Stresses in Albino Rats

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ABSTRACT

Objective: To compare the steroidogenesis by the adrenal gland of rats between control, acute, and chronic heat exposed groups

Methodology: This was an experimental study, conducted in IBMS, DUHS. Seventy-two male rats of adult age (12 ± 1 weeks / weight ranges 200 ±50 gms.) were selected for the study and divided into three large groups A, B, and C (each experimental group consisted of 10 rats while control group consisted of 8 rats). All rats were acclimatized at a temperature of 22 ± 2°C for two weeks. Group A was considered as control to the chronic and acute heat exposed group. It had four subgroups, while each group comprised eight animals. Group B rats were divided into three subgroups: each group contained 10 animals and were exposed to 35 ± 2°C for two hours daily for two weeks, four weeks and six weeks respectively. Group C consisted of 10 male rats after acclimatization of two weeks and they were exposed to the high ambient temperature of 40 ± 2^{0}C (once during daytime for 60 minutes). All the rats were sacrificed on completion of the respective period of heat exposure alongwith their respective controls. The animals were dissected through midline thoraco-abdominal incision. Thoracic cavity was exposed after the dissection of sternum and direct cardiac puncture was done to collect blood for the serum analysis of corticosterone by ELISA technique. Statistical analysis was done by using the software SPSS version 16. t-test, ANOVA and Kruskal Wallis were applied for comparison between the group variables.

Results: The comparison of serum corticosterone level, done between acute and all chronic groups with their respective control groups, showed highly significant p-value of <0.0001.

Conclusion: Highly significant increase was observed in the serum corticosterone after heat exposure in acute as well as in all chronic groups as it was secreted by the adrenal gland.

Key words: chronic stress, acute stress, heat, adrenal gland, corticosterone

عنوان: سفید چوہوں میں شدید اور دائمی اقسام کے دباؤ کے دوران ہونے والے Steroidogenesis کا تقابلی جائزہ۔

مقصد: چوہوں کے کنٹرول، شدید اور دائمی گروپس کو گرم درجہ حرارت میں رکھ کر ان کے ایڈرینل غدود میں ہونے والے Steroidogenesis کا تقابل کرنا۔

مطالعہ کا خاکہ: یہ ایک تجرباتی مطالعہ تھا جو کہ IBMS اور DUHS میں کیا گیا۔

طریقہ: اس مطالعے میں بہتر بالغ مرد چوہوں کا انتخاب کیا گیا (1±12 ہفتے عمر وزن 50±200 گرام) جنہیں تین بڑے گروپس A، B اور C میں تقسیم کیا گیا۔ (ہر تجرباتی گروپ میں 10 چوہے رکھے گئے جبکہ کنٹرول گروپ میں 8 چوہے تھے۔) تمام چوہوں کو دو ہفتے کے لئے 2±22 سینٹی گریڈ درجہ حرارت کا عادی بنایا گیا۔

گروپ A کو کنٹرول بنایا گیا۔ اس میں چار ذیلی گروپس بنائے گئے۔ جن میں سے ہر گروپ 8 چوہوں پر مشتمل تھا۔

گروپ B میں تین ذیلی گروپس بنے جن میں سے ہر گروپ میں دس چوہے تھے جن کو روزانہ دو گھنٹے کے لئے 2±35 C درجہ حرارت میں بالترتیب دو، چار اور چھ ہفتے کے لئے رکھا گیا۔

گروپ C میں دس چوہے ہے جنہیں دو ہفتے عادی بنانے کے بعد دو گھنٹے کے لئے 2±40 C کے گرم ماحولیاتی درجہ حرارت پر دو گھنٹے کے لئے روزانہ ایک گھنٹے کے لئے رکھا گیا۔

تجربات کے اختتام پر تمام چوہوں کو ذبح کر دیا گیا۔ چوہوں کو سینے اور پیٹ کے درمیانی مقام سے چاک کیا گیا۔ سینے کی ہڈی چاک کر کے سینے کے خلا کو ظاہر کیا گیا اور دل میں براہ راست سوراخ کر کے corticosterone تجربے کے لئے خون اکٹھا کیا گیا۔ تجربہ ELISA طریقے سے کیا گیا۔

شماریاتی تجزیے کے لئے SPSS سوفٹ ویر کا ورژن 16 استعمال کیا گیا، ٹی-ٹیسٹ، ANOVA اور Kruskal Wallis کو تقابلی جائزے کے لئے استعمال کیا گیا۔

نتائج: تمام گروپس کے serum corticosterone levels کے مقابلے نے بہت اہم نتائج دیۓ ہیں۔ p-value <0.0001

حاصلِ مطالعہ: شدید گرم درجہ حرارت میں رہنے کے بعد تمام گروپس میں serum corticosterone کی مقدار خاصی بڑھی ہوئی پائی گئی۔

INTRODUCTION

Stress—"the non-specific response of body to any demand made upon it"[1] —is an integral part of modern life. It is thought to be the non-specific response to the stressor that always activates adrenal glucocorticoid and catecholamine's release[1]. Stress can be acute and chronic and in stressful conditions, adrenal gland is the major gland to secrete stress hormones by the activation of Hypothalamic Pituitary Adrenal (HPA) pathway. Stress can be produced in many ways causing different effects on different parts of human body. It can be produced by heat exposure, an important environmental hazard which is continuously producing adverse effects on health. Increasing heat in our surroundings is the result of not only the climate change phenomenon, but it is also produced by the daily use of domestic electrical appliances and advance electrical technologies. The rise in surrounding temperature can occur due to the emission of radiation from telecommunication towers[2].

Heat is an environmental as well as occupational hazard[3]. The populations at risk to thermal stress are those working in open fields with prolonged sun exposure[4]. Heat stress causes significant morbidity and occasional mortality in populations. Those at risk include farmers, athletes, firefighters, children, the elderly, and the disabled population[5,6].

Heat stress, whether acute or chronic, can adversely affect human health[7]. Chronic stress is a state of ongoing psychological arousal and threatens the body throughout life[8]. Chronic heat exposure affects different body organs especially the brain and is also accompanied by stress pathway activation[9]. The HPA axis is important for survival. Stress stimulus increases the release of corticotrophin releasing hormone from the Hypothalamic Paraventricular Nucleus (PVA), causing ACTH secretion from the anterior pituitary, which, in turn, stimulates the secretion of corticosteroid[10].

Stress increases the production of cortisol hormone from adrenal cortex under the influence of Adenocorticotropin hormone (ACTH), resulting in the rise of glucocorticoid level[11]. Under normal conditions, cortisol helps in the daily conversion of protein into energy, release of glycogen, and in counteracting inflammation, but continuous exposure to stress results in persisting high levels of cortisol that gradually tear the body. In tissues, cortisol directly affects fat storage and weight gain in stressed individuals[12,13]. Increased level of cortisol results in hypertension, hyperlipidaemia, and hyperglycaemia[14].

In this study, we compared the serum level of corticosterone in the chronic heat exposure group and acute heat exposure group with their respective controls. A significantly high level of serum steroid was observed.

METHODOLOGY

This was an experimental study. The experimental work was done in the animal house of Dow University of Health Sciences, Karachi. The study was done on 72 male albino Wistar rats of weight range 200gm±50gm.

The animals were divided into three main groups—A, B, and C. Group A and group B were divided into different subgroups; Group A was taken as control and further divided into four subgroups into A1, A2, A3, and A4, each consisting of eight animals. Group B was experimental chronic heat exposure group with three subgroups B1, B2, and B3 comprising 10 animals each, divided according to the experimental exposure of two weeks, four weeks and six weeks respectively. Group C was taken as acute heat exposure group consisting of 10 animals.

All the animals were acclimatized to the temperature of 22 ± 2^0C for a period of two weeks with adequate supply of food and water. All the animals of Group A were kept in similar environmental conditions during the period of experiment. Group B1, B2, and B3 were exposed to the temperature of 35 ± 2^0C[4], two hours daily for durations of two, four, and six weeks respectively. Group C consisted of 10 male rats. After acclimatization of two weeks, their weights and temperatures were recorded and then they were exposed to the high ambient temperature of 40 ± 2^0C (in daytime, once for 60 minutes).

All animals were sacrificed after their respective exposure. Dissection and direct cardiac puncture was done to collect blood for the serum analysis of corticosterone, poured directly into specific test tubes and put for centrifuge at 30 cycles to obtain adequate serum. Serum corticosterone levels were detected by the ELISA technique.

Statistical analysis was done by applying the t-test and Kruskal Wallis, non-parametric ANOVA to compare the groups.

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RESULTS

Serum corticosterone level, an important stress marker, increased not only in chronic stressful condition but also in acute stress. Acute and all chronic groups with their respective control groups were compared. In control group, the mean ±SD value of corticosterone was 114.38± 24.24 µg/ml while in chronic B1 subgroup, the mean ±SD value of serum corticosterone was 178.29±17.91 µg / ml. In chronic B2 subgroup, the mean ±SD value of serum corticosterone was 186.44 ± 57.99 µg / ml. In chronic B3 subgroup, the mean ±SD value of serum corticosterone was 200.44 ± 23.23 µg / ml and in acute exposed group (C group), the mean ±SD value of serum corticosterone was 181.04±21.82 µg / ml. When the means of acute and all chronic groups were compared, it showed p-value <0.0001. Highly significant increase was observed in serum corticosterone in acute as well as in all chronic groups.

Comparison of Serum Corticosterone Levels between Chronic, Acute and Control Groups (µg/ml)

Serum Corticosterone Level	Mean ± Standard Deviation	P Value
Acute: C group	181.04 ± 21.82	
Chronic: B1 subgroup	178.29 ± 17.91	
Chronic: B2 subgroup	186.44 ± 57.99	<0.0001
Chronic: B3 subgroup	200.44 ± 23.23	
Control (A1, A2, A3, and A4 subgroups)	114.38 ± 24.24	

Mean Serum Corticosterone Level
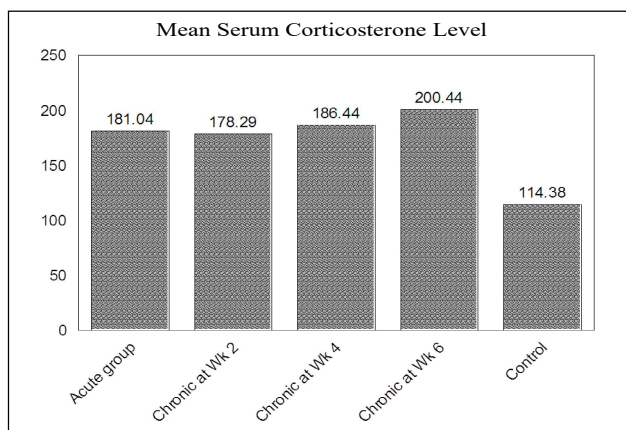
DISCUSSION

The adrenal gland responds immediately after the stress stimulus, whether acute or chronic. The gland respond differently to various types of stressors and these responses are also influenced by the duration of stress applied[15]. Heat is an important environmental stress, hazardous to human life and health, especially in those working in hot and humid climate.

In any stressful state, the glucocorticoid is an important hormone. In rats, corticosterone is released to maintain the body homeostasis after stress injury. In this study, the serum corticosterone was measured and found to be increased in both acute as well as chronic heat exposed groups. The highly significant change was observed in chronic B3 subgroup. These observations are supported by previous experiments which show significant increase in the serum corticosterone level after prolonged light exposure in female rats[16].

In another study, exposure to the chronic and acute noise stresses resulted in the secretion of high levels of corticosterone. These findings are concordant with the work of another researcher who found increased serum levels of corticosterone in the animals exposed to 12 hours noise stress[17]. The effect of sustained intensive noise on rats (two hours daily) showed that the ACTH and corticosterone levels in serum are increased after exposure[18]. Another experimental study discovered that when animals were subjected to continuous sound (130 dB 120), an immediate increase in corticosteroid excretion was reported[19].

A permanent elevation of cortisol by noise stress signifies the risk of diabetes (inhibition of glucose functions), osteoporosis (amplified protein and bone degradation), immunosuppression, hypertonia (increased BP, rising hormones), cardiovascular damage (increased cholesterol), myocardial infarction, gastric ulcers (increased secretion of gastric juices), etc[20].

Based on these results, it is suggested that persistent exposure to heat causes high level of the serum corticosterone level. Persistently high levels of cortisol cause adverse effects on human as well as animal health.

CONCLUSION

This study showed significantly high levels of serum corticosterone after chronic heat stress. More research is needed to find out genetic influences and the ultra-structural consequences of the process and post-exposure adverse effects of high serum corticosterone.

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ORIGINAL ARTICLE

Status of Vitamin B$_{12}$ Deficiency in Strict Lacto-Vegetarian Rural Population from Tharparkar, Pakistan

Suresh K Langhani[1], Loung V Umedani[2] and M Akbar Agha[3]

ABSTRACT

Objective: Prevalence of vitamin B$_{12}$ deficiency among vegetarians of Tharparkar, Pakistan

Methodology: An observational case control study was carried out after ethical approval from Dow University of Health Sciences (DUHS) in 2012. Two hundred subjects were conveniently selected from a Tharparkar village (100 strict lacto-vegetarians and 100 non-vegetarians). After a physical examination of the participants, blood samples were collected and sent for estimation of serum B$_{12}$ levels, full blood counts etc. The data from these groups were analyzed descriptively and statistically by SPSS-16.

Results: The mean age of the strict-vegetarian group was 30.5 years (± 8.3) and that of non-vegetarians was 30.1 years (± 9.2). Male to female ratio was 3.4:1.0. Vitamin B$_{12}$ deficiency was found in 83% of strict-vegetarians and 66% of non-vegetarians; anaemia was present in 36% vegetarians versus only 20% of non-vegetarians.

Conclusion: The vitamin B$_{12}$ deficiency was predominantly found in strict-vegetarians who also displayed alarming levels that could produce neuropathy. Carrying out homocysteine and methylmalonic acid levels is recommended alongside providing vitamin B$_{12}$ supplementations to the high-risk population of Tharparkar.

Key words: Vitamin B$_{12}$ deficiency, strict lacto-vegetarians, macrocytic anaemia, neuropathy, dietary habits, Tharparkar

عنوان: تھر پارکر پاکستان کے دیہی علاقوں میں گوشت نہ کھانے والے افراد میں وٹامن B12 کی کمی کی صورت حال۔

پس منظر اور مقصد: وٹامن B12 کی کمی دنیا کے سارے لوگوں میں عام ہے۔ اس وٹامن کا اہم وسیلہ جانوروں کا گوشت ہوتا ہے۔ گوشت نہ کھانے والے افراد میں اس وٹامن کی زیادہ کمی ہوسکتی ہے۔ اس تحقیق کا مقصد پاکستان کے صوبہ سندھ کے گوشت نہ کھانے والے افراد میں B12 کی کمی معلوم کرنا ہے۔

طریقہ: یہ عمومی بیو میڈیکل تحقیق صوبہ سندھ کے تھر پارکر کے ایک گاؤں میں 2012ء میں کی گئی۔ دو سو افراد جن میں سو افراد گوشت نہ کھانے والے اور سو گوشت کھانے والے شامل کئے گئے۔ جسمانی امتحان کے بعد خون کے نمونے جمع کئے گئے اور خون میں ذرات کی مقدار وغیرہ معلوم کرنے کے لئے لیبارٹری بھیج دئیے گئے۔

نتیجہ: گوشت نہ کھانے والے افراد کی اوسط عمر (8.3±)30.5 سال اور گوشت کھانے والے افراد کی اوسط عمر (9.2±)30.1 سال تھی۔ مردوں اور عورتوں کا تناسب 3.4:1.0 تھا۔ گوشت نہ کھانے والے افراد میں B12 کی کمی 83% اور گوشت کھانے والے افراد میں B12 کی کمی 66% حاصل ہوئیں۔ خون میں کمی کی مقدار 36% گوشت نہ کھانے والوں اور 20% گوشت کھانے والوں میں پائی گئی۔

خلاصہ: گوشت نہ کھانے والوں میں B12 کی مقدار انتہائی خطرناک حد تک کم پائی گئی جو Neuropathy پیدا کر سکتی ہے۔ ہم تجویز دیتے ہیں کہ ان افراد میں مزید تحقیق کے لئے homocystiene اور methylmalonic acid کی مقدار معلوم کی جائے اور تھر پارکر کے ان لوگوں کو وٹامن B12 کی اضافی مقدار فراہم کی جائے۔

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INTRODUCTION

Micronutrient deficiencies and their associated mortalities are very common globally. This is due to many factors like low socioeconomic status, lack of balanced diet, and social dietary traditions such as consuming a strict vegetarian diet. In Pakistan, the vegetarian population shows an appreciable incidence of low biochemical levels of micronutrients mainly vitamin B$_{12}$ and folate[1]. The biological role of vitamin B$_{12}$ and folate is of prime importance e.g. in maturation

of the blood cells and the stability of neuronal cells and their connections[2]. The deficiency of vitamin B$_{12}$ can cause severe and irreversible damage to brain and nervous system[2]. Therefore, the deficiency of this vitamin will result not only in anaemia but can also produce uncommon neurological and psychological manifestations. The prevalence of vitamin B$_{12}$ deficiency varies in different parts of the world. It is reported to be 20% in Mexico, 33% in India and 56% in the lacto-vegetarian group of the Northern tribal areas of Pakistan[3]. However, the deficiency of vitamin B$_{12}$ and folate is underestimated globally because of lack of awareness; nonavailability, and high cost of investigations. Vitamin B$_{12}$ is a water-soluble essential micronutrient of vitamin B family and has to be supplied in the diet, as gut microflora do not synthesize it but rather utilize it for their growth. This essential vitamin is only present in animal foods, therefore manifestations and consequences of its deficiency can occur earlier in those who are on restricted vegetarian diet or have malabsorption states. Many researchers have found that vegetarian diet is known to be deficient in vitamin B$_{12}$ content[4]. The normal value of serum vitamin B$_{12}$ is 205pg/ml to 770pg/ml. Value less than 205pg/ml should be considered as deficiency of vitamin B$_{12}$ and the resultant anaemia will be macrocytic. Because vitamin B$_{12}$ is stored in the liver for prolonged periods, so the manifestations of vitamin B$_{12}$ deficiency will appear within two years after complete cessation. Iron, vitamin B$_{12}$, and folic acid are needed for the production of red blood cells. If one or more of these is lacking, anaemia will develop. The normal haemoglobin level in females is between 11.5 to 15.5mg/dl and in males between 13.0 to 18.0mg/dl.

The objective of the present study was to screen the B$_{12}$ deficiency status for vitamin B$_{12}$ in healthy lacto-vegetarian and non-vegetarian population of Tharparkar village, Sindh. This study is of particular importance because the studied inhabitants of Tharparkar are known from the ancient past to be strict vegetarians according to their religious traditions. They are at high risk of becoming victims of the consequences of vitamin B$_{12}$ deficiency. The study did not measure folate levels because of its easy availability through both vegetable and animal dietary resources and the fact that folic acid deficiency alone in healthy vegetarians has never been documented in medical literature.

METHODOLOGY

This study was done at Dow University of Health Sciences, Karachi, Pakistan for a period of six months in 2012 after the approval of the Institutional Review Board of the Dow University of Health Sciences. This was a observational case control study conducted in limited resource settings. A total N=200 subjects (males

n=147; females n=53) were recruited from a village called Chachro, Tharparkar Sindh. Sample size was calculated by using Open-Epi (i.e. Epi-Info for 95% confidence interval and margin of error as ±2.4% with prevalence of 3% according to WHO recommendations). Subjects were divided into two groups i.e. n=100 strict lacto-vegetarians and n=100 non-vegetarians.

This particular location was selected because more than half of the inhabitants were known to be strict lacto-vegetarians by birth as required by their Hindu religion. The staple diet of these subjects is wheat, millet, vegetables, and cow's milk and its products. Other non-vegetarian population from the same area was recruited as matching controls. Before recruitment, preliminary visits of this village were made to raise awareness about vitamin B$_{12}$ deficiency and its consequences. The inclusion and exclusion criteria were set up. Apparently healthy adult subjects of both genders from 14 to 60 years of age were included with no presenting complaint except recent history of flu, fever and diarrhoea. The exclusion criteria comprised children younger than 14 years, history of blood transfusion, history of any vitamin B$_{12}$ intake in the last three months, parenteral vitamin B$_{12}$ administration in the last two years, history of persistent diarrhoea, recurrent worms infestations, and pregnancy.

Consent was taken in local language i.e. Sindhi and a signature or a thumb impression was secured if the subject was uneducated. Qualified physicians filled a questionnaire recording the anthropometric and demographic data, short medical history, dietary habits, co-morbids, and clinical examination findings.

Blood samples were collected with aseptic precautions by venipuncture using vacutainer. From each participant, 5cc blood was collected in sterile tubes containing silicone gel for estimation of vitamin B$_{12}$ by using the COBOS E 411 analyzer. Tubes were kept for 20-25 minutes for clot formation and getting serum after centrifugation at 1500 rpm for six minutes. Blood Peripheral Smear was prepared and fixed with 70% methanol. Silicone gel tubes were stored at 0 degrees centigrade. Samples were transported in ice boxes within 8-10 hours to Dow Diagnostic Research Laboratory, Ojha campus, Karachi.

The data was analyzed by SPSS version 20. The variables such as age, gender, peripheral smear morphology findings, and Serum B$_{12}$ levels were entered in SPSS-16. Pearson Chi Square test was carried out for checking the significance of biochemical variables like Serum B$_{12}$ etc. and for computation of all continuous variables. Frequency and percentages were calculated and compared for all categorical variables like marital status, gender etc. The p-value of less than 0.05 was considered statistically significant.

RESULTS

The population under study comprised of N=200 subjects (males n=147; females n=53. Male to female ratio was 3.4:1.0). For comparative analysis, we took equal number of subjects from each group i.e. 100 strict lacto-vegetarians and 100 non-vegetarian subjects. The mean age of strict lacto-vegetarian group was 30.5 years (SD ± 8.3) and a closely matching mean age was found for the non-vegetarian group i.e. 30.1 years (SD±9.2).

The study investigated the pattern of dietary intake of the individuals belonging to the two groups.

Clinical examination of the two studied groups showed a mixed pattern of symptoms and signs but the vegetarian group revealed general weakness as a notable and prominent symptom (i.e. 54% of vegetarian-subjects versus 24% non-vegetarians) *Figure 1*. The percentages of these clinical symptoms help to understand their relationship with the observed haematological parameters.
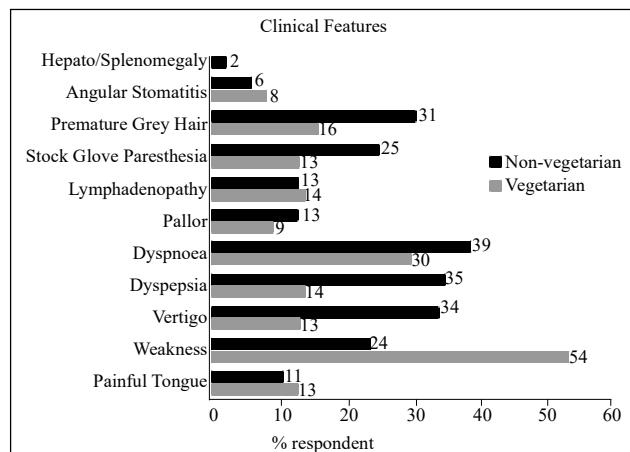

Figure 1: Assessment of clinical symptoms in strict lacto-vegetarian n=100 and non-vegetarian group, n=100.

The biochemical analysis showed that more subjects belonging to the strict vegetarian group were found to have low levels of vitamin B_{12} (i.e. <205ng/ml) than the non-vegetarians i.e. n=83 per 100 strict lacto-vegetarians as opposed to n=66 per 100 non-vegetarians, OR=9.4775, 95% C.1 (4.86, 12.94) and p <0.0001.

Table 1: Vitamin B_{12} distribution in study population, strict lacto-vegetarian n=100 and non-vegetarian group, n=100

	Vegetarian	Non - Vegetarian	Odds Ratio
Male			
Normal	14(20.6%)	23(29.1%)	Reference
Deficient	54(79.4%)	56(70.9%)	9.3913
Total	68	79	P<0.01
Female			
Normal	3(9.4%)	11(52.4%)	Reference
Deficient	29(90.6%)	10(47.6%)	8.7879
Total	32	21	P<0.05

Analysis based on gender was also carried out for the status of vitamin B_{12} in both groups. It showed that the strict vegetarian females were predominantly more predisposed to B_{12} deficiency (i.e. 90.6%) than the strict vegetarian males (i.e. 79.4%). However, only 47.6% non-vegetarian females showed B_{12} deficiency. These findings generated higher levels of Odds ratio as described in the Table 1.

Comparative analysis of the co-morbids present among the two groups was also carried out. Diabetes mellitus and acid peptic disease were observed to be more prevalent in the vegetarian group (i.e. 25 % of vegetarians were found to be diabetic in contrast with 16% non-vegetarians; acid peptic disease was found in 28% vegetarians versus 19% in non-vegetarian group). Using cross tabulation, more subjects from non-vegetarian group showed absence of major co-morbids i.e. 39% as compared to vegetarians i.e. 30%.

DISCUSSION

Multiple studies are carried out globally on various micronutrient elements[5]. Researchers and nutrition experts are highly interested in clinical presentations of micronutrient deficiencies mainly the vitamin B_{12} deficiency[6]. A major systemic review comprising many important search engines viz. PubMed, Medline, CINAHL plus, ERIC, Nursing and Allied Health Collection and Nursing/Academic Edition showed a maximum prevalence of vitamin B_{12} deficiency to be 86% with a preponderance associated with vegetarian population[7]. In some cases, food and drug interaction may cause vitamin B_{12} deficiency. In diabetic subjects, absorption of vitamin B_{12} is poor if they are taking metformin. This exposes and predisposes individuals with this common metabolic disorder to the development of macrocytic anaemia[8].

In the past, few clinical features related to anaemia and peripheral neuropathies were described in literature[9,10]. Now, vitamin B_{12} is considered important for many clinical disciplines like internal medicine, haematology, neurology, psychiatry, gastroenterology, gynaecology, geriatric medicine, pathology, and even general surgery because many clinical scenarios may occur because of unexpected low levels.

The present study is unique in the sense that it was done for the first time on a strict vegetarian population in a rural area of Pakistan. This area is inhabited equally by Muslims and Hindus; but the Hindus from this area are known to have been strict vegetarians by birth for many centuries. In most of the studies done previously, identifying a subject as a vegetarian may be considered problematic. Some people acquire vegetarianism later in life for any reason e.g. disliking the taste of meat products, fear of developing heart diseases, dyslipidaemia, or obesity etc[11-13]. These presumed vegetarians are predisposed to consuming animal products occasionally e.g. during Christmas, people

usually consume turkey meat which can supply a good amount of vitamin B_{12} which can be stored in the liver for 2-5 years[14]. In order to get more accurate results, the present study recruited only strict vegetarians from this area who have been vegetarians for generations because of religious restrictions.

In our study, the mean age of the vitamin B_{12} deficient vegetarians was 30 years which differs from the study done by Hashim and Tahir in Pakistan in 2006 who observed the mean age as 55 years[15]. This difference could be an indication of an early onset of etio-pathogenesis of B_{12} deficiency or could be due to our age-wise inclusion criteria (i.e. recruitment of subjects between 16-60 years). We did not include the paediatric population because vitamin B_{12} deficiency in this age group has different causes e.g. genetic factors and enzyme deficiencies[16]. Also, children are usually non-cooperative and reluctant to give blood samples. Older people were also excluded because of many factors related to this age group, viz. development of atrophic gastritis, anorexia, negligent attitude of caregivers, wrong selection of food, decreased absorption capacity of intestines, presence of co- morbids, and usage of multiple over the counters drugs[17].

In this study, male to female ratio was 1:2 in vegetarians and 3.4:1 in non-vegetarians. This is in contrast with most such studies done in different parts of the world in which gender was found to have made no difference[18]. This difference can be due to the local tradition of restricted exposure of women for medical examination.

As many as 23% subjects with positive history of addiction manifested definite lab evidence of macrocytic anaemia. These findings matched with the studies done before, like the one carried out by Piyathilake and his co- workers in 1995[19]. However, the role of the active substance used in smoking in relation to the pathogenesis of macrocytic anaemia needs further study. In the present study, the role of dietary habits in pathogenesis of macrocytic anaemia was the key factor in strict vegetarians.

Although, the study done by Sorfrizzi et al in 2003 showed that most of the cases of macrocytic anaemia are caused by the dietary deficiency of vitamin B_{12},[20] but most of the nutritional experts especially Indian investigators insist that strict vegetarians have enough vitamin B_{12} in their diets to avoid the consequences of its deficiency[21]. Therefore, this study tried to explore the facts in the vegetarian population of a locality in Pakistan.

The main typical dietary resource of vitamin B_{12} in the studied vegetarian population of Tharparkar is milk (0.45 to 0.60ìg/100ml), cheese (3.3ìg/100gm), yoghurt and cream (0.53 to 0.66ìg/100gm)[22]. Since the daily requirement of vitamin B_{12} for an adult is 400-600ìg, even a daily intake of about 200ml of milk would not meet the optimum B_{12} requirements in these individuals. In this study, overall 83% vegetarians were found to have vitamin B_{12} deficiency. On the other hand, only 66% non-vegetarians showed low levels of vitamin B_{12}. These findings clearly point to the significance and importance of dietary intake in genesis of macrocytic anaemia. In addition, this could also be due to overheating milk resulting in destruction of the vitamin. On the contrary, the non-vegetarians of Tharparkar usually eat chicken and occasionally mutton. In addition, they also consume various vegetables and dairy products.

The consequences of the vitamin B_{12} related anaemia were studied which showed that more vegetarian subjects (54%) gave a positive history of weakness and progressive fatigability as opposed to non-vegetarians who were lethargic (24%). Other features were less remarkable e.g. dyspnoea on exertion was noted in 30% of vegetarians and 39% of non-vegetarians, while vertigo and dyspepsia were noted in both groups in a few individuals. These finding are supported by the study done by Stable et al in 1990[23] which suggested that fatigability and lethargy are the most initial symptoms experienced by those who developed macrocytic anaemia. The study done by Louwman et al in USA in 2000 regarded these symptoms as a kind of warning before the frank development of macrocytic anaemia[24].

The population group investigated in this study can be considered highly predisposed to developing consequence of vitamin B_{12} deficiency. Women with low levels of vitamin B_{12} could become victims of neuropsychiatric problems and if pregnant have risk of the development of serious congenital anomalies e.g. newborns with neural canal abnormalities[25]. Children of lactating vegetarian mothers, who were exposed to low socioeconomic status, manifested neurodevelopmental delay and regression. This was observed in a study on 91 children age 9 years +/- 4 showing 17 (19%) children with vitamin B_{12} deficiency[26]. Some investigators have advised the vegetarian population with emphasis on suggestion to identify resources of vitamin B_{12}[27].

Statistical calculations carried out for analysis of vitamin B_{12} deficiency on the 200 apparently healthy subjects from this area showed odds ratio as 9.4 (95% CI 4.8) and p-value as <0.0001 which indicate existence of a highly significant level B_{12} deficiency in these individuals. The mean vitamin B_{12} levels was found to be 190.2 (normal <205.0ng/ml) in vegetarian and 226.7 in non-vegetarians which shows a preponderance of B_{12} deficiency among the vegetarian group. The correlation coefficient between vitamin B_{12} and age was positive i.e. 0.173 with significant p-value 0.0140. Our proposed hypothesis for the B_{12} deficiency quite satisfactorily matched with the results of clinical and statistical outputs. Other investigations should be carried out e.g. folic acid, serum TSH, serum iron, and particularly homocysteine and methylmalonic acid for proper assessment.

CONCLUSION

In the studied population of Tharparkar village, the vitamin- B_{12} deficiency was predominantly found in strict vegetarian subjects. Homocysteine and methylmalonic acid levels in serum or urine should be investigated; and that the vitamin B_{12} supplementation should be given to individuals from the high-risk areas of Tharparkar.

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ORIGINAL ARTICLE

Prevalance of Vitamin D Deficiency in Different Age Groups and Genders in Karachi: A Laboratory Study

Shazia Bhutto[1] and Naila Tariq[2]

ABSTRACT

Objective: This study aims to determine the prevalence of vitamin D deficiency and insufficiency in different age groups and genders in Karachi.

Methodology: Over a period of two months (September and October 2016), 323 samples were analysed in the department of clinical pathology at the Jinnah Postgraduate Medical Centre (JPMC), Karachi. Analysis of 25[OH]D was done by electrochemiluminescence Immunoassay technique.

Results: Out of the total 323 subjects, 228 were found to be deficient for vitamin D, the predominant age group for deficiency of vitamin D was between 20 and 50 years.

Conclusion: This study observed that females of age group 20 to 50 years were more predisposed to vitamin D deficiency.

Key words: Rickets, osteomalacia, osteoporosis, 25[OH]D

عنوان: کراچی میں مختلف عمروں اور جنس کے اعتبار سے وٹامن ڈی کی کمی کے پھیلاؤ کی موجودگی

تعارف: وٹامن ڈی ایک چربی میں حل ہونے والا وٹامن ہے۔ اس کے آنتوں، گردوں اور پیراتھائرائڈ غدود پر عمل کے نتیجے میں خون میں کیلشیم اور فاسفیٹ برقرار رہتے ہیں۔ وٹامن ڈی کی ہڈیوں میں معدن سازی اور نشوونما کے لیے ضروری ہے۔ یہ وٹامن جلد پر سورج کی روشنی پڑنے سے اور غذائی ذرائع سے جسم کے اندر پیدا ہوتا ہے۔ وٹامن ڈی کی مناسب مقدار رکٹس اور ہڈیوں کے نرم پڑنے سے محفوظ رکھتی ہے۔ کیلشیم اور وٹامن ڈی مل کر بزرگوں کو ہڈیوں کے بھر بھرے پن سے بچاتے ہیں۔

مقصد: اس مطالعے کا مقصد کراچی میں عمر اور جنس کے اعتبار سے مختلف گروپوں میں وٹامن ڈی کی کمی اور نا کافی مقدار کے پھیلاؤ کا اندازہ لگانا تھا۔

طریقہ: جناح پوسٹ گریجویٹ میڈیکل سینٹر (JPMC) کے کلینیکل پیتھالوجی شعبے میں دو مہینے (ستمبر اور اکتوبر 2016ء) کے دوران 323 (تین سو تیئس) نمونے جانچے گئے۔ 25[OH]D کی جانچ الیکٹرو کیمیلیو منیسنس امیونو اسے طریقے سے کی گئی۔

نتائج: تمام 323 نمونوں میں سے 228 میں وٹامن ڈی کی کمی پائی گئی۔ سب سے زیادہ کمی والے نمونے بیس سے پچاس سال تک کی عمر کے لوگوں کے تھے۔

حاصل مطالعہ: اس مطالعے کی رو سے بیس سے پچاس سال تک کی عمر کی خواتین میں وٹامن ڈی کی کمی کا رجحان زیادہ پایا گیا۔

INTRODUCTION

During the last decade, the beneficial effects of vitamin D on different aspects of health outcomes have generated growing interest. Vitamin D is a fat-soluble vitamin, which plays an important role in bone mineralization and growth because of its remodeling action through osteoblasts and osteoclasts activity[1,2]. Vitamin D helps to maintain calcium and phosphate homeostasis through its action on intestines, bones, kidneys, and parathyroid[2,3]. This vitamin is endogenously synthesized from exposure of skin to sunlight and dietary intake of sources of vitamin D like milk, yogurt, egg yolk, cheese, fish, liver, fortified products, and supplements[2-4]. Vitamin D has a significant role in skeletal health, and prevention of muscle weakness, bone loss, falls and fragility fractures in the elderly. Together with calcium, it helps to protect from osteoporosis in older adults and females after menopause[5]. These health problems

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increase morbidity, decrease quality of life, and enhance the costs to health facilities.

Karachi is a densely populated city home to different socioeconomic groups with varied eating habits. Most of the population lives in closed houses and have little exposure to sunlight. For this reason, Karachi residents are at risk for developing vitamin D deficiency. Vitamin D deficiency is a global problem. An estimated 1 billion people have inadequate vitamin D levels, across all ethnicities and age groups[6,7]. Its deficiency and insufficiency has been documented as a frequent public health problem in Europe[8]. In a single centre analysis done at a U.A.E. hospital on individuals from 136 countries, 82.5% showed deficiency to insufficiency of vitamin D, out of whom the majority were females[9]. Vitamin D deficiency's reported prevalence in the Indian subcontinent is 70-100% in the general population[10]. A study reported in Lahore showed 90% of premenopausal females had deficient serum 25[OH] D levels[11]. Another study conducted at Aga Khan University in apparently healthy adults, showed deficient vitamin D in 69.9% and 21.1% insufficient serum vitamin D levels[12].

The purpose of our study was to achieve local data from a government tertiary care centre, where most of the low income people receive services. Therefore, this study is documenting the prevalence of vitamin D deficiency. The data generated will be helpful in making policy decision regarding essential evaluation of vitamin D.

METHODOLOGY

This was a cross-sectional study conducted at the Clinical Pathology department at the Jinnah Postgraduate Medical Centre (JPMC), Karachi over a period of two months from September to October, 2016.

Ethical Consideration

Ethical permission for this study has been taken from the ethical review committee of the JPMC. Consent and assent was acquired from respondents by means of informed consent form.

Sample Size

Calculated sample size was 323. Calculation done by using software open epi. At the confidence level of 95% and bound on the error of 5%, taking the prevalence of 70% from the previous study at AKU on vitamin D deficiency among apparently healthy adults[12].

Data Collection Method

Sample was collected by non-probability sampling technique. Individuals included in the study were residents of Karachi, male and female from age 01 year to 75 years. Patients diagnosed with metabolic bone diseases, diabetes, pregnant women, lactating mothers and those who are on vitamin D therapy were

excluded. After taking consent from subjects, the questionnaire recorded information on age, gender, co-morbidities, and vitamin D supplements intake. Specimens of venous blood were collected in lithium heparin tube. Serum was separated after centrifugation at 3000 RPM for five minutes. Serum marker 25(OH) vitamin D was measured on automatic analyzer, Cobas (Roche diagnostics), by electrochemiluminescence Immunoassay technique. Subjects were divided into three groups according to serum 25[OH]D concentration, deficient group identified at vitamin D levels 20 ng/ml, insufficient at 21-29 ng/ml, 30 ng/ml was considered sufficient[2]. Individuals were grouped in male and female. Another four groups were categorized according to age: first group from 1 year to 12 years, second from 13 to 30 years, third from 31 to 50 years and fourth group was from the age of 51 to 75 years. The statistical data was analyzed on SPSS. P value of less than 0.05 was considered significant.

RESULTS

Out of the total 323 subjects, 71 were males (22%) and 252 were females (78%). Among these, 228 (70.5%) were found deficient for serum vitamin D level, 32 (10%) subjects had insufficient vitamin D levels, other 63 (19.5%) individuals had vitamin D levels within normal limits. Within the deficient population, 183 (80%) were females and 45 (20%) were males. Out of the total female population, 72.6% were found to be deficient. By age groups, the vitamin D level was found to be deficient in 3% of respondents from 1 to 12 years of age; 43% in respondents from 13 to 30 years; 46% in the age group from 31 to 50 years; and 8% in 51 to 75 years olds. Highest prevalence of the deficiency was found in respondents between 31 and 50 years of age.

Table 1: Gender Versus Frequency

Gender	Frequency of Participants	Percent	No. of Deficient Participants	Deficiency by Gender (%)
Male	71	22	45	20
Female	252	78	183	80
Total	323	100	228	100

Table 2: Vitamin D Deficiency in Different Age Groups

Age Groups		Vitamin D Deficiency in Population (%)
Children	1–12 yrs	3
Young	13–30 yrs	43
Middle-aged	31–50 yrs	46
Old	51–75 yrs	8

Categories of Vitamin "D" Status with Frequency

Sufficient
19.5%

Insufficient
10%

Deficient
71.5%

deficiency and might cause early osteoporotic changes in this particular group.
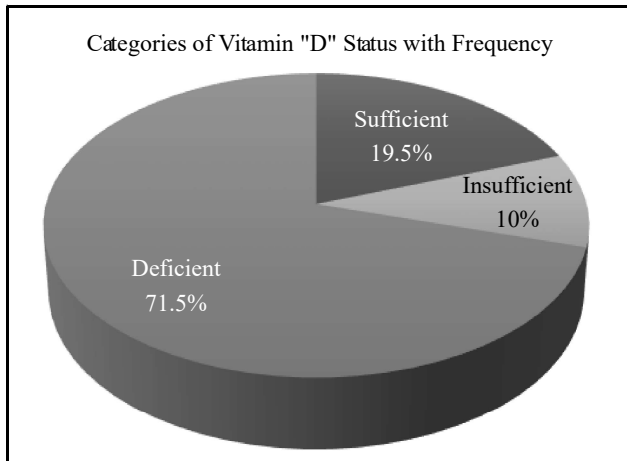
DISCUSSION

As seen in our study, deficient levels of vitamin D (20 ng/ml) were present in 70% and insufficient levels of vitamin D (21-29 ng/ml) were in 10% of the population. Overall, 80% of the study poipyulation was found to have deficient and insufficient vitamin D levels.These findings are comparable to those reported by other studies done in Karachi on vitamin D levels. As reported by Adil Sheikh et al., (84.3%) population were found deficient for Vitamin D[10]. Another study by Aysha Habib Khan et al, conducted at AKU, suggested a high prevalence (90%) of the deficiency and insufficiency in a group of healthy adult population living in Karachi, Pakistan[11]. Our study noted that 20% of population had sufficient vitamin D levels (30 ng/dL), which is comparable with other studies: 15.7%, 8.9%, reported by Adil Shaikh et. Al and Mansoor et al. respectively, while Zuberi et al has documented 8% sufficient vitamin D levels in population[12-14].

Our study also showed vitamin D deficiency predominantly in females from age group 13 to 50 years, and predominent deficiency observed in 20 to 50 yrs, which is in agreement with the study by Kashaf Junaid et. al that showed 73% vitamin D deficiency in women of child bearing age[11]. A study done in India reported that 90% of the population had insufficient vitamin D levels, 40% were deficient, and 10% had serum vitamin D levels within normal limits. The study also showed 28% of men and 52% of women deficient in vitamin D. These results also match with our study. These results also coincide with our study where females are 80% deficient and males 20%[15]. A study in the US has reported that 41.6% of the US population has vitamin D deficiency, which is comparable with our results[16].

CONCLUSION

The main observation from this study is that females of age group 20 to 50 years are more predisposed to vitamin D deficiency than adult males and children. This could be attributed to lack of dietary intake, inadequate exposure to sunlight leading to vitamin D

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Spirometric Evaluation of Asthmatic Children and Its Association with Biophysical Parameters: A Case Control Study

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ABSTRACT

Objective: The purpose of this study was to perform pulmonary function test in asthmatic children and to associate the results with biophysical parameters, and to compare these findings with those of normal healthy controls.

Methodology: A total number of 88 subjects (aged 6-14 years) of both genders were included. The study was carried out from October 2014 to April 2015. Cases were diagnosed to be asthmatic patients according to GINA guideline. Healthy non-asthmatics were recruited from a local school. A written and verbal consent was taken from the subject's guardians after explaining the study. The subjects' pulmonary function test included forced vital capacity (FVC), forced expiratory volume in 1st second (FEV1) and FEV1/FVC ratio. Biophysical parameters were age, gender, and BMI. Spirometry was done by vitalograph.

Results: Total 88 subjects were divided into two equal groups, which were matched for age and sex. Comparison of biophysical parameters showed lower values of BMI in cases than controls ($p < 0.001$). We did spirometry in both groups. Lower values of FVC, FEV1 and FVC/FEV1 were observed in asthmatic group ($p < 0.001$). We also found strong association of BMI with asthmatic state (OR = 0.961, $P < 0.001$). Double chances of asthma in males than females (OR = 2.141) and decreased chances of asthma with increasing age (OR=0.848) but both these findings were statistically insignificant ($p > 0.05$).

Conclusion: Study concludes that FVC, FEV1 and FEV1/FVC were significantly lower in asthmatic children than in healthy controls and BMI is strongly correlated with asthmatic state. Furthermore, spirometry should be considered as a routine assessment of children with bronchial asthma.

Key words: asthma, spirometry, asthmatic children, biophysical parameters

عنوان : دے کے مریض بچوں کی سپائرومیٹرک تشخیص اور اس کی حیاتی وطبیعی متعین مقداروں سے مطابقت :ایک کیس کنٹرول مطالعہ

مقصد:اس مطالعے کا مقصد دے کے مریض بچوں میں پھیپھڑوں کی کارکردگی کا تجزیہ کر کے ان کے نتائج کی حیاتی وطبیعی طور سے متعین مقداروں سے مطابقت دیکھنا اور ان نتائج کا صحتمند بچوں کے نتائج سے تقابل کرنا تھا۔

طریقہ: چھے سے چودہ سال کی عمر کے ۸۸ لڑکے اور لڑکیاں اس مطالعے میں شامل کیے گئے ۔ یہ مطالعہ اکتوبر ۲۰۱۴ سے اپریل ۲۰۱۵ تک کیا گیا ۔ کیسز میں GINA ہدایات کے مطابق دمہ تشخیص کیا گیا ۔ صحت مند بچے ایک اسکول سے لیے گئے ۔ تحقیق کے شرکاء کے سرپرستوں سے تحقیق کی وضاحت کرنے کے بعد زبانی اور تحریری رضا نامہ لیا گیا ۔ شرکاء کے پھیپھڑوں کی کارکردگی کے تجزیہ میں FVC،FEV1, اور FEV1/FVC کے ratios شامل تھے ۔ سپائرومیٹری وائٹلوگراف کے ذریعے کی گئی ۔ شماریاتی تجزیہ SPSS سافٹوئیر کے ورژن ۱۹ پر کیا گیا۔

نتائج: تمام ۸۸ شرکا کو عمر اور جنس کے لحاظ سے دو گروپس میں تقسیم کیا گیا ۔ حیاتی طبیعی حدود کے تقابل میں کیس گروپ کا BMI کنٹرول گروپ سے کم پایا گیا ۔ (p<0.001) دونوں گروپس میں سپائرومیٹری کی گئی ۔دے کے مریض گروپ میں FEV1 ،FVC, اور FVC/FEV1 کی کم تر مقدار کا مشاہدہ کیا گیا۔ BMI کی دے سے مضبوط شماریاتی مطابقت پائی گئی ۔ (OR=0.961, p<0.001)

لڑکوں میں دے کے امکان لڑکیوں سے زیادہ پایا گیا (OR=2.141)اور بڑھتی عمر کے ساتھ دے کے امکان کم ہوتے ہوئے تخمینہ کیے گئے ۔ مگر یہ دونوں نتائج شماریاتی اعتبار سے غیراہم تھے۔(p<0.05)

حاصل مطالعہ:اس مطالعے کے مطابق دے کے مریض بچوں میں FEV1 ،FVC, اور FEV1/FVC صحتمند بچوں کی نسبت خاصے کم پائے گئے ۔اور BMI کا دے کی کیفیت سے مضبوط تعلق پایا گیا ۔ اس کے علاوہ سپائرومیٹری کو دے کے مریض بچوں میں معمول کا امتحان سمجھا جانا چاہیے۔

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INTRODUCTION

Asthma affects 300 million people worldwide. It is an important cause of hospitalization among children under 15 years of age. In the United States, asthma is the most common cause of childhood emergency department visits, hospitalizations, and absent school days[1]. It is thought to be caused by a combination of genetic and environmental factors[2]. Its diagnosis is usually based on the pattern of symptoms, response to therapy over time and spirometry[3]. It is clinically classified according to the frequency of symptoms, forced expiratory volume in 1st second (FEV1), and peak expiratory flow rate[4]. Asthma may also be classified as atopic (extrinsic) or non-atopic (intrinsic) where atopy refers to a predisposition toward developing reactions[5]. Asthma is affecting (1-18%) of the population of different countries[6]. It is characterized by variable expiratory airflow limitation. Lung function may vary between completely normal and severely obstructed in the same patient[7]. Forced expiratory volume in 1st second (FEV1) from spirometry is more reliable than peak expiratory flow (PEF) but a reduced ratio of FEV1 to FVC indicates airflow limitation. The FEV1/FVC ratio is normally greater than 0.75 to 0.80, and usually greater than 0.90 in children. Any values less than these suggest airflow limitation[8]. Asthma is the result of chronic inflammation of the airways which results in increased contractibility of the surrounding smooth muscles[9].

METHODOLOGY

This case control study was conducted during October 2014 to April 2015 in the Department of Physiology, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, in collaboration with the National Institute of Child Health (NICH), Karachi. Approval was taken from Institutional Ethical Review Board (IERB) of NICH, Karachi, for conducting the research (IERB No: 09/2014).

A total number of 88 children were included in this study. Forty-four non-asthmatic healthy children were recruited from a local school as controls and 44 diagnosed asthmatic children from NICH as cases. A detailed history, questionnaire, and written consent was taken from every subject's guardians. The age of the children was between 6 and 14 years and the asthmatic children were diagnosed and classified according to the Global Initiative for Asthma guidelines. The children with chronic pulmonary disease e.g. cystic fibrosis, bronchiectasis were excluded from the study. The information of age, gender, forced vital capacity FVC (L), and forced expiratory volume in 1st second FEV1 (L), FVC (L) and FEV1 / FVC (%) were collected

from each participant. Pulmonary function test was carried out by vitalograph compact. The BMI categorization was defined according to the WHO percentile references according to age, which were as follows:

Severely overweight ($\geq 97^{th}$ percentile), overweight (90th to 97th percentile), normal (5th to $< 90^{th}$ percentile), underweight (3rd to 5th percentile) and severely underweight ($< 3^{rd}$ percentile) (WHO, 2007)[10]. Spirometry was performed by vitalograph compact spirometer model 6600, Ennis company, Ireland. It was done according to the user manual.

RESULTS

Table 1 describes the comparison of biophysical parameters between cases and controls. The control subjects included 25 boys and 19 girls and asthmatic children were 26 boys and 18 girls (Figure 1). The mean age of control group was 10.84±1.73 years while the mean age of case group was 10.06±2.23 years. Median BMI of controls was 50th percentile and median BMI of cases was 10th percentile, which was found to be highly significant (p<0.001) (Table 1).

Table 1: Comparison of Biophysical Parameters between Control and Case Groups

Variables	Control (n=44) Mean± SD	Case (n=44) Mean ± SD
Age (years)	10.84±1.73	10.06±2.23
Gender:		
Female	19(43.2%)	18(40.9%)
Male	25(56.8%)	26(59.1%)
BMI percentile*	50^{th}(25-92.5)	10^{th}(1.5-25)**

* Median (interquartile range)
**Statistically highly significant p- value < 0.001

Table 2 shows the comparison of pulmonary function test between healthy controls and asthmatic children. Mean values of FVC (L), FEV1 (L) and FEV1/FVC% were significantly lower than normal healthy controls (p < 0.001).

Table 2: Comparison of Pulmonary Function Tests Between Control and Case Groups

Variables	Control Mean±SD	Case Mean±SD
FVC(L)	2.54±0.52	1.37±0.50**
FEV1(L)	2.33±0.52	1.15±0.45**
FEV1/FVC (%)	91.25±4.25	84.27±12.50**

**Statistically highly significant p- value< 0.001

Table 3 is the multiple binary logistic regression analysis of the relationship between biophysical parameters and asthma. This analysis indicates that the chances of asthma are lower with increased BMI and this is statistically significant (OR = 0.961, P< 0.001). This

table shows a two times increase in the chances of developing asthma in males than females (OR =2.141) and decrease chances of asthma with increasing age (OR=0.848) but both these findings are statistically insignificant (p>0.05).

Table 3: Association between Asthmatic State and Age, Sex, BMI in Multiple Binary Logistic Regression Analysis

Parameter	Coefficient of B	Significance	OR
Male	0.761	0.287	2.141
Age (years)	-0.165	0.380	0.848
BMI (percentile)	-0.40	<0.001	0.961

Statistically highly significant p- value < 0.001
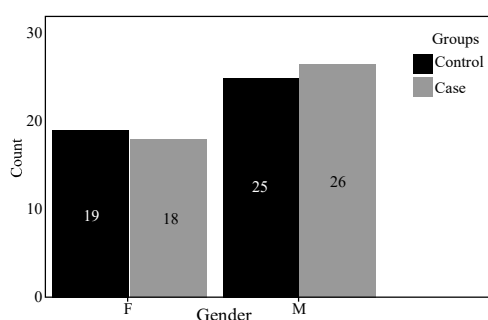

Figure 1: Gender Distribution in Control and Case Groups

DISCUSSION

This study compared pulmonary function tests of normal, healthy, non-asthmatic children with registered diagnosed asthmatic children. Eighty-eight (88) subjects were enrolled in this study. These subjects were divided into control (healthy non-asthmatics) and case (asthmatics) groups equally. Both groups were matched for age and sex.

The comparison of pulmonary function test between controls and cases found statistically significant lower values of FVC, FEV1 and FEV1/FVC% in cases than in controls.

The comparison of BMI in control and case groups found lower BMI in cases than controls, which was statistically significant. In a nine-year European longitudinal study, de Marco et al[12]. showed no difference in baseline BMI across asthma severity groups using GINA severity classification.

In multiple binary regression analysis, we noticed 26% decreased chances of asthma with increased BMI. However, Minto Porter et al.[13], had found that increased BMI was associated with increased chances of asthma. In the study by Taylor et al.[11] obese subjects with asthma were more likely to report continuous symptoms, miss more workdays, use short acting beta agonists,

use inhaled corticosteroids, and use any controller medication according to GINA guidelines. Also, obese were more likely to have severe persistent asthma.

In binary regression model, younger age was found to be associated with increased asthmatic state. Alyasin et al.[14] also showed in univariate analysis the relationship between asthmatic state and age, sex, and vitamin D concluding that younger age and lower vitamin D levels were associated with significantly increased odds of asthmatic state.

In our study, we noticed increased chances of asthma in males as compared to females, which was not reported by Brehm et al.[15] in asthma management program study.

CONCLUSION

Study concludes that FVC, FEV1 and FEV1/FVC were significantly lower in asthmatic children than in healthy controls and BMI is strongly correlated with asthmatic state. Furthermore, spirometry should be considered as a routine assessment of children with bronchial asthma.

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Certain types of articles, such as guidelines produced by governmental agencies and professional organizations, may need to reach the widest possible audience. In such instances, editors sometimes choose deliberately to publish material that is also being published in other journals, with the agreement of the authors and the editors of those other journals. Secondary publication for various other reasons, in the same or another language, especially in other countries, is justifiable, and can be beneficial, provided all of the following conditions are met.

1. The authors have received approval from the editors of both journals; the editor concerned with secondary publication must have a photocopy, reprint, or manuscript of the primary version.

2. The priority of the primary publication is respected by a publication interval of at least one week (unless specifically negotiated otherwise by both editors).

3. The paper for secondary publication is intended for a different group of readers; an abbreviated version could be sufficient.

4. The secondary version faithfully reflects the data and interpretations of the primary version.

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- Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publications—International Committee of Medical Journal Editors

ORIGINAL ARTICLE

Prevalence of First Permanent Molar Caries among 8 to 12 Years Old School-Going Children Living in Dammam, Kingdom of Saudi Arabia

Soban Qadir Khan[1], Imran Farooq[2], Aws Saleh ArRejaie[3], Abdul Khabeer[4]
and Faraz Ahmed Farooqi[1]

ABSTRACT

Objective: The aim of the study was to determine the prevalence of caries in first permanent molars (FPM) among children aged from 8 to 12 years.

Methodology: This cross-sectional study was performed from May to November 2014 on school-going children living in Dammam, Saudi Arabia. A total of 540 children aged between 8 and 12 years were randomly selected from different schools of Dammam, out of whom 322 were boys and 218 were girls. The number of carious FPM in each jaw was noted separately.

Results: The prevalence of caries in FPM among 540 examined children was found in 269 (49.8%). Among these 269 children, 109 (40.5%) children had only one carious molar, 75 (27.9%) children had caries in two molars, 43 (16%) children had three carious molars, and 42 (15.6%) children had caries in all four FPM. There was no statistically significant link between prevalence of carious FPM and gender. Caries prevalence with increase in age was found to be statistically significant (P-value = 0.00).

Conclusion: Dental health care professionals should be concerned about the findings of nearly 50% untreated carious FPM. Multiple factors can contribute to the high prevalence such as socioeconomic status, culture, and easy access to cariogenic food. Irrespective of the exact cause, an early intervention by educating children in schools about the etiological factors and consequences of dental caries is highly desirable.

Key words: First permanent molars (FPM), caries, school-going children

عنوان: دمام سعودی عرب کے 8 سے 12 سال تک کے بچوں میں پہلی مستقل داڑھ میں کیڑے لگنے کا نفوز۔

مقصد: اس تحقیق کا مقصد 8 سے 12 سال کے بچوں میں پہلی مستقل داڑھ میں کیڑے لگنے کا پھیلاؤ معلوم کرنا تھا۔

طریقہ: مئی تا نومبر 2016ء کے درمیان دمام سعودی عرب کے بچوں میں ایک عمومی جائزہ تحقیق کی گئی۔ پانچ سو چالیس آٹھ سے بارہ سال کی عمر کے بچے دمام کے مختلف اسکولوں سے بلاتفریق اس تحقیق میں شامل کئے گئے۔ ان میں 322 لڑکے اور 218 لڑکیاں شامل تھیں۔ ان بچوں کی پہلی مستقل داڑھ کا معائنہ کیا گیا۔

نتیجہ: ان 540 بچوں کے معائنہ میں 269 (49.8%) بچوں کی داڑھ میں کیڑے لگنے کا نفوز پایا گیا۔ ان 269 بچوں میں 109 (40%) بچوں کی ایک داڑھ 75 (27.9%) بچوں کے ایک داڑھ میں کیڑے لگنے کے پھیلاؤ اور جنس میں کوئی شماریاتی فرق نہیں پایا گیا۔ جبکہ عمروں میں اضافہ اور کیڑے لگنے کے اضافہ میں شماریاتی ہم ربطی پائی گئی۔

خلاصہ: بچوں میں تقریباً 50% بلا علاج کیڑے لگے دانتوں کی زیادہ مقدار کی وجہ معاشی ساجی اور دانتوں کو متاثر کرنے والے کھانوں کی آسانی سے دستیابی ہو سکتی ہے۔ اس سوال کو چھوڑتے ہوئے کہ اس کی وجوہات کیا ہیں، اسکولوں میں بچوں کے دانتوں کا مستقل معائنہ ہونا چاہیے اور دانتوں میں کیڑے لگنے میں کمی لانے والے طریقہ کا استعمال ہونا چاہیے۔

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INTRODUCTION

First permanent molars (FPM) most commonly erupt at the age of 6 or 7 years[1]. Due to this early eruption, FPM undergo challenges from occlusion and external environment such as acids and microorganisms at an early stage[2]. Moreover, their anatomy and location also leaves the FPM most susceptible to dental caries[3,4]. Hescot and Rolan (1993) report that 4.9% of FPM are carious at the age of six years[3]. According to McDonald

(1992), during the early stages of eruption, the occlusal surface of FPM is most susceptible to caries, as only the occlusal surface is exposed to the oral cavity[4]. Other reports have suggested that the mesial surface of FPM is more prone to caries as compared to the mesial surfaces of other teeth[5], due to the mesial contact of FPM with the carious distal surface of second deciduous molar. Due to this caries contact, the chances of caries in FPM reportedly increases 15 times[6], however, the studies backing this statement are not strong.

Recently, a change in trend in the prevalence of dental caries has been observed in different parts of the world. Improvements in gingival health and reduced caries prevalence have been reported in many developing countries[7]. These improvements can be attributed to increase in awareness of dental hygiene including proper brushing techniques, flossing and use of mouth washes[8]. According to the World Health Organization (WHO), the goal for the year 2000 was to achieve a DMFT (decayed, missing, and filled teeth) score of 3 or less at the age of 12 years, while for 2010 the goal was set to a DMFT score of 1[9].

A study performed in China by Yu Xue et al. in 2015 reported a mean DMFT score of 1.3 ($\pm$1.59) for FPM[10], while another study completed in Iran, reported a DMFT score of 1.9$\pm$1.6. Various studies done in different regions of Saudi Arabia have also reported increased caries prevalence in FPM in primary school children. A study from Jeddah, a western city of Saudi Arabia, reported a DMFT score of 2.5 in children aged between 9 and 11 years[11]. Another study performed in 7–11 years old school children of the southern region of Saudi Arabia, showed a DMFT score of 2.74$\pm$1.18[12].

The prevention of dental caries remains an important challenge for dentists and other oral health care personnel, as dental caries can have an impact on an individual's diet leading to poor health[8]. Moreover, the cost of treatment for dental caries is relatively high, therefore efforts are required to prevent dental caries. Currently, there is a lack of information regarding the prevalence of dental caries in FPM in the eastern regions of Saudi Arabia. Therefore, the aim of our study was to determine its prevalence in children aged between 8 and 12 years.

METHODOLOGY

This cross-sectional study was performed during May to November 2014 to investigate the prevalence of FPM caries among school going children living in Dammam, eastern region of Kingdom of Saudi Arabia (KSA). Ethical approval for the study was obtained from Institutional Review Board (IRB) of the University of Dammam.

A total of 540 children from different private and government schools located in Dammam, KSA, were randomly selected. Their ages varied from 8 to 12 years. Two stage sampling technique was used to select the required sample. Out of these 540 children, 322 were boys and 218 girls. Informed consent was obtained from the heads of the schools before starting the study. Two trained examiners separately checked the students while a trained assistant recorded data. Examinations were performed on school chairs in rooms with sufficient light to enable acceptable intraoral visualization. All participants were examined with disposable mirrors, disposable probes, and disposable tweezers alongwith disposable masks and gloves. Cotton was used to dry the teeth. A form was developed under the WHO guidelines for the diagnosis of dental caries[13]. Form included various demographic details of the participants like age, gender, nationality with information about brushing habits and a dental chart for recording DMFT. Intra-examiner reliability was tested by using Kappa statistics. The level of agreement between the examiners was 0.89, which was under the acceptable consistency expected among the examiners[14].

Statistical analysis

Statistical Package for Social Sciences (SPSS, version 19.0, SPSS Inc., Chicago, IL, USA) was used for data entry and analysis. In descriptive statistics, bar diagrams were used to present the children's demographics. Student's t-test was used to check statistical significance between the prevalence and the ages of the participants. Chi-square test was employed to compare the caries prevalence with gender and brushing habits. The level of significance was set at $p < 0.05$.

RESULTS

The age of the participants varied from 8 to 12 years. There were more boys in the study than girls: 322 (59.6%) boys and 218 (40.4%) girls. The prevalence of caries in FPM among 540 examined children was found to be 49.8%. Among 269 children who had caries in FPM, 109 (40.5%) had caries in only one molar, 75 (27.9%) had it in two molars, 43 (16%) in three molars and 42 (15.6%) had caries in all four. Caries was observed to be more prevalent in the lower jaw than in the upper jaw. Forty-seven (17.5%) children out of 540 were found to have caries in the upper jaw, 117 (43.5%) in the lower jaw, and 105 (39%) had caries in both jaws.

The prevalence of carious teeth among boys was 50% while it was a little higher in girls (52.7%) (Figure 1).
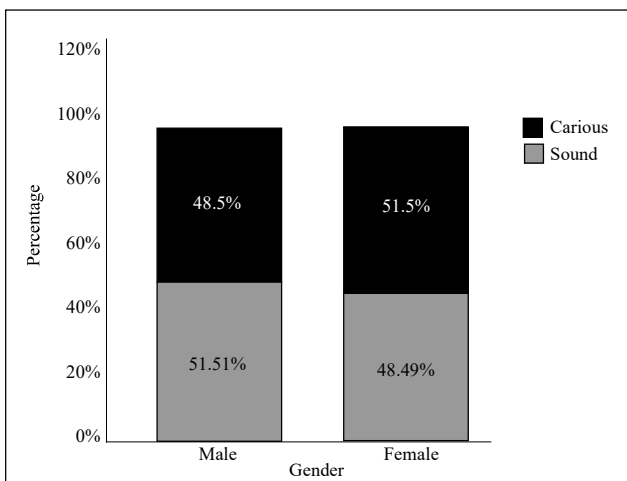
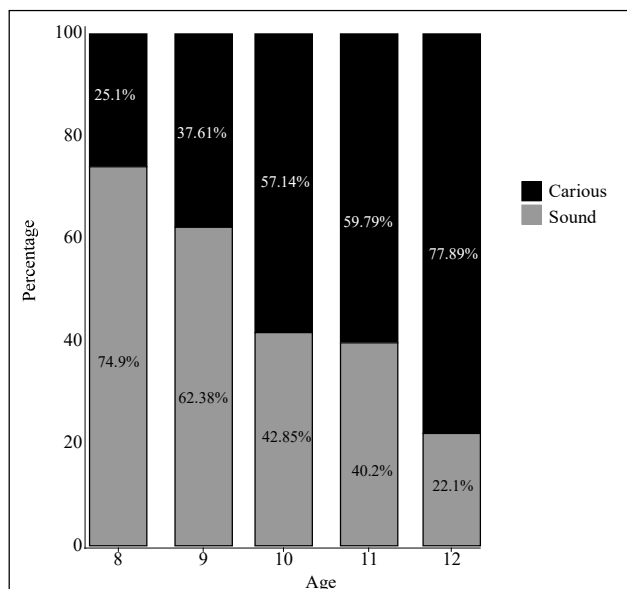Figure 1: Number of Carious and Sound Teeth among Males and Females

Figure 2: Number of Carious and Sound Teeth at Various Ages

However, there was no statistically significant difference in the prevalence of carious teeth according to gender. With the continuous increase in ages, it was observed that the prevalence of caries also increased (Figure 2). The prevalence of caries among 8-years-old children, was found to be 25.9% only. In 9-year-olds it increased to 38.5%; in 10- year-olds it rose to 58.9%; In 11-year-olds it was 61.9%; and in 12-year-olds, it became 78.9%. That continuous increase in prevalence according to age was found statistically significant as well (p-value = 0.00).

The children were also asked about their brushing habits and 81% (n=438) reported brushing daily. When asked about the frequency of brushing, 49.2% students reported brushing twice a day while 39% brushed only once a day. The rest reported brushing more than twice in a day.

DISCUSSION

The present study assessed the prevalence of dental caries in FPM of children. The FPM were selected for evaluation as they are the largest teeth in the oral cavity, bear maximum occlusal load, and are also the first permanent teeth to appear in the oral cavity, thus establishing that they are exposed to the external environment adversities for the longest period as compared to the other permanent teeth[2].

It can be observed from the results of this study that in 49.8% students (269 of 540), one or more FPM were affected from caries. A similar study carried out in Japan reported a caries prevalence of 50% among 11-12 years old children whereas a study from Pakistan, reported a caries prevalence of 30.6% among 8-12 years old school children[16,17]. The prevalence of FPM caries reported in our study is less than the one reported from a previous study carried out in Jeddah, Saudi Arabia which established that 75.5% (326 of 432) 9-12 years old children had one or more FPM affected from dental caries[15]. Compared to similar international studies, the results of the present study indicate a higher prevalence of FPM caries in Saudi Arabia than in the other parts of the world. Many reasons such as differences in socioeconomic status, culture, and easy access to cariogenic food could be put forward to explain this trend. Irrespective of the exact cause, an early intervention by educating children in the schools about the etiological factors and consequences of dental caries is highly desirable.

The results of the current study also indicate that 552 FPM of 540 children were affected from caries; lower jaw being more affected (n = 342) than the upper jaw (n = 210). This result is similar to the results reported from a study carried out in Karnataka, India where it was also observed that mandibular arch had more caries prevalence than the maxillary arch[18], which could be due to inappropriate indirect visualization. Another study carried out to observe caries prevalence among 7-10 years old children from Abha, Saudi Arabia also reported that mandibular molar was more affected than the maxillary molar[19]. The probable reason for mandibular FPM being more affected from caries could be their complex anatomy (presence of deep pits and fissure, and large anatomical crown size) and early eruption time as compared to the maxillary FPM[19]. Therefore, the use of fluoride and application of fissure sealants on susceptible teeth could lead to a decrease in the prevalence of dental caries[20].

The results of the present study indicate that prevalence of FPM caries was directly proportional to the increase in the age of the participants. This finding is similar

to the findings of a few previous studies which were also aimed at detecting the prevalence of FPM caries[15,19]. The possible reason for this finding could be that the FPM of older children have had longer exposure to acidic environments since their eruption, leading to more demineralization, as compared to the FPM of the younger children.

In the present study, the students were also enquired about their brushing habits and 81% reported that they brush their teeth daily. This could be the reason for relatively low caries prevalence of FPM reported in this study from Saudi Arabia as compared to the previously carried out studies from this country[15,19]. Data regarding the frequency of tooth brushing shows that only 49.2% were brushing twice a day. It is recommended to introduce educational programmes at schools aimed at emphasizing the importance of regular tooth brushing and the role of fluoride in prevention of caries in order to make the children more aware and conscious about their oral health.

The results of the present study show the prevalence of dental caries in FPM of children aged 8-12 years. The limitations of the present study include small sample size and the study being conducted in only one region. Furthermore, inclusion of questions about the children's eating habits could also help in determining the reasons of caries prevalence in FPM. Future studies with greater sample sizes conducted in various regions of Saudi Arabia could help in establishing a clearer picture of the prevalence of caries in FPM in children and necessary steps could be taken to decrease their prevalence.

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Importance of Serum Iron and Total Iron Binding Capacity as Important Tools in the Diagnosis of Iron Deficiency Anaemia

Fauzia Hashmi[1] and Naila Tariq[1]

ABSTRACT

Objective: To evaluate the importance of serum ferritin in the diagnosis of iron deficiency anaemia as compared to other diagnostic parameters

Methodology: Serum iron and Total Iron Binding Capacity (TIBC) was evaluated among three groups with group-A comprising male patients (n=150), group-B comprising females (n=150) and group-C designated for pregnant women (n=150). All groups were falling in the age group of 20 to 40 years. These patients were from medical and Obstetrics/ Gynae OPD of JPMC, Karachi. The subjected parameters were analyzed on instruments Beckman Coulter and Selectra using Colorimetric method.

Results: The results indicated that in group A, 48/150 (32%) participants showed less value of serum iron from the normal range (50-168 ug/dl) while their TIBC was reported to be inversely proportional to serum iron deficiency, elevated from the normal recommended range for males and females (250-450ug/dl) in 29/48 (19.3%) cases. Similarly, in group B, 56/150 (37%) cases were positive for serum iron deficiency while TIBC was raised in 41/56 (73.2%). Group C reported lower value of serum iron in 83/150 (55.3%) participants and raised TIBC in 62/83 (74.6%).

Conclusion: On the basis of the above findings, it is concluded that the estimation of serum iron and TIBC gives accurate analysis and these are important tools in the diagnosis of Iron Deficiency Anaemia.

Key words: Serum iron, TIBC, iron deficiency anaemia

عنوان: خون کی کمی کی تشخیص میں فولاد اور TIBC کے امتحان کی اہمیت۔

پس منظر اور مقصد: خون میں 11.5g/dl سے کم فولاد کی مقدار انیمیا کہلاتی ہے۔ یہ دنیا بھر میں سب سے عام پائی جانے والی غذائی کمی ہے۔ پاکستان میں 30 فیصد مرد اور 70 فیصد خواتین اس کمی کا شکار ہیں۔ اس معاملے کا مقصد دوسرے تشخیصی امتحانات کے مقابلے میں سیرم فیریٹن کی اہمیت کا اندازہ لگانا تھا۔

طریقہ: شرکاء کو تین حصوں میں تقسیم کرکے سیرم فولاد اور TIBC کو ناپا گیا۔ حصہ (الف) مردوں پر مشتمل تھا (n=150)، حصہ (ب) میں خواتین تھیں (n=150)، اور حصہ ج حاملہ خواتین (n=150) پر مبنی تھا۔ تمام شرکاء کی عمر 20 سے 40 سال کے درمیان تھی۔ یہ سب جناح ہسپتال کے طبی اور زنانہ OPD میں آنے والے مریض تھے۔ تمام نمونوں کو بیکمین کولٹر اور سلیکٹر آلات پر کلری میٹرک طریقے سے جانچا گیا۔

نتائج: نتائج کے مطابق حصہ الف کے 150 میں سے 48 (32%) شرکاء میں سیرم فولاد کی مقدار عمومی مقدار (50-168ug/dl) سے کم پائی گئی جبکہ ان 48 میں سے 29 افراد (19.3%) میں TIBC بڑھا ہوا پایا گیا۔

اسی طرح حصہ ب میں 150 میں سے 56 شرکاء میں فولاد کی کمی پائی گئی۔ حصہ ج میں 83 شرکاء (55.3%) میں یہی کمی موجود تھی۔ حصہ ب کے (73.2%)41/56 شرکاء میں TIBC بڑھا ہوا تھا جبکہ حصہ ج میں (74.6%)62/83 شرکاء میں بھی TIBC بڑھا ہوا پایا گیا۔

حاصل مطالعہ: اس مطالعے سے پتہ چلا کہ سیرم فولاد اور TIBC کے امتحان انیمیا کی صحیح تشخیص میں اہم کردار ادا کرسکتے ہیں۔

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INTRODUCTION

Iron deficiency (ID) is the insufficiency of the total content of iron in the body. It occurs when ID is severe enough to reduce erythropoiesis. In developed countries, Iron Deficiency Anaemia occurs in about 2 to 5% of

men and women[1]. In men and women, Iron Deficiency Anaemia is the most commonly caused by gastrointestinal blood loss[2]. Other causes include malabsorption, blood donation, haematuria, and dietary deficiency. In women, it is usually due to menstrual blood loss, increased demand in pregnancy, breast-feeding, and dietary deficiency or malabsorption most commonly caused by celiac disease. It is estimated that about 11% of women aged between 20 and 49 years have Iron Deficiency[3].

It is a commonly recognized form of nutritional deficiency, prevalent among infants and young children as hypochromic microcytic anaemia[4].

Globally, the most significant contributor to the onset of anaemia is iron deficiency. It is generally assumed that 50% of the cases of anaemia are due to iron deficiency but the proportions may vary among population groups and in different areas according to the local conditions. The main risk factors for Iron Deficiency Anaemia include a low intake of iron, poor absorption of iron from diets high in phytate or phenolic compounds, and period of life when iron requirements are especially high (i.e. growth and pregnancy.)[5]

The objective of this study is to assess the significance of serum iron and TIBC in the diagnosis of iron deficiency anaemia as compared to other diagnostic parameters.

METHODOLOGY

Experimental Design

Serum iron and TIBC was analyzed in three groups comprising group-A for male patients (n=150), group-B for females (n=150) and group-C for pregnant women (n=150).

Sampling

Patients from medical OPDs and Obstetrics/Gynae department of JPMC, Karachi were selected for the diagnosis of Iron Deficiency Anaemia. This was a cross-sectional study and sampling was done by convenience (non-probability).

Laboratory Analysis

Laboratory investigations of the cases were done to determine the level of haemoglobin, serum ferritin, serum iron level, total iron-binding capacity (TIBC), unsaturated iron-binding capacity, and percentage saturation of transferrin (Fe/TIBC%). Mean and standard deviation were calculated for haemoglobin percentage, Serum Ferritin, Serum Iron, TIBC, UIBC, and Fe/TIBC percentage.

RESULTS

The present results indicated that the group-A had less value of serum iron while contrary TIBC was inversely proportional to serum iron deficiency that was elevated from the normal recommended range. Likewise group-B showed positive cases for serum iron deficiency followed by group-C. Beside this, TIBC values were found to be raised in female patients (group-B) followed by group-C, as shown in table 1.

Table 1: Interpretation of Results among Various Groups

Groups	No. of Patients	No of Low Serum Iron Cases (ug/dL)	No of Raised TIBC Cases (ug/dL)
A	150	48 (32%)	29 (19.3%)
B	150	56 (37%)	41 (73.2%)
C	150	83 (55%)	62 (74.6%)

DISCUSSION

Iron Deficiency Anaemia is one of the most common anaemias seen worldwide affecting about two billion people. However, in developed countries, it accounts for about 3-6% of adult men and post-menopausal women and is a common cause of referral to gastroenterology clinic[6].

Using specimens from our outpatient population, we determined the utility of the serum iron/ TIBC test for detecting decreased iron stores, which may be associated with simple iron deficiency or iron-deficiency anaemia. As shown in results, where the concentration of iron is decreased, TIBC is increased. The detection efficiency of the transferrin saturation value is as good as that of the ferritin test. This inference is in concordance with a study conducted by Frank and Stephen[7]. Our study was in close agreement with the study of Vena et al[8] that demonstrated the importance of serum iron, TIBC, and ferritin test as important tools in the diagnosis of iron deficiency anaemia. Wendell[9] also indicated this in his study on macro and micro methods for the determination of Serum Iron and Iron-Binding Capacity. But contrarily, serum iron is useful for the diagnosis of iron depletion states especially when used in combination with transferrin and transferrin saturation (Bogen et al[10]). While Goddard et al[1] describe transferrin/ TIBC to be significantly increased in iron depletion states, it is decreased in inflammatory states leading to anaemia secondary to chronic inflammation.

CONCLUSION

On the basis of the above findings, it is concluded that the estimation of serum iron and TIBC gives accurate analysis and these are important tools in the diagnosis of Iron Deficiency Anaemia. This also helps to take necessary interventions in planning, treating, and thus preventing the risk of adverse events.

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- Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publications—International Committee of Medical Journal Editors

ORIGINAL ARTICLE

Association Between Incidental Diagnosis of Adenomyosis on Histopathology to the Clinical Symptomatology

Saadia Akram[1], Muhammad Waqas Nisar Ahmed[1], Farhana Sami[1], Mohammad Aamir Mirza[2], Samina Rizvi[3], Rizwana Barakzai[1] and Syed Mehmood Hasan[1]

ABSTRACT

Objectives: To determine association between incidental diagnosis of adenomyosis on histopathology and clinical symptomatology

Methodology: This hospital-based cross-sectional study was conducted at Lady Dufferin Hospital, Karachi, Pakistan. A structured standardized proforma was used to collect data between January 2014 to Dec 2016 from the pathological laboratory data on surgical hysterectomies. The data comprised of clinical, physical, and histological examination. The analysis was made on association between incidental diagnosis of adenomyosis on histopathology and the clinical symptomatology and diagnosis.

Results: Two hundred and seventy-four abdominal hysterectomies were studied. Out of 274 study subjects, 97 cases (35.4%) were diagnosed as adenomyosis after histopathological examination. Among the study subjects, the mean age was 42.77 + 6.96. Seventy-nine cases (81.44%) were fertile and had children. There was a history of abortion in 16 cases (16.49%). The commonest clinical symptom associated with adenomyosis was menorrhagia in 38 cases (39.17%). Clinically, adenomyosis was diagnosed in only 5 (5.15%) women. History of hormonal therapy (primolut) was available in only three cases (3.09%). Not a single case (0%) showed association of primary infertility with adenomyosis. Our study found no clear association between adenomyosis and any specific histological type and stage of endometrium.

Conclusion: This hospital-based study showed that adenomyosis is a neglected diagnosis and is mostly incidentally diagnosed in various cases of clinically diagnosed dysfunctional uterine bleeding. Since it is found to be much more common than clinically expected, a proper investigation to exclude adenomyosis prior to surgery is recommended by various diagnostic modalities.

Key words: Adenomyosis, incidental adenomyosis, clinical symptomatology of adenomyosis, diagnosis of adenomyosis

عنوان: Histopathology کے ذریعے Adenomyosis کی اتفاقی تشخیص اور طبی علامانی تشخیص کا تعلق۔

مقصد: Histopathology کے ذریعے Adenomyosis کی اتفاقی تشخیص اور طبی علامانی تشخیص کے تعلق کو معلوم کرنا۔

طریقہ: یہ تحقیق ایک عمومی جائزے کے تحت لیڈی ڈفرن ہسپتال میں کی گئی۔ اس تحقیق میں 274 مریض شامل کیے گئے۔ مریض آسانی کے اصول کے تحت لیکن مقصد کو سامنے رکھتے ہوئے لیے گئے۔ جنوری 2014 ء سے دسمبر 2016 ء تک کے Surgical Hysterectomies مریضوں کی معلومات pathological laboratory سے پہلے سے تیار شدہ سوالنامہ کے ذریعے اندراج کی گئیں۔ یہ معلومات طبی جسمانی اور علوم تر قیات سے متعلق تھیں۔ شماریاتی تجزیہ کے ذریعے Adenomyosis کی اتفاقی تشخیص بذریعہ نظریاتی علم امراض سے علامانی تشخیص کا تعلق معلوم کیا گیا۔

نتیجہ: دوسو چوہتر کے نمونہ کا تجزیہ کیا گیا۔ ان 274 میں 97 (35.4%) نمونوں میں Adenomyosis کی تشخیص کی گئی۔ تشخیص کی ہوئی عورتوں کی اوسط عمر 47.72±6.96 تھیں۔ ان عورتوں میں 79 (81.44%) میں تو پیدا کی اہمیت موجود تھی اور ان کی اولاد بھی تھیں۔ سولہ (16.49%) عورتوں کا اسقاط حمل ہو چکا تھا۔ ان میں طبی علامات اور Adenomyosis سے متعلق (39.12%) عورتوں میں menorrhagia میں پائی گئیں۔ طبی طور سے صرف 5 (5.15%) عورتوں میں Adenomyosis پایا گیا۔ ہارمون کا علاج صرف 3 عورتوں میں کیا گیا تھا۔ کسی بھی نمونے میں بانجھ پن Adenomyosis کے ساتھ نہیں پایا گیا۔ تحقیق میں Adenomyosis اور تسخیاتی (histopathological) قسم اور بابت ندم کے درجات میں کوئی تعلق نہیں پایا گیا۔

حاصل مطالعہ: ہسپتال میں کی کئی تحقیق یہ ثابت کرتی ہے کہ Adenomyosis ایک ایسا مرض ہے جسے طبی ماہرین عام طور پر تشخیصی عمل میں نظر انداز کر دیتے ہیں۔ اس مرض کی تشخیص زیادہ تر جراحی اور جراحی کے بعد اتفاقی طور پر ہوتی ہے جبکہ مختلف تشخیصی کمیٹ کی مدد سے جراحی سے پہل ہی اس مرض کا سراغ لگایا جا سکتا ہے۔

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INTRODUCTION

Adenomyosis, a pathological condition initially coined as "adenomyoma" was provided by the German pathologist Carl von Rokitansky in 1860, who found endometrial glands in the myometrium and subsequently referred to this finding as "cystosarcoma adenoids uterinum".[1]

In 1972, the modern definition of adenomyosis was provided by Bird who stated that "Adenomyosis, a pathological condition, which is a benign invasion of endometrium into the myometrium, producing a diffusely enlarged uterus that microscopically exhibits ectopic non-neoplastic, endometrial glands and stroma surrounded by the hypertrophic and hyperplastic myometrium." [2]

Adenomyosis is associated with a range of clinical presentations like prolonged menstrual cramps, spotting between periods, menorrhagia, longer menstrual cycles than normal, blood clots during menstrual bleeding, dyspareunia, tenderness in the abdominal area, and post-menopausal bleeding[3-5].

Adenomyosis is not a life-threatening condition. Many treatment modalities like anti-inflammatory medications, hormonal treatments, endometrial ablation, uterine artery embolization, MRI-guided focused ultrasound surgery (MRgFUS), and hysterectomy are available. However, hysterectomy is the most common and conventional surgical management in current clinical setups[6,7].

METHODOLOGY

This was a hospital-based cross-sectional study conducted at the Lady Dufferin Hospital, Karachi, over a period of three years from January 2014–December 2016.

This study included all women undergoing elective abdominal hysterectomy with or without salphingo-oopherectomy.

Information regarding age, menstrual history, obstetric history, presenting signs and symptoms, hormonal therapy and clinical diagnosis was recorded on a proforma. Specimens were received in 10% formalin solution. Detailed gross examination was done. Representative tissue sections were taken and processed. Blocks were prepared with the help of moulds. Four to five micron thick sections were cut with the help of a microtome and stained with routine hematoxylin and eosin stain (H&E stain) for microscopic examination.

All slides were examined and reported by consultant histopathologist. Keeping in view the different histopathological variables, all specimens were re-examined by the same histopathologist to rule out any human and systemic errors.

The data was collected and analyzed on Social Package for Statistical Analysis (SPSS) Windows version 22.

Criteria used for the diagnosis of adenomyosis were:

1. Endometrial glands or stroma or both at one low power (3mm) depth from the basal endometrium

2. Plump/hypertrophied smooth muscle fibers immediately surrounding the endometrial tissue deep in the myometrium[8,9]

RESULTS

Two hundred and seventy-four abdominal hysterectomies were studied.

Out of 274 study subjects, 97 cases (35.4%) were diagnosed to have adenomyosis after histopathological examination.

Among the study subjects, the mean age was 42.77 + 6.96.

Seventy-nine cases (81.44%) were fertile and had children. There was a history of abortion in 16 cases (16.49%).

The common clinical symptoms associated with adenomyosis were menorrhagia in 38 cases (39.17%), polymenorrhagia in 16 cases (16.49%), pain in 9 cases (9.27%), menorrhagia with pain in 7 cases (7.21%), irregular bleeding in 5 cases (5.15%), and post-menopausal bleeding in 4 cases (4.12%). (Table 1)

Table 1: Major Complaints in Study Samples Diagnosed as Adenomyosis (n=97)

Major Complaints	Adenomyosis	
	Total cases n=97	100 %
Pain	09	9.27
Menorrhagia	38	39.17
Polymenorrhagia	16	16.49
Pain + Menorrhagia	07	7.21
Post Menopausal Bleeding	04	4.12
Irregular Bleeding	05	5.15
Others	18	18.55
Total	97	100

Clinically, adenomyosis was diagnosed in only 5 (5.15%) women. Otherwise dysfunctional uterine bleeding 39 (40.20%), leiomyoma in 16 cases (16.49%) and endometriosis in 4 cases (4.12%) were clinical interpretations which were later on diagnosed on

histopathological examination as adenomyosis. (Table 2)

Table 2: Clinical Diagnosis in Histopathologically Proven Adenomyosis (n=97)

Clinical Diagnosis	Number of Cases	
	n=97	100 %
Leiomyoma	16	16.45
Endometriosis	04	4.12
Dysfunctional Uterine Bleeding	39	40.20
Ovarian Mass	04	5.15
Adenomyosis	05	5.15
Leiomyoma	16	16.45
Others	29	29.89
Total	97	100

History of hormonal therapy (primolut) was available in only three cases (3.09%).

Not a single case (0%) was found showing association of primary infertility with adenomyosis.

Our study found no clear association between adenomyosis and any specific histological type and stage of endometrium. All possible types of histological stages of endometrium were found in our study sample.

DISCUSSION

In this study on adenomyosis, we analyzed two hundred and seventy-four subjects in order to find the most common signs and symptoms and the clinical interpretations in cases which were later diagnosed as adenomyosis on histopathological examination. In our study, we found that the mean age of the participants was 42.77. In study by Taran et al. (2013), 70 to 80% of women undergoing hysterectomy for adenomyosis are in their fourth and fifth decade of life which is in agreement with our study[10,11].

Our study found a history of abortion in 16.49% of cases. The study by Kathryn M. Curtis et al. (2002), also supports association of one or more abortions with adenomyosis[12,13].

Menorrhagia was found to be the most common clinical symptom in our study. However, polymenorrhagia, pain, menorrhagia with pain, irregular bleeding, and post-menopausal bleeding were other frequent clinical symptoms reported. The study by Azziz (1989) also supports our finding that menorrhagia and dysmenorrhea are chief clinical symptoms frequently found in adenomyosis[14,15].

No association between primary infertility with adenomyosis was found in our study, however, Matalliotakis et al. (2005), support the association between adenomyosis and primary infertility[16,17].

Our study found history of hormonal therapy (primolut) in only three cases. However, hormonal therapy is a well established tool for the management of adenomyosis before hysterectomy as presented by Farquhar et al. (2006)[18,19]

There could be two possible reasons for the presence of low number of hormonal therapy history in our study. The first one being delay in approaching the medical clinic due to lack of awareness, poor guidance, and lack of education of the patients. The second reason is related to medical practitioners. The unfortunate fact is that in many clinical setups, physicians do not focus on treating adenomyosis with hormonal therapy or any other non-invasive treatment, and use hysterectomy as the first line of management to treat adenomyosis.

In our study, andenomyosis was the clinical diagnosis in only 5.15% patients. Most of the patients were inaccurately clinically interpreted as dysfunctional uterine bleeding, leiomyoma, endometriosis, and ovarian mass cases by medical practitioners. These patients were later on diagnosed to have adenomyosis after histopathological examination. The study work by Owolabi and Strickler (1977), Anwar Ali (2005) and Albers et al (2004), also support our study outcome that most of the patients of adenomyosis are clinically misdiagnosed and the histopathological examination after hysterectomy shows the real diagnosis to be adenomyosis[20-22].

CONCLUSION

This hospital-based study shows that adenomyosis is a neglected diagnosis and mostly incidentally diagnosed in various cases of dysfunctional uterine bleeding. Since it is found to be much more common than clinically expected, a proper investigation to exclude adenomyosis through various diagnostic modalities prior to surgery is recommended.

Limitation of Study: The study population was from one private tertiary care hospital and therefore is a unicentre analysis.

Conflict of Interest: The authors declare no conflict of interest for this study.

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Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as the use of P values, which fails to convey important quantitative information. Discuss the eligibility of experimental subjects. Give details about randomization. Describe the methods for and success of any blinding of observations. Report complications of treatment. Give numbers of observations. Report losses to observation (such as dropouts from a clinical trial). References for the design of the study and statistical methods should be to standard works when possible (with pages stated) rather than to papers in which the designs or methods were originally reported. Specify any general-use computer programs used.

Put a general description of methods in the Methods section. When data are summarized in the Results section, specify the statistical methods used to analyze them. Restrict tables and figures to those needed to explain the argument of the paper and to assess its support. Use graphs as an alternative to tables with many entries; do not duplicate data in graphs and tables. Avoid nontechnical uses of technical terms in statistics, such as "random" (which implies a randomizing device), "normal," "significant," "correlations," and "sample." Define statistical terms, abbreviations, and most symbols.

- Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publications—International Committee of Medical Journal Editors

Endoscopic Evidence of Helicobacter Pylori in Dyspeptic Patients

Saleem Shahzad[1], Syed Masroor Ahmad[1], Zeeshan Ali[1], Shabnam Naveed[1], Syed Adnan Ali[2], Aqeel Ahmad Sheikh[1] and Saba Khan[1]

ABSTRACT

Objective: The aim of this study was to find out the prevalence of H. pylori severity in dyspeptic patients.
Methodology: This descriptive, retrospective, observational study was carried out at Ward VII of Jinnah Postgraduate Medical Centre (JPMC) after reviewing the record of one hundred patients, aged from 13 to 70 years, who presented in medical OPD or were admitted in Ward VII with complaints of dyspepsia for more than six weeks. After detailed history and physical examination and investigations, hepatitis B, C, and chronic liver disease were ruled out.
Results: The mean age of participants was 37.44 ± 12.29 years. Sixty-six per cent participants were women. Mild inflammation was found in 75% of the cases, out of which 49% were with moderate activity, 50% were found to be H. pylori positive, 31% had mild to moderate colonization,19% had moderate to severe colonization and 39% had pit abscesses. Out of male samples, 79.4% were found with mild inflammation. In 47.1% male samples, activity was mild to moderate. In females, 39.4% were found with mild to moderate colonization in H. pylori.
Conclusion: This study concludes that 31% dyspeptic patients presenting in JPMC had mild to moderate colonization for H. pylori.

Key words: Endoscopy, helicobacter pylori, dyspepsia

عنوان: بدہضمی کے مریضوں میں ہیلی کوبیکٹر پاکوری کے جراثیم کی موجودگی کا اینڈوسکوپک ثبوت ۔

پس منظر اور مقصد: بدہضمی کو بڑی آنت کی جسمانی یا فعلی خرابی کا نتیجہ بتایا جاتا ہے ۔ بے شمار مطالعوں میں بدہضمی کو ایک عام شکایت پایا گیا ہے ۔ اس مطالعے کا مقصد بدہضمی کے شکار مریضوں میں پاکلوری کے جراثیم کی موجودگی اور شدت کا پتہ چلانا تھا ۔

طریقہ: یہ تحقیق ریکارڈ کی بنیاد پر ایک بیانیہ تحقیق تھی جو کہ JPMC کے وارڈ نمبر سات میں کی گئی ۔ اس میں 13 سے 70 سال کی عمر تک کے ایسے 100 مریضوں کا مطالعہ کیا گیا جو چھ ہفتے سے زائد دنے والی بدہضمی کی شکایت کے ساتھ OPD آئے یا وارڈ میں داخل ہوئے ۔ ان مریضوں سے مرض کی تاریخ لینا اور جسمانی امتحان کرنے کے بعد ہیپاٹائٹس بی ،سی اور جگر کی شدید بیماری کے امکان کو رد کیا گیا ۔

نتائج: تحقیق کے شرکاء کی اوسط عمر 37.44±12.29 سال تھی ۔ ان میں سے 66 فیصد خواتین تھیں ۔ ہلکی سوجن 75 فیصد شرکاء میں پائی گئی ۔ ان میں سے 49 فیصد میں درمیانے درجے کی شدت (moderate activity) پائی گئی ۔ پچاس فیصد میں ایچ ۔ پاکوری مثبت طور پر پایا گیا ۔ 31 فیصد میں ہلکے سے درمیانے درجے colonization پائی گئی ۔ 19 فیصد میں درمیانے سے شدید درجے کی colonization پائی گئی اور 39 فیصد میں ناسور پائے گئے ۔ مردوں میں سے 79.4 میں ہلکی سوجن موجود تھی ۔ 47.1 فیصد مردوں میں ہلکے سے درمیانے درجے کی سرگرمی تھی ۔ خواتین میں 39.4 فیصد میں ہلکے سے درمیانے درجے کی colonization پائی گئی ۔

حاصل مطالعہ: اس مطالعے کے مطابق JPMC میں آنے والے بدہضمی کے مریضوں میں سے 31 فیصد میں ہلکے سے درمیانے درجے کی ایچ ۔ پاکلوری colonization پائی گئی ۔

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INTRODUCTION

Dyspepsia is defined as a syndrome thought to have originated from anatomic or functional disorder of the upper G.I. Tract[1]. A study in Canada revealed that 40% of the general population had dyspepsia as a common condition, making up approximately 7% of all patients visiting family physicians. This syndrome

produces upper gastrointestinal symptoms like epigastric pain or discomfort and may also include heartburn, acid regurgitation, excessive burping/belching, abdominal bloating, feeling of abnormal or slow digestion, early satiety or nausea. Rome-III criteria defines dyspepsia as one or more of the following three symptoms for three months within the initial six months of symptoms onset: Postprandial fullness, early satiety, and epigastric pain and burning[2]. Studies show that these symptoms are often divided into subgroups on the basis of dysmotility, ulcer, reflux or unspecified dyspepsia, although the clinical significance of this subdivision is unclear[3].

The second point of focus in this study is H. pylori infection that causes dyspepsia. Around half of the world population is infected with H. pylori, especially in developing countries[4]. The transmission route of H. pylori is still not clear though studies have documented data indicating oral-oral, gastric-oral, and fecal-oral as main transmission routes. If left untreated, H. pylori infection can be lifelong, even though clinical disease can occur decades after initial infection[4]. Studies also show that gastric inflammation ulcers have correlation with the progress of gastric cancer.

Generally, H. pylori infection can be diagnosed invasively and non- invasively. In a few patients with dyspepsia or symptoms of weight loss, endoscopy is required to determine the underlying disease. Describing endoscopic techniques to diagnose H. pylori, Cohen H. et al. (1997) advise that findings like gastroduodenal ulceration and/or antral nodularity indicate H. pylori infection. Endoscopic gastric biopsies, however, direct to a perfect diagnosis of H. pylori on histopathology[5].

A 2002 study by Tytgat GNJ has shown a relationship between mucosal abnormalities and symptom presentation. It suggests endoscopy as the initial line of investigation for clinically related abnormalities and for proper diagnosis and biopsy[6].

A study on gastric mucosa biopsies in dyspeptic patients to see H. pylori infection, advises that patients with chronic active gastritis, heartburn, dyspepsia, and possibility of H. pylori infection should be ruled out with diagnostic assays based on biopsy specimens and serology. Biopsy specimens by Giemsa stain are the 'gold standard' for detecting the presence of the bacteria[7].

METHODOLOGY

This descriptive, retrospective, observational study was carried out at Ward VII of JPMC after reviewing the record of one hundred patients presenting from 1st July to 31 December, 2016. All adult patients aged 13 to 70 years who visited medical OPD Ward VII or were admitted in Ward VII with complaints of dyspepsia for more than three months after medical treatment, were included in the study after giving formal informed consent. All patients aged 13-70 with dyspeptic symptoms, who had not improved with PPI for more than three months, were included in the study. Patients who improved with PPI or had other associated diseases, were excluded from the study. Sample size was estimated using open EPI sample size calculator version 3.01, after inserting the 40% prevalence of dyspepsia (3) at 10% margin of error and 95% confidence interval, we got n=93 for this study. These patients were evaluated by consultants and postgraduate doctors. Data was extracted from the patient's files and stored and analyzed using SPSS version 19. Descriptive analysis were done and mean standard deviation (SD) was calculated for age and count and percentages were reported for sex, inflammation, presence of H. pylori, pit abscesses activity, atrophy, and metaplasia.

RESULTS

The mean age of one hundred studied samples was 37.48 years with standard deviation of 12.29. Minimum age was 20 years and maximum was 63, so the range of age data was 43. In our study, more than 70% of the cases were found with ages less than or equal to 40 years. Sixty-six per cent data was received from females. In 55% of cases, specimens were antral, and 75% cases were found with mild inflammation. Forty-nine per cent of cases were found with mild to moderate activity. Over all, H. pylori was present in 50% of cases. Out of these, 31% were found to have mild to moderate colonization and 19% had moderate to severe colonization. Only 3% cases were found with atrophy and 7% had metaplasia. Pit abscesses were present in 39% of cases (Table 1).
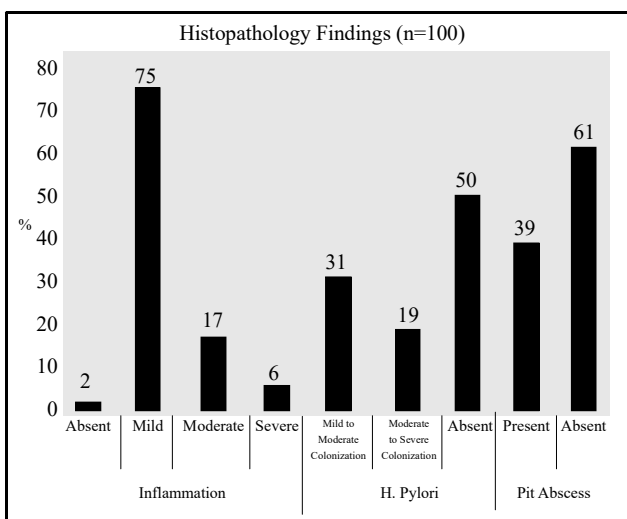
Table 1: Endoscopic and Histopathology Findings among Patients (n=100)

Characteristics		N	%
Gender	Male	34	34.0
	Female	66	66.0
Specimen	Antral	55	55.0
	Both	45	45.0
Inflammation	Absent	2	2.0
	Mild	75	75.0
	Moderate	17	17.0
	Severe	6	6.0
Activity	Mild to Moderate	49	49.0
	Moderate to Severe	23	23.0
	Normal	28	28.0
H. pylori	Mild to Moderate Colonization	31	31.0
	Moderate to Severe Colonization	19	19.0
	Absent	50	50.0
Atrophy	Present	3	3.0
	Absent	97	97.0
Intestinal Metaplasia	Present	7	7.0
	Absent	93	93.0
Pit Abscess	Present	39	39.0
	Absent	61	61.0

Results of the study show that mild inflammation was found in 79.4% cases while it was absent in 5.9% male cases. In men's samples, 47.1% cases were observed to have mild to moderate activity. In women's samples, 39.4% H. pylori cases were found with mild to moderate colonization. Atrophy was present in 8.8% males and totally absent in females. Intestinal metaplasia was found in 6.1% female cases and pit abscesses were present in 45.5% female cases. (Table 2)

Table 2: Classification of Endoscopic and Histopathology Findings with Gender (n=100)

Endoscopic and Histopathology Findings		Gender			
		Male (n=34)		Female (n=66)	
		n	%	n	%
Inflammation	Absent	2	5.9	-	-
	Mild	27	79.4	48	72.7
	Moderate	5	14.7	12	18.2
	Severe	-	-	6	9.1
Activity	Mild to Moderate	16	47.1	33	50.0
	Moderate to severe	7	20.6	16	24.2
	Normal	11	32.4	17	25.8
H. pylori	Mild to Moderate Colonization	5	14.7	26	39.4
	Moderate to Severe Colonization	2	5.9	17	25.8
	Absent	27	79.4	23	34.8
Atrophy	Present	3	8.8	-	-
	Absent	31	91.2	66	100.0
Intestinal Metaplasia	Present	3	8.8	4	6.1
	Absent	31	91.2	62	93.9
Pit Abscess	Present	9	26.5	30	45.5
	Absent	25	73.5	36	54.5

DISCUSSION

In our study, half of the dyspeptic patients were found to have H. pylori infection proven by gastric biopsy. This result is consistent with the findings of the study by Talley NJ et al. (1997) which showed that gastroduodenal dysfunction could be related with H. pylori infection[8].

Our data shows 31% dyspeptic patients to have mild to moderate colonization of H. pylori. In a study, McColl K et al. (1998) performed endoscopy in younger patients with uncomplicated dyspepsia. They found many dyspeptic patients to have H. pylori infection and related gastric or duodenal ulcer. The study advocates that H. pylori eradication is favourable for cases with non-peptic ulcers[9].

Blum AL et al. (1998) have shown that 33% of people in developed countries had frequent dyspeptic symptoms or dyspepsia, while many of them did not have any chronic peptic ulceration, reflux oesophagitis, a malignant condition, or other definite disease. Peptic ulcer diseases have strong association with H. pylori infection. Therefore, eradication of H. pylori prevents reversion of peptic ulcers, until reinfection occurs[10]. In this study, we suggest that H. pylori eradication can be administered in non-responding dyspeptic patients. In our study, dyspeptic patients who did not improve with PPI were subjected to endoscopy. Study done in Denmark by Bytzer P. et al. (1994) recommends treating dyspepsia empirically with H_2-blocking drug and endoscopy if the patient does not improve. Bytzer P. et al. compared two strategies for the treatment of dyspepsia: the first group was treated based on prompt endoscopy; while the second group was treated empirically with H_2-antagonist drugs. They concluded that "Prompt endoscopy is a cost-effective strategy in dyspeptic patients with symptoms severe enough to justify the current practice of empirical H_2-blocker treatment[11]".

In the present study, 75% patients were found to have mild gastritis but only 17% had moderate gastritis. This is comparable to a study done in Kenya by Ogutu EO et al. in 1993 on patients older than twelve years with symptoms of dyspepsia. The investigators did upper gastrointestinal endoscopy to see the mucosal pathology related to dyspeptic symptoms. Gastric mucosal biopsies were taken to see H. pylori infection using different methods e.g. culture, histology and the rapid urease test. Normal mucosa was a common endoscopic finding accounting for 34.2%, while 31.7% showed gastritis and 29.2% were observed to have duodenal ulcer[3,12].

CONCLUSION

This study shows that 31% of the samples had mild to moderate colonization of H. pylori. It is suggested that in cases not responding to PPI treatment, anti-H. pylori therapy may be administered.

Limitation: In the present study, purposive sampling was used for the selection of patients, so the study results could not be generalized for the population.

Recommendation: This cross sectional study could be helpful in generating hypothesis for further investigation in dyspeptic patients and endoscopic findings of population in Karachi.

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ORIGINAL ARTICLE

Frequency and Clinico - Pathological Profile of Patients with Leukaemia; Tertiary Care Centre Experience from JPMC Karachi

Erum Amir[1], Naila Tariq[2] and Amir Sharif[3]

ABSTRACT

Objective: The aim of this study was to analyze the trends in the frequency rates of four major types of leukaemias and to .determine the clinico - pathological profile of the patients visited to the oncology department of a tertiary care centre in Karachi.

Methodology: It is a retrospective, cross-sectional study conducted in the oncology and clinical pathology units of one of the largest tertiary care centres in Karachi, from January 2015 -December 2016. A total of 350 known cases of leukaemia were taken with confirmative diagnosis supported by bone marrow biopsy trephine, FISH, RT-PCR immunohistochemistry, cytogenetics and other base line investigations like CBC, LFTs, urea creatinine, LDH, ALP.

Results: The present study reveals that 350 cases of leukaemia ranging between 15 and 60 years, were seen at the JPMC clinical pathology/ oncology unit. Acute leukaemia was more commonly found i.e. 218 (62.3%) than chronic leukaemia i.e. 132 (37.7%). The ratio of acute and chronic leukaemias was 1.7:1. Male:Female ratio was 2.3:1. Acute leukaemias were subdivided into 116 (33%) cases of ALL and 102 (29%) cases of AML. Chronic leukaemia was further subdivided into 108 (31%) cases of CML and 24 (7%) cases of CLL. Four cases were of Myeloproliferative Disorders with JAK2 mutations out of which three were of polycythemia-vera and one case of essential thrombocytosis.Two cases of transformation from MPD to AML were seen.. The major presenting complaints were fever 78 %, general weakness 62%, bleeding diathesis 40%, shortness of breath 7% and weight loss 35%. The most common sites of bleeding were mucosal bleeds from nose, gums, gastrointestinal tract, and genito-urinary tract. Physical examination revealed splenomegaly 41%, lymphadenopathy 37%, hepatomegaly 32%. Anaemia was the most common finding affecting 259 patients (74 %). The mean haemoglobin was 7.2 g/dl (range 6.3-8.5) g/dl. TLC was 76.5x 10/l (range 44 to 140). The mean platelets count was 90x10/l.

Conclusion: We concluded that the clinico-epidemiological features of adult leukaemias differ considerably from those seen in the developed world. In our study, the most common of all leukaemias was ALL 62.3%. In contrast, AML was reported to be the most prevalent acute leukaemia in the US. CLL was the least common accounting for 7% of all cases. The mean age of presentation was 61 years. CML was 4.3 times commoner than CLL with a younger age at presentation (median age was 30 years) in contrast to CLL.

Key words: Leukaemias, acute lymphoblastic leukaemia, acute myeloblastic leukaemias, chronic lymphocytic leukaemia, chronic myeloid leukaemias

عنوان: خون کے سرطان کی مریضوں کی تعداد اور ان کا طبی اور علم الامراضی (Pathological) خاکہ۔

مقصد: اس مطالعہ کا مقصد کراچی کے ایک تیسرے بلند درجے کے ہسپتال کی شعبہ سرطان کی علمیہ وعیہدہ کثرت تعداد کی شرح اور علم الامراضی خاکہ تشکیل دینا تھا۔

طریقہ: کراچی کے ایک تیسرے بلند درجے کے ہسپتال کے جنوری 2015ء تا دسمبر 2016ء کے دکارڈ کی تجزیہ کی بنیاد پر ایک عمومی مطالعہ کیا گیا۔ تین سو پچاس (350) خون کے سرطان کے مریضوں کو اس تحقیق میں شامل کیا گیا۔ ان سب کے درج ذیل طبی معائنہ ہو چکے تھے۔ بنیادی کے گودے کی Fish، biopsy trephine، RT-PCR immunohistochemistry،cytogenetics اور دیگر مروجہ امتحان جیسے CBC،Urea Cretinina LDHC اور ALP۔

نتائج: 62.3% مریضوں میں شدید خون کے سرطان جبکہ 37.7% مریضوں میں دائمی خون کا سرطان پایا گیا۔ اس طرح سے شدید اور دائمی خون کا سرطان کا تناسب 1:1.7 تھا۔ شدید خون کے سرطان کے 108(31%) مریض CML کے مریض اور

102 AML(29%) سرطان کے مریض تھے۔ 37.7% (62.3%) جبکہ امریکہ میں AML سب سے زیادہ پایا جانے والا شدید لیوکیمیا ہے۔ ہمارے مطالعے کے مطابق CLL سب سے کم پایا گیا جس کے صرف 7% تیسرے طے CML کے مریض دیگر مریضوں کی نسبت کم عمر اور CLL سے 4.3 گنا زیادہ پائے گئے۔

حاصل مطالعہ: اس مطالعے سے یہ نتیجہ برآمد ہوا کہ اس خطے میں پائے جانے والے خون کے سرطان کی اقسام اور ملکک شکایات ترقی یافتہ ممالک کے حالات سے کافی مختلف ہیں۔ ہمارے مطالعے کے مطابق ALL سب سے زیادہ پایا جانے والا لیوکیمیا تھا۔

INTRODUCTION

Leukaemia is a malignant disease of haematopoietic tissue characterized by the accumulation of abnormal white blood cells (neoplastic or leukaemic) in the bone marrow, leading to bone marrow failure, a raised white cell count (leucocytosis) and infiltration in other organs resulting in organomegaly e.g. liver, spleen, lymph nodes, brain[9].

In 1945, Bennet made the initial description of leukaemia as a clinical entity in Scotland and Germany. The etiology of leukaemia is still not completely understood. The most common factors contributing in development of leukaemia are oncogene mutations and tumour suppressor gene alteration, host factors, and environmental factors. Leukaemias are broadly classified as Acute and Chronic leukaemias which are further classified as acute lymphoblastic (ALL), acute myeloblastic (AML), chronic lymphocytic (CLL), and chronic myeloid leukaemias (CML)[8,12].

According to data published in SEER (Institute of Surveillance, Epidemiology, End Results) programme's fact sheet of 2015, leukaemias accounts for 9.5% of all newly diagnosed cancer cases in the US, whereas data in the UK shows 3% prevalence. In the West, leukaemia strikes more in adults than in children (10:1), while in our region, acute leukaemias especially acute lymphoblastic variant is more common. Data in the West shows both adults and children to be more or less equally involved. Gender wise distribution is more or less the same in the West and in our region. In the West, male to female incidence ratio is 2.1:1 whereas in Pakistan unfortunately there is no cumulative data so far for haematological malignancies[8,9]. No cancer incidence data from Pakistan has been published on provincial or regional levels in the last five decades. No proper cancer registry programme exists in the country, however, institutional data were seen in a few places only documenting a certain period.

Rationale of Study: An intensified effort is needed to collect, organize, and analyze the institution-based data and to develop a comprehensive system of a cancer registry on the national level. Cancer registration is vital to monitor trends in incidence, prevalence, clinico-pathological profile, mortality /morbidity and etiological assessment of different variants of leukaemias. This data will help the authorities in policymaking, resource allocation, tracking progress, and the over all development of cancer control programmes in our country. Monitoring the trends in cancer incidence and prevalence is one of the most important factors for the development of a strong national surveillance system.

Aims and Objectives: The aim of the study was to assess the frequency of various types of leukaemias and to observe the clinicopathological spectrum and changing trends of the commonest types of leukaemias seen in the Oncology unit of the JPMC.

METHODOLOGY

Study design: It is a retrospective, cross-sectional, institution-based study.

Duration and Place of Study: The study was conducted in a tertiary care hospital, JPMC in collaboration with the oncology and the clinical pathology departments from January 2015 to December 2016.

Methodology: All diagnosed cases of leukaemias in both genders above the age of 15 years were included in the analysis. Patients below the age of 15 years and patients with other associated malignant disorders were excluded.

Based on the criteria above, 350 cases were included in the study. The case sheets of the patients were reviewed, especially regarding sex, age at diagnosis, risk factors, occupation, and chief complaints. The diagnosis was confirmed on complete blood count, peripheral smear, bone marrow and trephine biopsy, immunohistochemistry, cytochemistry, and karyotyping. Findings of bone marrow aspiration, trephine biopsy, and special stains were interpreted in the light of the clinical history, examination, and peripheral smear findings. FAB Classification of leukaemias was applied for subtyping.

RESULTS

The present study showed that 350 cases in age group of more than 15 years and above in both genders were registered in oncology unit of JPMC from January 2015 to December 2016. Out of these 350 patients, 259 (74%) were males and 91 (26 %) were females with male to female ratio of 3:1.

Acute leukaemias were maximum in number i.e. 218 (62.3 %) as compared to chronic leukaemias. Out of these, 113 (52%) were acute lymphoblastic leukaemia

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(ALL) and 105 (48%) were acute myeloid leukaemia (AML). Gender-wise distribution showed that out of a total of 113 patients of ALL, 83 were males (74%) and 30 (26%) were females, whereas AML cases comprised 65 (62%) males and 40 (48 %) females. In total 132 patients of chronic leukaemias, 106 (80%) had CML among whom 72 (68.5%) were males and 33(32.5%) were females. Chronic lymphocytic leukaemias (CLL) constituted 20% i.e. 26 patients out of whom 20 (77%) were males and 6 (33%) were females.

The clinical spectrum comprised of major complaints. Fever was reported in 78% of cases, generalized weakness in 63% cases, bleeding diathesis in 40% cases, shortness of breath in 7% and weight loss in 35% cases.

The most common bleeding sites were mucosal bleeds from nose, gums, GIT, and the genitourinary tract. Physical examination revealed splenomegaly in 41%, lymphadenopathy in 37% cases, and hepatomegaly in 32% cases. On laboratory analysis, anaemia was the commonest finding affecting 259 (74%) patients with the mean Hb of 7.2g/dl (Range 6.3-8.5), the mean TLC 76.5x10/L (Range 44-140), and the mean platelet count as 90x10/L.

Table 1: Prevalence of different types of leukaemias

Types of leukaemias	Total cases	Percentage
69% ALL?	113	33
AML	106	30
CML	105	30
CLL	26	7
TOTAL	350	100

Table 2: Gender distribution in leukaemia subtypes

	Male	Female	Total
ALL	83(74%)	30(26%)	113(33%)
AML	65(62%)	40(38%)	106(30%)
CML	72(69%)	33(31%)	105(30%)
CLL	20(77%)	6(33%)	26(7%)
TOTAL	240(69%)	110(31%)	350(100%)

Table 3: Clinical profile of leukemic patients

complaints	%
Fever	78
Bleeding diathesis	40
Body ache /weakness	63
Hepatomegaly	32
Splenomegaly	41
Lymphadenopathy	37
Shortness of breath	7

DISCUSSION

Haematological malignancies are common in Pakistan, different studies have been conducted covering various aspects of individual haematological malignancies in the past. In industrial countries, cancer is the second most important cause of mortality accounting for 9.5% of all deaths, next to cardiovascular disease which causes 21 % of mortality[12,13]. In Pakistan, different kinds of blood cancers are diagnosed per year with approximately 8000 cases annually. Whereas in Karachi, the annual reported incidence was about 800 cases[12,14]. In our study, male to female ratio is more pronounced at 3:1 as compare to the study of Dastagiri et al where male to female ratio is 1.2 :1[16]. In our findings, the most prevalent types of leukaemias are acute leukaemias (62.3%), as compared to chronic types, thus in concordance with the studies by Rajab ali et al, Khalid et al and et al[7,8,10]. According to our institution-based study from 2015 to 2016, leukaemias were the fourth most common malignancy in females, on the basis of registration records. Data reported in SEER showed AML as the most prevalent acute leukaemia in adults, i.e. three times more prevalent than ALL, according to the cancer factsheet[9].

In our study, ALL prevalence is 1.2 times more than AML similar to the data reported by Khalid et al. Our study showed age incidence of AML in the third decade of life comparable with Rajab Ali et al who report age incidence to be >50 years and Kakepoto et al in which average age incidence for AML was 40 years. ALL age incidence is more or less the same as given in literature i.e. Second to third decade of life (7;10). In the West, CLL is the predominant type alongwith chronic leukaemias, in contrast with our study which showed the least number of CLL cases (7%)[10]. Leukaemias were most commonly seen in the Sindhi population especially those belonging to the rural areas followed by Karachi and Balochistan, for which no evident reason was known except for somewhat similar environment and lifestyles.

Limitations of Study: There were a number of limitations in this study. It was a retrospective, institution-based study. The data was taken from files and case sheets of patients filled in by different residents, hence lacked uniformity. There was no follow up of patients to monitor progress. Institution-based studies also do not account for the number of similar cases within the community.

CONCLUSION

This study showed a significant increase in adult patients of acute leukaemias with lymphocytic type comprising 33.7%, which is not in concordance with the data from the West showing increasing trends in myeloid leukaemias. However, the chronic variety showed increase prevalence of CML 31% as compared to CLL which was in contrast with western data. Therefore, our study concludes that ALL and CML are more prevalent types seen in younger age groups, making it necessary to enhance diagnostic and treatment tools in our setup. The data from this cross-sectional, institution-based study can be used as baseline information to establish an institution-based and population-based registry of haematological malignancies in the region for healthcare and research purposes.

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Abstracts: For original articles a structured abstract of 250 words should include the following subheadings: Objectives (may include background in one line if pertinent), Materials/Subjects/Patients and Methods (place and duration of study, design, patients/respondents, brief methodology), Results (key findings only), Conclusion. For review articles and case reports the abstract should be unstructured with not more than 150 words. The abstracts should also include Urdu translation however if author(s) are unable to do so, then the editorial board of the journal will provide the translation service free of cost.

Introduction: For original research articles the introduction should be fully referenced with not more than three paragraphs and a maximum of 15 pertinent references. The introduction should end with the rationale of the study leading to the research question/study objectives. For case reports the introductory section should include a maximum of 10 pertinent references and end with the objective and reason of presenting the case.

Methods: This section should include setting, study design, sampling technique and sample size estimation. In studies of diagnostic accuracy, the methodology should be detailed and include the inclusion and exclusion criteria of patients involved in the study together with information on patient recruitment. Textual re-use of portions of an author's previous work in the methods section will be considered, providing that an explanatory note is included with appropriate referencing: "The methods are exactly as published in the previous publication....."

Statistical Analysis: The author(s) should adequately describe or reference all statistical procedures used in a paragraph at the end of the methods section. It is expected that the statistical tests used are appropriately selected and applied, with an indication of the related assumptions and how they have been tested. The ambiguous use of statistical terms should be avoided such as random with the meaning of haphazard, correlation instead of association, etc. Exact p-values and confidence intervals are to be used. Standard guidelines must be used in reporting the results of clinical trials, studies assessing diagnostic tests, etc. The statistical software package used must be specified and properly referenced.

Tables: Tables should be double spaced on a separate sheet of paper. Do not submit tables as photographs. Number tables consecutively in the order of their first citation in the text and supply a brief "stand-alone" title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in the heading. Explain in footnotes all non-standard abbreviations that are used in each table. For footnotes use the following symbols, in this sequence *, †, ‡, §, **, ††, ‡‡, §§ etc. Bar graphs and pie charts should only be used where absolutely indicated and should be provided in pattern. If the author is interested to print the graphs in color, he/she has to pay color printing. Where possible the information should be presented in table format.

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Conclusion and Recommendations: Conclusions should directly relate to the objectives of the study and not

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References to journal articles should include, in this order: (1) authors, (2) title, (3) journal name (as abbreviated in Index Medicus, if not included in Index Medicus journal title should be given in full), (4) year, (5) volume, (6) page numbers (start and end). Volume and edition numbers and specific page numbers should be included when appropriate. Secondary references are not acceptable. The author is responsible for the accuracy and completeness of the references and for their correct textual citation. When a citation is referred to in the text by name, the accompanying reference must be from the original source. Upon acceptance of a paper all authors must be able to provide the full paper for each reference cited upon request at any time up to publication. Failure to do so may result in the paper being withdrawn from the journal. Example of correct reference form is given as follows:

Journal: Khan NB. Treatment needs for dental caries in schoolchildren of Riyadh: A-follow-up study of the Oral Health Survey of Saudi Arabia. Saudi Med J 2003; 24 (10): 1081-1086.

Al-Nozha MM, Al-Maatouq MA, Al-Mazrou YY, Arafah MR, Khalil MZ, Khan NB, et al. Diabetes mellitus in Saudi Arabia. Saudi Med J 2004; 25(11): 1603-1610.

Book Chapter: Thilander B. Ronning O. Introduction to Orthodontics. Stockholm: Gothia; 1995. p. 43-49.

Review Articles should include an extended bibliography up to 100 references. Original Articles and Case Reports should include up to date references, preferably not exceeding 40 and 15 respectively. Brief Communication should include a maximum of 5 references.

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