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Rational Drug use and Essential Drug Concepts

Akbar Waheed

Rational drug therapy is defined as "administration of the right drug indicated for the disease, in right dose through an appropriate route for a right duration"following are the reasons for irrational use of drugs:[1,2,3,4,5,6]

- Lack of information about drugs
- faulty training of medical graduates
- Absence of role models
- Lack of diagnostic facilities
- Demand from patient – prompt and quick
- Patient load
- Promotional activities of drug companies
- Exaggerated claims by drug companies
- Lack of patient doctor communication
- Ineffective rules and regulations

There are so manyhazards of irrational useof drugs, some of these hazards are given below:[7,8,9]

- Ineffective and unsafe treatment
- Over treatment
- Under treatment
- Prolongation of ailment
- Loss of patient doctor confidence

Following are examples of some common types of irrational uses:

Type I Drug	Type II Drug used for same[8]
Chlorpheniramine Paisa = 00.05	Terfenadine Rs = 08.00
Nalidixic Acid Rs = 02.32	Norfloxacin Rs = 11.00
Bandrofluzaide Paisa = 00.12	Indapamide Rs = 03.00
Cimetidin Rs= 06.05	Ranitidine Rs = 10.50
Aspirin Paisa = 00.05	Piroxicam Rs = 03.75
Diazepam Paisa =00.20	Bromazepam Rs = 02.07

Some general principles for rational prescribing are [6,7,8,10]

- Need for drug therapy
- Choice of drug:
  - Efficacy of drug
  - Safety of drug
- Cost effectiveness
- Mono drug therapy
- Drug combinations (only if essential e.g chemotherapy of cancerT.B.etc)
- Dose of the drug (optimum doses should be used)
- Dosage form/route of administration (parenteral route should only be used when
- required and in emergencies)
- Duration of therapy(not so long and not too short)
- Patient's compliance

Following are some suggestion for developing national strategies for promoting rational use of drugs.[11,12,13,14]

- EBM guidelines to be developed
- Essential drug lists / treatment of choice should be published by MoH.
- Auditing of prescription by drug and therapeutic comittees of MoH.
- Subject should be taught at UG level at medical colleges
- Continuing medical education (CME) for doctors.
- Public education through family physicians / PMDC/medical institutes.
- Avoidance of perverse financial incentives by drug marketing companies.
- Appropriate and strict drug regulations by MoH.

Doctor's participation should be a pre requisite for changes in behaviour of irrational Prescribing andthe objectives should be:

- To identify factors which hinder rational drug therapy
- To foster the concept of essential drugs in order to reduce the cost of health caredelivery
- To tailor prescribing to the needs of individual patients
- They should know the advantages of an essential drug list, such as cost effectiveness control, management, purchase, storage and distribution
- Factor responsible for irrational therapy with

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reference to patients such as his socioeconomic status, social taboos and beliefs, simultaneous treatment form different systems of medicine.

- Prescribing irrational drug combinations & formulationby physicians.

There is a need to find remedial measures to overcome irrationality at various levelsthat what is the reason for the popularity of certain drugs orcombined products which are irrational & expensive, & to find out/suggest measures to curb their unethicalpromotion. Remedial measures suggested include:[14,15]

- Patient education.
- Improvement in diagnostic facilities.
- Making essential drugs readily available at all times.
- Continued medical educations of physicians/ monitoring and feedback from prescription data
- Training at undergraduate and graduate level

The public should be educated about the harmful effects of drugs, especially of self-medication. Patient education is the responsibility of the prescriber/members of health team. Patient should be explained about the drug prescribed, dose, and duration of therapy, possible side effects, implications of missing dose/ or discontinuation of therapy.[16]Emphasis is needed on preventive aspects of health rather than curatives one.

Some reasons for use of irrational drugs include:[14]

- Easy availability without prescription
- Ignorance of harmful effects.
- Misleading advertisements.
- Attractive incentives for marketing / prescribing.

Advertisement for a drug influence young doctor a lot, some prescribers will look to advertisements for science on which to base their choice and marketers will provide it. The most important step in preserving the profession's integrity is to explain at under graduate/postgraduatelevels how marketing works. Doctors themselves can be taught to look at advertisement critically.[4,5]

Conclusion:

The profession needs to be alert not subverted.

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ORIGINAL ARTICLE

Irbesartan; An Antihypertensive Drug Reduces LDL-Cholesterol in Diet Induced Hyperlipidemic Rabbits

Sabeen Shakir[1], Akbar Waheed[2], Noureen Hafeez[3]

ABSTRACT

Objective: This study on animal model was designed to explore the LDL-Cholesterol lowering effect of an antihypertensive drug Irbesartan.

Study Design: Randomised controlled experimental study.

Place and Duration of Study: The study was conducted in the animal house of National Institute of Health (NIH), Islamabad. Biochemical analysis of rabbit's serum was carried out in the department of chemical pathology, Army Medical College, Rawalpindi from February 2013 to June 2013.

Materials and Methods: Eighteen rabbits were divided into three groups (group A, group B and group C) of six rabbits each. Group one was labelled as normal control. The other two groups (B and C) were made hyperlipidemic by feeding them with high cholesterol diet. Of these, group B was taken as hyperlipidemic control and group C as treatment group. Serum LDL levels were estimated at three different occasions i.e. baseline, after giving high cholesterol diet to hyperlipidemic groups and after 30 days of giving irbesartan to treatment group.

Results: Serum analysis for the estimation of LDL-Cholesterol of all the groups was done and their means were calculated and compared with the base line values using SPSS Version 20. Irbesartan treated group showed a marked reduction in serum LDL cholesterol in comparison with the hyperlipidemic control group.

Conclusion: It is concluded that Irbesartan an antihypertensive agent, has also the ability to markedly reduce raised serum LDL cholesterol levels.

Key Words: *Hyperlipidemia, Irbesartan, LDL-Cholesterol.*

Introduction

Cardiovascular diseases (CVD) have become a major problem nowadays and are responsible for 76 percent of deaths and disabilities due to myocardial infarction, atherosclerosis and stroke.[1] The risk of cardiovascular events is strongly associated with high blood pressure as well as high blood cholesterol levels.[2] There are different groups of drugs used to lower blood pressure.[3] Likewise there is separate class of drugs to reduce elevated blood cholesterol levels having its own mechanism of action.[4] Therefore patients with cardiovascular diseases require drugs from both these classes i.e. antihypertensives as well as antihyperlipidemics to manage two major determinants of CVD i.e. hypertension and hyperlipidemia.[5]

Lipids are the essential biomolecules, divided into five different classes i.e. chylomicrons, VLDL-Cholesterol, IDL-Cholesterol, LDL-Cholesterol and HDL-Cholesterol. Among these, low density lipoproteins (LDL-Cholesterol) in normal range is necessary for innumerable normal healthy body functions.[6] However the elevated levels of LDL molecules loaded with cholesterol, get accumulated on the walls of the arteries and cause various fatal and nonfatal cardiovascular abnormalities.[7]

Irbesartan, an antihypertensive drug, is a novel, orally active, noncompetitive angiotensin receptor antagonist, specific for angiotensin 1 receptor subtype.[8] The drug has a stronger capability to lessen the risk of combined major cardiovascular events (stroke, atherosclerosis, heart failure, angina, myocardial infarction, peripheral arterial disease and death).[9]

Irbesartan is metabolized in the liver by glucuronide conjugation and oxidation.[10] A big fraction of irbesartan i.e. 80 percent remains unchanged and possess pharmacological activity whether given

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orally or intravenously.[11] Only 6 percent of circulating drug gets converted into inactive glucuronide conjugate. The rest of the metabolites are pharmacologically inactive and are excreted via urine or bile.[12]

Angiotensin II is a potent vasoconstrictor in vascular smooth muscles.[13] It synthesizes in a sequential step of conversion of angiotensinogen in the presence of renin to angiotensin I and then to angiotensin II. This angiotensin II then acts on angiotensin 1 (AT1) receptor subtype, stimulates it and causes vasoconstriction. It also promotes synthesis and secretion of aldosterone by stimulating the adrenal cortex thereby decreasing sodium excretion and increasing potassium excretion.[14] Irbesartan inhibits the action of angiotensin II and promotes vasodilatation, by selectively binding to AT1 receptor subtype and blocking it noncompetitively.[15] Irbesartan also antagonizes the effects of aldosterone. Irbesartan has been reported to be a peroxisome proliferator activated receptor (PPAR) alpha activating agent. PPAR alpha is a nuclear transcription receptor, which regulates the expression of genes involved in fatty acid oxidation and energy homeostasis.[16]

Irbesartan has a good safety profile with least or no adverse reactions.[17] Unlike angiotensin converting enzyme (ACE) inhibitors, the only complication seen with the use of angiotensin receptor blockers (ARB's) is mild angioedema but it is extremely rare.[18]

It is obvious from a large number of clinical trials that there is 30 percent decline in the risk of development of CVD by pharmacologically lowering serum LDL cholesterol.[19] Therefore, in addition to controlling high blood pressure in patients with cardiovascular disease, it becomes necessary to improve plasma lipid biochemistry.[20] This study explores the antihyperlipidemic property of irbesartan to cope with the two major risk factors of cardiovascular diseases.

Materials and Methods

This randomized controlled study was conducted in the animal house of National Institute of Health (NIH), Islamabad. Biochemical analysis of rabbit's serum was carried out in the department of chemical pathology, Army Medical College, Rawalpindi, from February 2013 to June 2013, after approval from the Ethics committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College.

Eighteen healthy adult domestic breed rabbits (Oryctolagus Cuniculus) having a weight of 1.5 to 2.0 kg were selected. They were of mixed breed both males and non-pregnant females. Animals under 1.5 years of age were not included in the experimental study. Standard laboratory conditions were maintained in animal house of National Institute of Health and rabbits were provided with controlled environment assuring twelve hours day and night cycle and an average temperature of 24°C. Rabbits were acclimatized for one week prior to the study.

Diet formula for animals used in the study was composed of cholesterol powder (1g/day) mixed in 1g/day of wheat bran along with routine diet of rabbits (gram whole,carrots, cucumbers, seasonal fruits) and was in strict compliance with the guidelines for the care of laboratory animals NIH Islamabad. Feeding of cholesterol powder and drugs was ensured by giving them mixed in small pellets of wheat bran after four hours fast before giving the routine diet.

Cholesterol powder one gram per day was added to the diet of the two experimental groups (group B and group C) excluding the rabbits of group A (Normal control group). All the rabbits were given tap water ad libitum for drinking.

The rabbits were randomly assigned into three groups of six animals each. The study period comprised of a total of twenty weeks after one week period for acclimatization. Animals were weighed prior to giving high cholesterol diet on day zero. Blood samples were taken on three different occasions as follows.

1. Baseline samples were collected on day zero, before starting the high cholesterol diet.
2. After 120 days of feeding on high cholesterol diet.
3. At the end of the study, on completing the treatment course for a period of 30 days.

The rabbits (n=6) in two of the experimental groups (group B and group C) were given high cholesterol diet followed by Irbesartan (40mg/kg) once daily according to the following schedule.

Group A (normal control; n=6) was the control group and received normal diet consisting of gram whole, wheat bran, green fodder, seasonal fruits and water

ad libitum for 150 days as it was normal control group and fed on normal cholesterol free diet for the whole study period. Group B (hyperlipidemic control; n=6) animals received cholesterol powder (1g/day) mixed in a diet comprising of grain whole, wheat bran, green fodder and seasonal fruits (cucumber, carrots and apples) for 120 days. Cholesterol powder was excluded from the diet for the next 30 days. Rabbits were also given tap water ad libitum for drinking.

Group C (hyperlipidemic+irbesartan; n=6) animals received the high cholesterol diet (1g/day) as per group B for 120 days and then fed on normal/routine diet without cholesterol along with Irbesartan (40mg/kg) once daily via gavage for a period of 30 days.

Fasting whole blood (4 ml) samples (n=6.0) were drawn from the tip of the ear of each animal with the help of a 5cc syringe. All the samples were transferred to separate plain clot activator tube and were let to clot at room temperature for at least 30 minutes. The samples were then centrifuged at 4500 rounds per minutes for 10 minutes. Serum was separated via an automatic micropipette for estimation of serum cholesterol, serum triglycerides and serum HDL-Cholesterol labelled accordingly in order to calculate serum LDL by using Friedwald's equation.[21]

[LDL Cholesterol mmol/L] = [Total Cholesterol] - [HDL Cholesterol] - [Triglyceride] / 2.20

The results of serum analysis of LDL-Cholesterol were established as means + standard error of mean. The difference between the two observations was derived using SPSS Version 20. The difference was taken as significant for a p value of 0.05.

Results

Serum LDL

The differences in means of LDL-Cholesterol values were calculated among normal control and hyperlipidemic control as well as among hyperlipidemic and treatment group.

Group A (normal control) showed unchanged levels of serum LDL when recorded on day zero, day 120 and day 150 i.e. 1.64±0.5, 1.64±1.1 mmol/L and 1.64±0.8 mmol/L as shown in table, p=NS.

The values of serum LDL of group B (hyperlipidemic control). Serum LDL levels on day 120 were increased significantly as compared to day zero, i.e. 3.45±1.1 mmol/L versus 1.61±1.1 mmol/L with p=0.0005. but remained unchanged on day 150 in comparison to day 120. When compared with normal control group A, a significant rise was observed in group B and group C on day 150.

Group C (Irbesartan), when compared with group B (hyperlipidemic control) to assess the post treatment reduction in serum LDL levels on day 150, a statistically remarkable decline was recorded, i.e. group C (Irbesartan) showed mean values of 1.29±0.2 mmol/L in comparison with group B (hyperlipidemic control) showing a mean of 3.46±0.3 mmol/L on day 150, p value for group C (Irbesartan) was 0.005 as shown in table I.

Table: Comparison of serum LDL levels among group A, group B and group C on day 0, 120 and 150 in rabbits (n=6)

No. of Days	Group A	Group B	Group C
Day 0	1.64±0.5	1.61±1.1	1.63±1.1
Day 120	1.64±1.1	3.45±1.1	3.46±0.3
Day 150	1.64±0.8	3.45±0.2	1.29±0.2
P value	0.005		

1n=6, Results are expressed as mean ± SEM (Standard c Error of Mean)

Discussion

In our study we found a highly significant reduction (28%) in serum LDL cholesterol with irbesartan. This favors the dual role of irbesartan, i.e. an anti hypertensive drug serving as an anti hyperlipidemic drug. Shimamura et al also demonstrated the similar results in rats as their experimental model.[22] This was also revealed by Derosa et al after conducting a 12 months clinical study on 188 patients with metabolic syndrome that irbesartan has the ability to significantly reduce serum LDL cholesterol levels compared with the baseline. This role of an antihypertensive drug renders it special in respect of serum LDL cholesterol from other drugs that are specific for the treatment of hypertension.

Unlike irbesartan, when a long term therapy is conducted for primary or secondary prevention of atherosclerotic complications, angiotensin converting enzyme inhibitors have no effect on serum LDL-C.[23] Other classes of antihypertensive drugs including beta blockers, diuretics and calcium channel blockers would rather increase the serum LDL cholesterol levels.[24] However irbesartan does not cause any undesired derangement in the plasma LDL cholesterol after long term use and this was also

demonstrated by Kirk, (1999), although blood pressure lowering efficacy is same as those of other antihypertensive drugs.[25]

Our study effects concerning serum LDL-Cholesterol strongly support the previous study performed on cholesterol fed rabbits.[26] In their study, they reported the lipid lowering effect of irbesartan and losartan. They concluded that irbesartan treatment is associated with significant reduction in plasma LDL levels compared to base line. An open multidrug comparison trial was carried out by Stella et al to study the variation in response of different ARB's on plasma lipid profile.[27] They demonstrated that angiotensin receptor blockers (ARB's) action on different indices of lipid profile are not same.[28,29,30]

This conflict with Rong et al and Shimamura et al could be a reflection of use of different species, as we had rabbits instead of rats while Shimamuraet al presented a clinical study. The other prospect may include the reason that animal model of Ronget al was genetically obese rats whereas we used high cholesterol diet induced hyperlipidemic rabbits. Duration of treatment could be another possibility of insignificant reduction of total cholesterol as we gave the treatment for 30 days whereas treatment period of Rong et al was 49 days.Shimamura et al gave significant results after 90 days treatment.

In the light of these findings it is clearly evident that irbesartan reduced the serum low density lipoprotein cholesterol (LDL-C) in high cholesterol diet fed rabbits. For this reason irbesartan administration can be helpful to decrease the risk of developing cardiovascular diseases because besides its antihypertensive property, irbesartan has an additional subsidy of lowering serum LDL-Cholesterol which is evident from this study. Further studies are warranted to explore the mechanism of lowering serum LDL by irbesartan in rabbits.

Conclusion

Irbesartan, an efficient antihypertensive drug, is highly effective in lowering serum LDL cholesterol. So when one has to deal with the risk factors, irbesartan with dual function, exhibiting both antihypertensive and antihyperlipidemic actions with least or no side effects can serve the humanity by treating initial hypertension as well as by reducing serum LDL-Cholesterol. As medication safety is a recognized indicator of quality of care, irbesartan can be considered to prevent and treat life threatening cardiovascular problems safely for long duration serving at the same time for correcting both hypertension and hyperlipidemia due to high serum LDL levels.

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ORIGINAL ARTICLE

Hepatoprotective Effect of Aqueous Extract of *Chichorium Intybus* Roots on Isoniazid Induced Hepatotoxicity

Amanat Ali[1], Adnan Jehangir[2], Farhana Ayub[3]

ABSTRACT

Objective: To determine the hepatoprotective effect of aqueous extract of *Chichorium intybus* roots in isoniazid induced hepatotoxicity in adult male mice.

Study Design: Experimental study.

Place and Duration of Study: Study was conducted from 15th of January to 15th of March 2015 at National Institute of Health Sciences (NIH) in collaboration with Riphah Institute of Pharmaceutical Sciences (RIPS).

Materials and Methods: Forty four Balb/c albino mice were divided randomly in to two groups, Group A (n=12) a control group and Group B (n=32) ,was given isoniazid 50mg/kg body weight orally once daily along with normal diet and water for 30 days to develop hepatotoxicity. Initially 2 mice from both groups were taken to check the ALT level on day 0. Isoniazid induced hepatotoxicity was confirmed by raised serum ALT levels in a mid-cycle sample of 10 mice from the Group B on day 30 mice (n= 10). After development of hepatotoxicity mice from Group B were further divided into two groups C and D. Group B1 (n=10) were given aqueous extract of *Chichorium intybus* roots at a dose of 200mg/kg/day and Group B2 (n = 10) at a dose of 400mg/kg/day orally for a duration of 30 days. On day 60 serum ALT of all the mice of Group B1, Group B2 was estimated to determine the hepatoprotective effect of aqueous extract of *Chichorium intybus* roots in Group C and D.

Results: Isoniazid produced severe hepatotoxicity as depicted by raised alanine aminotransferase (ALT) levels. ALT levels were decreased in Group B1 and B2.

Conclusion: Aqueous extract of *Chichorium intybus* roots has significant hepatoprotective effects.

Key Words: *Chichorium Intybus, Drug Induced Liver Injury (DILI), Hepatoprotoxicity, Isoniazid.*

Introduction

Across the globe most of cases of tuberculosis occur due to Mycobacterium tuberculosis.[1] Before the invention of antibiotics tuberculosis was a leading cause of death in both Eastern and Western nations.[2]According to the statistics Pakistan is ranked 4th amongst the multi-drug resistant cases of tuberculosis, approximately 3 million deaths per annum have been recorded with increased frequency of new cases.[3] All major drugs used for the treatment of tuberculosis i.e. isoniazid, rifampicin and pyrazinamide have hepatotoxic effects.[4] Drug induced liver injury (DILI) caused by the anti-tuberculous drugs varies from 2.0-28%.[5] Drug induced liver injury is clinically manifested by the raised liver enzymes. The most sensitive hepatic injury indicator alanine aminotransferase (ALT) level was measured to see the hepatotoxicity in all groups.[6] Isoniazid is the main antibiotic used for longer duration for the treatment of tuberculosis.[7] Acetyl hydrazine, a metabolite of isoniazid which on bio-activation leads to hepatotoxicity.[8] Plants have been a source of medicinal importance throughout the history.[9] *Chichorium intybus* commonly known as chicory, has been used as a medication in gastrointestinal and inflammatory diseases, whole plant has got valuable phytochemicals in it however roots contain essential components of therapeutic significance.[10] *Chichorium intybus* roots has got hepatoprotective,antioxidant[11,12] anti-inflammatory[13] antimicrobial[14,15] anti-hyperglycemic[16,17] immunostimulant[18,19] tumor inhibitory properties.[20] Traditional medicines and herbs have been used locally in the market and scientific study has not be explored to see the active

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principles and phytochemicals. Current research was aimed to see the active principle in the herb and to support it biochemically. Rationale was to explore the scientific evidence of the active ingredients helpful in preventing DILI in patients on antituberculous drugs. The objective of the present study was to explore the hepatoprotective effect of aqueous extract of *Chichorium intybus* roots in dose dependent manner on isoniazid induced hepatotoxicity.

Materials and Methods

An experimental randomized control study was carried out at Riphah Institute of Pharmaceutical Sciences (RIPS) and National Institute of Health Sciences (NIH), Islamabad. Forty four Balb/c male and healthy albino mice weighting 30-50 grams with normal ALT levels were taken for the study and were acclimatized for one week in the NIH animal house under standard facilities and were given normal diet and water ad libitum.

Initially, 44 mice were randomly divided in to two groups, Group A (n=12) which was given normal diet and tap water ad libitum, Group B (n=32) was given isoniazid 50mg/kg body weight orally once daily along with normal diet and water for 30 days to develop hepatotoxicity.[21,22] On day 0 blood samples of two mice from each group were taken through cardiac puncture. After 30 days mid cycle samples of 10 mice from Group B were taken, ALT levels were performed to see establishment of hepatotoxicity. After confirmation of hepatotoxicity mice from Group B were further divided in to two groups, Group B1 n=10 which was given aqueous extract of *Chichorium intybus* roots at a low dose of 200mg/kg/day[23] and Group B2 which was given aqueous extract of *Chichorium intybus* roots at a high dose of 400mg/kg/day [23] orally for a duration of 30 days. On termination day i.e. day 60th blood samples were taken from the both experimental Groups B1 and B2 for evaluation of ALT levels.

Chichorium intybus was identified by herbarium department, Quaid-e-Azam University, Islamabad. Aqueous extract of Chichorium intybus roots was prepared at RIPS, Islamabad by using fine homogenized powder of dried chicory roots which were mixed with distilled water, the whole solution was boiled for 2 hours and after cooling was sifted through filter paper. The aqueous extract was formed by using vacuum rotary evaporator and was frozen dried.[24]

Results were compiled and data was entered into SPSS 17 was used for statistical analysis. Tuckey's multiple comparison test to observe group mean differences. A p-value of <0.05 was considered as statistically significant.

Results

Serum ALT levels were significantly raised (p<0.01) in Group B treated with isoniazid as compared to Group A. *Chichorium intybus* roots extract significantly reduced (p<0.01) serum ALT level in Group B1 and Group B2 in comparison to Group B.

Table I: Tukey's multiple comparisons test between study Groups

Groups	Mean Difference	Significant	Summary
Group A vs Group B	-156.8	Yes	****
Group A vs Group B1	-59.47	Yes	**
Group A vs Group B2	-44.13	Yes	*
Group B vs Group B1	97.33	Yes	****
Group B vs Group B2	112.7	Yes	****
Group B1 vs Group B2	15.33	No	Ns

ANOVA summary
F 35.52
P value <0.0001
P value summary ****
Are differences among means statistically significant? (P <0.05) Yes

Discussion

In the present study mice were treated with isoniazid at 50mg/kg resulted with significant elevation in serum ALT levels. Group B1 and B2 received aqueous extract of *Chichorium intybus* roots resulted in significant improvement of ALT levels in a dose dependent manner. Our study in accordance with study carried out by El-Sayed et al in 2015 which showed antioxidant activity of *Chichorium intybus* in CCl4 induced hepatotoxicity.[14] similarly our study is in correlation with another study performed by Atta et al. showing hepatoprotective effect of *Chichorium intybus* extract when given with methanolic extract of Zinger Officinale.[25] Similar results have been

found in the study performed by Li et al. on hepatoprotective effect of *Chichorium intybus* in CCl4 induced hepatotoxicity in rat model.[26]

Previously studies have been done on exploring hepatoprotective effect of *Chichorium intybus* in combination with medical compounds like silymarin and other herbal compounds and extracts. No dose dependent study was done individually on aqueous extract of *Chichorium intybus* roots extract which guides us about the submaximal, ceiling effect and toxicity. Our study confirms the individual hepatoprotective effect of aqueous extract of *Chichorium intybus* roots.

Further studies are needed to determine molecular mechanism of inulin which is the major active principle of the *Chichorium intybus* roots. In addition a higher dose and different routes of administration can be tried to see the same effect.

Conclusion

Aqueous extract of *Chichorium intybus* roots have significant hepatoprotective effect on isoniazid induced hepatotoxicity.

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ORIGINAL ARTICLE

Knowledge, Attitude and Practices of Barbers about Hepatitis B&C Transmission in Islamabad

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ABSTRACT

Objective: To determine the knowledge, attitude and practices of barbers about hepatitis B&C transmission in Islamabad.

Study Design: A descriptive cross-sectional survey.

Place and Duration of Study: Study was conducted on barbers (street barbers and shop owner barbers) placed in different sectors of urban areas of Islamabad from September 2012 to March 2013.

Materials and Methods: A KAP survey (knowledge, Attitude and Practice Survey) was done. One hundred and twenty six barbers were selected through non-probability sampling technique. A pre-tested structured questionnaire was used to collect the data. The data was analysed through SPSS version 17. Descriptive statistical analysis was carried out in order to document frequencies and percentages.

Results: It was found that only 39% of the barbers had knowledge about different aspects of Hepatitis B&C (sterilization of instruments before using on next client ,mode of transmission, sign and symptoms, treatment, vaccination and which organ is effected most).

Conclusion: There is a huge gap in knowledge about hepatitis B&C transmission amongst barbers which highlights the importance of health education and different awareness raising campaigns to target this issue and bridge the gap.

Key Words: *Barbers, Hepatitis B, Hepatitis C, KAP Survey.*

Introduction

Developing world is facing a burden of epidemics of blood borne diseases. These diseases increase the morbidity and mortality, ultimately resulting in heavy burden on national economics and individual level.[1,2] Globally, each year around 2 billion people are infected with the hepatitis B virus (HBV), of which more than 350 million have chronic HBV infections.[1,2] An estimated, more than 180 million people worldwide are infected with hepatitis C virus (HCV) and 3–4 million are newly infected each year.[3] It is a blood borne infection transmitted by infected blood and blood products through transfusions, contaminated needles, vertical transmission, unsafe sex and reuse of razors by barbers.[4] The prevalence of Hepatitis B & C infection worldwide in general population is around 10–15%, and majority of the cases are seen in rural population as compared to the urban.[5,6] Unfortunately, Pakistan has a high prevalence rate of Hepatitis B and C with a constant rise in the number of cases.[7,8] Some of the major reasons for the constant rise in number of infected cases may be because of lack of health care facilities, low socio economic class, poor political commitment and most important among them is the lack of education and awareness about the transmission of these infectious diseases.[9]

There are many risk factors of hepatitis B&C. Any person who has an exposure and not been vaccinated can become infected. Risk factors include sharing infected needles in drug users, sharing razors/blades, transmission of infection from mother to her child, unprotected sexual contact with infected person, tattooing or body piercing, equipment not properly sterilized, transfusions of infected blood and individuals subjected to medical or dental interventions if equipment is not properly sterilized.[10] Mostly cases of Hepatitis remain undiagnosed, because of non-specific symptomatology. The common symptoms of

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hepatitis are loss of appetite, weakness, low grade fever, muscle aches or joint pains, vomiting and abdominal pain.[11]

The "Barber" profession has a very old history. The historical records of barbers indicate that they have important role in the community.[12] Barbers at that times were considered as the medicine men and the scholars of their religion, they belonged to the groups who offer their services for bloodletting, circumcision, extraction of teeth and different types of minor operations. With the development of health sector, their role has been limited to hair cutting and shaving only.[13,14]

Control of chronic diseases like hepatitis B&C are very difficult in countries where health facilities are expensive and not easily accessible, especially for low socio economic group. On average the cost of treatment for these diseases is beyond the affordability of an average earning citizen of Pakistan.[14]

Almost everyone is utilizing the barber' services in our society. This may be a potential source of infectious diseases like Hepatitis B & C. Taking into consideration this threat of viral infections particularly Hepatitis and AIDS, linked to this occupation; awareness amongst barbers holds a significant importance. Positive attitude and right practices of the barbers would significantly decrease the prevalence of these infections.[6,13] Main theme of the current study is to assess the knowledge about the role of barbers in spreading hepatitis B&C infection. The outcome of this current study will motivate the health educators, community developers and non-governmental organization to identify gaps and barriers in knowledge, attitude & practices of barbers with the impacts on disease transmission. The novelty of this research is the inclusion of street barbers who, so far neglected in different studies.

Materials and Methods

A descriptive KAP (knowledge, attitude and practice) survey was carried out among barbers in urban areas of Islamabad from 1st September, 2012 to 30th March 2013. The calculated sample size was 126 using WHO Sample Size Calculator by keeping 95% confidence interval, proportion (P) 9% and precision (d) 0.05 (5%) using non-probability purposive sampling technique. The data was collected by the researcher assisted by Capital Development Authority.

This study was carried out in different sectors of Islamabad. The barbers selected for this study were both shop owners and street barbers. The purpose of selecting these both types of barber was to represent the different socio-economic group of the community. The data was collected after informed consent from the barber's shops and street barbers and all those barbers /shop owners were excluded who did not give the consent.

The purpose and importance of the study was explained to each respondent and data confidentiality was assured. The data was collected using a structured closed ended questionnaire in Urdu language to avoid language barrier. At the completion of the questionnaire, health education session was given to barbers regarding their own protection as well as the protection of their customers. The data was analyzed using SPSS version 17.0.

Results

There were two types of barbers selected for the study, 104 (82.5%) of them were shop barbers whereas 22 (17.5) were street barbers working in green belt areas sitting at places adjacent to different markets. The mean age of the barbers was 34.4 years + 12.2. Out of the total 34 (27.0%) did not acquire any school education while 1 (.8%) can read and write, 43 (34.1%) got primary education, 33 (26.2%) middle, 13(10.3%) till matric and only 2(1.6%) were educated to intermediate and above. It was found that 50(39%) of barbers had knowledge of Hepatitis B & C as a major disease, 17(13.5%) had some knowledge and 59(46.8%) were totally unaware of the disease.

Discussion

The present study carried out to assess the knowledge of barbers regarding Hepatitis B & C and their current practices. The study revealed that there is a huge gap in knowledge about Hepatitis B & C among barbers, showing more than 85% were not aware of the signs and symptoms of this disease, and almost 80% did not know that they are vulnerable to the infections.[6]

The prevalence of HBV and HCV has been widely investigated in many occupational groups, but relatively few data are available on the prevalence in barbers who are involved in the transmission of these infections and are at elevated risk of exposure

Table I: Knowledge of Barbers about Hepatitis B & C

Knowledge items		Frequency	Percent (%)	p-value
Knowledge of disinfection of instruments after every customer	Yes	50	39.7	0.033
	No	17	13.5	
	Don't know	59	46.8	
Knowledge about spread of hepatitis B & C	Blood transfusion	2	1.6	0.012
	Sexual contact	2	1.6	
	Unclean blades	24	19	
	Unsterilized Surgical and Dental equipment	21	16.7	
	Don't know	77	61.1	
Knowledge about sign and symptoms of Hepatitis B & C	Headache	3	2.4	0.001
	Jaundice	4	3.2	
	General weakness	9	7.1	
	Don't know	107	87.3	
Which body organ is affected	Liver	17	13	0.001
	Don't know	109	87	
Knowledge about treatment	Yes	64	50.8	0.001
	No/Don't know	62	49.2	
Knowledge about vaccination for Hepatitis C	Yes	45	36	0.001
	No/ Don't know	81	64	

About 51% of respondents knew that Hepatitis B & C are treatable diseases and there main source of information was television.

Table II: Attitudes of Barbers about Hepatitis B & C

Attitude of Barbers	Yes (%)	No (%)
Screening against Hepatitis B & C	22 (17.4)	104 (82.5)
Vaccination status of barbers against Hepatitis B & C	9 (7.14)	117 (92.8)
Should we use new blades/razors for every customer	92 (73.0)	34 (26.9)
Registration of the barber shop	13 (10.3)	113 (89.6)

Table III: Practices of Barbers about Hepatitis B & C

Practices of Barbers	Yes (%)	No (%)	p-value
Hand washing	97 (76.9)	29 (23.0)	0.035
Use of disinfectants	41 (32.5)	85 (67.4)	0.002
Reuse of blades/razors	71 (56.3)	55 (43.6)	0.053
Use of antiseptics	84 (66.6)	42 (33.3)	0.014
Disposal method of blades	83 (65.8)	43 (34.1)	0.000

to blood borne pathogens. In our study, 17.5% of mobile barbers and 82.5% shop barbers had evidence of current or past HBV infection (all were unvaccinated against HBV), similar to a previous study in Rabat region of Morocco, in 2007, which showed that traditional barbers 1.9% and 1.7% respectively had active HBV and 1.1% of barbers and 1.3% of clients had chronic HCV.

In Casablanca region same type of study conducted in 2001 among barbers showed that they did not have the knowledge about the concept of infectious risk linked with blood, particularly of hepatitis B and C and HIV; majority of them had not been vaccinated. In this survey different tests were applied in which Syphilis serology found positive in 7%. Hepatitis B virus found positive in 2% and Hepatitis C virus found positive in 5% barbers.

In Islamabad and allied cities, a study was carried out by private university students which showed that barbers knowledge about Hepatitis B & C was very poor which is one of the key health issues of any developing country endorse the findings of current study.[12,13] The study found out that 13% of barbers had knowledge about Hepatitis B&C affects liver and transmitted through infected razors.

Some studies[14-15] showed that barbers who are relatively young had better knowledge about the hazards of using old blade which was not the case in our study. Similarly, in our study no relationship was seen between the working experience of the barbers and increased knowledge about hepatitis B & C.[16] The findings of current study obviously indicate that more efforts are required for the awareness regarding health issues for both barbers and their customers to decrease the prevalence of these infections. There are several studies showing that barber shops are important places for spreading of Infectious diseases like Hepatitis B&C and HIV/AIDS.[6] A study conducted in Italy showed strongest association with barber shop shaving for HBV and

with tattooing for HCV cases.[17] While researching on the knowledge and practices of barbers in India[16], it was found that large proportion of mobile barbers working in green belt areas are totally ignored. These mobile barbers are more unaware about the transmission of infectious diseases particularly through different instruments used by them. Similar findings were depicted in our study too, only 19% barbers were aware that infectious diseases spread through their instruments and 62% had no knowledge altogether. In a related study conducted in Italy noted that 90% of barbers washed hands after each customer handling, 66% did not change the towel after each customer. In addition, this study indicated that circumcision or minor surgeries also conducted by these barbers in rural areas.

A study conducted in Sivas region of Turkey "Prevalence of Hepatitis B and C virus infection" explained that Hepatitis B&C infection can be spread through razors, different type of barber's instruments and other related possessions, the findings consistent with the current research.

It is important to realize that more efforts are required to raise awareness amongst barbers by launching different types of awareness programs. For better planning and implementation of adequate health promotion and intervention measures for controlling hepatitis B, it is important for both health care providers and policy makers to know the real burden of chronic Hepatitis B in region and population specific groups.[18] The health officials and authorities must develop appropriate strategies and monitor systems to restrict the barbers not fulfilling the pre-requisites for registration. Large scale efforts and resources are considered necessary to meet these goals. This information is comparable to our study in order to complement the conclusion and recommendations drawn from this study.

Conclusion

The current study highlighted the gap between knowledge & practices of barbers regarding HBV & HCV working in various sectors of Islamabad. Majority of barbers did not know the extent of risk to themselves and their customers are facing due to their lack of awareness about infectious disease spread through their casual attitude and practices. Various awareness raising programs and campaigns are required involving mass media at national level. The competent authorities must be involved through which all barbers of Islamabad region should be registered, properly vaccinated and tested for Hepatitis B & C on annual basis cost-effectively.

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ORIGINAL ARTICLE

Point Mutation in Factor V Leiden G1691A and Factor II G20210A and Effect on Coagulation Profile and Frequency of Recurrent Spontaneous Abortions among Sudanese Women

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ABSTRACT

Objective: To investigate the effect of point mutation in FV Leiden G1691A and FII G20210A gene on coagulation and recurrent spontaneous abortion (RSA) among Sudanese women.

Study Design: This was retrospective case control study.

Place and Duration of Study: The study was carried out from Aug 2012 to Dec 2014 at Omdurman Maternal Hospital, Sudan.

Materials and Methods: The study included hundred pregnant females with a history of recurrent spontaneous abortion as (case group) and ninety five healthy reproductive Sudanese women as (control group). The data was collected with the help of structured questionnaire and direct interview to collect information. Identification of point mutation in factor V Leiden G1691A and factor II G20210A gene by polymerase chain reaction was performed; Coagulometer was used for the measurement of PT and APTT. Odds Ratio and the 95% confidence interval (95%CI) were calculated for the presence of mutation between cases and controls and analyzed by SPSS program, version 17.0.

Results: The Heterozygous alleles G/A in factor V gene was 8.0% in all cases related with three, four and five times of recurrent abortion and 6% was found in control group. Heterozygous alleles of factor II G/A Prothrombin time (PT) and partial thromboplastin time (PTT) in women with Recusant Spontaneous Abortion (RSA) were not affected significantly (P=0. 93 and P=0.69).

Conclusion: Based upon the results it is concluded that the point mutation in factor V Leiden G1691A and factor II G20210A might play a role in recurrent spontaneous abortion loss among Sudanese women. However these point mutations do not affect the coagulation profile.

Key Words: *Factor V Leiden G1691A, Factor II G20210A, RSA, Sudanese Pregnant Women.*

Introduction

Recurrent pregnancy loss (RPL) is defined as three or more consecutive pregnancy losses before the 24th week of gestation .The modern definition, however, is the spontaneous loss of 2 or more consecutive pregnancies before 20 weeks of gestation.[1] Recurrent pregnancy loss is an experience which can be very painful for the couple. Most of the miscarriages occur in the first trimester and affect about 15% of all recognized pregnancies.[2] RPL has many possible causes that can be categorized as genetic abnormalities, hormonal and metabolic disorders, uterine anatomic abnormalities, infectious causes, immune disorders and thrombophilic disorders.[3] Hereditary thrombophilias are a group of genetic disorders of blood coagulation resulting in a hypercoagulable state, which in turn can result in abnormal placentation. Early in pregnancy this may manifest as spontaneous loss.[4] Factor V Leiden (FVL) and prothrombin gene (G20210A) mutations are the most common types of hereditary thrombophilias. Most carriers of this mutation do not develop any clinical signs and remain undiagnosed because these conditions result

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in a small absolute risk of clinically significant thrombosis.[5] Factor V Leiden is a single point mutation involving a guanine to adenine transition at position 1691 in exon 10 of the factor V gene, which leads to the synthesis of a variant factor V molecule.[6] The prothrombin G20210A mutation involves guanine to adenine substitution at nucleotide 20210 of the prothrombin gene.[7] FV Leiden and factor II G20210A mutations are associated with increased production of thrombin and risk of venous thrombosis.[8] Also Factor V Leiden mutation is found to be the most common inherited thrombotic risk factor associated with RPL its frequency in whites varies from 3% to 8% and 1 in 1000 are homozygous. It is rare in African Americans, Asians and Native Americans.[9] The incidence of genetic prothrombotic mutations in women with unexplained pregnancy loss was examined in various studies: some of these studies supported the association.[10,11] While others reported no association.[12,13] The present retrospective case control study was conducted to evaluate the FV Leiden G1691A and FII G20210A mutations and their affect on some coagulation profiles (PT and PTT) among women with a history of three or more consecutive pregnancy losses and healthy controls. This is the first study that investigated FV Leiden G1691A and FII G20210A alleles and genotype distributions in the Sudanese females with habitual RPL.

Materials and Methods

This was retrospective case control study. The genomic DNA samples of one hundred and ninety five Sudanese women who recruited and followed at Omdurman Maternal Hospital were screened from Aug 2012 to Dec 2014. One hundred cases having a history of RPL were compared with ninety five healthy reproductive Sudanese women as control group with a history of two or more successful live birth. Cases and controls were tested for the FV Leiden G1691A and FII G20210A. Genomic DNA was extracted from 3–5 ml of EDTA anti-coagulated blood by salting.[14] DNA was extracted from the blood samples using Master pure DNA purification kit for blood GF-1 Blood DNA Extraction Kit, 50 PREPS (cat. No. GF-BD-050, Vivantis Technologies Sdn. Bhd., Malaysia). FV Leiden G1691A and FII. a 345-bp genomic DNA fragment encompassing a part of the prothrombin gene that contains the mutation was amplified by PCR using specific primers Forward (5'TCT AGA AAC AGT TGC CTG GC-3') and Reverse primer (5'ATA GCA CTG GGA GCA TTG AAG C-3). And 267-basepair (bp) segment of the factor V gene was amplified used specific forward primer (5'TCA GGC AGG AAC AAC ACC AT-3') and reverse primer 5'GGT TAC TTC AAG GAC AAA ATA CCT GTA AAG CT3. The reaction program was as follows: Denaturation at 94°C for 30 seconds, annealing at 51°C for 30 seconds, extension at 72°C for 30 seconds for 35 cycles and 72 °C for 5 minute.[15] A master mix was prepared by adding Nuclease free water,10x buffer,dNTP,tow primers,Mgcl2,Taq DNA polymerase and DNA, the mixture was loaded into thermocycler according to the specific Temperature profile. The working solution of 1X TBE is prepared from the stock solution (1 L) which contains the following: 89 mM Tris base (108 gm), 89 mM boric acid (55 gm) 40 ml of 0.5M EDTA, adjust pH to 8.0.1.5% agarose was prepared from 1x TBE, and 5µl PCR products was loaded by mixing PCR products with 1µl loading dye, run on the gel for 30 mins and visualized on UV transllimantor. Factor V Digested with 10 µl of DNA restriction enzyme MnI1 at 37°Cfor 18 h, subjected to 2% low melting point agarose and Prothrombin product (10 µL) was digested with 20 U of Hind III, at 37°C for 16 h, and loaded into 2% low melting point agarose gel, eletropherosed at 90 volts for 60 mins .

Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages where appropriate. Odds Ratio (OR) and the 95% confidence interval (95%CI) were calculated for the presence of mutation between cases and controls and analyzed by SPSS prograrmme (version: 17.0). Data were analyzed using the Chi-square test to compareson the prevalence of MTHFR mutation between patients and controls (The test considered significant when P.value <0.05).

Ethical consent was obtained from ethical committee at Hospital of Omdurman Maternity Hospital (Sudan).

Results

The participants included 195 women subjects. Out of them, 100 had a history of 3 or more events of recurrent fetal loss (abortion, miscarriage or still birth). Their mean age± SD was 25 ± 4. Ninety five

women were healthy the mean age of was 30 ± 4. The prothrombin time PT (p=0.93) and PTT (p=0.69) were normal among all women with RPL and controls. Factor V Leiden mutation distribution showed higher prevalence among study participant with RPL as compared to control group. The mutation was detected in 8 out of 100 (8.0%) and 6 out of 94 controls (6.4%). P- Value =0.66, Odds Ratio=1.28, 95% CI (0.42 to 3.84) The prevalence of heterozygous FVL mutation in recurrent miscarrige women was found to be 8 % but in control it found to be 6.4%. Mutant allele (A) was seen only in 4 % of the cases. Frequency of mutant allele (A) was 3.2 % and G allele occurred with a frequency of 96.8 % among controls. These results are statistically insignificant between the cases and controls group.

Prevalence of the Prothrombin gene was 3% among cases with P- Value =0.091.but no mutant gene was detected among control group. According to the genotyping in cases showed (Heterozygotes, 3.0%; Homozygotes, 97.0%), Alleles G (98.5%) and Alleles A (1.5%) while in controls group show normal homozygous G/G (100%) and Alleles G (Alleles G). No significant association between cases carriage any of this mutation and risk with recurrent pregnancy miscarriage (Table II). The cases group was divided into subgroups based on time of recurrent abortion from second to eight times of repeated miscarriage. Our data indicates that factor V gene mutation was most frequent in women with recurrent miscarriage. Prothrombin mutation was found only among women with three time recurrent miscarriages with 100% and MTHFR present in three, four and five times of recurrent miscarriage women with equal percentage 33.3% for each (Table III).

Table I: Frequency of factor V (Leiden) mutation among cases of recurrent pregnancy loss compared to controls

Genotype	Patients N (%)	ControlsN (%)	P-value	OR (95%CI)
Heterozygous G/A	8(8.0)	6(6.4)	0.66	1.28(0.42 to 3.84)
Normal homozygous G/G	92(92.0)	88(93.6)		
Alleles G	192(96.0)	182(96.8)	0.67	0.76(0.27 to 2.33)
Alleles A	8(4.0)	6(3.2)		

Table II: Frequency of Prothrombin mutation among cases of recurrent pregnancy loss compared to controls

Genotype	Patients N (%)	Controls N (%)	P-value	OR (95%CI)
Heterozygous G/A	3(3.0)	0	0.091	0
Normal homozygous G/G	97(97.0)	94(100)		
Alleles G	194(98.5)	188(100)	0.089	0
Alleles A	3(1.5)	0		

Table III: Frequency of factor V (Leiden) and Prothrombin related to times of recurrent pregnancy loss

Times of RPL	Factor V		Prothrombin	
	Positive	Negative	Positive	Negative
Twice	0	8(8.8)	0	8(8.3)
Three times	3(37.5)	57(62.6)	3(100)	57(59.4)
Four times	4(50.0)	16(17.6)	0	20(20.8)
Five times	1(12.5)	6(6.6)	0	7(7.3)
Six times	0	1(1.1)	0	1(1.0)
Seven times	0	1(1.1)	0	1(1.0)
Eight times	0	2(2.2)	0	2(2.1)

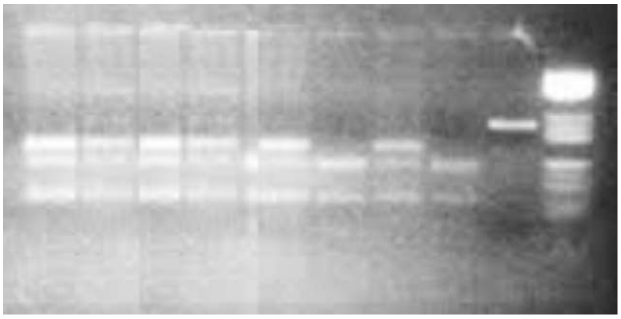

Fig 1: PCR amplification of FVL gene mutation

Digestion of factor v gene with MnI1 enzyme on 2% agarose gel disolved in 1X TBE buffer, stanied with ethidium bromide , Lane 1 molecular weight marker 50 bp, lane2 undigested PCR products lane 3 and 5 were hetrozygous mutant (AG), Lane 4,6,7 and 8 ,9 and 10 were Wild typ (AA), The 267 bp DNA products digested with MnI1.

Fig 2: PCR amplification of Prothrombin gene mutation

Digestion of prothrombin gene with Hind III on 2% agarose gel disolved in 1X TBE buffer, stanied with ethidium bromide , Lane 1 molecular weight marker 100 bp, lane 2 (322 bp) , mutant(AA), control ,lane 3 and 5 were hetrozygous mutant (GA), Lane 4,6 and 7 were Wild type (GG), lane 8 undigested(345 bp).

Discussion

One hundred Sudanese women suffering from RPL as compared to ninety five healthy women. Because inherited thrombophilia has been implicated as a possible cause of RPL.[16] Gene defects frequently associated with RPL were prothrombin A20210G and factor V Leiden reported in many studies.[17] Due to their important roles in the coagulation pathway, this study was conducted to investigate the association between genetic polymorphisms of Factor V and Factor II G20210A among women experiencing RPL. The frequency of polymorphic A allele was more prevalent in RPL patients (8%) than in controls (6.4%) and the G allele was less prevalent in RPL patients (98%) than in controls (100%).The prothrombin G20210A mutation our result revealed that the mutation not common among recurrent spontaneous aborted Sudanese women they were found Heterozygous G/A alleles with frequency 3% and did not found any mutated gene among control group. The frequency of polymorphic A allele was prevalent in RPL patients (1.5%) and the G allele was less prevalent in RPL patients (98.5%) than in controls (100%). our finding was consistence with Altintas et al, 2007[18] , Freire et al[19], Sottilotta et al [20] and Dalmaz et al[21] but it was inconsistent with Mello et al[22], Behjati et al[23] , and Bagheri et al.[24] Prothrombin time (PT) and partial thromboplastin time (PTT) in women with RPL in this study were not affected significantly (P=0. 93 and P=0.69) respectively this is similar to the normal results reported by Ghulam, et.al., (2014) among Sixty three pakistanian women with history of three spontaneous abortions in their first three months of pregnancy.[25] Also our finding in PT and PTT were consistence with Salamat et al[26] and Shahida et al. [27] The normal result of PT and PTT in women with V Leiden G1691A, factor II G20210A because the patient with these mutations makes fibrin at same rate as a person with normal factor V. It's just that later on, when the body tries to turn factor V off, the factor V Leiden patient will keep on making fibrin. In future sample should be increased and study may extent to other provinces in Sudan to cover up more races and tribes and make the study more accurate and precised.

Conclusion

In our study we found that the Polymorphism in V Leiden G1691A and FII G20210A mutation do not increase risk for RPL in tested population and there are no any affect of these mutations in the prothrombin time (PT) and partial thromboplastin time (PTT).

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ORIGINAL ARTICLE

Effect of Zinc on Salt Induced Impaired Remodeling in Long Bones of Rats

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ABSTRACT

Objective: To determine the effect of zinc on salt induced bone damage in rats.

Study Design: Laboratory based randomized control trial.

Place and Duration of Study: The Anatomy department of Islamic International Medical College, Rawalpindi, hosted the conduction of research with the cooperation of National Institute of Health, Islamabad. The study commenced on 17th September 2015 and completed on 17th March 2016.

Materials and Methods: Forty five female Sprague Dawley rats, 10-12 weeks old were used in the study. The animals were randomly divided into 3 groups. The rats in experimental group A fed on high salt diet (8%NaCl) whereas animals in experimental group B were given high salt diet supplemented with zinc (50mg/kg/day) for eight weeks however, the diet of control group was not tempered with. Blood samples were drawn at the start of intervention through tail vein and at the end of the experiment by intracardiac puncture for hormonal assay. All rats were dissected, left humeri and femora were removed, decalcified and five micrometer (µm) sections were obtained after tissue processing. Tissues were stained with Haematoxylin and eosin (H&E) for histological parameters. The quantitative data was analyzed by using Statistical Package for Social Sciences (SPSS) version 21 and was expressed as Mean + S.D.

One Way Analysis of Variance (ANOVA) followed by Post hoc tukey test was applied for inter group comparison of parameters. T-test was applied for intragroup comparison of values. Result having p-value <0.05 was considered statistically significant.

Results: Marked histological changes were identified in the experimental groups. These changes were of greater severity in high salt diet group as compared to the zinc supplemented group in which reverse beneficial effects were observed. Fall in serum calcium and alkaline phosphatase levels were deemed substantial in group A with respect to group B.

Conclusion: Zinc has a Protective role against High salt exposed diet induced damage on the histomorphology of bone tissue.

Key Words: *Cortical Bone, Hypercalciuria, Osteoblast, Salt, Zinc.*

Introduction

The world is under continuous threat of increase diet-related non-communicable ailments.[1] Unbalanced and excessive salt intake is often closely associated with development of hypertension and other cardiovascular diseases.[2] However, awareness regarding relationship of zinc to sodium induced osteoporosis is still in a gray area. Despite of previous researches, precise associations of the trace elements with bone health are not clear as yet. Inverse of negative balance between bone formation and resorption has been evaluated with the help of trace elements.[3]

Low bone mass is a silent epidemic of the 21st century and figures are set to increase worldwide. Considering the elements which affect bone metabolism is of utmost importance for the prevention of osteoporosis. Although nutrition is an important determinant of bone health, but the effects of the micronutrients is little understood.[4]

Bone is a systematized tissue which acclimates and changes according to certain factors and its organization varies due to diverse functional requirements.[5] The net result of unaltered healthy bone mass is sustained by a balanced bone formation and resorption activity.[6] Imbalance results in a progressive metabolic ailment called osteoporosis,[7] becoming a public health problem,[8]

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upsetting 200 million people worldwide.[9] Characterized by lessened structural integrity and proneness to fractures, it is more prevailing than myocardial infarct, breast cancer and stroke[10] It is imperative to explore and develop nutritional strategies for osteoporosis prevention as the life threatening outcomes and increase in annual cost associated with disease morbidity requires a quick fix.[11]

Salt being most ubiquitous of food flavorings[12] and a known risk factor for osteoporosis,[13] imposes hazards on human wellbeing. High urinary excretion of calcium with increase salt intake leads to impaired bone health.[14]

Human population has exceeded the daily limit of 2000 mg of Na /day as recommended by WHO.[15] Different communities have different intakes (Western 2300-4300 mg Na/day , Asian 5300mg-6000mg of Na/ day)[16] Sodium in this range is adversely affecting people including osteoporosis, hypertension , increase urinary tract stones and stroke.[17]

It took 75 years to realize that zinc is a crucial trace element[18] although it has been used therapeutically in Ayurveda but its nutritional significance in public health was recognized recently.[19] As it is a vital element[20] and human body contains only 2-3 grams. even a small deficiency is a disaster.[21] Zinc can be a hidden link for the prevention of osteoporosis due to its regulatory role in bone metabolism.[22] It has the ability to stimulate the differentiation and proliferation of osteoblasts and inhibiting osteoclast like cells formation from bone marrow[22] Zinc ,by stimulating apoptotic cell death of mature osteoclasts can inhibit bone resorption and have direct positive effect on bone metabolism.[23] Other than bones which act as a zinc sink zinc is stored in muscles and skin.[24] So free available quantity is negligible and only food source can be utilized when required[25] to prevent conditions like bone loss, gastric ulcers[26] night blindness.[27]

Therefore, this experimental study will highlight the potential benefits of Zn supplementation in reducing bone loss more accurately and eventually will give desired awareness to masses regarding positive link between zinc and bone health.

Materials and Methods

The study was a laboratory based randomized control trial carried out in the Anatomy department of Islamic International Medical College Rawalpindi. It was initiated after the approval of the Ethical Review Committee. The research was carried out with the collaboration of National Institute of Health (NIH) Islamabad and Army Medical College. It took six months to complete this study. Inclusion criteria were forty five, 12 weeks old, adult female Sprague Dawley rats weighing 250-300g. Pregnancy, male rats and any evident pathology were also considered as exclusion factors.

Forty five rats grouped by using random number table method, selected by non-probability convenient sampling, were randomly divided in to three groups (15 animals in each group) and were allowed to adjust in well aired new environment in a temperature range of 20-26°C. The rats in group A (N=15) were given diet having 8% NaCl[28] for eight weeks. Rats in group B (N=15) were given high salt diet supplemented with zinc at a dose of 50mg/kg body weight.[29] The rats of group C (N=15) served as controls, they were given standard laboratory diet. Water was provided ad libitum. The dose of NaCl and Zinc was set based on previous studies.

Dissection was done after eight weeks. Blood was drawn through intracardiac puncture for assessing serum calcium and alkaline phosphatase (ALP) level at the end of intervention. The left humeri and femora of rats were removed and immediately fixed in 10% neutral buffered formaldehyde for 2 days. Decalcification was performed using aqueous solution of 5-10% nitric acid for 24-48 hours. Transverse sections from the mid diaphysis were obtained, processed and embedded in paraffin wax to form blocks. Five µm[30] thick sections were obtained by mounting blocks on rotary microtome. Haematoxylin and eosin was used for histological study of specimen.

Cortical bone thickness of diaphysis of humeri and femora was measured with the help of ocular micrometer. The thickness of cortical bone was measured by counting the number of divisions of eye piece of linear ocular micrometer, placed perpendicularly from underneath the periosteum to endosteum. Cortical bone width of opposite side was measured in a same manner per section under 4X objective and results were averaged.

Parametric data was analyzed by using Statistical

Package for Social Sciences (SPSS) version 21. Quantitative data was expressed as Mean + S.D. One Way Analysis of Variance (ANOVA) followed by Post hoc tukey test was applied for inter group comparison of parameters.t-test was applied for intra group comparison of values. Result having p-value <0.05 was considered statistically significant.

Results

Mean thickness of the humeral cortical bone was 53.766±9.066 µm in control group C, 53.666±7.596µm in experimental group B and lowest of all, 41.8000±15.254 µm in experimental group A. The results were significant (p<0.05) amongst different groups (Table I) (Fig 2, 3).

The difference between group C and A was 11.966 µm, being highly significant (p=0.014).The result between group C and B was insignificant (p=1.000) with difference of 0.100µm. The mean cortical thickness of group B was greater than group A with difference of -11.866µm (p<0.05) (Table II) (Fig 1).

Mean thickness of the femoral cortical bone was 44.600±8.437µm in control group C, 39.366±10.677µm in experimental group B and lowest of all, 30.433±9.350µm in experimental group A. The results of difference in cortical bone thickness were significant between groups (p<0.05).

The difference between group C and A was 14.166 µm, the result was highly significant (p=0.001).The insignificant difference of 5.233µm (p=0.300) was recorded between group C and B. The mean of thickness was greater in group B than group A difference being -8.933µm (p=0.036).

Mean random initial and final serum calcium was 8.680±0.90333 mg/dl and 8.5333±0.9559mg/dl in control group C, 7.826±0.6123 mg/dl and 7.153±1.364mg/dl in experimental group A and 8.666±0.952 mg/dl and 8.816±0.9635mg/dl in experimental group B Initial calcium levels revealed p-value of 0.010 whereas the final levels were different in all groups (p=0.004).The mean difference between initial and final value in Control group C was 1.3800, 0.3466 in experimental group A and -1.0333 in experimental group B. Decrease in calcium level was highly significant between experimental group C and A

(p=0.004), insignificant (p=0.672) between group C and B and there is significant result between group A and group B (p=0.038) (Table IV).

Initial and final mean serum alkaline phosphatase level was 487.800±51.669 U/L and 478.066±53.620 U/L in control group C, 466.200±45.874U/L and 349.9333±56.0484 U/L in experimental group A and 486.066±47.373 U/L and 416.666±62.009 U/L in experimental group B (Table III) (Fig 5).

Initial Serum alkaline phosphatase showed inconsequential value in all the groups (p=0.405) whereas the final levels were significant (p=0.000) The mean of difference between initial and final value in Control group C was 9.7333 U/L,116.2666 U/L in experimental group A and 69.4000 U/L in experimental group B.

Comparison among groups demonstrated the highest decrease in alkaline phosphatase level between group C and A being 109.2000 U/L with significant value (p=0.000).The mean of decrease between group C and group B was 42.4666 U/L (p=0.021) which was less group A and B -66.7333 U/L (p=0.038

Table I: Multiple comparison of cortical bone thickness among all groups of Humerus and Femur by Post Hoc Tukey test

	Humerus			Femur		
Groups	C	A	B	C	A	B
Mean value	53.766	41.800	53.666	44.600	30.433	39.366
Std. Deviation	9.066	15.254	7.596	8.437	9.350	10.677
SEM	2.341	3.938	1.961	2.178	2.414	2.757
p-value	0.006*			0.001*		

Table II: Mean Cortical Bone thickness in Humerus and femur (µm) of all groups

	Humerus			Femur		
Groups	C vs. A	C vs. B	A vs. B	C vs. A	C vs. B	A vs. B
Mean Difference	11.966	0.100	-11.866	14.166	5.233	-8.933
p-value	0.014*	1.000	0.015*	0.001*	0.300	0.036*

*p<0.05

Discussion

Bone acclimates and changes under the influence of certain elements and its organization varies due to diverse functional requirements.[5] The healthy bone mass is sustained by a balanced between bone

Fig 1 : Cross-section of Humerus diaphysis of A13 showing decreased cortical bone thickness (CBT). H&E, X4.

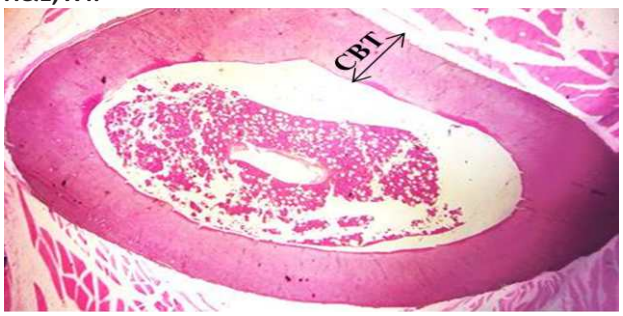

Fig 2: Cross-section of Humerus diaphysis of B7 showing increased cortical bone thickness (CBT). H&E, X4.

Table III: Initial-final serum calcium (mg/dl) and Alkaline Phosphatase (U/L) level of all groups

Parameter	Group	Initial level	Final level	Std. Deviation	*p*-value
	Group A	7.8267± .61233	7.1533± 1.3642	0.9346	.000*
	Group B	8.6667± .95219	8.1867± .96353	1.6249	.l02
	Group C	8.680± 0.903	8.533± 0.9553	0.2587	0.000*
	p-value	0.010*	0.004*	-	-
	Group A	466.20000± 45.8743	349.93333± 56.04847	74.5419	.833
	Group B	486.0667± 47.3730	416.6667± 62.0099	58.11190	.083
	Group C	487.800± 51.6695	478.0667± 53.6209	22.6604	0.000*
	p-value	0.405	0.000*	-	-

*p < 0.05

Table IV: Multiple comparison of final calcium (mg/dl) and Alkaline Phosphatase (U/L) level

Parameter	Groups	Mean Difference	*p*-value
Serum Calcium (mg/dl)	Group C vs. Group A	1.3800	.004*
	Group C vs. Group B	0.3466	.672
	Group A vs. Group B	-1.0333	.038*
Serum Alkaline phosphatase (U/L)	Group C vs. Group A	109.20000	.000*
	Group C vs. Group B	42.46667	0.021*
	Group A vs. Group B	-66.73333	.038*

*p < 0.05

formation and resorption activity.[6] Life style, genetic and dietary factors have impact on its prevalence. Although dietary factors have limited influence but are nonetheless crucial because they modulate the achievement of maximum peak bone mass and subsequent better bone health. By developing nutritional strategies for osteoporosis prevention, the annual cost and debilitation associated with its morbidity can be lessened.

The present study focused on determining the beneficial effects of zinc on high salt diet induced bone damage in long bones of rats by observing microscopic quantitative and biochemical parameters. The results suggested that zinc supplementation can prevent the high salt induced deleterious effects on bones.

ALP is a marker enzyme of osteoblast activity[31] reevaluated by Ahmed[32] who documented the decrease in calcium, ALP levels and subsequent impaired bone integrity after salt loaded diet. Decrease in the osteoblast activity due to salt overload can be the reason of low ALP levels. Furthermore, decline in the ALP activity has been demonstrated in animal models of experimental induced osteoporosis.[33] As > 99% of Na and 95% of the calcium are reabsorbed in the kidneys, it is speculated that impaired renal function may be responsible for Na induced calciuria and temporarily depress calcium levels.[14] Substandard kidney function also causes hypophosphatemia and fall in 1, 25 (OH) 2 D3. All these events lead to less intestinal absorption of calcium as well as decrease availability to bones.[16] Reduction in the biomarkers of bone formation (ALP) and significant increase in the biomarkers of bone resorption has been observed due to high PTH secretion secondary to low calcium levels and consequently increase in bone remodeling.[16] In line with other publications, Creedon[34] also observed the decrease in calcium levels due to sodium induced increase urinary excretion of calcium. As a compensatory mechanism, the PTH secretion increases which causes calcium mobilization from bones at the expense of bone loss.[15]

As Zn is a cofactor of ALP[22] which is an enzyme expressed by osteoblasts close to the blood vessels and is a valuable index for bone tissue development. Administration of zinc results in increase of enzyme

activity indicating enhance osteoblastic activity.[35] Increase in levels of Calcium and ALP with significant difference ($p<0.05$) in the present study is also validated by Otsuka[36] who observed increase in levels after measured zinc discharge on bone mineral density from injectable Zn-containing B-Tricalcium Phosphate. It could be attributed to intensified differentiation of osteoblastic cells to raise ALP activity.[37] Zinc plays an important role in preventing osteoporosis by stimulating bone formation, reported by Ma[31] by demonstrating increase in calcium and ALP in the femoral-diaphyseal and metaphyseal tissues. Decrease in calcium content by bone resorbing factors can be prevented by zinc supplementation.[19] Our outcome is in agreement with above results, further firming up my research.

Cross section of long bones reveals four different bone types: periosteum, cortical bone, endosteum and cancellous bone. Femur diaphysis is mainly composed of compact bone[38] and cancellous bone forms a very thin layer on the inner aspect of diaphysis of long bones.[5] The cortical bone thickness is an important parameter to evaluate bone quality and strength[39] so in the present study the bone damage is assessed by measuring the cortical bone thickness in cross sections of mid diaphysis. It is revealed that humerus and femur of control group has maximum thickness of 50.7um and 44.6um respectively followed by experimental group B who took salt and zinc supplementation whereas the lowest dimensions are found in experimental group A fed on high salt diet.

My results are in harmony with the work of Ahmed[32] who observed decline in the thickness of cortical bone of rats. He anticipated that high salt intake can be related with increased plasma levels of creatinine, urea, phosphate and potassium due to deranged kidney function which finally led to bone changes. Furthermore increased serum phosphate inhibits 1αhydroxylase and produced fall in 1, 25(OH) 2 D3. As a result intestinal absorption of calcium is decreased with subsequent increase in PTH secretion leading to increase osteoclastic activity. Degenerative changes in osteoblasts, osteocytes and hyperactivity of osteoclasts results in inaccurate bone remodeling with decrease in cortical bone thickness.[40] Changes in bone remodeling which is mediated by bone cells, increased osteoclastic activity and multiple resorption cavities can be the reason of decrease in the thickness of cortical bone.[41] My result is in conformity with the results of all above periodicals sharing a common point that salt intake results in osteoporosis with decrease in cortical bone thickness.

Increase in cortical bone thickness after zinc supplementation in experimental group B is documented in the present study. As many published studies has confirmed that zinc has positive role in improving bone health , it is further strengthened by Brzoska[22] who reported the shielding effect of zinc diet on bone homeostasis. He postulated that increase in the bone alkaline phosphatase activity may be due to zinc adequacy. Increase in the osteocalcin level produced by osteoblasts after zinc supplemented diet might have resulted in increase in cortical bone thickness.[42] Zinc is required for growth of osteoblasts and zinc showed decreased was bone resorption.[43]

Conclusion

This research indicates that zinc supplementation can be considered an appropriate dietary strategy to reduce risk of osteoporosis. Cortical bone thickness, alkaline phosphatase activity and calcium levels were considerably increased after zinc administration showing that zinc has protective role against high salt induced impaired remodeling in long bones of rats.

Recommendations

Effects of high salt diet can be studied for longer period of time to assess significant gross changes in long bones of rats. Effects of highs salt and zinc can be observed on the osteocytes apoptosis to evaluate their role in development and prevention of osteoporosis. Comparison of high salt diet induced effects can be studied between male and female rats to assess the difference in the degree of damage.

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ORIGINAL ARTICLE

Growth Failure in β-Thalassemia major Patients Undergoing Repeated Transfusions

Shazia Ali[1], Sarwat Jahan[2]

ABSTRACT

Objective: To determine the effects of iron overload on Height, Body Mass Index (BMI), Hemoglobin and Serum Ferritin levels in beta thalassemia major patients undergoing regular blood transfusion.

Study Design: Case control study.

Place and Duration of Study: It was carried out at Quaid-e-Azam University, Islamabad in collaboration with Jamila Sultana Foundation Rawalpindi, Thalassemia House Rawalpindi and Pakistan Institute of Medical Sciences (PIMS), Islamabad from 5th January 2010 to 5th December 2014.

Materials and Methods: Total 300 individuals were included in the study out of which 200 were Beta thalassemia major patients and 100 were controlled matched for age and gender with the thalassemic group. They were further divided into 4 groups of <13 years female, ⩾13 years female, <13 years male and ⩾13 years male (each having 50 thalassemic and 25 control). Height, BMI, Hemoglobin and serum Ferritin levels were determined. Non parametric (Spearman) co-relation co efficient was used to find the correlation between BMI and Ferritin and Hb levels. Data was analyzed through Graph Pad Prism 5.01. P<0.05 was considered statistically significant.

Results: All groups had reduced Height, BMI, Hb and high Ferritin levels as compared to the control groups. Significantly positive (P<0.001) correlation of BMI with Hemoglobin and serum Ferritin levels were observed in thalassemic females of ⩾13. While <13 years thalassemic males had significant (P<0.01) negative correlation of BMI with Hemoglobin.

Conclusion: Our study revealed that beta thalassemic patients had reduced height and BMI, associated with high levels of serum ferritin and low hemoglobin.

Key Words: *Body Mass Index, Ferritin, Height, Hemoglobin, Thalassemia Major.*

Introduction

Thalassaemia major is a hereditary hemolytic disorder which is treated with repeated blood transfusions. About 240 million beta thalassemia carriers are present all over the world.[1] Every year about 100,000 children are born with the disease of thalassemia. On diagnosis of a child with thalassemia homozygous there is a lifelong sequence of blood transfusion every three weeks along with chelation therapy and facing complications due to iron overload and transfusion transmitted infections.[2] These transfusions maintain a hemoglobin level higher than 9.5 gm/dl as anemia effects the normal growth and development of these patients.[3,4]

Despite the fact that blood transfusions are mandatory for the treatment of patients suffering from anemia, repeated transfusions lead to iron overload as human beings do not have the ability to remove the extra accumulated iron.[5] Increased intestinal absorption of iron further worsens the condition, due to iron overload.[6] The iron gets deposited in various organs like liver, heart and endocrine glands which lead to various types of endocrinopathies like hypogonadism and diabetes mellitus which lead to retarded pubertal development in thalassemia major patients.[3]

Underweight and under-nutrition may lead to loss of energy and susceptibility to injury and infection, under-function of multiple endocrine systems, as well as distorted body image and other psychological problems.[7] There is increased prevalence of bone disease in patients suffering from thalassemia major as compared to normal individual.[8,9,10] The bone growth depends on the sex steroids which regulate

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bone maturity. Thalassemia major patients suffer from hypogonadism and fail to achieve their peak bone mass due to the bone disease which develops during the course of their disease .[9,11]

Serum Ferritin levels above 1000 ng/mL are considered as an iron overload.[12] The levels of serum Ferritin vary among patients getting multiple transfusions.[13] However, the cutting level at which iron toxicity and organ damage takes place is still not identified.[14]

The biochemical screening such as serum Ferritin and Hemoglobin levels are of paramount importance in all beta thalassemia patients in pediatric and adolescent age groups. These levels should be detected and treated for preventing pubertal delay in such individuals which has not been recognized in our part of the world in view of their pubertal growth. Therefore, present study was done to determine the effects of iron overload on Height (cm), BMI (Kg/m2), serum Ferritin (ng/mL) and Hemoglobin (gm/dl) levels along with exploration of the correlation of BMI with serum Ferritin (ng/mL) and Hemoglobin (gm/dl) levels of beta thalassemic patients of pubertal age group undergoing repeated blood transfusions with chelation therapy.

Materials and Methods

A case control study was carried out at Quaid-e-Azam University, Islamabad in collaboration with Jamila Sultana Foundation Rawalpindi, Thalassemia House Rawalpindi and Pakistan Institute of Medical Sciences (PIMS), Islamabad from 5th January. 2010 to 5th December 2014. The patients selected for the study were diagnosed as beta thalassemia major according to Hemoglobin electrophoresis. These patients were on regular blood transfusions with chelation therapy (desferroxamine injections). Patients suffering from any blood disorder other than beta thalassemia major or any other pathology besides spleen and liver enlargement or hepatitis B and C were not included. Total 300 individuals out of which 200 were patients suffering from beta thalassemia major and 100 were control matched for age and gender.

The age of thalassemic patients along with their corresponding control included in the study was between 8 to 22 years. Informed consent and a detail proforma including history and clinical examination were filled on patients visit to the thalassemia center for blood transfusion with chelation therapy.

Height in centimeter and Weight in kilogram were measured and BMI was calculated according to the following formula.[8]

$$BMI = \frac{\text{Weight in kilogram}}{\text{Height in meters}^2}$$

The blood samples from controlled individuals were collected in hospital and blood from thalassemic patients were collected when they came for their routine blood transfusions with chelation therapy. For collection of blood sample, the sampling area was cleaned with a spirit swab. Blood sample of (3ml) was drawn from the right median cubital vein of both female and male patients and control individuals. Blood was then collected in labeled serum separator tubes containing Ethylene diamine tetra acetic acid (EDTA). The blood samples were centrifuged at 3000 rpm for 10 minutes, and serum separated was stored at 2 - 80C until analyzed. Quantitative measurement of Hemoglobin (gm/dl) was done by and serum Ferritin was measured by using Ferritin (FTL) ELISA (Enzyme-Linked Immunosorbent Assay) technique.

Mean ± SEM of data was calculated and analyzed through Graph Pad Prism 5.01. Comparison amongst BMI, Hemoglobin and serum Ferritin levels with the control group was done by using unpaired t-test. Non parametric (Spearman) co-relation co efficient was used to find the correlation between BMI and Ferritin and Hb levels. $P<0.05$ was considered statistically significant in both cases.

Results

The results of present study for the following variables are:

Age

Mean ± SEM of age in male and female patients of <13 years was 10.3± 0.20 years. Male patients ⩾13 years had Mean ± SEM of age 16.7 ± 0.42 years, whereas the female patients of ⩾13 years had Mean ± SEM of age 17.8 ± 0.70 years.

Height (cm)

All four groups of thalassemia patients showed significantly reduced ($P<0.001$) height in comparison with their corresponding control group. Comparison of height (cm) of male and female thalassemic patients with their corresponding control of different age groups is represented in Fig 1.

BMI (Kg/m2)

Comparison of Body Mass Index (Kg/m2), in control

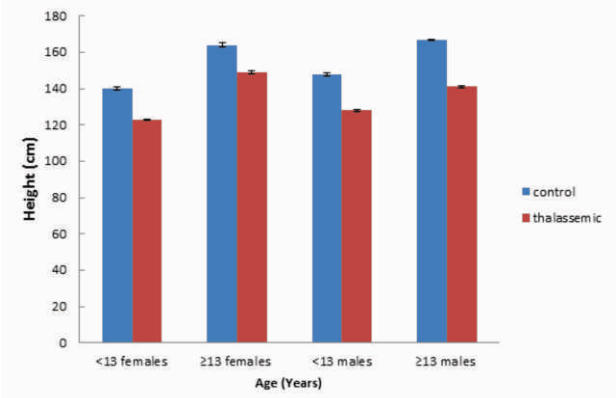

Fig 1: Mean ± SEM of height (cm) of female and male thalassemic patients with their corresponding control of different age groups. ***=P<0.001(value vs corresponding control)

and thalassemic female and male patients of different age groups is shown in Fig 2. All four groups of thalassemia patients showed significant reduction (P<0.001) in BMI on comparison with their corresponding control group.

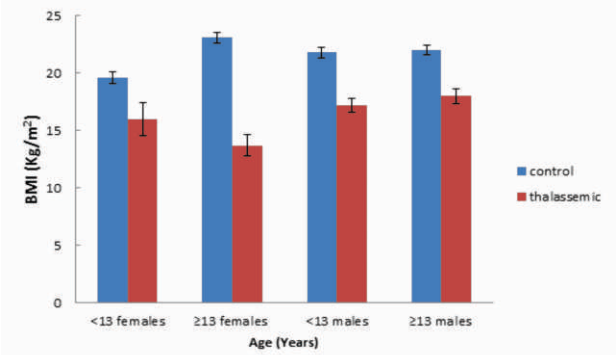

Fig 2: Mean ± SEM of BMI (Kg/m2) of female and male thalassemic patients with their corresponding control of different age groups. ***=P<0.001(value vs corresponding control)

Hemoglobin levels (gm/dl)

All four groups of thalassemia patients showed significantly reduced (P<0.001) hemoglobin levels on comparison with their corresponding control group. Comparison of Hemoglobin (gm/dl) of female and male thalassemic patients with their corresponding control of different age groups are presented in Fig 3.

Serum Ferritin levels (ng/mL)

Comparison of serum Ferritin (ng/mL) of female and male thalassemic patients with their corresponding control of different age groups are shown in Fig 4. All four groups of thalassemia patients showed significantly raised (P<0.001) serum ferritin levels in comparison with their corresponding control group.

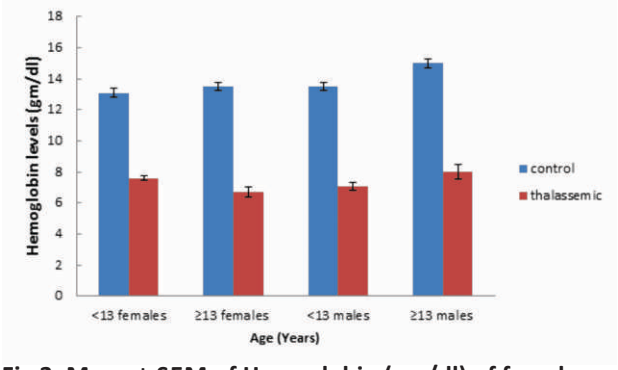

Fig 3: Mean ± SEM of Hemoglobin (gm/dl) of female and male thalassemic patients with their corresponding control of different age groups. ***=P<0.001, value vs corresponding control

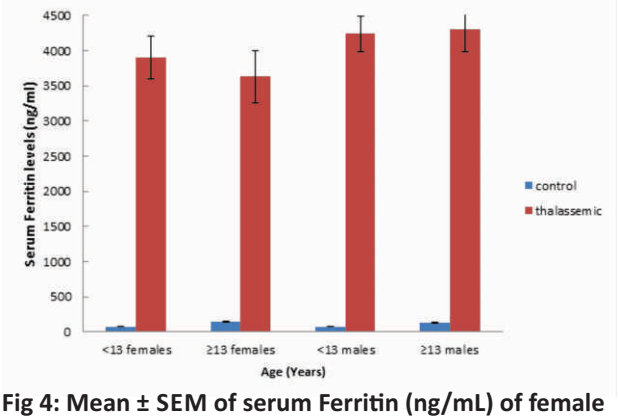

Fig 4: Mean ± SEM of serum Ferritin (ng/mL) of female and male thalassemic patients with their corresponding control of different age groups. ***=P<0.001, value vs corresponding control

Correlation of BMI (Kg/m2) with Hemoglobin (gm/dl) levels

While thalassemic females of ⩾ 13 years, BMI (Kg/m2) had a significant (P<0.001) positive correlation with Hemoglobin (gm/dl), (r=0.558). While thalassemic males of <13 years had a significant (P<0.001) negative correlation of BMI (Kg/m2) with Hemoglobin (gm/dl) levels (r=-0.374).

On calculating correlation of BMI (Kg/m2) with Hemoglobin (gm/dl) in <13 years thalassemic males it was concluded that there was a significant (P<0.01) negative correlation of BMI (Kg/m2) with Hemoglobin (gm/dl) levels (r= -0.374). Correlation of BMI (Kg/m2) with Hemoglobin (gm/dl) in control and thalassemic female and male patients in different age groups is represented in Table I.

Correlation of BMI (Kg/m2) with Serum Ferritin (ng/mL) levels

Correlation of BMI (Kg/m2) with, serum Ferritin (ng/mL) in control and thalassemic female and male

patients in different age groups is shown in Table I. Thalassemic females of ⩾ 13 years had a significant (P<0.001) positive correlation with serum Ferritin (ng/mL) levels ® 0.498).

Table I: Correlation of BMI (Kg/m2) with serum Ferritin (ng/mL) and Hemoglobin (gm/dl) levels in thalassemic female and male patients of different age groups

Gender	Age (Years)		Groups	Hemoglobin (gm/dl)	Ferritin (ng/mL)
Females	< 13	BMI (Kg/m^2)	Thalassemic n=50	-0.110	0.192
	≥ 13		Thalassemic n=50	0.558***	0.498***
Males	< 13		Thalassemic n=50	-0.374**	-0.188
	≥ 13		Thalassemic n=50	-0.238	0.127

**=P<0.01, ***=P<0.001, value are considered significant.

Discussion

In our study we observed that patients suffering from thalassemia major presented with reduced height and weight which was associated with increased serum ferritin and low hemoglobin levels. Najaf et al., (2008) research revealed that 70% of the males and 73% of female thalassemic patients of 10–27 years suffered from short stature.[15] While Li et al. (2002) observed short stature in 29.7% of patients. The iron overload leading to endocrinopathies, chronic anemia, zinc and folate deficiencies can lead to short stature.[16] These findings are in accordance to our study results in which we observed reduced height in all four groups of thalassemic males and females patients. Therefore, close observation of growth in such individuals can lead to early detection of such findings can be managed to their full extent so, that the individual achieve their normal adult height.[17,18]

Patients with thalassemia major are exposed to many growth abnormalities as an outcome of the disease or due to the adverse effects of chelating therapy which they receive on regular basis as described.[19] Work done by Ali and Hamdollah, (2004) on thalassemic patients revealed that reduced BMI was more apparent in greater than 10 years of age, 20 which are similar to our study results. Thalassemic males of <13 and ⩾ 13 years and thalassemic females of ⩾ 13 years in our study had reduced BMI as compared to the control group. The explanation to these results can be that endocrinopathies which appear as a result of iron overload and development of side effects due to prolong use of chelation therapy can be chief contributing factors in development of underweight thalassemic patients.[20] Deena et al. (2014) also showed similar results of 18 (30%) patients who had low BMI of more than 12 years of age.[21] This finding is indicating that low BMI is highly dependent on disease progression and are in accordance with our present findings.

Viprakasit et al. (2001) explained that frequent blood transfusions normally reestablishes the normal growth spurt.[22] However, despite frequent blood transfusions the adolescent growth spurt is often delayed, except if rigorous iron chelation treatment is commenced at an early age in life.[23] Previous studies on thalassemic patients revealed that average age of 12 ± 8 years occasionally suffered from growth failure as 77.4% of these patients had normal BMI.[24] Although these results are contrary to our study findings where low BMI and reduced height was detected.

Shalitin et al. (2005) also observed that thalassemic patients receiving effective chelation therapy in prepubertal years still developed short stature with significantly raised serum Ferritin levels.[25] But these finding were contrary to results obtained by De Sanctis et al. (1994) who detected no significant difference in final height between patients who started chelation therapy during adolescence with high serum Ferritin level and those who started chelation therapy during childhood with low serum Ferritin levels.[17]

Hegazi et al. (2013) observed a significantly low Hb levels and red blood cell count along with significant increase in the mean serum levels of iron and Ferritin in thalassemic patients as compared with control groups.[26] These findings are in accordance with results obtained by Charles and Linker, (2005); who also reported that Hb levels in thalassemic patients are significantly lower than control.[27] These results are similar to our study findings as all thalassemic groups had low Hb levels as compared to the control groups.

Hegazi et al. (2013) carried out a study on thalassemic male and female patients of 4-18 years of age, where there was a significant increase in the mean serum levels of iron and Ferritin in thalassemic patients as compared to control groups.[26] Similarly, Abdulzahra et al. (2011); work also revealed that iron indices were markedly increased in thalassemic

patients, and the mean serum level of Ferritin were also raised as compared to control group.[28] Similarly, in our study high serum Ferritin levels were observed in all four thalassemic groups as compared to the control groups which was similar to the results reported by Adil et al. 2012, suggesting that increased serum Ferritin levels are related to short stature and endocrinopathies.[24]

Conclusion

In beta thalassemic patients growth disturbance or delay is main clinical feature that affects the life and wellbeing of such individuals. Our study has revealed that patients with beta thalassemia suffer from reduced height, BMI which is enhanced in patients having high levels of serum Ferritin (ng/mL) and low Hemoglobin (gm/dl).

Under-nutrition and complications of thalassemia such as tissue hypoxia and side effects of chelating therapy with desferrioxamine effect the patients with iron overload. Therefore, lifelong care and management of such patients is mandatory which requires significant cost for proper treatment and ruling out other factors like various hormones that might play a role in development of short stature.

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ORIGINAL ARTICLE

How do Physical Therapy Teachers Perceive 'Professionalism' in Pakistani Context?

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ABSTRACT

Objective: The objective of the study was to determine that how Physical therapy teachers perceive 'Professionalism' in Pakistani Context?

Study Design: This was a qualitative case study.

Place and Duration of Study: The study was conducted in five different universities from five different cities of Pakistan from 10th of January to 25th June 2013 at different time.

Materials and Methods: Semi structured Interviews were conducted with 15 Physical Therapy teachers of 05 different universities from 05 different cities of Pakistan. The Physical therapy teachers with at least 5 years teaching experience in a university were included for data collection. The interviews were audio recorded; data verbatim transcribed and analyzed manually by open coding and in-Vivo coding.

Thematic analysis was done in order to identify different elements of 'Professionalism' in Physical Therapy teachers through finding the patterns in the data. Member checking was done by three researchers in the study in order to validate the data.

Results: After thematic analysis based on emerging patterns from transcribed data, following important elements about 'professionalism' in the order of priority were identified; expert in subject with knowledge and skill, behavior and attitude towards the students, patients and staff, Autonomy, serving and benefits to the community and accountability in health services, compassion, moral reasoning, reflective thinker, acquainted with professional ethics, trustworthiness, honest and team based approach.

In response to the question about the professionalism in 'Pakistani culture, the faculty responses in the order of priority were as follows; lack of resources, proper jobs, moral support, poor salary packages and financial support and religion boundaries, requirements of the community and cultural hurdle and poor context in the society, lack of governing body, council, responsibility and accountability.

Conclusion: It is concluded that for professionalism expertise in subject's domain & skills is the most important element. Besides that behavior and attitude towards students and patients are the key components of professionalism. It is concluded that in Pakistani culture, there is lack of professional behavior in physical therapy teachers/faculty. Majority of the institutes don't concentrate on professionalism.

Key Words: *Culture, Context, Faculty, Professionalism.*

Introduction

Physical therapy is an emerging field of health sciences in Pakistan. Twenty two universities offer undergraduate and graduate programs in this discipline. To understand the 'Professionalism' as core competency and phenomenon in real life practice of PT's teaching is very important. It is because PT teachers educate physical therapists. Also PT has a close andprolongs interaction with patients during rehabilitation. It has been observed that there is increased evidence about professionalism in medical litetrature.[1]

In North America and Europe the International experts have been instrumental in defining professionalism. The professionalism is also describedbythe General Medical Council's publication as the duties of a doctors, who providesgood health care, based on an updated continuous professional education, clinical teaching and bed side training, relationships with patients, and colleagues.[2]

In Pakistancurrently 60 academic institutions are offering 5 years entry level Doctor of PT Program (DPT). Fifty seven institutions have been developed in past 15 years. There were only three academic

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institutions till 2000.[3] Therefore need and demand of knowledgeable, skilled and professional PT Teaching staff has been increased in last 15 years. Currently there are more than 1000 qualified Physical therapy professionals in both academic and clinical institutions. [4]

Professionalism is one of the three key areas of interest and importance for a PT teacher, along with knowledge and skill. It is the behaviors and attitudes of Physical therapist teachers which isimportant and role models for their students during teaching and training sessions. In medical and health sciences, professionalism is of global interest and the reason for this is the failure of traditional method of teaching due to non-professional behaviors and attitudes of teachers. The global awareness is continuously increasing about the teaching of professionalism to students of health and medical sciences disciplines. [5]

China has made great changes in their education system since 2008 and mostly upgraded thestandards in health professional education, along withnew addition of integrated courses and teaching methods.[6] The present study was designed to determine the core elements that describe 'professionalism' among the PT teachersin Pakistani culture. The objective of the study was to determine how Physical therapy teachers perceive 'Professionalism' in Pakistani Context?

Materials and Methods

This was Qualitative case study. The case study strategy is used to understand the social phenomenologyin order to retain the holistic and meaningful characteristics of real life events.[7]Semi structured Interviews were conducted with 15 Physical therapy teachers of 05 different universities from 05 different cities of Pakistan, from 10th of January to 25th June 2013 at different time. The universities included were, Riphah International University Islamabad and Lahore, Lahore University, GC University Faisalabad, University of Sargodha, and Foundation University. Consent was taken from faculty; no conflict of interest raised. The ethical approval was taken from Riphah Research Ethical Committee before the data collection.

The Physical therapy teachers with at least 05 years teaching experience in a university were included for data collection.The interviews were taken in English language. The interviews were audio recorded; data verbatim transcribed and analyzed manually by open coding and in-Vivo coding. A code can be defined as, "a word or a short phrase that metaphorically assigns a salient, essence catching, and/or redolent attribute for a portion of language-based or visual data". There are more than twenty different types of codes and with one single data set it is not necessary to use all of them. The data was analyzed two times and coding was done in two cycles. In first cycle single word to sentence in the paragraphs were interpreted as the code. Open coding and In-Vivo coding was done. In the second cycle constant comparison in data sets of different respondents, cross case results was done in order to form the categories and themes.

Thematic analysis was done in order to identify the different elements of 'Professionalism' in Physical Therapy through finding the patterns in the data. This helps in answering the research question. Member checking was done by three researchers in the study in order to validate the data.Mainly data was analyzed at 'manifest level'.

Results

After thematic analysis which was done manually, the following important elements of 'professionalism' in the order of priority were identified; Expert in Subject and domain in knowledge and skill, Behavior and Attitude towards the students/patients/staff, Autonomy, serving and benefits to the community and accountability health services, Compassion, moral reasoning, reflective thinker, acquaint with professional ethics, trustworthiness, honest and team based approach.

The perception of professionalism in 'Pakistani context' the faculty responses in the order of priority were; lack of resources, proper jobs, moral support, poor salary packages, financial support, religion boundaries, requirements of the community, cultural hurdle, poor context in the society, lack of governing body, council, responsibility and accountability. Other responses in orders are lack of knowledge professional ethics traininghonesty and teaching skills, also lack of literature access to literature, planning and team work. Also there is curriculum fault and limited global perception.

The importance of professionalism for a Physical therapy teacher, most of the participants responded; 'it is one of the most important competency to be

Q: How will you perceive professionalism in Pakistani context? Following themes were identifiedin order of similarities.	
1	Lack of resources,proper job, moral support, poor salary package & financial support and religion boundaries.
2	Requirements of the community hurdle and poor context in society
3	Lack of governing body,council, responsibility and accountability.
4	Lack of knowledge & teaching skills.
5	Lack of Professional ethics.
6	Lack of trainings.
7	Lack dishonesty.
8	Lack of literature or access to literature.
9	Lack of planning.
10	Curriculum fault.
11	Lack of team work.
12	Limited global perception.

demonstratedby most ofPhysical therapy teachers'.
The level of professionalism for a Physical therapy teachers in Pakistani context, maximum responded that; 'it is one of the most important competency but very fewteachers practice act of professionalism'.

Q: In a question about the most important elementsof professionalism in PT, the following word phrase were identified on thematic analysis	
1.	Accountability
2.	Knowledge of ethical standards
3.	Lifelong learning
4.	Honesty
5.	Integrity
6.	Reflective practice
7.	Social responsibility
8.	Trust worthiness
9.	Autonomy
10.	Professional integrity
11.	Altruism
12.	Compassion
13.	Excellence

In response to question that how much our PT institutions concentrate on teaching professionalism, the respondent said; “PT institutions pay very less attention to professionalism. Very few said that PT institutions concentrate on professionalism”.
Globally, our Physical Therapy faculty members stand in order of priorities are below the level or substandard as compare to developed countries, the reasons for this is that, there is lack of ethical practice, lack of research and evidence based practice,lackofhigh quality education and talent equal or even more in initial phase of development of good theoretical background. There is no council, infrastructure, non-satisfactory level and lack of communication.
The responses to improve professionalism in PT teachers were; proper training in research and teaching and excellence and teaching skills, conferences of national as well as international level, international level exposure, professional ethics and access to latest literature, establishment of council, approved service structure and salary package, continuous professional development , uniform curriculum regular updatation, specialized practice in PT, institution to institution collaboration, society awareness programme and vision development.

Q. The responses of the question; what strategies should be adopted to improve professionalism in PT Teachers in Pakistani Context in you perception.	
1	Proper training in teaching and research training and excellence and teaching skills
2	Conferences of national as well as international levels.
3	International exposure.
4	Professional ethics and access to latest literature.
5	Establishment of council.
6	Approved service structure and salary package.
7	Continuous Professional development.
8	Uniform curriculum and need to be updated regular.
9	Specialized practice in PT.
10	Institution to institution collaboration.
11	Society awareness programme.
12	Vision development.

The possible outcomes after achieving good level of professionalism, the following were identified; improve PT service and its quality and application of latest skills with evidence based practice, professional teacher and good researcher, benefits of students, community and patient, enhance quality of knowledge, professional ethical practice, recognition, responsibility, improve autonomous practice and international standards.

Discussion

In our study the important elements of 'professionalism' in the order of priority were

Q: What will be the possible outcomes after achieving good level professionalism in f PT Teachers in Pakistani Context?	
1	Improve PT service and its quality and application of latest skills with evidence base practice.
2	Professional teacher and good researcher , Benefit to students, community and patients
3	Enhance quality of knowledge.
4	Professional ethical practice.
5	Recognition
6	Responsibility.
7	Improve autonomous practice.
8	International standard.

identified; Expert in subject and domain in knowledge and skill, Behavior and Attitude towards the students/patients/staff, Autonomy, serving and benefits to the community and accountability health services, Compassion, moral reasoning, reflective thinker, acquaint with professional ethics, trustworthiness, honest and team based approach. In 2012 Byszewski A et al in their study said "role modeling is single most important aspect of professionalism".[8]

A systematic review done by Passi V et al in 2012, in which they studied that there are 5 main themes for supporting the development of professionalism in medical students. These include curriculum design, student selection, and teaching and learning methods, role modeling and assessment methods.[9] In our study there are five components of professionalism which are more important for our PT teachers in Pakistan. These are accountability, ethical knowledge, Lifelong learning, honesty, autonomy and reflection.

In 2012 a study conducted by Cruess SR and his colleagues on teaching professionalism and concluded that teaching professionalism requires cognitive base from each and every teaching community,which is a definition of profession, the attributes of the professional, and the relationship of medicine to the society which it serves. These should be taught explicitly. The substance of professionalism must become part of each physician's identity and be reflected in observable behaviors. Professionalism should be taught as "an ideal to be pursued" rather than as a set of rules and regulations.[10] According to our study after achieving good level professionalism in PT Teachers in Pakistani Context; there will be improvement in teaching as well as PT services, which ultimately would have a very good impact on community.

Hur Y in 2009 studied that medical professors need to encourage their students to increase their elevation. He studied on 31 core elements, significant perception gap were found in 28 elements. The 31 core elements were divided form 3 major domains – including professional knowledge, clinical skills, and professional attitude-all contained perception gaps, and professors' ratings generally were higher than those of the students, a noteworthy observation.[10] According to our study, strategies should be adopted to improve professionalism in PT teachers in Pakistani context the responses were proper training in research and teaching and training and excellence in teaching skills, conferences of national as well as international level, international level exposure, professional ethics and access to latest literature, establishment of council, approved service structure and salary package continuous professional development, uniform curriculum regular updatation, specialized practice in PT, institution to institution collaboration, society awareness programme and vision development.

CahalinLPin 2012 conducted a study onLinda Crane Lecture on Professionalism as a Core Values in PT. The objective was to highlight the professionalism of Linda Crane with examples of the methods appropriate for physical therapist and to develop their own professionalism. She used a tool developed by the American PT Association (APTA), named professionalism assessment tool.[11] The study was conducted on small sample size of professionals due to lack of time with Physical Therapist. In future for generalized results large sample size with more universities are to be involved.

Conclusion

It is concluded that for professionalism expertise in subject's domain & skills is the most important element. Besides that behavior and attitude towards students and patients are the key components of professionalism. It is concluded that in Pakistani culture, there is lack of professional behavior in physical therapy teachers. Majority of the institutes don't concentrate on professionalism.

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ORIGINAL ARTICLE

Perception of Parents about Dentistry as a Career Option for their Children

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ABSTRACT

Objective: To explore the perceptions of Pakistani parents about dentistry as a career option for their pre medical group children.

Study Design: Qualitative study.

Place and Duration of Study: Rawalpindi, Islamabad, Mirpur AJK and Lahore, 30th December 2014 to 15th August 2015.

Materials and Methods: Constructivist grounded theory approach was used as methodology. Data was collected from parents of pre-medical students (FSc, A-level) using purposive and convenient sampling method. In-depth semi-structured interviews were used as data collection tool. Data was analyzed using Constant comparative method for thematic content analysis. Computer Aided Qualitative Data Analysis Software NVivo was used for data analysis and management.

Results: Five major themes including, awareness in society, value in society, job opportunities, knowledge and balanced personal and professional life emerged from the data. Themes were broadly classified as sociocultural, socioeconomic and personal themes and helped in conceptualizing and generating "butterfly theory of career choice". Majority of parents perceived dentistry career as having lack of awareness and social acceptance.

Conclusion: In developing countries such as Pakistan, awareness of oral health is lacking and scope of dentistry is not as much as in foreign countries. So, parents overwhelmingly prefer medicine as a career choice for their children, rather than dentistry which they think is not as valuable in the society.

Key Words: *Achievement Related Perceptions, Career Development, Career Option, Congruence, Family Influence, Perceptions.*

Introduction

Parents play an important role in children's career guidance and career selection from Various standpoints and influence their career choices both intentionally and unintentionally.[1] Research has shown that parents greatly influence their child's career selection[2,3] and many studies in literature have explored impact of parent's involvement on children's achievements and career selection as a general.[4] Some parents encourage their children by providing them financial and moral support to explore career options available and find out the best career fit for them, as opposed to other parents trying to live out their own unrealized career dreams through their children.[5] It is pertinent to mention that since many studies have explored perceptions of parents about their children's career as a general but no study could be found that has been done specifically in perspective of dentistry as a career.

This study is important to understand preferences and insecurities of parents about future careers of their children especially in perspective of dentistry. It has been observed that parents in Pakistan usually insist to get their children admitted in medicine rather than dentistry and long counseling sessions are required to convince them for taking admission in dentistry. It has also been observed that parents are at times authoritative and play a critical role in career choices, which can affect children's academic achievements. Therefore it was thought important to uncover the hidden insights of parents and to know their point of view regarding vocation of their children.

This research will help to generate a substantive theory explaining the abovementioned behavior of Pakistani parents and ultimately developing a system

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model to be used for conducting career counseling sessions. It is assumed that results and predictive information from this study would be used for supporting and advising students and families, to get enrolled in a program according to current trends. The main objective of the study was to explore the perceptions of Pakistani parents about dentistry as a career option for their pre medical group children.

Materials and Methods

This study utilized qualitative research approach and constructivist grounded theory research methodology. Reasons for selecting this research design were twofold. First was the intention to know in depth, the concerns and insecurities of the parents while selecting a career for their children. Second, was the aim to generate a substantive theory that will explain the observed phenomenon in a specific context.

Study was conducted in twin cities of Rawalpindi and Islamabad, Lahore and Mirpur AJK over a period of eight months, 30th December 2014 to 15th August 2015. Purposive sampling was done initially and parents of pre medical group students were included in the study. Later, convenience sampling method was used to include parents of students studying in eight different higher secondary schools including five private and three government schools (Roots IVY School Rawalpindi, City School Islamabad, Beacon house School system Islamabad, Federal College Islamabad, Sir Syed College Rawalpindi, Kashmir model College Mirpur, Army public School Rawalpindi and Lahore grammar School Islamabad).Those who volunteered themselves to participate in the interview via publicity of research project through personal meetings were also included in the study. Parents who were doctors or dentists himself/herself were not included in the study. Similarly parents who had children already studying in medicine or dentistry were also excluded from the study. Different venues were used for interviews according to the availability of participants, including Roots DHA-1 School premises, residence of participants, researcher's own residence, Garrison sports complex, Mirpur Public Park and other public places. Constructivist grounded theory approach was used for data collection and analyses.[6] Both data collection and analysis occurred simultaneously throughout the study, Insight from initial data collection and analysis led to subsequent data collection. A semi-structured question guide was developed using "AMEE guide 87 developing questionnaire"[7] as reference (Table 1).

Table I: Summary of semi-structured question guide

Sequence	Questions
Engagement questions Research question 1	1. How do you perceive successful career with respect to your children? 2. What expectation do you have from your children's future profession?
Exploration questions Research question 2	1. How do you perceive BDS as a career option for your children? 2. How do you perceive MBBS as a career option for your children? 3. Are there any social pressures/threats influencing career choice decisions?
Exit questions Research question 3	1. How good do you think is the standard of medical and dental education in Pakistan? 2. How will you describe knowledge, skills and attitudes of Pakistani dentists?

We pilot tested questions with faculty members of our institute before commencement of study for clarity and unambiguity. Their input helped in evaluation and Questions were further refined and elaborated.

Semi-structured, in-depth, one-to-one interviews were used as data collection tool. A mix of both in person and telephonic interviews were conducted by principal researcher. Interviews were audiotaped, and with the field notes were also taken to increase credibility of data. The duration of interviews varied considerably depending upon the respondent's interest and ranged from 15 to 40 minutes. Participants were interviewed in both English and Urdu language, which later was transcribed in English by researcher herself, having command on both Urdu and English languages. Interviews were done in confidentiality. For reporting purposes, and to protect participants' identities, each participant was assigned a number.

The total sample size was 18 based on data saturation. Data collection procedure took three months and included interviews followed by transcription of each interview.

Exploratory thematic content analysis was done

using constructivist grounded theory approach to find out pertinent concepts and emergent themes. Initially manual analysis using three cycle open, axial and selective coding was done to have a basic idea about categories, concepts and themes embedded in the data (Fig 1). Later Computer Aided Qualitative Data Analysis Software (CAQDAS) was used for data analysis. Data compiled in field notes and responses of the respondents were transcribed verbatim and then were imported into NVIVO version10. Analysis was done by making nodes and child nodes. Open coding was done for identification of themes. Themes that emerged from the data were coded using tree nodes. Coding comparison queries were run for interlinking of different themes. We made comparisons between and across empirical data,

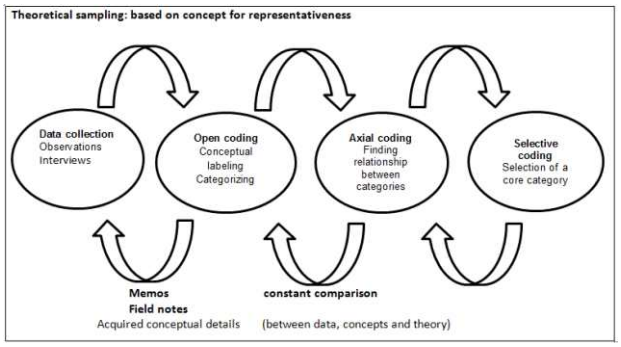

Fig 1: Data analysis procedure

concepts and categories in order to reach higher levels of abstraction and conceptualization. On the basis of constant comparison, concepts and categories were identified, which resulted in formulation of substantive theory, "the Butterfly theory". Formal theory could not be extracted from this substantive theory as the research was conducted in limited area in Pakistan.

Presentation and visualization of results was ensured using NVivo data tables, flow charts and models. Qualitative summaries were generated to help interpret the data according to issues and themes analyzed.

Results

Of the 18 survey participants, most respondents were in the 33 to 52 age bracket and belonged to middle and high socioeconomic status. All of the fathers surveyed were employed and generally belonged to professions including Army, Law, education and business. Whereas 40% of mothers surveyed were employed and associated with teaching profession.

A total of 552 open codes were created (Table 2), which were merged into bigger (axial) codes or

Table II: Total number of codes and references created from data

Name	Codes	References
Interviewee 1	43	246
Interviewee 2	37	123
Interviewee 3	36	146
Interviewee 4	33	84
Interviewee 5	30	60
Interviewee 6	25	54
Interviewee 7	28	52
Interviewee 8	30	67
Interviewee 9	30	73
Interviewee 10	31	71
Interviewee 11	31	75
Interviewee 12	24	59
Interviewee 13	36	84
Interviewee 14	28	82
Interviewee 15	30	69
Interviewee 16	23	36
Interviewee 17	18	27
Interviewee 18	39	88
-	**552**	-

Table III: Perception of parents about dentistry: categories created from data and references

conceptual units showing perceptions of parents about dentistry as positive and limiting points in comparison to medicine (Table 3).

Name	Sources	References
Career Option Medicine or Dentistry	18	191
Dentistry	17	87
Dentistry limiting points	16	64
Dental graduates are not doctors	4	10
Dentistry only second choice	5	6
Inferiority complex in dental students	4	6
Its attraction is not that much	1	2
Lack of awareness	10	22
Lack of job opportunities	2	3
Less career progression than medicine	1	1
Less parent's satisfaction	1	1
Limited knowledge	4	4
Low merit than medicine	5	6
Dentistry positive points	5	19

Balanced family life	4	5
Dentistry is good for girls	3	7
Less working hours	2	2
No emergencies	2	2
Specialization in itself	1	1
Medicine	17	104
Medicine limiting points	2	4
Emergencies	1	1
Needs further specialization	1	1
No family life	2	2
Medicine positive points	16	78
Better educational way towards CSS	1	4
Better Future	8	14
Easy to open up clinic	1	3
Opportunities are more, and scope is not limited	5	6
Professional security	2	2
Challenging task	2	3
High merit	2	4
Important profession	1	2
Life savers	1	1
More valuable in our society	13	37
Good marriage proposals after medicine	3	9
Having good place in life	1	1
Its big as compared to dentistry	2	2
More Knowledge	3	4
Parent's satisfaction	1	1
Respect in society	1	3
Only medical graduates are doctors	4	5
Versatile field	2	3

Table IV: General description of themes

Categories	Themes
Sociocultural themes	1. Awareness in society 2. Value in society
Socioeconomic themes	Job opportunities
Personal themes	1. Knowledge 2. Balanced professional and personal life

Fourteen categories were identified about perceptions of parents about dentistry as a career and five main themes emerged from data including Awareness in society, Value in society, Job opportunities, Knowledge and Balanced professional and personal life (Fig 2).

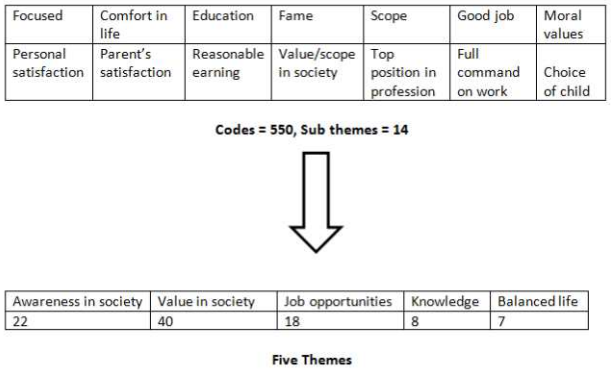

Fig 2: Description of Sub themes and themes in data analysis

These themes were broadly classified as sociocultural themes (theme 1 and 2), socioeconomic themes (theme 3) and Personal themes (theme 4 and 5).

Themes were presented as a perception model, "the butterfly model" due to its appearance as a butterfly (Fig 3).

Most frequently discussed theme determining career choice was value and merit of profession in the society.

A representative statement from one of the interviews is as follows:

"So first of all respect and value of doctors in the society is more as compared to dentists, which is a trend in Pakistan" (Participant 14).

The second perception which influenced career decision was awareness of a particular profession in the society. A representative statement from one of the interviews is as follows:

"Awareness of dentistry is less in people and its importance and nuisance is not much in society." (Participant 3).

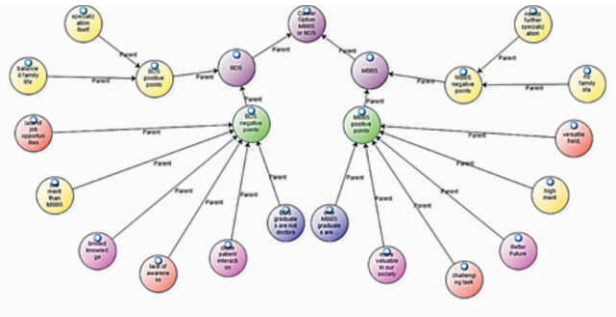

Fig 3: Butterfly model of career perceptions

Most of the participants mentioned that awareness of oral health and dentistry is low in society. Especially in small cities and villages, people do not

know about dentists and they give quacks and dentists equal status so they cannot appreciate importance of dentistry as a career in our society.

The third commonly found theme was earning that is related to the socioeconomic status within the society. Eighty percent of participants find dentistry career as having lack of Job opportunities.

A representative statement from one of the interviews is as follows:

"Because as a doctor everybody needs u, younger and elders, will come to your clinic from morning to evening and there are more vacancies for medical doctors in government hospitals and only few seats for dentists" (participant 8).

The personal themes included knowledge and then balanced professional life. A representative statement from one of the interviews is as follows.

"I like medicine more for my child, because in dentistry they study only specific area but medicine is a broad subject, and they have more knowledge" (Participant 16).

"For my son I will also choose dentistry because he can spend time with his family and can spend comfortable life" (Participant 4).

Slight differences were found between perceptions of participants demographically. Parents living in Mirpur (a small city in peripheral Pakistan) did not appreciate dentistry as an important career while parents living in Rawalpindi, Islamabad and Lahore (major cities) at least perceived dentistry as an important and latest field.

Discussion

The present study looked at the perceptions of parents about dentistry as a career for their children. Parents generally want their child to have the best place in the society; they want to see them at the top of the pyramid, a finding in accordance with one of the previous studies.[8] The parents do not consider dental graduates as doctors and its attraction in society as career option is not found substantial according to the present study. Generally parents consider dental surgery as second option that is if their child is unable to get admission in medicine. They do not feel proud by announcing their child's admission in dentistry due to its low merit in medical colleges as compared to medicine. Since parents are convinced about lack of acknowledgement and value given to dentistry as profession in our society they do not prefer it as career choice for their children. There is no supporting data available internationally on parental perceptions about dentistry; to be compared to results of the present study but the literature supports value, status and scope of dentistry internationally.[9] This finding is in contrast to other parts of the world where awareness of dentistry is high, for instance USA and European countries[10,11] and they prefer dental profession due to social and economic status offered by this profession. The present study also found economic status an important constituent of career choice decision which is lacking in dental profession. Different studies have explored this factor as indicator of successful career.[12] This finding is again in contrast to a previous study which concluded that dentistry is perceived as a profession which provides financially lucrative, contained career in healthcare, with professional status, job security and opportunity to work flexibly.[13]Participants are generally convinced that dentists have only limited knowledge and skill (particular to head and neck area) while they want to see their children having full command and knowledge of human body. Finally although parents think of dentistry as a career that offers opportunity to spend balanced professional and personal life with no emergency calls and extra duty hours, but they still are not ready to give it first choice, again due to dominancy of above mentioned factors.

Constant comparison and interlinking of themes helped conceptualizing and generating a substantial theory that was grounded in the data. Theory is given the name "butterfly theory" after the name of thematic representation model.

Key findings are:

1- In Pakistani culture, parents do not prefer dentistry as a career option for their premedical group children.

2- Parents want their children to have value, fame and reasonable earning in the society, which they think is lacking in the dentistry career.

3- Lack of oral health awareness is an important factor in limiting choice of dentistry as a career.

Butterfly appearance of career preference model metaphorically explains the phenomena. Successful career is like a beautiful butterfly, with different colors of fame, respect, money, knowledge,

satisfaction and comfort. Doctors look like career butterflies to majority of parents in Pakistan.

Another explanation of butterfly model is that career is also like a butterfly going from one job to other like a butterfly goes from one flower to other until personal satisfaction is achieved. In present study, research model indicates one wing of butterfly as having all the colors of positive points regarding dentistry and opposite wing having equal limiting points for not opting dentistry as career choice, while its tentacles exhibit umbrella points which are indicators of any successful career.

This research helped to understand perceptions that influence parental insecurities and reservations about dentistry career and could be addressed by first conducting career counseling sessions for pre medical students. Secondly, dentistry awareness programs could be introduced at government and regulatory body level, in schools and colleges to educate students on their career choices especially finding a gap in job saturation.

Conclusion

Parents do not prefer dentistry as a career option for their children. They perceive dentistry as a career with lack of social acceptance and low value in our society. Sociocultural and socioeconomic aspects are found dominant factors while selecting career. Lack of job opportunities and career progression make parents insecure about financial stability of their children and they consider dentistry only as second choice in case of inability to get admission in medicine. They attribute all these factors to lack of awareness about oral health and dentistry as a profession in Pakistan.

Acknowledgement

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LETTER TO THE EDITOR

Food-added Monosodium Glutamate does not induce Changes in the Ovaries

Ajinomoto Group is one of the leading global producers of the flavor enhancer monosodium glutamate (MSG). Since early 20th century, Ajinomoto has gathered a substantial amount of data related to MSG safety and use. Based on that database, we are commenting here-below on the recent article by Abbasi et al., entitled "Effect of Vitamin C on monosodium glutamate (Ajinomoto) induced changes in the ovary of rats" (JIIMC 11(2), 2016, 66-70).

The article described a rat study with MSG and vitamin C. The authors extensively speculated that MSG ingestion may cause harmful effects on human female fertility and ovarian functions. We argue that the study by Abbasi et al. was burdened by methodological problems and a lack of reproducibility. In addition, we conclude that the authors neglected glutamate metabolism in mammalian bodies in interpreting the results.

1) The doses of MSG or the mode of MSG treatment were not described, thus one cannot conclude if there was any relevance to human nutrition. If MSG was mixed into the experimental diet, the authors needed to explain how that was achieved since conventional rat diets are granulated. If MSG was applied in drinking water, the authors needed to describe how taste was masked.
2) No data on diet intake, water intake or body weight were provided, but the authors mentioned that the control group was characterized by a lower terminal mean body weight when compared to body weight of MSG–treated rats. Therefore, the observed ovarian differences could have been attributed to the changes in body weight and not to the treatments per se. In other words, in the absence of body weight information, it is impossible to toxicologically interpret the observed changes.
3) It is not clear where the tested MSG was obtained from, who was the producer and whether it contained impurities or other substances which may have affected the observed results.
4) The authors indicated that "AJINOMOTO" was a common name of all MSG used in Pakistan. Indeed, "AJINOMOTO" is a trademark registered by Ajinomoto Co., Inc. in more than 170 countries, including countries in Central and South Asia. However, while the "AJINOMOTO" is one of the most popular seasoning brands world-wide, it is not the only MSG brand on the market. Mentioning "AJINOMOTO" brand name in a title of a scientific article without describing the source of the tested MSG, or its purity, was disparaging and academically unjustified.
5) As the authors mentioned, a molecule of MSG contains glutamate and sodium. However, sodium intake from MSG was not controlled for even though the authors attributed all observed changes to glutamate alone. One cannot preclude that at least some effects were attributable to sodium. In that respect, we note that there was no information on how control rats were treated or what control diet was composed of. Adult rats ingest standard diet at approximately 12% of their body weight, thus we suppose the studied female rats ingested daily approximately 30 – 40 g of a chaw diet. If that diet was based on milk casein, as is usually the case, it contained 10% glutamate, so the rats were eating 3 – 4 g of glutamate from the diet alone without MSG added (i.e., 1). No attention was given to that "diet-contained" glutamate source.
6) Authors extensively speculated on MSG use in humans. Adult humans ingest about > 10 g glutamate per day from a normal diet. This volume includes 0.5 – 1.0 g per day of glutamate added to food as a flavor enhancer, whether in a form of MSG or included in other condiments rich in glutamate (bouillon cubes, soy sauces, mushrooms etc.). In simple words, MSG is only a small portion of ingested glutamate. Considering that all food free glutamates are metabolized identically, it is disproportionate to speculate solely on MSG – especially if the speculation is based on a rodent study only.
7) Importantly, histopathological evaluation of the tissues was not described. Specifically, was the evaluation done visually only; and were the persons conducting the observations blinded?

8) Finally, the authors selectively used references and omitted scientific papers on the lack of dietary MSG effect on reproductive functions (2-4). Instead, the authors used pharmacological studies with MSG or non-scientific articles published online (See Ref. 17 in the original article). At this point, it is appropriate to mention that less than 5% of orally ingested glutamate from food (including MSG) is absorbed from the gut into the systemic circulation. The rest is used as an oxidative substrate by the intestinal mucosa (5-8). Other food components, which are inevitably ingested along with food-added glutamate, further suppress circulating glutamate levels (9-10) and therefore increasing blood glutamate levels by food-added MSG is extremely difficult. In the absence of a high circulating glutamate, any changes in ovarian physiology are impossible to attribute to food-derived glutamates, such as MSG.

Key Words: *Monosodium Glutamate, Metabolism, Ovaries.*

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COMMENTS BY AUTHOR

Effect of Vitamin C on MSG induced Changes in the Ovaries of Rat

- The dose of MSG was 0.08 mg per kg body weight.[1-3] And it was mixed in their pallet diet. Daily diet intake of one rat is 10-12grams. Weight of one rat is approximately 300grams. So the weight of fifteen rats in experimental group A was 15 * 300 = 4500gm/4.5kg. The estimated dose of MSG for fifteen rats was 0.08 * 4500 = 0.036grams. Approximate dose of MSG per rate per day will be 0.024grams. The dose of MSG for four weeks was 0.024 * 30 = 0.72grams.
- High quality MSG free from impurities was obtained from Asia Scientific Traders, Rawalpindi manufactured by Zinef Company China.
- I followed the tradition in academic literature where MSG is commonly known as Ajinomoto.[4-6]
- The major component of MSG is glutamate that is 78%[7] and literature showed that glutamate is harmful component of MSG not the sodium.5 Indeed glutamate is a major component of protein rich food like tomatoes, fermented beans, soya sauce and fish sauce.
- Histopathological evaluation was done microscopically under the supervision of histopathologist.

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INSTRUCTIONS FOR AUTHORS

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Tables and illustrations should be merged within the text of the paper, and legends to illustrations should be typed on the same sheet. Table should be simple, and should supplement rather than duplicate information in the text; tables repeating information will be omitted. Each table should have a title and be typed in double space without horizontal and vertical lines on an 8 ½" x 11' paper. Tables should be numbered consecutively with Roman numeral 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 foot notes and when they first appear in text. When graphs, scattergrams, or histogram are submitted, the numerical data on which they are based should be supplied. All graphs should be made with MS Excel and be sent as a separate Excel file even if merged in the manuscript. For scanned photographs highest resolution should be used.

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

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REFERENCES

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ABSTRACT

Abstracts of original article should be in structured format with following sub-headings: i. Objective, ii. Design, iii. Place & Duration of study iv. Materials & Methods, v. Result, vi. Conclusion. Four elements should be addressed: why did you start, why did you do, what did you find and what does it mean. Why did you start in the objective. What did you do constitutes the methodology and could 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; our 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 do not required 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.

MATERIALS AND 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 names(s), dose(s) and route(s) of administration.

RESULTS

These should be presented in 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.

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