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EDITORIAL

PROMOTING INNOVATIVE RESEARCH THROUGH COLLABORATIONS BETWEEN ACADEMIA AND INDUSTRY

Fazal Ghani

Continued exploration of the health-care science by conducting innovative and health-care outcome based research has been the main reasons for the globally dominating role played by countries of the developed world. These activities have made their health-care industry un-matching in terms of innovations, technologies and products. A great deal of such innovation and development has been possible through active collaborations among the academic research faculty, clinicians, industries and health ministries. In fact a major proportion of the finances involved in such research activities has been provided by the industrial sector. Despite some unwanted dimensions of such collaborative relationship, they have in general provided an opportunity to researchers and scholars by facilitating their continued engagement in research activities.^{1,2}

Unfortunately, our local industry, in this regard, is not only scarce and weak but it is also not cognizant of the importance of investing in Research & Development (R&D) related work. The only involvement of the local pharmaceutical sector is up to a level not encompassing any research that could lead to the development and practical implementation of new molecules of pharmaceutical relevance.³⁻⁵ This poor situation prevailing in our medical institutes and universities has been mainly because of the government decision taken in the late 70s that led to the taking-over of medical education and its funding from main universities by the local health ministries. As a result, our medical colleges and its attached teaching hospitals have remained simply technical training and certifying places and treatment providing centers with no emphasis put on research and innovation. Thus, during this entire period they remained as non research intensive medical centers where interest of the concerned faculty in research had to end with the achievement of career promotion target.⁶

Recently with the re-establishment of independent medical universities and their funding by the Higher

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Education Commission (HEC) Pakistan, it has become a mandatory requirement for these universities to build their existing research and development capacity in terms of diversity and quality of their faculty and institutional infra-structure as well as to provide an environment that is conducive and intensive for research. They had to do all this very urgently and rephrase so that their status is raised beyond the simple awarding of certificates leading to practice licensure of the graduating medical students. In fact our medical universities and its institutes should come up as places of excellence for providing health-care manpower capable to perform multidimensional duties including innovative health-care and health-system research. By ensuring science and research in our medical institutes, we will not only facilitate new inventions, products and technologies but will also provide a base for the practice of medicine that is evidence based.⁷

One way to achieve the desired research objectives is that medical universities and institutes in this country have to attract the industry and to convince it to invest in their research and development work. This will only be possible if they could show the industry their research strength and science base. They should also have the potential and ability to educate the local industry about the reality that rapid exploration of scientific advances is the key to survival. Both parties (university faculty and industry) must be aware of the several ethical concerns resulting as a consequence of such academia – industry (A-I) relations. The ever-rising incidence and complexity of these issues have intensified the oversight of research conducted under such A-I collaborations.

Keeping in mind the above concerns relating to A-I collaborations, it is possible to build such multiple and mutually beneficial research collaborations between medical academia and industry. These will surely pave the way for the development of new medical products and technologies. These collaborations will enhance the research infra-structure within the institutions as well as their intellectual credibility and reputation. These will also facilitate the provision of additional income for them through royalties, licensing and sales of their research discoveries. In fact research support from the industry has facilitated research that would

not have been otherwise possible.^{8,9}

Any innovative research carried out in a medical university or institute and its further advancement generally require the help of industry. The industry or a corporate body is better able to translate research findings, new discoveries and innovative concepts into profitable products and technologies. Upon translation of a discovery into practice, further “proof of concept” studies as well as its further updating will be needed. Obviously, more money for such continued research work will be required. Thus the need for support from the industry shall always exist within our universities so that they remain actively engaged in innovative research.

Apart from the availability of faculty and research infrastructure, the establishment and maintenance of collaboration with industry requires additional considerations. The university and its faculty should have earned guaranteed reputation for data confidentiality and research activities. They should have review and ethics boards at the level of institution (IREB) as well as university (UREB). To attract the industry, the available faculty should not only be sufficient but also diverse and well trained for doing the intended research with minimal cost. The faculty should have received advance training and skills necessary for negotiating and addressing issues related to research contracts, protocols, budgets, payment terms, liability, schedules and timelines, data dissemination publication, royalties and patents. In fact the faculty should be well-aware of all the problems and issues that may arise during the course of such A-I relations.¹⁰ Obviously, a few of the existing faculty in our medical universities have been adequately trained for dealing with these issues. It is thus hoped that the topic of A-I Collaboration will be made an integral part of postgraduate training programmes in our medical universities.

To sum up, there is a lot that need to be done to obtain maximum benefits of the A-I relations. The Higher Education Commission (HEC) Pakistan is generously supporting conferences, seminars and workshops that could raise the level of awarness among the industry and academia. It has also set up an industrial liaison secretariat for the purpose.¹¹ The objective is to develop an effective cooperation between academia and industry and to capitalize on the ever increasing international demand for products and processes. On one side it has launched new programs in the academic

institutions to support the discovery of new knowledge and enhancement of the skilled workforce. On the other side, industry is being informed to identify their needs according to the changing circumstances so that intellectual capital and emerging technologies are brought together in such a way that will promote economic growth and an improved quality of life. Medical universities should get maximum support of the kind as offered by the HEC. At the same time, it is also for the HEC to tie the funding of universities with a minimum amount of money earned through A-I collaborations and innovations.

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A HISTOLOGIC STUDY OF CARBIMAZOLE-INDUCED HYPERPLASIA OF ADENOHYPHYSIS WITH PROTECTIVE ROLE OF THYROXINE IN MALE ALBINO RATS

Khalida Perveen¹, Naheed Khan², Muhammad Rafique¹, Syed Samiullah¹ and Anjum Naqvi³

ABSTRACT

Objective : To assess the frequency and degree of pituitary hyperplasia in albino rats made hypothyroid by Carbimazole, and the association of severity of pituitary enlargement, and the response to treatment with thyroxine. To determine the microscopic changes occurring in Beta basophil cells (Thyroid stimulating hormone & Adreno corticotrophin hormone producing cells) of Anterior Pituitary gland by giving an anti-thyroid drug, Carbimazole and Carbimazol plus Thyroxin on Anterior pituitary glands of male albino rats with increasing time period.

Design: Experimental study

Place: Anatomy Department, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi.

Methods: Forty five healthy, young adult male albino rats were selected for the study. They were distributed into 3 main groups of 15 rats each. Group A served as control while Group B received injection Carbimazole 6 µgm/G body weight subcutaneously daily. Group-C were treated with injection Carbimazole 6 µgm/G body weight subcutaneously plus injection Thyroxin 5 µgm intraperitoneally daily for their respective period of treatment. Each group was further subdivided into three sub-groups according to the period of treatment they received i.e. 2, 4 & 6 weeks at the end of which animals were sacrificed. The Pituitary glands were dissected out after processing and staining (Wilson-Ezrin method). The tissues were subjected to detailed micrometric examination.

Result: The results are based on changes in morphometric study of number and diameter of Beta Basophil cells in anterior Pituitary gland. Mean value of number of Basophils were increased significantly ($P < 0.001$) in group B (Carbimazole treated) i.e 151.0 ± 3.38 than group A (control) i.e 82.2 ± 3.48 . While in group C (Carbimazole plus Thyroxin treated) the number of cells were decreased i.e 117.6 ± 3.83 than group B but were more than group A, Beta Basophil cell size (diameter) was also increased significantly ($P < 0.001$) in group B i.e 17.64 ± 1.06 than group A 14.45 ± 3.28 .

In group C Beta Basophil cell size was 16.16 ± 2.02 , which was more than group A but less than group C. The number and size of Beta Basophils in group C was significant ($P < 0.05$), when compared with corresponding controls.

Conclusion:. In conclusion these results strongly suggest that Carbimazole-induced hyperplasia and hypertrophy of Anterior pituitary gland may be prevented by simultaneous treatment with Thyroxin. Long standing treatment with Carbimazole in Hyperthyroid patients as in Graves disease should accompany, small doses of Thyroxin as well, to avoid the enlargement of Anterior Pituitary gland during their treatment.

Keywords:. Pituitary, Adenohypophysis, Carbimazole, Hyperplasia, Thyroxin.

INTRODUCTION

Pituitary hyperplasia has been known for a long period of time and yet remains poorly understood.¹

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Pituitary hyperplasia due to primary hypothyroidism and presents growth arrest causing mass on MRI can regress with thyroxine therapy and symptoms be resolved.²

Regulation of the thyroid gland by the hypothalamo-pituitary axis depends on feedback loops. The thyroid gland secretes triiodothyronine (T3) and thyroxine (T4). Hypothyroidism arises through iodine

deficiency, autoimmunity or surgery: treatment is by replacement of T3 and T4. Hyperthyroidism is also an autoimmune disease, commonly Graves' disease. Treatment is by selective destruction of thyroid cells by radioiodine. Alternatively thioureylenes (carbimazole) are used to inhibit iodination of thyroglobulin, thereby reducing T3 and T4 synthesis.³

Many cases of Thyroid hyperplasia resulting from prolonged primary hypothyroidism have been reported.^{4,5} Thyrotroph hyperplasia is usually reversible with the institution of thyroxine replacement therapy. Thyrotroph releasing hormone is likely to be a major player initiating thyrotroph proliferation.^{1,4}

Pituitary hyperplasia is defined as a non neoplastic increase in one or more functionally distinct types of pituitary cells. The acini though expanded, remain intact.^{5,6}

The anterior pituitary gland integrates the repertoire of hormonal signals controlling thyroid, adrenal, reproductive, and growth functions. The pituitary gland responds to complex central and peripheral signals by two mechanisms. First trophic hormone secretion is exquisitely controlled to regulate homeostasis. Second, developmental or acquired pituitary signals may elicit plastic pituitary growth responses, consisting of either hypoplasia, hyperplasia or adenoma formation.⁷

Any cell population within the pituitary gland can undergo hyperplasia and when prolonged may progress to adenoma formation.^{8,9} Pituitary enlargement occurs in primary hypothyroidism due to pituitary thyrotroph hyperplasia.¹⁰ Untreated primary hypothyroidism has long been recognized as causing enlargement of the pituitary gland and sella turcica.¹¹ The pituitary enlargement, representing hyperplasia or adenomatous transformation of pituitary thyrotropic cells in response to primary thyroid deficiency.¹² Patients with longstanding primary hypothyroidism may have pituitary enlargement visible on MRI or computed tomography.¹³ Thiomides-methimazole- and propyl thiouracil are major drugs for the treatment of thyrotoxicosis. In the United Kingdom Carbimazole which is converted to methimazole in vivo is widely used. Methimazole is about 10 times more active than propyl thiouracil.¹⁴

The principal hormones of the thyroid gland are the iodine containing amino-acid derivative of thyroxine (T4 and T3). Patients with hypothyroidism are usually treated with thyroxine (levothyroxine) only, although both thyroxine and tri-iodothyronine are secreted by normal thyroid gland.¹⁵ In any patient with pituitary gland enlargement, primary hypothyroidism must be excluded. Circulating thyroxine normally serves as a negative feedback on release of thyrotropin-releasing hormone by the hypothalamus. If the thyroid gland secretes insufficient quantities of thyroxine, levels of thyrotropin-releasing hormone will increase. This, in turn, results in thyrotroph hyperplasia and pituitary gland enlargement.^{16,17}

MATERIALS AND METHODS

This study was conducted in Department of Anatomy, BMSI, JPMC, Karachi during the period 2004--2005. The animals used for this experimental study were adult male albino rats. A total of 45 animals of 190-250 G were selected for this experimental study and maintained on balanced laboratory diet.

All the animals were divided into three groups A, B and C each group comprising 15 animals. Each group is further divided with three sub-groups based on the period of treatment 2, 4 & 6 weeks respectively, each sub-group comprising of five animals.

The animals of group-A served as normal control received injections of normal saline 1c.c daily for 2, 4 and 6 weeks respectively, and animals of group-B were treated with injection Carbimazole 6 µgm/G body weight subcutaneously daily for 2, 4 and 6 weeks respectively.

The animals in group-C were treated with injection Carbimazole plus injection Thyroxine 5 µgm in 0.9% NaCl intraperitoneally daily for their respective period of time.

They were sacrificed according to time period of treatment under ether anaesthesia.

Pituitary glands were fixed in Zenker formal (Helly's solution) for 4-6 hours. After tissues processing, Five micron thick sections were cut on rotatory microtome and mounted on glass slides. These were stained with Wilson-Ezrin method.

In Wilson-Ezrin method (PAS, Orange G and

Methylene blue) beta basophil cells of anterior pituitary were counted with the help of ocular counting reticule. The size of beta basophil cells was measured with the help of ocular micrometer scale and counting reticules.

The statistical significance of differences of various quantitative changes between Carbimazole and Carbimazole plus Thyroxine treated and control rats were evaluated by student ‘t’ test.

OBSERVATIONS AND RESULTS

The present study was designed to observe the effects of Carbimazole and Carbimazole plus Thyroxine on the number and size of Basophils particularly Beta Basophils in anterior pituitary gland at variable time intervals.

The observation and results of the present study demonstrated that Carbimazole is effective in producing hyperplasia in the anterior pituitary gland and reversion of hyperplasia by giving thyroxine in experimental animals.

Mean values of Basophil numbers were recorded and shown in table-1. When comparing group B with group A there was statistically significant increase ($P < 0.001$) in numbers of Basophils, as seen in Fig-1 (control), Fig-2 (treated with Carbimazole) and Fig-3 (treated with Carbimazole and Thyroxine).

Table 1: Mean* Basophil Cell Count of Animals in Different Groups at Variable Time Period

Group	No. of Subject	Basophil Counts Durations		
		2 Week	4 Week	6 Week
Control (A)	n=5	80.8 ± 4.16	80.8 ± 5.0	82.2 ± 3.48
Carbimazole (B)	n=5	129.6 ± 9.69	184.8 ± 9.86	151.0 ± 3.38
Carbimazole + Thyroxine (C)	n=5	116.2 ± 3.02	142.8 ± 4.93	117.6 ± 3.83

*Mean ± Standard Error

P value = 0.001 means statistically highly significant.

Note: Microscopy was performed under high power i.e 40x objective and 8x ocular with the help of ocular micrometer scale and counting reticule.

A stage micrometer was used for the calibration of

the ocular micrometer scale and ocular counting reticule. The stage micrometer used in the study had a scale of 1mm length divided into 100 parts. So each division measured 10 µm. The ocular micrometer used in this study had 100 divisions. The reticule used had five squares along both the ‘x’ and ‘y’ axes. The ocular micrometer was placed in right eyepiece of microscope and reticule in the left eyepiece.

Table 2: Mean* Beta Basophil Cell Count of Animals in Different Groups at Variable Time Period

Group	No. of Subjects	Beta Counts Durations		
		2 Week	4 Week	6 Week
Control (A)	n=5	28.6 ± 1.69	30.6 ± 2.29	34.6 ± 3.34
Carbimazole (B)	n=5	80.4 ± 9.04	110.06 ± 10.42	91.0 ± 2.21
Carbimazole + Thyroxine (C)	n=5	55.4 ± 9.84	79.2 ± 2.08	67.0 ± 1.52

*Mean ± Standard Error

Mean values of Beta Basophil numbers were recorded and shown in table 2. There was significant increase ($P < 0.05$) in Beta cell count while comparing group A with group C. The highly significant increase ($P < 0.001$) in Beta cell count when comparing group A with groups B as shown in Graph-1.

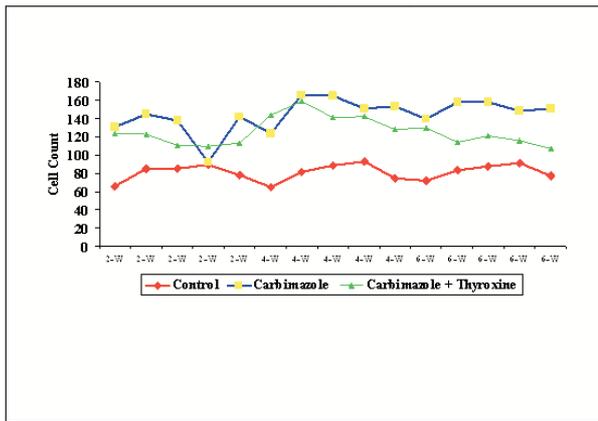
Table 3: Mean* Basophils Size (microns) Count of Animals in Different Groups at Variable Time Period

Group	No. of Subject	Basophil Counts Durations		
		2 Week	4 Week	6 Week
Control (A)	n=5	13.78 ± 2.90	14.04 ± 2.36	14.45 ± 3.28
Carbimazole (B)	n=5	16.94 ± 5.77	17.5 ± 6.04	17.64 ± 1.06
Carbimazole + Thyroxine (C)	n=5	13.22 ± 3.84	14.91 ± 0.44	16.16 ± 2.02

*Mean ± Standard Error

P value = 0.001 means statistically highly significant.

Mean values of Beta Basophil Size were recorded and shown in table-3 when comparing group A with group B there was statistically highly significant increase ($P < 0.001$) in size of Beta Basophils. Significant increase ($P < 0.05$) in size of cells comparing group C with group A. When comparing group C with group B, highly significant increase ($P < 0.001$) in size of cells were noted as shown in Graph-2.



Graph - 1 : Beta Basophil Cell Count in Control , Carbimazole and Carbimazole + Thyroxine Groups

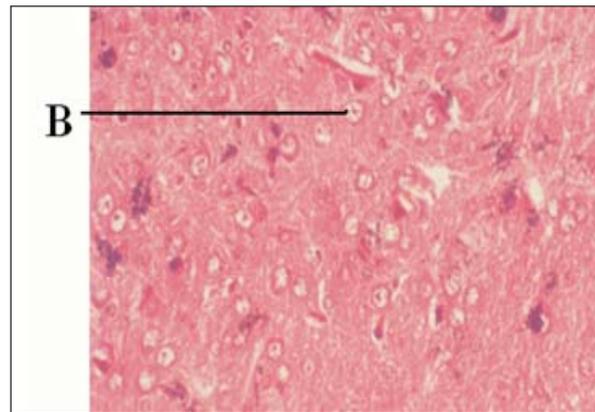
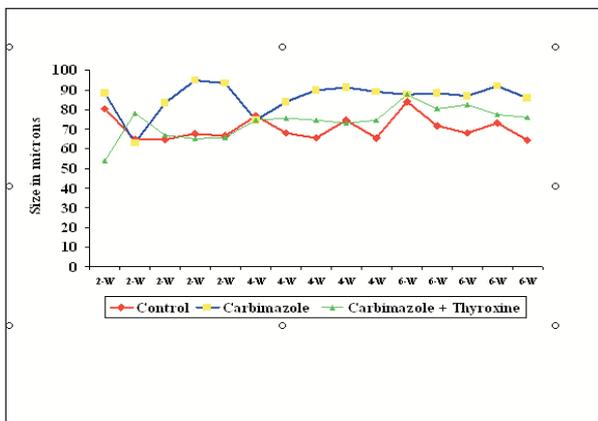


Figure - 2 : PAS-Orange-G, Methylene Blue (Wilson Ezrin Method) stained 5 μm thick section of pituitary gland showing increased number of basophils (B), after four weeks Carbimazole treatment in rat. (Photomicrograph under high power x 40)



Graph - 2 : Size of Basophils (microns) in Control, Carbimazole and Carbimazole + Thyroxine Groups

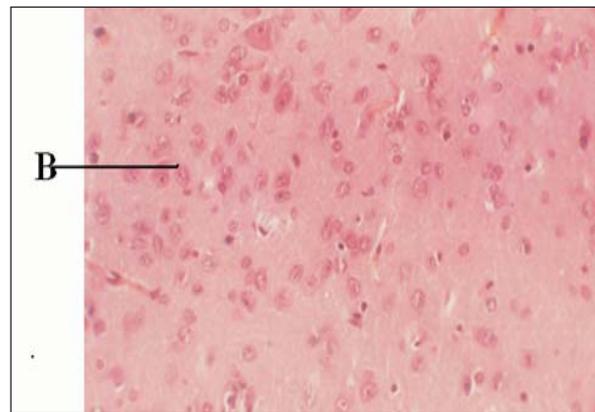


Figure - 3 : PAS-Orange-G, Methylene Blue (Wilson Ezrin Method) stained 5 μm thick sections of pituitary gland showing increased number of basophils (B) after six weeks Carbimazole plus Thyroxine treatment in rat. (Photomicrograph under high power x 40)

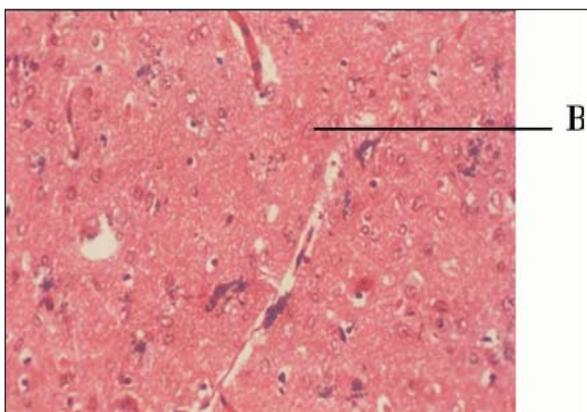


Figure - 1 : PAS-Orange-G, Methylene Blue (Wilson Ezrin Method) stained 5 μm thick section of the pituitary gland showing basophils (B) in control rat. (Photomicrograph under low power x 40)

DISCUSSION

This study was designed according to Inauwa and Williams in 1995 who produced hypothyroidism in rats by administering Carbimazole and then treated them with Thyroxine.¹⁸ They observed the effects of these drugs on the uterine horns. In this study the effects of two drugs (Carbimazole and Thyroxine) were observed on the adenohypophysis of male albino rats. The present study demonstrates the acute reversal of pituitary hyperplasia after Thyroxine therapy. Total number of Basophil cells in Wilson Ezrin method and high magnification, increased markedly in group-B (Carbimazole treated) as compared to

age matched controls, but they were less increased after the combination of Carbimazole with Thyroxine in group-C. However these cells were more than control group.

The effect on pituitary basophils of introducing thyroxine daily injection of 5 µg of L-thyroxine shows regressive changes in the central group of basophil cells. Thyroxine is the treatment of choice in hypothyroidism. Thyroxine was first isolated in the crystalline form, from a hydrolysate of thyroid by Kendall in 1915. The principal hormones of the thyroid gland are the iodine containing aminoacid derivative of thyronine (T4 and T3).

Dose of thyroxine is 1.7µg/kg/day with requirements falling to 1 µg/ kg/day in the elderly (Hueston, 2001).¹⁹

This study matches with the study (Pioro EP et al 1988). Primary hypothyroidism may also produce pituitary enlargement secondary to thyrotroph hyperplasia and present with a sellar mass.²⁰ Although laboratory and radiologic abnormalities of pituitary enlargement may resolve after corrective thyroid therapy. Histologic examination revealed thyrotroph hyperplasia. Thyrotroph hyperplasia probably results from lack of negative feedback of thyroid hormone upon the anterior pituitary, which is probably due to hypothalamic release of thyrotropin-releasing hormone (TRH). Pituitary hyperplasia is characterized by increased proliferation of a single cell type, which may be focal, nodular, or diffuse. There is an absolute increase in numbers of specific cells, with pituitary enlargement visible on MRI. Pituitary hyperplasia may range from modest cell type increases to large glandular expansion with grossly altered tissue architecture and morphology.²¹ Specifically, corticotroph hyperplasia may be associated with Crooke's hyaline changes, and thyrotroph hyperplasia, with periodic acid Schiff-positive lysosomes. Rarely, pituitary hyperplasia may be of primary origin, and is usually secondary to extrinsic signals. Normal pituitary height as assessed by MRI is up to 9 mm in healthy subjects, while adolescent females tend to have larger pituitary glands.²²

This study also correlates with the study performed by (Shlomo Melmed 2003), who states that target hormones (sex, adrenal steroids, and thyroid hormones) exert powerful negative feedback

inhibition of their respective trophic hormone gene transcription and hormone secretion, as well as suppression of pituitary growth. Failure of target glands (thyroid, adrenal, and gonads) leads to loss of negative feedback inhibition and resultant compensatory hyperplasia of the respective pituitary trophic hormone cells. Thus, longstanding primary hypothyroidism, hypogonadism, or hypoadrenalism may be associated with a clinically enlarged pituitary gland visible on MRI, with involution of the gland occurring after appropriate target hormone replacement and restoration of negative feedback.⁷

There was a marked increase in beta cell count in group-B, same was also increased in group-C which was more than controls but less marked than group-B. So we conclude that increase in Basophil count was due to Beta basophils which include Corticotrophs and Thyrotrophs. This increase in Beta count was due to positive feedback produced by lower level of circulating thyroid hormones as a result of Carbimazole treatment. This feedback stimulates thyrotrophs for more production of TSH by thyrotrophs. The increase was more in Carbimazole treated group, than in Carbimazole plus Thyroxine treated group but increase was more than control. In conclusion these results strongly suggest that Carbimazole-induced hyperplasia and hypertrophy of pituitary gland may be prevented by simultaneous treatment with Thyroxine. Thyroxin along with Carbimazole is more effective, to make euthyroid and to prevent the hyperplasia of anterior pituitary gland..

The Electron microscope study should be performed for the cellular details for their more and clear differentiation which were not performed because of its non-availability.

CONCLUSION

The present study supports the view that the animals were made hypothyroid with Carbimazole treatment, and that Thyroxin in support to the treatment reversed the changes in adenohypophysis.

In conclusion, these results strongly suggest that Carbimazole-induced hyperplasia and hypertrophy of Anterior pituitary gland may be prevented by simultaneous treatment with Thyroxin. Long standing treatment with Carbimazole in Hyperthyroid patients as in Graves disease should accompany, small doses

of Thyroxin as well to avoid the enlargement of Anterior Pituitary gland during their treatment. The association between pituitary gland enlargement and hypothyroidism should be kept in mind when pituitary hyperplasia is detected on MRI, before unwarranted and drastic interventions are initiated.

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TOXIC EFFECTS OF LEAD ON THE GERMINAL EPITHELIUM IN MALE ALBINO RATS

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ABSTRACT

Objective: To determine the toxic effects of lead on the germinal epithelium of testes of albino rat.

Study Design: Experimental study.

Place and Duration of Study: Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, from August 2003 to December 2005.

Methods: Forty adult Albino rats selected for the study were divided into two groups; group A, received injection normal saline 1 ml intraperitoneally daily for eight weeks. Group B received lead chloride in a dose of 10 mg / kg body weight intraperitoneally daily. The testes were removed and fixed in Bouin's fluid for 24 hours. They were dehydrated in ascending strength of alcohol and the paraffin blocks were made. Four μm thick tissue sections were obtained, stained with PAS Iron Hematoxylin method and the morphometric study was done. Student's T-test was used for statistical analysis.

Results: Student's T test was used to determinate significance; P value = 0.05 was taken significant. Mean \pm SEM diameter of seminiferous tubules was 291.92 ± 1.11706 mm and 198.54 ± 1.67282 mm in groups A and B respectively after eight week of treatment. Mean diameter of seminiferous tubule of group B was decreased significantly ($P < 0.0001$) as compared to groups A. Mean \pm SEM thickness of germinal epithelium was 96.19 ± 1.01215 mm and 50.69 ± 1.20064 mm in groups A and B respectively after eight week of treatment. Mean thickness of germinal epithelium of seminiferous tubules of group B was decrease significantly ($P < 0.0001$) as compare to group A.

Conclusion: Heavy metal lead present in environment had direct toxic effects on male germinal epithelium and produced damaged on male germinal epithelium.

Keywords: Lead chloride, Albino rats, Seminiferous tubules and Germinal epithelium.

INTRODUCTION

Most of the heavy metals are toxic to the human body. The oldest and most important heavy metal which is toxic to the most of organs of body is lead.

Lead poisoning may affect body organs for several years even in the absence of continued exposure. Reproductive toxicity is an important feature of lead toxicity. During exposure, lead accumulates in testis tissue dependent upon the dose. Lead toxicity induces

a significant increase in apoptotic cell death in the seminiferous tubules of young growing rats. It is also associated with disruption of spermatogenesis, histoarchitecture and lowered enzyme activities in testis.¹

Accumulated data suggests that there is a close relationship between declining reproductive health and environmental pollutants like fluoride (F) and lead (Pb).² Reproductive dysfunction induced by fluoride (F) and lead (Pb) has distinct morphological

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and biochemical features such as disorganized germinal epithelia, giant cells in the lumen, decreased sperm quality, and low androgen levels.^{3,4} Earlier investigations indicate that high lead exposure can also reduce sperm quality, decrease sperm count and motility, besides sperm morphology.^{5,6} The previous altering studies suggest that disturbance of energy metabolism plays an important role in reducing sperm activity and blocking sperm maturation.^{7,8} In recent years, there are increasing reports indicating low sperm quality caused by F and Pb alone. Epidemiological investigations indicate that F pollution is accompanied by enhanced Pb levels in drinking water.^{9,10}

The diminution of semen quality due to occupational exposure of heavy metals is a major health concern around the world.¹¹⁻¹³ Lead exposure and moderate lead absorption produces alteration in fertility with decreased production in spermatozoa in the battery factory workers probably due to the direct toxic effect of lead on germinal epithelium of testis during spermatogenesis.¹⁴⁻¹⁶ Blood lead levels also inversely correlated with sperm count and viability.¹⁷ Reduction in sperm motility, count, density, and low antioxidant profile along with increase incidence of sperm abnormality and sperm membrane lipid per oxidation was prevalent after occupational lead exposure.^{18,19}

Occupational exposure to heavy metal lead, caused toxicity in industrial workers of smelters, acid battery plants, lead production units, and storage battery plants etc.²⁰⁻²¹ Lead exposure and moderate lead absorption produced alteration in fertility with decreased production of spermatozoa, probably due to the direct toxic effect of lead on germinal epithelium of testis during spermatogenesis.²²⁻²⁵ Among different abnormalities, significant reduction in total motile sperm proportion, count, viability, forward progression, sperm kinetics, teratogenicity (morphological abnormalities) of spermatozoa were reported along with elevated blood lead levels after exposure to lead.²⁶⁻²⁸ Recently it has also been reported that lead exerted some deleterious effects on testicular steroid genesis indirectly by decreasing serum level of gonadotropin.²⁹

The objective of this study was to determine the

toxic effects of lead on the germinal epithelium of albino rats.

MATERIAL AND METHOD

The experimental study was carried out at Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, from August 2003 to December 2005. Forty adult male albino rats between the ages of 90 to 120 days were obtained from Animal House, BMSI and JPMS. They were maintained at food and water ad libitum. The animals were divided into two groups A and B, each group consisted of 20 animals. The animals were kept in separate cages.

Group A: Served as Control Group and divided into four subgroups (A1, A2, A3 & A4), based on the period of treatment (1, 3, 5 and 8 weeks), Each subgroup consisted of five animals. This group received injection Normal Saline 1 ml. intraperitoneally daily for their respective period of treatment.

Group B: Lead Group was also divided into four subgroups (B1, B2, B3 & B4) based on the period of treatment (1, 3, 5 and 8 weeks). Each subgroup consisted of five animals. This group received Lead Chloride in dose of 10 mg/kg body weight in distilled water, intraperitoneally daily for eight weeks.³⁰ On the day of completion of treatment, the animals were sacrificed under deep Ether anesthesia. The scrotal sacs were identified and opened. Testes, vas deferens and blood vessels were excised.

TISSUE TREATMENT: The testes were fixed in Bouin's fluid for 24 hours, after that they were cut longitudinally into two equal halves and again post fixed in fresh Bouin's fluid for next 24 hours. The tissues were dehydrated in the ascending strengths of alcohol, cleared in xylene. Infiltrated and embedded in paraffin wax, the tissue blocks were made, and were cut into 4 m thick sections with the help of rotatory microtome. The sections were mounted on albumenized glass slides and stained with PAS-Iron Hematoxylin. Morphometric study of germinal epithelium was measured with the help of ocular

micrometer scale under light microscope.

The level of significance (P) was calculated by the help of student's t-distribution table and the P value was read against the table degree of freedom (d. f.). The significance level was considered as $P = 0.05$

All the calculations were done utilizing computer software, "SPSS 15.0" in Window 2000 XP.

RESULTS

The diameters of seminiferous tubules were recorded at different time intervals in different groups as shown in Table-I. The diameter of seminiferous tubules was shown in Figure 1. The diameter of germinal epithelium was decreased from 291.92 ± 1.17906 mm (group A) to 198.54 ± 1.67282 mm (group B). There was statistically significant decrease in diameter of seminiferous tubules ($P < 0.001$) in group B as compared to group A after eight weeks of treatment. The thicknesses of germinal epithelium of seminiferous tubules were recorded at different time intervals in different groups as shown in Table-II. The thickness of germinal epithelium of seminiferous tubules is given in Figure-2. The thickness of germinal epithelium was decreased from 96.19 ± 1.01215 mm (group A) to 50.69 ± 1.20064 mm in group B. There was statistically significant decrease in thickness of seminiferous tubules ($P < 0.001$) in group B as compared to group A after eight weeks of treatment.

DISCUSSION

Many recent studies have indicated an increasing prevalence of various abnormalities of reproductive system in human males. There is growing concern about the considerable decrease in sperm density over the last 50 years in general population worldwide^{31,32}

The present study is based on the fact that the trace elements or heavy metals produce male genital system abnormalities and damage the germinal epithelium producing oligospermia, asthenospermia, teratozoospermia and azoospermia, which account for 20–25% of cases.³³

One of the important trace element, lead was used for the study. Lead intoxication and its effects on male germinal epithelium and male reproductive system has been focussed in many pervious studies.^{33, 35} But the morphometric study of seminiferous tubules in respect to diameter of seminiferous tubules and thickness of germinal epithelium is still lacking. The study was designed to observe the morphometric changes that appeared after the lead accumulation in testes of albino rats. Lead was used in a dose of 10 mg/kg body weight intraperitoneally; the same dose was used by Batra and coworkers.³⁵ The heavy metal was given in injectable form so that accurately calculated doses of solutions can be administered to animals. This was against the study of researchers Antnio & co. workers (2004)³⁴ and Batra & his colleagues 1998, 2004).^{30,35}

The decrease in diameter of seminiferous tubules in lead treated group was due to the degeneration of collagen fibers and germinal epithelium. The degenerated germinal epithelium collected in the lumen of seminiferous tubules as slough. The degeneration of interstitial tissues resulted in widening of interstitial spaces between the seminiferous tubules. This finding can be correlated with finding of Batra et al (1998, 2004).^{30,34}

The thickness of germinal epithelium decreased in the lead treated group and this was due to loss of series of cells comprising of germinal epithelium, ranging from spermatogonia to spermatozoa with final reduction in the numbers of mature sperms. This finding correlated with finding of Antnio & colleagues (2004) Batra et al. (1998, 2004).^{30, 34}

Reduced width of germinal epithelium, which was seen in this study seems to be due to damage of germinal epithelial cells which was also reported by other researchers, Barta & coworkers (2001, 2004).^{30,36} The number of Spermatogonia and spermatocytes was almost intact in lead exposed animals; this reduction may be mainly due to decrease of number of spermatids Batra et al. (2004).³⁰ Lead induced apoptosis of germinal cells, reported by Adhikari &

his colleagues (2001) may be a possible mechanism for loss of germinal epithelium (Adhikari et al., 2001).³⁷Batra and coworkers (2001) observed, dose depended reduction in the activity of two major enzymes in the testis, alkaline phosphatase and Na-K ATPase, in lead exposed animals, which is another probable mechanism of lead induced reproductive toxicity (Batra et al., 2001).³⁶

According to the study there was reduction of germinal epithelium width and number of sertoli cells. The number of spermatogonia and primary spermatocytes was lower than control as demonstrated by Han et al., (1997).³⁸

CONCLUSION

Based on this study, it is concluded that lead severely damages the germinal epithelium and causes degeneration of collagen fibers which results in shrinkage of seminiferous tubules with decreased diameter of seminiferous tubules. At the same time the direct toxic effects of lead on germinal epithelium reduces thickness of germinal epithelium. The presence of lead in environment has opened the new era for further study in human.

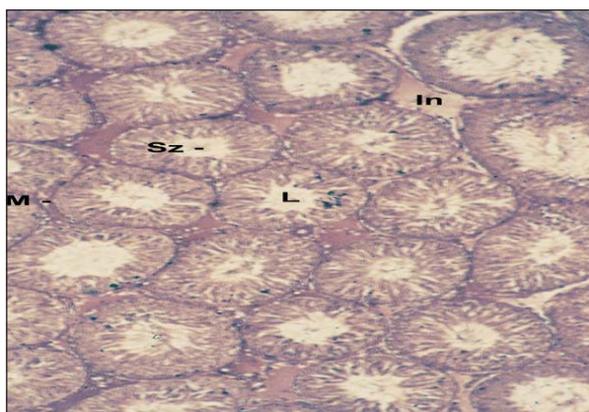


Figure – 1: Photomicrograph of PAS-Iron Hematoxylin 4 mm thick stained section of testis showing regular compact arrangement of seminiferous tubules with intact BM, lumen of tubules contained spermatozoa in control albino rat, low magnification

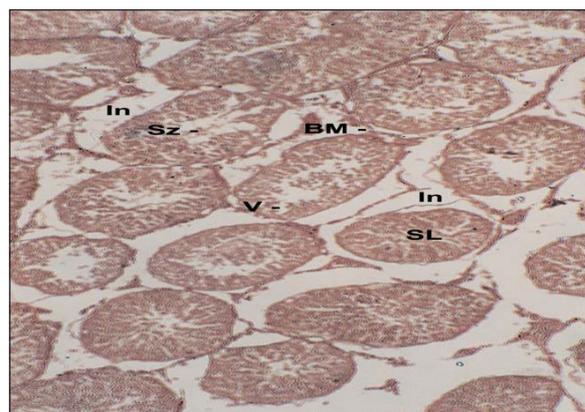


Figure – 2: Photomicrograph of PAS-Iron Haematoxylin stained 4 mm thick section of testis showing shrunken seminiferous tubules with mark widening of interstitial space, distorted BM, and lumen of tubules contained slough, after eight weeks in lead treated albino rat, at low magnification.

Table 1: Mean* diameter (μm) of seminiferous tubules of albino rats in different groups at variable time interval

Group	First week (1)	Third week (2)	Fifth week (3)	Eight week (4)
A	271.48 \pm 3.15837	279.11 \pm 1.78070	283.6 \pm 1.64112	291.92 \pm 1.17906
h = 20	h = 5	h = 5	(h = 5)	(h = 5)
B	234.25 \pm 3.10299	229.19 \pm 1.69550	211.46 \pm 2.07906	198.54 \pm 1.67282
h = 20	h = 5	h = 5	(h = 5)	(h = 5)
P- Value	A1 VS B1 0.020	A2 VS B2 0.008	A3 VS B3 0.002	A4 VS B4 0.000

* Mean \pm SEM (Mean of 5 animals in a subgroup)
P-Value \leq 0.05 is significant

Table 2: Mean* Thickness of Germinal Epithelium (m) of seminiferous tubules of albino rats in different groups at variable time interval

Group	First week (1)	Third week (2)	Fifth week (3)	Eight week (4)
A	82.56 \pm 1.00945	88.21 \pm 0.97324	90.41 \pm 1.22951	96.19 \pm 1.01215
h = 20	h = 5	h = 5	(h = 5)	(h = 5)
B	70.99 \pm 0.93983	66.89 \pm 0.92875	56.79 \pm 0.737962	50.69 \pm 1.20064
h = 20	h = 5	h = 5	(h = 5)	(h = 5)
P- Value	A1 VS B1 0.020	A2 VS B2 0.008	A3 VS B3 0.002	A4 VS B4 0.000

*Mean \pm SEM
P value \leq 0.05 means statistically significant.

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SERUM AND SALIVARY MINERALS IN DENTAL CARIES

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ABSTRACT

Background: Dental caries is a multifactor disease, affecting people of all ages. Inorganic mineral of serum and saliva can also have protective role in dental caries. This study was carried out to evaluate and compare the possible role of salivary and serum factors like pH, adequate level of calcium, phosphate and fluoride in dental caries.

Methodology: A Total of 100 subjects aged 10-40 were selected. Decayed, missed and filled teeth (DMFT) were used as indices for scoring the dental caries and were distributed or divided into 4 groups on the basis of DMFT indices as 4-8 (Group I), 9-16 (Group II), 17-24 (Group III) and more than 25 (Group IV), while the control subjects had DMFT index equal to or less than 3. pH, calcium, phosphate, fluoride and lactic acid were analyzed in saliva and serum.

Results: Patients of dental caries showed significantly low levels of calcium, phosphate, fluoride ($P < 0.001$) and significantly high level of lactic acid ($P < 0.001$) in all the groups as compared to control subjects. Prominent significant changes were observed in different groups. The salivary and serum pH, calcium, fluoride, phosphate and lactic levels were found to be significantly changed among the patients having dental caries.

Conclusion: It can be concluded from the findings of present study that the adequate levels of calcium, phosphate and fluoride in saliva as well as serum are responsible for the significant deposition of these minerals in plaque which greatly reduces the developmental caries in the adjacent enamel.

Keywords: Serum, Saliva, Calcium, Phosphate, Fluoride, Lactic acid, Dental caries.

INTRODUCTION

Dental caries is a multifactorial disease, which has affected people throughout the ages.^{1,2} Many constituent of serum and saliva, both organic and inorganic have potentially protective role. These include pH, calcium, phosphate, fluoride ions and

bicarbonate buffer systems.³⁻⁵ Epidemiological studies have supported the view that raised level of calcium, phosphate, and Fluoride in plaque might inhibit dental caries.⁶⁻⁹ It is commonly thought that the organic acid produced in dental plaque is responsible for caries, but this is partly true because it is a complex effect of pH, calcium, phosphate and fluoride, which brought about minerals dissolution.¹⁰ In low concentration, fluoride alone partially inhibits the net dissolution of enamel and the production of acid by plaque organisms, while demineralization

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requires the presence of calcium and phosphate.¹¹⁻¹³ The present study was done to estimate and compare the salivary and serum calcium, phosphate, and fluoride in the patients of dental caries and to see and compare their levels with the severity of disease and control.

MATERIALS AND METHODS

A total of 100 subjects aged 10-40 years were selected from the Department of Dentistry, Jinnah Postgraduate Medical Centre and from the Out Patient Department of Fatima Jinnah Dental Hospital Karachi, Pakistan. The subjects were not suffering from any systemic illness and were not taking any caries preventive regimen like fluoride toothpaste, fluoride rinses or NaF/calcium tablets. Subjects who gave improper history about missing teeth or suffering from any type of Xerostomia or having any oral inflammatory problems were not included in the study.

Dental examination was done with the assistance of dentist under natural light source. Decayed, missed and filled teeth (DMFT) were used as index for scoring the dental caries.¹⁴ All subjects were distributed into 5 groups (Table 1) each having twenty individuals. Group 1 with DMFT index 4-8, group 2 with DMFT index 9-16, group 3 with DMFT index 17-24 and group 4 with DMFT index more than 25, while the control subjects have the DMFT index equal or less than 3.

About 10 mL of unstimulated mixed saliva was obtained from the individual 2 hours after the breakfast and 10 minutes after mouthwash with deionized water several times. Saliva was collected in 10 cc disposable plastic syringes after removing the piston and blocking the needle. Syringes were then labeled, covered and transferred in icebox to the laboratory. The saliva samples were centrifuged for 15 minutes at 3000 rpm. The clear supernatant of saliva was separated and labeled tubes were stored

at -20°C.

Approximately 10 mL of venous blood sample was drawn after applying a tourniquet, followed by proper aseptic precautions with a sterile disposable plastic syringe without any anticoagulant. A drop of blood was put on the electrode of pH meter from the nozzle of syringe carefully for blood pH determination. Half mL of blood was immediately put into sterile bottle containing 0.5 mg of EDTA (Ethylene Diamine Tetra Acetic acid) powder, shaken gently and stoppered. This blood was used within 24 hours for the estimation of lactic acid.

The blood in the syringe was covered, labelled and transferred in an ice box to the laboratory. Blood sample was centrifuged for 15 minutes at 3000 rpm. The hemolyzed samples were discarded. The supernatant layer of serum was then separated and poured in labeled glass bottles and stored in deep freezer at -20°C.

The salivary and serum pH were measured electrometrically with the glass electrode by digital pH meter HI 8014 (Hanna Instrument, USA). After calibration and temperature adjustment the bulb of glass electrode was immersed in a drop of sample and pH was noted from the screen of digital pH meter.

The calcium was estimated calorimetrically by using kit (Ref # 995936) supplied by Quimica Clinical Aplicada SA Aposta Spain. Inorganic phosphate was measured by colorimetric method using kit, cat # KC 120 supplied by Clonital Italy. Fluoride was also measured by colorimetric method using alizerine and zirconium dye. The fluoride was analyzed by the Magregian, Haier method cited by Farber,¹⁵ in which the fluoride reacts with dye lake, dissociating a portion of it into a colorless complex anion (ZrF₆) and the dye. As the amount of fluoride increased, the color produced becomes progressively lighter or different in hue depending on the reagent used. The student's "t-test" was used to compare the salivary and serum pH, calcium, phosphate and fluoride among the control and diseased groups.

RESULTS

One hundred individuals were divided into five groups according to their DMFT index (Table 1). The base line comparison of mean values of age, DMFT, index and number of tooth brushing per day (Table 2) shows a significant decrease in number of brushing and significant increase in DMFT index in all groups when compared to control.

The comparison between salivary and serum pH, calcium, fluoride, phosphate and lactic acid levels is given in Table 3. According to the findings of present study, the serum pH, calcium, fluoride and lactic acid levels were significantly high ($p < 0.01$) where as serum phosphate levels were found significantly low ($p < 0.01$) in all patients of dental caries as compared to saliva in same patients.

DISCUSSION

The role of salivary and serum pH, calcium, phosphate and fluoride in dental caries has been the point of interest since the mid of this century by many oral hygienist in the field of oral biochemistry. The early work of Stephan¹⁶, regarding the estimation of salivary pH had showed that the pH of saliva remained below the critical level of 5.5 in caries patients, than those without dental caries. The saliva exert its major influence on caries initiation by means of plaque formation rather than by direct contact on the tooth surface, they showed that plaque pH fall was greater in dental caries susceptible subjects. However this study did not show any significant change in the blood pH with the progression of disease.^{17, 18} The calcium ions are present normally in dental plaque bound to matrix and other proteins attracting phosphate and fluoride as counter ion, other phosphate and fluoride occurs intracellularly.¹⁹ All three ions occur as an inorganic mineral in serum and are in continuous exchange phase with the saliva over the dental plaque. This is responsible for the “pool” or “reservoir” of calcium, phosphate and fluoride in

dental plaque and also maintains their saturation. These observations are quite identical with this study as levels of serum calcium, phosphate and fluoride are significantly low in dental caries patient in comparison to the control.

Our study quite clearly gives the information that there is significant difference in salivary and serum calcium, phosphate and fluoride as the disease process advances (Table 3). This observation is in complete agreement with the study carried out by Pearce¹⁰ who explained that salt dissolution is governed by the concentration of calcium, phosphate and OH-ions in the surrounding fluid. These results are also supported by the research study of previous investigators who explained the process of dental caries on the basis of ionic product and solubility product. They explained that these ions are the main constituent of the enamel apatite lattice. The crystals formed in the presence of fluoride dissolved more slowly in acid as they have lower intrinsic rate of dissolution²⁰ particularly of fluoride are taken up during remineralization and the crystals formed in the presence of fluoride are large, dense and more perfect.²¹ Another observation made in this study was that, the rate of remineralization was raised in the presence of fluoride in early carious lesion at those time when the pH has risen so that remineralization is the dominant process. The investigations also demonstrated the antibacterial property of fluoride as it has a tendency to bind with the active metal of enzyme system e.g. in case of enolase, an enzyme that require magnesium which can be inhibited up to 100% by fluoride with the level of 95 ppm in the solution.

CONCLUSION

It is concluded that salivary and serum pH, calcium, phosphate, fluoride, and lactic acid variations in which are greatly influenced by the progression of dental caries. These minerals found in both the fluids and deposited in plaque greatly reduce the

development of experimental caries in the adjacent enamel^{22, 23} because it tends to maintain the saturation of plaque fluid with respect to enamel mineral at low pH. This saturation is a combined result of reduced plaque pH depression due to the acid neutralizing properties of apatite, and the high concentrations of calcium, phosphate and fluoride leached into plaque fluid by acids. Secondly, these results support the findings of Geddes^{20,24} that total plaque acid production does not correlate well with

plaque pH following incubation with sugar, and thirdly, lead us to predict that pH measurement alone is inadequate to assess the potential cariogenicity of plaque. Rather, the degree of under saturation of plaque fluid with respect to enamel mineral is the principal factor to be considered. The levels of these influential factors in both the characteristic body fluids; saliva and serum have variations to be maintained during the treatment against the progression of the disease.

Table 1: Distribution of control and patients in groups. (According to the DMFT index)

Group	DMFT index	Distribution of subjects	Sex	
			Male	Female
Control (n=20)	= 3	20	13	7
Group – I (n=20)	4-8	20	11	9
Group – II (n=20)	9-16	20	11	9
Group – III (n=20)	17-24	20	10	10
Group – IV (n=20)	=25	20	10	10

Table 2 : Baseline comparison personale data of the control and patients.

Group	Age (years)	DMFT Index	Brushing (No. of times/day)
Control (n=20)	23.9 +1.623	1.35 +0.208	2.05 +0.05
Group – I (n=20)	27.75 +1.680	6.3* +0.291	1.6* +0.11
Group – II (n=20)	28.25 +1.769	12.15* +0.099	1.05* +0.135
Group – III (n=20)	31.7* +1.818	19.8* +0.47	0.5* +0.114
Group – IV (n=20)	31.95* +1.59	26.95* +0.364	0.15* +0.08

* P < 0.01 as compared to control

Table 3: Comparison of serum and salivary pH, calcium, fluoride, phosphate and lactic acid in groups

Group	pH		Calcium		Fluoride		Phosphate		Lactic acid	
	Saliva	Serum	Saliva	Serum	Saliva	Serum	Saliva	Serum	Saliva	Serum
I	6.948 ±0.217	7.407* ±0.006	7.425* ±0.363	9.72* ±0.128	0.036 ±0.014	2.295* ±0.317	19.70 ±0.529	4.03* ±0.099	6.03 ±0.315	11.765* ±0.809
II	6.39 ±0.088	7.417* ± 0.005	4.24* ±0.342	9.1* ±0.127	0.024 ±0.015	1.615* ±0.713	16.28 ±0.655	3.59* ±0.047	8.815 ±0.412	15.32* ±0.695
III	5.845 ±0.175	7.419* ±0.004	2.3* ±0.201	8.6* ±0.139	0.012 ±0.004	0.76* ±0.044	13.05 ±0.400	3.05* ±0.032	13.27 ±0.43	18.14* ±0.794
IV	5.05 ±0.734	7.418* ±0.005	2.38* ±0.177	7.95* ±0.115	0.013 ±0.004	0.58* ±0.069	10.44 ±0.328	2.295*±0 .059	17.27 ±0.444	22.875* ±0.956

* P < 0.01 as compared to control

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ROLE OF POSTOPERATIVE MEDICAL TREATMENT IN THE MANAGEMENT OF ALLERGIC FUNGAL SINUSITIS

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ABSTRACT

Objectives: To evaluate the effectiveness of postoperative medical treatment in the management of allergic fungal sinusitis with orbital and/ or skull base erosion.

Study design: -Descriptive study.

Place and Duration of Study: This study was conducted in the Department of ENT and Head & Neck Surgery, Khyber Medical College & Khyber Teaching Hospital Peshawar between January 2002 and April 2007.

Patients and Method: Eighteen cases of allergic fungal sinusitis with orbital and/ or skull base erosion were selected for the study. Demographic Data like, name, age, sex, address, clinical features, labs and imaging studies were recorded, clinical data including the pre and post-operative medical treatment, operative findings and postoperative results, recurrence of disease were recorded. All patients were divided into three groups on the basis of postoperative medical treatment.

Results: Study revealed that Allergic Fungal Sinusitis (AFS) is a disease of younger age, mainly occurring in 2nd & 3rd decade of life, with male to female ratio 1:1.25. The recurrence was 100% in group-1 who were on oral antifungal therapy postoperatively. The recurrence of disease was 40% in patients of group-2 who were on topical nasal steroid, antihistamine and saline irrigation postoperatively. The recurrence rate was much lower 11% in group-3 patients who received oral and topical nasal corticosteroids postoperatively.

Conclusion: - AFS is a disease of young immunocompetent adults. Surgical debridement and drainage combined with topical and oral corticosteroids can lead to resolution of disease in majority of the cases and prevent recurrences. Antifungal medication has no role in the treatment of allergic fungal sinusitis.

Keywords: Allergic fungal sinusitis, Skull base erosion, Orbital erosion, Role of corticosteroids, Antifungal medication

INTRODUCTION

Allergic fungal sinusitis (AFS) is a relatively recently defined pathological entity. Allergic fungal sinusitis (AFS) was first described in early 1980, when Millar & others noticed a clinical entity of sinus disease that was similar in many ways to allergic

bronchopulmonary aspergillosis (ABPA).^{1,2} The pathological features of AFS have been known for two decades. Earlier, the condition was misdiagnosed for sinus mucocele, invasive fungal infections, undifferentiated neoplasm and fungal mycetoma.³ The incidence of allergic fungal sinusitis (AFS) in cases of chronic rhinosinusitis (CRS) treated surgically has been approximately 5-10%.⁴ Patients with non-invasive form have intractable sinusitis

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that fails to respond to repeated courses of antibiotics and surgical procedures.

Bent and Kuhn published the diagnostic criteria of AFS in 1994, 1)- nasal polyposis, 2)-allergic mucin, 3)-CT Scan findings consistent with CRS, 4)-fungus found in histopathologic analysis or culture and 5)-type-1 hypersensitivity as determined by history, serology or positive skin test. DeShazo and Swain also proposed diagnostic criteria.⁶ Some patients may present with clinical and histopathological features similar to AFS but without fungal hyphae in allergic mucin by both special stain and culture.⁷ This has recently been described a distinct clinicopathologic entity by Ferguson as eosinophilic mucin rhinosinusitis (EMRS).⁸

Patients with AFS have a high incidence of bone erosion of the skull base and orbit, which is best demonstrated on CT scanning.⁹ The incidence of bone erosion has considerable variation in different regions, races and gender. Wise et al demonstrated the erosion rate of 44.7% in AFS patients.¹⁰ The ophthalmologic findings are said to occur in as many as 18.3% of cases, varying from proptosis and diplopia to impaired vision and rarely sudden visual loss.¹¹ The presence of bony erosion of the skull base and orbital in AFS has been well documented in the literature.

Surgery has emerged as a universally accepted component and first step of the treatment. However, surgery alone without other adjuvant therapy frequently leads to recurrence of the disease. Being relatively recently discovered, there are many controversies regarding medical management of the disease. The postoperative medical therapies may include systemic corticosteroids, antifungal agents, or immunotherapy.^{12, 13}

This study was done to see the postoperative clinical response of different modalities of medical treatment in AFS with orbital and /or skull base erosion.

MATERIALS AND METHODS

This is a descriptive study conducted in the

department of Otorhinolaryngology, Head and Neck Surgery, Khyber Teaching Hospital Peshawar a tertiary care, referral center between January 2002 and April 2007. The sampling was by convenience. Eighteen (18) diagnosed patients of allergic fungal sinusitis with orbital or base of skull erosion were included in this study.

Inclusion Criteria; Allergic fungal sinusitis with bone erosion.

Exclusion Criteria; Allergic fungal sinusitis without erosion of bone. Allergic fungal sinusitis without fungal hyphae on staining .All Other form of sinusitis both invasive and non-invasive.

Based on CT Scan findings patients were diagnosed as having orbital and/ or skull base erosion.

The medical records of 18 patients were analyzed in terms of clinical profile, investigative profile (ophthalmologic findings, CT Scan, MRI), operative findings and postoperative results in terms of nasal symptoms, headache and visual improvement. All patients were evaluated for common conditions that could contraindicate the use of oral corticosteroids or any other medication.

All these patients underwent surgery, which included complete removal of allergic fungal mucin from involved sinuses and creating wide access to these sinuses for ventilation and postoperative care. The surgical approach was based on the extent of the disease according to the findings of CT Scan. All the allergic mucin and polyp/sinus mucosa removed was sent for histopathological examination. Pathologist was requested for special fungal staining such as PAS (Periodic acid-Schiff) and GMS (Gomori methanamine silver stain) or KOH preparation. Postoperatively, all patients received antibiotics for one week.

On the basis of postoperative medical treatment received, patients were divided into three groups.

Group-1; patients who received oral antifungal postoperatively for 3 months.

Group-2; patients who received postoperatively

topical nasal steroids and antihistamine for 3 months

Group-3; patients who received topical and oral corticosteroids treatment for three months postoperatively Follow up; the minimum follow up was up to 8 months.

The follow up period ranged from 8 to 84.4 months.

Details of postoperative treatment, relief in symptoms, examination finding and recurrences if any and side effect of drugs if any, appropriate treatment given that is, uses of antifungal, topical or systemic corticosteroids were recorded.

RESULTS

On the bases of the Schubert diagnostic criteria 18 patients of allergic fungal sinusitis with orbital and/or skull base erosion were selected.¹⁴ Only 7 cases presented with recurrence. The clinical features, age, sex, extent of disease, number of previous surgeries and incidence of recurrence are shown in the Table-1 and 2.

Table 1:

S.No.	Clinical Features	No.	%
1	Nasal obstruction	17	94%
2	Nasal discharge	16	89%
3	Rhinorrhoea	16	89%
4	Facial pain	08	44%
5	Headache	09	50%
6	Polyposis	17	94%
7	Proptosis	16	89%
8	Telecanthus	02	11%
9	Impaired Vision	02	11%
10	Previous surgeries	07	39%

Table 2:

Number of Patients	Sex	Sex		Recurrence	Extend of disease in recurrence		Sex ratio in recurrence	
		M	F		Unilateral	Bilateral	M	F
Group-1	Four	1	3	Four (100%)	2	2	1	3
Group-2	Five	2	3	Two (40%)		2		2
Group-3	Nine	5	4	One (11%)		1	1	

The male to female ratio was 1:2, while among the recurrent cases two were male and 5 were female. The youngest was 11 years and the oldest was 45 years (average 19 years). This study revealed that AFS is a disease of young adults, mainly occurring in the 2nd & 3rd decade of life. The disease was unilateral in thirteen (72%) of 18 patients and bilateral in 5 patients. Among the recurrent 7 cases 4 patients had bilateral & 2 had unilateral disease. Fungal hyphae were seen in allergic fungal mucin on histopathological examination in all cases. Cultures were performed in only 5 cases, in which *Aspergillus* was isolated. 13 cases presented with nasal allergy and one was a known case of bronchial asthma. History of previous sinonasal surgeries for rhinosinusitis with polyposis was elicited in eight (39%) cases.

DISCUSSION

With heightened awareness and sophisticated laboratory diagnostic techniques and imaging, an increasing number of reports are being published. A full consensus among rhinologists and immunologists worldwide concerning diagnostic criteria for AFS is still awaited. We employed Schubert criteria¹⁴ for diagnosis of AFS in our patients. This study describes the effectiveness of postoperative medical treatment in minimizing the recurrence of disease and improving the symptomatology with which patients presented. All patients in our series were immunocompetent and young. The mean age at presentation was 20 years and 83% were in 2nd & 3rd decade of life, which is similar to studies reported in the literature.^{6,16}

The male to female ratio of 1:1.25, has also been reported by Scott C Manning.¹⁵ Conversely Thahim K et al and Richard D deshazo found male predominance in their study.^{6,16}

In our study the disease was unilateral in 13 (73%) patients and bilateral 5 (18%) patients. Bent & Kuhn⁵, Sohail et al¹⁹ and Thahim et al¹⁶ also reported unilateral predominance in allergic fungal sinusitis. On the other hand Bradley Marple¹⁷ found 51% bilateral disease in 45 patients. The recurrence is more common in female in the study and in that group of patients

having bilateral disease; five of 7 patients had bilateral disease in recurrent cases.

Regarding management of AFS, adequate sinus surgery is a universally accepted component and the first step in the treatment of any patient with AFS¹⁴. Although no studies are available to compare different techniques, it has been suggested that limited functional endoscopic sinus surgery has been associated with higher rates of AFS recurrence than more aggressive surgical procedures that remove all dysfunctional obstructive hypertrophic/ polypoidal sinus mucosa as well as all inspissated allergic mucin.^{4,18} Aims of surgical treatment regardless of surgical techniques are complete removal of all allergic mucin and fungal debris, permanent drainage and ventilation of the affected sinuses while preserving the integrity of the sino-nasal mucosa and access for postoperative care. Keeping in mind the aims of surgical treatment, we adopted more radical approaches in surgery. Endoscopic sinus surgery with preservation of structures is popular procedure in most centers.²⁰

In Group-1 patient's recurrence was observed in all cases (100%). Similar poor responses were observed in different studies.^{4,17,21,22,28,30} The use of topical and systemic antifungal agents is controversial. No indication exists for the use of toxic antifungal agents to treat noninvasive fungal sinusitis. Since AFS is thought to be an allergic (hypersensitivity) disease caused by extrinsic fungal antigen rather than a true infection, systemic antifungal should be ineffective.^{14, 24} Itraconazole, a relative benign oral antifungal agent was used in four patients postoperatively in our study with no beneficial effects. However in some cases antifungal agents were added to oral corticosteroids in the treatment of allergic fungal sinusitis with additional clinical benefits.^{25,26}

In Group-2 patient's recurrence was observed in two cases (40%) that received nasal corticosteroids and antihistamine postoperatively. Topical intranasal steroids spray, antihistamine and saline irrigation have minimal side effects and are commonly used in postoperative management of ASF. However the duration and effectiveness of steroids sprays in AFS

has not been proven scientifically.^{4,13,18,27}

In this study Group-3 patients who underwent surgery followed by oral and topical corticosteroids showed very good response, regarding relief from symptoms and recurrence. In this group of patients only one case (11%) presented with recurrence. Use of oral corticosteroids postoperative in AFS is well established and supported by many studies.^{4,17,18,28,29} Although no fully controlled studies have been published for any treatment for AFS, the origin of corticosteroids therapy for long-term management of AFS arose directly from the analogy of AFS to ABPA. The potent anti-inflammatory and immunomodulatory effect of corticosteroids appears to be well suited to control recurrence of disease. Bent and Kuhn³⁰ noted eventual universal recurrence of AFS in their patients who were not receiving systemic corticosteroids. Schubert and Goetz¹⁷ studied the role of systemic corticosteroids in the postoperative management of AFS, demonstrating a significant decrease in recurrence of AFS, much improvement in relief of symptoms and quality of life in patients who received prolonged courses of postoperative systemic steroids. Systemic steroids have been used successfully in cases of recurrence of AFS, but the potential side effects limits their use and the dosing regime and length of therapy remain unclear. A modified protocol of systemic corticosteroids regime for AFS from that used for ABPA has been suggested and used by Schubert and Goetz¹⁸ in their cases.

This study did encounter some limitations. The time taken to transport the specimen to the histopathology and mycology laboratory could not strictly be observed. Because of budget constraints, fungal culture and fungal-specific IgE and IgG, which are good screening tools, were not performed.

CONCLUSION

AFS is a disease of young immunocompetent adults. Skull base & orbital erosion is seen in majority of the cases. Surgical debridement and drainage combined with topical steroids and oral steroids can lead to resolution of disease in majority of the cases and prevent recurrences. Antifungal medication has

no role in the treatment of allergic fungal sinusitis. Allergic fungal sinusitis should be considered in all patients with refractory chronic sinusitis.

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COMPARATIVE STUDY OF BONE MINERAL DENSITY IN HIGH AND LOW INCOME SCHOOL CHILDREN

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ABSTRACT

Objective: To compare the bone mineral density [B M D] and associated dietary and physical activity factors in low and high socio economic status (SES) groups of children.

Design: This is a cross sectional, prospective, descriptive study.

Setting: This study was conducted at two different socio-economic level schools. The low socio-economic school was Major Sultan Shaheed Primary School in North Nazimabad, Karachi, while the high socio-economic school was Horizon School in Gulshan-e-Iqbal Karachi.

Material and Methods: This study was conducted in September 2007. The performas were filled & the tests were done during one week. One hundred children of age 10—12 years of both sexes were selected randomly from two schools of Karachi representing the two different socioeconomic classes. Fifty children each were recruited from either school. Each group included 25 from either sex. A questionnaire was designed to include basic demographic features, weight, height, BMI, dietary habits and physical activities. BMD was measured at the heel using a portable quantitative ultrasound (QUS) densitometry machine. The results were expressed as T-score. The data was analysed on SPSS version 11.5. For data comparison and calculation of significance ANNOVA and ETA were used.

Result: The mean T-scores in high SES group were significantly lower at -2.82 than the low SES group which was -2.36 (p- value < 0.05). This is despite the fact that the high SES group had a significantly higher BMI and better overall dietary calcium and other minerals consumption. However, the high SES group children also consumed significantly higher quantities of carbonated soft drinks and chocolates. The high intensity activities were comparable in both groups (p-value 0.83). However, the moderate intensity activities were observed 2.5 folds more frequently in low SES than in high SES group.

Conclusion: Lower BMD values were noted in high SES group of children despite having higher BMI and better overall mineral consumption. This could be because of concomitantly higher consumption of carbonated soft drinks and other junk food that may hinder calcium and minerals absorptions and sedentary lifestyle observed in high SES group children.

Keywords: Bone mineral density, Children, Physical activity, dietary factors.

INTRODUCTION

Bone mineral density (BMD) reflects the strength of the bones in the body. It increases during childhood and adolescence until peak bone mass is reached at maturity - usually until the age of 25-30 years. Peak

bone mass and subsequent bone losses are important determinant of osteoporosis later in life especially in the postmenopausal women and elderly people of either sex. Due to this reason osteoporosis is also sometimes regarded as a 'paediatric disease with geriatric consequences'. Many factors adversely affect the BMD in early life such as bad dietary habits, lack of physical activity, low weight, less exposure to sunlight. Effective awareness programmes highlighting these factors could help

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to achieve the optimum peak BMD at an early age thereby reducing the future risk of osteoporosis.

In adults, BMD is often measured to diagnose osteoporosis and is the only reliable test available to predict future fracture risk in an individual. In children, however, BMD measurement is generally restricted as a research tool. Rarely, though it can be clinically required to diagnose rare childhood osteoporotic states.

Different devices can be used to measure BMD.¹ These include quantitative ultrasound (QUS), quantitative computed tomography (QCT),²⁻⁴ dual energy X-ray absorptiometry (DEXA) and peripheral DEXA etc. DEXA scan is currently the gold standard method for clinical use for the diagnosis and monitoring of osteoporosis due to its high precision, reproducibility and low radiation exposure. The QUS although has limited clinical application, could be useful for research and in situations where mass screening is required as it is radiation free and has a convenient portable instrument. The heel QUS has close correlation with DEXA scan especially at hip^{2,3} and to a lesser extent at lumbar spine⁴ QCT on the other hand is expensive and uses higher radiation.⁵⁻⁷

Dietary nutrients that play an important role in bone health are calcium, phosphorus, magnesium, potassium and some vitamins such as vitamin D and vitamin K.⁸⁻¹¹

Many research studies showed that physical activities positively affects bone mineralization in children. Recent health guidelines suggest that children should indulge in 60 minutes of moderate to severe physical activity every day.¹²⁻¹⁶ On the other hand, certain factors adversely affect bone mass e.g. anticonvulsant and steroid medicines¹⁷⁻¹⁹ diseases like Crohn's disease²⁰⁻²⁴ and Celiac disease and genetic factors.²⁵⁻²⁷

SUBJECTS AND METHODS

A total of 100 children of age 10—12 years of both sexes were selected randomly from two schools of Karachi on the basis of convenience. The low SES children were represented by a school in North

Nazimabad area which provides free education to the children of labour class. The high SES group children were represented by another school in the Gulshan-e-Iqbal area which caters affluent class children & had a fee structure of Rupees 1500 per month. Fifty children each were selected from both schools. Each group included 25 from either sex. A questionnaire was designed to include basic demographic features, weight, height, BMI, dietary habits and physical activities. The questionnaire was filled by a single interviewer, spending approximately 15 minutes for each interview. Different food and beverages were quantified by using photographs of food items and other house hold measures. High intensity activities were classified as those sports which require running, jumping and high impact activities such as cricket, football and athletics etc. The moderate intensity activities included casual strolling, racquet games, hide and seek, marble games etc. Every child's bone mineral density was measured at the heel using Hologic Sahara 'Quantitative Ultrasonic Densitometry' (QUS). This instrument uses calcaneal QUS measurements of broadband ultrasound attenuation (BUA) and speed of sound (SOS). A stiffness index is calculated from BUA and a SOS value, which is then converted to a T-score, technically termed as-'estimated-T-score'. Pre-interview verbal consents were obtained from the parents. The study was approved by the ethical review board. For data transformation and analysis SPSS version 11.5 was used. For data transformation of dietary habits selected commands were used to calculate the mineral (calcium, phosphorus, potassium and magnesium) consumed during a week in milligram (mg) which was then divided by seven to calculate the mineral consumption in mg per day.

Physical activity data was transformed into SPSS as high intensity activities and moderate intensity activities in minutes per day (again this was calculated by summing up activities during a whole week and dividing by seven). The data was analysed by comparing mean values of mineral consumption, consumption of carbonated drinks and chocolate (as servings per day), BMI, BMD and physical activities using ETA. The significance was calculated by ANNOVA table.

RESULTS

The mean T-scores was significantly lower at -2.82 in high SES group than the low SES group which was -2.36 (p- value < 0.05). This is despite the fact that the mean BMI in high SES group was higher at 16.49 as compared to low SES group which was 15.01. (Table I).

Table 1: Mean weight, height, BMI and BMD in both groups of 10-12 years age.

Variables	Socio-Economic Status		
	High SES (Mean)	Low SES (Mean)	Significant
Standard – Weight for age 81.4 (kg)	79.2	61.3	.000
Standard – Height for age 56.8 (inch)	57.6	53.5	
BMI*** (kg/m2)	16.49	15.01	.004
BMD** (T-score)	-2.82	-2.36	.006

* S.E.S = Socio Economic Status

** BMD = Bone Mineral density

*** BMI = Body Mass Index

The consumption of mineral intake in both groups was generally lower than the RDA (recommended daily allowance) except for phosphorus intake in either sex and calcium intake in girls in both groups which were above the RDA recommendations. The mean consumption of all minerals was, in general, better in high SES group than in the low SES group. The mean calcium intake was 602.30 ± mg/day and 484.79 ± mg/day respectively for boys and 734.42 mg/day and 574 mg/day respectively for girls of high and low SES groups. The mean phosphorus consumption was 1165.67 mg/day and 837.96mg/day respectively for boy and 1013.79 and 1073.89 for girls in high and low SES groups. Table 2 shows complete details of other minerals consumed in different subgroups

Table 2: Mineral Consumption

	Gender	RDA** (mg).	High S.E.S*	Low S.E.S*	Significance
Total Calcium (mg)/day	Boys	700	602.30	484.79	.023
	Girls	500	734.42	574.77	
Total Phosphorus(mg)/day	Boys	700	1165.67	873.96	.228
	Girls	500	1013.39	1073.89	
Total Potassium (mg)/day	Boys	2000	2022.60	1538.42	.001
	Girls	2000	1889.99	1672.09	
Total Magnesium (mg)/day	Boys	300	265.61	251.46	.546
	Girls	300	247.18	243.18	

* S.E.S = Socio Economic Status

** RDA = Recommended Dietary Allowances

The mean consumptions of carbonated soft drinks and chocolate were 0.32 and 0.16 servings per day respectively in high SES group whereas none consumed in low SES group which was a highly significant finding (Table 3).

Table 3: Intake of food items that hinder mineral absorption

Food items	High S.E.S.* (mean)	Low S.E.S.* (mean)	Significant
Total Carbonated soft drink's serving / day	0.32	.00	.000
Total Chocolates serving / day	0.16	.00	.000

* S.E.S = Socio Economic Status

The high intensity activities were 50.90 min/day in high SES group and 48.71 min/day in low SES group which were not statistically different (p-value 0.83). However, the duration of moderate intensity activities were 13.81 min/day in high SES group as compared to 33.41 min/day in low SES group (Table 4).

Table 4: Mean duration of physical activities.

Physical Activity	High S.E.S*	Low S.E.S*	Significant
High Intensity activities	50.90 minutes / day	48.71 minutes / day	0.83
Moderate Intensity activity	13.81 minutes / day	33.41 minutes / day	0.00

* S.E.S = Socio Economic Status

This means that high SES group children were two and a half fold less active than their low SES counterparts– again a highly significant finding.

DISCUSSION

This study shows lower BMD in high SES group children - a result that is somewhat unprecedented. The higher BMI in high SES group was expected to be positively associated with BMD as observed in Lebanese²⁸ and Indian²⁹ studies. Similarly, high dietary mineral contents, as observed in the high SES group, were expected to be associated with a higher BMD.⁸⁻¹¹ But our results are contrary to that. There could be several reasons for these contradictory results. Firstly, the high SES group also consumed carbonated drinks which are known to hinder calcium absorption from the gut. Mc Gartland C et al. have shown that carbonated soft drinks consumption is inversely related to BMD in adolescence.³⁰ Secondly, the 2.5 fold lesser duration of physical activity (in moderate category) in high SES group could have resulted in diminished bone mineralization and consequently lesser BMD.³¹⁻³⁴

Thirdly, the high SES children were less exposed to sunlight by being relatively more house bound due to sedentary activities such as computer and TV watching etc.

Another interesting observation was that the high SES group had a standard mean height whereas low SES children had a below standard mean height. This could be because of ethnic differences i.e. majority (54%) of the children in high SES group were from 'Urdu speaking' families as opposed to low SES group in which majority (54%) were from 'Punjabi speaking' families.

Another important point noted in this study was that the children of both the groups were not consuming recommended dietary allowances (RDA) of minerals except phosphorus intake which was above the RDA in both groups and calcium intake of girls which was

also above the recommended dietary allowances in both groups. This means that further efforts are required to educate all concerned regarding blending a balanced diet for the children especially boys. It is however encouraging to note that the calcium intake in girls was adequate in both groups.

For reasons of general familiarity and popularity we expressed the BMD values as a 'T-score' rather than gm. per cm². T-score is automatically calculated by an in-built programme in the QUS machine. Contrary to the adult population the T-score values of osteoporosis and osteopenia do not apply to children because of a different quality of bone which is more resilient, flexible and possesses a normal architecture. However it does represent the overall current bone mineral deposits which could have implications for the future.

This study has some limitations. Although the true scientific approach demands to find the relation between dependent and independent variables by considering one at a time while others are kept constant. This ideal approach was not practically possible for this study as the number of independent variables were quite high as compared to samples collected. A good approximation was made by taking the means of the results and ignoring the less significant factors. The results thus obtained were satisfactory enough for this level of research and can be confidently used in future studies. We recommend that serum levels of mineral i.e. calcium, phosphorus, magnesium, alkaline phosphatase and vitamin D levels be measured along with markers of bone turnover be used to correlate with BMD changes in different groups.

CONCLUSION

The low SES group children had a significantly higher BMD values despite having a lower BMI, lower calcium and mineral and similar high intensity activities. This could possibly be attributed to a two and a half folds more frequent physical activities in

moderate cadre and lack of consumption of carbonated drinks and chocolates which may hinder calcium absorption from the gut. School administration should actively discourage consumption of carbonated drinks within their premises. They should organise events such as seminars, debates and health awareness walks etc. To encourage activity levels amongst the children. Further studies are recommended using serum levels of bone biochemistry including serum vitamin D levels and markers of bone turnover.

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PSYCHIATRIC MORBIDITY IN CHILDREN REPORTING AT A TERTIARY CARE HOSPITAL

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Objective: The study is designed to assess the psychiatric morbidity in children reporting at psychiatry OPD, of National Institute of Child Health (NICH), Karachi

Study Design: Simple descriptive study

Place and Duration of study: This study was conducted during the period of two years from January 2005 to January 2007 at National Institute of Child Health (NICH) Karachi.

Patients and methodology: All consecutive patients who reported in child psychiatry clinic were enrolled in this study if they fulfilled the inclusion criteria having age of 3 years to 15 years of either sex. Cases excluded in the study were psychiatric presentation associated with physical illness and symptoms related to drug side effects. The facility received direct referrals from other professional colleagues, and from parents, school teachers who have been sensitized to child psychiatric issues. Semi-structured interview based on DSM- IV along with an open ended slot of question were used for evaluation. Data regarding demographic characteristics, referral source, reasons for referral and diagnostics based on clinical judgment were collected.

Results: Five hundred and seventy new cases were inducted in this study over a period of two years. Males outnumbered females with the ratio of 1.7:1. Majority (42.28%) of children were in the age range from 11-15 years. Standard deviation of age was 6.8 years for male and 3.5 years for female children. Most (69.6%) of the referrals were made from professionals working in pediatric medicine. The most common reason for referral was disruptive behaviour (26.32%), followed by behavioural problem with fits (15.79%) and physical over activity (7.89%). Other presenting complaints (reasons for referral) included slow learner, self injurious behaviour, aggression, speech delay, unexplained physical symptoms, bed wetting, emotional problems poor attention and tics etc.

Mental retardation with behavioural problem was the most frequent (36.14%) provisional diagnosis. About 16.14% of cases related to seizure disorder with behavioural problem while 10.53% were attention deficit hyperactivity disorder (ADHD). Other diagnoses made were depressive illness, conversion disorder, functional enuresis and autism.

Conclusions: After mental retardation, one third of cases comprised of seizure disorder with behavioral problem, attention deficit hyperactivity disorder (ADHD), and depressive illness. It is therefore recommended that professionals at primary health care level should be trained to identify psychiatric illness in children so that early intervention, proper referral and effective management may be possible.

Keywords : Child psychiatry, Mental health, Disruptive behaviour

INTRODUCTION

In Pakistan, like many other countries the current scarcity of child mental health services is reflected in non-availability of epidemiological evidence. Lately both community and clinical researches in

child mental health have shown that emotional and behavioral problems are on rise in our country.¹⁻² It is worth mentioning that Mental Health Ordinance 2001 stressed to provide separate psychiatric facility for children and adolescents. This clearly reflects the need for child mental health care services in Pakistan.³

Worldwide literature review shows that mental health problems among children in the age range of 3-15 years are 5-15%.⁴ In the past year the studies have

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been carried out in developing countries exploring the prevalence of child psychiatric disorders. In a study from Bangladesh, with a sample size of 922, in 5 to 10 years old children, a 15% prevalence for any ICD-10 diagnosis was found.⁵ Another study carried out in India indicated a prevalence rate of 12.5% among children aged 0-16 years.⁶ Epilepsy, speech and other behavioral problems constitute substantial proportion in child psychiatric disorders in Pakistan.⁷

Mental illness is considered a major social stigma. It might be due to misconception, illiteracy, and indifference, which may complicate with social customs of the society. Many people consult faith healers before visiting mental health professionals. Under these circumstances, professionals faced with the task of developing mental health services for children in Pakistan are confronted with a number of challenging issues.

All over the world, child psychiatric illnesses are regarded as serious but treatable conditions, however if left unattended, most of these result in poor parenting, disintegration of family life and deteriorating moral values and are considered as precursors of adult psycho-pathology.⁸⁻⁹ The importance of early detection of emotional and behavioral problems is being increasingly recognized worldwide. However, until now there has been little systematic research into childhood psychiatric disorders in the developing countries.¹⁰ This paper is an attempt to highlight the different psychiatric presentations in children. It would in turn enable us to recognize and deal effectively with children who have psychiatric disorders.

PATIENTS AND METHODOLOGY

This study was conducted during the period of two years from January 2005 to January 2007 at National Institute of Child Health (NICH), Karachi which is the largest tertiary care children hospital in the country. This hospital caters services not only for Karachi where it is located but also to neighboring provinces. An informed consent was obtained from guardian, explaining them purpose and nature of

study. Reassurance was also given regarding the confidentiality of information.

Sample consisted of all new patients aged 3-15 year attending the clinic in the two year period. Patients excluded from the study included; psychiatric presentation associated physical illness, behavior related to drug side effects, age less than three years and older than fifteen years. Demographic data included age, sex, source of referral and relationship with the accompanying person. Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) based semi-structured interview was used for evaluation. For suggestive mentally retarded children informal clinical assessment was carried out. Presenting complaints and/or reasons for referral were recorded and provisional diagnoses were made. Statistical analysis was done through SPSS computer software (version 10.0) and presented as percentage.

RESULTS

Five hundred and seventy patients were enrolled in psychiatry OPD. Of them 63.3% (n=361) were males and 36.6% (n=209) were females. Male female ratio was 1.7:1. Most of children reported (42.2% n =241) fell in age group ranges between 11-15 years. Mean age of male and female children were 9.2 years \pm 6.8 and 3.6 \pm 3.5 years respectively.

Majority (69.6% n=397) of referrals were from medical professionals. Self-referrals constituted 18.9% (n=108) of the total sample. Other referrals included schools (6.1% n=35), siblings and relatives (5.2% n=30) and miscellaneous sources (5.44%) (Table I).

Most of children (65.94%) visited psychiatry OPD with their mothers and 22.71% came with their fathers. A very small proportion of children visited the clinic with both the parents (8.47%) and relatives (2.5%). Majority (94.7% n=540) children belonged to Karachi and a small number were from adjoining areas (5.6% n=30) in this sample.

The reasons that most commonly led to the referral were Disruptive behavior (26.32% n=150) while epileptic fits with behavioral problems (16.6% n=95)

and physical over activity (7.89% n=45) were also referred to psychiatry OPD. Other presenting complaints included slow learner, self injurious behavior, aggression, speech delay, unexplained somatic symptoms, bed wetting, and emotional problem etc. (Table 2)

Mental retardation with behavioral problem was the most frequent (36.14% n=206) provisional diagnosis made. About (16.6% n=95) of cases were related to behavioral problems with seizure disorders, while (10.5% n=60) were diagnosed as attention deficit hyperactivity disorders (ADHD). Other diagnoses included depressive illness, conversion disorder, functional enuresis, tic disorder, and conduct disorder. While in 7.04% (n=40) cases no diagnosis (where diagnosis could not be possible due to variable complaints) was made. (Table 3).

Table 1: Gender vs Age

Age in year	Male		Female		Total	
	N	%	N	%	N	%
3-5	61	10.70%	43	7.54%	104	18.25
6-10	167	29.30%	58	10.18%	225	39.47
11-15	132	23.16%	109	19.12%	241	42.28
Total	360	63.16%	210	36.84	570	100.00

Table 2: Reasons for referral (Top ten)

Age in year	Gender					
	Male n	%	Female n	%	Total n	%
Disruptive behavior	105	18.42	45	7.89	150	26.32
Behavioral problems with fits	56	9.82	34	5.96	90	15.79
Physical over activity	36	6.32	9	1.58	45	7.89
Slow learner	19	3.33	16	2.81	35	6.14
Self injurious behavior	20	3.51	10	1.75	30	5.26
Aggressive behavior	20	3.51	16	2.81	36	6.32
Speech delay	15	2.63	10	1.75	25	4.39
Unexplained Physical symptoms	13	2.28	10	1.75	23	4.04
Bed Wetting	15	2.63	13	2.28	28	4.91
Emotional Problem	10	1.75	15	2.63	25	4.39

Table 3: Provisional (probable) diagnosis (Top ten)

Age in year	Gender					
	Male n	%	Female n	%	Total n	%
Mental retardation with behavioral problem	116	20.35	90	15.79	206	36.14
Seizure disorder with behavioral problem	78	13.68	17	2.98	95	16.67
ADHD	42	7.37	18	3.16	60	10.53
Depressive illness	22	3.86	16	2.81	38	6.67
Conversion disorder	20	3.51	4	0.70	24	4.21
Functional enuresis	11	1.93	13	2.28	24	4.21
Emotional disorder	10	1.75	7	1.23	17	2.98
Psychogenic vomiting	9	1.58	7	1.23	16	2.81
Autism	6	1.05	3	0.53	9	1.58
Organic psychosis	5	0.88	4	0.70	9	1.58

DISCUSSION

This descriptive study represents the profile of attendee at the child psychiatric clinic of a major tertiary care center over a period of two years precisely reflecting the source of referral and pattern of psychiatric presentation observed at the outpatient clinic in a tertiary care children hospital during January 2005 to January 2007.

The findings suggest that majority of children were referred with varied complaints suggestive of disruptive behavior, and mental retardation with behavioral difficulties. A study conducted at a private university hospital in Karachi has also shown similar results with aggressive behavior being the most common reason for referral. However in contrast to our study, attention deficit hyperactivity disorder (ADHD) was most frequent diagnosis made. Another study carried out at child guidance clinic Delhi, (India) revealed that mental retardation was on top (20.61%) among 300 children which is inconsistent with our study.¹² Regarding gender, findings of the present study manifest a large proportion of male children (63.16, n=210) reported in our OPD which is in accordance with study carried out in other countries.^{13,14} In relation to age, it was noted that more than 80 percent reported psychiatric problems were school age children (6-15 year). Another study revealed that higher prevalence of psychopathology

has been reported in a school based study of emotional and behavioral problem amongst 5-11 year school children in Karachi.² This finding is similar to that of other studies.¹⁵⁻¹⁷ This might be due to the fact that in older children, psychiatric illness may be recognizable by parents or family physician. Also probably a wrong perception that small children do not suffer from mental illness or possibly denial attitude by parent contribute to delayed consultation. All that may attribute to under reported cases in early childhood period.

The limitations of study should be borne in mind which includes relatively small sample size, single centre study and data based on clinical diagnosis. It is recommended that further studies should be carried out on risk factors in causation of childhood psychiatric illness.

CONCLUSIONS

A variety of psychiatric problems were observed in children population. After mental retardation, one third of cases comprised of seizure disorder with behavioral problem, attention deficit hyperactivity disorder (ADHD), and depressive illness. A sizable number of children were referred to the clinic by non-psychiatry professionals including pediatricians and teachers which points the need to train our colleagues involved in primary health care as well as teachers and parents to be able to at least identify those children in need of mental health attention, and to be able to make appropriate and timely referrals.

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IMPORTANCE OF THYROID PROFILE IN FERTILITY

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ABSTRACT

Objective: Present study was conducted to see the effect of thyroid profile in different menstrual problems and their effects on fertility.

Study Design: Prospective study

Methodology: A total of eighteen hundred and seventy eight patients reported during the year 2006-8, for the present study to see the role of thyroid hormone in fertility. Out of them, 580(30.9%) males and 1298(69.1%) were females with mean age 35±5 yr and 32±5 yr respectively.

Results: All data analysis were performed using statistical package SPSS version 12.0 (SPSS, Inc., Chicago IL, USA). Mean T3, T4 and TSH levels were found to be 0.85, 0.94 and 0.56. However, significant correlation was found between the thyroid profile and fertility ($r=0.535$ at $p<0.01$ with 99% CI).

Conclusion: The contribution of thyroid profile in fertility and menstrual disturbances are substantial. Therefore, it is recommended to know the thyroid status in these cases to minimize the problem.

Keywords: Thyroid profile, Fertility, Hypothyroidism, Hyperthyroidism.

INTRODUCTION

Fertility is influenced by the moral attitudes of society. The understanding of the causes of infertility has made enormous progress during the past 20 years.¹ Infertility is recognized and defined as a public health problem and is the manifestation of one or more pathological conditions either of female or male origin.² The optimal approach in the management of infertility requires that the timing and method of the routine investigation are beneficial for the couple by avoiding both under- and over treatment.

Unfortunately, infertility is a disorder in which the diagnosis and consequently reliable treatments are frequently unduly and excessively delayed.³ Significant awareness has occurred in the diagnosis and, more importantly, in the treatment of reproductive disorders over the past decade but the overall incidence of infertility has remained stable.⁴ In this regard, thyroid plays an important role in the reproductive activity of males and females. Disorders of thyroid function interfere with several aspects of reproduction.^{5,6} Thyroid hormones are essential for normal growth, sexual development and reproductive function.⁷ Thyroid dysfunction is prevalent in the population and affects many organs including gonads, thus interfering with the reproductive physiology. Hypothyroidism and hyperthyroidism are considered

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to give rise to menstrual irregularities. However, sub-clinical hypothyroidism is also associated with ovulatory dysfunction. Thyroid dysfunction is quite prevalent in the population and affects many organs including the males and females gonads, interferes with human reproductive physiology, and reduces the chance of conception.⁸

Hypothyroidism results in to an insufficient production of the thyroid hormone and can cause a delay in the onset of puberty or incomplete isosexual precocity in girls and increased testis volume in boys without adrenarche.⁷ Severe and prolonged hypothyroidism in male during childhood may be associated with permanent abnormalities in gonadal function.⁹ Approximately 2-5% of all women in the reproductive age group suffer from hypothyroidism.¹⁰ The disease is associated with cycle disturbances / menstrual such as oligomenorrhea and amenonhea. Low levels of thyroid hormone can interfere with ovulation, which impairs fertility.^{11,12} These observations show a link between hypothyroidism and infertility. Present study was conducted to see the effect of thyroid profile in different menstrual problems and infertility.

PATIENTS AND METHODS

Study design

A Prospective study was conducted during the year 2006-8 on 1878 patients, reported at diagnostic centre Karachi, presenting for the various causes such as amenorrhea, menorrhagia, dysmenorrhagia in females and infertility in both males and females.

Serum assay

Collected samples were measured using ELISA based technology (Monobind kit method, USA) for the estimation of serum T3, T4 and TSH levels. The reference values were 0.6-1.85 ng/ml for T3, 5.0-13.0 ng/dl, for T4, and 0.4–7.0 miu/ml for TSH,

Statistical analysis

All data analysis were performed using SPSS version 12.0 (SPSS, Inc., Chicago IL, USA). Mean T3, T4

and TSH levels and correlation with their presentation (menorrhagia, amenorrhea, and infertility) were observed.

RESULTS

Among 1878 cases, 580(30.9%) males and 1298(69.1%) were females; with mean age was 35±5 yr and 32±5 yr respectively. The mean T3, T4 and TSH levels were found to be 0.85, 0.94 and 0.56. The distributions of different presentation (menorrhagia, amenorrhea, and infertility) of the individuals are 78.2% infertile, 20.1% menorrhagia and 1.7% found to be amenorrhea (Fig-1). The levels of T4 and TSH significantly correlated to each other ($r = 0.377$ at $p < 0.01$), while TSH levels is substantially correlated with hypothyroidism ($r = 0.592$ at $p < 0.01$), (Table 1). On the other hand, infertility is found in high TSH levels ($r = 0.535$ at $p < 0.01$), and at low T4 levels ($r = 0.422$ at $p < 0.05$), (Table-2).

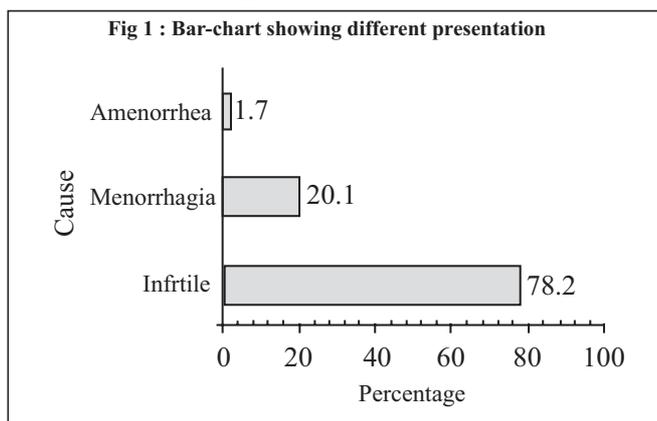


Figure 1: Bar-chart showing different presentation

Table 1: Correlation between thyroid profile, hyperthyroidism and hypothyroidism

	T ₃	T ₄	TSH	Hypothyroid	Hyperthyroid
T ₃	1.000	0.535**	0.106	0.684	0.728
T ₄		1.000	0.377**	0.366**	0.922
TSH			1.000	0.592**	0.178*
Hypothyroidism				1.000	0.030
Hyperthyroidism					1.000

* $p < 0.05$
** $p < 0.01$

Table 2: Correlation between presentation of the individual and thyroid profile:

	T ₃	T ₄	TSH	Menorrhagia	Amenorrhoea	Infertility
T ₃	1.000	0.555**	0.106	0.622	0.722	0.626
T ₄		1.000	0.377**	0.426*	0.389*	0.422*
TSH			1.000	0.329*	0.428*	0.535**
Menorrhagia				1.000	0.962	0.866
Amenorrhoea					1.000	0.881
Infertility						1.000

* p<0.01

** p<0.05

DISCUSSION:

Infertility is one of the major social problems of the new millennium worldwide, which affects women's health and leads to social and psychological disturbances in their life especially in our population. The percentage of couples experiencing infertility involves all regions of the world¹³, and in some countries it may be growing.¹⁴ There could be many reasons for this phenomenon (spread of sexually transmitted diseases, pushing back the age at which reproduction attempts are begun, environmental factors interfering for example with sperm production, etc.).

Conception involves spatiotemporally regulated endocrine, cellular and molecular events. Generally, measurement of serum TSH level among infertile women is employed for detection of hypothyroidism. High serum TSH level and presence of anti-thyroid antibodies was the major risk factors of infertility (Table 1, 2). Thyroid disease can interfere with the process of getting pregnant; by ovulation and irregular menstruation.¹⁵ Male infertility was observed in 21% among the Makkans population with correlated abnormalities of gonadotrophins, thyroid stimulating hormone, and thyroid and testosterone hormone, they reported 35% cases of hypothyroidism while hyperthyroidism was found to be 14%, where as 28% of thyroid abnormality constituted an independent infertile group.¹⁶ Hypothyroidism may also be associated with an increased frequency of menstrual period in patients with mild to moderate

thyroid failure, and a lack of menstruation (amenorrhoea) when hypothyroidism is severe.

In our study, significant correlation of serum TSH level, menorrhagia, amenorrhoea and infertility were found at p<0.01 (Table-2). Similar study was conducted in Belgium on a cohort of 438 infertile couples, 45% females were identified as infertile; endometriosis (11%), tubal disease (30%) and ovarian dysfunction (59%). However, Male infertility was diagnosed in 38% and idiopathic infertility in 17% of the couples.¹⁷ The origin of infertility is similarly due to male or female factors; the causes are multiple. Female factors account for 46.7% of infertility. Male factors account for 19.0% of infertility. Male and female factors combined cause 18.2% of fertility. The etiology is unknown in 11.2%, and other causes are identified in 5.2%. About 25% infertility and 15% menstrual cycle disorders result from thyroid dysfunction. This prevalence of sub-clinical hypothyroidism has also been reported in population of Pragu.¹⁸ Therefore, thyroid function must be examined in female with unclear infertility or menstrual problems.¹⁹

CONCLUSION:

The contribution of thyroid in fertility and menstrual disturbances are substantial. So, it is recommended to know the thyroid status in these cases. Further more, thyroid profile estimation cannot be ignored in the infertile couples. Awareness of the thyroid status in the infertile couple is crucial, because of its significant, frequent and often reversible or preventable effect on infertility. These abnormalities reverse after restoration of euthyroidism.

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COMMENTS

ROLE OF GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF)/FILGRASTIM AS AN ADJUNCT IN CHRONIC HEPATITIS C MANAGEMENT

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ABSTRACT

Drug-induced hematotoxicity is the commonest reason for reducing the dose or withdrawing interferon (IFN) therapy in a case of chronic hepatitis C thus depriving the patient of a possible cure. Traditionally, severe neutropenia has been considered an absolute contraindication to start antiviral therapy. Since the advent of adjunct therapy with Granulocyte-colony stimulating factor, the same is not true any more. Some recent landmark studies have used this adjunct therapy to help avoid antiviral dose reductions. Although, addition of this adjunct therapy has been shown to significantly increase the overall cost of the treatment, if the infection is cured at the end of the day, this extra cost is worth bearing. Although, more studies are needed to refine the true indications of this adjunct therapy, determine the best dose regimen, quantify the average extra cost and validate that whether or not the addition of this therapy increases the sustained virologic response rates achieved, the initial reports are encouraging. Therefore, although not recommended on routine basis, some selected patients may be given the benefits of these factors. In this article, a review of the current literature on this subject is given followed by few suggested recommendations at the end to help develop local guidelines.

Keywords: Chronic Hepatitis C. Neutropenia. Hematopoietic growth factors. Granulocyte-colony stimulating factor.

INTRODUCTION

Ribavirin(RBV)-induced hemolytic anemia and Interferon(IFN)-induced neutropenia are two well known side effects of antiviral therapy in HCV-infected patients. Some studies have estimated that these side effects are responsible for dose reductions in almost 40%^{1,2} of the subjects with consequent 10-20%³⁻⁵ reductions in the virologic responses achieved. One study incriminated pegylated interferon (PEG-IFN) as directly responsible for suppressing hematopoiesis in all three cell lineages.⁶ Cirrhosis with hypersplenism, history of blood cell count drop

with prior antiviral therapy, lower baseline cell counts (Hb level 13 g/dl; neutrophil count 2900/mm³; platelet count <170,000/mm³) at the initiation of antiviral therapy, obesity and old age are considered to be the major risk factors for the development for hematotoxicity with RBV and IFN.⁷ Based on these observations, there is a renewed interest in the use of hematopoietic growth factors (HGFs) in patients undergoing antiviral therapy. Only a few studies have so far been done on the pros and cons of HGFs use, and data is still considered insufficient to recommend their routine use.⁸ This review article aims to discuss the current trends in the rationale, protocols and pros and cons of granulocyte-colony stimulating factor (G-CSF) use as an adjunct in the management of chronic hepatitis C. For this review, a literature search

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was made on 20/Oct/08 on PubMed, MEDLINE and EMBASE databases (key words: chronic hepatitis C, neutropenia, hematopoietic growth factors and granulocyte-colony stimulating factor), the public Web site of the US Food and Drug Administration and Erythropoiesis-stimulating agents (ESAs) manufacturers, and safety advisories. American⁹ and Canadian⁸ consensus guidelines on the management of chronic hepatitis C were also consulted.

DISCUSSION:

G-CSF is a 175 amino acid, highly purified, non-glycosylated protein produced by recombinant technology in a lab strain of *E. Coli* by the addition of a gene for the Granulocyte colony-stimulating factor.¹⁰ It induces neutrophil production,¹¹ differentiation¹² and release from the bone marrow.¹³ Significant increase in the neutrophil counts can be observed within 24hrs of G-CSF administration. It also appears to cause selected end-cell functional activation including enhanced phagocytic ability.¹⁴ Other cell lines are affected by negligible proportions, if at all. Neutrophil levels usually normalize within 1-7 days (average 4 days). Studies, however, have not shown any survival benefit. It appears that no effect – positive or negative - is produced on disease progression and despite the fact that neutropenia is common, infective episodes are extremely rare in treated HCV patients.

G-CSF is primarily used in patients with nonmyeloid cancers (myeloid haemopathy is a contraindication to use G-CSF) undergoing bone-marrow-suppressive cytotoxic chemotherapy, or in patients undergoing myeloablative therapy before bone marrow transplantation. The aim is to reduce the incidence, severity and duration of neutropenia. Besides avoiding/correcting neutropenia, an additional effect of G-CSF is mobilization of the hematopoietic progenitor cells into the peripheral blood. These peripheral blood progenitor cells (PBPC) may then be harvested and infused into patients undergoing cytotoxic chemotherapy, either alone or in addition to bone marrow transplantation with consequent rapid and more adequate hematological recovery.

Because of the known benefits of G-CSF in neutropenia patients, it has been tried in some recent studies in HCV-infected patients undergoing IFN therapy. The commonest cause of interferon dose reductions in HCV-infected patients is IFN-induced neutropenia.⁵ Some 30-50% of the subjects develop neutropenia within 1-2 weeks of starting the therapy.³⁻⁵ The frequency appears to be higher with PEG-IFN as compared to the non-PEG-IFN.^{6,15} G-CSF has been tried in some studies⁷ with reasonable results to avoid IFN dose reductions.

The current recommendation⁸ is to reduce IFN dose if neutrophil count falls to $<0.5 \times 10^9/L$, and discontinue it if it falls to $<0.3 \times 10^9/L$. The minimum effective dose of PEG-IFN appears to be $1 \mu g/kg/wk$. If despite reducing the PEG-IFN dose to the minimum effective level, neutrophil counts of $<0.5 \times 10^9/L$ persist, G-CSF therapy may be considered.

G-CSF is commercially available in the form of sterile, clear, colorless, preservative-free liquid for parenteral administration. The product is available in single use vials and prefilled syringes containing either 300 mcg or 480 mcg Filgrastim at a filled volume of 1.0 mL or 1.6 mL, respectively. Suggested starting dose regimen of G-CSF is $300 \mu g$ SQ once weekly and then adjusting the dose as per response/requirement. The aim should be to maintain a neutrophil count of $\approx 1000 \text{ cells}/\mu L$ (return to the pretreatment level is not the aim). Most patients adequately respond to a G-CSF dose of $300 \mu g$ SQ once weekly, whereas $1/3^{\text{rd}}$ cases require dose adjustments. Some patients may require up to $480 \mu g$ Filgrastim SQ thrice weekly; others may only need $150 \mu g$ Filgrastim SQ once weekly. Complete blood counts should be asked twice or thrice weekly and response to therapy judged. After the adequate neutrophil count is achieved, IFN dose can be increased to the optimum level. Once started, adjunct G-CSF therapy may be required till the end of the treatment. In one study⁷, the median duration of G-CSF therapy was 20 weeks (range 9–45). No international consensus currently exists on the lower cut-off value of neutrophil count after which the risk of development of serious infections is high enough to warrant initiation of G-CSF therapy.¹⁶⁻¹⁷

Whether or not neutropenia increases the risk of infection, is also debatable. One study showed an average fall of 34% in the neutrophil count with no documented or suspected bacterial infection.¹⁸ Another study demonstrated that infections neither correlate with the nadir of neutrophil count (<1,000 or >750/mm³) nor with the magnitude of neutrophil count fall from the baseline.¹⁷ This is in contrast to the observations made in the immunodeficient cirrhotics¹⁹, HIV carriers²⁰ and liver-transplant patients²¹ in which prolonged neutropenia has been associated with the development of bacterial infections warranting cessation of antiviral therapy. The frequency of development of superadded bacterial infection, secondary to neutropenia appears to be lower in blacks.¹⁸ These patients also have an intrinsic low white cell count prior to starting treatment. Thus the lower cut-off value ought to be lower in blacks. Interestingly, as demonstrated by Puoti et al, the frequency of non-respiratory infections may increase with PEG-IFN therapy, independent of the neutrophil count.²²

G-CSF is considered contraindicated in patients with known hypersensitivity to E coli-derived proteins including Filgrastim or any of its components.

This drug is generally well tolerated. Common side effects include bone/muscle aches, nausea and vomiting. The frequency of bone/muscle aches can be reduced by giving G-CSF either 2 days before or 2 days after interferon injection.²³ Rarely, splenomegaly and spontaneous splenic rupture have also been reported with G-CSF use. Thus, any patient reporting with left upper abdominal and/or shoulder tip pain should be evaluated for an enlarged spleen or splenic rupture. Neutropenic patients receiving G-CSF, if develop, fever, dyspnoea or lung infiltrates, should be evaluated for the possibility of adult respiratory distress syndrome (ARDS). Development of ARDS warrants immediate cessation of G-CSF therapy till the resolution of the symptoms. There are conflicting reports regarding the cost effectiveness of HGF therapy. One study reported an increase in the final cost by 43% with adjunct biotherapy with EPO and G-CSF,⁷ whereas another study suggested that since HGF therapy increases

therapeutic compliance, improves quality of life, and avoids complications of chronic liver disease, compared to the standard care, it is cost effective.²⁴ A cost analysis using a decision analysis model demonstrated that G-CSF use in HCV-infected genotype 1 cases is cost effective, especially when given in a dose of 300µg SQ once weekly.²⁵ Most published, cost-effectiveness studies assume that, once started, patients continue to take HGF's for the remaining hepatitis C therapy. In significant percentage of patients' withdrawal of HGF therapy may be possible much earlier without negatively affecting the SVR rates. This makes HGF therapy even more cost-effective.

CONCLUSION:

Despite the data being limited, it appears that HGF therapy improves the quality of life (QOL) across many domains (physical, mental and social).^{26,27} Due to lack of SVR data, no respectable association recommends the routine use of HGF therapy, based on the current evidence. It is quite reasonable to believe that, adjunct therapy with HGFs where indicated, helps avoiding antiviral dose reductions and attain optimum adherence (defined as the administration of bitherapy in an optimum dose i.e. PEG-IFN =1 g/kg/wk and RBV =10.6 mg/kg/day for more than 80% of the prescribed duration). The possible net effect may be, attainment of higher SVR rates, although more studies are needed to validate it. Studies have shown that HGF therapy is generally well tolerated. Further studies are needed to determine the lower cut-off values of neutrophil count after which G-CSF therapy should be started. More studies are also needed to establish the right dosages and cost effectiveness of HGF therapy.

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BILIOUS VOMITING IN CHILDREN - A PLEA FOR EARLY SURGICAL CONSULTATION

Sir,

We would like to share our experience with our colleagues on a very important aspect. A 3½ years old boy weighing 12 kg was referred from a paediatric unit for surgical opinion. He had a history of bilious vomiting and abdominal distention off and on for the last 2½ years. He was treated for these complaints by different general practitioners and pediatricians for this length of time. Physical examination revealed upper abdominal distention with visible peristalsis. Barium meal & follow through examination revealed partial obstruction at the level of lower jejunum with massively dilated proximal intestine (Photograph – I)

A laparotomy was performed which revealed massively dilated proximal jejunum with change in the size of gut at the level of distal jejunum (Photograph – II). Enterotomy was carried out which showed a mucosal web with small eccentric opening (Photograph – III). Mucosal web was excised and side to side anastomosis performed. Post operative recovery was uneventful.

Jejunioileal atresias and stenosis are major causes of neonatal intestinal obstruction.¹⁻³ Most newborns with intestinal obstruction present with bilious vomiting. Bilious vomiting in the neonate should be considered secondary to a mechanical obstruction until proven otherwise, and emergency surgical evaluation is warranted in every newborn with this symptom.^{4,5} Most of the patients with intestinal stenosis create diagnostic difficulty. Intermittent partial obstruction or failure to thrive may be the initial symptoms that may subside without treatment. Most of these babies eventually develop complete intestinal obstruction.⁶⁻⁸

These lesions should be distinguished from other causes of neonatal intestinal obstruction which include malrotation with or without midgut volvulus, intestinal duplication, and internal herniation etc.⁹ Meticulous history taking, physical examination and radiological investigations are the most useful elements in differentiating this condition.¹⁰

Our patient had bilious vomiting, abdominal distention with visible peristalsis off & on for the past 2½ years and he has been seen by many physicians and pediatricians but nobody investigated this patient or sought a surgical advice. There are reports which showed delayed diagnosis for years in such cases.^{7,11} Early surgical opinion and investigation of patients with bilious vomiting would help in early diagnosis of these cases.

Our purpose of reporting this case is to draw attention of our colleagues to take early surgical opinion in patients having bilious vomiting with or without abdominal distention, so that these cases should be diagnosed and treated as early as possible to decrease morbidity and mortality associated with delayed diagnosis and treatment of this condition.



Figure 1: Barium meal & follow-through examination showing partial obstruction at distal jejunum with dilatation of proximal loops.

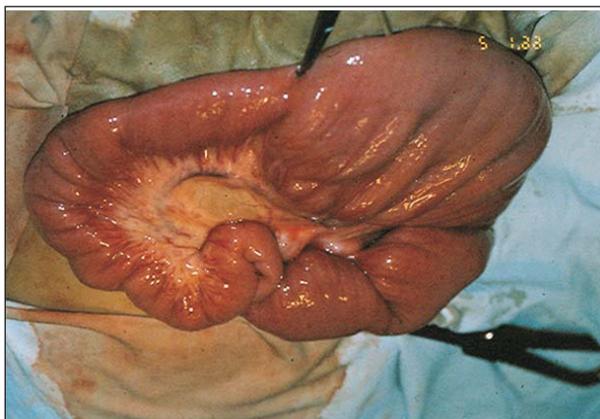


Figure 2: Operative view showing massively dilated proximal jejunum with abrupt change in the size of intestine

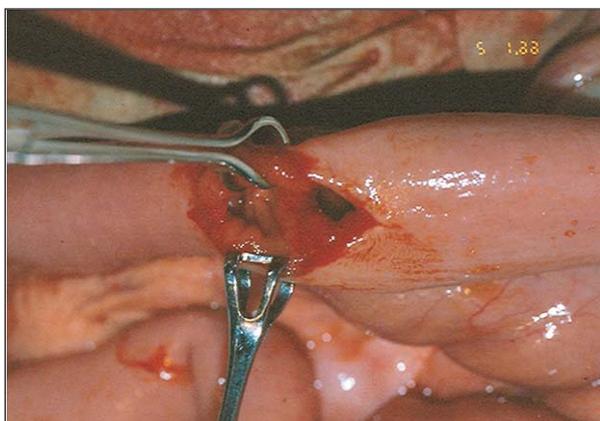


Figure 3: Operative view: Enterotomy showing mucosal web with small eccentric opening

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GUIDELINES ON GOOD PUBLICATION PRACTICE BY COMMITTEE ON PUBLICATION ETHICS (COPE)

Why the guidelines were developed

These guidelines should be useful for authors, editors, editorial board members, readers, owners of journals, and publishers.

What they aim to do

These guidelines are intended to be advisory rather than prescriptive, and to evolve over time. We hope that they will be disseminated widely, endorsed by editors, and refined by those who use them.

1. Study design and ethical approval

Definition

Good research should be well justified, well planned, appropriately designed, and ethically approved. To conduct research to a lower standard may constitute misconduct.

Action

- (1) Laboratory and clinical research should be driven by protocol; pilot studies should have a written rationale.
- (2) Research protocols should seek to answer specific questions, rather than just collect data.
- (3) Protocols must be carefully agreed by all contributors and collaborators, including, if appropriate, the participants.
- (4) The final protocol should form part of the research record.
- (5) Early agreement on the precise roles of the contributors and collaborators, and on matters of authorship and publication, is advised.
- (6) Statistical issues should be considered early in study design, including power calculations, to ensure that the statistical data is adequate as per the study design there are neither too few nor too many participants.

(7) Formal and documented ethical approval from an appropriately constituted research ethics committee is required for all studies involving people, medical records, and anonymised human tissues.

(8) Use of human tissues in research should conform to the highest ethical standards, such as those recommended by the Nuffield Council on Bioethics.

(9) Fully informed consent should always be sought. It may not always be possible, however in such circumstances, an appropriately constituted research ethics committee should decide if this is ethically acceptable.

(10) When participants are unable to give fully informed consent, research should follow international guidelines, such as those of the Council for International Organizations of Medical Sciences (CIOMS).

(11) Animal experiments require full compliance with local and national, ethical, regulatory principles, along with local licensing arrangements. The International standards vary.

(12) Formal supervision, which is usually the responsibility of the principal investigator, should be provided for all research projects: this must include quality control, frequent review and long term (may be up to 15 years) retention of all records and primary outputs.

2 Data analysis

Definition

Data should be appropriately analysed, but inappropriate analysis does not necessarily amount to misconduct. Fabrication and false data, are misconduct.

Action

(1) All sources and methods used to obtain and analyse data, including any electronic pre-processing, should be fully disclosed; detailed explanations should be provided for any exclusions.

(2) Methods of analysis must be explained in detail, and referenced if they are not in common use.

(3) The post hoc analysis of subgroups is acceptable, as long as this is disclosed. Failure to disclose that the analysis was post hoc, is unacceptable.

(4) The discussion section of a paper should mention any issues of bias which were considered, and explain how they have been dealt with in the design and interpretation of the study.

3. Authorship

Definition

There is no universally agreed definition of authorship, although attempts have been made. At least the authors must take responsibility for a particular section of the study.

Action

(1) The award of authorship should balance intellectual contributions to the conception, design, analysis and writing of the study against the collection of data and other routine work. If there is no task that can reasonably be attributed to a particular individual, then that individual should not be credited with authorship.

(2) To avoid disputes over attribution of academic credit, it is advisable to decide early in the planning of a research project for credit to authors, as contributors, and who will be acknowledged.

(3) If professional writers employed by pharmaceutical companies, medical agencies, or other parties have written the paper, their names should be included, and any conflicts of interest should be declared.

(4) All authors must take public responsibility for the content of their paper. The multidisciplinary nature of many research work can make this difficult, however, this can be resolved by disclosure of individual contributions.

(5) Careful reading of the target journal's "Advice to Authors" is advised in the light of current uncertainties.

(6) Authors should be vigilant about allowing their name to be used on a piece of work to add credibility to the content.

4 Conflicts of interest

Definition

Conflicts of interest arise when authors, reviewers, or editors have interests that are not fully apparent and that may influence their judgements on what is published. They have been described as those which, when revealed later, would make a reasonable reader feel misled or deceived.

They may be personal, commercial, political, academic or financial. "Financial" interests may include employment, research funding, stock or shared ownership, payment for lectures or travel, consultancies and company support for staff.

Action

(1) Such interests, where relevant, must be declared to editors by researchers, authors, and reviewers.

(2) Editors should also disclose relevant conflicts of interest to their readers. If in doubt, disclose.

(3) Editors should also consider disclosing to readers their own conflicts of interest and those of their teams, editorial boards, managers, and owners.

(4) Sometimes conflicts of interest may be so much that publication will not be possible or people (for example, reviewers or editors) may have to be excluded from decisions on publication.

5. Peer review

Definition

Peer reviewers are external experts chosen by editors to provide written opinions with the aim of improving the study.

Working methods vary from journal to journal, but some use open procedures in which the name of the reviewer is disclosed, together with the full or "edited" report.

Action

- (1) Suggestions from authors, as to who might act as reviewers are often useful, but there should be no obligation on editors to use those suggestions.
- (2) The confidentiality in the assessment of a manuscript must be maintained by expert reviewers, and this extends to reviewers' colleagues who may be asked (with the editor's permission) to give opinions on specific sections only.
- (3) The submitted manuscript should not be retained or copied.
- (4) Reviewers and editors should not use the data, arguments, or interpretations, unless they have the authors' permission.
- (5) Reviewers should provide speedy, accurate, courteous, unbiased and justifiable reports.
- (6) If reviewers suspect misconduct, they should write in confidentiality to the editor.
- (7) Journals should publish accurate descriptions of their peer review, selection, and appeal processes.
- (8) Journals should also provide regular audits of their acceptance rates and publication timings.

6. Redundant publication

Definition

Redundant publications are papers, without full cross reference, and share the same hypothesis, data, discussion points, or conclusions.

Action

- (1) Published studies should not be repeated unless further confirmation is required.
- (2) Previous publication of an abstract during the proceedings of meetings does not preclude subsequent submission for publication, but full disclosure should be made at the time of submission.
- (3) Re-publication of a paper in another language is acceptable, provided there is full and prominent

disclosure of its original source at the time of submission.

- (4) At the time of submission, authors should disclose details of related papers, even if in a different language, and similar papers are in press for publication.

Dealing with misconduct

A Principles

- (1) The general principle confirming misconduct is intention to give impression of being true although it is not true.
- (2) The review for misconduct must therefore focus, not only on the particular act or omission, but also on the **intention** of the researcher, author, editor, reviewer or publisher involved.
- (3) Deception may be by intention, by reckless disregard of possible consequences, or by negligence. Therefore, it is implicit, that "best practice" requires complete honesty, with full disclosure.
- (4) Codes of practice increase awareness and can never be exhaustive.

B Investigating misconduct

- (1) Editors should not simply reject papers that raise questions of misconduct. They are ethically obliged to pursue the case. However, knowing how to investigate and respond to possible cases of misconduct is difficult at times.
- (2) (COPE) is always willing to advise, but for legal reasons, can only advise on anonymous cases.
- (3) The editor should decide about the action to be taken

C Serious misconduct

- (1) Editors must take all allegations and suspicions of misconduct seriously. However, they must recognise that usually they do not have either the legal legitimacy or the means to conduct investigations into serious cases.
- (2) The editor must decide when to inform the employers of the accused author(s).

(3) Investigation of accusations require some evidence and a format as it is undesirable for editors to conduct enquiries involving experts which makes the credibility of the author questionable.

(4) If editors are presented with convincing evidence of serious misconduct by authors perhaps by reviewers, they should immediately pass on this information to the employers.

(5) If accusations of serious misconduct are not accompanied by convincing evidence, editors should confidentially seek expert advice.

(6) If the experts raise serious questions about the research, editors should notify the employers.

(7) If the experts find no evidence of misconduct, the editorial processes should proceed in the normal way.

(8) If presented with convincing evidence of serious misconduct, where there is no employer to whom this can be referred, and the author(s) are registered doctors, cases can be referred to the General Medical Council, like PMDC.

(9) If, however, there is no organisation with the legitimacy and the means to conduct an investigation, the editor may decide that the case is sufficiently important to warrant publishing something in the journal. Legal advice will then be essential.

(10) If editors are convinced that an employer has not conducted an adequate investigation of a serious accusation, they may feel that publication of a notice in the journal is warranted. Legal advice will be essential.

(11) Authors should be given the opportunity to respond to accusations of serious misconduct

D Less serious misconduct

(1) Editors may decide whether or not to involve employers in less serious cases of misconduct, such as redundant publication, deception over authorship,

or failure to declare conflict of interest. Sometimes the evidence may speak for itself, although it may be wise to appoint an independent expert.

(2) Editors should remember that accusations of even minor misconduct may have serious implications for the author(s), and it may then be necessary to ask the employers to investigate.

(3) Authors should be given the opportunity to respond to any charge of minor misconduct.

(4) If convinced of wrongdoing, editors may wish to adopt some of the sanctions outlined below.

E Sanctions

Sanctions may be applied separately or combined. The following are ranked in approximate order of severity:

(1) A letter of explanation (and education) to the authors, where there is a genuine misunderstanding of principles.

(2) A letter of reprimand and warning as to future conduct.

(3) A formal letter to the relevant head of institution or funding body.

(4) Publication of a notice of redundant publication or plagiarism.

(5) An editorial giving full details of the misconduct.

(6) Refusal to accept future submissions from the individual, unit, or institution responsible for the misconduct, for a stated period.

(7) Formal withdrawal or retraction of the paper from the scientific literature, informing other editors and the indexing authorities.

(8) Reporting the case to the General Medical Council, or other such authority or organisation which can investigate and act with due process.



INSTRUCTIONS TO AUTHORS

The JDUHS agrees to accept manuscripts prepared in accordance with the "Uniform Requirements submitted to the Biomedical Journals" published in the British Medical Journal 1991; 302:334-41. Revised February, 2006.

INSTRUCTIONS TO AUTHORS

All materials submitted for publication should be sent exclusively to the Journal of the Dow University of Health Sciences. Work that has already been reported in a published paper or described in a paper sent or accepted elsewhere for publication should not be submitted. However, a complete report following publication of a preliminary report, usually in the form of an abstract, or a paper that has been presented at a scientific meeting, if not published in full in a proceeding or similar publication, may be submitted. Press reports of meetings will not be considered as breach of this rule, but such reports should not be amplified by additional data or copies of tables and illustrations. In case of doubt, a copy of the published material should be included with a manuscript to help the editors decide how to deal with the matter. Dissertation or thesis-based articles should be reformatted according to the instructions to authors.

ETHICAL CONSIDERATIONS

If tables, illustrations or photographs, which have already been published, are included, a letter of permission for re-publication should be obtained from author (s) as well as the editor of the journal where it was previously published.

Written permission to reproduce photographs of patients, whose identity is not disguised, should be sent with the manuscript; otherwise the eyes will be blackened out.

MATERIAL FOR PUBLICATION

The material submitted for publication may be in the form of an Original research, a Review Article, evidence-based reports, Special article, Commentary, Short communication, a Case Report, Recent Advances, New techniques, view points on Clinical/Medical Education, Adverse Drug Reports or a Letter to the Editor. Original articles should normally report original research of relevance to clinical medicine, and may appear either as papers or as short communications. The paper should be of about 2000 words, with no more than six tables or illustrations; short communications should be of about 600 words, with one table or illustration and no more than five references. Clinical case reports and brief or negative research findings may appear in this section.

Review article should consist of structured overview of some relatively narrow topics providing background and recent development with reference to the original literature. An author can write a review article only if he/she has written a minimum of three original research articles and some case reports on the same topic.

Letters should normally not exceed 400 words, have no more than 05 references, and be signed by all the authors; preference is given to those that take up points made in contributions published in the journal. **Editorials are by invitation.**

Authors should keep one copy of their manuscript for reference, and send three copies (laser copies or inkjet, photocopies are not accepted)

to the Managing Editor, Journal of the Dow University of Health Sciences. The author should also submit an electronic copy of the manuscript typed in MS Word. Any illustrations or photographs should also be sent in duplicate. People from outside Pakistan can also e-mail their manuscript.

Each manuscript should include a title page (containing e-mail address, fax and phone numbers of the corresponding author), abstract, text, acknowledgements (if any), references, tables, and legends. Each component should begin on a new page, in the following sequence: title page; abstract and at least three key words; text; acknowledgements; references; tables (each table, complete with title and footnotes, should be merged in the manuscript); and legends for illustrations.

The manuscript should be typed in double spacing as a single column on 8 1/2" X 11" (21.5cm X 28.0 cm) white bond paper with one inch (2.5cms) margin on both sides. It should not exceed 3000 words, excluding tables and references. There should be no less than 20 or more than 40 references in an Original Article and no less than 40 or more than 60 in a Review Article. If prepared on a word processor / computer, the diskette, properly protected, or CDs should be sent with the manuscript.

TABLES AND ILLUSTRATIONS

Tables and illustrations should be merged within the text of the paper, and legends to illustrations should be typed on the same sheet. Tables should be simple, and should supplement rather than duplicate information in the text; tables repeating the information will be omitted. Each table should have a title and be typed in double space without horizontal and vertical lines on an 8 1/2" X 11" (21.5 X 28.0 cms) paper. Tables should be numbered consecutively with Roman numerals in the order they are mentioned in the text. Page number should be in the upper right corner. If abbreviations are used, they should be explained in footnotes and when they first appear in text. When graphs, scatter grams, or histograms are submitted, the numerical data on which they are based should be supplied. All graphs should be merged in the manuscript. For scanned photographs highest resolution should be used.

S.I. UNITS

System International (S.I) Unit measurement should be used. All drugs must be mentioned in their generic form. The commercial name may, however, be mentioned within brackets, if necessary.

FIGURES AND PHOTOGRAPHS

Figures and photographs should only be sent when data cannot be expressed in any other form. They must be unmounted, glossy prints in sharp focus, 5" X 7" (12.7X17.3 cms) in size. They may be in black and white or color. Negatives, transparencies, and X-ray films should not be submitted. The number of the figures, the name of the author(s) should be printed on the back of each figure/photograph.

The top of the figure must be identified by the author. These figures and photographs must be cited in the text in consecutive order. Legends must be typed on the same paper. Legends for photomicrographs should indicate the magnification, internal scale and the method of staining. Photographs in published articles will not be returned.

REFERENCE NUMBERING AND FORMAT

References should be numbered in the order in which they are cited in the text. At the end of the article, the full list of references should give the names and initials of all the authors (**If the authors are more than 6, then et al. should be followed after the 6th name**). The author(s) names are followed by the title of the article; title of the journal abbreviated according to the style of the Index Medicus (see "List of Journals Indexed," printed yearly in the January issue of Index Medicus); year volume and page number; e. g.: Hall RR. The healing of tissues by CO₂ laser. *Br J Surg*: 1971;58:222-5. Reference to books should give the names of editors, place of publication, publisher, and year. The author must verify the references against the original documents before submitting the article.

PEER REVIEW

Every paper will be read by the staff editors of the editorial board. The papers selected will then be sent to one or more external reviewers. If statistical analysis is included, further examination by a statistician will be carried out.

ABSTRACT

Abstracts of original article should be in structured format with following sub-headings:

i. Objective, ii. Design, iii. Patients & Methods, iv. Result, v. Conclusion. Four elements should be addressed: why did you start? what did you do? what did you find? and what does it mean? Why did you start is the objective. What did you do constitutes the methodology and should include design, setting, patients or other participants, interventions, and outcome measures. What did you find is the results, and what does it mean would constitute your conclusions. Please label each section clearly with the appropriate sub-headings. Structured abstract for an original article should not be more than 250 words.

Review article, case report and other requires a short, unstructured abstract. Commentaries and short communications do not require abstract.

INTRODUCTION

This should include the purpose of the article. The rationale for the study or observation should be summarized; only strictly pertinent references should be cited; the subject should not be extensively reviewed. Data or conclusions from the work being reported should not be presented.

METHODS

Study design and sampling methods should be mentioned. Obsolete terms such as retrospective studies should not be used. The selection of the observational or experimental subjects (patients or experimental animals, including controls) should be described clearly.

The methods and the apparatus used should be identified (with the manufacturer's name and address in parentheses) and procedures described in sufficient detail to allow other workers to reproduce the results. References to established methods should be given, including statistical methods; references and brief descriptions for methods that have been published but are not well-known should be provided; new or substantially modified methods should be described, giving reasons for using them, and evaluating their limitations. All drugs and chemicals used should be identified precisely, including generic name (s), dose (s), and route (s) of administration.

RESULTS

These should be presented in a logical sequence in the text, tables, and illustrations. All the data in the tables or illustrations should not be repeated in the text; only important observations should be emphasized or summarized.

DISCUSSION

The author's comment on the results, supported with contemporary references, including arguments and analysis of identical work done by other workers. A summary is not required. Brief acknowledgement may be made at the end.

CONCLUSION

Conclusion should be provided under separate heading and highlight new aspects arising from the study. It should be in accordance with the objectives.

REPRINTS

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