Vol. 27, No.2. February,2016

ISSN 1029 - 385 X

MEDICAL FORUM MONTHLY

APNS Member | CPNE Member | ABC Certified

RECOGNISED BY PMDC & HEC

Journal of all Specialities

"Medical Forum" Monthly Recognised and Indexed by

- PMDC with Index Pakistan No. 48 Since 1998
- HEC Since 2009
- Pakmedinet Since 2011
- Medlip (CPSP) Since 2000
- PASTIC & PSA Since 2000
- NLP Since 2000
- WHO, Index Medicus (IMEMR) Since 1997
- EXCERPTA MEDICA, Netherlands Since 2000
- EMBASE SCOPUS Database Since 2008
- Registered with International Serials Data System of France bearing ISSN No. 1029-385X Since 1992
- Registered with Press Registrar Govt. of Pak bearing No. 1221-B Copr. Since 2009
- ABC Certification Since 1992
- On Central Media List Since 1995
- Med. Forum Published from Lahore Since 1989
- Peer Review & Online Journal
- Electronic Publication of Journal Now Available on website: www.medforum.pk

MEDICAL FORUM MONTHLY

ISSN 1029 - 385 X

APNS Member | CPNE Member | ABC Certified

Peer Review Journal | Online Journal | Published Since 1989

e-journal available on: www.medforum.pk

Medical Forum Recognized and Indexed by

PMDC-IP-0048 (1998), HEC-Y-Category (2009), Pastic and PSA, Isd (2000), Medlip, Karachi (2000), NLP, Isd (2000), Pakmedinet, Isd (2011), Excerpta Medica, Netherlands (2000), EMBASE Scopus Database (2008), Index Medicus (IMEMR) WHO (1997), ABC Certification, Govt. of Pak. (1992), Central Media list, Govt. of Pak (1995), Press Reg. No.1221-B Copr (2009)

Business Manager Nayyar Zia Ch.

Legal Advisors Jan Muhammad Bhatti, Kh. Ejaz Feroz (Barrister), Kh. Mazhar Hassan & Firdous Ayub Ch. (Advocates)

Published By Dr. Nasreen Azhar
Gohawa Road, Link Defence / New Airport Road,
Opposite Toyota Motors, Lahore Cantt. Lahore.
Mob. 0331-6361436, 0300-4879016, 0345-4221303, 0345-4221323
E-mail: med_forum@hotmail.com, medicalforum@gmail.com
Website: www.medforum.pk

Printed By Syed Ajmal Hussain
Naqvi Brothers Printing Press, Darbar Market, Lahore

Rate per Copy Rs.1500.00

Subscription Rates

Annually

Pakistan	Rs.15000.00
USA & Canada	US$ 500.00
China & Japan	US$ 450.00
United Kingdom	US$ 450.00
Middle East	US$ 400.00

Med. Forum, Vol. 27, No.2 February, 2016 **ISSN 1029-385-X**

Recognized by PMDC **Recognized by HEC**

CONTENTS

Editorial

Original Articles

Editorial

Death By Cholesterol?

Dr. Mohsin Masud Jan
Editor

A new health advisory shows replacing all foods containing cholesterol from your diet does not make the amount of cholesterol in the blood go down

Cholesterol is not a nutrient of concern for overconsumption. This statement essentially reverses previous recommendations.

The point made is that replacing all foods containing cholesterol from a diet does not make the amount of cholesterol in the blood go down. That said, more important than the total amount of cholesterol in our blood from a heart artery problem is the amount of the 'good' cholesterol. And the amount of the good cholesterol in our blood depends more on genetics than diet.

As such limiting cholesterol intake does not make sense. Also, people try and fill themselves up with sugary foods and carbohydrates especially of the refined sort when they avoid fatty foods. The reason is simple, fatty foods 'satiate' or fill a person up more easily than non-fatty foods and a sated person does not get hungry too soon.

The other problem with removing cholesterol rich fat from food is that the prepared, processed and fast food suppliers all try and add more sugar and salt to their foods to make them taste better. Increased amount of refined sugar and refined starches play an important role in the recent 'epidemic' of adult type diabetes. High salt/sodium possibly contributes to high blood pressure.

So it is not avoiding cholesterol in food that is all bad but rather it is what such foods are replaced with that is of concern. In DGAC, the Dangerous Goods Advisory Council, most of the observations are interesting but do not really bring any new ideas out. Below I will present two sentences/paragraphs that in my opinion are of importance especially to people in Pakistan.

"The overall body of evidence examined by the 2015 DGAC identifies that a healthy dietary pattern is higher in vegetables, fruits, whole grains, low or non-fatty dairy, seafood, legumes and nuts; moderate in alcohol (among adults) lower in red and processed meats, and low in sugar sweetened foods and drinks and refined grains." The report adds that: "Current research also strongly demonstrates that regular physical activity promotes health and reduces chronic disease risk."

The report also says about Estimated Average Requirement (EAR) that: "Nutrient data from a representative sample of the US population ages two and older indicate that: vitamin A, vitamin D, vitamin E, folate, vitamin C, calcium, and magnesium are under consumed relative to the EAR."

The first recommendation mentioned above has been a well-established part of dietary advice for many years. However for a country where close to a majority of people barely fulfill their recommended daily needs for caloric intakes, especially women and children, the above recommendations provide two important pieces of information. First, that diets high in animal fats and 'red meat' that so many of the poor and even some in the middle class long for are not necessarily good for them.

The other important point is that less expensive forms of calories and protein like unrefined grains, vegetables and legumes (daals) on the whole form a better basis for a healthy diet. As I have said before and I will say again, eat whatever you want but mostly plants. And for those that can afford cooking oils of their choice, oils from vegetable origins (olive oil, canola oil, etc.) are better than oils of animal origin.

Generally oils that are liquid at room temperature (for people in Lahore-room temperature means 20 degrees centigrade, give or take.) are better than those that are solid at room temperature. However, it is important to remember the old adage, everything in moderation.

One thing that needs to be considered by the well-to-do is that eating cows and other animals that are bred for this purpose is just very bad. Cattle breeding requires areas for producing feed that in cattle breeding countries can produce deforestation and diversion of available agricultural land towards producing cattle feed. Besides that, breeding cattle stresses water supplies and produces ecological damage.

As the world population increases and more people are able to afford meat, the damage to the environment from cattle breeding will become more pronounced. Eventually people will have to make a choice between eating meat or letting most of Bangladesh disappear into the Bay of Bengal. Eventually all forms of meat including that from chicken, pork or even llamas will also become progressively expensive. And sadly even the oceans are being overfished so eventually all forms of 'animal' proteins are going to become difficult to procure.

But then human ingenuity and modern technology has already made the Malthusian forecasts of a world running out of food a bit premature. And here I would like to throw in a concept that most of my coreligionists might find slightly blasphemous. Do animals have rights?

The second paragraph I quoted from the DGAC related to the 'under consumption' of micronutrients like vitamins and minerals of tremendous importance to normal and healthy function of the human body. For

decades physicians have advised patients that those who eat a 'proper' diet do not require 'dietary supplements'. But the DGAC report clearly presents data suggesting that many if not all 'Americans' are consuming less of these vitamins and minerals than they should. This is even more so true of people in Pakistan including even the well-off that can eat whatever they want.

The addition of Iodine to salt virtually eliminated enlargement of thyroid glands (goiter). The same can be said of adding Vitamin D and Calcium to foods like milk and bread that virtually eliminated 'Rickets'. But even so as we all live longer, even in Pakistan it is important that if we really start eating unprocessed foods as suggested by the DGAC, we might start running out of these micronutrients. So, for those that can afford them, a decent multivitamin pill taken every day is not a bad idea.

And in young women especially those who might get pregnant, Iron and Folate supplements are absolutely necessary. And for those that cannot afford them especially among children and pregnant women, provision of many of these micronutrients has to be a public health imperative.

If we ate what we should and if we also exercised incessantly we should live forever. Sadly, all such attempts at longevity are often cut short by cancer that but for a few is essentially a matter of pure chance. But, more about that another time.

Original Article

To Study the Frequency of Obesity Among Female Students of Nishtar Medical College, Multan

1. Nasrin Siddique 2. Qaiser Mahmood 3. Affan Qaiser 4. Farhan Jamil 5. Nazish Butt

1. Assoc. Prof. of Community Medicine, 2. Assoc. Prof., Medical Unit-IV, 3. PG Trainee of Medicine, 4. PG Trainee of Community Medicine, 5. House Officer, NMC / Nishtar Hospital, Multan.

ABSTRACT

Objectives: 1. To find out obesity frequency among female students of Nishtar Medical College by measuring their Body Mass Index. 2-To create awareness about hazards of obesity among them.

Study Design: Observational / Cross sectional study.

Place and duration of study: This Study was carried out at Nishtar Medical College, Multan from October 2015 to December, 2015.

Materials and Methods: A sample of 250 female students was taken by convenient sampling technique,50 students from each class were included in the study. After taking informed consent, all the desired in formations were collected by developing a structured questionnaire. Excel and SPSS software were used to analyze data.

Results: Out of the total 250 students, 80(32%) were obese and 170(68%) were non-obese. Among the obese students, 24(30%) were from final year and 13(16.25%) were from first year MBBS class. There was very high percentage of obese students 59(73.75%) belonging to middle socioeconomic class. Most of the students 42 (52.5%) were consuming 2100-2500 calories/day. Among the obese students only 10(12.5%) were doing exercise daily. 22 (27.5%)students had positive family history of obesity. About 13(16.25%) students were consuming fast food once daily and 46(57.5%)were taking 2-3 times per week.

Conclusion: Obesity is a significant problem among female medical students. The main risk factors identified were middle economic status, lack of exercise , overall sedentary life style, with high calories and fast food consumption.

Key Words: Obesity, BMI, Female Medical Students

Citation of article: Siddique N, Mahmood Q, Qaiser A, Jamil F, Butt N. To Study the Frequency of Obesity Among Female Students of Nishtar Medical College, Multan. Med Forum 2016;27(2):3-6.

INTRODUCTION

Obesity is the most common type of malnutrition in both developed and developing countries and effecting all age groups among both male and females. It is now so common that it has replaced under nutrition.[1]

Obesity is the second most common cause of death in the United States where 300000 deaths occur annually due to overweight and obesity which can be prevented. Mortality rates are high among obese than the normal persons. Obesity is a risk factor for many diseases particularly cardiovascular disease, non insulin dependent diabetes mellitus, sleep disturbances-[2], few types of benign and malignant conditions, e.g. Breast, colon endometrial, prostate and joints problems.[3]

Overweight and obesity epidemic is growing gradually worldwide and in the United States 34.9% of adults aged 20 years or older are obese.[4] In USA , abdominal obesity is more in females.[5] In Indonesia the obesity in females is found to be 15%.[6] Pakistan ranked 9th out of 188 countries in terms of obesity. The overall prevalence of overweight and obesity is 18.5% and 27.85% respectively in general population of Pakistan.[7]

In Arabian countries, traditional / cultural restrictions in lifestyle choices available to women are the main source for increased rates of obesity. Because of these limitations, females have limited access to supporting / exercise activities. In Sri Lanka, the prevalence of obesity in females is 8.5%.[8]

Overweight and obesity prevalence is also increasing among medical students and doctors than general population. It is very critical situation as they are the role models for the general public to follow for healthy weight and diet.

According to a study conducted in doctors in Pakistan, prevalence of obesity is 28.2%. [9]

Among many factors which influence body weight gain, sustained energy imbalance due to more intake than its expenditure is very strong determinant of overweight and obesity.

Studies have shown that age is strongly associated with obesity. The highest prevalence of obesity is seen in 50 – 59 years age group for both genders.[10]Greatest increase in weight is seen in less educated women .This is true for most of the developing countries. The etiology of obesity is multifactorial. Economic growth,

Correspondence: Dr.Nasrin Siddique,
Assoc. Prof. of Community Medicine, NMC / Nishter Hospital, Multan.
Cell No.: 0322-6106099
E-mail: dr.nasrin.qaiser@gmail.com

urbanization and subsequent changes in lifestyle are among the factors driving the global obesity epidemic .[11]

The environmental facilitators for mass obesity include greater availability of food (freezers and fast food culture) , changing dietary composition (more fat, more refined sugars , more alcohol) , changing eating styles (more snacks than regular food , excessive soft drinks , chocolates) which encourage overeating in relation to physiological needs. More than 600 genes, markers and chromosomal regions have been associated with human obesity.[12] Overconsumption is considered a crucial factor in the development of obesity[13]. An important factor which leads to obesity is 'fast food' consumption on daily basis.[14] A typical fast food meal can contain 2000 kcal and 84 g of fat. Nibbling between meals contribute to the obesity in some females who are found of cooking and of others who work in kitchens. Studies also show that females who take fruits frequently in their diet have low BMI as compare to females who take fruits less frequently.[15,16] Malnutrition in early life is believed to play an important role in the rising rates of obesity in the developing countries.

Studies have also shown that more use of vehicles even for short distances is responsible for the increase prevalence of overweight and obesity.[17] This is perhaps the main cause of increased weight in medical students because they have to do more work in short period of time. No study has yet been conducted in female medical students in Multan. The results of the study will help to develop strategies for prevention of obesity in them so that they can be able to prevent obesity and its adverse effects in general population

MATERIALS AND METHODS

It was an Observational Cross-Sectional study carried out at Nishtar Medical College, Multan during the period October 2015-December 2015.The Objectives of the study were to find out the obesity frequency among female medical students and to create awareness among them about hazards of obesity. A sample of 250 female students was taken by convenient sampling technique,50 students from each class were included in the study. After taking informed consent, all the desired in formations were collected by developing a structured questionnaire. EXCEL and SPSS software were used to analyze data.

Inclusion criteria was female medical students of Nishtar Medical College. Exclusion criteria was students of any other college and male students of Nishtar Medical college. All selected students were questioned regarding dietary pattern and lifestyle behaviors .Obesity was estimated by measuring height and weight and by calculating BMI in kg/m^2. Percentage was calculated for obesity and correlation analysis was done between various variables with statistical significance taken at P <0.5..

RESULTS

According to BMI criteria, 170(68%) students had normal weight while 80(32%) students were obese in varying degree of obesity. (table -1)

Among the obese students,13(16.25%) belonged to first year class and 24(30%) were of final year MBBS class.(table-2) There was very high percentage of obese students59(73.75%) belonging to middle socio economic class than upper class16(20%) and lower class 05 (6.25%).(table-3)

Among the 80 obese students, 06(7.5%) students were taking 2600& above calories/day and majority 42 (52.5%) were consuming 2100-2500 calories, while 24(30%) were taking 1600-2000 calories and 08(10%) were consuming 1000-1500 calories/day(table-4)

Among the obese students , only 10(12.5%) were doing exercise daily, 15(18.75%) were performing exercise2-3times/ week, 22(27.5%) were doing seldom, and 33(41.25%) were not doing any exercise.(table-5)

22 (27.5%)students had positive family history ,while 58(72.5%) had negative family history of obesity.(table-6)

About 13(16.25%) students were consuming fast food once daily and 46(57.5%)were taking 2-3 times per week. 16(20%) were in seldom category and only 05(6.25%) answered never. (Table-7) About 69(86.25%)were using vehicle daily and 11(13.75%) were not in habit of using vehicle. (table -8).

Table No.1: Frequency distribution of obesity on the basis of BMI

Obesity	Number	%age
Non-obese	170	68
Pre-obese	45	18
Obese-I	30	12
Obese-II	5	2
Obese-III	0	0

80(32%) students were obese in varying degree of obesity.

Table No.2: Relationship of obesity on the basis of study of year.

Year of study	Number	%age
1st Year	13	16.25
2nd Year	11	13.75
3rd Year	14	17.5
4th Year	18	22.5
Final Year	24	30

Among the obese students,24(30%) belonged to final year and 13(16.25%) were of first year MBBS class.

Table No.3: Relationship of obesity with socio-economic status.

Socio-economic status	Number	%age
Upper class	16	20
Middle class	59	73.75
Lower class	05	6.25

Very high percentage of obese students 59(73.75%) belonged to middle SE class

Table No.4: Frequency distribution of calories intake.

Calories	Number	%age
1000-1500	08	10
1600-2000	24	30
2100-2500	42	52.5
2600 & above	06	7.5

Most of the students 42(52.5%) were consuming 2100-2500 calories/day

Table No.5: Relationship between obesity & exercise.

Exercise	Obese	
	Number	% age
Daily	10	12.5
2-3times/week	15	18.75
Seldom	22	27.75
Never	33	41.25

Among the obese students , only 10(12.5%) were doing exercise daily

Table No.6: Relationship of obesity with family history.

Family history	Number	%age
Yes	22	27.5
No	58	72.5

22(27.5%) students had positive family history of obesity.

Table No.7: Relationship of obesity with fast food consumption.

Fast food consumption	Number	%age
Daily	13	16.25
2-3 times/week	46	57.5
Seldom	16	20
Never	05	6.25

46(57.5%) students were taking fast food 2-3times/week

Table No.8: Relationship of obesity with vehicle use.

Vehicle use	Number	%age
Daily	69	86.25
Seldom	11	13.75

About 69(86.25%) students were using vehicle daily

DISCUSSION

Obesity is both an individual clinical condition and is increasingly viewed as a serious public health problem. The mechanism by which obesity develops is unknown. The occurrence of obesity is the combined results of interaction between individual biology and environment Various studies in Pakistan predict prevalence of obesity 37%,but obesity is underestimated in pakistan.[18]

In this study out of 250 students,80(32%) were obese and out of the 80 obese students, mostly 24(30%) belonged to final year class. Various studies also revealed incidence of obesity increases with age. In Pakistan in relation to age prevalence is 37% in 25-44 years of age and 40% in 45 -64 Years[19].

Prevalence of obesity was maximum in middle socio-economic class irrespective of residential area, 59(73.75%) students were obese in varying degree in contrast to other studies which give evidence of high BMI in low socioeconomic class, in underdeveloped countries. Lack of education, preference to purchase cheap fast food ,marry to low income status, all evidences are in favor of obesity in low income group.[20]

Regarding calories , most of the students 42(52.5%) were taking between 2100-2500 calories/day. It is well approved in all national and international studies that obesity is strongly associated with consumption of high calories food.[21] Exercise status showed only 10(12.5%) students were doing exercise daily and 33(41.25%) students never do exercise. Many studies show an association of physical activity and obesity.[22] , According to a global survey currently most important leading cause of obesity is sedentary life style alone or in combination with other factors.[23] Pakistanis do not meet the guide line for physical activity index in comparison to other countries.[24]

58(72.5%) students had negative family history while 22(27.5%) had positive family history of obesity. Internationally studies claimed that the risk of obesity is 6 to 8 times higher with positive family history but an article written by Boucherd 2007, argues not always we see the above mentioned rule of family history until other co factors are also present.[25]

Maximum percentage of students 46(57.5%) were consuming fast food 2-3 times per week. Some studies showed that obesity is directly proportional to sweets and fast food consumption, [14] but many interventional and cross sectional studies do not show strong correlation of obesity with sweets and fast food consumption. They found obesity only in susceptible peoples.[26]

Studies have also shown that increased use of vehicles even for short distances is responsible for the increase in prevalence of overweight and obesity.[14] In this study 69(86.25%) students were using vehicle daily.

Different researches are in favor of that not a single cause is responsible for obesity. Many factors along with susceptible personality lead to epidemic of obesity globally.

CONCLUSION

The results of this study suggest that obesity was frequent among female medical students. The main risk factors of obesity identified in this study were middle socio economic status, lack of exercise , overall sedentary life style, with high calories and fast food consumption. The high and rising trends of obesity among female medical students demand attention and urgent health promotion and prevention strategies to

address this problem. Physical educational activity should be encouraged and made a part of medical curriculum.

Recommendation:

- Encouraging balanced diet throughout the life cycle.
- Promoting healthy eating behaviors in life. Use of fruits and vegetables at least five serving per week, eating breakfast daily, encouraging family meal and limiting portion size.
- Limiting television and other screen time.
- Promoting moderate to vigorous physical activity for at least 60 minutes per day.
- Limiting consumption of sugar sweetened beverages and fast food.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Park K. Epidemiology of chronic non-communicable disease and conditions. In: Bhanot BM, editor. Parks textbook of preventive and social medicine. Jabalpur;2013. p.334-82.
2. Papaetis GS, Papakyriakou P, Panagiotou TN. Central obesity, type 2 diabetes and insulin: exploring a pathway full of thorns. Arch Med Sci 2015;11(3):463-82.
3. Haslam DW, James WP. "Obesity". Lancet 2005;366(9492):1197-209.
4. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the united states 2011 – 2012. JAMA 2014;311(8): 806-14.
5. Ford ES, Li C, Zhao G, Tsai J. Trends in obesity and abdominal obesity among adults in the united states from 1999-2008. Int J Obes 2011;35(5): 736-43.
6. Mihardja L, Soetrisno U. Prevalence and determinant factors for overweight and obesity and degenerative diseases among young adults in Indonesia. JAFES 2012;27(1):77-81.
7. Aslam M, SaeedA, Pasha GR, Altaf S. Gender difference of body mass index in adults of Pakistan; A case study of Multan city. Pak J Nutr 2010;9(2):162-66.
8. Jayatissa R, Hossain SM, Gunawardana S, Ranbanda JM, Gunathilaka M, De Silva PC. Prevalence and association of overweight among adult women in Sri Lanka; a national survey. SJDEM 2012;2(2):61-8.
9. Mehmood S, Najjad MKR, Ali N, Yousaf N, Hamid Y. Predictors of obesity among postgraduate trainee doctors working in a tertiary care hospital of public sector in Karachi, Pakistan. J Pak Med Assoc 2010;60(9):758-61.
10. Usutu Y, Ugurlu M, Kasim I, Aslan O, Aksoy YM, Egici MT, et al. High prevalence of obesity in Tokat, a northern province of Turkey. JPMA 2012;62(5):435-40.
11. Malik VS, Willett WC, Hu FB. Global obesity: trends, risk factors and policy implication. Nat Rev Endocrinol 2013;9(1):13-27.
12. Gahagan S. Overweight and obesity. In: Kliegman RM, St Geme JW, Stanton BF, Schor NF, Behraman RE, editors. Nelson textbook of paediatrics. Philadelphia: WB Saunders Company; 2011.p.179-88.
13. Pandit R, De Jong JW, Vanderschuren LJ, Aden RA. Neurobiology of overeating and obesity: The role of melanocortins and beyond. Eur J Pharmacol 2011;660(1):28-42.
14. Fortin B, Yazbeck M. Peer affects, fast food consumption and adolescent weight gain. J Health Econ 2015;42:125-38.
15. Thompson AK, Minihane AM, Williams CM. Trans fatty acids and weight gain. Int J Obes 2011;35(3):315-24.
16. Ahmed J, Laghari A, Naseer M, Mehraj V. Prevalence of and factors associated with obesity among Pakistani school children: a school-based, cross-sectional study. EMHJ 2013;9(3):242-7.
17. Chaudhry MA, Ahmad F, Ashraf MZ. Frequency of overweight and obesity in students of medical college of Lahore. Ann Pak Inst Med Sci 2012; 8(2):137-40.
18. Aslam M, Saeed A, Pasha GR, Altaf S. Gender differences of BMI in adults of Pakistan, a case study of Multan city. Pak J Nutrition 2010;9 (2):162-166.
19. Nathan DJ. The Obesity Pandemic - Implications for Pakistan. J Pak Med Assoc 2002;52:342.
20. Monteiro CA, Conde WL, Barry M, Popkin. Independent Effects of Income and Education on the Risk of Obesity in the Brazilian Adult Population. J Nutrition 2001;131:881-886.
21. Streib, Lauren.. World's Fattest Countries 2007."Forbes. http://www.forbes.com/2007/02/07/ worlds -fattest-countries- forbeslife-cx_ls_0208 worldfat_2.html.
22. Bell JA, Hamer M, Batty GD, Singh-Manoux A, Sabia S, Kivimaki M. Combined effect of physical activity and leisure time sitting on long-term risk of incident obesity and metabolic risk factor clustering. Diabetologia 2014;57(10):2048-56.
23. Chau JY, Van der Ploeg HP, Merom D, Chey T, Bauman AE. Cross-sectional associations between occupational and leisure-time sitting, physical activity and obesity in working adults. Prev Med 2012;54(3-4):195-200.
24. Iqbal F, Naz R. Obesity in Pakistan. Pak J Med Res 2001;4(1).
25. Bouchard C. The biological predisposition to obesity, beyond the thrifty genotype scenario. Int J Obesit 2007;31:1337-9
26. Gibson S. Sugar-sweetened soft drinks and obesity, a systematic review of the evidence from observational studies and interventions. Nutrition Res Rev 2008; 21(2), 134-47.

Original Article

Inguinal Hernia's Repair

Tension-Free Mesh Repair of Inguinal Hernias; The Lichtenstein Technique – A Feasible Option

Mohammad Ayub Jat
Asstt. Prof. of Surgery, Ghulam Muhammad Maher Medical College, Sukkur. Sind

ABSTRACT

Objective: The objective of study is to determine the feasibility, safety and effectiveness of Lichtenstein technique the tension free mesh repair of inguinal hernias and also to record the early and late postoperative complications of hernioplasty.
Study Design: Prospective study
Place and Duration of Study: This study was carried out at the Department of Surgery, Unit-I Glulam Muhammad Maher Medical College Teaching Hospital, Sukkur from May 2009 to April 2012.
Materials and Methods: Total 210 male patients with inguinal hernias admitted through OPD as an elective cases were included in this study .Exclusion criteria was patients with obstructed inguinal hernia came in emergency department. Clinically 20% patients had bilateral and 80 % had one sided inguinal hernias. Mean age of the patient was 45 years (range, 18– 70 years) .189 patients (90%) were operated under spinal anesthesia while remaining 21 patients (10%) under general anesthesia .All patients were follow-up for the period of 2 years in the out –patient department .
Results: The present study showed the early and late postoperative complications (morbidity = 8%) such as retention of urine in 4 patients (2%), Hematoma in 3 patients (1.5%), seroma formation in 2 patients (1 %) , Cord induration in 3 patients (1.5 %) and postoperative neuralgia in 4 patients(2%). After a follow-up for 2 years, there was no evidence of recurrence or mesh rejection.
Conclusion: This study showed that Lichtenstein tension- free hernioplasty is a simple, feasible and safe technique for repair of inguinal hernias with a low recurrence rate and low morbidity .
Key Words: Inguinal hernias, Lichtenstein tension free hernioplasty, Recurrence

Citation of article: Jat **MA. Tension- Free Mesh Repair of Inguinal Hernias; The Lichtenstein Technique – A Feasible Option. Med Forum 2016;27(2):7-9.**

INTRODUCTION

Hernia repair is one of the common surgical procedure performed in general surgical practice .Recurrence following repair of inguinal hernias without mesh is a common problem so to reduce the incidence of recurrence it is recommended that apply a mesh for repair of inguinal hernias. Tension –free hernioplasty, a term coined by Irving L. Lichtenstein, MD, began in June 1984 at the Lichtenstein Hernia Institute.[2] This technique is a tension free repair of inguinal hernias with a low recurrence rate.
The studies by stoppa, et al[3] and by Lichtenstein [4], as well as the innovation of laparoscopic hernia repair[5,6]showed that tension free hernioplasty has many advantages, such as simplicity, effectiveness, safety, rapid returned to normal activities and low recurrence rate . Recurrence, the ultimate nightmare to health care costs and pose a further economic burden .Berliner noted that follow –up of hernia is notoriously difficult; the dissatisfied patient will seek medical services elsewhere.[7]

Correspondence: Dr. Mohammad Ayub Jat
Asstt. Prof. of Surgery, Ghulam Muhammad Maher Medical College, Sukkur. Sind
Cell No.: 0300-3144503
E-mail: dr.ayubjat@yahoo.com

MATERIALS AND METHODS

The present prospective study was conducted in the Department of Surgery, unit-I at Ghulam Muhammad Maher Medical College Teaching Hospital, sukkur during the three years period from May 2009 to April 2012.
Inclusion criteria was 210 male patients with inguinal hernias admitted in surgical ward through OPD. Exclusion criteria was obstructed inguinal hernia came in emergency department. .On admission, all patients were clinically evaluated and findings were recorded on proforma. The mean age of patient was 45 years (range, 18–70 years). Clinically 20% patients had bilateral and 80 % had one sided Inguinal hernias. There were 95 (45%) right-sided hernias and 73 (35%) left sided hernias. In 189 patients (90%) hernioplasty was done under spinal anesthesia whereas 21 patients (10%) under general anesthesia. All operative findings and steps of operations were recorded in operative notes.

In Lichtenstein technique a polypropylene mesh (6 x 11 cm) was kept in the floor of the inguinal canal and sutured with 2/0 prolene. It is important to keep the mesh slightly relaxed and not to taut to compensate for the patient stand up and to compensate for the inevitable future shrinkage of approximately 20% .[8]To minimizing recurrence the mesh extending 2cm medial to pubis tubercle and 3 cm lateral to the internal ring . 13 All patients were given 1 doses of cefotaxime sodium 1 g intravenous as prophylactic antibiotics at the time of induction and then 2 dose postoperatively with interval of 8 and 16 hours. Postoperative analgesia was given in the form of paracetamol or NSAIDS. The duration of the hospitalization was 2 -3 days. Post operatively all patients were urged to attend the follow –up in out- patient department at 3 months, 6 months and then yearly .

RESULTS

Total of 210 male patients with inguinal hernias admitted in surgical ward through OPD were included. The mean age of the patient was 45 years (range, 18 – 70 years). The early postoperative complications occurred (morbidity = 8 %) such as acute urinary retention in 4 patients (2%), Seroma in 2 patients (1%) , hematoma in 3 patients (1.5 %) , Cord induration in 3 patients (1. 5 %) and postoperative neuralgia in 4 patients (2 %) patients .The Late complications such as Sinus formation , ischemic orchitis, and testicular atrophy were not seen in any case .After a follow up for 2 years there was no evidence of recurrence or mesh rejection as showed in table 1 .

The average operating time was 40 minutes (range 30 – 60 minutes). The operative findings were recorded i.e indirect hernia in 60 % of cases (126) , direct in 37 % (78) and of the pantaloons (mixed) type in 3 % (6) as shown in Table -2.

Table No.1: The early and late postoperative complications of hernioplasty .

Name of complication	No of patients	Percentage
Early		
Retention of urine	4	2 %
Seroma formation	2	1 %
Wound hematoma	3	1.5 %
Cord induration	3	1.5 %
Postoperative neuralgia	4	2 %
Late		
Testicular atrophy	0	0 %
Sinus formation	0	0 %
Recurrence	0	0 %
Mesh rejection	0	0 %

Table No.2: The distribution of hernia according to clinical and operative findings.

Types of hernia	Total patients n = 210	Percentage
On Clinical finding		
Rt sided hernias	95	45 %
Lt sided hernias	73	35 %
Bilateral hernias	42	20 %
On operative finding		
Indirect hernias	126	60 %
Direct hernias	78	37 %
Pantaloons hernias	6	3 %

DISCUSSION

Hernia repair is the common surgical procedure performed in the surgical practice.6Advanced surgical techniques, using prosthetic materials prolene mesh have significantly improved outcomes of patients. Edgardo Bassini (1844-1924) is credited for developing and performing the first modern hernioplasty. He had a clear insight into the anatomy and physiology of the inguinal region .His landmarks series ignited the enthusiasm of surgeons worldwide .[9] In reality the authentic Bassini operation includes deliberate and complete dissection to expose the anatomy in its entirety ,a repair in which the internal oblique muscle ,the transverses abdominal muscle ,and the transversalis fascia (Bassini triple layer) were approximated to both the femoral sheath and the enrolled edge of the inguinal ligament with interrupted sutures . [10] Pre –Bassini, best centers in Europe and North America reported mortality rates of 7% and recurrence rate after one year was 30-40 % .[11] Bassini s astounding result earned him the name of " Father of Modern Herniorraphy . The period 1880 to 1890 can justifiably be termed "the decade of hernia surgery ".He reported 206 cases with zero mortality and 5 years follow –up a recurrence of 2.7 %. 11 In 1953 Earle shouldice modified the pristine Bassini repair with less than 1% recurrence at 10 years .[12] It has become the " gold standard " worldwide , by which other hernioplasties are compared .The shouldice technique employs complicated double breasting repair , ,time consuming and stiches under tension cause pain with all movements and restricts physical activity for several weeks . Randomized trials shows recurrence rate of 4- 10 % and increase in the incidence (40 %) of femoral hernias following the shouldice repair. [13] Despite its popularity in the "80 and '90s less than 20 % consultant surgeons were employing this technique in the U.K.[14] The Lichtenstein technique is very simple ,effective, is associated with a very low recurrence rates (ranging from 0 to 2 % in the literature) and can be performed under local or regional anesthesia.[15,16] The method is simple, can be performed by all the surgeons – even those without special interest in hernia surgery. In this study the overall morbidity was 8% and recurrence rate was 0 %. In other studies

by G H Sakorafas, et al, and M Aurangzeb the overall morbidity was 2.5%, 12 % and recurrence rate (0.2 %), 0 % respectively.[17,18] Lichtenstein reported an infection rate of less than 0.3% so the belief that the risk of infection is high is unfounded .[19]

CONCLUSION

The present study showed that Lichtenstein tension free hernioplasty is a simple, safe, and feasible option with a very low recurrence rate and low morbidity. Therefore this technique can also be performed by non-hernia specialist General Surgeon.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Lichtenstein IL, Shulman AG, Amid PK. The cause, prevention, and treatment of recurrent groin hernia. Surg Clin North Am 1993;73(3):529-44
2. Read RC. A review: The role of protease – antiprotease imbalance in the pathogenesis of Herniation and Abdominal Aortic Aneurysm in certain smokers. Postgraduate Gen Surg 1999 :14; 161 -165.
3. Stoppa R, Petit J, Henry X. Unsutured Dacron prosthesis in groin hernias. Into Surg 1975;60(8): 411- 2.
4. Liechtenstein IL, Shulman AG, Amid PK, et al. The tension free hernioplasty. Am J Surg 1989; 157(2):188-93.
5. Popp LW. Endoscopic patch repair of inguinal hernia in a female patient. Surg Endosc 1990;4(1); 10-12.
6. Ram Shaw BJ, Tucker JG, Duncan TD. Laparoscopic herniorraphy; a review of 900 cases. Surg Endosc 1996;10:255
7. Berliner RC. An approach to groin hernia. Surg Clin North Am 1984;64(2)197-213.
8. Shouldice EE. The treatment of hernia .Ontario Med Rev 1953:20:670-684.
9. Bassini E. Nuevo method per la cura radicale dell hernia inguinal. Attic Conger Associ Med Ital 1887;2:179-182.
10. Waltz GE. The operation of Bassini as described by Attillio Catherina. Surg Gynecol Obstetric 1989;168(1):67- 80.
11. Lucas –Championniere J. Chirurgie Operatoire C. ure Radicale des Hemics. Paris France Ruff ET Cie; 1892.
12. Glassow Inguinal hernia repair using local anesthesia. Ann R Cull Surg Engle 1984;66(6): 382-387.
13. Amid PK, Shulman AG, Lichtenstein IL. Femoral hernia resulting from inguinal herniorraphy; The plug repair. Contemp. Surg 1991;39:19-24.
14. Morgan M, et al. Are current techniques of inguinal hernia repair optimal? A survey in the United Kingdom. Ann R Coll Surg Eng 1992;73: 341-345.
15. Kurzer M, Belsham PA, Karuk AE. The L Lichtenstein repair. Surg Clan North Am 1998;789 (6):1325-46.
16. Goldstein HS. Selecting the right mesh. Springer-Verlag 1999;3(1):23-26.
17. Sakorafas GH, Halikias L, et al. Open tension free repair of inguinal hernias; the Lichtenstein technique. BMC Surg 2001;1(3).
18. Aurangzeb M. Tension –free mesh Hernioplasty: A Review of 96 cases. JPMI 2011;18(1).www.jpmi. org.pk/index.php/jpmi/article/download/851/760
19. Lichtenstein IL. Herniorraphy: a personal experience with 6321 cases. Ann J Surg 1987;153 (6):553-9.

Original Article

Fatty Liver in Diabetic

Non-Alcoholic Fatty Liver Disease in Type 2 Diabetes: Effect on Diabetic Control and Lipid Profile

1. Umar Farooq Dar 2. Tasneem Majeed 3. Naima Umar 4. Ujala Nayyar

1.Asstt. Prof. of Community Medicine, GMC, Gujranwala 2. Asstt. Prof. of Pathology, FJMC, Lahore, 3. Asstt. Prof. of Physiology, GMC, Gujranwala 4.Provincial Surveillance Officer, Polio Eradication Initiative Punjab.

ABSTRACT

Objective: To determine the frequency of non-alcoholic fatty liver disease in patients with type 2 diabetes and to compare the diabetic control and dyslipidemia in diabetic patients with and without non-alcoholic fatty liver disease.

Study Design: Cross sectional study.

Place and Duration of Study: This study was conducted in Outpatient Department of Medicine, Jinnah Hospital, Lahore from 31st August 2013 to 28th February 2014.

Materials and Methods: Both male and female with age ranging from 30-60 years having type 2 diabetes for more than 5 years duration were included. Two hundred and sixty diagnosed diabetes patients presenting to outpatient department were enrolled. Screening was done for NAFLD on the basis of ultrasonography. Sampling for HbA1C and lipid profile was done. Data was analyzed using SPSS 17.

Results: Proportion of NAFLD was quite high i.e. 70%. Seventy five patients (28.8%) had abnormal triglyceride level and 72 patients (27.7%) had raised serum cholesterol. Low density lipoprotein was abnormally high in all individuals and high density lipoprotein was low in all individuals. One hundred and six (40.8%) diabetics patients had good control while rest has poor control and 135 patients (51.1%) were obese.

Conclusion: Proportion of NAFLD was quite high in diabetic patients. We should screen every patient for NAFLD as it may reduce the co morbidity.

Key Words: Type II diabetes, Nonalcoholic fatty liver disease, Dyslipidemia, Obesity, Glycosylated Hemoglobin

Citation of article: Dar UF, Majeed T, Umar N, Nayyar U. Non-Alcoholic Fatty Liver Disease in Type 2 Diabetes: Effect on Diabetic Control and Lipid Profile. Med Forum 2016;27(2):10-13.

INTRODUCTION

In 2010 the diabetes world prevalence is about 6.4% among adults (aged 20-79 years) with 285 million adults affected. This will increase by 2030 to 7.7% and 439 million adults. In developing countries between 2010 and 2030, number of adults with diabetes will be increased by 69% and in developed countries there will be 20% increase.[1] Same is the case with Non-alcoholic fatty liver disease (NAFLD) which in western countries is the most common cause of chronic liver disease and has reached epidemic proportions. In western countries, NAFLD is present in approximately 20-30% of adults in general population and among persons who are obese or have diabetes its prevalence increases to 70-90%.There is also increased risk for the development of advanced fibrosis and cirrhosis in such patients.[2]

Type 2 diabetes mellitus (T_2 DM) and NAFLD both are linked with harmful outcomes of the other, T_2 DM is a threat for advanced liver disease and liver allied death in patients with NAFLD, whereas in individuals with T_2 DM, NAFLD may be the sign of cardiovascular risk and mortality[3]. The severity of liver disease is linked with the presence of multiple metabolic abnormalities such as fibrosis accompanying non-alcoholic steatohepatitis (NASH), cryptogenic cirrhosis and ultimately with the hepato cellular carcinoma (HCC). Although the major cause for excess mortality in T_2 DM is cardiovascular risk, the danger of advanced liver disease should no longer be under estimated[4]. In general population of Pakistan the prevalence of NAFLD is 18%. There is a high risk of atherosclerosis in patients with NAFLD regardless of classical cardiovascular risk factors and metabolic syndrome[5].

In a study to determine the prevalence and the metabolic impact of NAFLD in 120 patients with type 2 diabetes mellitus, Seventy three (60.8%) patients had fatty liver whereas forty seven (39.2%) had no fatty liver on ultrasonography. When both groups i.e. first with diabetes and NAFLD and second diabetic group without NAFLD were compared, the outcome levels in first and second group respectively came out as BMI 30.17±3.92 and 23.7±2.55, HbA1c 8.29±088 and 7.02±0.47, Total Cholesterol 198.12±47.6 and

Correspondence: Dr. Umar Farooq Dar,
Asstt. Prof. of Community Medicine, Gujranwala Medical College, Gujranwala.
Cell No.: 03214035099
E-mail: umardar84@gmail.com

158.53±41.84 (mg/dl), TG 244.8±128.9 and 117.49±39.29 (mg/dl),LDL 120.1±48.6 and 90.72±34.63 (mg/dl) and LDL 120.1±48.6 and 90.72±34.63 (mg/dl) All the differences were significant showing poor diabetic control and dyslipidemia.[6-8] To decrease liver fat accumulation and prevent the development of non-alcoholic steatohepatitis, cirrhosis or hepatocellular carcinoma and their associated complications, this category of patients should be advised regular checking of blood glucose levels and liver function tests.[9-10]

All the available studies for Pakistani population which differs from developed nations in lifestyle and dietary habits a hall mark of treatment of NAFLD and T2DM are done on smaller population groups i.e. ranging from 100 to 120 patients. Current study will replicate above findings in a larger population. Early detection of NAFLD and dyslipidemia in diabetics will subsequently help prevent morbidities related to atherosclerosis and fatty liver.

MATERIALS AND METHODS

This cross sectional survey was conducted in Outpatient Department of Medicine, Jinnah Hospital, Lahore over a period of 6 months from 31st August 2013 to 28th February 2014. Both male and female with age ranging from 30-60 years having type 2 diabetes for more than 5 years duration were included. Patients with positive HBsAg, Anti HCV, ANA, abnormal TSH determined and a body mass index above 30 kg/m2, history of cerebrovascular accidents and acute or autoimmune hepatitis were excluded. Two hundred and sixty diagnosed diabetes patients presenting to outpatient department were enrolled. Screening was done for NAFLD on the basis of ultrasonography. Included patients were called next day with a 12 h overnight fast. Screening was done for NAFLD on the basis of ultrasonography. Sampling for HbA1C and lipid profile was done by a trained nurse under aseptic conditions. Body mass index was calculated and treated as effect modifiers as it had a non-proportionate effect on both diabetes and NAFLD. On basis of NAFLD screening the participants were divided into two groups i.e. one with NAFLD and other with no NAFLD and subsequently compared. Sampling for HbA1C and lipid profile was done. Data was analyzed using SPSS 17. Chi square and Independent sample t-test were used to analyze the categorical and numerical data respectively. P ≤0.05 was considered to be statistically significant.

RESULTS

There were 136 (52.3%) were male while 124 (47.3%) were female with mean age of the patients were 43.5±8.5 years. Nonalcoholic fatty liver disease was present in 70% individuals. Seventy five patients (28.8%) had abnormal triglyceride level, 72 patients (27.7%) had raised serum cholesterol, low density lipoprotein was abnormally high in all individuals and high density lipoprotein was low in all individuals. One hundred and six (40.8%) diabetics patients had good control while rest has poor control and 135 patients (51.1%) were obese (Table 1). When gender was compared with NAFLD there came out a non-significant difference (Table 2). Similarly there was non-significant association between triglycerides and serum cholesterol with NAFLD (Tables 3-4). However fatty liver disease was significantly associated with un-controlled diabetes when compare to patients with good control. Nonalcoholic disease found more in obese diabetic patients (Tables 5-6)

Table No.I:Demographic information of the patients

Variable	No.	%
Gender		
Male	136	52.3
Female	124	47.7
Non Alcoholic Fatty Liver Disease		
Yes	182	70.0
No	78	30.0
Triglycerides level		
Normal	185	71.2
Abnormal	75	58.8
Cholesterol level		
Normal	188	72.3
Abnormal	72	27.7
Low Density Lipoprotein level		
Normal	260	100.0
Abnormal	-	-
High Density Lipoprotein level		
Normal	-	-
Abnormal	260	100.0
Obesity		
Non-obese	125	48.1
Obese	135	51.9

Table No.2: Comparison of gender according to non-alcoholic fatty liver disease

Gender	Non-alcoholic fatty liver disease	
	Yes	No
Male	95	41
Female	87	37

Using chi square, p value = 0.883 (Non-significant)

Table No.3: Comparison of triglycerides according to non-alcoholic fatty liver disease

Triglycerides	Non-alcoholic fatty liver disease	
	Yes	No
Normal	135	50
Abnormal	47	28

Using chi square, p value = 0.109 (Non-significant)

Table No.4: Comparison of cholesterol according to non-alcoholic fatty liver disease

Cholesterol	Non-alcoholic fatty liver disease	
	Yes	No
Normal	128	60
Abnormal	54	18

Using chi square, p value = 0.257 (Non-significant)

Table No.5: Comparison of HbAIC according to non-alcoholic fatty liver disease

HbAIC	Non-alcoholic fatty liver disease	
	Yes	No
Good	61	45
Poor	121	33

Using chi square, p value = 0.01 (Significant)

Table No.6: Comparison of obesity according to non-alcoholic fatty liver disease

Obesity	Non-alcoholic fatty liver disease	
	Yes	No
Obese	122	13
Non-obese	60	65

Using chi square, p value = 0.001 (Significant)

DISCUSSION

Clinical and pathological commodity named non-alcoholic fatty liver disease (NAFLD) histologically has features that are similar to alcohol induced liver injury, but patients of NAFLD has no history of alcohol intake.[1-5] Histologically it encloses a spectrum that categorises from fat deposition in liver cells without associated inflammation or fibrosis (Simply hepatic steatosis) to hepatic steatosis with necrosis and inflammatory element (Steato-hepatitis) with or without concomitant fibrosis.[12-21] The last entity referred as non-alcoholic steato-hepatitis (NASH) in upto 20% of patients may develop into cirrhosis.[7,11,]

Current study has revealed different aspects of NAFLD. There is almost equal distribution of male and female in our diabetic sampled population showing equal effect of growing epidemic of diabetes in our developing countries. Patients' mean age was 43.5 years ranging from 30-60 years showing early start of diabetes. Diabetes is more affecting our younger generation. So there is a need of preventing programs to counter this menace. Currently we are facing double burden of disease both infectious and noninfectious. We are still unable to cater the patients coming with chronic liver disease and here we find a rising prevalence of NAFLD and associated diabetes.

Proportion of NAFLD was quite high i.e. 70% such a high percentage is alarming as in non-diabetic individuals it is about 15%, so all the diabetic should be screened for presence of fatty liver. Sampled population lipid profile including fasting cholesterol, triglycerides, low and high density lipoproteins was assessed. Cholesterol and triglycerides level were abnormal in one third population proximately but on the other hand hundred percent individual have abnormal low density protein and abnormal high density protein.[22-26]

CONCLUSION

Proportion of NAFLD was quite high in diabetic patients and ultrasonography which ideally should be by liver biopsy. We should screen every patient for NAFLD as it may reduce the co morbidity.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Research and Clinical Practice 2010;87 (1):4-14.
2. Mohammadi A, Sedani HH, Ghasemi-Rad M. Evaluation of carotid intima-media thickness and flow-mediated dilatation in middle-aged patients with nonalcoholic fatty liver disease. Vascular Health and Risk Management 2011:7 661–665.
3. Smith BW, Adams LA. Nonalcoholic fatty liver disease and diabetes mellitus: pathogenesis and treatment. Nature Reviews Endocrinol 2011;7: 456-65.
4. Bugianesi E. Diabetes and NAFLD: Why is the Connection Important?, in Non-Alcoholic Fatty Liver Disease: A Practical Guide In: Farrell GC, McCullough AJ, Day CP, editors. Wiley-Blackwell: Oxford UK; 2013.
5. World Gastroenterology Organization Global Guidelines Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis June 2012.
6. Luxmi S, Sattar RA, Ara J. Association of Non Alcoholic Fatty Liver with type 2 Diabetes Mellitus. J Liauq Univ Med Health Sci 2008; 188-93.
7. Amarapurkar DN, Hashimoto E, Lesmana LA, Sollano JD, Chen PJ, Goh KL. How common is non-alcoholic fatty liver disease in the Asia Pacific region and are there local differences? J Gastroenterol Hepatol 2007; 22: 788-93.
8. Fan JG, Li F, Cai XB, Peng YD, Ao QH, Gao Y, et al. The importance of metabolic factors for the increasing prevalence of fatty liver in Shanghai factory workers. J Gastroenterol Hepatol 2007; 22: 663-8.
9. Amarapurkar D, Kamani P, Patel N, Gupte P, Kumar P, Agal S, et al. Prevalence of non-alcoholic fatty liver disease: population based study. Ann Hepatol 2007;6(3):161–3.
10. Wagenknecht LE, Scherzinger AL, Stamm ER, Hanley AJ, Norris JM, Chen YD, et al. Correlates and heritability of nonalcoholic fatty liver disease in a minority cohort. Obesity 2009;17:1240–6.

11. Söderberg C, Stål P, Askling J, Glaumann H, Lindberg G, Marmur J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatol 2010;51: 595–602.
12. Chitturi S, Farrell GC, Hashimoto E, Saibara T, Lau GK, Sollano JD, et al. Nonalcoholic fatty liver disease in the Asia-Pacific region: definitions and overview of proposed guidelines. J Gastroenterol Hepatol 2007; 22: 778-87.
13. Ramesh S, Sanyal AJ. Evaluation and management of non-alcoholic steatohepatitis. J Hepatol 2005; 42: S2-12.
14. Akinci E, Dogan NO, Gumus H, Akilli NB. Can we use serum gamma-glutamyl transferase levels to predict early mortality in stroke? Pak J Med Sci 2014;30(3):606-10.
15. Gurbuzer N, Gozke E. Gamma-glutamyl transferase levels in patients with acute ischemic. Stroke 2014; 170626.
16. Korantzopoulos P, Tzimas P, Kalantzi K, Kostapanos M, Vemmos K, Goudevenos J, et al. Association between serum γ-glutamyltransferase and acute ischemic nonembolic stroke in elderly subjects. Archives of medical research. 2009; 40(7):582-9.
17. Kim SY, Guevara JP, Kim KM, Choi HK, Heitjan DF, Albert DA. Hyperuricemia and risk of stroke: A systematic review and meta-analysis. Arthritis Care & Research 2009;61(7):885-92.
18. Shimizu Y, Imano H, Ohira T, Kitamura A, Kiyama M, Okada T, et al. γ-Glutamyl transpeptidase and Incident Stroke Among Japanese Men and Women The Circulatory Risk in Communities Study (CIRCS). Stroke. 2010;41(2): 385-8.
19. Katsiki N, Papanas N, Fonseca VA, Maltezos E, Mikhailidis DP. Uric acid and diabetes: Is there a link? Current pharmaceutical design. 2013;19(27): 4930-7.
20. Fujiyoshi A, Miura K, Hozawa A, Murakami Y, Takashima N, Okuda N, et al. γ-Glutamyl transferase and mortality risk from heart disease and stroke in Japanese men and women: NIPPON DATA90. CVD Prevention and Control. 2010; 5(1):27-34.
21. Weikert C, Drogan D, di Giuseppe R, Fritsche A, Buijsse B, Nothlings U, et al. Liver enzymes and stroke risk in middle-aged German adults. Atherosclerosis 2013;228(2):508-14.
22. Ayhan Basturk Z, Kunutsor SK, Apekey TA, Khan H. Liver enzymes and risk of cardiovascular disease in the general population: A meta-analysis of prospective cohort studies. Cardiovascular psychiatry and neurology. 2014;236(1):7-17.
23. Aries MJ, Elting JW, De Keyser J. Cerebral autoregulation in stroke: a review of transcranial Doppler studies. Stroke 2010; 41:2697.
24. Matarin M, Singleton A, Hardy J, Meschia J. The genetics of ischaemic stroke. J Intern Med 2010; 267:139-43.
25. Simard JM, Kent TA, Chen M. Brain oedema in focal ischaemia: molecular pathophysiology and theoretical implications. Lancet Neurol 2007; 6:258-63.
26. International Stroke Genetics Consortium (ISGC), Wellcome Trust Case Control Consortium 2 (WTCCC2), Bellenguez C. Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke. Nat Genet 2012; 44:328-34.

Original Article

Malaria in Dengue Patients

Malia in Dengue

1. Naresh Kumar Seetlani 2. Pyar Ali 3. Asif Ali

1. Asstt. Prof. of Medicine, Dow Medical College, DUHS, Karachi 2. Asstt. Prof. of Medicine, Dow Medical College, DUHS, Karachi 3. Registrar of Medicine, Civil Hospital, Karachi

ABSTRACT

Objective: To determine frequency of malaria in dengue patients

Study Design: Cross sectional study

Place and Duration of Study: The study was carried out in the Department of Medicine, Civil Hospital Karachi from June 2008 to December 2008.

Materials and Methods: 274 dengue patients with Ig M antibody positive results were included in the study after informed consent Malaria parasite peripheral film and malarial ICT (immunochromatography) test of all these dengue patients were performed in order to detect the presence of co-infection with malaria. Age and sex of the patients were also noted.

Results: In the study, out of 274 dengue patients, 189 (69%) were males and 85 (31%) were females. Mean age was 31.01± 14.83 years. 41 (15%) dengue patients had concomitant malarial infection. Out of these 41 dengue-malaria co-infected patients, 29 (10.6%) had plasmodium vivax; 8 (2.9%) had plasmodium falciparum and 4 (1.5%) had both falciparum + vivax.

Conclusion: Patients with dengue fever are predisposed to have concomitant malarial infection. Hence, these must be investigated for malaria as undiagnosed and untreated malaria can increase the morbidity and mortality of the dengue patients.

Key Words: Dengue, Malaria, Thrombocytopenia

Citation of article: Seetlani NK, Ali P, Ali A. Malaria in Dengue Patients. Med Forum 2016;27(2):14-16.

INTRODUCTION

Malaria parasite and dengue virus are two different organisms, transmitted by two different types of mosquitoes i.e. female anopheles and aedes aegypti respectively. Despite the difference in causative organisms and the causative vector species, dengue-malaria co-infection is not uncommon worldwide[1].

It remains unclear whether the dual malarial and viral infections[6-12] are independent of each other or the immunodeficiency state caused by the viral infections leads to the reactivation of the dormant malarial hypnozoites in the liver, resulting in relapse of malaria.

Dengue is the most common flaviviral disease transmitted globally. Dengue virus has four distinct serotypes (DEN 1, DEN 2, DEN 3 and DEN 4). It is transmitted by Aedes aegypti mosquito.[13]

Dengue cases can be divided into probable and suspected cases. Probable case of dengue includes patients having fever, nausea, vomiting, petechial rash, bone and joint pains, hemorrhagic manifestations, leucopoenia and thrombocytopenia. Confirmed case of dengue fever requires isolation of dengue virus or dengue antibodies from serum, cerebrospinal fluid or autopsy tissues of the affected patients.[13]

In our country, diagnosis of dengue fever is mostly confirmed by demonstration of anti dengue IgM or IgG antibodies in the serum of the affected patients by ICT (immunochromatography).

Malaria is a parasitic infection caused by four species of genus plasmodium: falciparum, vivax, ovale and malariae. Malaria is transmitted by the female anopheles mosquito.[14]

Life cycle of malaria parasite has different stages of development including sporozoites, merozoites, hypnozoites, trophozoites and schizonts. Malarial parasites, especially plasmodium vivax and ovale remain dormant in the liver in the form of hypnozoites and these hypnozoites may reactivate any time even after years to cause relapse of malaria.[14]

Diagnosis of malaria is mostly done by thick and thin film microscopy for malaria parasite. The well recognized limitations of malarial peripheral film include poor quality of microscopy, particularly at the peripheral level. The accuracy of malarial microscopy is absolutely dependent on the microscopist reading malarial peripheral film. He may be a Professor involved in teaching and research or simply an inadequately trained technician involved in malaria microscopy. To overcome such limitations and for counter checking results of malarial microscopy, malarial immunochromatography (ICT) test is done.[8]

Apart from dengue virus, co-infection of malaria is also seen with other viruses like Human immunodeficiency virus[6], hepatitis A, B, C, D, E virus[7-8], Ebstein Barr virus,[9] parvo virus B19,[10] and herpes virus[11-12] etc.

It remains unclear whether the dual malarial and viral infection is independent of each other or the immunodeficiency state caused by the viral infection

Correspondence: Dr Naresh Kumar Seetlani,
Asstt. Prof. of Medicine, Medical Unit II Dow Medical College, Civil Hospital Karachi
Cell No.: 0345-3962509
E-mail: drnaresh2004@yahoo.com

leads to the reactivation of the dormant malarial hypnozoites in the liver, resulting in relapse of malaria.

A previous study was conducted in a tertiary care hospital in Karachi which showed 26 (23.1%) patients out of 78 were concomitantly positive for malaria and diagnosis was based on malarial thin and thick films only.[1]

While, this study has done malarial thin and thick film microscopy along with malarial ICT for more accurate diagnosis of malaria and included 274 patients which is a good number as compared to the previous study.

MATERIALS AND METHODS

This is a cross sectional study conducted in department of Medicine Civil Hospital Karachi after ethical permission from June 2008 to Dec 2008 for a period of six months. Patient of more than 08 years of age of either sex having seropositive (Dengue IgM and ICT method) status for dengue were selected by non probablity purposive sampling, while probable or suspected cases of dengue fever (not serologically confirmed by the presence of IgM antibodies) were excluded from the study.

Sample size was calculated from a previous study using open EPI website and the data was analysed through SPSS v.16. The frequency and percentage were computed for gender and malarial infection in dengue patients. Mean and standard deviation were estimated for age. Stratification was done to control effect modifier like age and gender to observe the effect on the outcome variable through chi-square test. $p< 0.05$ was taken as significant.

Operational Definition

Dengue: Patients with dengue IgM antibody positive results are considered to have dengue infection.

Malaria: Patients with malarial peripheral film or malarial ICT positive results are considered to have malarial infection.

RESULTS

In our study (Table 1), out of 274 dengue patients, 189 (69%) were males and 85 (31%) were females. Mean age was 31.01± 14.83 years. 41 (15%) dengue patients had concomitant malarial infection. Out of these 41 dengue-malaria co-infected patients, 29 (10.6%) had plasmodium vivax; 8 (2.9%) had plasmodium falciparum and 4 (1.5%) had both falciparum + vivax.

Table No.1: Type of infections and age-descriptive analysis

Type of infection	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Only Dengue	233	30.62	14.191	.930	28.79	32.45	12	74
Falciparum	8	30.00	16.562	5.855	16.15	43.85	18	50
Vivax	29	32.93	18.908	3.511	25.74	40.12	8	55
Falciparim+Vivax	4	42.00	16.000	8.000	16.54	67.46	18	50
Total	274	31.01	14.831	.896	29.25	32.77	8	74

DISCUSSION

This study included 274 dengue IgM antibody positive patients which were tested for concomitant malarial infection by both MP peripheral film and MP ICT methods.

In this study, out of 274 dengue patients, 189 (69%) were males and 85 (31%) were females. Mean age was 31.01± 14.83 years. 41 (15%) dengue patients had concomitant malarial infection. Out of these 41 dengue-malaria co-infected patients, 29 (10.6%) had plasmodium vivax; 8 (2.9%) had plasmodium falciparum and 4 (1.5%) had both falciparum + vivax.

Malarial parasite and dengue virus are two different organisms, transmitted by two different types of mosquitoes i.e. female anopheles and aedes aegypti respectively. Despite the difference in causative organisms and the causative vector species, dengue malaria co-infection is not uncommon worldwide[1-5].

In 2009, a previous study titled as 'clinical features, diagnostic techniques and management of dual dengue and malaria infection' was conducted in a tertiary care hospital in Karachi and it showed that out of 78 dengue positive patients, 26 patients (23.21%) were concomitantly infected with malaria and the diagnosis of malaria was based on MP peripheral film only.[1]

However, this study utilizes malarial thin and thick film microscopy along with malarial ICT method for more accurate diagnosis of malaria. Moreover, the sample size of this study is 3.5 times the previous study.

Currently, diagnosis of dengue fever by ICT or ELISA costs around one thousand Pakistani rupees from majority of the private labs. Moreover, dengue is a self-limiting infection that requires only symptomatic treatment in majority (but not all) of the patients.

This study gives the frequency of malaria in dengue patients but many patients presenting to the physicians with low platelet count and lab proven malaria are just treated with antimalarials without investigating for the presence of underlying dengue infection even during dengue epidemics. This is mainly to decrease the burden of the cost of the investigations. This situation creates a limitation for our study as the patients diagnosed and treated as 'malaria only' might actually have the concomitant dengue infection.

Dual dengue-malaria infection increases the risk of morbidity and mortality among the patients. Hence, treating physicians must not miss the presence of malaria in dengue patients and vice versa.

CONCLUSION

Patients with dengue fever are predisposed to have concomitant malarial infection. Hence, these must be investigated for malaria as undiagnosed and untreated malaria can increase the morbidity and mortality of the dengue patients.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Abbasi A, Butt N, Sheikh QH, Bhutto AR, Munir SM, Ahmed SM. Clinical features, diagnostic techniques and management of dual dengue and malaria infection. J Coll Physicians Surg Pak 2009; 19(1):25-9.
2. Carme B, Matheus S, Donutil G, Raulin O, Nacher M, Morvan J. Concurrent dengue and malaria in cayenne hospital, French Guiana. Emerg Infect Dis 2009;15(4):668-71.
3. Kaushik RM, Varma A, Kaushik R, Gaur KJ. Concurrent dengue and malaria due to Plasmodium falciparum and P. vivax. Trans R Soc Trop Med Hyg 2007;101(10):1048-50.
4. Bhalla A, Sharma N, Sharma A, Suri V. Concurrent infection with dengue and malaria. Ind J Med Sci 2006;60(8):330-1.
5. Ward DI. A case of fatal Plasmodium falciparum malaria complicated by acute dengue fever in East Timor. Am J Trop Med Hyg 2006;75(1):182-5.
6. Suri V, Bhalla A, Sharma N, Jain S, Varma S. HIV immunosupression and malaria: is there a correlation? Indian J Med Sci 2006;60(9):376-9.
7. Ouwe-Missi-Oukem-Boyer O, Ndouo FS, Ollomo B, Mezui-Me-Ndong J, Noulin F,Lachard I, et al. Hepatitis C virus infection may lead to slower emergence of P. falciparum in blood. PLoS One 2011;6(1):e16034.
8. Bansal R, Kadhiravan T, Aggarwal P, Handa R, Biswas A, Wali JP. Plasmodium vivax and hepatitis E co-infection--a rare cause of malarial jaundice. Ind J Gastroenterol 2002;21(5):207-8.
9. Chêne A, Donati D, Guerreiro-Cacais AO, Levitsky V, Chen Q, Falk KI, et al. A molecular link between malaria and Epstein-Barr virus reactivation. PLoS Pathog 2007;3(6):e80.
10. Ingrassia F, Gadaleta A, Maggi P, Pastore G. Plasmodium falciparum malaria and Parvovirus B19: a case of acute co-infection. BMC Infect Dis 2010;10:87.
11. Chêne A, Nyle´n S, Donati D, Bejarano MT, Kironde F, Wahlgren M, et al. Effect of Acute Plasmodium falciparum Malaria on Reactivation and Shedding of the Eight Human Herpes Viruses. PLoS One 2011;6(10): e26266.
12. Regunath H, Shivashankara KN, Sundeep KB, Bhaskar AP. Reactivation of Herpes zoster in an adult with Plasmodium infection. J Vector Borne Dis 2008;251–3.
13. Dengue guide lines for diagnosis, treatment, prevention and control. New ed. WHO. 2009.
14. Current medical diagnosis and treatment, chap 32 fifty one ed. Mc Graw Hill; 2012.

Original Article

Rhinosinusitis

Allergic Fungal Rhinosinusitis: Microbiologic and Pathologic Review

1. Abdul Latif Kakar 2. Khalid Mahmood 3. Muhammad Iqbal

1. Assoc. Prof. of ENT, Head & Neck Surgery, BMC/H, Quetta 2,3. Asstt. Profs. of ENT CMC/Chaudhary Rehmat Ali Memorial Trust Hospital, Lahore

ABSTRACT

Objective: To observe the various aspects of allergic fungal rhinosinusitis (AFRS).
Study Design: Prospective Study
Place and Duration of Study: This study was conducted at Department of Head and Neck Surgery, Civil Hospital Quetta and Chaudhary Rehmat Ali Memorial Trust Hospital, Lahore from 1st January 2015 to 30th June 2015.
Materials and Methods: Sixty seven patients suffering from chronic rhinosinusitis with polyps were included in this study. They were assessed clinically, computed tomography and histopathologic and mycologic monitoring. Depending on the presence or absence of allergic mucin and mycelial elements in the sinus, the patients were studied for different parameters.
Results: The mean age of 36.4 years with ranged from 14 to 51 and male to female ratio was 1.6:1. Out of these presumed 67 AFRS patients, 8 had positive fungal cultures. Remaining 59 patients with EM either had negative fungal cultures and these patients were thought to have insufficient evidence for a pathologic diagnosis of AFRS.
Conclusion: For the diagnosis of AFRS, the detection of fungal elements and allergic mucin should be considered.
Key Words: Allergic fungal, Chronic rhinosinusitis, Pathologic review

Citation of article: Kakar AL, Mahmood K, Iqbal M. Allergic Fungal Rhinosinusitis: Microbiologic and Pathologic Review. Med Forum 2016;27(2):17-20.

INTRODUCTION

Allergic fungal sinusitis (AFS) or rhinosinusitis (AFRS) is a form of polypoid chronic rhinosinusitis that is believed to be due to hypersensitivity to fungal antigens.[1,2] Allergic fungal rhinosinusitis (AFS) is thought to be a part of the disease spectrum of chronic rhinosinusitis, which affects between five to fifteen per cent of the population.[3] Allergic fungal sinusitis and eosinophilic mucin rhinosinusitis can easily be misdiagnosed and treated as chronic sinusitis, causing continuing harm.[4] Forms of chronic rhinosinusitis, such as allergic fungal rhinosinusitis (AFRS), could mimic malignant features.[5] Fungal rhinosinusitis (FRS) is an important infection of paranasal sinuses, which encompasses two main categories; invasive and noninvasive forms according to histopathological findings.[6] Fibroblasts are implicated in tissue remodeling and recruitment of inflammatory cells in chronic rhinosinusitis (CRS).[7]

Plonk and Luong[8] demonstrated that key role of cytokines imitative from respiratory epithelial cells, as well as interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin, in the orchestration of both innate and adaptive T helper 2 immune responses that are vital components of the immunopathology of chronic rhinosinusitis with nasal polyposis and AFRS.[8] AFRS was seen to be more aggressive in children with increased fungal load when compared with adults.[9] Our understanding of the pathogenesis and treatment of allergic fungal rhinosinusitis (AFRS) continues to evolve.[10] The spectrum of fungal involvement in CRS runs from benign colonisation to potentially life-threatening invasive disease.[11]

Correspondence: Dr. Abdul Latif Kakar,
Assoc. Prof., ENT Head & Neck Surgery Department,
Bolan Medical College/Civil Hospital, Quetta
Contact No.: 0333-7859657
E-mail: kakar617@gmail.com

MATERIALS AND METHODS

This prospective cross-sectional study was carried out at Department of Head and Neck Surgery, Civil Hospital Quetta and Chaudhary Rehmat Ali Memorial Trust Hospital, Lahore from 1st January 2015 to 30th June 2015. Sixty seven patients with CRS (of >3 months' duration) with nasal polyposis were included. Those patients who have apparent immune-compromised status or with histologic documentation of invasive fungal disease were not included. The detailed medical history along with clinical examination including preliminary nasal endoscopy was carried out in all patients. Aspirin sensitivity was assessed from history alone. Bronchial asthma was thought in those patients who were under the care of a pulmonologist and were on bronchodilator therapy. Patients were examined for follow-up during the study period. All patients underwent computed tomography (CT) of the paranasal sinuses and orbit in the axial and coronal planes, total leukocyte count, differential leukocyte count, absolute eosinophil count, and fasting blood sugar level estimation. All surgically/endoscopically

excised sinus mucosa and intrasinus debris were equally divided into two halves. One half was used for histopathologic monitoring, and the other half was used for mycologic examination. The histopathologic examination was carried out for the presence or absence of extramucosal allergic mucin, eosinophil clusters, Charcot-Leyden crystals, fungal hyphae, and possible mucosal invasion by fungal hyphae. The portion of surgically excised specimen used for mycologic examination was collected in sterile normal saline

RESULTS

The mean age of the patients was 36.4 years ranged from 14 to 51 years with male to female ratio was 1.6:1 (Table 1). Positive culture test results indicate that age group up to 20 years is the most prone to AFRS (14.45%) whereas the age group above 40 years is the least affected. Average of patients that suffer from AFRS is 10.35%. AFRS was diagnosed in 8 patients. The diagnosis of AFRS was established using pathologic criteria by either the presence of eosinophilic mucin (EM) containing fungal forms on histologic examination using a GMS stain or the presence of EM without histologic evidence of fungi but with positive fungal cultures. Out of the presumed 67 AFRS patients, 8 had positive fungal cultures. The remaining 59 patients with EM either had negative fungal cultures and these patients were considered to have insufficient evidence for a pathologic diagnosis of AFRS (Table 2).

Table No.1: Demographic statistics of the patients (n = 67)

Variable	No.	%
Age (years)		
Upto 20	28	41.8
21- 40	22	32.8
> 40	17	25.4
Gender		
Male	40	59.7
Female	25	40.3

Table No.2: Summary of culture test results

Age (years)	Samples tested	Positive culture	Negative culture
Upto 20	28	4	24
21- 40	22	3	19
> 40	17	1	16
Total	67	8	59

DISCUSSION

Allergic fungal rhinosinusitis (AFRS) is a type of chronic rhinosinusitis in which patients classically suffer from nasal polyps, type I IgE-mediated hypersensitivity, characteristic findings on computed tomography scans, eosinophilic mucin, and positive fungal stain.[12,13] Allergic fungal sinusitis (AFS), also referred to as allergic fungal rhinosinusitis (AFRS), is a noninvasive, eosinophilic form of recurrent chronic allergic hypertrophic rhinosinusitis.[14]

The interaction between fungi and the sinonasal tract results in a diverse range of diseases with an equally broad spectrum of clinical severity. These conditions may be discussed under two major headings: non-invasive disease (localized fungal colonization, fungal ball and allergic fungal rhinosinusitis) and invasive disease (acute invasive rhinosinusitis, chronic invasive rhinosinusitis and granulomatous invasive rhinosinusitis).[15] Aspergillus sp. or dematiaceous species were the most common fungi isolated in AFS while Aspergillus sp. was most common in FB and AIFRS.[16] In other studies Aspergillus ssp. were the most prevalent followed by Bipolaris sp. and Curvularia.[17] The presence of eosinophils in the allergic mucin, and not a type I hypersensitivity, is likely the common denominator in the pathophysiology of AFS.[18] The presence of allergic mucin is not unique to allergic fungal sinusitis, but rather is the result of a process that could have other etiologies.[19] Local IgE specific for a range of antigens has been identified in sinus and inferior turbinate tissue in patients with allergic fungal rhinosinusitis.[20] The total IgE concentration was significantly lower in patients with fungal presence in sinuses. Multiple elevations of fungal IgE are adequate diagnostic evidence of these fungi when fungal cultures and histologic examinations are negative in diagnosing AFS.[21] Nasal polyps occurred more frequently in patients with fungal presence in sinuses.[22] In AFRS patients, fungal antigens stimulated T-cell activation, inducing a predominantly the immune response. Healthy controls expressed an inhibitory cytokine IL-10 when exposed to these fungal antigens, possibly serving as a protective response.[23]

Recent studies demonstrate a central role of cytokines derived from respiratory epithelial cells, including interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin, in the orchestration of both innate and adaptive T helper 2 immune responses that are important components of the immunopathology of chronic rhinosinusitis with nasal polyposis and AFRS.[8] In AFRS and EMCRS patients, only fungal-specific CD4(+) T-cell proliferation occurred; hence, a lack of CD8(+) T-cell proliferation and activation in the presence of sinus eosinophilic mucus in these patients, regardless of fungal allergy, is a novel finding. This raises the question whether a dysfunctional CD8(+) T-cell response predisposes to ineffective clearance and accumulation of fungi in the sinuses of susceptible patients.[24]

Patients with AFS and HSD have HLA-DQB1 *03 alleles as a risk factor for disease, with AFS having the highest association.[25] Allergic fungal rhinosinusitis is a disease of young, immunocompetent individuals.[26] Demographic and socioeconomic factors may affect

AFRS presentation and treatment.[27] Proper diagnosis of AFS and differentiation from the other forms of both noninvasive and invasive fungal rhinosinusitis requires strict adherence to published diagnostic criteria.[28] Nasal obstruction, nasal discharge, nasal allergy and proptosis were the most common presentations.[29,30] Visual symptoms, proptosis, headaches, and increased nasal symptoms, especially in association with bony erosions on sinus computed tomography, suggest allergic fungal sinusitis and its complications in patients with chronic rhinosinusitis and nasal polyps. Patients with allergic fungal rhinosinusitis may present with a complication of the disease as the first symptom.[31]

The diagnosis of AFRS depends on history, the characteristics of CT scanning, pathology, mycologic and immunologic test.[32] The presence of more than one genotype in clinical samples illustrates the possibility that persons may be colonized by multiple genotypes and that any isolate from a clinical specimen is not necessarily the one actually causing infection.[33]

Acute invasive FRS showed unilateral pacifications of the sinonasal cavity, perisinus fat infiltration and/or bone destruction. Chronic invasive FRS demonstrated mass like hyper-attenuating soft tissue, with bony destruction. The soft tissue changes were hypointense on T1 and markedly hypointense on T2-weighted images. In allergic FRS, hyper-attenuating soft tissue causing paranasal expansion due to allergic mucin was observed on CT. Fungus ball presented as a hyper-attenuating lesion with calcifications within a single sinus.[34]

CONCLUSION

Allergic fungal rhinosinusitis may be caused by variety of fungi, of which dematiaceous species is most common.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Ryan MW, Clark CM. Allergic fungal rhinosinusitis and the unified airway: the role of antifungal therapy in AFRS. Current Allergy Asthma Reports 2015;15(12):75.
2. Melzer JM, Driskill BR, Clenney TL, Gessler EM. Sublingual Immunotherapy for Allergic Fungal Sinusitis. Ann Otol Rhinol Laryngol 2015; 124(10):782-7.
3. Mistry SG, Kumar BN. The value of antifungal therapy in allergic fungal rhinosinusitis. Rhinol 2014;52(1):9-18.
4. Uri N, Ronen O, Marshak T, Parpara O, Nashashibi M, Gruber M. Allergic fungal sinusitis and eosinophilic mucin rhinosinusitis: diagnostic criteria. The J Laryngol Otol 2013;127(9):867-71.
5. Meccariello G, Deganello A, Mannelli G, Bianco G, Ammannati F, Georgalas C, et al. Allergic fungal rhinosinusitis infiltrating anterior skull base and clivus. Auris, Nasus, Larynx 2013;40(4):405-8.
6. Nazeri M, Hashemi SJ, Ardehali M, Rezaei S, Seyedmousavi S, Zareei M, et al. Fungal rhino sinusitisin in tehran, iran. Iranian J Public Health 2015;44(3):374-9.
7. Carroll WW, O'Connell BP, Schlosser RJ, Gudis DA, Karnezis TT, Lawrence LA, et al. Fibroblast levels are increased in chronic rhinosinusitis with nasal polyps and are associated with worse subjective disease severity. Int Forum Allergy Rhinol 2015;9:43-5.
8. Plonk DP, Luong A. Current understanding of allergic fungal rhinosinusitis and treatment implications. Curr Opin Otolaryngol Head Neck Surg 2014;22(3):221-6.
9. Patro SK, Verma RK, Panda NK, Chakrabarti A. Understanding paediatric allergic fungal sinusitis: Is it more aggressive? Int J Pediatr Otorhinolaryngol 2015;79(11):1876-80.
10. Patadia MO, Welch KC. Role of immunotherapy in allergic fungal rhinosinusitis. Curr Opin Otolaryngol Head Neck Surg 2015;23(1):21-8.
11. Krishnan KU, Agatha D, Selvi R. Fungal rhinosinusitis: a clinicomycological perspective. Ind J Med Microbiol 2015;33(1):120-4.
12. Lanza DC, Dhong HJ, Tantilipikorn P, Tanabodee J, Nadel DM, Kennedy DW. Fungus and chronic rhinosinusitis: from bench to clinical understanding. Ann Otol Rhinol Laryngol 2006; 196:27-34.
13. Laury AM, Wise SK. Chapter 7: Allergic fungal rhinosinusitis. Am J Rhinol Allergy 2013;27 Suppl 1:S26-7.
14. Doellman MS, Dion GR, Weitzel EK, Reyes EG. Immunotherapy in allergic fungal sinusitis: The controversy continues. A recent review of literature. Allergy Rhinol (Providence). 2013;4(1): e32-5.
15. Callejas CA, Douglas RG. Fungal rhinosinusitis: what every allergist should know. Clinical and experimental allergy. J Br Soc Allergy Clin Immunol 2013;43(8):835-49.
16. Montone KT, Livolsi VA, Feldman MD, Palmer J, Chiu AG, Lanza DC, et al. Fungal rhinosinusitis: a retrospective microbiologic and pathologic review of 400 patients at a single university medical center. Int J Otolaryngol 2012;2012:684835.
17. Elmorsy S, El-Naggar MM, Abdel aal SM, Abou-elela MA. Sinus aspirates in chronic rhinosinusitis: fungal colonization of paranasal sinuses, evaluation of ICAM-1 and IL-8 and studying of immunological effect of long-term macrolide therapy. Rhinol 2010;48(3):312-7.

18. Ponikau JU, Sherris DA, Kern EB, Homburger HA, Frigas E, Gaffey TA, et al. The diagnosis and incidence of allergic fungal sinusitis. Mayo Clinic Proceedings 1999;74(9):877-84.
19. Lara JF, Gomez JD. Allergic mucin with and without fungus: a comparative clinicopathologic analysis. Arch Pathol Lab Med 2001;125(11): 1442-7.
20. Wise SK, Ahn CN, Schlosser RJ. Localized immunoglobulin E expression in allergic rhinitis and nasal polyposis. Curr Opin Otolaryngol Head Neck Surg 2009;17(3):216-22.
21. Stewart AE, Hunsaker DH. Fungus-specific IgG and IgE in allergic fungal rhinosinusitis. Otolaryngology - head and neck surgery. J Am Acad Otolaryngol Head Neck Surg 2002;127(4): 324-32.
22. Gawlik R, Czecior E, Jarzab J, Grzanka A, Cichecka E, Sowa P, et al. Frequency of IgE-dependent hypersensitivity to moulds in patients with chronic rhinosinusitis with polyps. Postepy Dermatol Alergol 2014;31(3):159-63.
23. Luong A, Davis LS, Marple BF. Peripheral blood mononuclear cells from allergic fungal rhinosinusitis adults express a Th2 cytokine response to fungal antigens. Am J Rhinol Allergy 2009;23(3):281-7.
24. Pant H, Macardle P. CD8(+) T cells implicated in the pathogenesis of allergic fungal rhinosinusitis. Allergy Rhinol (Providence) 2014;5(3):146-56.
25. Schubert MS, Hutcheson PS, Graff RJ, Santiago I, Slavin RG. HLA-DQB1 *03 in allergic fungal sinusitis and other chronic hypertrophic rhinosinusitis disorders. J Allergy Clin Immunol 2004;114(6):1376-83.
26. Marfani MS, Jawaid MA, Shaikh SM, Thaheem K. Allergic fungal rhinosinusitis with skull base and orbital erosion. J Laryngol Otol 2010;124(2):161-5.
27. Wise SK, Ghegan MD, Gorham E, Schlosser RJ. Socioeconomic factors in the diagnosis of allergic fungal rhinosinusitis. Otolaryngology - head and neck surgery. J Am Acad Otolaryngol Head Neck Surg 2008;138(1):38-42.
28. Schubert MS. Allergic fungal sinusitis: pathophysiology, diagnosis and management. Med Mycol 2009;47 Suppl 1:S324-30.
29. Zakirullah, Nawaz G, Sattar SF. Presentation and diagnosis of allergic fungal sinusitis. JAMC 2010; 22(1):53-7.
30. Karthikeyan P, Nirmal Coumare V. Incidence and presentation of fungal sinusitis in patient diagnosed with chronic rhinosinusitis. Indian journal of otolaryngology and head and neck surgery. Assoc Otolaryngol Ind 2010;62(4):381-5.
31. Bozeman S, deShazo R, Stringer S, Wright L. Complications of allergic fungal sinusitis. Am J Med 2011;124(4):359-68.
32. Zhang G, Ni C, Xu Y, Zhou H. [Allergic fungal rhinosinusitis: one case and literature review]. Lin Chuang Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2010;24(11):496-7, 501.
33. Rudramurthy SM, de Valk HA, Chakrabarti A, Meis JF, Klaassen CH. High resolution genotyping of clinical Aspergillus flavus isolates from India using microsatellites. PloS one 2011;6(1):e16086.
34. Ilica AT, Mossa-Basha M, Maluf F, Izbudak I, Aygun N. Clinical and radiologic features of fungal diseases of the paranasal sinuses. J Computer Assisted Tomography 2012;36(5):570-6

Original Article

Doppler of DI Epigastric Artery

Effect of Body Mass Index on Accuracy of Doppler Ultrasonographic Detection of Dominant Perforator of Deep Inferior Epigastric Artery Perforator Flap

1. Ammara Rabbani 2. Kamran Khalid

1. Women Medical Officer, 2. Asstt. Prof., Jinnah Burn & Reconstructive Surgery Center, Jinnah Hospital / AIMC, Lahore.

ABSTRACT

Objective: The objective of this study is to find the frequency of correct identification of the dominant perforators by pre-operative Doppler ultrasonography in deep inferior epigastric artery perforator flap in relation to increasing body mass index (BMI).

Study Design: Descriptive cross-sectional study

Place and Duration of Study: This study was carried out in Jinnah Burn & Reconstructive Surgery Centre, Lahore from 4th June 2013 to 4th June 2015.

Materials and Methods: A total of 46 patients fulfilling the inclusion criteria were enrolled from Jinnah Burn & Reconstructive Surgery Centre, Lahore. An informed consent was taken for color Doppler ultrasonography, operation and to gather information for study purpose. Pre operative BMI was calculated by dividing weight in kilogram (Kg) by height in meter square (m^2) and placed into two group ; group 1 with BMI <30 (normal to overweight) had 33 patients (71.74%) and group 2 BMI ≥30 (obese), 13 patients (28.26%). Preoperative Doppler USG was done for localization of dominant perforator in periumbilical region of Deep inferior epigastric artery and distance from umbilicus was measured in centimeters (cm). Per operative distance of dominant perforator confirmed and measured in centimeters along the radius of umbilicus.

Results: Forty six patients aged between 32-46 years with mean 39.3±3.5 years were included. In group 1 all 33 (76.7%) had correctly identified dominant perforators, whereas all the 3 (100%) patients whom the per operative perforator location fall out of 1cm range of pre-operative Doppler localization fell in group 2 (obese patients) showing clinically significant p- value 0.004.

Conclusion: It is concluded that Color Doppler USG is a safe and reliable imaging technique but increasing BMI effects the accuracy of pre-operative mapping of dominant perforator of DIEP flap.

Key words: Deep inferior epigastric artery perforator (DIEP) flap, Dominant perforator, Pre-operative Doppler ultrasonography, Body mass index

Citation of article: Rabbani A, Khalid K. Effect of Body Mass Index on Accuracy of Doppler Ultrasonographic Detection of Dominant Perforator of Deep Inferior Epigastric Artery Perforator Flap. Med Forum 2016;27(2):21-24.

INTRODUCTION

Breast reconstruction is an integral component of breast cancer treatment. Newer more refined surgical techniques are being evolved, in order to improve the appearance and feel of autologus reconstructed breast with minimal donor site morbidity.[1]

The era of autologous breast reconstruction began with the Transverse Rectus Abdominus Muscle (TRAM) flap. In 1982 Carl Hartrampf for the first time used pedical TRAM flap for autologus breast reconstruction based upon superior epigastric artery.[2] It was in 1973, Taylor and Daniel coined the term "Free Flap" to describe the distant transfer of many island flap by microvascular anastomosis.[3,4] Due to great work done by Ian Taylor on the vascular territory of the flaps , he documented many of the more common free flap still in use today.[5]

Perforator flaps are the new in the evolution of soft-tissue flaps. These flaps allow the transfer of the patient's own skin and fat in a reliable manner, and have minimal donor-site morbidity.[6] So Deep Inferior Epigastric Artery Perforator (DIEP) flap is a technique which is both an evolution and modification of Transverse Rectus Abdominis Muscle (TRAM) flap for breast reconstruction. In 1989 Koshima and Soeda for the first time used the skin and fat of lower abdomen sparing the rectus muscle and used it for reconstruction other than breast.[7] Finally in 1993 Dr Allen and Treece used DIEP flap for autologous breast reconstruction.[8] Blondeel further improved the understanding and

Correspondence: Dr. Kamran Khalid,
Asstt. Prof. Jinnah Burn & Reconstructive Surgery Center, Jinnah Hospital/Allama Iqbal Medical College, Lahore
Cell No.: 0321-4426322
E-mail: drkamrankhalid@hotmail.com

popularized the use of DIEP flap in autologus breast reconstruction.[9,10]

Deep inferior epigastric artery has a significantly variable vascular anatomy.[11,12] It has two set of branching system lateral branch (dominant in 54%) and medial branch (dominant in 18%). However in 28% of the cases deep inferior epigastric artery has a central course with centrally located perforators.[13] So pre operative imaging has an important role for the assessment of vessels of DIEP flap for microvascular autologous breast reconstruction.

A number of imaging modalities are available starting from simplest to most sophisticated investigation like hand held Doppler, color Doppler duplex ultrasound scanning, traditional angiography, computed tomographic angiography (CTA), magnetic resonance imaging and angiography (MRI/A) for pre operative vascular assessment.[14]

The ideal imaging modality should give valuable information about the course and caliber of perforating vessels, it should be reproducible, inexpensive, and readily available with low radiation dose.[14]

In developed countries sophisticated and invasive investigations e.g. CT Angiography are used for perforator localization and course. Due to limited availability and affordability, an imaging technique i.e. color Doppler ultrasound that is almost as accurate as CT angiography, non invasive, affordable and available all time can be used to achieve the same purpose .This study in Our population will evaluate color Doppler ultrasound for locating dominant perforators based on size, location, intramuscular and subcutaneous course in comparison to BMI that are important for surgery outcome and flap survival.

MATERIALS AND METHODS

This descriptive cross-sectional study was carried out Jinnah Burn & Reconstructive Surgery Centre, Lahore within two years from 4th June 2013 to 4th June 2015. A total of 46 patients fulfilling the inclusion criteria were enrolled from Jinnah Burn & Reconstructive Surgery Centre, Lahore. They were divided into two groups; group 1 with BMI <30 (normal to overweight) had 33 patients (71.74%) and group 2, BMI ≥30 (obese) had 13 patients (28.26%). Female patients aged between 20-50 years, undergoing breast reconstruction were included for the study. Patients with diverication of recti, peri and per umbilical hernia on clinical examination or ultrasonography, mid line infraumbilical scar, previous history of abdominoplasty/ liposuction were excluded. An informed consent was taken for color Doppler, operation and to gather information for study purpose. Pre-operative BMI was calculated; weight in kilogram (Kg) divided by height in meter square (m^2) and placed into two groups; group 1 BMI <30 and group 2 BMI >30. Preoperative Doppler USG was done for localization of dominant perforator in periumbilical region of deep inferior epigastric artery flap and distance from umbilicus was measured in centimeters. Per-operative distance of dominant perforator confirmed and measured in centimeters along the radius of umbilicus and documented as correctly identified or not (Figs. 1-4). The defect was created on chest wall and internal mammary vessels dissected out in the 2nd and 3rd intercostals space for microvascular anastomsis and flap insetting done. Donor site closed primarily and drains placed. Post operatively free flap monitoring and vital monitoring was done for 5 days. Drains were removed on 2-3rd day depending on the outcome. Patient mobilized on 2nd post operative day. First dressing was changed on 5th post-operative day. Uneventful, patients were discharged on 8th post-operative day. Data was recorded and patients were divided in 2 groups. Correct identification of dominant perforator was labeled as per operational definition.

RESULTS

In group 1 all 33 (76.7%) had correctly identified dominant perforators, where as all the 3 (100%) patients whom the per operative perforator location fall out of 1cm range of pre operative Doppler USG localization fell in group 2 obese patient showing clinically significant p-value 0.004.

Table No. 1: Crosstab between BMI & correct identification of dominant perforator

BMI	Correct identification of dominant perforator	
	Yes	No
Normal to overweight (BMI <30)	33	-
Obese (BMI ≥30)	10	3
Using chi square test, p value=0.004 (Significant)		

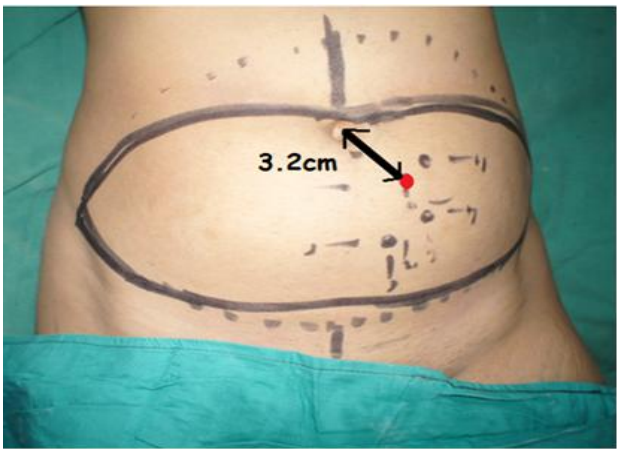


Figure No. 1: Pre-operative Doppler ultrasonographic marking of perforators

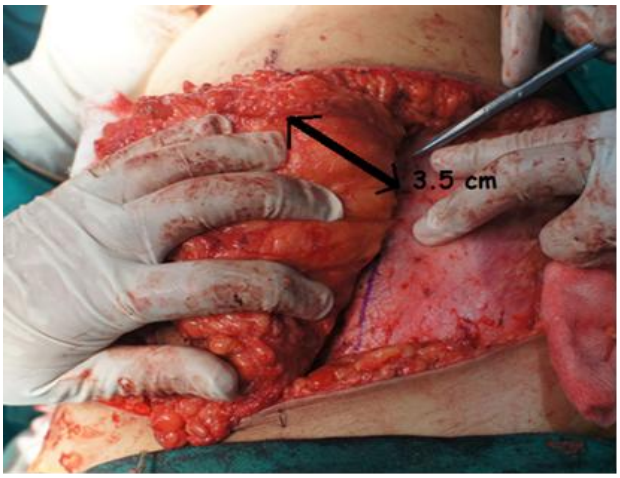


Figure No.2 2: Per-operative localization of dominant perforator

Figure No. 3: Final flap insetting

Figure No. 4: Three months follow up picture

DISCUSSION

Color Doppler ultrasonography is noninvasive and has no radiation exposure. Its can give valuable information on flow characteristics of perforators and the main vessels. From this, the quality of vessels and their suitability can be assessed. Studies have shown perforators with diameter greater the 0.5 mm can be accurately detected by color Doppler USG with a true-positive rate of more than 90 percent.[15] Thus, several surgeons still choose this method of imaging despite recent advances in computed tomographic angiography and magnetic resonance angiography. It has a small probe, so can show vessels in a small area only.

So, the color Doppler ultrasonography which is readily available and also cost effective, when combined with other conventional techniques i.e. hand held Doppler can give high predictive value. The only drawback of these are they are operator dependant and not reproducible. A study conducted by Seidenstucker et al[16] shows a single perforator identified by both pre-operatively color flow duplex ultrasonography and at the time of intra-operative flap harvest as the dominant perforator was identified in 36 of 46 flaps (78.3%). The study also showed that in 45 of 46 patients (97.8%) the intra-operative perforator chosen at the time of flap harvest was identified as one of the pre-operative perforators marked by duplex ultrasonography.[16] In our study combination of hand held Doppler and color Doppler ultrasonography has shown a high predictive value of correct identification of pre operative and per operative dominant perforator in 43 out of 46 patients (93.48%). Also all the single or multiple perforators in a single row with diameter >1mm, within 6cm radius of umbilicus were included in flap harvest in order to increase the flap perfusion. This shows that combination of conventional handheld Doppler and color Doppler ultrasonography with help of professional expertise can give valuable information on perforator vessels of new perforator flaps with high true predictive value comparable to MDCT or MRA and also higher than any of the conventional techniques used alone.

The musculocutaneous perforators of deep inferior epgastric artery supplying the lower abdominal skin have a variable course through the abdominal fat after they exit from the anterior rectus sheath. So the patients included in the study were divided into two groups based upon the BMI, group 1 with BMI less the 30 and group 2 with BMI more then 30. The data analysis and cross tabulation of correct identification of the dominant perforator with BMI has shown a significant effect of BMI on perforator identification and course through the fat. Out of 46 patient 3 had incorrectly identified dominant perforator location on per operative dissection and all the 3 patients belonged to group 2 (BMI >30). This shows that in obese patients Doppler ultrasonography has decreased sensitivity for correct identification of dominant perforator confirmed with per operative findings.

CONCLUSION

In our target population Color Doppler USG is a safe and reliable imaging technique, with professional expertise present and combined with other conventional methods i.e. hand held Doppler it provides an accurate

pre-operative mapping of dominant perforator of DIEP flap in normal or slightly overweight patients which is comparable to CT angiography, but in Obese patients its sensitivity decreases and one should consider other modalities like MRA for such patients.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. LoTempio MM, Gerald GL, Allen RJ. Breast Reconstruction with DIEP and S/IGAP, Marzia Salgarello. Breast Reconstr Current Tech 2012; 11:129.
2. Hartrampf CR, Scheflan M, Black PW. Breast reconstruction with a transverse abdominal island flap. Plast Reconstr Surg 1982;69(2): 216-25.
3. Daniel RK, Taylor GI. Distant transfer of an island flap by microvascular anastomoses. Plas Reconstr Surg 1973;52: 111-7.
4. Taylor GI, Daniel RK. The free flap: composite tissue transfer by vascular anastomoses. Aust NZ J Surg 1973; 43: 1-3.
5. Taylor GI, Daniel RK. The anatomy of several free flap donor site. Plas Reconstr Surg 1975; 56(3): 243-53.
6. Granzow JW, Levine JL, Chiu ES, LoTempio MM, Allen RJ. Breast Reconstruction with Perforator Flaps. Plast Reconstr Surg 2007; 120: 1.
7. Koshima I, Soeda S. Inferior epigastric artery skin flaps without rectus abdominis muscle. Br J Plast Surg 1989;42(6):645–8.
8. Allen RJ, Treece P. Deep inferior epigastric perforator flap for breast reconstruction. Ann Plast Surg 1994;32(1):32–8.
9. Blondeel PN, Boeckx WD. Refinements in free flap breast reconstruction: the free bilateral deep inferior epigastric perforator flap anastomosed to the internal mammary artery. Br J Plast Surg 1994;47(7):495–5.
10. Blondeel PN. One hundred free DIEP flap breast reconstructions: a personal experience.Br J Plast Surg 1999;52(2):104–11.
11. Kikuchi N, Murakami G, Kashiwa H, et al. Morphometrical study of the arterial perforators of the deep inferior epigastric perforator flap. Surg Radiol Anat 2001;23(6):375–81.
12. Heitmann C, Felmerer G, Durmus C, et al. Anatomical features of perforator blood vessels in the deep inferior epigastric perforator flap. Br J Plast Surg 2000;53(3):205–8.
13. Blondeel PN, Morrison CM, Allen RJ. The deep inferior epigastric artery perforator (DIEAP) flap. In: Neligan PC, editor. Plastic surgery. 3rd ed. 2013.p.436.
14. Pratt GF, Rozen WM, Chubb D, Ashton MW, Alonso-Burgos A, Whitaker IS. Preoperative Imaging for Perforator Flaps in Reconstructive Surgery, Ann Plast Surg 2012; 69: 100-110.
15. Zhang Y. Application of Multidetector-Row Computed Tomography in Propeller Flap Planning, PRS 2015;27: 712-5.
16. Seidenstucker K, Munder B, Richrath P, Rybacki G, Horch RE, Beier JP, et al. A prospective study using color flow duplex ultrasonography for abdominal perforator mapping in microvascular breast reconstruction. Med Sci Monit 2010;16(8): MT65-70.

Original Article

Protective Effects of Chronic Methylcobalamin Administration against Lithium Carbonate Induced Purkinje Cell Toxicity in Albino Rats

1. Khalid Saeed 2. Tazeen Kohari

1. Asstt. Prof. of Physiology, 2. Asstt. Prof. of Anatomy, Liaquat College of Medicine and Dentistry, Karachi

ABSTRACT

Objective: The effect of Lithium on central nervous system is well known but due to paucity of literature on scavenging effect of Lithium, this study was undertaken to see Lithium toxicity on cerebellar Purkinje neurons.

Study Design: Experimental study.

Place and Duration of study: This study was carried out at Animal House, JPMC, Karachi from April 2012 to June 2012.

Materials and Methods: Fifteen male albino rats of 195-245 grams were selected and divided into three major groups A, B, and C. Each major group consisted of 5 animals. Time period of this study was12 weeks. Group A served as control group which was given normal healthy lab diet and B was the Lithium Carbonate-treated group. Group C received Lithium carbonate in powder form and injection Methylcobalamin (B12) intraperitoneally. Lithium carbonate was given at a dose of 20mg/kg/day to group B and C for 12 weeks, and Methylcobalamin was injected at a dose of 200µg/kg/day/bwt. Purkinje cell count was performed with a counting reticule under light microscope.

Results: The present study concluded that Lithium carbonate when administered the 12 weeks cause the significant decrease of Purkinje cell count and Methylcobalamin restored the cerebellar cell count.

Conclusion: In the light of this study it was concluded that that Lithium carbonate causes significant permanent loss of permanent Purkinje cell neuron but Methylcobalamin provided neuroprotective effect and restored the cell count.

Key Words: Methylcobalamin, Lithium Carbonate, Toxicity

Citation of article: Saeed K, Kohari T. Protective Effects of Chronic Methylcobalamin Administration against Lithium Carbonate Induced Purkinje cell Toxicity in Albino Rats. Med Forum 2016;27(2):25-27.

INTRODUCTION

Lithium the bridge element[1] is an alkali metal found in group 1A, of the periodic table. The true use of Lithium carbonate as a drug was by Johan's Cade in 1949.[2] Now for the past five decades it is used for Bipolar disorder, it is also used to treat schizophrenia, cycloid psychosis[3] and major depression. In 1970 the Food and Drug Administration (FDA), United States of America[4] approved the Lithium as a treatment for mania. In a study it was observed that lithium carbonate inhibits glycogen synthase activity causing apoptosis. Glycogen Synthase enzyme is an evolutionary conserved ubiquitous serine / threonine protein kinase. It is abundant in the neurons and neuroglia. GSK-3 suppresses the activity of several prominent pathways such as Wnt signaling pathway, phosphoinositol 3 protein kinases and neurotropic pathway. GSK-3 are implicated in fundamental brain functions cytoskeletal stabilization axonal growth cone collapse, cell adhesions, synaptic plasticity and memory formation. Dysregulation of GSK-3 is implicated in schizophrenia, Alzheimer's, attention deficit hyperactivity disorder BPD and lithium inhibits GSK-3.[5,6] The highest concentration of inhibition of GSK-3 were found in cerebellum. The cerebellum is an important factor in the brain motor system. Cerebellar cortex contains 8 types of neurons and among them the purkinje cells provide the sole output of the cerebellar cortex. Immunohistological examination of GSK-3 mutant mice revealed significant degrees number of purkinje cells.[7]

It is due to such observations great concern has been documented to discourage long term use of Lithium until it is indicated. Methylcobalamin is a vitamin B-12 analogue and is necessary for the maintenance[8] of central nervous system.

B-12 analogue was discovered by British Chemist Baron Alexander[9] and later it was discovered biologically active alkyl cobalamin in 1962 by Guest. It occurs as dark red crystals or crystalline powder. Vitamin B12 has been used in the therapy of trigeminal neuralgia, MS, neuropathies, psychiatric disorders, poor growth or nutrition.[10] B12 participates in one carbon atom unit metabolism and hence is essential for methylation of DNA and proteins because B12 is

Correspondence: Dr. Tazeen Kohari,
Asstt. Prof. of Anatomy, Liaquat College of Medicine and Dentistry, Karachi
Cell No.: 03232967849
E-mail: tazeenk67@gmail.com

required for homocysteine metabolism by serving as a co-factor for methionine synthase, homocysteine, and non-conversion of methionine results in oxidative stress thereby by increasing the oxidative in the endothelial cells, so B12 is necessary to eliminate the oxidative stress.[11]

It is essential for cell growth and replication. It promotes neurite growth neuronal[12] survival and these effects are mediated by methylation cycle. Mecobalamin is used in spinal cord and peripheral nerve injuries in which it restores DNA synthesis by DNA methylation.[13]

MATERIALS AND METHODS

This study was conducted in the department of anatomy and practical work of research was conducted at Basic Medical sciences Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC), Karachi. For this study 15 male Albino rats of 195-245 grams of weights were selected for study. They were kept under observation for 7 days prior to commencement of study. The animals were randomized into four experimental groups each comprising of 5 rats. Group was labeled that A, B, and C, the treatment period was 12 weeks. Group A served as control and group B received Lithium Carbonate, taken from Adamjee Pharmaceuticals, at a dose of 20 mg/kg/day in powder form mixed in flour pellets and group C received Lithium carbonate injection Methylcobalamin 200mcg/kg/day IP.[14,15] The standard laboratory Chow was available and libitum. The albino rats were decapitated. The brain was taken out and the cerebellum was separated from the rest of the brain. The cerebellum was preserved in formal saline for 24 hours the tissue was fixed and than four micrometers thick sections of the tissue were prepared. The Purkinje cell count was done by counting reticule was done for all the three groups at 12 weeks. Data collected was analyzed using students t-test. Results were expressed as mean, SEM. P<0.001 was considered statistically highly significant. All calculation was done by utilizing computer software SPSS 16.

RESULTS

The Purkinje cell count of group B animals at 12 weeks was significantly decreased P<0.001 with control group A.

Table No.I: Mean* Purkinje cell count of Albino rats in control group, Lithium treated group B and Lithium and Methylcobalamin group C at 12 weeks.

Group	12th Week		
	Mean	S.D	SEM
Control (A)	69.5	0.40	0.18
Lithium (B)	34.9	0.23	0.10
Lithium B-12 (C)	66.3	0.24	0.11

The above result shows a highly significant P-value <0.001 decreased Purkinje cell count in Lithium treated group B and a restoration of Purkinje cell count in group C which is Lithium and Methylcobalamin treated group.

Statistical Analysis of Purkinje cell count between Groups

Group	P-value
B vs. A	< 0.001***
C vs. A	<0.001***
C vs. B	<0.001***

***highly significant P-value <0.001

DISCUSSION

Lithium is a 27th most abundant ubiquitous element present in the earth crust to the extent of about 0.006%.[16] Ingestion of Lithium in the body causes several damaging effect on the body organs like brain, heart and kidney nervous system is the primary target organ of Lithium toxicity nearly 50% of all neuron of the brain allocated in this region so it may be vulnerable to injury.[17] Lithium administration caused Glycogen synthase kinase (GSK) inhibition, which increased translation of nuclear factor of activated T-cells C-3/4 (NFAT C-3/4) transcription factors to the nucleus leading to increased Fas ligand. Fas ligand cases apoptosis by binding to surface receptor as a consequence there is an activation of caspases-3 which causes cellular degradation.[18] It is of utmost important to note the levels of Lithium induced apoptosis, were highest in rat cerebellum. It is suggested that neuronal cell death due to lithium is due to the reason that it causes decrease action potential peak amplitude[19] and the amplitude of after potential following a single action potential and decreases action potential and re-polarization phase. Although depolarization results from increased Na^+ influx the effect of lithium on action potential re-polarization and after potential suggests that lithium acts to decrease outward potassium current.

Given that mammalian CNS, has limited regenerative capacity, it is of utmost importance,[20] to limit the damage than novel therapeutic strategies. Evidence suggests that caspases-3 is the key enzyme in neuronal apoptosis which is inhibited by methylcobalamin decreasing cell damage. Methylcobalamin promotes DNA repair and growth of cell.

In a study conducted by Zhang,[21] it was reported that Methylcobalamin at such an important factor restoring the normal function of DNA because it causes methylation of DNA and proteins.

It restores delayed synaptic transmission and diminished neurotransmitters[22] to normal and promotes myelination due to synthesize of lecithin, it promotes axonal transport and regeneration. Mecobalamin restores end plate potential induction in rats, by increasing nerve fiber excitability. It causes mitosis of schawn cells and incorporation of amino acids in to protein fraction of crushed sciatic nerve in rats.[23]

It was observed in our study that the Purkinje cell count was decreased in group B as compared to group C and Methylcobalamin restored the Purkinje cell count.

CONCLUSION

Our present study suggests that chronic administration of Methylcobalamin protects the Purkinje cell neuron and restores there number. So B-12 analogue ameliorates the toxic effect of Lithium carbonate.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Gupta PK. Pharmaceutical chemistry. In: Remington, the science and practice of pharmacy, 21st ed. Philadelphia; 2012.p.365.
2. Wikipedia, free encyclopedia: Lithium. 2009; Available at: http://en-wikipedia.org/wiki/ Lithium.
3. Baldessarini RJ. Psychosis and Anxiety. In: Goodman and Gilman's the pharmacological basis of therapeutics. 19th ed. New York: McGraw Hill; 1996.p.399-460.
4. Fortune city; Lithium mineral and drug, 2006. Available at: http:// www.fortunecity. com/campus/ psychology/781/Lithium.html.
5. Kaidanovich-Beilin O. Abnormalities in brain structure and behavior in GSK-3 mutant mice.
6. Stambolic V, Ruel L, Woodgett R. Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signaling in intact cells. Cur Biol 1996; 6(12):1664-68.
7. Gomez-Sintes R, Lucas JJ. NFAT/Fas signaling mediate the neuronal apoptosis and motor side effects of GSK-3 inhibition in a mouse model of lithium therapy. J Clin Invest 2010;120(7): 2432-45.
8. Katzung BG. Anti-psychotic agents and Lithium in: Basic and clinical Pharmacology. 9th ed. New York: McGraw Hill; 2004.p.475-79.
9. Lotha G. Vitamin B 12. http://www.britannica. com/print/topic/631051, 2012.
10. Brunton LL, Lazo JS, Parker KL. Goodman and Gilman's the pharmacological basis of therapeutics. 11th ed. New York: McGraw Hill; 2006.p.1454-58.
11. Sutherland C, Leighton I, Cohen P. Inactivation of glycogen synthase kinase-3β by MAP kinase-activated protein kinase-1 (RSK-2) and p70 S6 kinase; new kinase connections in insulin and growth factor signaling. Biochem J 1993;296: 15-19.
12. Okada K, Tanaka H, Temporin K, Okamoto M, Kuroda Y, Moritomo H, et al. Methylcobalamin increases Erk1/2 and Akt activities through the methylation cycle and promotes nerve regeneration in a rat sciatic nerve injury model. Exper Neurol 2012;222(2):191-203.
13. Sikander BJ. JCI-folate regulation of axonal regeneration in the rodent CNS. http://www. jci.org/articles/view/400000, 2012.
14. Kolachi GM. Toxic effects of lithium carbonate of proximal tubules of rat kidney: A morphometric study. MC 2008;14(3).
15. Licht RW, Larsen JO, Smith D, Braendgaard H. Chronic lithium treatment with or without haloperidol fails to affect the morphology of the rat cerebellum. Eur Neuropsychopharmacol 2003; 173-176.
16. Shaikh BA. The radioprotective role of Methylcobalamin on cerebella cortex on young albino rats. M Phil thesis BMSI JPMC Karachi 2010.
17. Arai H, Vecchio-Sadus A. Toxicity of lithium to humans and the environment – a literature review. Ecotoxicol Environ Safety 2008;70:349-56.
18. Friedlander RM. Apoptosis and caspases in neurodegenerative diseases. N Engl J Med 2003; 348: 1365-75.
19. Morgan JM. http://humboldt.edu/jmmorgan/ lith_s05.htm, 2005.
20. Birch CS, Brasch NE, McCaddon A, Williams JHH. A novel role for vitamin B12: cobalamins are intracellular antioxidants in vitro. Free Rad Biol Med 2009; 47: 184-88.
21. Zhang YF, Ning G. Mecobalamin. Expert Opin. Investig. Drugs 2008;17(6): 953-64.
22. Kikuchi M, Kashii S, Honda Y, Tamura Y, Kaneda K, Akaike A. Protective effects of Methylcobalamin, a vitamin B12 analog, against glutamate-induced neurotoxicity in retinal cell culture. Invest Ophthalmol Visual Sci 1997;38(5): 848-54.
23. Yamatsu K, Yamanishi Y, Kancko T, Ohkawa I. Pharmacological studies on degeneration and regeneration of the peripheral nerves. Folia Pharmacologica Japonica 1976;72(2):269-78.

Original Article

Impact of Putative Bacteriocins against Multidrug Resistant Clinical Isolates

1. Samyyia Abrar 2. Saba Riaz

1. PG Student, Deptt. of Microbiology and Molecular Genetics 2. Asstt. Prof. of Microbiology and Molecular Genetics, University of the Punjab, Lahore

ABSTRACT

Objective: To use one of such mechanism like bacteriocins, produced by Lactobacilli activities against pathogens.
Study Design: Analytical / observational study
Place and Duration of Study: This study was carried out at Gulab Devi Chest Hospital, Lahore from November 2012 to January 2013.
Materials and Methods: This study included 203 clinical samples. Multidrug resistant clinical isolates were selected on the basis of their MAR (Multiple antibiotic resistances) index, antibiotic susceptibility testing, methicillin resistant Staphylococcus aureus (MRSA) with oxacillin disc, double disc synergism and combination disc test. Plasmid isolation, conjugation was performed. Well-Diffusion assay was used for screening of putative bacteriocins produced by Lactobacillus strains against MDRs. Physiological characterization of antimicrobial compounds and protein estimation was analyzed.
Results: Twenty five strains were selected based on MAR index (>0.2). In which 6 MRSA and 19 extended spectrum beta-lacatmases (ESBL) producers were further proceeded for antimicrobial activity with putative bactericins. Plasmid was easily transferred their resistance by the process of conjugation. Five bacteriocins were obtained from Lactobacillus strains isolated from commercial products. These bacteriocins showed a strong anti-bacterial activity against selected MDRs. Decrease in zone sizes was observed when putative bacteriocins were treated with heat, SDS (Sodium dodecyl sulfate) and Protinase k. Putative bacteriocins produced by Lactobacilli exhibit significant antibacterial activity against MDRs. SA1 has high antibacterial activity with high protein content of 13mg/ml.
Conclusion: Putative bacteriocins produced by Lactobacilli exhibit significant antibacterial activity against selected MDRs. MDRs have ability to transfer their resistance to other bacteria. The peptidal component of these bacteriocins can be used as an alternative therapy. Proper hospital policies require minimizing the horizontal spread of MDRs. Hence, it is necessary to purify the antibacterial molecule out of putative bacteriocin for further analysis.
Key words: MDRs, Bacteriocins, Lactobacilli, Antibiotic Resistance, MAR, Antibacterial activity

Citation of article: Abrar S, Riaz S. Impact of Putative Bacteriocins against Multidrug Resistant Clinical Isolates. Med Forum 2016;27(2):28-32

INTRODUCTION

Antibiotics are becoming non-responsive against many bacteria from last three decades. This is because of excessive, indiscriminate use of drugs in agriculture, health sector and even in veterinary medicine.[1] This misuse of antibiotics is creating troubles in treating the different infections, hence increasing the rate of morbidity and mortality.[2] The un-prescribed utilization of the antibiotics is considered as major source behind the high level of drug resistance. Horizontal gene transfer has contributed a lot in the spread of such infections. Nosocomial and community acquired infections are the consequences of this uncontrolled usage of drug.[3] To combat with such issue, much work is being done on the use of natural products as antimicrobial agents.[4] The use of medicinal plants as antimicrobial therapy is very common and an old age practice. Most of the antibiotics available today come from natural origin particularly from microbial sources.[5] Microbial solutions to the microbial problems are one of the best possible solutions to this problem. Lactobacilli are the well-known friendly bacteria for their beneficial activities against pathogens. The antagonistic activity of lactic acid bacteria is debatable due to different substances such as hydrogen peroxide, diacetyl, lactic acid and bacteriocin.[6] The bacteriocins are antimicrobial peptides, naturally produced by Lactic Acid Bacteria (LAB). Several bacteriocins producing strains have been isolated from raw and fermented products.[7] Bacteriocins produced by these strains have been assessed for potential application as therapeutic agents. Anionic lipids are present in abundance in the cellular membranes; antibacterial peptides interact with those lipids, thereby initiating the pore formation in the membranes of susceptible cells.[8]

In the present work, the multidrug resistant strains (MDRs) were isolated and then biochemically characterized. Phenotypic and genotypic

Correspondence: Dr. Saba Riaz,
Asstt. Prof. of Microbiology and Molecular Genetics,
University of the Punjab, Lahore
Cell No.: 03214530648
E-mail: saba.mmg@pu.edu.pk

characterization was performed for confirmation of MDR. Putative bacteriocins was isolated from five Lactobacilli and checked for the nature of bacteriocins by different treatments.

MATERIALS AND METHODS

Two hundred and three clinical samples were analyzed during November 2012 to January 2013 in Gulab Devi Chest Hospital, Lahore. Samples include pus, sputum, blood, body fluids etc. Strain identification was done by using biochemical profiling and analytical profile index (API) according to the guidelines provided by Clinical and Laboratory Standards Institute (CLSI).[9]

Antibiotic susceptibility testing was performed using the modified Kirby-Bauer disk diffusion method according to CLSI guidelines. The antibiotic disks (HiMedia, Mumbai, India) usedwere Ampicillin (10µg), Piperacillin + Tazobactam (100/10 µg), Ceftriaxone (30 µg), Cefotaxime (30µg), Ciprofloxacin (5 µg), Norfloxacin (10 µg), Cephtazidime (30 µg), Amikacin(30 µg), Gentamycin (10 µg), Cotrimoxazole (5µg), Imepenem (10 µg), Meropenem (10 µg), Clindamycin (2 µg), Nitrofeurontoin (300µg), Oxacillin (1 µg), Vancomycin (30 µg) and Augmentin (10 µg). Multiple antibiotic resistance index (MAR) is helpful in analyzing health risk, and is used to check the antibiotic resistance. The MAR Index of an isolate is defined as "a/b", where "a" represents the number of antibiotics to which the isolate was resistant and "b" represents the number of antibiotics to which the isolate was subjected.

A disc of augmentin (20 µg amoxicillin + 10 µg CLA) was placed on the surface of Muller Hinton (MH), then discs of cefpodoxime (30 µg), CAZ (30 µg) and CTX (30 µg) were kept around it in such a way, that each disc was at distance ranging between 20 and 22 mm from the augmentin disc (centre to centre). The plate was incubated at 37°C overnight.

CAZ and CTX disc with and without clavulanic acid were placed on the agar at a distance more than 30mm. After that, plates were incubated at 37°C and observed zone of inhibition after 24hours. If the zone of inhibition increased upto 5 mm with the addition of clavulanic acid than the strain was reported to as positive for combination disc (CD) test. Plasmid isolation was done by rapid mini-prep isolation method. Finally the bands were visualized in 0.7% agarose gel with 0.5 mg/ml of ethidium bromide in 0.5 X TAE buffer.

Conjugation is the process by which DNA is transferred from one bacterial cell to another. The mechanism requires a direct contact between a donor and a recipient cell. Escherichia coli K12 and DH5α strains were used as donor strains. Donor and recipient were grown overnight at 37°C in N-broth supplemented with respective antibiotics. Conjugation mixtures were prepared by mixing Donor and Recipient in the ratio of 1:9, 1:1 and 9:1 respectively. Mating mixtures were incubated at 37°C for 18-24 hours. After incubation, 50µl of conjugation mixture was plated on N- Agar plates (containing respective antibiotics that were used as markers). Results were recorded after 18-24 hours of incubation at 37°C.

Samples were prepared by making dilutions of yoghurt, cheese and probiotic sachets. 50µl of 10^{-4}dilution of each sample was plated on MRS agar[10]. Biochemical tests were performed for the identification of strains and phylogenetic analysis was used for confirm identification

Isolated strains were characterized morphologically, microscopically as well as biochemically. Growth at different temperatures (15, 25 and 37ºC), acid tolerance test (pH 2, 3, 5, 7 and 9) and acid production was evaluated in which supplementation of media with different sugars normally present in diet was used. Minimal medium was supplemented with 4% sugars (glucose, lactose and sucrose).[11] Strains were inoculated in MRS broth. After specific incubation, broth culture from each tube was transferred to eppendorf. Supernatant was obtained by centrifugation at 10,000 rpm for 10 min. Addition of 5 mol l^{-1} of NaOH was used as neutralizing agent.[12] Cell free extract was then used to check inhibitory effect against characterized MDRs.[13] Wells were cut in swabbed plates and 100µl of the cell free supernatant of the Lactobacillus strains were placed into each well. Plates were incubated at 37ºC for 24 hours.[14] A clear zone of inhibition of at least 2mm was reported positive inhibitory activity.[15]

Putative bacteriocins were treated with SDS, high temperature and proteinase k enzyme [16].Cell free supernatant was treated with 20% SDS (sodium dodecyl sulphate) for 5mints, 5 µl of proteinase k for 2hrs and heated at 80ºC for 15 minutes. After treatment, 100µl of supernatant was placed in the agar wells and incubated at 37 ºC for 24 hrs. After incubation, zone sizes of treated and untreated extract were measure.

Protein content present in the antibacterial compounds of selected strains was estimated by the bradford method. Protein concentration was determined by comparing the curve with the standard curve of BSA.

16SrRNA sequencing was performed, phylogenetic analysis was established and tree was constructed by MEGA4 software. The sequenced data was refined and submitted to Gen Bank, and the accession numbers were obtained.

RESULTS

Two hundred and three clinical strains were analyzed. 135 member of Enterobacteriaceae were confirmed by API. Enterobacteriaceae confers resistance against ampicillin, amikacin, cephtazidime, gentamycin, augmentin, cotrimoxazole ceftriaxone, cefuroxime, ciprofloxacin and tazocin. Selected Staphylococcus aureus strains were resistant to augmentin, ampicillin,

cephradine, ciprofloxacin, gentamycin, ceftriaxone, cefuroxime, clindamycin, imepenum, oxacillin. When the gender based study was conducted it was observed that Escherichia coli resistance was higher in female. In Staphylococcus aureus, resistance in female was about 92% and only 23% in males. Overall resistance of antibiotics in Staphylococcus aureuswas 79% which was quite higher.MAR value ranges from 0.2 to 0.95 among different strains (Table 1).

Total 17 suspected ESBLs strains were tested. Among them 10 strains showed synergism of antibiotic CAZ with Amoxicillin. 4 strains showed Synergism of CTX with AMC. This indicated that 47% strains were determined ESBLs by this technique. Total 17 Suspected strains were tested for ESBLs character. 11 strains indicated positive test towards combination of CTX and CAZ with Clavunate. 65% were determined ESBLs positively by this test (Fig. 1). Plasmids of Selected strains were extracted and all of the tested strains possessed plasmid DNA. All the strains that harbor plasmid DNA were tested for conjugation and these strains indicated transfer of plasmids in the recipient strains. Total 15 strains were isolated on MRS Agar. Samples used were Yoghurt, cheese as well as commercial probiotic sachet. Out of 15 isolated strains 10 were from Yoghurt sample, 2 from cheese sample and 3 were obtained from probiotic sachet and they were named as SY1-10, SC1, 2 and SA1-5 respectively. Antibacterial compound was stable at high temperature treatment but significant decrease in zone sizes was observed when treated with 20% SDS and 5µl of Proteinase k (Fig. 2). This decrease in zone sizes indicated the proteinaceous nature of the compound.

Table No.1: Multiple antibiotic resistance (MAR) value of bacterial strains (n = 25)

MAR values	Number of strains (%)
0.2 – 0.4	16
0.41 – 0.6	20
0.61-0.8	36
0.81-1.0	28

DNA sequencing was done for selected strains. NCBI nucleotide blast was used to determine homology of consensus sequence. Phylogenetic tree was formulated. It was observed that the Blast results for strain SA1 and SA3 showed 99% similarity with Lactobacillus casei while SC1 and SY1 both showed 99 to 100% similarity with Lactobacillus paracasei. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.00532341 is shown. Accession no for strain SA1=KC967209, SA3=KC967210, SC1=KC967211 and SY1= KC967212 (Fig. 3).

Out of 15 isolated strains 10 appeared as non-motile, gram positive bacilli with negative results for catalase production as well as cytochrome oxidase production. Moreover, these strains showed maximum growth at 25º C and 37 ºC but growth was also observed at 15º C for some strains. All strains were stable at basic pH 5, 7 and 9butonly 8 strains were stable at basic as well as acidic pH (2 and 3). Acid production was also observed for some strains when different carbohydrate sources were used. Generally high production of acid was observed with 4% sucrose as a carbohydrate source, with the exception of SA2 (Table 2).

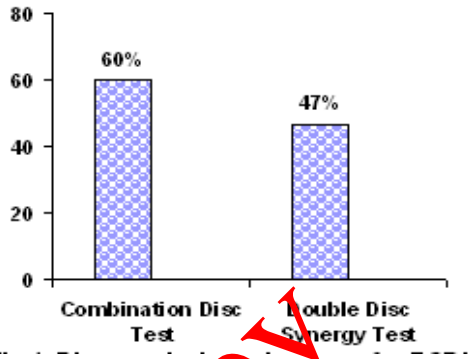


Figure No.1:Phenotypic detection tests for ESBLs

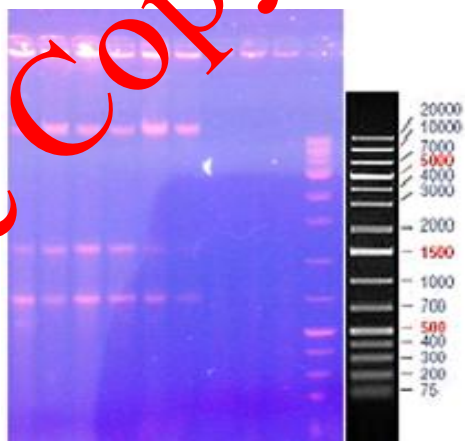


Figure No.2: Plasmid DNA extraction

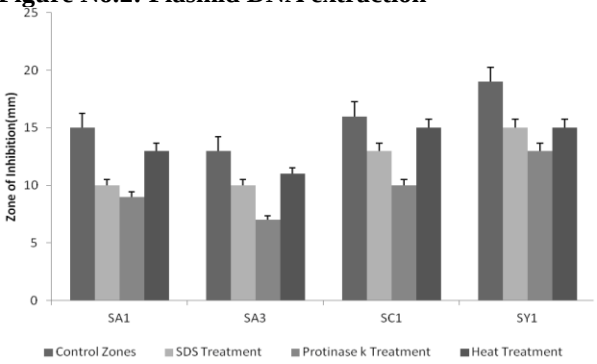


Figure No. 3: Effect of different treatment on antibacterial compound

All the characterized bacterial strains were observed for their growth inhibition activity against different MDRs. Only five strains showed significant activity against selected MDRs. Strains obtained from yoghurt SY1 were most effective against gram negative strains specifically Enterobacteriaceae. While, for gram

positive strains including Staphylococcus aureus and Streptococcus pyogenes, most effective strains were those obtained from cheese and probiotic sachet, SC1 and SA1.

Comparison of the optical density of antibacterial compounds with standard curve indicated that all the strains showed different protein contents. SY1 contain 10 mg/ml while SC1 contain 7mg/ml in its antibacterial compound (Table 2).

Table No.2: Characterization of lactic acid bacteria

Strain name	Production of acid value			Growth at Different Temperatures				Growth at Different pH				Protein Content in Antibacterial Compound	
	Sucrose	Glucose	Lactose	15ºC	30ºC	37ºC	45ºC	6.8	5.0	3.0	2.0	OD at 595nm	Conc. (mg/ml)
SA1	40.18	34.65	37.22	+	++	++	+	++	++	+	+	1.45	4.0
SA2	40.81	45.23	45.04	-	++	++	+	++	++	-	+	1.40	2.0
SA3	36.24	36.41	34.67	+	++	++	+	++	++	++	++	1.43	3.0
SC1	42.70	39.94	35.60	+	++	++	-	++	++	-	-	1.50	7.0
SY1	43.42	36.12	40.36	-	-	++	-	++	+++	++	+	1.55	10
SY2	37.95	34.37	37.67	-	-	++	-	++	+++	++	+	1.53	8.0

DISCUSSION

A few years ago availability and discovery of new life saving drugs was considered as like vanishing the sorrows of the developing world. But, in western world bacterial strains are becoming highly resistant to the antibiotics that were used previously. Few decades ago, focus was particularly on MRSA (Methicillin Resistant Staphylococcus aureus) and vancomycin-resistant Enterococcus spp. Multidrug resistance in gram negative bacteria is going in an alarming situation.[17]

Here, higher resistance towards augmentin was reported (>90%) but in another study > 34.4% resistance has been reported. NOR had higher resistance profiles, similar results were observed in another study in which 18.5% resistance for this antibiotic among different clinical isolates.[18] This increase in bacterial resistance towards these antibiotics may be due to specific genetic makeup of the pathogens, antibiotics utilization including the widespread use of systemic antibiotics, less information about the utilization of antibiotics.[19] Horizontal gene transfer contributes towards the spontaneous spread of an epidemic which is predominantly due to mobile genetic elements. Other contributing factors may include improper dosage, misuse of antibiotics for non-bacterial infections, self-medication, extended duration of therapy, globalization as well as migration.[20]

Physiological characterization of isolated strains showed that these strains have ability to grow on large scale as they grow effectively at 25ºC and 37ºC growth was also observed at 15ºC. Another important criterion for these strains is to the tolerance of acidic environment of our GI tract that is as low as 1.5[21,22] and it is usually observed when a person is under fasting condition.[23] A probiotic source is considered as good if it remains stable at pH as low as 3. When these strains were grown at lower pH i.e. 2 and 3, reduction in their growth was observed but still they tolerated the extreme pH. High resistance to temperature and stability at low pH, makes these compounds more useful even inhuman gastrointestinal tract.[24] Protein content of the antibacterial compound effects directs to its antibacterial activity. This is an indirect indicator that the antibacterial compound is a protein product.

It has been predicted that in very near future antibiotic resistance will make healthcare professionals helpless towards effective therapies for bacterial infections. Consequently, there is an urgent need to search for unconventional antibiotics. Interest in peptide antibiotics has increased greatly during the past decade, as these are believed to be very potent and are biologically and chemically very diverse. They show higher activity and higher specificity towards their target. Moreover, they have few toxicological problems and their accumulation in organs is not observed quite often. There is few drug-drug interaction problems have been observed for peptide antibiotics.[25]

The antagonistic activity of the putative compounds secreted by the strains exerts either inhibitory or bactericidal activity. But, for the in vivo utilization of these bacterial strains it is necessary to know the process by which the bacteria is producing specific compound or compounds.

CONCLUSION

Putative bacteriocins produced by Lactobacilli exhibit significant antibacterial activity against selected MDRs. MDRs have ability to transfer their resistance to other bacteria. The peptidal component of these bacteriocins can be used as an alternative therapy. Proper hospital policies require minimizing the horizontal spread of MDRs. Hence, it is necessary to purify the antibacterial molecule out of putative bacteriocin for further analysis.

Acknowledgement: Authors wish to express their gratitude to staff of Gulab Devi Chest Hospital for their kind help in assistance of research.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. McDermott PF, Walker R, White DG. Antimicrobials: modes of action and mechanisms of resistance. Int J Toxicol 2003;22:135-43.
2. White GD. Antimicrobial resistance in pathogenic Escherichia coli from animals. Emerg Infec Dis 2006; 44:145–66.
3. Pfaller MA, Jones RN, Marshall SA. Inducible amp C beta-lactamase producing gram-negative bacilli from blood stream infections: frequency, antimicrobial susceptibility, and molecular epidemiology in a national surveillance program (SCOPE). Diag Micr Infec Dis 1997; 28:211-9.
4. Riaz S, Faisal M, Hasnain S. Antibiotic susceptibility pattern and multiple antibiotic resistances (MAR) calculation of extended spectrum β-lactamase (ESBL) producing Escherichia coli and Klebsiella species in Pakistan. Afr J Biotechnol 2011; 10:6325-31.
5. Saleem M, Nazir M, Ali MS, Hussain H, Lee YS, Riaz N, et al. Antimicrobial natural products: an update on future antibiotic drug candidates. Nat Prod Rep 2010; 27:238–254.
6. Remiger A, Eijsink MA, Ehrmann. Purification and partial amino acid sequence of plantaricin 1.25ααα and 1.25βββ, two bacteriocins produced by Lactobacillus plantarum TMW1.25. . App Microbiol 1999; 86:1053-8.
7. Rodriguez E, Gonzalis B, Gaya P, Nuñez M, Diversity of bacteriocins produced by lactic acid bacteria isolated from raw milk. Int Dairy J 2000;10:7-15.
8. Moll GN, Konings WN, Driessen JM. Bacteriocins: mechanism of membrane insertion and pore formation. Anton van Leeuw J Microb 1999;76:185–98.
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Twenty-first informational supplement; M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA, 2011.
10. De Man JC, Rogosa M, Sharpe ME. A medium for the cultivation of Lactobacilli. J Appl Bacteriol 1960; 23:130-35.
11. Bosch M, Molina JN, Audivert S, Bonachera MA, Alemany AS, Fuentes MC, Cuñé J. Isolation and characterization of probiotic strains for improving oral health. Arch Oral Biol 2011; 2691:413-24.
12. Nawaz SK, Riaz S, Hasnain S. Screening for anti-methicillin resistant Staphylococcus aureus (MRSA) bacteriocin producing bacteria. Afr J Biotechnol 2009; 8:365-8.
13. Schillinger U, Lucke FK. Antibacterial activity of Lactobacillus sake isolated from meat. Appl Environ Microb 1989; 55:1901-6.
14. Ivanova I, Kabadjova P, Pantev A, Danova S, Dousset X. Detection, purification and partial characterization of a novel bacteriocin, substance produced by Lactoccouslactis subsp. lactis b14 isolated from Boza-Bulgarian traditional cereal beverage. Biocatalysis 2000; 41: 47-53.
15. van Reenen CA, Dicks LMT, Chikindas ML. Isolation, purification and partial characterization of plantaricin 423, a bacteriocin produced by Lactobacillus plantarum. J Appl Microb 1998; 84: 1131–7.
16. Rajaram G, Manivasagan P, Thilagavathi B, Saravanakumar A. Purification and Characterization of a Bacteriocin Produced by Lactobacillus lactis Isolated from Marine Environment. In. J F Sci Tech 2009; 2: 138-44.
17. Rice LB. The clinical consequences of antimicrobial resistance. Curr Opin Microbiol 2009; 12: 476–81.
18. Guay DR. An update on the role of nitrofurans in the management of urinary tract infections. Drugs 2001; 61: 353–64.
19. Harbarth S, Samore MH. Antimicrobial resistance determinants and future control. Emerg Infect Dis 2005; 11:794–801.
20. Sharma S. Antibiotic resistance in ocular bacterial pathogens. Indian J Med Microbiol 2001;29: 218-22.
21. Lin WH, Hwang CF, Chen LW and Tsen HY. Viable counts, characteristic evaluation for commercial lactic acid bacteria products. Food Microbiol 2006;23: 74-81.
22. Huang Y, Adams MC. In vitro assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria. Int J Food Microbiol 2004; 91: 253-60.
23. Fernandez MF, Boris S, Barbes C. Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. J Appl Microbiol 2003; 94: 449-55.
24. Russel JB, Mantovani HC. Thebacteriocins of ruminal bacteria and their potential as an alternative to antibiotics. J Mol Microbiol Biotechnol 2004;4:347–55.
25. Rehman S, Khanum, A. Isolation and characterization of peptide(s) from Pisum Sativum having antimicrobial activity against various Bacteria. Pak J Bot 2001; 43: 2971-78..

Original Article

Oral Biology of Medical Students

Perception Study of Relevance of Oral Biology Amongst 1st Year BDS Students, Dental House Officers & Post-Graduate Trainees in DUHS

1. Farzeen Shafiq Waseem 2. Noor ul Hira Aftab 3. Asmat Jameel 4. Amynah Charnia

1. Asstt. Prof. of Oral Biology, DIMC, DUHS, Karachi 2. Msc. Trainee of Oral Biology, Dr. Ishratulebad Khan Institute of Oral Health Sciences, DUHS, Karachi 3. Assoc. Prof. of Operative Dentistry, JM&DC, Karachi 4. Prof. and Head of Deptt. Of Oral Biology, Dr. Ishratulebad Khan Institute of Oral Health Sciences, DUHS, Karachi

ABSTRACT

Objectives; To investigate the significance of Oral biology course in dentistry amongst 1st year BDS, interns and postgraduate students.

Study Design: Cross sectional study

Place and Duration of Study: This study was carried out at Dow University of health sciences from 1st April 2015 to 1st July 2015.

Materials and Methods: This study was conducted on 75 1st year BDS students, 25 house officers & 100 postgraduate trainees of Dow University of Health Sciences. A questionnaire based on a likert scale was distributed among 200 participants. The response rate was 100%. 5 questions were asked with five options. The main focus was to know whether Oral Biology being the basic foundation of dentistry is adequate enough to be taught for one year only. Data was analyzed on SPSS version 22.

Results: 65% strongly agreed and 34% agreed that Oral Biology is relevant subject for general dentistry. Whereas 47.5% disagree and 9% strongly disagree that Oral Biology is one of the pillars in dentistry. 68.5% strongly agree and 25% agree that Oral Biology is a building block in medical and dental sciences. 24% agree, strongly agree12% and remaining 59% were unaware with the fact about the knowledge of oral biology is applicable to clear the aptitude test. Majority of the students agreed that the knowledge of the oral biology is essential for their clinic practice in future (Strongly agree-44% Agree-48.5%).

Conclusion: Oral biology is the relevant subject to the general dentistry.

Key Words: Oral Biology, relevance, dental students, subject knowledge

Citation of article: Waseem FS, Aftab NH, Jameel A, Charnia A. Perception Study of Relevance of Oral Biology Amongst 1st Year BDS Students, Dental House Officers & Post-Graduate Trainees in DUHS. Med Forum 2016;27(2):33-36.

INTRODUCTION

Dental sciences play an essential role in education of dentistry.[1] It includes obtaining ethical knowledge, exposing clinical knowledge and different researches on which diagnosis and patient's treatment are based[2]. It is the matter of concern that the dental community and the system of basic dental sciences education is not accentuated, in particular dental sciences is always absolved not getting an access. Formerly dental education has evidenced that the students give preference to the clinical dentistry and not to the basic sciences as their profession which is the matter of concern.[3]

According to American Association of Oral Biologists, Oral Biology is defined as a discipline that deals with the understanding of the development structure and function of oral tissues in health and diseased conditions[4]. It is a basic dental science subject with the vast divergent in the scientific era including the development of molecular biology and genetics, microbiology and immunology, biochemistry, biophysics, craniofacial biology and development, pharmacology and physiology.[5] With the new advancement in dentistry, the addition of forensic odontology, dentists are getting awareness regarding forensic odontology and it will be the major part of dentistry in future. Because of the solitary coalescence of tissue types, functions of the mouth and craniofacial complex it has the connection with every field of dentistry, clinical as well as basic dental sciences[6].

The importance of oral biology has been overlooked for a long period of time in the dental curriculum. With the recent advancement in the molecular and genetic levels it has now become impossible to deny that oral biology is the vital part of all dental subjects in any manner[7]. For the good clinical practice it is very important to know the development, anatomy, morphology of the

Correspondence: Dr. Asmat Jameel
Assoc. Prof. of Operative Dentistry, JM&DC, Karachi
Cell No.: 0333-2133570
E-mail:asmat.jameel@gmail.com

tooth. In order to justify their profession, clinicians should treat patients to the best of their knowledge that can only be achieved by improving the basic knowledge & studying the subject in depth.[8]

The aim to conduct this study was to investigate the perception of relevance of Oral Biology amongst 1st year BDS students, house officers and post graduate trainee at Dow University of health sciences. Moreover, to know that whether Oral Biology subject is relevant for the general dentistry or serving as a bridge in between the clinical & basic sciences and helping post graduate students.

MATERIALS AND METHODS

It is the cross sectional based study which was conducted on 1st year BDS students, house officers & post graduate trainees of Dow University of health sciences. A total of 200 questionnaires were distributed among the participants in which 75 forms were filled voluntarily by 1st year BDS students 25 by interns and 100 by the post graduates and collected by hand. Duration of the study was four months starting from 1st April 2015 to 1st July 2015

A short questionnaire based on Likerts scale was formulated. The questionnaire was close ended. It comprised of five questions. In order to survey the viewpoint of the respondents five options were given to answer each query from strongly agree/agree to strongly disagree/disagree to don't know. 1st question was about the relevance of Oral biology to the general dentistry, the second was about the opinion of Oral biology subject as bridging to fill the gap between clinical & basic sciences. Third question was about the importance of oral biology as the foundation course. The fourth question was on the perception of Oral biology as a future clinical professional training. In the last question they were asked about the role of Oral biology in the entrance test & post grad level.

Data was analyzed using SPSS 22 and chi-square goodness of fit test was applied.

RESULTS

It can be observed from the Table-1 that the most of subjects agreed (SA-65% ,A-34%) with the fact that Oral Biology is relevant subject for general dentistry, only 1% were not in favor about the connection between Oral Biology and general dentistry (p-value<0.05%). Regarding time period of teaching Oral Biology, varied feedback was seen with more than half of the students disagree (DA-47.5%, SD-9%), believing that oral biology should acquire strong position in dentistry and for more than one year of BDS.

For further evaluation the separate frequency Table 2 is given so that the distribution could be seen individually. The importance of Oral Biology in building the base between the medical and dental sciences dominantly it is evident in Table 1 (SA-68.5% ,A-25%) with negligible people disagree(DA-2.5%,SD-2%). Majority of the students agreed to the fact that knowledge of the Oral Biology is essential for their clinical practice in future (SA-44%, A-48.5%), insignificant amount of students (SD-2.5%) 1% were unaware of it .Last question was addressed about the Oral biology course knowledge to determine whether it is helpful for clearing basic aptitude post graduate test (MDS/ MSC.DS/FCPS PART 1/M.PHIL), the data shows that the a total of 36% agreed (A-24%, SA-12%) and 59% were unaware of it.

Table No.1: Number & percentage of respondent showing perception and relevance amongst undergraduates and post graduates of DUHS

Question	A	SA	DA	SD	DN	P -value
Q1.Do you consider oral biology relevant for general dentistry?	34%	65%	1%	0%	0%	<0.05%
Q2- Do you think Oral biology is enough teaching at 1st year BDS ?	29%	11.5%	47.5%	9%	3%	<0.05%
Q3- Do you think Oral biology is important subject that helps in building the base for medical & dental sciences?	25%	68.5%	2.5%	2%	2%	<0.05%
Q4- Do you consider Oral biology relevant for your future clinical training?	48%	44%	2.5%	4%	1%	<0.05%
Q5- Do you think Oral biology course knowledge was helpful for clearing basic aptitude post-graduatetest (MDS/MSC.DS/FCPS PART 1/M.PHIL)	24%	12.5%	4%	0%	59.5%	<0.05%

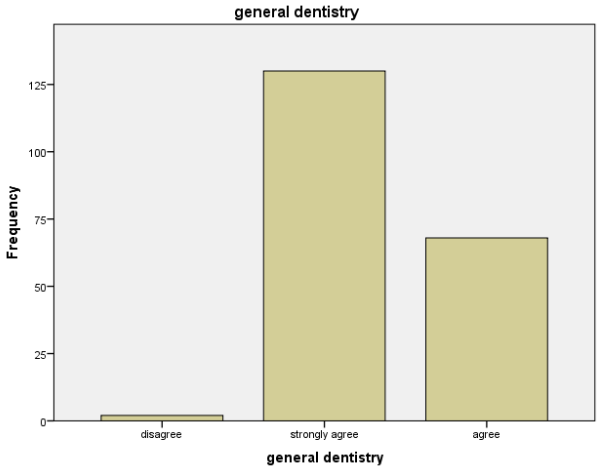
A-agreed which is the neutral , SA- strongly agreed, DA- Disagree, SD- strongly disagree, DN- don't know

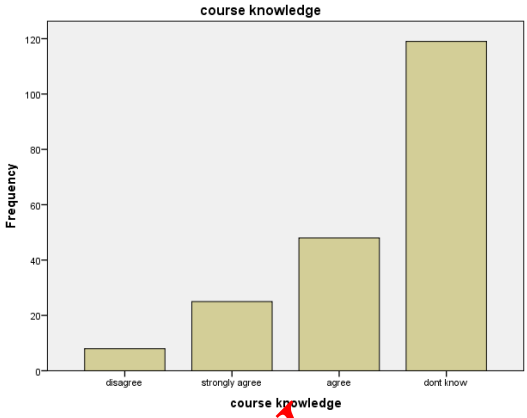
Chi square testing represents p value of less than 0.05% which shows the result is significant (no opinion answers excluded)

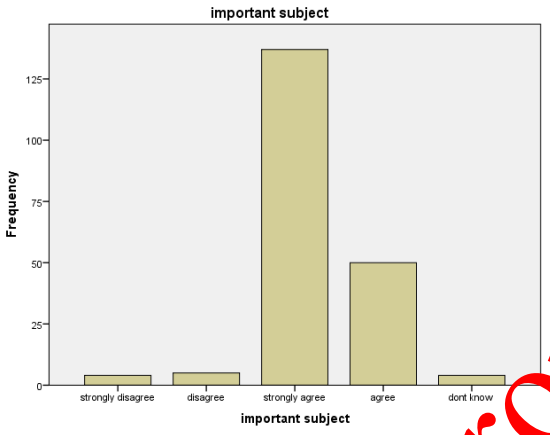
Table No.2: Evaluation of frequency

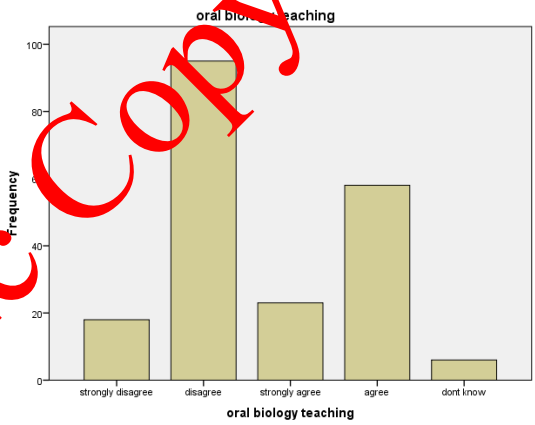
	general dentistry	oral biology teaching	important subject	clinical training	course knowledge
Chi-Square	122.920[a]	131.950[b]	333.150[b]	231.150[b]	143.080[c]
Df	2	4	4	4	3
Asymp. Sig.	.000	.000	.000	.000	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 66.7
b. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 40.0.
c. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 50.0









DISCUSSION

The clinical expertise in dentistry is dependent on the firm ground provided by the knowledge of basic biological concepts.[9] Among the basic subjects taught in dental curriculum the subject of utmost importance is Oral Biology as it ligands the basic medical subjects with the other dental basic and clinical subjects[10]. The aim of the present study was to evaluate the students' perspective at different level of the education starting from undergrads along with house officers and post graduates. The results obtained demonstrate convincingly that all groups perceived Oral Biology relevant to dentistry, including dental education and clinical practice .The results interestingly revealed that the significance of the Oral Biology course increases with the advancement of the education. This is in accordance with the study conducted by Schevan BA[11]. This apparent change is obvious when they enter some post graduate program and shift towards deeper learning and understanding of the subject.

Oral Biology is taught in Pakistan in second year or first year, when students foremost adopt a superficial or strategic learning approach just to pass the examinations. The need of developing the research/problem-based learning (PBL) to produce deep learning rather than 'spoon feeding' and assessing the students still remains the greatest challenge in the field of dental education[12].

Relevance of oral biology to dentistry could further be increased if it is not taught independently but as an integrated subject with clinical sciences, which has been proposed previously by Gotjamanos.[13] Clinical subjects teach the students to recognize and apply narrative therapeutics.[14]

In a study by Dr Imran Farooq[15] found out that in this era students have begun to emphasize on the basics with the clinical subjects. The recent research advancements in the medical and dental fields are now treated as biological sciences, as they share the integrated scope regarding etiology diagnosis knowledge treatment of many diseases[16]. In short, when treating patients, relationship with the structures and tissues involved must be considered, the importance of Oral Biology in dentistry can be well accepted.[17]

It has been suggested that oral biology must be incorporated in vertical integration rather than horizontal to improve the clinical relevance of the subject[18,19]. The same was shown in our research when 47.5% of total students strongly disagreed that teaching one year teaching of oral biology is sufficient. Moreover, 44% agreed and 48%strongly found the course is relevant for their clinical training.

Oral Biology like dentistry should emphasize on the Forensic Odontology also[20]. Regarding the query made to the under graduate, house officers and post graduate that whether Oral biology helped them in clearing their aptitude test, among them undergrads did not know whereas post graduates and house officer agreed that the subject helped them in clearing the milestone but lacking is still there.

CONCLUSION

- Dental students, house officers and post graduate students all agreed with the fact that Oral Biology being the relevant subject. Though the more positive response was registered by the seniors as their progression in dental education and training matures the vision of the students.
- Furthermore there is a need for the vertical integration of Oral biology and clinical dentistry combined with a problem-based approach to learning gives the students opportunities to use and adapt their knowledge to common odontological problems and situations.
- It is therefore necessary to conduct further studies in particular, to compare different Oral Biology teaching programs established at various dental institutions and relate those to students' perceptions and experiences to get be more accurate results and to develop an interest in the subject which has been lacking since decades.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Al-Jandan BA, Farooq I, Khan SQ. Students' perspectives on the relevance of internet-based educational videos in dental education. J Taibah Univ Med Sci 2015;10(3):288–292
2. DePaola1D, Howell H, Baker G. Research and the dental student. Eur J Dent Educ 2002;6(3):45–51
3. Kieser J, Herbison P, Waddell N, Kardos T, Innes P. Learning in oral biology: a comparison between deep and surface approaches. NZ Dent J 2006;102: 64–68.
4. The American Association of Oral Biologists. Curricular guidelines for oral biology. J Dent Educ 1984;48:269–273.
5. Gotjamanos T. Oral biology and the early introduction of clinical dentistry in the dental curriculum. J Dent Educ 1986;50:208–212.
6. Baghdady T M, Carnahan H, Lam E W N, Woods NN. Integration of Basic Sciences and Clinical Sciences in Oral Radiology Education for Dental Students. J Dental Educ 2013;77(6):757-763.
7. Kieser J, Herbison P. Student learning and the teaching-research nexus in oral biology. Eur J Dent Educ 2001;5:60–62.
8. Rossomando EF, Moura M. The role of science and technology in shaping the dental curriculum. J Dent Educ 2008;72:19-25.
9. Suddick RP. The current status and impact of oral biology in dental education. J Dent Educ 1976;40: 662–671
10. Kieser J, Kardos T, Higgins A, Herbison P. Context rich problems in oral biology teaching. Eur J Dent Educ 2002;6:114–120
11. Scheven BAA. Perceived relevance of oral biology by dental students. Eur J Dent Educ 2012;16:64-72.
12. [1] Lust KS, Appleton J, Stevenson H. Basic science knowledge of dental students on conventional and problem-based learning (PBL) courses at Liverpool. Eur J Dent Educ 2001;5:148-154.
13. Gotjamanos T .Integration of basic biological sciences and clinical dentistry in the dental curriculum. A clinically orientated approach to teaching oral and dental anatomy Aust Dent J 1990;35(3):290-3
14. Baum BJ. Can biomedical science be made relevant in dental education? A North American perspective. Eur J Dent Educ 2003;7:49-55.
15. Farooq I and S. Ali. Comparison of the perceived relevance of oral biology reported by students and interns of a Pakistani dental college Eur J Dent Educ 2014; 18(Suppl. 4): 203–206
16. Entwistle NJ. Approaches to learning and perceptions of the learning environment. Higher Educ 1991;22:201-204.
17. Chavez V A.The current role of Oral Biology in Dentistry. Joralres 2015;58.
18. Fry H, Ketteridge S, Marshall S. Understanding student learning. A handbook for teaching and learning in higher education: enhancing academic practice. London: Kogan Page;1999.p.21–40,82.
19. Kieser J, Herbison P, Waddell N, Kardos T, Innes P. Learning in oral biology: a comparison between deep and surface approaches. NZ Dent J 2006;102: 64–68.
20. Yip HK, Barnes I. Learning in dental education. Eur J Dent Educ 1997;1:54–60.

Original Article

Breast Self-Examination

Assessment of Factors Associated with Practice of Breast Self-Examination in a Tertiary Care Health Setting In Karachi

1. Farhat Jaleel 2. Riffat Jaleel 3. Syeda Sakina Abidi 4. Masood Javaid

1. Assoc. Prof. of Surgery, 2. Assoc. Prof. of Obst. & Gynae, 3. PG Trainee of Surgery, 4. Asstt. Prof. of Surgery, DIMC / DUHS, Karachi.

ABSTRACT

Objectives: (1) To determine the knowledge, attitude and practice towards breast self-examination (BSE) in tertiary care health setting in Karachi. (2) To assess factors associated with practice of BSE

Study Design: Cross sectional study

Place and Duration of Study: This study was carried out at the Dow University Hospital, Dow International Medical College and Dow Medical College / Dow University of Health Sciences Karachi from July to December, 2014.

Materials and Methods: Study participants were medical students, interns, residents, consultants, as well as female patients and their lady attendants visiting General Surgical Out Patient Department. A questionnaire was developed including demographic details, knowledge about BSE, attitude towards and practice of BSE. These questionnaires were filled in by interns and residents of Department of General Surgery. Interviews were done in privacy after taking verbal consent.

Data were entered and analyzed using SPSS version 16. Categorical variables about knowledge, attitude and practice are presented as frequencies. Predictors of BSE ie., age groups, level of education, occupation, income, marital status, personal history of benign breast disease and family history of breast cancer are also presented as frequencies. Their association with ever practice of BSE is determined by using chi square test.

Results: Total of 729 women were interviewed to achieve a sample size of 500 positive responders, who had heard about BSE, which was 68.6%. Further questioning was done from these women.

Majority of participants were young, 74% being less than 30 years of age. Around half of them were graduates and 21% were post-graduates. Sixty four percent respondents belonged to medical profession, while 22.2% were housewives and 13.8% had other professions. Majority belonged to high income group. 61.4% were married and 11.4% had family history of breast cancer. 29% respondents knew correct age to start BSE. Proposed frequency was stated correctly by 49.6%, appropriate time by 42.6%. Around 42% women said they knew how to perform and 36% could verbally explain the procedure correctly. More than 96% women thought that BSE was useful and should be practiced. Only 39.2% women claimed that they practiced BSE, while just 63 of the total 500 women interviewed, were doing it regularly. Level of education, profession, income, marital status and family member with breast cancer were found to be significant factors associated with BSE practice.

Conclusion: Although overall awareness about BSE was average but correct knowledge and actual performance were poor. Medical profession, graduate level of education, handsome income, married status and family history of breast cancer were associated with BSE practice.

Key Words: Breast Self-Examination, Breast Cancer, Predictors

Citation of article: Jaleel F, Jaleel R, Abidi SS, Javaid M. Assessment of Factors Associated with Practice of Breast Self-Examination in a Tertiary Care Health Setting In Karachi. Med Forum 2016;27(2):97-40.

INTRODUCTION

Breast cancer is the commonest malignancy affecting women worldwide.[1,2] It is the leading cause of morbidity and mortality among all female cancers.[3] An estimated 502,000 women die each year from this deadly disease.[2] Prevalence of breast cancer is on the rise, more so in developing countries.[4] A hundred percent rise has been noted in the last decade in Nigeria.[3] Crude incidence of breast cancer in Asian countries is reported between 21.3 to 52 per 100,000 population.[5] Roughly 1 in 26 women have a lifetime chance of suffering from breast cancer.[6] Moreover, the age of onset is decreasing and cancer in younger women tends to be more aggressive.[7]

Late presentation of patients in third or fourth stage disease is common in developing countries. Lack of awareness, socio-economic constraints, shyness, fear, paucity of widespread diagnostic facilities and misdiagnosis are main factors responsible for low survival rates.[6] It is well known that early detection can considerably improve the outcome. Breast self

Correspondence: Dr. Farhat Jaleel,
Assoc. Prof. of Surgery, DIMC / DUHS, Karachi.
Cell No.: 03002163383
E-mail: farhatjaleel@yahoo.com

examination (BSE), clinical breast examination, ultrasound and mammography are recommended screening methods.

BSE is visualization and palpation of the breasts by one's own self for lump, shape, texture, size and contour. American College of Obstetricians & Gynecologists and American Cancer Society recommends that it should be performed monthly, starting at age of 20 years.[8]BSE has lately been questioned as an effective screening tool as it has not shown to aid in reducing mortality.[9,10]Nevertheless it is simple to perform, time saving and costless method with no associated pain and no risk of radiation.[11] It encourages women to be aware of their own health, as they can be the best persons to identify any change in their body. If widely used, BSE can be of immense help in low resource countries like Pakistan.

This study was planned to gain baseline data about knowledge, attitudes and practice of women of varying educational levels. We further wanted to explore the factors associated with practice of BSE. By highlighting these aspects, we can plan steps and guidelines to promote this method of screening.

MATERIALS AND METHODS

This cross sectional study was conducted in Dow University Hospital, Dow International Medical College and Dow Medical College / Dow University of Health Sciences Karachi. Duration of study was six months between August to December, 2014. Study participants were medical students, interns, residents, consultants, as well as female patients and their lady attendants visiting General Surgical Out Patient Department. A questionnaire was developed including demographic details, knowledge about BSE, source of knowledge, attitude towards acquiring knowledge and practice of BSE and reasons for not doing so. These questionnaires were filled in by interns and residents of Department of General Surgery. A total of 729 females were approached to reach a number of 500, who had heard about BSE. Interviews were done in privacy after taking verbal consent.

Data were entered and analyzed using SPSS version 16. Categorical variables about knowledge, attitude and practice are presented as frequencies. Factors associated with BSE practice i.e., age groups, level of education, occupation, income, marital status, personal history of benign breast disease and family history of breast cancer are also presented as frequencies. Their association with ever practice of BSE is determined by using chi square.

RESULTS

Total of 729 women were interviewed to achieve a sample size of 500 positive responders, who had heard about BSE, which was 68.6%. Further questioning was done from these women.

Majority of participants were young, 74% being less than 30 years of age. Around half of them were graduates and 21% were post-graduates. Sixty four percent respondents belonged to medical profession; 36.6% medical students and 27.4% doctors, while 22.2% were housewives and 13.8% had other professions. Majority belonged to high income group. 61.4% were married and 11.4% had family history of breast cancer.

Table No.1: Frequency Of Knowledge, Attitude, Practice About BSE

Variables		Response	Number	%age
Knowledge	Heard about BSE (n=729)	Yes	500	68.6
		no	229	31.4
	(n=500) Age to start BSE	20 years	145	29
		After menarche	201	40.2
		After pregnancy	64	12.8
		After menopause	47	9.4
		Any other	43	8.6
	Proposed frequency	Daily	51	10.2
		Monthly	248	49.6
		Yearly	91	18.2
		Whenever get time	65	13
		Any other	45	9
	Appropriate time	Before periods	90	18
		After periods	213	42.6
		No specific time	151	30.2
		Any other	46	9.2
	Know how to perform	Yes	213	42.6
		Partly	209	41.8
		No	78	15.6
	Can explain procedure	Yes	184	36.8
		partly	222	44.4
		No	94	18.8
Attitude	Should be done	Yes	482	96.4
		No	18	3.6
	Useful method	Yes	484	96.8
Practice	Do perform	No	16	3.2
		Yes	196	39.2
		No	304	60.8
	How often (n=196)	Regularly monthly	63	32.1
		Regularly weekly	5	2.6
		irregularly	128	65.3

When asked about which age to start BSE, 29% gave correct answers. Proposed frequency was stated correctly by 49.6%, appropriate time by 42.6%. around 42% women said they knew how to perform and 36% could verbally explain the procedure correctly. More than 96% women thought that BSE was useful and

should be practiced. Only 39.2% women claimed that they practiced BSE, while just 63 of the total 500 women interviewed, were doing it regularly (Table 1). Table 2 shows the different responses of women who were not performing BSE despite awareness. Commonest reason was not knowing the technique properly.

Table 3 shows the significance of various factors which could predict the practice of BSE. Level of education, profession, income, marital status and family member with breast cancer were found to be significant.

Table No.2: Reasons for non practice of BSE. Total number = 304

Responses	n	%
Did not know technique	86	28.3
Not important	26	8.6
Fear of detecting lump or cancer	48	15.8
Don't get time	48	15.8
Don't have symptoms	64	21.1
Feel shy / embarrassed	48	15.8

Table No.3: Factors associated with BSE practice

Variables		Total N(%)	BSE N(%)	p
Age	< 30	371(74.2)	149(76)	0.261
	31-40	86(17.2)	37(18.9)	
	41-50	26(5.2)	6(3.1)	
	51-60	13(2.6)	3(1.5)	
	60	4(0.8)	1(0.5)	
Level of education	< matric	59(11.8)	1(0.5)	0.000
	Matric	30(6)	4(2)	
	Intermediate	105(21)	30(15.3)	
	Graduate	245(49)	124(63.3)	
	Postgraduate	61(21.2)	37(18.9)	
Profession	Housewife	111(22.2)	12(6.1)	0.000
	Medical student	183(36.6)	85(43.4)	
	Doctor	137(27.4)	79(40.3)	
	Other	69(13.8)	20(10.2)	
income	< 10,000	36(7.2)	1(0.5)	0.000
	10-30,000	61(12.2)	9(4.6)	
	31-50,000	49(9.8)	15(7.7)	
	51-100,000	138(27.6)	65(33.2)	
	1-2 lac	145(29)	69(35.2)	
	2 lac	71(14.2)	37(18.8)	
Marital status	Single	184(36.8)	45(23)	0.000
	Married	307(61.4)	145(74)	
	Divorcee / Widow	9(1.8)	6(3)	
Personal history of breast disease	Yes	5(1)	3(1.5)	0.338
	No	495(99)	193(98.5)	
Family history of breast cancer	Yes	57(11.4)	30(15.3)	0.027
	No	443(88.6)	166(84.7)	

DISCUSSION

A National study from Lahore suggests that Pakistani women tend to present with malignant breast disease at comparatively younger age. They found 20% of breast lumps to be malignant.[12]It is postulated that if all women examine their breasts monthly and visit healthcare professional timely for clinical examination and mammography, it may be possible to prevent breast cancer progression in 95% cases.[13]

The present study was conducted amongst women attending a private tertiary care setup and medical students / professionals. This explains why majority of participants had heard about BSE. In a study conducted in Saudi Arabia, 91.2% women employees and relatives in a teaching hospital were aware of BSE.[14]In another study involving Ethiopian teachers, 52% were aware of BSE.[15] In our study, around half of women responded positively to frequency, appropriate timing, knowledge about how to perform and could verbally explain the technique. But only few knew the correct age to start. In a study of mixed population in Iran, 67.2% knew correct frequency and 41.8% knew proper timing.[16]

Our participants exhibited a very positive attitude,> 96% considered it was a useful method and should be performed. This is consistent with findings in study by Demirkiran F, et al in Turkey.[17] But even with this reassuring attitude, very few of our participants were actually performing regular BSE. In Iranian women study 5[illegible].5% performed BSE[16], while Abolfotouh MA, et al reported that 41.6% women performed BSE.[14] Unfortunately, our results match with a study of market women in Nigeria, 21.8% of whom were practicing BSE.[8]

Our study showed that women who were not performing BSE despite awareness did so because they did not know the technique properly. Other responses like 'don't know importance' and 'don't have symptoms' point to incomplete knowledge. In the Saudi women study, 54.9% said they did not know technique, while 24.5% said they did not trust their own findings.[14] We studied factors associated with positive BSE practice. We have found that level of education, medical profession, high income, being married and having a family member with breast cancer were significant predictors. In the fore mentioned study of Saudi women, family history of breast cancer was significantly associated with BSE performance.[14] Amongst the Ethiopian teachers, important predictors were good knowledge, perceived susceptibility to cancer, perception that cancer is severe and feeling benefited from BSE.[15]In a study from Western Massachusetts involving various ethnic groups, it was found that despite a positive attitude towards BSE, Vietnamese women were less likely to perform BSE regularly and low health literacy scores was important factor.[18] In a Nigerian rural setup, level of education of family head, type of family whether nuclear or extended, breast cancer in family and smoking status were found to be significant predictors for BSE performance.[19] Mohammadi E, et al have described a computer vision technique to calculate the percentage

of the palpated blocks in BSE. Using this real time evaluation women can perform BSE in private without any human supervision.[20]

We suggest that awareness in general population should be enhanced using various forms of media. Furthermore, awareness can be provided and technique taught to females attending antenatal and postnatal clinics. Limitation of our study was that the participants do not represent true general population of Pakistan. More and larger studies are needed in this regard.

CONCLUSION

Although overall awareness about BSE was average but correct knowledge and actual performance were poor. Medical profession, graduate level of education, handsome income, married status and family history of breast cancer were found to influence BSE practice.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. World Health Organization. Breast cancer prevention and control Geneva, Switzerland: WHO:2013. http//www.whoint/cancer/detection/breast/en/.
2. Peter Nde F, Assob JCN, Kwenti TM, Njunda AL, Tainenbe TRG. Knowledge, attitude and practice of breast self-examination among female undergraduate students in the University of Buea. BMC Research Notes 2015;8:43. Doi 10.1186/s13104-015-1004-4
3. Oladimeji KE, et al. Knowledge and Beliefs of Breast Self-Examination and Breast Cancer among Market Women in Ibadan, South West Nigeria. PLoS ONE 10(11):e0140904. Doi:10.1371/journal.pone.0140904
4. Azage M, Abeje G, Mekonnen A. Assessment of Factors Associated with Breast Self-Examination among Health Extension Workers in West Gojjam Zone, Northwest Ethiopia. International Journal of Breast Cancer 2013, Article ID 814395. http://dx.doi. org/10.1155/2013/814395
5. Akhtari-Zavare M, Juni MH, Ismail IZ, Said SM, Latiff LA. Barriers to breast self examination practice among Malaysian female students: a cross sectional study. SpringerPlus 2015;4:692.
6. Doshi D, Reddy BS, Kulkarni SS, Karunakar P. Breast Self-examination: Knowledge, Attitude and Practice among Female Dental Students in Hyderabad City, India. Ind J Palliat Care 2012; 18(1):68-73
7. Karayurt O, Ozmen D, Cetikaya AC. Awareness of breast cancer risk factors and practice of breast self examination among high school students in Turkey. BMC Public Health 2008;8:359.
8. Obaji NC, Elom UM, Nwigwe CG, Ezeonu PO, Umeora OUJ. Awareness and Practice of Breast Self-Examination among Market Women in Abakaliki, South East Nigeria. Ann Med Health Sci Res 2013;3(1):7-12.
9. Blais J. Breast self examination. Editorial. Canadian Family Physician 2002;48.
10. Yang RJ, Huang LH, Hsieh YS, Chung UL, Huang CS, Bih HD. Motivations and reasons for women attending a Breast Self-Examination training program: A qualitative study. BMC Women's Health 2010;10:23. http://www.biomedcentral.com/1472-6874/10/23
11. Suh MAB, Atashili J, Fuh EA, Eta VA. Breast Self-Examination and breast cancer awareness in women in developing countries: a survey of women in Buea, Cameroon. BMC Research Notes 2012;5:627. http://www.biomedcentral.com/1756-0500/5/627
12. Nazeer MA, Ahmad R, Ali M, Ali M, Saleem R, Suleman B. The Frequency of Benign & Malignant Breast Lesions at a Tertiary Care Hospital in Lahore. Pak J Med Health Sci 2012;6(3):570-2.
13. Petro-Nustas W. Health-related behaviours and lifestyle factors of patients with breast cancer. Cancer Nurs 2002;25(3):219-29.
14. Abolfotouh MA, BaniMustafa AA, Mahfouz AA, Al-Assiri MH, Al-Juhani AF, Alaskar AS. Using the health belief model to predict breast self examination among Saudi women. BMC Public Health 2015;15:1163.
15. Birhane N, Mamo A, Girma E, Asfaw S. Predictors of breast self-examination among female teachers in Ethopia using health belief model. Archives of Public Health 2015;73:39.
16. Marzouni HZ, et al. Women's Awareness and Attitude Toward Breast Self-Examination in Dezful City Iran, 2013. Iran Red Crescent Med J 2015;17(11):e17829.
17. Demikiran F, Balkaya NA, Memis S, Turk G, Ozvurmaz S, Tuncyurek P. BMC Public Health 2007;7:96.
18. Armin J, Torres CH, Vivian J, Vergara C, Shaw SJ. Breast self-examination beliefs and practices, ethnicity and health literacy: Implications for health education to reduce disparities. Health Educ J 2014;73(3):274-84.
19. Amoran OE, Toyobo OO. Predictors of breast self-examination as cancer prevention practice among women of reproductive age-group in a rural town in Nigeria. Niger Med J 2015;56(3):185-9.
20. Mohammadi E, et al. Real-Time Evaluation of Breast Self-Examination Using Computer Vision. Int J Biomedical Imaging 2014. http://dx.doi.org/10.1155/924759.

Original Article

Toxic Effects of Lithium

Detrimental Effect of Lithium Carbonate on Cerebellar Purkinje Neurons in Albino Rats

1. Tazeen Kohari 2. Naheed Najmi

1. Asstt. Prof. of Anatomy, 2. Assoc. Prof. of Operative Dentistry, Liaquat College of Medicine and Dentistry, Karachi

ABSTRACT

Objective: To observe the damaging effect of Lithium carbonate on Purkinje neuron in the light of available literature so this study was under taken to see the toxic effects of Lithium carbonate on permanent Purkinje neuron.

Study Design: Experimental study

Place and Duration of study: This study was carried out at Animal House, BMSI, JPMC, Karachi from April 2012 to June 2012.

Materials and Methods: Thirty male albino rats of 190-240 grams were selected and divided into two major groups (control) A and B (Lithium Carbonate-treated) comprising of 15 animals each. According to the time period of the study which was 2 weeks, 6 weeks and 12 weeks. Group A served as control group which was given normal diet and B was given Li2CO3 in powder form mixed in flour. Lithium carbonate was given at a dose of 20mg/kg/day for two, six and twelve weeks. At the end of 2nd, 6th and 12th weeks the cerebellum was removed and fixed in 10% formal saline. After processing in paraffin five micron thick section were prepared for Purkinje cell count.

Results: Group A revealed normal Purkinje cell count but the mean values of the Purkinje cell count in group B was highly significantly decreased p-value<0.001 as compared to group A.

Conclusion: Our study concludes that acute and chronic ingestion of lithium carbonate at therapeutic level is detrimental to the survival of Purkinje cerebellar neuron and patients who are prescribed lithium carbonate should be monitored carefully.

Key Words: cerebellum, Purkinje, neuron, Lithium carbonate.

Citation of article: Kohari T, Najmi N. Detrimental Effect of Lithium Carbonate on Cerebellar Purkinje Neurons in Albino Rats. Med Forum 2016;27(2):41-44.

INTRODUCTION

The word Lithium comes from Greek word "Lithos"[1], which means stone, with symbol "Li" [2]. Lithium is the gold standard, mood stabilizer [3]. Its salt is useful in treatment of bipolar [4] depressions. Lithium carbonate is often referred to as an anti-mania drug but in many parts of the world, it is considered as the stabilizer of mental status because of its role in preventing mood swings with bipolar affective disorder.[5] It is used for other disorders, such as cycloid psychosis, major depression, cluster headache [6], and schizophrenia.[7] The Food and Drug Administration of USA approved lithium as preventive or prophylactic treatment for depressive illness.[8] A cohort study conducted in United Kingdom for the usage of lithium as a mood stabilizer determined that increment in prescribing lithium for women was 33% and 24.1% for men in the year 1995 to 2009. [9]

It has multiple effects on biological processes. The enzyme inositol monophosphatases is a potential target for lithium's damaging effect. It was found that GSK-3 inhibition increased translocation of nuclear factor of activated T-cells, ¾ (NFAT C3/4) transcription factors to the nucleus leading to increased Fas ligand (FASL) levels and Fas activation which causes cell death (apoptosis), and it is worth noting that levels of lithium-induced apoptosis was highest in cerebellum.[10] Lithium effects have been investigated in detail in the brain, intestine, liver and thyroid functions.

De Cerqueira[11] et al. had reported cerebellar degeneration secondary to Lithium carbonate ingestion. The neuropathological manifestations reported were loss of Purkinje and granule cell leading to cerebellar atrophy. [12] Central neuronal damage by lithium is due to increase in glutamate secretion which causes increase sequestration of calcium ions in the mitochondrial cisternae resulting in disruption and distortion of mitochondria in the neurons, these changes are accompanied by nuclear clumping. As neuronal cell degeneration causes release of reactive oxidant species.[13] The lipid peroxidation of the cerebellar cortex leads to decrease in concentration of the antioxidant enzymes, resulting in DNA fragmentation and cell death of cerebellar neurons with a decrease in the gray matter of cerebellar cortex.[14, 15]

Correspondence: Dr. Tazeen Kohari,
Asstt. Prof. of Anatomy, Liaquat College of Medicine and Dentistry, Karachi
Cell No.: 03232967849
E-mail: tazeenk67@gmail.com

Many studies have been done on cerebellar degeneration by various agents and radiation, but my study was undertaken to evaluate the cytotoxic effect of Lithium carbonate on Purkinje neurons in the Purkinje layer of cerebellar cortex in albino rats.

MATERIALS AND METHODS

This study was conducted in the Animal House affiliated with Basic Medical sciences Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC), Karachi. For this study 30 male albino rats of 190-240 grams of weights were used. They were kept under observation for 7 day prior to commencement of study. The animals were randomized into two experimental groups comprising of 15 animals each according to the time period of the study that is 2 weeks, 6 weeks and 12 weeks respectively. Group A served as control and group B received Lithium carbonate Adamjee Pharmaceuticals at a dose of 20mg/kg/day[16,17] in powder form mixed in flour pellets for 2 weeks, 6 weeks and 12weeks. The standard laboratory Chow and tap water were available ad libitum at the end of each time period the animals were sacrificed under ether anesthesia. The cerebellums were removed by parietal bone approach. The tissue was fixed and processed for haemotoxylin and eosin staining. Morphomeetric examination was performed under light microscope and the results were recorded and tabulated.

Data collected were analyzed using students t-test. Results were expressed mean ± P-<0.001 and P-<0.05 was considered statistically significant. All the calculations were done by utilizing computer software SPSS 16 through Microsoft excel in windows.

RESULTS

The mean Purkinje cell count was highly significantly (P<0.001) decreased as compared to control group A table 1 and the Purkinje cell count of group B was highly significantly decreased at 6th wks than 2nd weeks and highly significantly decreased at 12th week than 2nd week.

Table No.I: Mean* Purkinje cell count (mm) in different group of albino rats.

	N	2nd week			6th week			12th week		
		Mean	S.D	SEM	Mean	S.D	SEM	Mean	S.D	SEM
A	15	24.2	4.44	1.98	24.9	2.3	1.03	26.9	1.90	0.85
B	15	16.0	1.00	0.45	11.4	0.55	0.24	8.2	0.84	0.37

Mean*±SEM

Statistical analysis of Mean* weekly Purkinje cell count in different group of albino rats

P-value		
2wk Vs 6wk	2wk Vs 12wk	6wk Vs 12wk
0.551	0.392	0.331
0.002	0.001	0.001

Significant** Highly Significant***

Stats analysis of major group comparison of P-value

P-value	B Vs A	0.001	0.001	0.001

Significant** Highly Significant***

DISCUSSION

Since 1949 Lithium carbonate has been in use for Bipolar disorder. Its therapeutic effects are undeniable but its adverse effects cause neurological manifestations[18]. The cerebellum is an important component of the hind brain, involved in the control of locomotion and balance. The cerebellar cortex contains eight types of neurons: Purkinje, granule, stellate, basket, Golgi, Lugaro, unipolar brush cells and candelabrum cells. Purkinje cells provide the sole output of the cerebellar cortex and are the pivotal element around which the cerebellar circuit is organized.[19]

Lithium salt therapy entails neurologic damage in persisting form. Due to lithium injection cerebellar features tend to be the most prominent .[20] The most frequent clinical feature is a permanent cerebellar syndrome and it was reported that lithium disrupt calcium hemostasis in Purkinje cell. Cerebellar syndrome has also been described after short term lithium medication. Neuropathological studies have demonstrated neuronal loss and spongiosis in the cerebellum.[21] Persisting neurologic damage follows lithium salt therapy. There are usually signs of damages at multiple sites, but cerebellar features tend to be most prominent.[22] Lithium carbonate causes neuronal Purkinje cell death due to lipid peroxidadtion which causes an increase imbalance in antioxidant enzymes which are superoxide dismutase (SOD), Catalase (CAT) and glutathione synthetase (GST), there by leading to excessive generation of free radicals hence resulting in enhanced oxidative stress.[23]

Our study showed a marked decrease in the Purkinje cell density in the Li2CO3 treated group B animals as compared to control group A. This is an agreement with Kaidanovich et al., whose work showed that lithium

ingestion causes a decrease number of the Purkinje cell in the Purkinje cell layer and this may be due to the reason that lithium causes inhibition of Glycogen Synthase Kinase-3. GSK-3 is an evolutionary conserved ubiquitous serine / thionine protein kinase. It is abundant in the neurons and neuroglia. GSK-3 acts downstream to suppress the activity of several prominent pathways such as Wnt signaling pathway, phasphoinositol 3 protein kinases and neurotropic pathway. GSK-3 is implicated in fundamental brain functions cytoskeletal stabilization axonal growth cone collapse, cell adhesions, synaptic plasticity and memory formation.

The decrease in Purkinje cell count in albino rats due to Lithium administration was also studied by Vijaymohan et al (2010). They have in their researches emphasized the fact that decreased in the concentration of the antioxidant enzymes, causes release of ROS.[24] This results in the decrease ATP production in the mitochondria and Purkinje neuron. This whole process causes excessive secretion of calcium ion resulting in fragmentation and death of Purkinje neurons.

The observations of decrease Purkinje cell density was also reported by Grignon[25] et al (1996). Cerebellar syndrome has also been described after short term as well as chronic lithium as is seen in our study.

CONCLUSION

Our study concludes that acute and chronic ingestion of lithium carbonate at therapeutic level is detrimental to the survival of Purkinje cerebellar neuron and patients who are prescribed lithium carbonate should be monitored carefully.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Wikipedia, free encyclopedia: Lithium. 2009; Available at: http://en.wikipedia.org/wiki/ Lithium.
2. O'Leary D. Lithium. http://143.239.128.67/ academic/chem/dolchem/ html/elem003.html.
3. Mohandas E, Rajmohan V. Lithium use in special population. Ind J Psychiat 2007;49(3): 211-8.
4. Baldessarini RJ. Psychosis and Anxiety. In: Goodman and Gilman's the pharmacological basis of therapeutics. 19th ed. New York: McGraw Hill; 1996.p.399-460.
5. Katzung BG. Anti-psychosic agents and Lithium in: Basic and clinical Pharmacology. 9th ed. New York: McGraw Hill;2004.p.475-79.
6. Robert E. Lithium medication, 27 Oct 2010.
7. Avasthi A, Grover S, Aggarwal M. Research on mood stabilizers in India. Ind J Psychiat 2010; 52(Suppl1): S355-S361.
8. Fortune city; Lithium mineral and drug, 2006. Available at: http://www.fortunecity.com/campus/ psychology/781/Lithium.html.
9. Hayes J, Prah P, Nazareth I, King M, Walters K, Peterson I, et al. Prescribing trends in bipolar disorder: cohort study in the UK database 1995-2009. Plos One 2011;6(12): e28725.
10. Gomez-Sintes R, Lucas JJ. NFAT/Fas signaling mediates the neuronal apoptosis and motor side effects of GSK-3 inhibition in a mouse model of lithium therapy. J Clin Invest 2010;120(7): 2432-45.
11. De Cerqueira ACR, Dos Reis MC, Novis FD, Bezerra JMF, De Magalhaes GC, Rozenthal M, et al. Cerebellar Degeneration Secondary to Acute Lithium Carbonate Intoxication. Arq Neuropsiquiatr 2008;66(3-A):578-580.
12. Neithammer M, Ford B. Permanent Lithium-induced cerebellar Toxicity: Three Cases and Review of Literature. Movement disorders 2007.
13. Kikuchi M, Kashii S, Honda Y, Tamura Y, Kaneda K, Akaike A. Protective effects of Methylcobalamin, a vitamin B12 analog, against glutamate-induced neurotoxicity in retinal cell culture. Invest. Ophthalmol Visual Sci 1997; 38(5): 848-54.
14. Tathagat A. Nadeem S. Mishra M. Choudhary DK, Advani JU, Tripathi S. Antioxidant status in lithium induced cerebellar toxicity in rat central nervous system. Department of Biotechnology, Nims Institute of engineering & technology and department of Obstetrics and Gynaecology Nims Medical college, Nims University, Shobha nagar, Jaipur (Rajasthan) India 303121.2012.
15. Bhalla P, Chadha VD, Dhawan DK. Neuroprotective effects of zinc on antioxidant defense system in Lithium treated rat brain. Indian J Experi Biol 2007;45:954-958.
16. Licht RW, Larsen JO, Smith D, Braendgaard H. Chronic lithium treatment with or without haloperidol fails to effect the morphology of the rat cerebellum. European Neuropsychopharmacology 2003;173-176.
17. Kolachi GM. Toxic effects of lithium carbonate of proximal tubules of rat kidney: A morphometric study. MC 2008;14(3).
18. Katzung BG. Anti-psychotic agents and lithium. In: Basic and clinical pharmacology. 9th ed. New York: McGraw Hill;2007.p.475-79.
19. Ito M. Signal processing in cerebellar Purkinje cells. Physiol Bohemoslov 1987;36(3):203-16.
20. Donaldson IM, Cuningham J. Persisting neurologic sequelae of lithium carbonate therapy. Arch Neurol. 1983;40(12):747-51.
21. Andrades M, Ritter C, Moreira JC, Dal-Pizzol F. Oxidative parameters differences during non-lethal

and lethal sepsis development. J Surg Res 2005; 125:68-72.
22. Dethy S, Manto M, Bastianelli E, Gangji V, Laute MA, Goldman S, et al. Cerebellar spongiform degeneration induced by acute lithium intoxication in the rat. Neurosci Lett 1997;224(1):25-8.
23. Rizvi MIH. Investigation on the Acute and chronic effects of acuoustic stress on B lymphocytes of white pulp of spleen in Albino rats and with antidepressment Drug 2008.
24. Vijaymohan K, Malaika J, Shyamala CS. Effect of Sobatum against Lithium-Induced Oxidative Damage in Rats. Jouranl List. J Young Pharm 2010;2(1).
25. Grignon S, Bruguerolle B. Cerebellar lithium toxicity: a review of recent literature and tentative pathophysiology. Therapie 1996;51(2):101-6.

Original Article

Opponensplasty

Outcome of Hand Function after Extensor Indices Proprius Opponensplasty

1. Kamran Khalid 2. Farrukh A Khalid 3. Moazzam N Tarar

1. Asstt. Prof. of Plastic Surgery, 2. Senior Registrar of Plastic Surgery, 3. Prof. of Plastic Surgery, Jinnah Burn & Reconstructive Surgery Center, Jinnah Hospital, Allama Iqbal Medical College, Lahore

ABSTRACT

Objective: To assess the functional improvement in the hand after opponensplasty.
Study Design: Descriptive study
Place and Duration of Study: This study was conducted at Plastic Surgery Department Jinnah Hospital, Lahore from October, 2005 to March, 2015.
Materials and Methods: A total of 40 patients with loss of opposition of thumb were included in the study. Their opposition was lost either due to median nerve or both median and ulnar nerve injury or directly damaged thenar muscles. Patients in whom there is restricted range of thumb motion or scarring in the route of tendon transfer were excluded from the study. After taking informed consent Extensor Indices Proprius tendon transfer was done. Post-operatively cast was given for 4 weeks and protective splintage was applied for another 2 weeks. Improvement in the hand function was recorded at conclusion of study.
Results: Four patients lost follow-up. Out of 36 patients, 26 (72%) patients showed good results, 6 (17%) showed fair results and 4 (11%) showed poor results. 25 patients (69%) had good Kapandji score, while 7 (20%) had fair and 4 (11%) had poor score. Mean DASH score was 24. Overall patient's satisfaction was very good. Most of the patients hand function improved significantly.
Conclusion: Our study results strongly suggest Opponensplasty with Extensor Indices Proprius is an excellent procedure for restoring opposition in patients with median or both median and ulnar nerve injury at wrist.
Key words: Opponensplasty, EIP transfer, loss of opposition, distal median nerve injury, DASH score, Kapandji score.

Citation of article: Khalid K, Khalid FA, Tarar MN. Outcome of Hand Function after Extensor Indices Proprius Opponensplasty. Med Forum 2016;27(2):45-48.

INTRODUCTION

The human hand is specially adapted for the prehensile functions of pinch and grip.[1,2] The thumb imparts such precision, efficiency and diversity to these functions of hand. Importance of thumb lies in its position and ability to oppose other digits (opposition).[3,4] Opposition is a complex movement consists of palmar abduction, pronation, and adduction of thumb. The motor branch of the median nerve innervates the abductor pollicis brevis (APB), opponens pollicis, and superficial head of the flexor pollicis brevis (FPB).[5, 6]

Loss of opposition is a major disability.[1,7] It is the most significant presenting complaint of median nerve palsy patients. Other causes include combined median and ulnar nerve, brachial plexus injuries, traumatic injury to muscles and tendons and some neurological conditions.[8-11] Opponensplasty is a type of tendon transfer which is done to restore the opposition of the thumb [10] and is required for pinch grip and other fine functions of the hand.[12,13] Several procedures are employed to achieve opposition.

Steindler performed the first opponensplasty in 1919 by transferring the radial slip of flexor pollicis longus (FPL) to the dorsal base of the thumb proximal phalanx.[14] Several more options have since been developed for opposition transfers including extensor indices proprius (EIP)[8,15] flexor digitorum superficialis (FDS)[13,16,17] and palmaris longus (PL).[18]

In our study we used the technique of EIP transfer for thumb opposition. We are presenting a series of 40 patients who underwent EIP transfer for restoring opposition of the thumb. The study was conducted to assess the functional improvement in the hand after opponensplasty.

MATERIALS AND METHODS

This study was conducted at Plastic Surgery Department, Jinnah Hospital, Lahore. The study period was from October 2005 to March 2015. Patients with median nerve injury at wrist, patients with both ulnar and median nerve injury at wrist and traumatic loss of thenar muscles directly resulting in loss of opposition were included. Those patients with restricted range of thumb movement, scarring and wound in the route of tendon transfer and absent or damaged extensor indices

Correspondence: Dr. Kamran Khalid,
Asstt. Prof. Jinnah Burn & Reconstructive Surgery Center, Jinnah Hospital /Allama Iqbal Medical College, Lahore
Cell No.: 0321-4426322
E-mail: drkamrankhalid@hotmail.com

proprius tendon were excluded. During this period 40 extensor indices proprius (EIP) opponensplasties were performed on 40 patients. An informed consent was taken, under general anesthesia and tourniquet control EIP tendon was identified through a small incision over the metacarpophalangeal joint of the index finger. EIP was transected through this incision, which is ulnar to the Extensor digitorum communis (EDC) tendon. The distal end was reef with the EDC tendon. EIP tendon was then retrieved; the route of the tendon was identified by traction over the distal end, 2-3 small incisions were made along the course of tendon. A small incision is then made in the area of the pisiform and the tendon passed subcutaneously around the ulnar border of the forearm. It is most important at this time to develop subcutaneously a large enough tunnel so that the entire muscle bulk of the extensor indicis proprius lies against the subcutaneous border of the ulna. After the tendon was brought out through the small incision in the area of the pisiform, a subcutaneous tunnel was made across the palm of the hand to the area of the metacarpophalangeal joint of the thumb and the tendon is passed through the tunnel into this incision. The abductor pollicis brevis (APB) tendon was identified. EIP was woven with the APB and the capsule of MCP joint under maximum tension with thumb in maximum palmar abduction.

Wrist was immobilized in a neutral position with thumb in maximum palmar abduction. Cast was applied for 4 weeks. After the removal of cast, physiotherapy was started (Figs. 1-3). Protective splint was applied for another 3 weeks.[19] Motor and sensory re-education was done along with physiotherapy. Monthly follow-up was done. The average follow-up was 27 months (range, 6 to 58 months). The difference between the Active abduction angle (AAA) and Resting abduction angle (RAA) is the Active range of abduction (ARA) of the thumb. An evaluation score of 5 was allocated to ARAs greater than 45°, score of 4 to ARA between 41-45°, score of 3 to ARA between 36-40° and a score of 0 to ARAs less than 35°. Opposition tendon transfer that restores adequate abduction and rotation would enable pulp-to-pulp pinch and those with limitation would achieve only pulp-to-side or key pinch. An evaluation score of 2 was allocated to pulp-to-pulp pinch and a score of 1 for key pinch. Pinch strengths were measured with a handheld pinch gauge Baseline® Mechanical Pinch Gauge (TheraTek Medical Inc., Seattle, Washington). The average of 3 consecutive measurements was used to calculate the pinch power as a percentage of that of the normal opposite hand. Pinch strengths were graded good when greater than 50%, fair when between 21% and 50%, and poor when less than 20% of the unaffected hand. Evaluation scores of 3, 2, and 0 were allocated to good, fair, and poor pinch strengths, respectively.

RESULTS

There were 35 male and 05 female patients. 30 of the hands were dominant and 10 non-dominant. The mean duration of injury was 20.5 months (range 06 to 35 months). The average age at the time of surgical repair was 25 years (range 07 to 52 years). There were no wound complications like infection or wound dehiscence. There was no tendon avulsion or tendon rupture. Four of the patients lost follow up either due to change of address or phone number and one died due to some other cause. Sixteen patients had ARA greater than 45°, 10 patients had ARA between 41°-45°, 6 patients had ARA between 36°-40° and 4 patients had ARA less than 35°. Thirty four patients had pulp to pulp pinch while 2 patients had pulp to side or even no pinch at all. In 25 patients the pinch strength was good, 7 patients had fair while 4 patients had poor pinch strength. Eighty four percent of the patients were satisfied with their results while 16% were not (Table 1).

Table No.1: Outcome evaluation of thumb opposition

Outcome	Evaluations system result[20]	Kapandji Score	DASH Score
Good	72%	69%	30.5
Fair	17%	20%	28.0
Poor	11%	11%	36.0
Mean			24.0

DISCUSSION

This procedure is known since 1956, well before Burkhalter who was credited with the technique, he rediscovered it in 1973. It was first done by Santiago Chouhy-Aguirre of Buenos Aires.[8] This transfer is an easy and quick procedure, may be performed either early or late in the management of peripheral nerve injury, without reducing flexor strength and without major morbidity of the donor finger. The donor site is usually preserved after combine median and ulnar nerve injury. There are no late complications and the over-all return of opposition of the thumb is satisfactory. Burkhalter[8] published his series of sixty five cases with 88% excellent or good results. The etiologies of these patients were fire arm injury or lacerated wounds as compared to our patients with majority of electrical injury patients.

Mehta et al[21] did 25 EIP opponensplasty in combined median and ulnar nerve palsy in leprosy patients and had good results in these cases. They revealed some difficulty of EIP being short in some case to reach its new insertion and had proximal interphalangeal joint contractures in few cases. We didn't encounter the problem of EIP being short and we feel proper release of muscle is the key and complication of proximal interphalangeal joint contractures may be avoided by stump repair.

Anderson et al[22] did EIP opponensplasty in 39 patients with variable aetiology and did adjuvant procedures in 29 cases. The results were similar to our study with 88% excellent and good results and 12% fair and poor result.

Andrew et al[3] did Abductor digiti quinti-opponensplasty (Huber's procedure)[23] in 15 patients with 80% excellent or good result, had claimed advantages of intrinsic muscle which has good amplitude and direction of pull, automatic correction of tension of muscle and good cosmetic appearance of the hand than other opponensplasties. The aetiologies of these cases were lacerations of median nerve alone or neurological diseases. This is a good option but for our indications of combined ulnar and median nerve injury patients and with electrical injury to hand this is not a valid option.

Palmaris longus (PL) tendon known as Camitz procedure[24] with flexor retinaculum pulley is also another option; Jung et al[9] did this in 11 cases of post carpel tunnel syndrome palsied thumb with good results, the author did a modification of standard Camitz (PL) opponensplasty by making a pulley in ulnar side remnant of flexor retinaculum. It is good option but has limitations in high median nerve palsy, electrical injury or lacerations of wrist with flexor tendon injury.

Median nerve injury spares Flexor digitorum superficialis of ring finger (FDS-R) (Bunnel's procedure)[25,26] which is used by Anderson et al[27] in their study in 116 patients out of 166 and used EIP in the rest; he showed good results using FDS-R in less pliable hands and good results with EIP in supple hands. The ulnar nerve injury along with median nerve injury rules out this option.

Many other options like Flexor carpi radialis[28], Extensor carpi ulnaris to extensor pollicis brevis[29], Brachioradialis[1,30] were used at different times with varying degree of success.

The limitations of this study are the pre-operative pinch strength and DASH score availability for comparison with the post-operative results. Secondly we initially did not follow Burkhalter's instructions on EIP insertion in patients with combine median and ulnar nerve injury which result in thumb MCP joint hyperextension in some cases which was later corrected.

CONCLUSION

Our study results strongly suggest opponensplasty with Extensor Indices Proprius is an excellent procedure, either performed as definitive procedure or as an adjuvant with nerve repair/graft. The main usefulness of the procedure at the present time seems to be in the median nerve injury and in the combined median and ulnar nerve injury, either high or low. The extensor indicis proprius has satisfactory amplitude, strength, and does not require a tendon graft to obtain satisfactory opposition of the thumb. The procedure is quick and easy to learn with minimal donor site morbidity.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Bindra RR, Bhandarkar DS, Taraporvala JC. Opponensplasty--an experience of twenty-three cases using three techniques. J Postgrad Med 1990;36(1):9-12.
2. Cho DY, Seo JG, Yang SB, Lee SC. Opponensplasty. J Korean Orthop Assoc 1990; 25(4):1263-8.
3. Sahoo JD, PatraPN, Rath S. Immediate post-operative active mobilization following opponensplasty. Ind J Occup Ther 2008;XL(3): 65-70.
4. Davis TRC. Median Nerve Palsy. In: Green DP, editor. Operative Hand Surgery, 3rd ed. New York: Churchill Livingstone; 1993.p.1131–59.
5. Wan SH, Wong TC, Yip TH, Ip FK. Clinical experience of open carpal `tunnel release and Camitz operation in elderly Chinese patients. Hong Kong Med J 2007;13(5):348-52.
6. Upton J, Taghinia AH. Abductor digiti minimi myocutaneous flap for opponensplasty in congenital hypoplastic thumbs. Plast Reconstr Surg 2008; 122(6):1807-11.
7. Skie MC, Parent T, Mudge K, Dai Q. Kinematic Analysis of Six Different Insertion Sites for FDS Opponensplasty. Hand (NY) 2010;5(3):261-6.
8. Burkhalter W, Christensen RC, Brown P. Extensor Indicis Proprius Opponensplasty. J Bone Joint Surg Am 1973;55(4):725-32.
9. Park IJ, Kim HM, Lee SU, Lee JY, Jeong C. Opponensplasty using palmaris longus tendon and flexor retinaculum pulley in patients with severe carpal tunnel syndrome. Arch Orthop Trauma Surg 2010;130(7):829–34.
10. Jones NR, Khiabini KT. Tendon Transfers in Upper Limbs. In: Mathes SJ, Hentz VR, editors. Plastic Surgery, 2nd ed. Philadelphia: Sunders Elsevier;2006..p.453-88.
11. Yip SL, Yen CH, Chan WL, Leung HB, Mak KH. Pick-up test: an indication for opponensplasty in patients with severe carpal tunnel syndrome. J Orthop Surg (Hong Kong) 2009;17(2):183-6.
12. Wissinger HA, Singsen EG. Abductor digiti quinti opponensplasty. J Bone Joint Surg Am 1977;59(7): 895-8.
13. Bunnell S. Reconstructive Surgery of the Hand. Surg Gynae Obstet 1924;39:259-74.
14. Steindler A. Operative treatment of paralytic conditions of the upper extremities. J Orthop Surg 1919;1:608-24.
15. Aguirre SC, Caplan S. Sobresecuelas de lesion alta e irreparable de nerviosmediano y cubital, y

sutratamiento [Spanish]. La Presa Medica Argentina 1956;43:2341-46.
16. Royle ND. An operation for paralysis of the intrinsic muscles of the thumb. JAMA 1938; 111:612-13.
17. Thompson TC. A modified operation for opponens paralysis. J Bone Joint Surg Am 1942;24(A): 632-40.
18. Foucher G, Malizos C, Sammut D, Braun FM, Michon J. Primary palmarislongus transfer as an opponensplasty in carpal tunnel release. A series of 73 cases. J Hand Surg [Br] 1991;16(1):56-60.
19. Rockwell WB, Stem CA. Tendon Transfers. In: Guyuron B, Eriksson E, Persing JA, editor. Plastic Surgery, 1st edn. Philadelphia: Sunders Elsevier; 2009. p. 1139-49.
20. Kapandji AI. Clinical evaluation of the thumb's opposition. J Hand Ther 1992;5:102-8.
21. Mehta R, Malaviya GN, Husain S. Extensor indicis opposition transfer in the ulnar and median palsied thumb in leprosy. J Hand Surg Br 1996;21(5): 617-21.
22. Anderson GA, Lee V, Sundararaj GD. Extensor indicis proprius opponensplasty. J Hand Surg Br 1991;16(3):334-8.
23. deRoode CP, James MA, McCarroll HR Jr. Abductor digit minimi opponensplasty: technique, modifications, and measurement of opposition. Tech Hand Up Extrem Surg 2010;14(1):51-3.
24. Camitz H. IJber die Behandlung der Oppositions Ã¤hmung. Acta Chir Scandinavica 1929;65:77-81.
25. Riordan DC. Tendon transplantations in median-nerve and ulnar-nerve paralysis. J Bone Joint Surg Am 1953;35-A(2):312-20.
26. Kozin SH, Ezaki M. Flexor Digitorum superficialis opponensplasty with ulnar collateral ligament reconstruction for thumb deficiency. Tech Hand Up Extrem Surg 2010;14(1):46-50.
27. Anderson GA, Lee V, Sundararaj GD. Opponensplasty by extensor indicis and flexor digitorum superficialis tendon transfer. J Hand Surg Br 1992;17(6):611-4.
28. Gahankari D, Tambwekar S, Rana RE. Flexor carpi radialis opponensplasty. Br J Plast Surg 1996; 49(6):401-3.
29. Kessler I. Transfer of extensor carpi ulnaris to tendon of extensor pollicis brevis for opponensplasty. J Bone Joint Surg Am 1969; 51(7):1303-8.
30. Henderson ED. Transfer of wrist extensors and brachioradialis to restore opposition of the thumb. J Bone Joint Surg Am 1962;44-A:513-22.

Guidelines & Instructions

Guidelines and Instructions to Authors

The Journal MEDICAL FORUM agrees to accept manuscripts prepared in accordance with the Uniform Requirements submitted to the Biomedical Journals published in the British Medical Journal 1991;302:334-41. Revised in February 2006.
Medical forum is a Peer Reviewed Journal of all Specialties. Recognized by PMDC, HEC and Indexed by WHO, EXCERPTA MEDICA, SCOPUS Database, Pakmedinet, National Library of Pakistan, Medlip of CPSP and registered with International serials data system of France.

Basic Requirement

The material submitted for publication should be forwarded containing;
1) 3 Hard copies of Laser Print.
2) 1 Soft copy on a CD.
3) Letter of Undertaking with Authors Name, Address, Mobile Numbers, Degrees, Designations, Department of Posting and Name of Institution.

ORIGINAL ARTICLE: It should be of 2000 to 3000 Words, not more than 6 Tables or Figures and at least 20 References but not more than 40.

REVIEW ARTICLE: It should be of 3000 Words with at least 40 References but not more than 60.

SHORT COMMUNICATIONS OR CASE REPORTS: It should be 600 Words with one Table or Figure and 5 References.

LETTER TO EDITOR: It should be 400 Words with 5 References.

TITLE OF THE ARTICLE; Accurate, Effective and Represent the main message of Article.

ABSTRACT

In Original Article, It should consist of the following seven subheadings: **Objective, Study Design, Place and Duration of study, Materials & Methods, Results, Conclusion & Key Words** and should not more than 250 Words.

The second part consists of Introduction, Materials and Methods, Results, Discussion, Conclusion and References

References should be entered in text Vancouver Style in ascending order and in shape of numbers & superscript (e.g. [1,2,3,4])

INTRODUCTION

The start of the introduction should be Relevant. Reasons and Importance of the study should be clear. Give only strictly pertinent References and do not include data or conclusions from the work being reported.

MATERIALS & METHODS

The Population taken for the study should be uniform and Sample selection criteria should be reliable. Inclusion & Exclusion criteria should be clearly specified.

RESULTS

Present yours results in a logical sequence in the Text, Tables, Illustrations, figures and Graphs.

DISCUSSION

Emphasize the new and important aspects of the study and conclusions that follow from them.

CONCLUSION

In this link write the goals of the study.

RECOMMENDATIONS

When appropriate may be included.

ACKNOWLEDGMENTS

List of all contributors who do not meet the criteria for Authorship, such as a person who provided purely technical help, writing assistance or department chair who provided only general support. Financial & Material support should be acknowledged.

REFERENCES

It should be in the **Vancouver style**. References should be numbered in the order in which they are cited in the text. At the end of the article, the full list of references should give the names and initials of all the authors. **(if the authors are more than 6, then et al should be followed after the 6th name).** Vancouver Style should be used like' The healing of tissues by C02 laser. Br J Surg 1971;58:222-5.

CONTACT: 66-R, Phase-VIII, Defence Housing Authority, Lahore.
Mob. 0331-6361436, 0300-4879016, 0345-4221303, 0345-4221323
E-mail. med_forum@hotmail.com,
medicalforum@gmail.com
Website: www.medforum.pk