Editoiral:

SMOG: A SERIOUS PUBLIC HEALTH PROBLEM

Iram Manzoor

SMOG is a type of threatening air pollution which is declared as Public Health Emergency in South East Asia recently.[1] The most hit areas are Pakistan, India and China.[2] This problem of SMOG has recently gained much significance in Pakistan due to the involvement of a larger area as compared to areas affected in the last 70 years. Recent projects of coal as source of energy, high rates of emissions from unmonitored industries, a large number of vehicles on road, increasing trends of deforestation to construct new roads and recently the burning of crops remnants has added fuel to the fire.[3] Lack of Public transport system has led to 9% increase in the vehicles as compared to statistics of last five years.[4] Pakistan, India and Bangladesh emit highest number of hydrocarbons in their fuel emissions as compared to other SAARC (South Asian Association for Regional Cooperation) countries.[5]

SMOG not only affects the physical health of inhabitants in terms of respiratory & eye infections but also affects the mental and social health aspects.[6] Fine particulate matter of fewer than 2 microns can be inhaled directly into the lungs causing increasing hospitalization rates in the SMOG hit areas.[7] Inability to commute and fulfilling the social and domestic commitments also affect the mental health. Increase the number of accidents related to SMOG not only causes a high mortality pattern but also affects the social and economic state of the country.[8] It is high time to think about preventive measures so we can reduce the burden of physical, mental and social impact on citizens of Pakistan.

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The government of Pakistan should now focus on community planning to reduce the number of unmonitored factories, less involvement of Coal-based energy projects, improvement of the public transport system and establishment of strong regulatory authorities that can target emissions and gas productions in air. Government has taken initiatives for control of this problem but as individuals, targeting tree implantation at household level, regular checkups of automobiles, Carpooling for transport and informing the law enforcing agencies about irregularities can significantly contribute in reduction of this public health issue.[9] Increasing public awareness among masses by use of mass media is also a good strategy which has produced long-lasting effects in china.[10]

India has recently introduced fines on illegal crop burning. The government of India has also introduced an odd-even scheme allowing an only odd number of cars on one day on-road and even number on the other day. Commercial trucks have been banned in cities. Construction has been stopped to deal with the acute condition. The need of the hour is that we should not only focus on acute measures but should have a long term plan for dealing with this important public health issue.

REFERENCES:

1. Louf R, Barthelemy M. Scaling: lost in the smog. Environment and Planning B: Planning and Design. 2014;41(5):767-69.
2. Chen R, Zhao Z, Kan H. Heavy smog and hospital visits in Beijing, China. American Journal of Respiratory and Critical Care Medicine. 2013;188(9):1170-1.
3. Qiang L, Ping L. An analysis on the causes of large-scale smog in China and policy recommendation. Journal of Graduate

School of Chinese Academy of Social Sciences. 2014;5:63-8.
4. Elsom D. Smog alert: managing urban air quality. Routledge; 2014 Apr 4.
5. Abas N, Kalair A, Khan N, Kalair AR. Review of GHG emissions in Pakistan compared to SAARC countries. Renewable and Sustainable Energy Reviews. 2017;80:990-1016.
6. Arif F. SMOG: Causes, Effects and Preventions. Annals of King Edward Medical University. 2016;22(4).
7. Kim KH, Kabir E, Kabir S. A review on the human health impact of airborne particulate matter. Environment international. 2015;74:136-43.
8. Samuelsson A. Cars, motoring and sustainable movement (s). InAcsis (Advanced Cultural Studies Institute of Sweden) conference 2013 On the Move, June 11-13, Session:(Auto) mobility, communication and spatial change. 2013:86.
9. Shi H, Wang Y, Huisingh D, Wang J. On moving towards an ecologically sound society: with special focus on preventing future smog crises in China and globally. J Clean Prod. 2014;64(1):9-12.
10. Wang Y, Sun M, Yang X, Yuan X. Public awareness and willingness to pay for tackling smog pollution in China: a case study. Journal of Cleaner Production. 2016;112:1627-34.

Original Article:

BENEFICIAL EFFECT OF VITAMIN D SUPPLEMENTATION ON WEIGHT AND BMI OF MICE TAKING HIGH-FAT DIET

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ABSTRACT:

Objective: Obesity is a serious health issue that is rising worldwide. Underdeveloped and developed countries both are becoming the culprits of this pandemic. Decreased levels of vitamin D and obesity are correlated. This study was planned to see whether vitamin D supplementation has any effect on weight and body mass index (BMI) of mice taking the high-fat diet.

Methods: This was a randomized control trial, conducted at the Physiology Department of Akhtar Saeed Medical and Dental College, Lahore from October 2017 to December 2017. Ninety (90) male mice were randomly divided into 3 groups. Each group had 30 mice. The total duration of the study was 6 weeks. Group A was the normal diet control group, Group B was the high-fat diet control group, Group C was high-fat diet and vitamin D taking test group. The weight of every mouse was recorded twice a week for 6 weeks by the electronic weighing machine. Initial and final nasoanal length of every mouse was taken and initial and final BMI was calculated. The difference in the nasoanal length and BMI was calculated and data was analyzed using SPSS version 20.

Results: Mean weight of group B mice increased significantly as compared to group A mice($p=0.005$). Mean weight of group C mice reduced significantly as compared to group B mice($p=0.028$). Mean weight of group A and C mice was not significantly different from each other ($p=0.822$).Mean BMI of group C mice reduced significantly as compared to group B mice ($p=0.002$). BMI of group A and B mice was not significantly different from each other ($p=0.330$). Difference of BMI between group C and A was also statistically insignificant ($p=0.111$).

Conclusion: Vitamin D prevents weight gain and increase in BMI of mice taking high fat diet.

Key Words: Obesity, Body Mass Index, Vitamin D

INTRODUCTION:

Obesity has emerged as an epidemic of the 21st century according to the WHO report.[1] WHO fact sheet reveals that about 300 million adults are obese worldwide.[2] Obesity is related to a number of disorders like diabetes, hypertension, various malignancies, chronic kidney disease, infertility and musculoskeletal problems.[3]

Vitamin D in diet or formed in the skin in the presence of sunlight is inactive. For it to be activated, it needs two hydroxylations, first hydroxylation in the liver and the second one in the kidney to form 1, 25 $(OH)_2D_3$ also called calcitriol.[4]

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It controls the transcription of many genes. Vitamin D is now being evaluated for its multiple roles. It has anti-inflammatory action, modulates our immune system and has anti-proliferative action too.[5]

Obesity is an alarming risk factor for various diseases like carcinogenesis, diabetes, hypertension, etc. Adipose tissue stores vitamin D and decreases its bioavailability. Vitamin D prevents the conversion of pre-adipocytes to mature adipocytes by regulating the gene expression of various transcription factors. However, these effects are different in different species. Adipose tissue is not merely a storehouse of fat, it also secretes many proteins and peptides which cause inflammation and produce comlications related to obesity. Obesity causes hypertrophy of the adipose tissue and thus its blood supply is compromised leading to hypoxia. This hypoxia initiates

inflammation and recruitment of macrophages to adipose tissues. The secretion of adiponectin by adipocytes is reduced and secretion of various inflammatory cytokines like interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), etc. Vitamin D inhibits chronic inflammation by decreasing production of inflammatory cytokines as evidenced by various mouse and human studies. Vitamin D inhibits nuclear-factor Kappa B (NF-KB) signaling pathway and mitogen-activated protein kinase (MAPK) signaling pathway, thus gene transcription is altered, and production of various pro-inflammatory substances is blocked.[6]

Previous studies, show that serum vitamin D levels are low in obese persons, and others show that low vitamin D levels are related to hyperlipidemia and obesity. Vitamin D levels have an inverse relationship with weight and body mass index (BMI).[7] However, further studies are required to have conclusive results.

As no cut off values for weight and BMI exist for underweight, normal and obese mice, we have compared the weight and BMI of the test group to that of control groups.[8]

MATERIAL AND METHODS:

Ninety healthy male mice were included in the study and they were randomly allocated into three groups, i.e, group A, B and C with 30 mice in each group. Group A was normal, control group given diet with normal constituents for 6 weeks duration. Group B was high-fat diet control group given a diet rich in fat constituents[9] for 6 weeks duration. Group C was test group, given high-fat diet and 100mg/kg/day oral dose of vitamin D for 6 weeks[10,11] (Table-1). The weight of every mouse was taken twice a week for 6 weeks by the electronic weighing machine. Initial and final naso-anal length of every mouse was taken and initial and final BMI was calculated. The difference in the naso-anal length and BMI was calculated and data was analyzed using SPSS version 20. The following formula was used to calculate BMI statistically.

BMI= weight in grams/ length in (cm)^2

p values ≤ 0.05 were considered statistically significant. p value > 0.05 is non significant.

Table -1 Grouping of mice, type of diet and supplementation

Groups	**Normal diet (**10% of kcal% fat)	**High fat diet** (60% kcal% fat)	**Vitamin D** 100ng/kg/day
Duration	**6 weeks**	**6weeks**	**6 weeks**
Group A	Given	Nil	Nil
Group B	Nil	Given	Nil
Group C	Nil	Given	Given

RESULTS:

There was significant difference of weight between the groups A, B, and C (one way ANOVA-Table 2) Mean weight of group B mice increased significantly as compared to group A mice(p=0.005-Table 3). Mean weight of group C mice reduced significantly as compared to group B mice(p=0.028-Table 4). Mean weight of group A and C mice was not significantly different from each other (p=0.822-Table 5).

There was significant difference of BMI between group A, B and C (P=0.003-Table 2).Mean BMI of group C mice reduced significantly as compared to group B mice (p=0.002-Table 4). BMI of groups A and B mice was not significantly different from each other (p=0.330-Table 3). The difference of BMI between groups C and A was also statistically insignificant(p=0.111-Table 5).

Table-2 Comparison of weight and BMI between Groups A (normal diet), B (high-fat diet)and C (high fat diet + vitamin D) (one way ANOVA)

Parameters assessed	**Group A (n=30)**	**Group B (n=30)**	**Group C (n=30)**	**p-value**
Weight change (grams)	7.17± 6.15	11.30± 4.57	7.93± 3.93	0.004*
BMI change (grams/cm^2)	0.07± 0.06	0.09± 0.06	0.05± 0.03	0.003*

Table-3 Comparison of weight and BMI between groups A(normal diet) and B (high fat diet) (Post Hoc Tukey's test)

Parameters assessed	Group A	Group B	p-value
Weight change (grams)	7.17±6.15	11.30±4.57	0.005*
BMI change (grams/cm^2)	0.07±0.06	0.09±0.06	0.330

Table-4 Comparison of weight and BMI between groups B(high fat diet) and C(high fat diet+ vitamin D) (Post Hoc Tukey's test)

Parameters assessed	Group B	Group C	p-value
Weight change (grams)	11.30±4.57	7.93±3.93	0.028*
BMI change (g/cm^2)	0.092±0.06	0.05±0.03	0.002*

Table-5 Comparison of weight and BMI between groups C(high fat diet+ vitamin D) and A(normal diet) (Post Hoc Tukey's test)

Parameters assessed	Group C	Group A	p-value
Weight change (grams)	7.93±3.93	7.17±6.15	0.822
BMI change (g/cm^2)	0.05±0.03	0.07±0.06	0.111

DISCUSSION:

There are various anthropometric parameters that are used to asses obesity in mice like weight, BMI, thoracic circumference and visceral fat.[12] In this study, only weight and BMI were measured. Three groups A, B, and C with 30 mice in each were assessed. Weight of group B mice increased significantly as compared to group A mice, while BMI was not raised significantly in group B mice as mice weight and length both increased proportionately. In group C, weight and BMI both were reduced significantly as compared to those of group B. However, between test group C and control group A, no significant difference in weight and BMI was found. Thus high fat diet increases weight and BMI of mice, whereas if vitamin D is given along with a diet rich in fat, weight and body mass index(BMI) both are reduced. But we can't consider vitamin D as an agent to prevent obesity in humans without the support of a lot of observational and interventional human studies.

In one study, mice who were fed diet high in fat and sugar along with vitamin D for 10 weeks showed reduced serum triglycerides levels, less hepatic steatosis, and reduced products of lipid peroxidation as compared to the group of mice who were fed diet high in fat and sugar content.[13] In another study, 8 weeks old mice were given a normal diet and high fat diet till 24 weeks of age, then one group was given normal diet and vitamin D supplementation and the other group was given high fat diet with vitamin D supplementation. Serum levels of 25(OH) D3, weight and BMI were assessed. Weight and BMI of both groups showed no significant difference.[14] In one study, vitamin D supplementation for 6 weeks in women who were obese and overweight resulted in the reduction of body weight and BMI.[15] A recent study in humans shows that vitamin D potentiates weight reduction in individuals who were taking a weight-reducing diet. It reduces weight and BMI.[16] In another systematic research, three groups of individuals were assessed, one group of obese individuals who were not undergoing any weight loss therapy, the other group on the weight loss therapy, and the third group with individuals after bariatric surgery. All groups were given oral vitamin D for 3 months. Normal serum levels of vitamin D were achieved. Weight and body mass index (BMI) were not changed significantly.[17] Further randomized controlled trials of longer duration should be carried out for the evaluation of the relationship between vitamin D and obesity in human beings.

CONCLUSION:

Vitamin D prevents weight gain and increases in BMI in mice taking high fat diet.

REFERENCES:

1. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. Physiological reviews. 2015 Dec 16;96(1):365-408.
2. Ofei F. Obesity-a preventable disease. Ghana medical journal. 2005 Sep;39(3):98-101.
3. Abdelaal M, le Roux CW, Docherty NG. Morbidity and mortality associated with obesity. Annals of translational medicine. 2017 Apr;5(7):161.
4. Nair R, Maseeh A. Vitamin D: The "sunshine" vitamin. Journal of Pharmacology & Pharmacotherapeutics. 2012 Apr;3(2):118.
5. Body JJ, Bergmann P, Boonen S, Devogelaer JP, Gielen E, Goemaere S, Kaufman JM, Rozenberg S, Reginster JY. Extraskeletal benefits and risks of calcium, vitamin D and anti-osteoporosis medications. Osteoporosis international. 2012 Feb 1;23(1):1-23.
6. Mutt SJ, Hyppönen E, Saarnio J, Järvelin MR, Herzig KH. Vitamin D and adipose tissue—more than storage. Frontiers in physiology. 2014 Jun 24;5:228.
7. Lagunova Z, Porojnicu AC, Lindberg F, Hexeberg S, Moan J. The dependency of vitamin D status on body mass index, gender, age and season. Anticancer research. 2009 Sep 1;29(9):3713-20.
8. Sandoval D, Cota D, Seeley RJ. The integrative role of CNS fuel-sensing mechanisms in energy balance and glucose regulation. Annu. Rev. Physiol.. 2008 Mar 17;70:513-35.
9. Oh JH, Kim J, Lee Y. Anti-inflammatory and anti-diabetic effects of brown seaweeds in high-fat diet-induced obese mice. Nutrition research and practice. 2016 Feb 1;10(1):42-8.
10. Cluny NL, Keenan CM, Reimer RA, Le Foll B, Sharkey KA. Prevention of diet-induced obesity effects on body weight and gut microbiota in mice treated chronically with 9-tetrahydrocannabinol. PLoS One. 2015 Dec 3;10(12):e0144270.
11. Moller S, Laigaard F, Olgaard K, Hemmingsen C. Effect of 1, 25-dihydroxy-vitamin D3 in experimental sepsis. International journal of medical sciences. 2007;4(4):190-95.
12. Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F,etal. Anthropometric parameters and markers of obesity in rats. Lab Anim.2003;41;111-19.
13. Kheder R, Hobkirk J, Saeed Z, Janus J, Carroll S, Browning MJ, Stover C. Vitamin D3 supplementation of a high fat high sugar diet ameliorates prediabetic phenotype in female LDLR−/− and LDLR+/+ mice. Immunity, inflammation and disease. 2017 Jun;5(2):151-62.
14. Seldeen KL, Pang M, Rodríguez-Gonzalez M, Hernandez M, Sheridan Z, Yu P, Troen BR. A mouse model of vitamin D insufficiency: is there a relationship between 25 (OH) vitamin D levels and obesity?. Nutrition & metabolism. 2017 Dec;14(1):26.
15. Khosravi ZS, Kafeshani M, Tavasoli P, Zadeh AH, Entezari MH. Effect of vitamin D supplementation on weight loss, glycemic indices, and lipid profile in obese and overweight women: a clinical trial study. International journal of preventive medicine. 2018;9:63.
16. Lotfi-Dizaji L, Mahboob S, Aliashrafi S, Vaghef-Mehrabany E, Ebrahimi-Mameghani M, Morovati A. Effect of vitamin D supplementation along with weight loss diet on meta-inflammation and fat mass in obese subjects with vitamin D deficiency: A double-blind placebo-controlled randomized clinical trial. Clinical endocrinology. 2019 Jan;90(1):94-101.
17. Bassatne A, Chakhtoura M, Saad R, Fuleihan GE. Vitamin D supplementation in obesity and during weight loss: A review of randomized controlled trials. Metabolism. 2019;92:193-205.

Original Article:

GENDER DIFFERENCE IN STRESS LEVELS AMONG MEDICAL AND NON- MEDICAL STUDENTS OF LAHORE

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ABSTRACT:

Objective: To compare the gender difference in stress levels among students of medical and non-medical institutions of Lahore

Methodology: This was a cross-sectional study conducted in medical students of Akhter Saeed Medical and Dental College, Lahore and non-medical students of Beacon House National University, Lahore from January 2018 to August 2018.A sample of two hundred and ninety- one student was collected by non- probability consecutive sampling technique which includes one hundred and fifty medical students and one hundred and forty-one non- medical students. Data was collected and analyzed using SPSS 24. To compare stress levels and gender differences in these levels, chi-square test was applied and p-value of less than and equal to 0.05 was fixed as significant.

Results: In this study 119 (40.9%) males and 172 (59.1%) female students participated. Among them, 150 (51.5%) were medical students and 141 (48.5%) were non-medical students. Out of 291 students, 46(15.8%) had severe stress. Results showed that 61(21%) students were smokers and 96(33%) were taking drugs. Sixty-seven students (23%) stated the reason of stress as the death of their family members in the last one year. A large proportion of students195 (67%) reported that they work harder and accomplish less, 188 (64%) had difficulty in sleeping and 206 (70.8%) had mood swings. One hundred and eighty-seven students (64.3%) felt frustration due to a lack of resources. One hundred and eighty-seven students (64.3%) give up their social life to succeed in university. The exam was the major cause of stress constituting 218 (74.9%) students. There was no significant difference between the stress level of medical and non-medical students (p=0.658) and no difference was observed in two genders (p=0.962).

Conclusion: Stress is prevalent in both medical and non-medical students irrespective of their career pathway. Both genders are affected by high-stress levels during academic years.

Key Words: Stress, Medical Students, Medical Education

INTRODUCTION:

The mental health of students in all fields of life is a global issue and it plays a significant role in any community worldwide.[1] According to WHO, a person could be termed as stressed when he/she shows variable combinations including low mood, lack of interest, disturbed sleep, loss of appetite and feeling of guilt.[2] The important indicators of mental health are depression, anxiety and stress level that increases the psychological morbidity among the students irrespective of their career choice.[3]

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Worldwide studies report that prevalence of depression among students varies globally showing the wide range of 1.4% to 73%.[4] A study conducted in Turkish medical students showed a high prevalence of depression as 27.1%, anxiety as 41.1%, and stress as 27%.[5] Moreover, high suicidal ideation from 4.9% to 35.6% was reported in a study conducted by the American Medical Association.[6]

A global survey revealed that the percentage of students in the U.K, seeking counseling for depression is 49%, for stress is 45% and for academic performance is 28%.[7]

In developing countries, stress is also prevailing as it is 20.9% in Nepali Medical students.[8] A study conducted in India showed 63.5% of stressed students.[9] While

in Bangladesh and Malaysia stress and anxiety disorder among students were found to be 54% and 41%, respectively.[10,11]

One of the most stressful fields of education is medicine because it is a highly demanding profession and requires enormous academic efforts. The constant sources of anxiety for medical students are extensive medical curricula, fear of failure and frequent examinations.[12] Medical students suffer deleterious effects of psychological stress which leads to poor academic performance**,** sleep disorders,[13] substance abuse and alcoholism[14] which, in turn, result in deterioration of relationships, marital problems and affect their future employment.[15]

The time period of an undergraduate student is very sensitive in his life and it plays a key role in developing systems and interventions to reduce mental problems.[16] Students having low socioeconomic status, family conflicts and rural background have a higher incidence of depression, anxiety and stress. Previous studies revealed morbidities due to anxiety and depression all over the world despite different educational courses.[17]

Stress is the major issue of medical students and this study is designed to develop a fair comparison between medical and non-medical students on stress issues that eventually be very helpful to prevent or overcome major stressors of student's life. The main objective of this study is to compare the level of stress among two genders and between medical and non-medical students.

MATERIAL AND METHODS:

A cross-sectional study was conducted in medical students of Akhtar Saeed Medical & Dental College Lahore & non-medical students of Beacon House National University, Lahore. Two hundred and ninety-one students were included in the sample through non-probability, consecutive sampling techniques. One hundred and fifty medical students and one hundred and forty-one non-medical students were recruited as participants from January 2018 to August 2018. Data was collected after IRB approval of Akhtar Saeed Medical and Dental College. A structured questionnaire was filled after informed consent through the interviewing technique. The questionnaire was developed and pretested on 10 students as a pilot run. Those students who were pilot tested were excluded while data collection for this study. Data were analyzed using SPSS 24. Chi-square test was applied to compare stress level among medical &non-medical students and p value were fixed at $\leq$ 0.05 to decide about the significance of results.

RESULTS:

There were 291 participants in total, out of which 119(41%) were males and 172(59%) were female, 141(48%) were of non-medical and 150(52%) were medical students. There were 61(21%) smokers, 96(33%) were drug abusers. and 195(67%), 75(25.8%) had undergone surgery in past, 67(23%) had the death of their family members, 86(29.6%) had the death of a close friend in last one year.

Table 1: Socio-demographic profile of Participants

Variables	Frequency (n)	Percentage (%)
Gender distribution of participants		
Male	119	40.9
Female	172	59.1
Degree sought		
Medical	150	51.5
Non-medical	141	48.5
Smokers		
Yes	61	21
No	230	79
Drug users		
Yes	96	33
No	195	67
Past Surgical History		
Yes	75	25.8
No	216	74.2
History of death of a family member		
Yes	67	23
No	224	77
History of death of a close friend		
Yes	86	29.6
No	205	70.4

On history regarding burnout, 195(67%) had this feeling that they work harder and accomplish less in terms of academic scores, 187(64.3%) complained about forgetting things, 176 (60.5%) suffering from physical complaints, 188(64.6%) invaded by sadness which cannot be explained, 103(35%) had difficulty in sleeping and 206(70.8%) had mood swings.

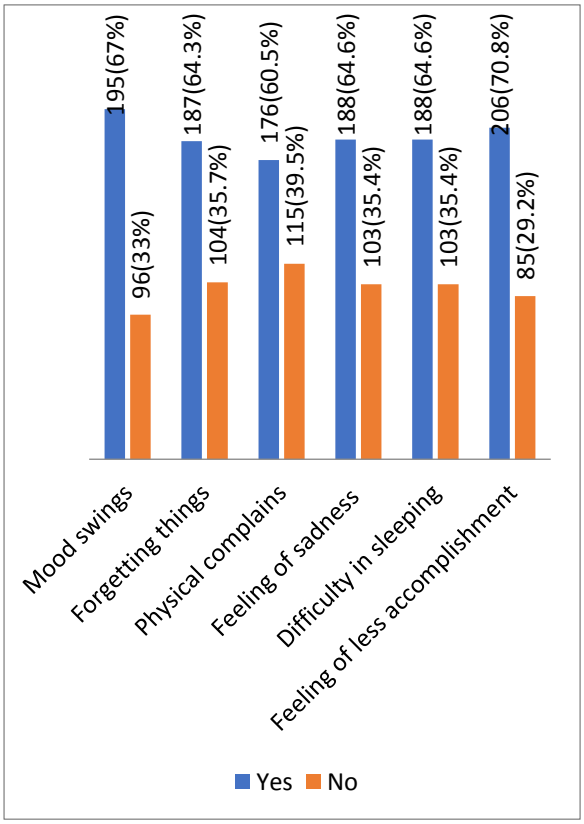

Figure 1: Information regarding burn out

The results of information regarding burn out of students indicated 162(55.7%) participants were stressed out because they thought that they were wrong if teachers do not agree with them,187(64.3%) felt frustrated due to lack of resources,178(61.2%)felt deprived of normal daily pleasure due to cost of undergraduate schooling, 187(64.3%)had given up much of their social life to succeed in university,151(51.9%) felt guilty if they take time off from their study to do something else for their selves.

Table 2: Reasons related to academics of getting stressed

Variables	Frequency (n)	Percentage %
Receiving negative comments		
Yes	167	57.4
No	124	42.6
Teachers or professors do not agree with you		
Yes	162	55.7
No	129	44.3
Lack of resources (internet, libraries)		
Yes	187	64.3
No	104	35.7
The cost of graduate/undergraduate school		
Yes	178	61.2
No	113	38.8
Giving up social life for academics		
Yes	187	64.3
No	104	35.7
Difficult to take time off for your self		
Yes	151	51.9
No	140	48.1

Upon further inquiry, 178(61.2%) were found to be stressed with fear of developing required skills, 140(48.1%) had unclear course objectives, 183(62.9%) were continuously stressed to participate in class, 218(74.9%) were stressed for studying for upcoming exams.

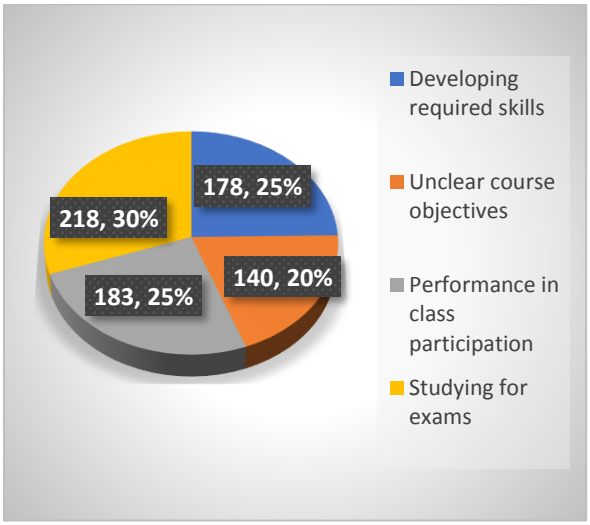

Figure 2: Information about academic stressors

Each stressor was allocated a number given on Likert scale and rating was done with mild, moderate and severe stress. Results showed that out of 291 participants, 89(30.6%) had no stress, 156(53.6%) had mild stress, 46(15.8%) had severe stress.

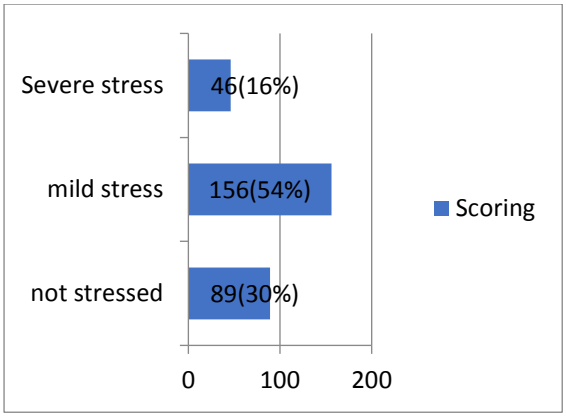

Figure 3: Scoring of stress levels

After the application of chi-square test, no significant difference was found between the two genders and their stress levels (p=0.962) and no difference was found in the stress level of medical and non-medical students (p=0.658). So, it was concluded that irrespective of gender and type of studies, students in undergraduate studies are generally stressed and the major reason is to cope with exams and peer pressure.

Table 3: Difference in stress levels between genders and professional degrees.

Variables	Scoring			p-value
	Mild	Moderate	Severe	
Gender				
Male	37 (31.1%)	64 (53.8%)	18 (15.1%)	0.962
Female	52 (30.2%)	92 (53.5%)	28 (16.3%)	
Degree sought medical				
Medical	43 (28.7%)	81 (54.0%)	26 (17.3%)	0.658
Non-Medical	46 (32.6%)	75 (53.2%)	20 (14.2%)	

DISCUSSION:

Globally medical students show a high prevalence of stress.[18] The academic performance and quality of life of the students are immensely affected by this psychological factor. Stress leads to a vital association between the individual and his environment. This interaction results in cognitive, emotional and behavioral alterations in case of any derangements.[19] This study shows that there is no significant association of gender and degree with stress. A study conducted at CMH Lahore also showed no association between stress level and gender in a sample of college students.[20] Another study conducted in India suggested that medical students were less stressed in comparison with non-medical students.[21] Similar findings were observed in this study which showed non-association between gender and type of undergraduate studies with stress level. This study showed that 21% of students were smokers and 33% were drug users. In a study conducted in France, a positive association was found between stress and regular use of alcohol[22], while in a study conducted in America, the prevalence of daily smoking among students in 4 years course was 87% and almost 50% in occasional smokers.[23] In this study, about 23% of students experienced recent trauma in the form of death of their family members which was a major cause of stress. In a study conducted in Thai medical school, 26.8% of students had stress due to family health problems.[24]

Lifestyle is badly affected by the stress. It has been noted that the young student population is always more susceptible to stressful life conditions to pursue higher professional education in a highly challenging environment.[25] About 70.8% of students in this study had mood swings and 64.6% reported the feeling of sadness. In another study, the prevalence of depressive symptoms among medical students was 12.9% and 2.7% of students had made suicidal attempts due to sadness.[26] In this study, 67% of students complained that they were working harder and were accomplishing less with 33% students having fear of future. In a study conducted among a group of Turkish medical students showed that students who were stratified with their education had lower stress levels.[27] In another study conducted at Surat, it was found that increased load towards exams and not getting expected marks were major stress factors.[28] In this study, 64.3% felt frustration due to lack of resources and 62% of students were stressed due to the

cost of undergraduate school. In a study conducted in Saudi Arabia, 25% of their students were facing financial problems.[29]

In this research, we have observed 35.4% of students cannot fall asleep due to stress. While a study conducted at the University of North Texas showed a significant portion of 9.5% of students met proposed DSM-5 criteria for chronic insomnia and sleeping difficulties.[30]

About 64.3% of students give up their social life due to the academic stressors. In a study conducted in Malaysia, 1.23% of students were stressed due to lack of time for their family and friends.[31] There were 51.9% of students who felt guilty if they took their time off from their study for themselves. In a study conducted at a Pakistan Medical School, 27.3% of students were unable to enjoy normal activities.[32] Stress relates to academic performance, pressure to succeed and post-graduate plans in students. The results of this study showed that no significant difference was observed in the stress level of medical and nonmedical students while a study conducted in Saudi Arabia among female students of medical and nonmedical institutions showed that students in med schools were much more stressed due to academic burden with p-value of less than 0.01.[33] In contrast to the results of this study, another published article in Korea showed that Nonmedical students had shown significantly higher stress levels when compared to their age fellow medical students($p=0.001$).[34] A study conducted in Kanpur India showed that stress level is much higher in students studying in professional colleges as compared to nonprofessional colleges.[35] To assess the gender difference in stress levels, both medical and nonmedical students were compared and it was observed that there was no significant difference in stress levels of both genders. A study published by the American Psychological association compared gender differences among medical and Law students and results showed that females were affected more with stress which included 30% medical students and 34% law students.[36] Similar results were shown by a study conducted in Egypt which showed that females had higher perceived stress levels but no significant difference was observed in two genders.[37] Similar results were obtained from Serbia.[38] Mental health issues interfere with the success of college students so, it is important that the colleges evaluate the mental health of their students regularly and launch prompt treatment programs to target their needs.[39]

CONCLUSION:

Medical and non-medical students face different types of stressors but the magnitude of stress remains approximately the same in both types of students. There is no gender difference among stress levels of medical and nonmedical students

Recommendations:

The solution lies in having a true perception of the emerging problem besides taking proper measures to alter the course of events earlier by providing support with adequate services. Counseling and preventive mental services should be an integral part of the routine clinical facilities.

Conflict of interest:

None

REFERENCES:

1. Adewuya AO, Ola BA, Aloba OO, Mapayi BM, Oginni OO. Depression amongst Nigerian university students. Social psychiatry and psychiatric epidemiology. 2006 1;41(8):674-78.
2. World Health Organization. Depression: A Global Public Health Concern, 2012. http://www.who.int/mediacentre/events/2012/wha65/journal/en/index4.html.
3. Woloschuk W, Harasym PH, Temple W. Attitude change during medical school: a cohort study. Medical education. 2004 ;38(5):522-34.
4. Prinz P, Hertrich K, Hirschfelder U, de Zwaan M. Burnout, depression and depersonalisation–Psychological factors and coping strategies in dental and medical

students. GMS Z Med Ausbild. 2012;29(1): Doc 10.
5. Bostanci M, Ozdel O, Oguzhanoglu NK, Ozdel L, Ergin A, Ergin N, et al. Depressive symptomatology among university students in Denizli, Turkey: prevalence and sociodemographic correlates. Croat med J. 2005 1;46(1):96-100.
6. Osama M, Islam MY, Hussain SA, Masroor SM, Burney MU, Masood MA, et al. Suicidal ideation among medical students of Pakistan: a cross-sectional study. Journal of forensic and legal medicine. 2014 1;27:65-8.
7. Moffat KJ, McConnachie A, Ross S, Morrison JM. First year medical student stress and coping in a problem-based learning medical curriculum. Medical education. 2004 ;38(5):482-91.
8. Sreeramareddy CT, Shankar PR, Binu VS, Mukhopadhyay C, Ray B, Menezes RG. Psychological morbidity, sources of stress and coping strategies among undergraduate medical students of Nepal. BMC Medical education. 2007 ;7(1):26.
9. Deb S, Strodl E, Sun J. Academic stress, parental pressure, anxiety and mental health among Indian high school students. International Journal of Psychology and Behavioral Sciences. 2015;5(1):26-34.
10. Sherina MS, Rampal L, Kaneson N. Psychological stress among undergraduate medical students. Medical Journal of Malaysia. 2004 ;59(2):207-11.
11. Abdulghani HM, AlKanhal AA, Mahmoud ES, Ponnamperuma GG, Alfaris EA. Stress and its effects on medical students: a cross-sectional study at a college of medicine in Saudi Arabia. Journal of health, population, and nutrition. 2011 ;29(5):516-22.
12. Shah M, Hasan S, Malik S, Sreeramareddy CT. Perceived stress, sources and severity of stress among medical undergraduates in a Pakistani medical school. BMC medical education. 2010 ;10(1):2.
13. Lemma S, Gelaye B, Berhane Y, Worku A, Williams MA. Sleep quality and its psychological correlates among university students in Ethiopia: a cross-sectional study. BMC psychiatry. 2012 ;12(1):237.
14. Ball S, Bax A. Self-care in medical education: effectiveness of health-habits interventions for first-year medical students. Academic Medicine. 2002 1;77(9):911-7.
15. Aktekin M, Karaman T, Senol YY, Erdem S, Erengin H, Akaydin M. Anxiety, depression and stressful life events among medical students: a prospective study in Antalya, Turkey. Medical education. 2001 4;35(1):12-7.
16. Arnett JJ. Emerging adulthood: A theory of development from the late teens through the twenties. American psychologist. 2000 ;55(5):469-80.
17. Smith CK, Peterson DF, Degenhardt BF, Johnson JC. Depression, anxiety, and perceived hassles among entering medical students. Psychology, health & medicine. 2007 1;12(1):31-9.
18. Almojali AI, Almalki SA, Alothman AS, Masuadi EM, Alaqeel MK. The prevalence and association of stress with sleep quality among medical students. Journal of epidemiology and global health. 2017;7(3):169-74.
19. Pulido-Martos M, Augusto-Landa JM, Lopez-Zafra E. Sources of stress in nursing students: a systematic review of quantitative studies. International Nursing Review. 2012;59(1):15-25.
20. Anjum A, Bajwa MA, Saeed R.2014. Sleep patterns; among medical and non-medical students of University of Lahore, 2010-11. The Professional Medical Journal 21(1):148-56.
21. Sheokand N, Kumar P. Social Determinants, Psychosocial Function and Mental Health among Medical and Non-Medical students. Journal of Disability Management and Rehabilitation. 2019;4(1):39-46.
22. Tavolacci MP, Boerg E, Richard L, Meyrignac G, Dechelotte P, Ladner J. Prevalence of binge drinking and associated behaviours among 3286 college students in France. BMC public health. 2016;16(1):178.
23. Tavolacci MP, Ladner J, Grigioni S, Richard L, Villet H, Dechelotte P. Prevalence and association of perceived stress, substance use and behavioral addictions: a cross-sectional study among university students in France, 2009–2011. BMC public health. 2013;13(1):724.
24. Saipanish R. Stress among medical students in a Thai medical school. Medical teacher. 2003;25(5):502-06.
25. Abdulghani HM, AlKanhal AA, Mahmoud ES, Ponnamperuma GG, Alfaris EA. Stress and its effects on medical students: a cross-sectional study at a college of medicine in Saudi Arabia. Journal of Health, Population, and Nutrition. 2011;29(5):516-522.

26. Dahlin M, Joneborg N, Runeson B. Stress and depression among medical students: A cross-sectional study. Medical Education. 2005;39(6):594-604.
27. Bayram N, Bilgel N. The prevalence and socio-demographic correlations of depression, anxiety and stress among a group of university students. Social Psychiatry and Psychiatric Epidemiology. 2008;43(8):667-72.
28. Solanky P, Desai B, Kavishwar A, Kantharia SL. Study of psychological stress among undergraduate medical students of government medical college, Surat. International Journal of Medical Science and Public Health. 2012;1(2):38-43.
29. El-Gilany AH, Amr M, Hammad S. Perceived stress among male medical students in Egypt and Saudi Arabia: effect of sociodemographic factors. Annals of Saudi Medicine. 2008;28(6):442-48.
30. American psychiatric Association (2012).DSM-5 development. Retrieved 2012.
31. Sherina MS, Rampal L, Kaneson N. Psychological stress among undergraduate medical students. Medical Journal of Malaysia. 2004;59(2):207-11.
32. Shaikh BT, Kahloon A, Kazmi M, Khalid H, Nawaz K, Khan N, et al. Students, stress and coping strategies: a case of Pakistani medical school. Education for Health-Abingdon-Carfax Publishing Limited. 2004;17(3):346-53.
33. Al-Dabal BK, Koura MR, Rasheed P, Al-Sowielem L, Makki SM. A comparative study of perceived stress among female medical and non-medical university students in Dammam, Saudi Arabia. Sultan Qaboos University Medical Journal. 2010;10(2):231-40.
34. Kim NC, Kim SH, Lhm HK, Kim JH, Jung HS, Park JC, Kim YS. Comparison of stress and life satisfaction between non-medical and medical college students. Korean J Psychosom Med. 2015;23(1):47-56.
35. Singh A, Singh S. Stress and adjustment among professional and non professional students. Industrial Psychiatry Journal. 2008;17(1):26-27.
36. Robotham D, Julian C. Stress and the higher education student: a critical review of the literature. Journal of further and higher education. 2006 May 1;30(02):107-17.
37. Amr M, El Gilany AH, El-Hawary A. Does gender predict medical students' stress in Mansoura, Egypt?. Medical education online. 2008;13(1):4481.
38. Backović DV, IlićŽivojinović J, Maksimović J, Maksimović M. Gender differences in academic stress and burnout among medical students in final years of education. Psychiatria Danubina. 2012;24(2.):175-81.
39. Beiter R, Nash R, McCrady M, Rhoades D, Linscomb M, Clarahan M, et al. The prevalence and correlates of depression, anxiety, and stress in a sample of college students. Journal of affective disorders. 2015;173:90-96.

Original Article

ASSOCIATION BETWEEN HIGH SERUM FERRITIN LEVELS AND MID-PREGNANCY GESTATIONAL DIABETES MELLITUS

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ABSTRACT

Introduction: Ferritin levels found to have an important role in the development of many syndromes during pregnancy. To compensate for the risk of low ferritin levels in pregnant females, usually iron and vitamin supplements have been prescribed. The use of these supplements may increase the ferritin level in normal pregnancy and can cause several syndromes including gestational diabetes mellitus.

Objective: To determine the association of high serum ferritin level with gestational diabetes mellitus

Material and methods: This Case-Control study was conducted at the Department of Obstetrics & Gynecology, Sheikh Zayed Hospital, Lahore for six months. Using Non-probability consecutive sampling 70 pregnant women were divided into cases and controls. Patients with high serum ferritin (149 µg / L) were calculated in both groups and then Odds ratio and Relative Risk were estimated to find an association between high serum ferritin levels and mid-pregnancy GDM.

Results: Patients with mid-pregnancy GDM had statistically significantly higher serum ferritin levels. (36.21 ± 59.07 µg/L) compared to controls (12.46 ± 13.87 µg/L) (t (68) = 2.316, p = 0.024) 15% GDM cases (n=35) had high serum ferritin level, while in the controls group (n=35) no change in the serum ferritin observed. Relative Risk estimates showed a doubling of the risk of having mid-pregnancy GDM with high serum ferritin concentration [RR=2.167 (95% CI = 1.66 – 2.81)]

Conclusion: High serum ferritin level is associated with GDM.

Key Words: Ferritin, Gestational Diabetes, Insulin Resistance

INTRODUCTION:

Anemia during pregnancy is prevalent world over but is much more severe in the developing nations like Pakistan. As per UNO reports, prevalence of anemia is about 56% in low socioeconomic groups.[1] Iron deficiency anemia is the commonest type of anemia in Pakistani population overall and is more prevalent among females.[2,3] Iron deficiency is the commonest cause of anemia in pregnancy which affects 54% of women in developing countries.[4] In under developed area of Khanewal District, 250 pregnant women (17 – 39 years of age) were studied for 6 months. Out of these, 55% were found anemic (83 % of these were moderately anemic: Hb 8-9.9g/dL).[5] A much larger study *(*enrolling 1,369 pregnant women at 20 to 26 weeks of gestation and followed to 6 weeks postpartum*)* on urban population of Hyderabad showed 90.5% of women being anemic according to WHO cut off of Hb<11g/dL.[6] In pregnancy, iron supplementation is routinely recommended all pregnant women[8] irrespective of their serum ferritin and/or haemoglobin (Hb) levels. Increasing evidence suggests that iron, a strong per-oxidant, influences glucose metabolism, even in the absence of significant iron overload.[9] Large prospective cohort studies found that dietary iron intake, particularly heme iron derived from meat, is associated with a significant increased risk of type II Diabetes.[10,11]

Serum ferritin levels (a biomarker of body iron stores) however have also been shown to be positively associated with diabetes risk,[12] hypertension,[13] the metabolic

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syndrome,[14] cardiovascular risk factors, and inflammation.[15] Although there have been several studies investigating the possible role of dietary and serum iron on glucose metabolism, only a few studies are available about these association during pregnancy.

Adverse effects of routine iron supplementation in Pakistani population has not been researched extensively. The rationale of this study was to find out association between high serum ferritin & risk of Gestational Diabetes Mellitus (GDM). On the basis of results recommendations could be made to abandon iron to all pregnant women routine and instead judicious iron supplementation to only severely anemic women(followed by close monitoring for GDM) can be made.

MATERIAL AND METHODS:

A case control study was planned in department of Gynae & Obstetrics in Sheikh Zayed Hospital Lahore from April to September 2012.

In this study 70 participants were enrolled between the age group of 20–35 years through non probability consecutive sampling. Out of these 70 participants, 35 females were controls, who were not pregnant & 35 females were taken as cases who were diagnosed case of gestational diabetes at more than 24 weeks with oral glucose tolerance test. If the BSL was more than 186 mg/dl after 1 hour of glucose tolerance test they were labelled as cases. Controls were without gestational diabetes mellitus. Serum ferritin was tested in both cases & controls**.** All those females were exluded who were known to have pre-pregnancy diabetes mellitus, diseases affecting glucose metabolism e.g. thyrotoxicosis, Cushing syndrome, hemochromatosis, Hypertensive or having Blood Pressure ≥ 140/90 mmHg on 2 consecutive occasions, current medical treatments (including hormonal preparations, corticosteroid therapy), and supplementation with micronutrients other than iron-on history.

A value of serum ferritin >149µg/L was considered as a high level. Females with high ferritin levels and diabetes were managed as per standard protocols.

All the information collected was entered into SPSS version 15.0 and was analyzed through its statistical package. Odd's ratio and Relative Risk was calculated to see the strength of the association of High Serum Ferritin Levels and GDM. Odd's ratio > 2 was taken as significant.

RESULTS:

In our study, the mean age of patients was 26.37±3.77 years of GDM cases and 28.00±4.39year of controls. The mean gestational age was 30.21±7.47weeks of GDM cases and 29.88±8.13weeks of controls. Among cases, there was 11 (31.4%) primigravida and 24 (68.6%) multigravida while among controls, there were 17 (48.6%) primigravida and 18 (51.4%) multigravida. The mean BMI of cases was 29.63±13.97kg/m^2 while of controls was 30.22±14.82kg/m^2. (Table 1)

The patients with gestational diabetes had statistically significantly higher serum ferritin levels (36.21±59.07µg/L) than non-GDM patients (12.46±13.87µg/L, p = 0.024). There was a significant association of high serum ferritin levels with GDM i.e. 14.3% vs. 0% and the risk of developing GDM is 12.80 times high in females with high ferritin levels. (Table 2)

Table 1: Comparison of Age and Serum Ferritin between cases and controls

	Group	
	Cases	Control
n	35	35
Age (years)	26.37±3.77	28.00±4.39
Gestational age (weeks)	30.21±7.47	29.88±8.13
Gravidity		
Primigravida	11 (31.4%)	17 (48.6%)
Multigravida	24 (68.6%)	18 (51.4%)
BMI (kg/m^2)	29.63±13.97	30.22±14.82

Table 2: High and Low Serum Ferritin Counts in Cases & Controls

		Group		Significance
		Cases	Controls	
Serum Ferritin Level (µg/L)		36.21± 59.08	12.46± 13.87	0.024
Ferritin level	High	5 (14.3%)	0 (0%)	OR= 12.80
	Low	30 (85.7%)	35 (100%)	95% CI (0.68 to 241.03)
Total		35	35	

DISCUSSION:

Gestational diabetes can be linked to free radical damage caused by high serum ferritin levels.[16-18]

In one of the studies of the Pakistani population, it was observed that the pregnant ladies of community have very low serum ferritin levels (reflecting low body iron stores). Mean serum ferritin levels found in this study simulate with those found in the study conducted at the Faculty of Health Sciences, Hazara University.[19]

Although overall ferritin levels were low regardless of presence and absence of gestational diabetes later on all patients with gestational diabetes diagnosed to have high ferritin levels; the same was found in the study of 128 ladies in Iran, and positive co-relation between serum ferritin, HbA1c and fasting plasma glucose levels was discovered.[20]

Bo et al in their Chinese cohort and Caucasian cohort confirmed the positive link between serum ferritin and gestational diabetes.[21,22,24] While iron deficiency is a defensive condition. [23]

Routine iron supplementation during pregnancy is necessary or toxic, is a highly controversial topic.[25] Another large prospective cohort study, which identified the existence between pre-pregnancy dietary heme iron intake and GDM.[26]

In this study, it was found out that a single woman among controls has higher than our threshold level of serum ferritin (i.e. 149 µ /L). This resulted in a "divide by zero" error while calculating the odds ratio. Thus the odds ratio was calculated after little adjustment (*adding 0.5 to all four values to prevent a divide by zero*). Relative Risk, however, is calculated using original counts as it is not affected by "divide by zero" error

By investigation of serum ferritin either before pregnancy or in early pregnancy, it is conceivable to order ladies in three groups: (a) those with low iron status (ferritin <30 µg/L) who either have or are in danger of developing iron deficiency and IDA; (b) those with intermediate iron status (ferritin 30–70 µg/L) and moderate danger of iron deficiency and IDA; (c) those with satisfactory iron status (ferritin >70–80 µg/L) with insignificant or no danger of iron deficiency. Healthy pregnant ladies having ferritin over 70–80 µg/L give off an impression of being in safe water concerning iron deficiency as their body iron sores are 500 mg or more, which is satisfactory to finish a pregnancy without taking iron supplements. Prophylactic iron supplementation along these lines gives off an impression of being sheltered in ladies with Intermediate and low iron status yet in those with pre-pregnancy sufficient iron stores alerts must be watched.

CONCLUSION:

Higher Serum ferritin levels are associated with increased risk of mid-pregnancy GDM. Owing to a great majority of Pakistani pregnant women having very low total iron body stores, general Iron prophylaxis during pregnancy seems to be a safe option in the vast majority. Pregnant women coming from a high socioeconomic group should have their serum ferritin levels done and iron prophylaxis initiated only if low ferritin count is found.

REFERENCES:

1. United Nations. Administrative Committee on Co-ordination. Sub-committee on Nutrition, International Food Policy Research Institute. 4th report on the world nutrition situation: nutrition throughout the life cycle. United Nations, Administrative Committee on Coordination, Subcommittee on Nutrition; 2000.

2. Pappas G, Akhtar T, Gergen PJ, Hadden WC, Khan AQ. Health status of the Pakistani population: a health profile and comparison with the United States. American Journal of Public Health. 2001;91(1):93-98.
3. Idris M. Iron deficiency anaemia in moderate to severely anaemic patients. Journal of Ayub Medical College Abbottabad. 2005;17(3).
4. Noronha JA, Bhaduri A, Bhat HV, Kamath A. Maternal risk factors and anaemia in pregnancy: a prospective retrospective cohort study. Journal of Obstetrics and Gynaecology. 2010;30(2):132-36.
5. Taseer IU, Mirbahar A, Safdar S, Awan Z. Anemia in pregnancy; Related risk factors in under developed area. Professional Medical Journal. 2011;18(1):1-4.
6. Baig-Ansari N, Badruddin SH, Karmaliani R, Harris H, Jehan I, Pasha O, Moss N, McClure EM, Goldenberg RL. Anemia prevalence and risk factors in pregnant women in an urban area of Pakistan. Food and nutrition bulletin. 2008;29(2):132-39.
7. Palma S, Perez-Iglesias R, Prieto D, Pardo R, Llorca J, Delgado-Rodriguez M. Iron but not folic acid supplementation reduces the risk of low birthweight in pregnant women without anaemia: a case–control study. Journal of Epidemiology & Community Health. 2008;62(2):120-24.
8. Rohra DK, Das N, Azam SI, Solangi NA, Memon Z, Shaikh AM, Khan NH. Drug-prescribing patterns during pregnancy in the tertiary care hospitals of Pakistan: a cross sectional study. BMC pregnancy and childbirth. 2008;8(1):24.
9. Rajpathak SN, Crandall JP, Wylie-Rosett J, Kabat GC, Rohan TE, Hu FB. The role of iron in type 2 diabetes in humans. Biochimica et Biophysica Acta (BBA)-General Subjects. 2009;1790(7):671-81.
10. Rajpathak S, Ma J, Manson J, Willett WC, Hu FB. Iron intake and the risk of type 2 diabetes in women: a prospective cohort study. Diabetes care. 2006 Jun 1;29(6):1370-6.
11. Shi Z, Yuan B, Qi L, Dai Y, Zuo H, Zhou M. Zinc intake and the risk of hyperglycemia among Chinese adults: the prospective Jiangsu Nutrition Study (JIN). The Journal of Nutrition, Health & Aging. 2010;14(4):332-35.
12. Forouhi NG, Harding AH, Allison M, Sandhu MS, Welch A, Luben R, Bingham S, Khaw KT, Wareham NJ. Elevated serum ferritin levels predict new-onset type 2 diabetes: results from the EPIC-Norfolk prospective study. Diabetologia. 2007;50(5):949-56.
13. Piperno A, Trombini P, Gelosa M, Mauri V, Pecci V, Vergani A, Salvioni A, Mariani R, Mancia G. Increased serum ferritin is common in men with essential hypertension. Journal of hypertension. 2002;20(8):1513-18.
14. Qi L, Van Dam RM, Rexrode K, Hu FB. Heme iron from diet as a risk factor for coronary heart disease in women with type 2 diabetes. Diabetes care. 2007;30(1):101-06.
15. Williams MJ, Poulton R, Williams S. Relationship of serum ferritin with cardiovascular risk factors and inflammation in young men and women. Atherosclerosis. 2002;165(1):179-84.
16. Van Campenhout A, Van Campenhout C, Lagrou AR, Abrams P, Moorkens G, Van Gaal L, Manuel-y-Keenoy B. Impact of diabetes mellitus on the relationships between iron-, inflammatory-and oxidative stress status. Diabetes/metabolism research and reviews. 2006;22(6):444-54.
17. Hallberg L, Hulthén L. High serum ferritin is not identical to high iron stores. The American journal of clinical nutrition. 2003 Dec 1;78(6):1225-6.
18. Crowe S, Bartfay WJ. Amlodipine decreases iron uptake and oxygen free radical production in the heart of chronically iron overloaded mice. Biological research for nursing. 2002 Apr;3(4):189-97.
19. Raza N, Sarwar I, Munazza B, Ayub M, Suleman M. Assessment of iron deficiency in pregnant women by determining iron status. Journal of Ayub Medical College Abbottabad. 2011;23(2):36-40.
20. Sharifi F, Ziaee A, Feizi A, Mousavinasab N, Anjomshoaa A, Mokhtari P. Serum ferritin concentration in gestational diabetes mellitus and risk of subsequent development of early postpartum diabetes mellitus. Diabetes, metabolic syndrome and obesity: targets and therapy. 2010;3:413-419.
21. Bo S, Menato G, Villois P, Gambino R, Cassader M, Cotrino I, Cavallo-Perin P. Iron supplementation and gestational diabetes in midpregnancy. American journal of

obstetrics and gynecology. 2009;201(2):158-e1.
22. Lao TT, Chan LY, Tam KF, Ho LF. Maternal hemoglobin and risk of gestational diabetes mellitus in Chinese women. Obstetrics & Gynecology. 2002;99(5):807-12.
23. Lao TT, Ho LF. Impact of iron deficiency anemia on prevalence of gestational diabetes mellitus. Diabetes Care. 2004;27(3):650-6.
24. Chen X, Scholl TO, Stein TP. Association of elevated serum ferritin levels and the risk of gestational diabetes mellitus in pregnant women: The Camden study. Diabetes Care. 2006;29(5):1077-82.
25. Milman N. Iron and pregnancy—a delicate balance. Annals of hematology. 2006;85(9):559.
26. Bowers K, Yeung E, Williams MA, Qi L, Tobias DK, Hu FB, Zhang C. A prospective study of prepregnancy dietary iron intake and risk for gestational diabetes mellitus. Diabetes care. 2011;34(7):1557-63.

Original Article

NEPHROPROTECTIVE EFFECT OF METHANOLIC EXTRACT OF DINOTHROMBIUM TINCTORIUM IN ALBINO RATS

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ABSTRACT:
Modern world has proven scientifically that medicines derived from animals are important tools in treating ailments today.
Objective: In current project, goal was to estimate the nephro-protective effects of methanolic extract of Dinothrombium tinctorium against carbon tetrachloride-induced nephrotoxicity.
Study design: It was a randomised control study.
Methodology: Aqueous methanolic extract (70% v/v) of Dinothrombium tinctorium (Dt.Cr) was arranged followed by subsequent evaporations. Renal toxicity was induced by CCl_4 (2 ml/kg, p.o) in paraffin oil on 7th day of experiment. Administration of methanolic extract of Dinothrombium tinctorium (300mg/kg body weight/day) orally sheltered the CCl_4 caused elevation of renal serum markers that include urea and creatinine. There was renal markers elevation in the CCl_4 alone treated animals.
Results: Administration of methanolic extract to CCl_4 encounter protection against the renal toxicity.
Conclusion: The findings thus suggested that this methanolic extract can be used as nephroprotective agent against CCl_4-induced renal toxicity in albino rats.

Key Words: Urea, Creatinine, Medicine

INTRODUCTION:

Renal ailments are threatening human life worldwide. Nephropathies nowadays are a big dilemma for the health professionals. Treatment options are limited as well as not much effective against renal diseases. According to World Health Organization (WHO) estimatation, 46% of all diseases and 60% deaths globally are because of renal hitches. The sixth leading cause of death globally is the renal ailment.[1]

Kidneys are continuously exposed to environmental toxins which eventually lead to various nephropathies.[2] Nephrotoxicants include carbon tetrachloride, non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics and carcinogens. They all have different sites of actions.[3]

In animal models, carbon tetrachloride (CCl_4), has extensively been employed to chemically induce renal injury.[4] Silymarin has been reported to have nephroprotective activity against toxins. As a herbal remedy against nephropathies, its extract from the seeds is being used traditionally.[5]

In modern era, Zootherapy provides an alternative treatment option among other known therapies applied globally. Chemicals from animal origin constitutes 8.7% of 252 essential drugs short-listed by the WHO.[6] In subcontinent, 9% of all traditional medicines come from 31 substances of animal origin.[7]

Traditionally, Dinothrombium tinctorium (Red Velvet Mite) extract has been used in the treatment of multiple medical ailments like paralysis, malaria, urogenital disorders and many other medical conditions.[8] It has antibacterial, antifungal and gastroprotective activity that have been established in previous many publications.[9] The current project was proposed to gauge the nephroprotective activity of methanolic extract of Dinothrombium tinctorium against CCl_4 induced nephrotoxicity in albino rats.

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MATERIAL AND METHODS:

This project was a randomised control trial and was conducted for 07 days at Pharmacology Department of Islamia University, Bahawalpur in 2017. Reagents used in current project included Diagnostic kits, Silymarin, Carbon Tetrachloride, distilled water, Digital electronic balance, Grinder, Vortex Mixer, Incubator, Centrifuge machine, Rotary Evaporator. All the chemicals were of analytical grade. The species Dinothrombium tinctorium identification was done by the zoology department, IUB. With the help of one kg of Red Velvet Mites, a coarse paste of Red Velvet Mites was waterlogged using 70% v/v aqueous methanol. It was carried for 03 days. Crude extract was extracted from filtrate after filtration by using Rotary Evaporator. Final extract was stored till further use[8]. In this study, 36 male albino rats were selected and separated into 06 groups each comprising of six animals. They were supported at a temperature (25±2°C) and humidity (55-55%) along with 12 hour light and dark cycle. Animals were given standard diet and tap water ad libitum. Acclimatization of subjects was done for seven days before the start of study[9]. Acute toxicity testing was carried out on 25 mice of both genders. They were randomly separated into 5 groups with 5 mice in each group. All the animals had overnight fast. Group1 was served with normal saline (10 ml/kg p.o) treated as normal control. 04 treatment groups were given oral methanolic extract of Dinothrombium tinctorium at increasing doses of 0.3, 1, 3, 5 g/kg respectively. Toxic effects like behavior with other animals, alertness, food intake, change in body weight and mortality were monitored strictly from zero hour till day 14. Carbon tetrachloride (2ml/kg p.o) was employed as nephrotoxic agent in male albino rats in order to assess the nephroprotective activity of methanolic extract of Dinothrombium tinctorium.[10] Division of animals with treatment plan during study is summarized in table #I. On 7th day, with a delay of 30 min after the respective treatments, CCL_4 was administered to all groups except control group to induce toxicity. Next day blood was collected to analyze it for renal markers by using standard kit methods.[11]

Table-1: Group Treatments for Calculation of Nephroprotective Action

Sets	Days (1-6)	Day (7)
Normal Control	Distilled water 4 ml/Kg	Distilled water 4 ml/Kg
Intoxicated	Distilled water 4 ml/Kg	Distilled water 4 ml/Kg + CCI4 (2 ml/Kg)
Rx. Set 1	Dt. Cr 30 mg/Kg	Dt. Cr 30 mg/Kg + CCI4 (2 ml/kg)
Rx. Set 2	Dt. Cr 100 mg/Kg	Dt. Cr 100 mg/Kg+CCI4 (2 ml/kg)
Rx. Set 3	Dt. Cr 300 mg/Kg	Dt. Cr 300 mg/Kg + CCI4 (2 ml/kg)
Control set	Silymarin 25 mg/Kg	Silymarin 25 mg/Kg + CCI4 (2 ml/Kg)

ANOVA with Bonferroni test was employed for analysis of data by using SPSS computer program and Mean ± S.E.M was used for expression of results. Significant (*) result values if $p<0.05$.

RESULTS:

Prepared extract, Dt. Cr, was screened for its phytochemical constituents as below in table-2.

Table-2: Phytochemical constituents of Dinothrombium tinctorium

Biochemical Constituents	
Alkaloids	+++
Carbohydrates	++
Flavonoids	++

(+ Sign indicates the presence and (-) sign indicates absence and number of signs shows the intensity)

Results of renal biomarkers showed significant decrease in their serum levels among groups treated with Dinothrombium tinctorium extract with different doses.

Table – 3: Serum Creatinine & Urea Levels in CCI_4-intoxicated albino rats.

Group Allocation	Serum Creatinine (mg/dL)	Urea (mg/dL)	p-value
Control (D/W 4ml/Kg)	0.50±0.05	28.10±2.2	<0.125
Intoxicated (CCI_4 2 ml/Kg)	1.79±0.05	86.40±4.45	<0.001*
Dt.Cr (30 mg/Kg) + CCI_4	1.56±0.04	74.63±4.22	<0.01**
Dt. Cr (100 mg/Kg) + CCI_4	1.24±0.08	46.03±1.6	<0.001**
Dt. Cr (300 mg/Kg) + CCI_4	0.79±0.04	35.47±2.99	<0.001**
Silymarin (25 mg/Kg) + CCI_4	0.65±0.03	33.45±2.9	<0.001**

*Statistically Significant

Acute toxicity studies showed that the extract used in study was practically non-toxic. It was also non nephrotoxic at selected given doses since the biochemical markers were in normal range.

DISCUSSION:

There are less number of modern medicine available for cure of renal diseases. Hence, the people have moved towards traditional treatment options for many years. Dinothrombium tinctorium was picked in current study due to its old-fashioned use in medical ailments.[9]

In current project, CCl_4-induced nephrotoxicity was carried out in male Wistar albino rats to observe its effects as nephroprotective agent. Our work was in line with previous studies who used same agent for induction.[10] Paradoxically, gentamicin was the inducing agent in other studies.[12]

Nephrotoxicity is impaired renal functions produced due to nephrotoxin drugs or other noninfectious agents.[13] Silymarin was used as control drug in current project to relate different strengths of Dinothrombium tinctorium extract as nephroprotective agent. It was used as standard drug in many old publications so our work was in line with past researchers.[5] Serum urea and creatinine levels were analyzed as biochemical renal markers.

Acute toxicity studies were carried out in current project in 25 mice. Strict surveillance for toxic behaviours for 24 hours and then daily for 14 days was conducted in our project. In other studies acute toxicity assay was done but for 24 hours and then daily for just 7 days.[11] Protocol adopted in current study regarding number of animals and groups was similar as adopted in one animals study to see different hepatoprotective effect of Fumairia indica plant extract but some modifications were made in our setting.[14] Different doses of extract were given to treatment groups in current study. In one previous work the plant extract at a dose of 50,100, 200 and 400 mg/kg body wt. exhibited orally to observe its nephroprotective effects. The extract at a dose of 30,100 and 300 mg/kg body wt. administered orally in current project to treatment groups respectively.[11]

Limitations:

Our study had a number of limitations like financial constraint and less resources. No histopathological study of renal tissue was done. Only renal function tests were done to assess nephroprotective effect of extract in present study. No similar study is available for comparison. It observed methanolic extract from animal origin as nephroprotective agent.

CONCLUSION:

The findings indicate that the methanolic extract of Dinothrombium tinctorium can be used as nephroprotective agent in albino rats.

REFERENCES:

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver

disease—meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016 Jul;64(1):73-84.
2. Chandan BK, Saxena AK, Shukla S, Sharma N, Gupta DK, Suri KA, Suri J, Bhadauria M, Singh B. Hepatoprotective potential of Aloe barbadensis Mill. against carbon tetrachloride induced hepatotoxicity. Journal of Ethnopharmacology. 2007 May 22;111(3):560-6.
3. Nirmala M, Girija K, Lakshman K, Divya T. Hepatoprotective activity of Musa paradisiaca on experimental animal models. Asian Pacific journal of tropical biomedicine. 2012 Jan 1;2(1):11-5.
4. Soliman AM, Fahmy SR. Protective and curative effects of the 15 KD isolated protein from the Peganum harmala L. seeds against carbon tetrachloride induced oxidative stress in brain, tests and erythrocytes of rats. Eur Rev Med Pharmacol Sci. 2011 Aug 1;15(8):888-99.
5. Gazak R, Walterova D, Kren V. Silybin and silymarin-new and emerging applications in medicine. Current medicinal chemistry. 2007 Feb 1;14(3):315-38
6. Costa-Neto EM. Animal-based medicines: biological prospection and the sustainable use of zootherapeutic resources. Anais da Academia Brasileira de ciências. 2005 Mar;77(1):33-43.
7. Mahawar MM, Jaroli DP. Traditional knowledge on zootherapeutic uses by the Saharia tribe of Rajasthan, India. Journal of Ethnobiology and Ethnomedicine. 2007 Dec;3(1):25-29.
8. Costa-Neto EM. Entomotherapy, or the medicinal use of insects. Journal of Ethnobiology. 2005 Mar;25(1):93-115.
9. George L, Padmalatha C, Ranjitsingh AJ, Dhasarathan P. Antifungl Efficiency of Haemolymph and Aqueous Extraction of Red Velvet Mite, T. Grandissimum. International Journal of Biology. 2011 Jan 1;3(1):111-114.
10. Khan, M.R. and Siddique, F., 2012. Antioxidant effects of Citharexylum spinosum in CCl4 induced nephrotoxicity in rat. Experimental and toxicologic pathology, 64(4), pp.349-355.
11. Qadir MI, Ali M, Saleem M, Hanif M. Hepatoprotective activity of aqueous methanolic extract of Viola odorata against paracetamol-induced liver injury in mice. Bangladesh Journal of Pharmacology. 2014 Apr 25;9(2):198-202.
12. Bienvenu KF, Cyril DG, Florian YB, Felix YH, Timothée OA. Evaluation of Nephroprotective Properties of Aqueous and Hydroethanolic Extracts of Crinum scillifolium against Gentamicin Induced Renal Dysfunction in the Albino Rats. Journal of Advances in Medicine and Medical Research. 2019 Jul 6:1-8.
13. Navarro VJ, Senior JR. Drug-related hepatotoxicity. New England Journal of Medicine. 2006 Feb 16;354(7):731-9.
14. Bhawna S, Kumar SU. Hepatoprotective activity of some indigenous plants. Int J Pharm Tech Res. 2009 Oct;4:1330-4.

Original Aritcle

REASONS OF NON-COMPLIANCE TO METFORMIN AMONG TYPE 2 DIABETICS ATTENDING DIABETIC CLINIC IN LAHORE.

Maryam Rashid[1], Neelofar Yousaf Warraich[1], Talha Laique[2], Kamil Shujaat[3], Sadaf Zawar[1], Aqsa Munir[1]

ABSTRACT

Objective: To identify the factors associated with non-compliance to metformin therapy in patients with type 2 diabetes mellitus.

Method: This was a cross-sectional study, carried out at Jinnah-Allama Iqbal Institute of Diabetes and Endocrinology (JAIDE) Lahore from April 2018 to June 2018.

In this study newly diagnosed type 2 diabetic patients were given metformin for the duration of three months and their compliance was observed over that period. Metformin was given starting with low dose of 500mg once daily and then after two weeks was titrated to optimum dose of 500mg twice daily and maximum dose range of 1500-2000mg per day. Multiple factors contributed to patient's fall out including co-morbidities, shifting to alternative medicine and insulin and GIT intolerance. Blood sampling for A_1C estimation was done by A1C analyzer (TD4611A TAI Doc). Research data was collected by questionnaires and patients were called up for follow-up through telephonic communication.

Results: Out of 260 patients, 200 continued their trial smoothly on metformin while 23% (n=60) were dropped out of the study. GIT intolerance contributed to the major reasons of discontinuing the drug accounting for 35% (n=21) of the patients. Other reasons for non- compliance included change of therapy to insulin (12%) due to uncontrolled raised glucose levels and alternative medicine (5%) by their own decision, deranged LFTs (10%) and RFTs (7%), refusal to the therapy (7%) and various domestic issues (10%).

Conclusion: Though metformin is the first line drug for treating type 2 diabetes mellitus (T2DM) but GIT intolerance to metformin is one of the major reasons that some patients are unable to tolerate the drug at all.

Key Words: Metformin, Type 2 diabetes mellitus, Liver function test

INTRODUCTION:

Type 2 Diabetes mellitus (T2DM) is a group of metabolic disorders characterized by hyperglycemia with classic symptoms of polyuria, polydipsia, loss of weight, tiredness, and fatigue, propensity for infections.[1] There is rapidly rising prevalence of Diabetese Mallitus worldwide and patients suffering from this disease are being expected to rise from 360 million in 2011 to as expected range of 550 million by the year 2030.

In various studies conducted by the Diabetic Association of Pakistan, 10% of the population ranging from 30 years or above are said to be suffering from T2DM. The estimated prevalence of T2DM in Pakistan is 6.7%.[2]

Hyperglycemia is investigated and diagnosed on the basis of blood sugar levels carried by; fasting or random blood sugar sampling, follow up OGTT and further glycated Hb (HbA_1c) is indicated =/> 6.5%. Other tests are also carried out according to clinical assessment (due to complications) e.g; lipid profile, kidney function tests, urine albumin- creatinine ratio (ACR), ECG.[1]

DM is a chronic disease and needs long term treatment which itself is a big challenge.[3] The main objective for the management of DM is to control the blood sugar levels by;

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healthy lifestyles, strict diet modification and therapeutic treatment such as oral hypoglycemic agents including mono drug therapy/combination drug therapy and insulin.[4]

Metformin, an insulin-sensitizing agent is a first-line drug used for the treatment of type 2 diabetes mellitus (T2DM).[5] The effects of metformin occur as a consequence of reduced hepatic glucose output primarily through inhibition of gluconeogenesis and to a lesser extent, due to glycogenolysis and increased insulin-stimulated glucose uptake in skeletal muscle and adipocytes. Metformin also increases insulin insensitivity.[5,6]

The use of metformin is associated with side-effects of the gastrointestinal (GI) tract in 20 - 30% of patients. Common GI symptoms are nausea, vomiting, diarrhea, dyspepsia, bloating, metallic taste, cramp-like abdominal pain with changes in gut motility which sometimes becomes uncontrollable[7, 8] and about 5% of diabetic patients prematurely discontinue the therapy due to these GI side effects.[9]

Despite its widespread use 35% of patients fail to reach initial target glycemic control with metformin.[10]Reasons of failure may be differently related either to the therapy or the patient itself leading to the poor compliance to the pharmacological treatment.[11]

The purpose of this study is to determine factors leading to non-compliance of metformin therapy and issues impeding the control of DM in Lahore, Pakistan so that quality based health-care services can be provided to the masses.

MATERIAL AND METHODS:

The sample size was calculated by WHO software based on S.K Lwanga and Lemeshow keeping the confidence (CI) level equal to 95% and the margin of error equal to 5%. Initially, a total of 260 patients with T2DM were included in the study.

Two hundred and sixty (260) patients with T2DM were enrolled in the study from Jinnah-Allama Iqbal Institute of Diabetes and Endocrinology (JAIDE) Jinnah Hospital, Lahore. The study was conducted for a duration of three months from April 2018 to June 2018. Patients were selected by convenient sampling. Patients were diagnosed with T2DM according to the criteria of the American Diabetes Association has any one of the criteria; fasting (8 hr or longer fast) glucose ≥ 126 mg/dl (≥ 7.0 mmol/liter)**,** two hours glucose ≥ 200 mg/dl (≥ 11.1 mmol/liter) during an oral glucose tolerance test (OGTT), non-fasting plasma glucose > 200 mg/dl or HbA_{1C} ≥ 6.5%.[12] Drug naïve patients were included in the present study with baseline A1C levels ranging between 7-9%. Out of 260, 200 patients continued their medication; however, 60 patients dropped out of the study.

Data was collected from these 60 patients using structured questionnaires comprising of age, sex, marital status, educational level, presence of other chronic diseases, the regularity of taking the medication, and follow-up.

Non-compliance was defined as missing more than one scheduled visit or discontinuation of medicine due to adverse effects or any other reason. Non-compliance was further assessed using the patients' self-report on how they had been taking their medication in the week preceding the interview and their regular attendance at the diabetic clinic. Further, patients were asked to recall if they had missed any doses of metformin on a day-to-day basis over a period of one week.[13]

GIT intolerance was defined as (questionnaire of side-effects filled by the doctor) the presence of at least one of the following gastro-intestinal symptoms: diarrhea, nausea, flatulence, abdominal pain, asthenia, and vomiting.

RESULTS:

In this study, 260 patients were enrolled initially .Out of which 200 were compliant and 60 were non compliant. These non-compliant patients were further observed for discontinuing the drug. The frequency

distribution of gender amongst compliant patients was 69% (n=138) females and 31% (n= 62) males (Fig 1). Whereas, in non-compliant patients 31% (n=19) were male and 69 % (n=41) were females (Fig 1). Male to female ratio was 1:3 and the median age was 59 years. Fig 2 shows that among non-compliant patients; 38% (n=23) were literate and 62% (n=37) were illiterate. Fig 3 shows the diverse reasons that made subjects to discontinue metformin. A considerable number of patients i.e, 35% (n=21) terminated the treatment because of GI adverse effects due to metformin. Patients who were shifted to insulin were second significant no i.e 12% (n=7). Deranged Liver Function Tests and Renal Function Tests comprise 10% (n=6) and 7% (n=4) of the patients respectively. Almost 8% (n=5) patients were shifted to alternative medicine while 7% (n=4) refused to participate. Domestic issues were also a great hindrance and almost 10% of patients refused treatment because of domestic issues while 3% of patients got pregnant and 8% (n=5) were non-adherent.

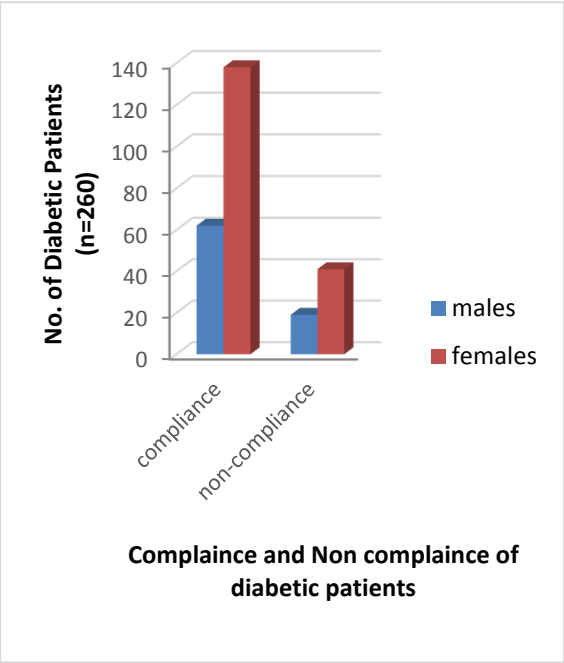

Figure 1: The gender distribution of the study population in compliance & non-compliance among T2DM patients (n=260)

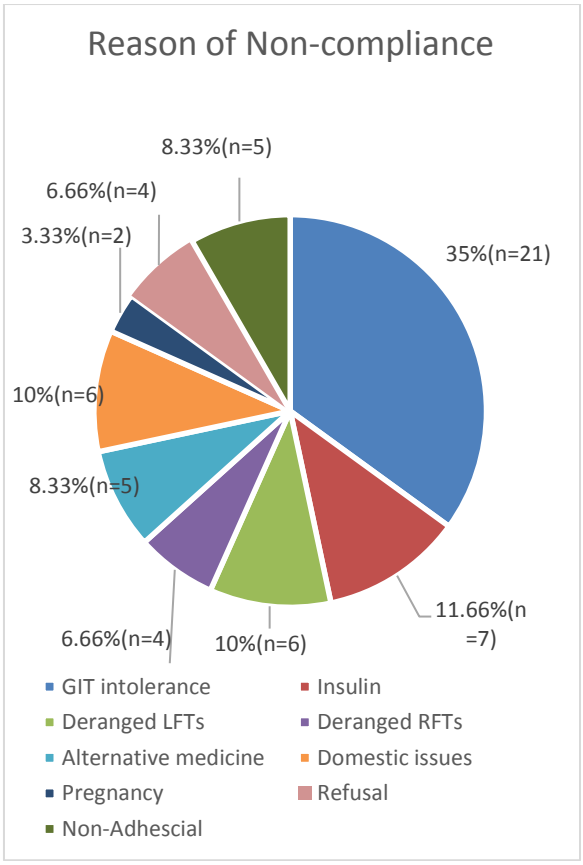

Figure 2: Pie chart showing reasons for non-compliance among the T2DM patients (n=60)

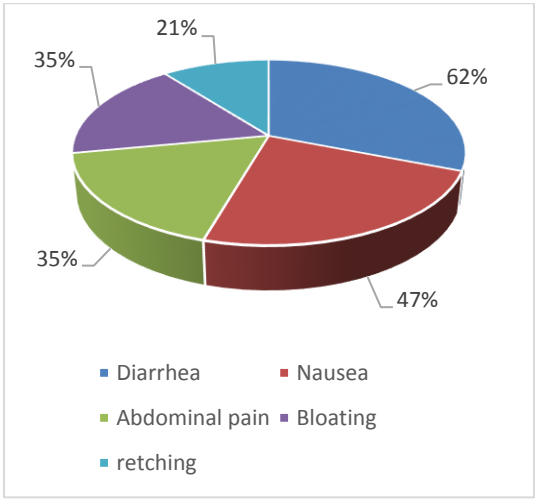

Figure 3: Frequency of GIT symptoms among 60 non- compliant T2DM patients (n=21)

DISCUSSION:

Type 2 diabetes mellitus (T2DM) is the most predominant form of diabetes[14] and needs long term therapy for adequate

glycemic control and to reduce the incidence of complications.

However, compliance of patients tend to decrease with time being lower in patients on long term medication that severely compromises the effectiveness of treatment.[15]

Patient non-compliance is a serious issue and has been reported worldwide.[16] It's not only limited to the failure to take medication but also in maintaining a healthy lifestyle, following a strict diet, going for proper follow-up visits including both regular tests and appointments with physicians.[17]

The usual first-line agent is considered metformin for the reduction of insulin resistance. It has proven benefits over other options of treatment, especially in overweight patients.[14] Other benefits include neither significant weight gain nor hypoglycemia and an improved lipid profile.[1]

According to some previous studies, there is considerable variation of metformin in maintaining adequate glycemic levels. Despite its extensive use, data shows that only 60-65% of patients successfully achieve the HbA1c target of less than 7% or adequate glycemic control with metformin.[18] This might be due to the adverse effects of metformin that may make it less palatable for patients. The most common adverse effect is gastrointestinal upset, occurring in 10.4–19.3% of patients, usually in the first few weeks of therapy. Although it appears that few patients discontinue therapy early in the course of treatment, a significant portion of patients continue to experience these effect even at 6 months.[19]

Measuring the compliance of diabetic patients is a complex issue. In this study, we aimed to explore the factors contributing to non-compliance of diabetic patients to metformin therapy. Newly diagnosed 260 patients with T2DM were enrolled initially and they were followed up

fortnightly. Out of which 200 diabetic patients followed all the requisites of treatment and achieved adequate glycemic control with HbA_1c range of 7% to 9% whereas remaining 60 patients failed to achieve adequate glycemic control and therefore labeled as non-compliant.

The major cause of non- compliance in most of the cases was early discontinuation of treatment due to GI adverse effects of metformin as the considerable number of patients i.e, 35% (n=21) terminated the treatment because of metformin. This observation was in accordance with previous studies in which patients were non-complaint to metformin treatment due to unwanted effects of metformin related to GIT upset.[20]

About 12% (n=7) of patients were shifted to insulin because of uncontrolled fasting glucose levels which were measured after every two weeks contributed to one of the factors of non-compliance to metformin therapy as discussed in a previous study.[21]

Deranged LFTs in 10% (n=6) and RFTs in 7% (n=4) of diabetic patients were seen which led to a change in antidiabetic therapy. Same results were discussed in a previous study in which metformin was contraindicated in patients with deranged LFTs and RFTs.[22]

The secret to success is adherence to the treatment plan advised by the physician but in our study, almost 8% (n=5) patients were shifted to alternative medicine such as Hikmat, spiritual healing (dum Darood), homeopathy owing to our socio-cultural factors further leading to complications of Diabetes Mellitus.[23] While 7% (n=4) straight away refused to participate in the treatment plan or follow the advice.

Domestic issues were also a great hindrance and almost accounted for 10% of patient non-compliance. Domestic issues included irregularity of follow-up due to financial issues, non-availability of transport followed by forgetfulness. Forgetfulness has been widely published as an important cause of irregularity of following up[24] further contributing to the non-compliance of the therapy in our study by up to 8% (n=5). These patients were called non-adherent to the treatment as they missed the prescribed doses may be due to lack of education and

good supervision. In addition to this 3% of the total number of patients got pregnant and stopped taking metformin.

In this study, we focused to understand the different reasons for non-compliance to metformin therapy through proper data collection so we can find proper solutions to eradicate the problems and educate the masses in detail about the disease to make the metformin therapy beneficial to the suffering patients.

CONCLUSION:

T2DM is a disease that requires long term therapy and a lot of patience for the compliance and success of the treatment. So we should try to avoid the factors discussed above leading to the non-compliance of the therapy by providing detailed and comprehensive patient education, support; whether it is financial or emotional and reassurance. Patients who are unable to visit clinics regularly should be provided transport facility or footstep visits by the administration. By solving these issues we can achieve control of presenting symptoms and complications and overcome noncompliance to metformin in T2DM.

REFERENCES:

1. Atkinson K. John Murtagh's general practice companion handbook 6th edition. Australian Journal of General Practice. 2016 Jul.
2. Gyawali B, Sharma R, Neupane D, Mishra SR, van Teijlingen E, Kallestrup P. Prevalence of type 2 diabetes in Nepal: a systematic review and meta-analysis from 2000 to 2014. Global health action. 2015 Dec 1;8(1):29088.
3. Hamine S, Gerth-Guyette E, Faulx D, Green BB, Ginsburg AS. Impact of mHealth chronic disease management on treatment adherence and patient outcomes: a systematic review. Journal of medical Internet research. 2015;17(2):e52.).
4. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nature Reviews Endocrinology. 2018 Feb;14(2):88-98.
5. Soumya D, Srilatha B. Late stage complications of diabetes and insulin resistance. J Diabetes Metab. 2011 Dec 25;2(9):1000167.
6. Consoli A, Gomis R, Halimi S, Home PD, Mehnert H, Strojek K, et al. Initiating oral glucose-lowering therapy with metformin in tyape 2 diabetic patients: an evidence-based strategy to reduce the burden of late-developing diabetes complications. Diabetes & metabolism. 2004 Dec;30(6):509-16.
7. Hermans MP, Ahn SA, Rousseau MF. What is the phenotype of patients with gastrointestinal intolerance to metformin? Diabetes & metabolism. 2013 Sep 1;39(4):322-9.
8. Dujic T, Zhou K, Donnelly LA, Tavendale R, Palmer CN, Pearson ER. Association of organic cation transporter 1 with intolerance to metformin in type 2 diabetes: a GoDARTS study. Diabetes. 2015 May 1;64(5):1786-93.
9. Tarasova L, Kalnina I, Geldnere K, Bumbure A, Ritenberga R, Nikitina-Zake L, Fridmanis D, Vaivade I, Pirags V, Klovins J. Association of genetic variation in the organic cation transporters OCT1, OCT2 and multidrug and toxin extrusion 1 transporter protein genes with the gastrointestinal side effects and lower BMI in metformin-treated type 2 diabetes patients. Pharmacogenetics and genomics. 2012 Sep 1;22(9):659-66.
10. Pawlyk AC, Giacomini KM, McKeon C, Shuldiner AR, Florez JC. Metformin pharmacogenomics: current status and future directions. Diabetes. 2014 Aug 1;63(8):2590-9.
11. Wens J, Vermeire E, Van Royen P, Hearnshaw H. A systematic review of adherence with medications for diabetes: response to cramer. Diabetes Care. 2004 Sep 1;27(9):2284-.
12. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care. 2013 Jan 1;36(Supplement 1):S67-74.
13. Khan AT, Lateef NA, Khamseen MA, Alithan MA, Khan SA, Ibrahim I. Knowledge, attitude and practice of ministry of health primary health care physicians in the management of type 2 diabetes mellitus: A cross sectional study in the Al Hasa District of Saudi Arabia, 2010. Nigerian Journal of Clinical Practice. 2011;14(1):52-59.

14. Pawlyk AC, Giacomini KM, McKeon C, Shuldiner AR, Florez JC. Metformin pharmacogenomics: current status and future directions. Diabetes. 2014 Aug 1;63(8):2590-9.
15. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes care. 2015 Jan 1;38(1):140-9.
16. AlHewiti A. Adherence to long-term therapies and beliefs about medications. International Journal of Family Medicine. 2014;2014:8.
17. Alsayed KA, Ghoraba MK. Assessment of diabetic patients' adherence to insulin injections on basal-bolus regimen in diabetic care center in Saudi Arabia 2018: Cross sectional survey. Journal of Family Medicine and Primary Care. 2019 Jun 1;8(6):1964-70.
18. Liao WL, Tsai FJ. Personalized medicine in type 2 diabetes. BioMedicine. 2014 Jun;4(2).
19. Salber GJ, Wang YB, Lynch JT, Pasquale KM, Rajan TV, Stevens RG, Grady JJ, Kenny AM. Metformin Use in Practice: Compliance With Guidelines for Patients With Diabetes and Preserved Renal Function. Clinical Diabetes. 2017 Jul 1;35(3):154-61.
20. Marczynski MA, Cortellazzi KL, Barberato-Filho S, Motta RH, Vieira AE, Quilici MT, Bergamaschi CD. Unsatisfactory glycemic control in type 2 Diabetes mellitus patients: predictive factors and negative clinical outcomes with the use of antidiabetic drugs. Brazilian Journal of Pharmaceutical Sciences. 2016 Dec;52(4):801-12.
21. Khunti K, Godec TR, Medina J, Garcia-Alvarez L, Hiller J, Gomes MB, Cid-Ruzafa J, Charbonnel B, Fenici P, Hammar N, Hashigami K. Patterns of glycaemic control in patients with type 2 diabetes mellitus initiating second-line therapy after metformin monotherapy: R etrospective data for 10 256 individuals from the U nited K ingdom and G ermany. Diabetes, Obesity and Metabolism. 2018 Feb;20(2):389-99.
22. Dmitri K, McFarlane Samy I, Sowers James R. Metformin: an update. Ann Intern Med. 2002 Jul 2;137(1):25-33.
23. Parivallal T, Anjana RM, Mohan V. Frequency of use of Indian systems of Medicine and homeopathy among diabetic patients in Chennai (CURES-80). Journal of Social Health and Diabetes. 2015 Dec;3(02):125-.
24. Khan AR, Lateef ZN, Al Aithan MA, Bu-Khamseen MA, Al Ibrahim I, Khan SA. Factors contributing to non-compliance among diabetics attending primary health centers in the Al Hasa district of Saudi Arabia. Journal of Family and Community Medicine. 2012 Jan;19(1):26-32.

Review Article

ROLE OF NEUROTRANSMITTERS IN THE HUMAN BODY

Hamid Javaid Qureshi

ABSTRACT:
A number of neurotransmitters are released in the body. Acetylcholine plays a role in the control of sleep and wakefulness, movements, memory and learning. Dopamine has a role in reward, behavior, and addiction. Hyperactivity of dopaminergic receptors is involved in some type of psychosis. Locus ceruleus and norepinephrine are involved in REM sleep. Serotonin is involved in the control of sleep, intake of food, remodeling of bone, reproductive behavior, emotional states, temperature and sensory perception. Substance P is the neurotransmitter in the slow pain pathway. Excessive glutamate receptor activation may give rise to Parkinson's disease and Alzheimer's disease. Degeneration of GABA secreting neurons results into Huntington's chorea. Brain histamine takes part in the regulation of wakefulness, sexual behavior, blood pressure, drinking, pain threshold and anterior pituitary hormones. Nitric-oxide takes part in the control of long term behavior and memory. Opioid neurotransmitters inhibit cerebral neurons involved in the perception of the pain.
Conclusion. It is concluded that neurotransmitters are involved in the regulation of many body functions and their disturbances lead to many diseases.

Key Words. Neurotransmitters, Catecholamines, Serotonin, Dopamine

INTRODUCTION:

Neurotransmitters have been the focus of research for the last many years. The role of important neurotransmitters has been reviewed.

Acetyl-choline is a small molecule neurotransmitter present in synaptic vesicles in nerve terminals of cholinergic neurones. Acetyl choline is formed by the reaction of acetyl – Co A with choline catalyzed by choline acetyl transferase. It is released from postganglionic parasympathetic nerve fibers, postganglionic sympathetic nerve fibers supplying sweat glands and also is the neurotransmitter at the neuromuscular junction. It is also present in preganglionic sympathetic and parasympathetic nerve endings. It is also released by gigantocellular neurons in upper brain stem. Corticostriate fibers release acetyl choline in caudate and putamen. Imbalance of acetyl choline and dopamine in caudate and putamen results into Parkinson's disease.[1] Acetyl choline plays a role in the regulation of sleep and wakefulness, movements, memory and learning.[2,3]

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Acetyl choline is removed from synapses by the enzyme acetyl cholinestrase. Cholinergic receptors are of two types; muscarinic and nicotinic. Muscarenic receptors are present on effectors supplied by parasympathetic postganglionic nerve fibers and sympathetic postganglionic fibers supplying sweat glands. Nicotinic receptors are present at synapses between preganlionic sympathetic and parasympathetic neurons and at muscle membrane in the neuromuscular junction.[4,5] Defects in cholinergic pathways in the brain are involved in senile dementia and Alzheimer's disease.[6] Treatment with long term acting anticholinestrase improves cognitive functions in these patients.[7]

Catecholamines

Catecholamines include norepinephrine, epinephrine, and dopamine.[7] Norepinephrine is released from postganglionic sympathetic nerve fibers, also secreted by the adrenal medulla. Norepinephrine secreting neurons are located in locus ceruleus.[8] Nerve fibers from locus ceruleus pass to the spinal cord, cerebellum, paraventricular nuclei and supraoptic nuclei of the hypothalamus, thalamus and neocortex.[9,10] Epinephrine is

secreted from the adrenal medulla.[1] Hypothalamus contains a high concentration of nor-epinephrine.[11]

Dopamine is secreted by nigrostriate fibers in caudate and putamen and is involved in motor control. Its deficiency leads to Parkinson's disease. Catecholamines are synthesized by hydroxylation and decarboxylation of the amino acid tyrosine. Norepinephrine is converted into epinephrine by phenyl ethanolamine –N– methyl transferase (PNMT).[2]

Catecholamines are metabolized by monoamine oxidase (MAO) and catechol-o-methyl transferase (COMT) into metanorepinephrine, metanephrrine and vanillylmandelic acid (VMA).[2] Monoamine oxidase inhibitors have been used in patients of depression.[6] In pheochromocytoma, excretion of VMA increases in the urine.[12] In the limbic subcortical region, dopamine is involved in emotion and behavior.[13] In human beings, with age, there is a gradual loss of dopamine receptors in basal ganglia. Hyperactivity of dopaminergic synapses may be involved in some types of psychosis.[7] Cocaine abuse leads to excessive dopamine activity.[13] Locus ceruleus and nor epinephrine are involved in REM sleep.[14]

Substance P. It is the neurotransmitter in the transmission of slow pain impulses from the periphery to the spinal cord. It is a polypeptide containing 11 amino acids. It is found in high concentration in migrostriatal system.[15] It is also present in the hypothalamus to be involved in neuroendocrinal regulation. It is released by nerve fibers involved in axon reflex.[2]

Serotonin:

Serotonin (5–hydroxytryptamine) is secreted by nerve fibers arising from raphe nuclei located at the junction of pons and medulla oblongata. Raphe nuclei are a part of the analgesia system. From raphe nuclei, nerve fibers project to the hypothalamus, the limbic system, neocortex, cerebellum and spinal cord.[2] Serotonin is involved in the control of sleep, intake of food, remodeling of bone, reproductive behavior and emotional states[6] body temperature and sensory perception.[7]

Glycine causes both excitation and inhibition in the brain and spinal cord. It is responsible for direct inhibition in the brain and spinal cord by increasing Cl^- conductance. The action of glycine is antagonized by strychnine. Glycine is involved in postsynaptic inhibition.[1,2]

Glutamate

It is the major excitatory neurotransmitter in the brain and spinal cord and is responsible for 75% of excitatory transmission in the central nervous system. In Kreb's cycle, alpha keto glutarate is converted into glutamate by the enzyme GABA transaminase. Another pathway for the formation of glutamate is that glutamine is converted into glutamate by the enzyme glutaminase.[2] Glutamate binds with glutamate receptors permitting Na^+ and Ca^{++} influx resulting into fast excitatory postsynaptic membrane potential (EPSP).[16,17] Glutamate accumulates in the infarcted area of the brain to produce excitotoxic damage and cell death. Excessive glutamate receptor activation may give rise to some neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease.[18,19]

Gamma-Aminobutyric Acid (GABA)

It is the major inhibitory neurotransmitter in the brain. Its formation involves decarboxylation of glutamate by the enzyme glutamate decarboxylase. Nerve fibers from caudate and putamen that pass to globus pallidus and substantia nigra secrete GABA at their nerve endings. Degeneration of GABA secreting neurons in caudate and putamen results into Huntington's chorea. It is metabolized by transamination of GABA to succinic semialdehyde and then to succinate in the citric acid cycle. GABA receptors are metabotropic that K^+ efflux and Cl^- influx to produce inhibitory postsynaptic membrane potential (IPSP).[20] GABA receptors are of 3 types; $GABA_A$,

$GABA_B$, and $GABA_C$.[21, 22] Benzodiazepines, Barbiturates increase Cl^- conductance produced by $GABA_A$ receptors. These drugs have antianxiety activity and are used as sedatives, muscle relaxant and anticonvulsant.[23]

Histamine:
Histamine secreting neurons are found in the posterior hypothalamus, their nerve fibers project to parts of the cerebral cortex and spinal cord. It is also present in gastric mucosa, mast cells and blood basophils. Histamine is formed by decarboxylation of histidine.[2]
There are 03 types of histamine receptors (H_1, H_2, and H_3). H_3 receptors mediate the release of histamine and other transmitters via a G-protein. Brain histamine is involved in wakefulness, sexual behavior, blood pressure, drinking, pain threshold and regulation of secretion of anterior pituitary hormones. Histamine H_2 receptors have a role in the regulation of cells of the immune system.[2]
Nitric oxide (Nitric oxide) secreted by nerve fibers in parts of the brain that control long term memory and behavior.[2] Its synthesis starts from arginine and this reaction is catalyzed by NO synthase. It activates guanalyl cyclase. It is not stored in vesicles but is synthesized on demand at postsynaptic sites.[2]
Nitric oxide is involved in transmission between inhibitory motor neurons of the enteric nervous system and gastrointestinal smooth cells. Also acts as a neurotransmitter in the brain.[7] Nitric oxide takes part in learning, development, penile and clitoral erection, sensory and motor modulations in cardiovascular system.[24]
Opioid peptide neurotransmitters are enkephalins, endorphins, and dynorphins. These are secreted by neurons in the central nervous system and intrinsic neurons of the gastrointestinal tract. These inhibit cerebral neurons and are involved in the perception of pain.
Opioid peptides bind to opioid receptors present in the brain and gastrointestinal tract.[25,26] These are present in substantia gelatinosa. When injected into the brain, these exert analgesia effect. These are metabolized by enkephalinase A, enkephalinase B and aminopeptidase.[2]

The feeling of pleasure on listening to music in due to the neurotransmitter dopamine released in the brain. There is also the release of endorphins and nitrous oxide while listening to music Endorphins result into an emotional response to music and nitrous oxide lead to vasodilation and blood pressure reduction.[27,28]

CONCLUSION:

It is concluded that neurotransmitters are involved in the regulation of many body functions and their disturbances lead to many diseases.

REFERENCE:

1. Hall JE. Nervous system In: Guyton and Hall textbook of medical physiology 13th ed. India, ELSEVIER, 2016; 734.
2. Barrett KE, Barman SM, Boitano S, Broooks HL. Learning, memory, language & speech. In: Ganong's. Review of Medical Physiology 24th ed. Boston. McGraw Hill. 2012; 283 – 90.
3. Tendon OP. Tripathi Y. Higher neural functions. In: Best & Taylor's Physiological Basis of Medical Practice. 13th ed. New Dehli. Wolters kluwer. 2012, 1212 – 14.
4. Dajas-Bailador F, Wonnacott S. Nicotinic acetylcholine receptors and the regulation of neuronal signalling. Trends in pharmacological sciences. 2004 Jun 1;25(6):317-24.
5. Dajas-Bailador F, Wonnacott S. Nicotinic acetylcholine receptors and the regulation of neuronal signalling. Trends in pharmacological sciences. 2004 Jun 1;25(6):317-24.
6. Van Den Pol AN. Neuropeptide transmission in brain circuits. Neuron. 2012 Oct 4;76(1):98-115.
7. Berne RM, Levy MN, Koeppen BM. Berne & levy physiology. Elsevier Brasil; 2008.
8. Sara SJ. The locus coeruleus and noradrenergic modulation of cognition. Nature reviews neuroscience. 2009 Mar;10(3):211.

9. Snell RS. The neurobiology of the neuron and the neuroglia. In. Clinical Neuroanatomy, 7th Ed. New Delhi. Wolters Kluwer, 2010;61
10. Goldstein DS, Robertson D, Esler M, Straus SE, Eisenhofer G. Dysautonomias: clinical disorders of the autonomic nervous system. Annals of internal medicine. 2002 Nov 5;137(9):753-63.
11. Sherwood L. Principals of Human Physiology 7th ed. New Dehli Cengage Learning, 2009; 205-8.
12. Kvetnansky R, Sabban EL, Palkovits M. Catecholaminergic systems in stress: structural and molecular genetic approaches. Physiological reviews. 2009 Apr;89(2):535-606.
13. Eisenhofer G, Kopin IJ, Goldstein DS. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Pharmacological reviews. 2004 Sep 1;56(3):331-49.
14. Onn SP, West AR, Grace AA. Dopamine-mediated regulation of striatal neuronal and network interactions. Trends in neurosciences. 2000 Oct 1;23:S48-56.
15. Hökfelt T, Pernow B, Wahren J. Substance P: a pioneer amongst neuropeptides. Journal of internal medicine. 2001 Jan;249(1):27-40.
16. Madden DR. Ion channel structure: the structure and function of glutamate receptor ion channels. Nature Reviews Neuroscience. 2002 Feb;3(2):91.
17. Popescu G, Auerbach A. The NMDA receptor gating machine: lessons from single channels. The Neuroscientist. 2004 Jun;10(3):192-8.
18. Jacob TC, Moss SJ, Jurd R. GABA A receptor trafficking and its role in the dynamic modulation of neuronal inhibition. Nature Reviews Neuroscience. 2008 May;9(5):331.
19. Amara SG, Fontana AC. Excitatory amino acid transporters: keeping up with glutamate. Neurochemistry international. 2002 Nov 1;41(5):313-8.
20. Owens DF, Kriegstein AR. Is there more to GABA than synaptic inhibition?. Nature Reviews Neuroscience. 2002 Sep;3(9):715-27.
21. Gassmann M, Bettler B. Regulation of neuronal GABA B receptor functions by subunit composition. Nature Reviews Neuroscience. 2012 Jun;13(6):380-94.
22. Sigel E, Steinmann ME. Structure, function, and modulation of GABAA receptors. Journal of Biological Chemistry. 2012 Nov 23;287(48):40224-31.
23. Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiological reviews. 2007 Oct;87(4):1215-84.
24. Widmair EP, Raff H, Strang KT. Consciousness, the brain and behavior. In: Vander's Human Physiology. The mechanism of body function. 12th ed. Boston. . McGraw Hill. 2011; 242-43.
25. Snyder SH, Pasternak GW. Historical review: opioid receptors. Trends in pharmacological sciences. 2003 Apr 1;24(4):198-205.
26. von Zastrow M. Opioid receptor regulation. Neuromolecular medicine. 2004 Feb 1;5(1):51-8.
27. Nizamie SH, Tikka SK. Psychiatry and music. Indian journal of psychiatry. 2014 Apr;56(2):128.
28. Van Den Pol AN. Neuropeptide transmission in brain circuits. Neuron. 2012 Oct 4;76(1):98-115.

Case Report

A RARE NASAL TUMOR WITH NEURAL AND MYOGENIC DIFFERENTIATION: BIPHENOTYPIC SINONASAL SARCOMA

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ABSTRACT:
Biphenotypic sinonasal sarcoma is a newly recognized mesenchymal tumor that has been included in the upgraded classification of sinonasal tumors by WHO. This is a low-grade spindle cell malignancy previously categorized as fibrosarcoma or peripheral nerve sheath tumor. We report a case of Biphenotypic sinonasal sarcoma arising in a middle-aged female. The patient came with the chief complaints of left nasal discharge and epistaxis. CT report was suggestive of left-sided sinonasal polyposis. After the surgery, histopathological and immunohistochemistry (IHC) analysis confirmed the diagnosis of Biphenotypic sinonasal sarcoma. This highlights the importance of IHC in this new entity to decrease the morbidity and mortality in such cases.

Key Words: Immunohistochemistry, Sarcoma, Mortality

INTRODUCTION:

Biphenotypic sinonasal sarcoma (BSNS) is a recently documented entity in the World Health Organization classification for head and neck tumors. This lesion is a rare form of malignancy arising primarily in the nasal tract.[1] This lesion, exhibiting characteristics of both neural and myogenic differentiation, carries a high preponderance for middle-aged females.[2] Researchers have found that fusion of paired box gene 3 (PAX3) and mastermind like transcription coactivator 3 (MAML3) genes give rise to this Biphenotypic tumor.[3] It has the predilection to quickly invade surrounding facial structures in an outward fashion typically towards each of the nostrils. The failure in early detection of this low-grade sarcoma and its infiltrative pattern makes it difficult to treat. Furthermore, attempts made at surgical removal of this lesion results in facial disfigurement.[4] The patient was a 50-year-old woman. She presented with a history of left nasal discharge for 01 year while bouts of epistaxis for last 01 month.

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CASE REPORT:

On examination, there was a widening and expansion of the left nasal cavity. Computed Tomography of Nose and Paranasal Sinuses (CT PNS) revealed a soft tissue expansile lesion in left-sided maxillary antrum filling maxillary sinus causing the widening of osteomeatal complex. The mass was noted to be extended up to the ipsilateral nasal cavity, ethmoidal air cell, sphenoidal and frontal sinuses. The left Cribriform plate was found to be thinned and eroded. However, there was no evidence of intracranial involvement. CT findings were suggestive of left-sided sinonasal polyposis. Laboratory investigations showed her lab values were in the normal range.

The patient was prepared for the surgical removal of the tumor. After surgery, the excised mass was sent to the Department of Histopathology at Akhter Saeed Medical and Dental College for confirmatory diagnosis.

The gross examination of the mass showed grey-white tissue fragments measuring approximately 1.2 cm in aggregate. While the histopathological examination of excised tissue section revealed a spindle cell neoplasm composed of fascicles and sheets of pleomorphic round to oval cells with uniform elongated nuclei. These cellular

fascicles were surrounded by delicate strands of collagen fibers. However, there were few areas that showed characteristic epithelial proliferation in small cystic spaces often forming glands. The intervening stroma revealed moderate chronic inflammatory cellular infiltrate and congested blood vessels. Mitotic figures were infrequent.

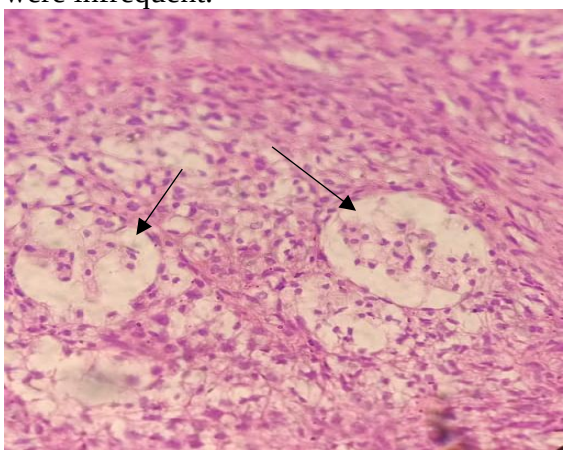

Fig 1: Photomicrograph shows cellular neoplasm with epithelial proliferation in small cystic spaces.

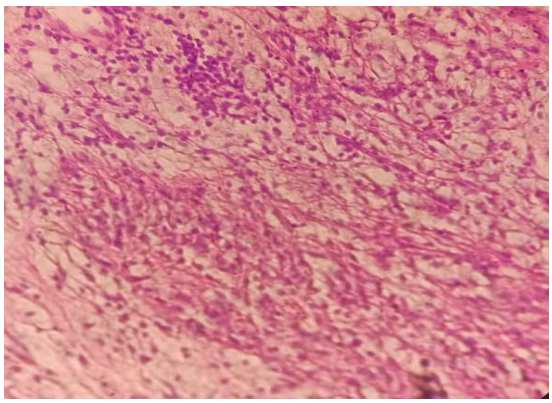

Fig 2: Photomicrograph shows cellular fascicles with intervening delicate collagen fibers.

On immunohistochemical studies, the tumor cells showed diffuse positivity for S-100 protein and only focal positivity for Smooth muscle actin (SMA). Whereas, expression of Desmin, Myogenin, Cytokeratin (CK) and HMB45 were found to be negative in the tumor. After a panel of immunohistochemical stains, a final diagnosis of Biphenotypic sinonasal sarcoma was made. The postoperative course was uneventful. The patient was referred to an oncologist for further treatment and follow-up.

DISCUSSION:

Biphenotypic sinonasal sarcoma is a low-grade, uncommon sarcoma that was first presented by Lewis et al [2] in 2012 as 28 cases of low-grade sarcoma with myogenic and neural differentiation which were negative for Synovial Sarcoma Translocated to X chromosome protein (SYT–SSX) chimeric transcript of synovial sarcoma.

The reported cases of BSNS show that it primarily affects women in the adult age group (24-78yrs) like our case.[2,5,6,7,8] Studies showed that patients present with nonspecific symptoms of nasal obstruction, like problems in nasal breathing, bouts of bleeding, pain and congestion in sinonasal areas the same as in our case. BSNS presents as a locally destructive tumor that involves multiple sinonasal sites, with the most commonly involved sites are superior nasal cavity and ethmoid sinus, and then by the sphenoid sinus. The invasion may occur beyond the sinonasal area, commonly into the orbit region (25% of cases) and via the cribriform plate (10% of cases). The CT scan findings of our patients were consistent with the previous literature and luckily with no evidence of intracranial involvement.[2]

Histopathologically, BSNS consists of an infiltrative, extremely cellular low-grade spindle cell lesion that has the long and slender tapered proliferation of uniform spindle cells, with syncytial borders, and nuclei with vesicular chromatin like in our case in which spindle cells are arranged in fascicles and sheets. Mitotic activity is low whereas necrosis is not a feature of BSNS. Commonly, there are entrapped respiratory epithelium as in our case epithelial proliferation in the form of glands and cystic spaces was seen.[2,7,9]

The immunohistochemical panel proposed by Rooper et al[9] is based on initial cases reported,[3,6,9] includes S100, SMA, β-catenin, desmin, SOX10, calponin, myogenin, factor XIIIa, and CK. BSNS cases mainly show at

least focal S100 expression[7,9] as well as of SMA and calponin. There could be a variable expression of factor XIIIa, desmin, myogenin, and negative expression for CK and SOX10. Immunohistochemical findings in this case were consistent with the previous data {positive for S-100, focal positivity for SMA, while negative expression of Desmin, Myogenin, Cytokeratin (CK) and Human melanoma black 45 (HMB45) were found in tumor cells}.

CONCLUSION:

BSNS was initially reported as a low-grade fibro-sarcoma or low-grade peripheral nerve sheath tumor prior to its description by Lewis et al.[2,10] However, cytogenetic studies augmented by immunohistochemistry (IHC), the BSNS has been finally recognized and designated as a separate entity. So this study highlights the absolute need for IHC and cytogenetic studies in such ambiguous cases for early detection and timely therapeutic management.

REFERENCES:

1. Thompson LD, Franchi A. New tumor entities in the 4th edition of the World Health Organization classification of head and neck tumors: nasal cavity, paranasal sinuses and skull base. Virchows Archiv. 2018;472(3):315-30.
2. Lewis JT, Oliveira AM, Nascimento AG, Schembri-Wismayer D, Moore EA, Olsen KD, Garcia JG, Lonzo ML, Lewis JE. Low-grade sinonasal sarcoma with neural and myogenic features: a clinicopathologic analysis of 28 cases. The American Journal of Surgical Pathology. 2012;36(4):517-25.
3. Wong WJ, Lauria A, Hornick JL, Xiao S, Fletcher JA, Marino-Enriquez A. Alternate PAX 3-FOXO 1 oncogenic fusion in biphenotypic sinonasal sarcoma. Genes, Chromosomes and Cancer. 2016;55(1):25-9.
4. Alkhudher SM, Al Zamel H, Bhat IN. A rare case of nasal biphenotypic sino-nasal sarcoma in a young female. Annals of Medicine and Surgery. 2019;37:4-6.
5. Wang X, Bledsoe KL, Graham RP, Asmann YW, Viswanatha DS, Lewis JE, Lewis JT, Chou MM, Yaszemski MJ, Jen J, Westendorf JJ. Recurrent PAX3-MAML3 fusion in biphenotypic sinonasal sarcoma. Nature genetics. 2014;46(7):666.
6. Huang SC, Ghossein RA, Bishop JA, Zhang L, Chen TC, Huang HY, Antonescu CR. Novel PAX3-NCOA1 fusions in biphenotypic sinonasal sarcoma with focal rhabdomyoblastic differentiation. The American Journal of Surgical Pathology. 2016;40(1):51.
7. Fritchie KJ, Jin L, Wang X, Graham RP, Torbenson MS, Lewis JE, Rivera M, Garcia JJ, Schembri-Wismayer DJ, Westendorf JJ, Chou MM. Fusion gene profile of biphenotypic sinonasal sarcoma: an analysis of 44 cases. Histopathology. 2016;69(6):930-6.
8. Powers KA, Han LM, Chiu AG, Aly FZ. Low-grade sinonasal sarcoma with neural and myogenic features—diagnostic challenge and pathogenic insight. Oral surgery, oral medicine, oral pathology and oral radiology. 2015;119(5):e265-9.
9. Rooper LM, Huang SC, Antonescu CR, Westra WH, Bishop JA. Biphenotypic sinonasal sarcoma: an expanded immunoprofile including consistent nuclear β-catenin positivity and absence of SOX10 expression. Human pathology. 2016;55:44-50.
10. Carter CS, East EG, McHugh JB. Biphenotypic Sinonasal sarcoma: a review and update. Archives of pathology & laboratory medicine. 2018;142(10):1196-201.