Editorial

SHIFT OF TRADITIONAL CURRICULUM TO INTEGRATED CURRICULUM: A DRASTIC STEP BY UNIVERSITY OF HEALTH SCIENCES (UHS), LAHORE

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The University of Health Sciences (UHS) Academic Council presided over by UHS Vice Chancellor Professor Dr. Ahsan Waheed Rathore, took a historic initiative on 17th February 2023, approving an integrated discipline-aligned curriculum model, replacing the traditional discipline-based curriculum with the preclinical and clinical divide. The new modular and integrated curriculum came into effect in medical colleges across Punjab on 1st March 2023.

Many medical institutes in Pakistan have already implemented the integrated curriculum, i.e., Agha Khan University, Ripha International University and Azad Jammu Kashmir Medical College (AJKM), to name a few. AJKMC established in 2011-12, is the first public sector medical institution, affiliated with the University of Health Sciences (UHS), Punjab, which adopted an integrated undergraduate medical curriculum, right from its inception.[1]

The traditional medical curriculum is focused primarily on memorized knowledge with a barricade between basic and clinical sciences, so that; the learners fail to recall the basic science knowledge upon entering the clinical phase. This disparity between basic and clinical curricula has been dealt with in the inception of integrated curricula; by developing comprehension with simultaneous clinical practice.[2]

Such a curriculum corroborates substantial associations between the disciplines to enhance knowledge; additionally, it provides such prospects for all the stakeholders, thus augmenting their creativity. Another achievement is the introduction of PERLs focussing on Professionalism, Ethics, Research, and Leadership skills to be assessed through portfolios. The introduction of Portfolios in the undergraduate curriculum in undergraduate medical education is a leaping step toward work-based assessment in the initial years of curriculum alignment.

Unlike traditional curriculum, an integrated curriculum stimulates the students to actively participate in learning a topic after understanding its significance, which is centered on the fundamental value of the disease under study. This, in turn, contributes to long-lasting recall and a profound comprehension of the topic. An integrated curriculum is comparatively much more organized curricula, allowing for more effective knowledge gain, skills acquisition, and development of the affective domain. The introduction of the Spiral curriculum in which modules are revisited with increasing depth and width also makes it unique, focussing more on the depth of undergraduate medical students.

A key question is the application of an integrated curriculum with several teaching methodologies. The answer lies in uniting the faculty as one team, instead of distinct faculty groups such as preclinical or clinical faculty. Therefore, problem-based learning (PBL), case-based discussions (CBD), Flip

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classrooms, and the introduction of Self-directed learning (SDLs) are learner-centered approaches, that concern the faculty members irrespective of their subject expertise. Furthermore, numerous teaching methodologies are prerequisites of an integrated curriculum, such as; lectures, seminars, tutorials, problem-based learning, case-based learning, bedside teaching, and clerkships. The clerkship model has also been introduced in year five by the University of health sciences.

Literature showed that curricula based on modules mandate learner to showcase greater maturity to be able to comprehend the linkages between the subjects without core knowledge of the subjects.[3] One of the major concerns of subject experts is that they are forced to reduce the content of these subjects, resulting in medical graduates with an inadequate theoretical foundation. Though educators might establish a transdisciplinary curriculum with a global context, yet, to embrace the standards and grading of distinct subjects, they focus on individual disciplines. The challenge does not lie, necessarily, in the planning and formulation phases of the curriculum, but more accurately, in its implementation. It is an irrefutable fact that no educational policy can be effectively implemented without reflecting and dealing with the educators' challenges, before commencing the decision-making and planning process of the curriculum.[4]

The majority of the faculty members are of the view that there is a deficiency of faculty development programs that could refine their skills, allowing them to acclimatize to advanced teaching approaches. In a systematic review, it is suggested that several formal as well as informal faculty development strategies comprising workshops, seminars, experiential learning, timely feedback, and effective peer coaching should be included in faculty training programs. Additionally, time restraint is a key challenge confronted by the faculty in curriculum implementation. A Modular Integration Committee must command the modular integration, monitored by a module coordinator at the implementation level. The infrastructure provides the foundation for the implementation of the integrated curriculum.[5] The integrated curriculum requires the participation of learners in the curriculum implementation, review, and assessments for them to take control of their learning and give meaningful feedback to the faculty and the administration.

To meet international standards, the adoption of an integrated curriculum is a necessity for our medical education system, however, for successful implementation of this curriculum, a preliminary survey to identify the deficient infrastructure as well as training requirements of faculty, as well as a pilot study, is essential to allow for a smooth transition. On the contrary, lack of expertise, as well as resistance to the transition by the faculty as well as the students, can bring many challenges in the implementation phase. Assessment plans, table of specifications, study guides, and an effective Department of medical education with a role in evaluation and monitoring, should be part of this initiative to provide feedback as well as a future projection of the goal set to be achieved.

AUTHOR'S CONTRIBUTION

AA: Manuscript writing
IM: Conception of idea and critical review

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Original Article

BEHAVIORAL CHANGES LINKED WITH ELECTRONIC ASSESSMENT ON UNDERGRADUATE STUDENTS AT THE UNIVERSITY COLLEGE OF MEDICINE AND DENTISTRY

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ABSTRACT

Background: Global lockdown occurred after the emergence of the COVID-19 health crisis. Courses and exams that used to be taken on campus are now being taken entirely online. The purpose of this research was to investigate how students feel about taking exams online from afar.

Material and Method: It was a descriptive cross-sectional study. To collect data from undergraduate medical and dental students, a pre-validated survey was distributed to the student body. It included questions about students' demographics as well as their experiences with stress and adjustments in coping strategies in the context of online exams. SPSS version 23 was used to analyze the data.

Results: Students' intake of high-energy drinks (57%) and soda drinks (60%) increased more in remote e-assessment compared to on-campus assessment, and the majority of students report that their caffeine consumption increased in remote e-exam compared to on-campus assessment. During the remote e-assessment, students reported a rise in their consumption of fast food and a decrease in their consumption of healthy foods. Half of the students surveyed said that they slept less and did less exercise when taking exams online. Seventy percent of students said they spent less time with their families because of exams taken at home. An increase in the use of analgesics (51%) and anti-stress medications (41%) was also observed during remote e-examination.

Conclusion: The results of the study showed that students rated taking exams remotely as more stressful than taking the same exams on campus. Students' eating habits, sleep schedules, and exercise routines suffered as a result of the distant e-exams.

Key Words: COVID-19, Smoking, Social media, Analgesics

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INTRODUCTION

Coronaviruses can result in an array of acute respiratory, hepatic, and neurological diseases ranging in severity.[1]

COVID-19 resulted in a health emergency across the world and resulted in a lockdown at the global level. Health services in the most affected countries were overwhelmed due to a massive increase in COVID-19 cases. To flatten the COVID-19 epidemiological curve most governments across the globe initiated a series of non-pharmaceutical interventions (NPI).[2] This global spread of the virus resulted in affecting the education sector badly. The institutions had to shift to remote education where possible. Few countries were

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able to shift to remote online education by using the latest technological tools. Data suggests that 99% of the formally enrolled students have been affected because of this shift and at the same time they are a part of this global experiment of online education in which various modalities have been deployed to continue their education.[3]

According to a survey conducted by the International Association of Universities across the world, more than 90% of educational institutes have shifted to remote teaching to continue educational activities during a pandemic.[4]

The most important challenge in this online transition was to organize assessments so that the students can progress through their studies.[5]

E- assessments were introduced to provide immediate feedback, which is a constant challenge for the teaching faculty.[6] E-assessments are considered to be an important component in distance education.[7] COVID-19 led to the development and adaptation of online technologies thus opening promising opportunities for e-assessments.[5] But even with exceptional information technology setup the chance of technical glitches during remote assessments is still a threat.[8] Initially, the e-assessments adopted by educational institutes were conducted in-campus[9] in which the students experienced similar exam settings, IT support as well as exam invigilation as in on-campus paper-based assessments.[5] But, remote E-exams mean that the student will appear in an E-assessment at home which comes with a lot of other challenges. More or less these challenges are similar to the routine E-exam but might be more challenging in remote settings.[5]

Technical hitches that are a threat to the exam validity and possible dishonest behavior among the students are possible challenges.[10] Moreover, it is difficult to assess practical knowledge and skills through remote exams, which in turn impacts achieving the purpose of learning.[11] UCMD adapted remote online e-exams as a mode of assessment to assess students' academic performance during online education during the COVID-19 pandemic. This study was conducted to explore the experiences of students during remote online exams. The study explores stress and associated factors along with its impact on students' dietary habits and lifestyles. The results of this study will help in reducing the stress-causing factors associated with remote online exams. The objective was to determine stress-related factors in remote and on-campus e-exams and to identify the behavioral changes associated with remote e-exams.

MATERIAL AND METHODS

The study was conducted in the University College of Medicine & Dentistry- The University of Lahore. The ethical review board of UCMD approved the study. A pre-validated questionnaire was distributed among the students. It was a descriptive cross-sectional study and a total of 950 students participated in the study. Only students of the University College of Medicine & Dentistry were included in the study. The data was collected using Google forms. The survey included 29 questions that aimed to assess the students' remote e-exam perceptions.

Exam-related stress factors included E-exam interface issues and connectivity issues) structure of the exam paper and preparedness. Teaching methods or courses not being covered properly and personal factors. The questions that explored the behavioral changes were dietary routine, sleeping pattern, smoking, and use of medications. The data was analyzed using SPSS version 21. Demographics were described using descriptive statistics. The association between remote e-exam stress and stress-related factors and behavioral changes was determined using Chi-square.

RESULTS

A total of 950 students participated in the study 73% were medical and 27% were dental students (Table-1). In the current study, 91% of students reported exam-related stress while only 7.47% expressed that they don't feel stress during examinations. Out of 91% almost one-third found remote E-Exam stressful, 19% of the data suggests in campus exams are more stressful whereas, 42% of students were of the view that both systems of exams are stressful (Figure-1).

Table-1: Discipline of participants

Discipline	Frequency(%)
MBBs	698(73.5%)
BDS	252(26.5%)

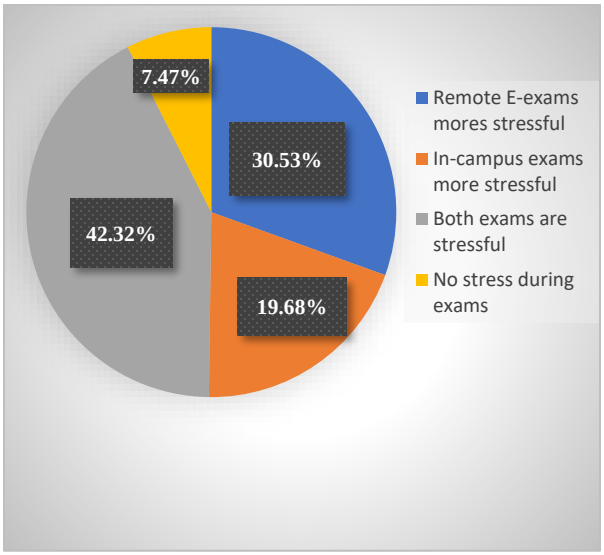

Figure-1: Students' experience of self-reported exam-related stress.

The study explored eleven different factors that may be responsible for stress during exams. There was a significant association between students' experience of stress during exams and all the studied factors.

78% (remote assessment) and 30% (in campus assessments) of the students reported Internet connection problems as major causative agents of stress.

Other causative stress factors in remote E-assessments were the exam question difficulty, freedom to navigate among the questions, teaching methods, form of exam, exam duration, and use of unfair means by other students. 59.2% (in-campus) and 41% (remote exams) students reported the exam question difficulty as one of the major factors. 70.6% (remote exams) and 29.4 % (in-campus) students considered the option to navigate between the exam questions as a factor for stress. Individual factors including students' grades were reported as the second and third most common factor that contributes to both remote & in-campus E-exams. Fellow students' dishonesty (61.3%) and exam setting (54.1%) were among the significant factors contributing to stress in remote E-exams.

Students reported a negative effect on their dietary patterns in remote assessments. 70% of the students reported that their caffeine intake rises in the remote e-exam in comparison with the campus assessment, Students' reported an increase in consumption of energy and soda drinks at 57% and 60% respectively in the remote e-assessment as compared to on-campus assessment. Students stated that their healthy food intake decreased by 43%. in the remote e-assessment and they increased fast food intake (65%) and high sugar content food (59.8%) during the remote-e exam. Half students labeled that their sleep duration was decreased during remote e-exams and they also conveyed that during remote- exams their physical activities were decreased (52.9%). 70% of students highlighted that the students spent lesser time with their family during remote- exams. The consumption of analgesics and anti-stress tablets was 51% & 41% and 57.1% of students thought that insomnia was also augmented during remote e-exam.

Table-2: Bivariate analysis of exam related stress

		Remote E-exams are more stressful	In-campus exams are more stressful	Both exams are stressful	No stress during exams	p-value
The technical problem associated with the e-assessment platform.	48.82%					<0.001
	Yes	67.3%	50.6%	59.6%	17.8%	
	No	32.7%	49.4%	40.4%	82.2%	
Internet connection problems associated with	44.92%					0.002
	Yes	78.6%	30.7%	47.3%	23.1%	
	No	21.4%	69.3%	52.7%	76.9%	
Exam duration (Time limit) associated with	47.65%					<0.001
	Yes	64.7%	52.5%	51.6%	21.8%	
	No	35.3%	47.5%	48.4%	78.2%	
Questions difficulty in	54.1%					0.005
	Yes	71.3%	59.2%	63.5%	22.4%	
	No	28.7%	40.8%	36.5%	77.6%	
Not studying the whole exam material in	48.82%					0.007
	Yes	60.9%	52.1%	58.4%	23.9%	
	No	39.1%	47.9%	47.6%	76.1%	
Mode of question navigation (open or close) in	50.5%					
	Yes	70.6%	53.2%	55.1%	23.1%	0.00
	No	29.4%	46.8%	44.9%	76.9%	
Whether the exam is more than one form (Includes MCQs & SEQs)	49.97%					0.0004
	Yes	63.4%	51.6%	59.6%	25.3%	
	No	36.6%	48.4%	40.4%	74.7%	
Teaching methods have not properly covered the course material	47.62%					<0.001
	Yes	67.2%	46.3%	55.3%	21.7%	
	No	32.8%	53.7%	44.7%	78.3%	
The grade is not what the student expects in	51.42%					0.001
	Yes	73.2%	52.8%	57.7%	22.0%	
	No	26.8%	47.1%	42.3%	78.0%	
use of unfair means by other students (cheating) in	41.4%					0.0008
	Yes	61.3%	35.7%	47.2%	21.4%	
	No	38.7%	64.3%	52.8%	78.6%	
Exam environment at home in	45.65%					0.0003
	Yes	54.1%	51.7%	52.0%	24.8%	
	No	45.9%	48.3%	48%	75.2%	

Table-3: Bivariate analysis of behavioral changes associated with e-exams

	Remote E-exams are more stressful	In-campus exams are more stressful	Both exams are stressful	No stress during exams	P value
Caffeine Consumption					<0.001
Increased	44.1%	46.1%	47.2%	25.4%	
Decreased	8.2%	9.9%	8.6%	25.4%	
No Change	22.6%	25.3%	24.0%	30.3%	
Not applicable	25.1%	18.7%	20%	35.9%	
High Energy Drinks					0.002
Increased	37.7%	35.1%	35.8%	20.7%	
Decreased	10.9%	14.4%	9.4%	11.1%	
No Change	23.4%	23.5%	24.9%	30.0%	
Not applicable	28.0%	27.1%	29.9%	38.1%	
Soda Drinks					0.004
Increased	32.2%	29.8%	26.8%	15.5%	
Decreased	15.7%	18.7%	15.2%	17.8%	
No Change	28.6%	28.0%	31.7%	31.1%	
Not applicable	23.5%	23.4%	26.3%	35.7%	
Eating healthy food					0.00
Increased	41.5%	23.4%	22.8%	20.5%	
Decreased	29.7%	46.1%	32.7%	22.7%	
No Change	22.9%	24.8%	31.7%	33.5%	
Not applicable	3.9%	5.7%	12.7%	23.3%	
Eating fast food					0.00
Increased	40.3%	50.3%	38.4%	23.8%	
Decreased	25.4%	18.6%	14.5%	18.3%	
No Change	24.3%	22.8%	30.6%	31.1%	
Not applicable	10%	8.2%	16.4%	26.8%	
Eating high sugar food					0.001
Increased	49.8%	47.3%	43.5%	26.6%	
Decreased	14.4%	18.6%	10.3%	14.4%	
No Change	24.6%	24.4%	30.4%	31.5%	
Not applicable	11.1%	9.7%	15.8%	27.5%	
Sleeping Hours					0.0006
Increased	33.9%	16.6%	13.4%	16.8%	
Decreased	48.4%	66.6%	59.5%	35.8%	
No Change	13.1%	12.2%	16.1%	18.8%	
Not applicable	4.6%	4.5%	11.1%	28.5%	
Exercise/sports					0.0009
Increased	24.0%	18.6%	10.2%	10.4%	
Decreased	52.9%	55.7%	51.5%	36.2%	
No Change	13.3%	14.7%	22.0%	24.3%	
Not applicable	9.8%	10.9%	16.7%	29.1%	

Smoking habits					0.0012
Increased	17.9%	13.6%	16.0%	11.9%	
Decreased	9.1%	11.8%	6.3%	8.2%	
No Change	8.1%	9.5%	11.9%	10.4%	
Not applicable	64.9%	65.2%	65.8%	69.5%	
Communications using social media					0.0004
Increased	40.9%	20.4%	22.5%	14.3%	
Decreased	32.4%	51.4%	37.7%	32.3%	
No Change	12.7%	16.0%	21.3%	20.9%	
Not applicable	13.9%	12.2%	18.5%	32.4%	
Time spent by this family					0.00
Increased	31.7%	10.8%	8.8%	8.9%	
Decreased	50%	69.3%	60.9%	42.9%	
No Change	9.9%	12.0%	16.5%	18.3%	
Not applicable	8.4%	7.9%	13.7%	29.8%	
Analgesics use					0.0005
Increased	31,6%	29.5%	26.6%	20.1%	
Decreased	14.0%	15.4%	13.1%	14.1%	
No Change	15.6%	15.3%	18.4%	16.4%	
Not applicable	38.8%	39.9%	41.9%	49.4%	
Have used medications to relieve stress					0.003
Increased	33.8%	32.6%	28.4%	19.4%	
Decreased	10.3%	10.1%	11.3%	12.7%	
No Change	13.7%	14.8%	16.5%	17.4%	
Not applicable	42.2%	42.4%	43.8%	50.5%	
Have used medications to relieve insomnia					0.0002
Increased	27.1%	23.5%	20.2%	14.9%	
Decreased	12.1%	12.9%	14.0%	12.5%	
No Change	11.7%	11.8%	12.7%	12.8%	
Not applicable	49.2%	51.8%	53.1%	59.7%	

DISCUSSION

Assessment is an essential element in education to evaluate the attainment of desired learning outcomes. Valid and reliable assessment is the backbone of a successful academic program.[12] E- Assessment has been adopted by many organizations across the globe. E-assessment has occupied a vital part in this digital revolution of the education system.[13] With the widespread of COVID-19, educationists have been required to adopt drastic actions to adapt the digital delivery of courses to aid students' teaching and learning.[14] This study intended to explore the association of stress with E-exam (remote and campus) and associated stress during remote & campus e-exams.

Almost one-third of the data reported remote E-Exam more stressful and 19% of the students considered campus exams to be more stressful. Previous research work has also highlighted stress related to e-Exams[15] especially Remote E-Exam when compared with campus E-exam[16] On exploration students' perceptions revealed internet connection problems as a major factor related to Remote E-Exam students' when compared to campus Exams.[17] It may be due to lack of good internet services across the country.[18]

Question difficulty was stated as the chief reason for exam-related stress in the majority (59.2%) of the students (in campus exam) as compared to 71.3% (remote E.exams).[17] The navigation mode was also described as one of the stress factors in 70.6% of students in remote E-exams as linked with 53.2% of students in campus exams. According to another study, Contrary to this students' performance is not affected by test time and elimination of navigation.[19] It has been reported in research that effective teaching, of course, can reduce stress in students.[20] Individual factors including individual grades were described as second and third most vital factor causing stress in remote E-and in-campus assessment respectively. Fellow students' dishonesty (61.3%) and exam setting (54.1%) have been reported as the significant factors increasing stress during remote E-exams. Examination cheating poses threats to the integrity and quality of higher education.[21] According to the study, the influence on dietary behaviors was negative. Students reported that their consumption of high-energy drinks (57%) and soda drinks (60%) increases in remote e-assessment, while students conveyed that their caffeine intake increased in remote e-exam in comparison to on-campus assessment (70 percent of the students conveyed this), and students reported that their consumption of remote e-exam. According to the findings of another piece of research, the most popular explanation for why people consume caffeine is "studying for exams."[22] In the context of a remote electronic assessment, the students thought that their preference for healthy food intake (43%) diminishes and that they prefer foods with a high sugar content (59.8%), namely fast food (65%). Another study has shed light on how variations in food behavior can be attributed to academic pressure.[23] The majority of the students said that the length of their sleep and the amount of time they spent being physically active reduced when they were taking remote e-exams. Changes in sleep habits may result from participation in remote learning.[24] The work-family balance was disrupted by remote online learning, and students spent 70 percent less time with their families during remote exams than they did during on-campus examinations. During the remote E.exam, participants reported the ingestion of analgesics (51%), as well as the intake of stress reduction (41%). The use of self-medication among medical students has been highlighted in a previous study and the present study highlights a rise in the use of self-medication during remote E.exams.[25]

CONCLUSION

According to the findings of the study, pupils were subjected to greater levels of anxiety and their conduct was negatively impacted as a result. This stress and behavioral alterations can be reduced by organizing earlier simulated training of remote e-assessment to offer an atmosphere that is free of tension associated with remote e-exams. A program designed to promote students' health and well-being should be made available in educational institutions. Such a program would help students better cope with the psychological and behavioral effects of stress.

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TA: Conceived the idea, introduction, methodology, and final review
MN: Data collection and results analysis
SM: Final review, discussion, writing and formatting
STF: Data analysis and discussion writing

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Original Article

EFFECT OF HYPERLIPIDEMIA ON FERTILITY PARAMETERS IN MALE ALBINO RATS

Maimona Tabbsum[1], Chaman Nasrullah[2], Maria Mufti[3], Babar Yasin[4], Saima Zareen[5], Fareha Saleem[6]

ABSTRACT

Background: Both industrialized and developing nations are experiencing an increase in lipid metabolic problems brought on by poor eating practices, which have an adverse effect on sperm quality and quantity. The oxidative stress, one of the most significant factors affecting the male gametes and linked to infertility, is a general characteristic of hyperlipidemia. This study aimed to determine the effects of a high fat diet on serum testosterone levels, sperm count and sperm motility in male albino rats.

Material and Methods: In this randomized control trial, 60 male albino rats were classified into two groups (groups A and B). Group A was a control group, and hyperlipidemia was induced in group B rats by adding 1% cholesterol and 15% palm oil to standard chow for four weeks. After four weeks, blood was taken through cardiac puncture for lipid profile and serum testosterone levels measurement. Semen was collected from cauda epididymis for sperm count and sperm motility. Data were analyzed by using SPSS version 20.

Results: In hyperlipidemic rats, serum testosterone, sperm count and sperm motility were significantly decreased with p-value= 0.03, 0.001, 0.028 respectively, compared to group A rats.

Conclusion: Decrease in testosterone, sperm count and motility after hyperlipidemia indicates its negative effects on male fertility

Key Words: Hyperlipidemia, High fat diet, Sperm count, Sperm motility

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INTRODUCTION

Infertility is one of the major health issues impacting 10-15% of couples of reproductive age.[1] Where a powerful characteristic is the reduction in semen quality. The "enemy of male fertility" has been described as obesity.[2] Nutrition can affect the sperm quality, positively or negatively, and this effect is influenced by dietary factors that are both qualitative and quantitative, such as the number of calories in each macronutrient and the specific carbohydrate, fatty acid and protein profiles.[3]

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Saturated fatty acid intake in excess and unhealthy high calorie diets hurt sperm quality, adversely influencing fertilisation. However , a balanced diet is linked to greater sperm quality, indicating that nutritional treatments may be crucial for maintaining male fertility.[4] Concentration, morphology, and motility are the three criteria used in a semen analysis, which is specifically used to evaluate and classify male infertility. These three characteristics have limits as indicators of sperm fertility.[5] Additionally, mounting evidence shows that changes in spermatogenesis, sperm function and endocrine profiles caused by lifestyle variables can impair male fertility.[6] Fast food deleteriously affects blood cholesterol, testosterone, sperm count, and quality. Lipid metabolic problems caused by unhealthy

eating habits are seen to be increased in both industrialised and developing countries. The quality of sperm and consequently, the fertilization process are negatively impacted by hypercaloric unhealthy diets and excessive consumption of trans and saturated fatty acids.[7] Dyslipidemia, or an imbalance in dietary lipids, has significantly increased the prevalence of obesity and overweight worldwide. The emergence of systemic oxidative stress, which is a known detrimental factor for the viability of male gametes and is related to infertility, is the main characteristic of dyslipidemia.[8] Male obesity causes the reproductive hormonal profile and semen production to diminish. Males who consume the HFD have more gonadal fat, which is thought to raise epididymal and testicular temperature and influence sperm count, maturation and storage. The enzyme aromatase, which transforms testosterone into oestrogen, is activated more frequently in excess fatty tissue. Oestrogen, through negative feedback, inhibits the release of GnRH, eventually decreasing FSH and LH levels. LH is required by Leydig cells for testosterone synthesis, while FSH has a crucial role in spermatogenesis. Also testosterone is the main androgen involved in the process of spermatogenesis in the testis , the resulting drop in testosterone levels leads to decreased sperm production. Low testosterone levels also appear to be associated with mitochondrial malfunction in Leydig cells and oxidative stress.[9,10] (Figure 1).

By disrupting gamete energy metabolism in animal models, high-fat diet intervention reduces the quality of sperm. Because HFD results in the production of high levels of reactive oxygen species which can destroy nearly all macromolecules, including DNA, protein, lipid, and carbohydrates, ROS have been linked to reduced testosterone levels, sperm motility and sperm count.[11]

Because of the high surge of dyslipidemia in young people in developing and underdeveloped nations, the harmful consequences of fat on reproductive processes should be considered.[12]

To determine the effect of high fat diet on serum testosterone levels, sperm count and sperm motility in male albino rats. Early start of metabolic disease is encouraged by adopting unhealthful eating habits, which may have unknown effects on reproductive function in later life.[13]

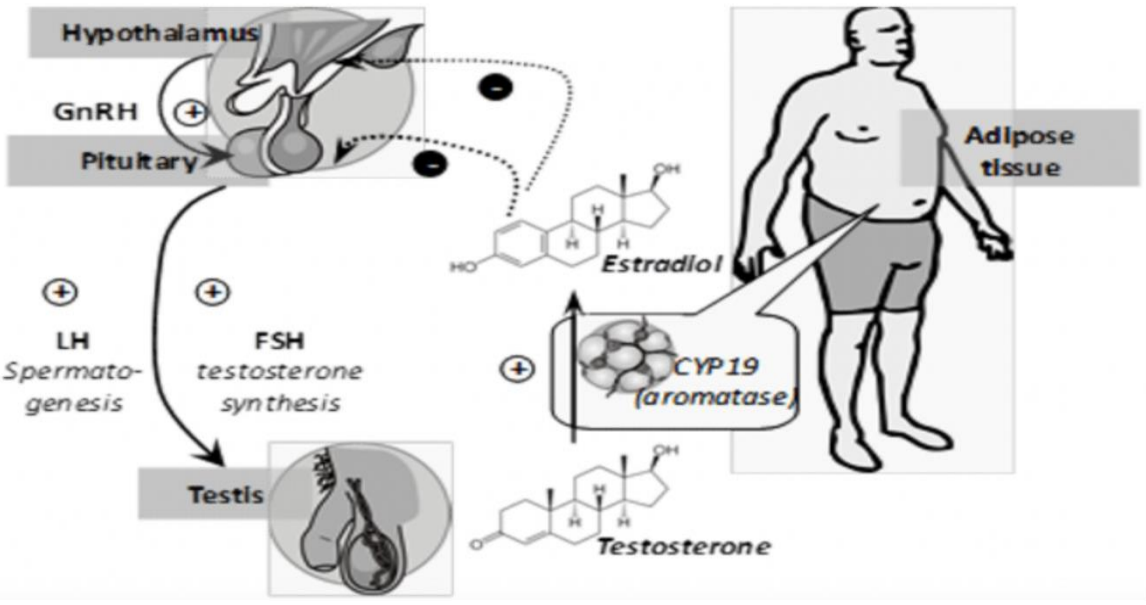

Figure-1: Increased adipose tissue is linked with decrease in testosterone levels and sperm count[9]

MATERIAL AND METHODS

The study was a randomised control trial. 60 male albino rats were divided into two groups, group A, control group (n=30) and group B, experimental group (n=30)

Animals were kept in the animal house of Akhtar Saeed Medical and Dental College Lahore. Animals were placed in cages, 10 rats per cage, for a minimum of one week for acclimatisation before the start of experiment. Animal house temperature was thermostatically maintained at 26±2˚C and a light/dark cycle.

Hyperlipidemia was induced in group B by addition of high cholesterol diet for four weeks[14]

Table-1: Composition of diet per 100g diet[14]

Composition	Normal diet	HFD
Yellow corn	60%	50.5%
Soybean	21%	17.3%
Fibres	11%	9.4%
Corn gluten	2%	1.4%
Nacl	0.5%	0.5%
Cholesterol	0%	1%
Palm oil	0%	15%

Blood samples were taken after four weeks from both groups through cardiac puncture to estimate lipid profile through calorimetric method and serum testosterone through ELISA. Semen was collected from cauda epididymis for sperm count and sperm motility.[15]

Data was analysed by using SPSS version 20. The mean and standard deviation of the variables were calculated. Significance of difference was determined by applying students t-test

RESULTS

The present study was performed to determine the effect of a high fat diet on serum testosterone, sperm count and motility.

Effect of high fat diet on lipid profile:

The difference of serum lipid profile parameters between group A (control) and group B (hyperlipidemic) was highly significant (p = 0.01) Table 2.

Table-2: Comparison of lipid profile

Parameters	Group A	Group B	p-value
Serum cholesterol mg/dl	210±8.48	293±21.9	0.001
Serum LDL mg/dl	125±3.86	169±6.95	0.001
Serum triglycerides mg/dl	195±7.73	216±16.9	0.001

Values are presented as mean ±SD

*p≤0.001, highly significant

Effect of high fat diet on serum testosterone, sperm count and sperm motility. After high fat diet supplementation, serum testosterone levels, sperm count and motility decreased significantly with p-value= 0.03, 0.001, 0.028 respectively, in group B compared to group A. Table 3.

There was a significant (p = 0.030) decrease in serum testosterone levels (3.22 ± 0.802) in group B as compared to group A (3.91±1.03).

Sperm count (79.24 ± 2.16) had highly significant (p=0.001) decrease in experimental group B in comparison to control group A (83.80±2.83)

Sperm motility (71 ± 4.31)showed significant decline (p=0.028) in group B as compared to group A (74±4.28)

Table-3: Comparison of serum testosterone, sperm count and sperm motility in Group A and Group B (mean ±SD)

Parameters	Group A	Group B	p- value
Serum testosterone (ng/ml)	3.91±1.03	3.22 ± 0.802	0.030**
Sperm count (Total No.X 10^6/ml)	83.80±2.83	79.24 ± 2.16	0.001*
Sperm motility (٪)	74±4.28	71 ± 4.31	0.028**

DISCUSSION

In the current study, we looked into how a high fat diet affected male rats' reproductive indices. Rats on the hyperlipidemic diet exhibited significantly higher levels of triglycerides, LDL, and cholesterol. Our key findings are that testosterone levels, sperm motility and sperm count significantly decreased in high fat diet fed rats.[16] According to one cross-sectional study conducted on 209 healthy males, eating more omega-6 and trans fatty acids, but less omega-3, is linked to worsening testicular functions, including lower levels of total and free testosterone and smaller testicles.[17] In 2019, You C et al, conducted a study in which there were two groups with 30 rats in each group, and 1% cholesterol diet was given to one of the group as exactly we did but in that study serum as well as intratesticular testosterone levels were measured at 4th, 8th and 12th weeks, the serum testosterone levels and intratesticular levels showed a decreasing pattern in rats who were treated with high cholesterol diet. So, their findings are consistent with those in our study.[18] One factor that may impact the outcomes is the composition and features of the high fat diet used in each trial. Males who consume the high fat diet have more gonadal fat, which is thought to impact sperm generation, maturation, and storage by raising testicular and epididymal temperature.[19]

An acknowledged risk factor for male infertility or subfertility is obesity. Male obesity affects endocrine hormone levels, including serum LH, testosterone, leptin, estradiol and FSH levels, as sperm function, specifically sperm motility, count, and morphology, according to several studies in humans and rats.[20] At the same time, testicular Leydig cells may begin to accumulate cholesterol due to dietary cholesterol. Although cholesterol is the main building block for testosterone production, having too much of it can be harmful because it triggers the endoplasmic reticulum stress response, downregulating the steroidogenic enzymes and reducing the in the amount of testosterone produced.[18] Ding N et al. had. Conducted a study to establish how a high fat diet could affect spermatogenesis, they found that one of the main reasons for the reduced sperm motility and production due to high fat diet is the development of abnormal gut microbiome. Epididymal inflammation, raised blood endotoxin and dysregulated testicular gene expression are likely the mediators of this phenotype.[21] Male reproductive potential may be significantly modified by diet. Therefore, it is important to emphasise daily nutritional exposure's function in maintaining or preventing male infertility. A strong positive correlation exists between sperm quality markers and a diet primarily plant-based and high in fish.[22] There are some limitations of this study. Serum FSH and LH levels and Malondialdehyde (MDA) levels should be measured. The effect of a hyperlipidemic diet on sperm morphology should also be analyzed.

CONCLUSION

High fat diet causes decline in serum testosterone, sperm count and motility.

Financial disclosure: None
Conflict of interest: None

AUTHOR'S CONTRIBUTION

MT: Conception of idea, data collection and literature review
CN: Data analysis and critical review
MM: Proof reading and critical review
BY: Proof reading and critical review
SZ: Manuscript writing
FS: Critical review

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Original Article

MINI HYDROCELECTOMY – A BETTER ALTERNATIVE IN THE MANAGEMENT OF MODERATE TO SEVERELY ENLARGED IDIOPATHIC HYDROCELE

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ABSTRACT

Background: Hydrocele is the most common benign scrotal swelling. Although different treatment options are tried two surgical techniques got popularity, Lords Plication and Jabuleys repair. To establish the role of Mini Hydrocelectomy in the management of moderate to severely enlarge idiopathic hydrocele.

Material and Methods: This study was conducted at Social Security Teaching Hospital, Lahore. All patients suffering from moderate to severe hydrocele, who needs surgical exploration were included in the study. These patients were followed for six months postoperatively. Five different variables, pain, haematoma, infection, the persistence of swelling and reoccurrence were measured in these patients postoperatively and in the follow-up period.

Results: A total of 132 male patients who were suffering from moderate to severe hydrocele were included in this study. Among these 47 patients had left-sided, 67 had right-sided while 18 patients had a bilateral hydrocele, 82 had moderately enlarged hydrocele and 50 had severely enlarged. Regarding pain 20 patients (15.27%) developed mild pain,107 (81.68%) moderate pain and 5 (3.82%) patients developed severe pain. Haematoma was developed in 5 (3.82%) patients. Only 3 patients (2.29%) suffered from wound infection. There was persistent scrotal swelling in 6 patients (4.58%). No patient was reported with reoccurrence.

Conclusion: In this study we tried to conclude that due to minimal manipulation and local trauma to the tissue, the overall complication rate is much less with more patient satisfaction.

Key Words: Pain, Wound infection, Antibiotic

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INTRODUCTION

The most common benign disease of the scrotum is hydrocele.[1,2] It is a collection of fluid between the two layers, parietal and visceral, of tunica vaginalis. The pathogenesis of hydrocele is described as it is due to an imbalance between the secretion of fluid and then its reabsorption.[3-5] On an etiological basis the hydrocele is classified as congenital and acquired.[1] The common causes of acquired hydrocele are infection in the intrascrotal region, some systemic or regional diseases, neoplasm and scrotal or inguinal injuries. However, the most

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common cause is idiopathy.[6] To make a diagnosis, a clinical examination and scrotal ultrasound are considered to be the first options.[7]

In adult males its incidence is about 1%, especially in those above 40 years of age.[8] A recent study, which was carried out in Sweden, states that the incidence of men with hydrocele who require medical assistance is 60 per 100,000 patients per year. Among these approximately 17 per 100,000 patients require an active management plan.[9] Although different surgical techniques are tried, for its management, such as aspiration of fluid or injecting sclerosing agents in the scrotal sac but two conventional surgical techniques are most common in the management of idiopathic hydrocele. One is the Jabuleys technique for aversion of the sac and the other is Lord's placation of tunica vaginalis. Due to durable success and low recurrence rate, both invasive procedures are acceptable for surgeons as well as patients.[10-12] However, like all invasive procedures, these procedures are also associated with a few post-operative complications. All these complications increase morbidity, reduce work hours and increase expenses.[13]

To reduce the morbidity of these procedures we selected this minimal access hydrocelectomy through a small scrotal skin incision in moderate to severely enlarged hydroceles. This fenestration of the sac causes direct contact with subcutaneous tissue which is rich in lymphatics.

MATERIAL AND METHODS

This study was conducted at Social Security Teaching Hospital, Lahore. All patients suffering from moderate to severe hydrocele, who needs surgical exploration, and reporting to the urology outdoor department between 1st January 2016 to 31st December 2021 were included in the study. The patients were above 40 years of age and diagnosed with idiopathic hydrocele. These patients were followed for six months postoperatively. The patients with reoccurrence, previous scrotal surgeries or neoplasm were excluded from the study. The patients with a fluid volume from 50 to 100 ml, measured on scrotal ultrasound, were labelled as moderately enlarged and more than this were as severely enlarged. Five different variables were measured in these patients postoperatively and in follow up period. These variables were pain, haematoma, infection, the persistence of swelling and reoccurrence. The pain was measured on a scale of 1 to 5 according to severity. Haematoma was assessed on examination and ultrasound while the infection was assessed clinically by fever, redness or pussy discharge from the wound. Persistent swelling and reoccurrence were assessed on ultrasound.

RESULTS

A total of 132 male patients who were above 40 years of age and suffering from moderate to severe hydrocele were included in this study. The age of patients was between 40 to 53 years with a mean age of 46.6 $\pm$ 3.9 years. Among these 47 patients had left sided, 67 had right sided while 18 patients had a bilateral hydrocele, 82 had moderately enlarged hydrocele and 50 had severely enlarged. Regarding pain none of the patients suffered from very mild or very severe pain, 20 patients (15.27%) developed mild pain,107 (81.68%) moderate pain and 5 (3.82%) patients developed severe pain. The pain subsided within a week and none of the patients complained later on. Haematoma was developed in 5 (3.82%) patients which were mild and subsided in a month. Only 3 patients (2.29%) suffered from wound infection and required antibiotic coverage for more than one week. There was persistent scrotal swelling in 6 patients (4.58%) which subsided gradually within six months. No patient was reported with reoccurrence, as confirmed on scrotal ultrasound, on six months of follow up.

Table-1: Post-operative complications and their frequencies.

	Mild	Moderate	Severe
Pain	20 (15.27%)	107 (81.865%)	05 (3.82 %)
Haematoma	05 (3.82%)		
Wound Infection	03 (2.29%)		
Persistence Swelling	06 (4.58%)		

Figure-1: Incision on a moderately enlarged hydrocele.

Figure-2: Wound showing sac

Figure-3: Excision of the sac.

Figure-4: Wound closure

DISCUSSION

Worldwide, the two most popular surgical techniques are widely accepted. These are the Lord's Plication and Jabuley's eversion of the sac.[10-12] People had tried other minimally invasive techniques but due to increased morbidity, these are abandoned. These minimally invasive procedures were less expensive but have an increased reoccurrence rate and poor satisfaction of the patients as compared to conventional hydrocelectomy procedures.[14] The objective of mini hydrocelectomy was to decrease the postoperative complications without compromising the efficacy of the procedure and the safety of the patient. Quick recovery and earlier return to work cause less financial cost also.[15]

Different people had tried different methods of minimal hydrocelectomy. People had tried traditional Lord's placation and Jabuleys inversion of sac through a small incision or a pull through technique in which a large sac is pulled through a small incision.[16,17] All these techniques are used through a small incision in which the sac of hydrocele was excised almost completely either by inversion or placation of the sac. Again these procedures have more morbidity, especially haematoma formation, as compared to mini hydrocelectomy.[17] In mini hydrocelecttomy we fenestrated the sac and put it in contact with the subcutaneous tissue of the scrotum, which is rich in lymphatics.

In the era of minimal access surgery, mini hydrocelectomy is a novel surgical

procedure for the management of hydrocele.[18] In it, through a 2-3 cm incision a small portion of the parietal layer of tunica vaginalis is removed. In our study the average time of surgery was 11 minutes. Almost all patients were satisfied with this surgical technique in their follow up period. Scrotal oedema was the most common complication (4.58%) which was settled within six months. As compared to Jabuleys inversion of sac in which the haematoma formation rate is 6.6%[19] our haematoma formation rate was 3.82% which was mild and settled within one week. A recent study mentioned that the overall complication rate is significantly less in mini hydrocelectomy.[20] In traditional surgeries of hydrocele there is a 4% chance of epididymal injuries while in mini hydrocelectomy it is almost nil because in this technique we do not, even, touch the epididymis. In traditional surgeries, there is more dissection of the sac resulting in oedema of scrotal skin and haematoma formation but in mini hydrocelectomy, these complications are also less. Our study also showed similar results as in studies conducted in different parts of the world. It also showed minimum post operative morbidity.

CONCLUSION

In this study, we tried to conclude that due to minimal manipulation and local trauma to the tissue, the overall complication rate is much less with more patient satisfaction. Moreover, early recovery leads to early joining at workplace, thus putting less financial burden.

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Conflict of interest: None

AUTHOR'S CONTRIBUTION

SH: Concept, design and writing
MAK: Manuscript writing
AH: Manuscript writing
MI: Manuscript writing
AAS: Critical review
NAG: Critical review

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Original Article

PREVALENCE OF PERCEIVED STRESS IN MEDICAL AND DENTAL STUDENTS DURING THEIR PROFESSIONAL STUDIES

Mamoona Shoukat[1], Nadia Ahmad[2], Ammara Ghafoor[3], Fariha Ahmad[4], Shamaila Ejaz[5]

ABSTRACT

Background: Stress is a common condition among students of all professional studies which not only adversely affects their mental and physical health but also affects their performance during studies. The current study is performed to assess the impact of daily routine stress on their education.

Material and Methods: 300 medical and dental students from first to final year between the age group 18- 25 years were selected and interviewed through an online perceived stress scale (PSS) questionnaire.

Results: In our study, 8.6% (26 out of 300) of medical and dental students have low-stress levels, 61% (185 out of 300) have moderate stress levels and 29% (89 out of 300) showed high perceived stress levels.

Conclusion: Medical and Dental students underwent moderate to high stress during their professional studies. Therefore, specific modifications are required in the professional curriculum so that a strong and healthy environment is provided to the students to reduce their stress levels. Multiple awareness and counseling sessions should be held throughout their course work that would help the students to cope with various challenges.

Key Words: Medical education, Curriculum, Awareness, Counseling

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INTRODUCTION

Many psychologists define stress as different challenges an individual faces which exceed his/her ability to cope with it.[1] Stress is considered a two-edged sword that can increase the performance of students to the peak or decrease to a very low.[2] Academic stress has been considered a major cause of many psychological problems in students.[3] According to the national crime record bureau of India, 1.8% of students committed suicide due to failing examinations.

In 2012 Lancet reported the highest rate of suicide among students between the ages of 15-29 years in India.[3] This number is increasing day by day. Although a major contributor to stress among students is studies and examinations but parental pressure, and peer or teacher pressure may also be a factor. Different parameters detect academic stress which includes depression, disturbed sleep, and anxiety which turns into frustration, conflict, and burden.[4]

A study conducted in Hong Kong showed that about 27% of students suffered from stress and 41% of students suffered from anxiety.[5] Furthermore, college is a new gateway for many students as they face a different social as well as educational environment. United States data shows that about 23% of the population is affected by continuous stress and it increases the risk of cardiovascular diseases, depression, and autoimmune disorders.[6] It is

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generallyconsidered that stress affects life negatively but some research has shown that stress may have a favorable outcome. This is because stress has multiple factors which are associated with positive outcomes.[7] The regions of the brain engaged in the regulation of stress include the subcortical, hypothalamus, hippocampus and brainstem. All these regions are under the control of the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axis.[8] Activation of the stress system resulted in behavioral and peripheral changes that affect the quality of life.[9] Many animal studies reported differences in hormonal and behavioral responses given by males and females after long or short-term stress.[8]

Therefore, the present study determines stress tolerance in medical and dental students. The result of this study will help identify various medical disorders that are correlated to psychological problems and hence helpful in timely treatment/intervention.

MATERIAL AND METHODS

A cross-sectional study was conducted on 300 medical and dental students of Sharif Medical and Dental College Lahore, for six months. Students of all professionals were included in the study except those having been diagnosed with psychiatric illness or any medical or systemic disorder secondary to psychiatric illness. The data was collected online via Google forms on an Authorized Questionnaire having 10 questions. The questionnaire included demographic details of the participants, their age, and stress-related questions which they experienced/observed in the last month.[10]

Stress levels will be scored by the perceived stress scale (PSS). The perceived stress scale is a classic assessment instrument having ten questions that indicate how often you will feel or think during the last month. Individual scores on the PSS will range from 0 to 40 with a higher score indicating a higher level of stress.

Scores ranging from 0- 13 would be considered low stress, scores ranging from 14- 26 would be considered moderate stress and scores ranging from 27- 40 would be considered high perceived stress.

RESULTS

A total of 300 medical and dental students of Sharif Medical Dental College were selected and the authorized questionnaire was given to each student. Among them 9% of students showed low stress, 61% had moderate stress and 29% showed high perceived stress (Figure 1). In the current study, 62.1% of females and 37.9% of males gave their consent and filled out the form and perceived stress test results are shown in Figure 2. Figure 3 shows the perceived stress test results of 59.3% of day scholars and 40.7% of hostilities. 46.55% of students answered that they were sometimes upset because of something that happened unexpectedly while 17.1% showed that they were upset very often. 34.6% of students felt that they were sometimes unable to control the things in their life while 10% showed they never had such feelings in their last month. 37.5% of students felt that they were sometimes nervous and stressed in the last month and 26.4% showed these feelings very often. 15.6% of students found that very often they could not cope with all the things they had to do while 8.2% never found that they could not cope with all the things. 16% of students found they were very often been angered because of things that were outside of their control and only 5.6% never had such feelings in the last month. 16.7% of students very often felt difficulties piling up so high that they could not overcome while 38.3% of students sometimes felt that they could not overcome their difficulties. 23.8% of students showed that they are not confident to handle their problems very often while 39% sometimes felt that they could not handle their problems. 43.5 % of students sometimes felt that things were going their way. 50.2% of students were able to control the irritations in their life and 43.5% of students sometimes felt that they were on top of things.

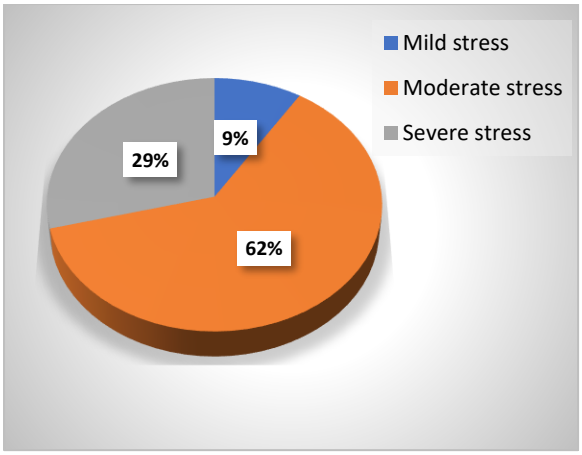

Figure-1: Showing mild, moderate, and severe stress among medical and dental students

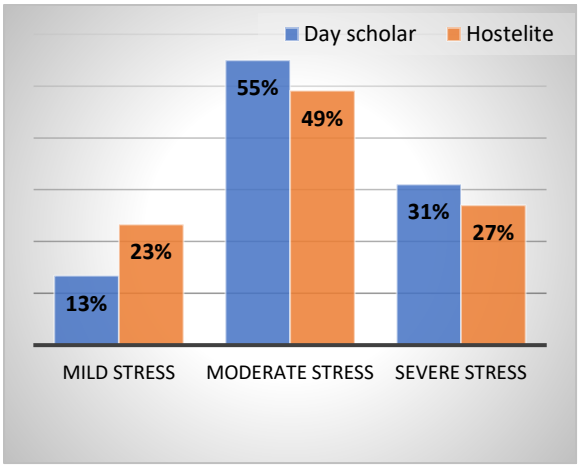

Figure-2: Graphical presentation showing male and female differences in mild, moderate and severe stress.

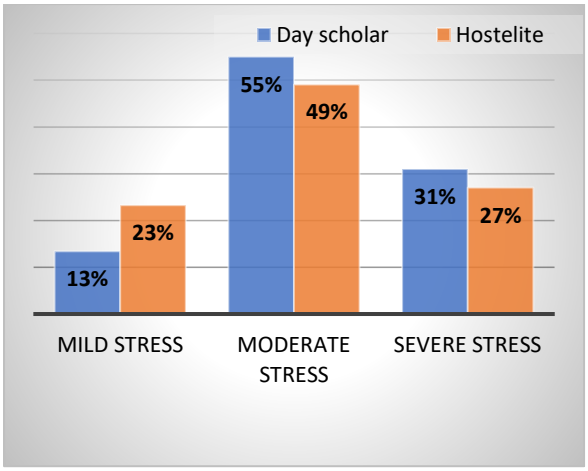

Figure-3: Graphical presentation showing day scholar and hostilities difference of mild, moderate, and severe stress.

DISCUSSION

Due to many expectations from internal and external sources, students particularly medical and dental face stress throughout their professional studies.[11] Continuous stress in adult age may cause various hormonal disturbances that lead to various physical and mental illnesses.[12] In this study 9% of students showed low stress, 61% showed moderate stress and 29% showed high perceived stress. These results correspond with a study conducted previously in which moderate stress was observed in nursing students in Poland.[13] Our estimates are lower in comparison to the Malaysian study where 55.5% of students showed stress. The difference in results among different students of different countries might be due to differences in the educational environment, competition among the students, and socio-demographic variation.[14] In this study, 39% of students were not confident in handling their problems which coincides with a study done by Lorenz Gotte et al. who showed that self-confidence is majorly lost by those who showed more stress and anxiety. Due to the subsequent release of various hormones like cortisol, the level of stress might be changed and self-decision capabilities might be affected.[15] Also continuous stress is in parallel relation with depression by releasing various neurotransmitters.[16] Previous studies showed that decreased or increased levels of these neurotransmitters have a direct effect on various systems of the body for example reproductive system by disturbing the gonadal hypothalamic-pituitary axis.[17] Keeping all these effects of stress it is the prime duty of institutions and training centers to provide a healthy and encouraging environment to the students which support their mental as well as physical health. It is because professional institutions and colleges are the places where young people of various backgrounds and various socioeconomic statuses come together.[18]

Like every other study, our study also has some strengths and limitations. The result of this study can be used for further research on depression and anxiety among students. A

comparison should be made among students of different professions. The perceived stress scale was used to measure the stress level which cannot be used for precise diagnosis but it can be used as a screening tool to determine stress among the students.

This study also highlighted that stress parallels professional studies which include yoga, life skills training, and psychotherapy. All these measures should be taken at the personal as well as institutional level, as social well-being is essential for the students particularly medical and dental as well as for the institution.[11]

CONCLUSION

Academic stress has become a common problem worldwide regardless of ethnicity, religion, or country due to peer or family pressure. The present study highlighted this problem among medical and dental students and concluded that about two third of them experienced moderate stress while one-third experienced high stress. Stress among the students could be minimized by introducing various management techniques like yoga and psychotherapy not only at the student level but at the institutional level also.

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AUTHOR'S CONTRIBUTIONS

MS: Collected and analyzed the data
NA: Reviewed and finalized the manuscript.
AG: Reviewed and finalized the manuscript.
FA: Review and formatting
SE: Review and formatting

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Original Article

QUANTIFICATION AND METHYLATION STATUS OF FREE CIRCULATING DNA BASED BIOMARKER IN DIABETIC PATIENTS FOR THE EARLY DIAGNOSIS OF DIABETES MELLITUS THROUGH LIQUID BIOPSY

Zunaira Kanwal[1], Zeeshan Arshad[2], Nazish Mehmood Aisha[3], Fiaz Ahmad[4], Zoha Khan[5], Syeda fiza[6]

ABSTRACT

Background: The prevalence rate of diabetes mellitus (DM) is becoming higher not only in developing countries but also worldwide. The commonly used tests for the diagnosis of DM lack standardization and sensitivity. DNA based free-circulating biomarkers are gaining attention as promising biomarkers for the diagnosis, prognosis, and progression of different diseases. In DM, β-cell destruction results in the release of free DNA into the plasma which can be quantified by different approaches. In our study, we have quantified and compared the level of total free circulating DNA and methylation status of preproinsulin (INS) DNA fragments levels in the plasma of diabetic patients and healthy controls.

Material and Methods: Our study included 45 diabetics and 45 age and sex-matched control individuals. We used Quantitative real-time PCR as a more reliable tool due to high specificity and sensitivity to determine the circulating copies of demethylated *INS*. The ROC curve was used to determine the sensitivity and specificity of the assay used for diagnosis purposes.

Results: In the plasma of patients, a relatively higher amount of free circulating DNA was observed as compared to the normal healthy person. ROC curve analysis showed strong discrimination potential of fcDNA concentrations for diabetic patient diagnosis with the area under the curve to be 0.794 (95% CI: 0.698-0.889; $P < 0.05$).

Conclusion: These assays may be used to detect the extent of destruction of β-cell death in DM and can give insights into progression and responses to the treatment used.

Key Words: DNA, Diabetes mellitus, Methylation, Preproinsulin

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INTRODUCTION

Diabetes mellitus has the highest rate of incidence and mortality globally and it will be the 7th dominant cause of death by the year 2030 as reported by the World Health Organization. It is reported that about 347 million people are suffering from diabetes around the globe.[1]

The prevalence rate of diabetes mellitus is the not highest in developing countries, but in the upcoming 25 years, they would encounter an elevation in the prevalence rate.[2] Asia has the leading prevalence rate.[3] In Pakistan, the incidence rate of glucose intolerance is approximately 22 % in urban areas and 17.1 % in rural areas.[4] The risk of diabetes onset increases two to six times in individuals with a family history of diabetes.[5] Various risk factors of type 2 diabetes include a family history of diabetes, overweight or obesity, unhealthy diet, high blood pressure, increasing age, physical inactivity, impaired glucose tolerance, and poor nutrition during pregnancy (International Diabetes

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Federation, 2001). Individuals who have stopped smoking possess the highest incidence rate to develop diabetes.[6] This is due to various factors such as lifestyle changes, genetics and environmental exposures. Growth hormones, cortisol, glucagon, epinephrine and some other hormones act in opposition to insulin activity.[7] diabetes mellitus is commonly diagnosed by different tests such as plasma glucose in fasting state, hemoglobin glycation and oral glucose tolerance test (OGTT).[8] OGTT possess some pitfalls such as complexity, lower reproducibility and high cost. It points out more individuals with diabetes than fasting glucose tests.[9] Tests employed for the determination of the type of diabetes or the assessment of the demand for insulin depend on the β-cell activity. These include C peptide levels and immune mediated β-cell devastation markers such as autoantibodies against pancreatic islet cells, glutamic acid decarboxylase, insulin levels and tyrosine phosphatase.[10] Antibody testing is limited due to availability, prognostic value and cost.[11] Cell free circulating DNA has become an attractive strategy for the detection of β-cell destruction in-vivo due to its ability to provide accurate and timely information about the health of cells. This strategy is gaining attention from scientists because it can be used to detect β-cell destruction without having to directly access the cells. It also offers a non-invasive approach, which makes it ideal for use in clinical settings. Furthermore, it can provide real-time data about the health of cells that can be used to inform treatment decisions and monitor disease progression.[12] Usually, free circulating DNA is secreted from the degraded cells and subsequently cleaved by endonucleases but the definite mechanism of release of free-circulating DNA is not obvious.[13] Endonucleases cut down the chromatin into smaller nucleosomal units.[14] Various clinical assays have been evolved for the detection of the destruction of cells in vivo which depend upon the identification of the nucleic acids, secreted by the lysed cells into the circulation.[15] Specific PCR dependent assays can be done to detect the presence of these molecules. Circulating DNA that is differentiated by the methylation pattern is used as a biomarker to identify the disease.[16] Then methylated and unmethylated positions can easily be differentiated. These positions can be quantified by PCR based assays and DNA sequencing.[17] A specific, sensitive and quantitative methylation specific PCR based assays are necessary for the detection of free circulating β-cell DNA.[15] Unmethylated circulating preproinsulin (*INS*) may be considered as a biomarker for β-cell death (15-16).[18] With the increasing prevalence of Diabetes mellitus, quantification of fcDNA and determination of methylation status of INS has become an attractive tool for screening and managing this disease. This method is important because it not only helps in early diagnosis but also provides valuable insight into the underlying causes and mechanisms of this chronic condition. Furthermore, it also helps in developing better strategies for treatment and monitoring. Thus, quantification of fcDNA and determination of methylation status of INS has become a crucial tool for screening and managing Diabetes mellitus.

MATERIAL AND METHODS

The cross sectional study was conducted in the year 2022 in the tertiary care teaching hospital in district Lahore. The population comprised 45 patients and 45 healthy controls with ages≥ 18 years and were permanent residents of Pakistan. The inclusion criteria for the patient series was a cytological or histological substantiated diagnosis of Type1 and Type2 Diabetes Mellitus as defined per WHO criteria.[19] The exclusion criteria were diagnosis of AIDS, pregnancy and cancer. A total of 45 control samples were selected by characteristics like the patients but having no clinical history of any type of diabetes mellitus. A 3ml peripheral blood sample from each patient and control in series was collected and immediately plasma was separated. DNA was isolated from the blood plasma using Abcam DNA Isolation Kit-Plasma/Serum

(ab156893) by following the instructions of the manufacturer.

The total concentration of fcDNA in both patients and healthy controls was determined by nanodrop spectrophotometer and the INS gene was used as a reference gene to measure the level of fcDNA in patients suffering from diabetes and healthy controls using real time PCR. After that, plasma fcDNA of all samples were subjected to bisulfite conversion by using EpiMark® Bisulfite Conversion Kit (# E3318S). This modified fcDNA was used as a template in quantitative PCR using primers reported in the literature. The conditions employed for thermal cycling were as follows: denaturation steps at 95 ºC for 10 min followed by 40 cycles of 95 ºC for 15-sec denaturation, annealing at 55 º C for 30 sec and extension for 30 sec at 72 ºC. All samples along with positive controls (*in-vitro* methylated leukocyte DNA) and negative controls (normal leukocyte DNA) were used. The leukocyte DNA from healthy controls was methylated using M. SssI methyltransferase (Thermo Scientific, USA) to generate the positive control.

SPSS for windows (version 21.0, USA) was used for the statistical analysis of data. A 2-tailed *P* value < 0.05 was considered significant statistically and box plots were generated to represent results. The diagnostic performance of selected makers was assessed by the receiver operating characteristic (ROC) curve and the respective area under the curve (AUC). The characteristics like specificity and sensitivity values having 95 % confidence intervals (CIs) were evaluated for biomarkers.

RESULTS

The plasma concentrations of fcDNA in the patient, as well as control subjects, were determined spectrophotometrically at 260 nm by using nanodrop. It is noticeable from figure 1 that four-fold higher median concentrations of fcDNA of diabetics as compared to control subjects were found. This is statistically significant ($P < 0.001$). Significantly higher concentrations of free circulating DNA were observed in the patient series as compared to the levels in the control series but there are some individuals in the patient series in which the levels are comparatively low. In the patient series greater variability of free circulating DNA was perceived. In the majority of them, higher levels were observed. Whereas there are some subjects in the patient series who possess low levels of fcDNA as shown in figure 1.

Table-1. Clinicopathological characteristics of patients and healthy controls

	Patients (45)	**Control (45)**
Gender		
Male	24	24
Female	21	21
Type of Diabetes		
Type 1	26	-
Type 2	19	-
Age (Years)		
20 or less	1	1
21-30	2	2
31-40	8	8
41-50	14	14
51-60	10	10
60 or above	10	10
BMI		
1-18.5	2	2
18.5-22.9	8	8
23-24.9	8	8
Above 25	27	27

The diagnostic performance of concentrations of fcDNA was evaluated by ROC (Receiver Operating Characteristic) curve analysis. The competence of this assay to differentiate patients from controls was determined by AUC. Closer the value to 1, the greater the diagnostic power. The respective area under the curve was found to be 0.864 (95% CI, 0.786-0.942, $p<0.05$), as shown in figure 2. It depicts the strong discrimination potential of fcDNA concentrations for diabetic patient diagnosis. It has been observed that a lower cut-off value enhances the specificity of the assay but at the cost of the sensitivity and vice versa. So, to favor the sensitivity of the assay, we select 640 ng/ml. as this test is 1st level test so it further needs confirmation, this

value let us minimize the risk of false negatives.

Free-circulating DNA and INS concentration was significantly correlated with BMI (γ = 0.206;
$p < 0.05$). The amount of fcDNA increased as the BMI increases. A significant correlation also present with age of the subject (γ = 0.311; $p < 0.05$). As the age grows the fcDNA levels may elevate. But no significant relation was observed between the onset of disease and gender.

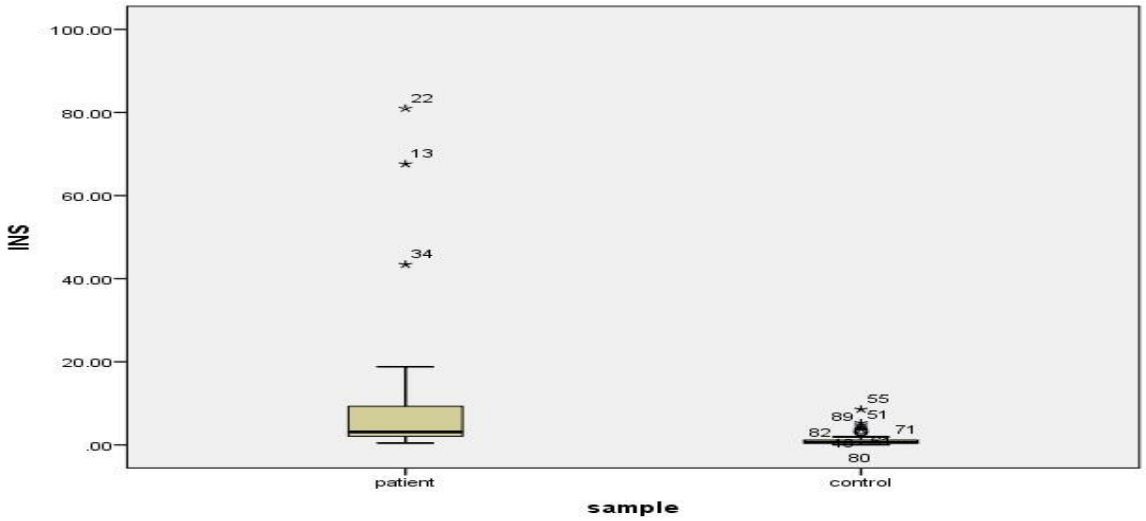

Figure-1: Box-Plot representation of fcDNA measured in ng/ml in diabetic patients and corresponding healthy controls by using *INS* as a marker. The upper and lower error bars show 90% of the values. The median is the solid line in the box

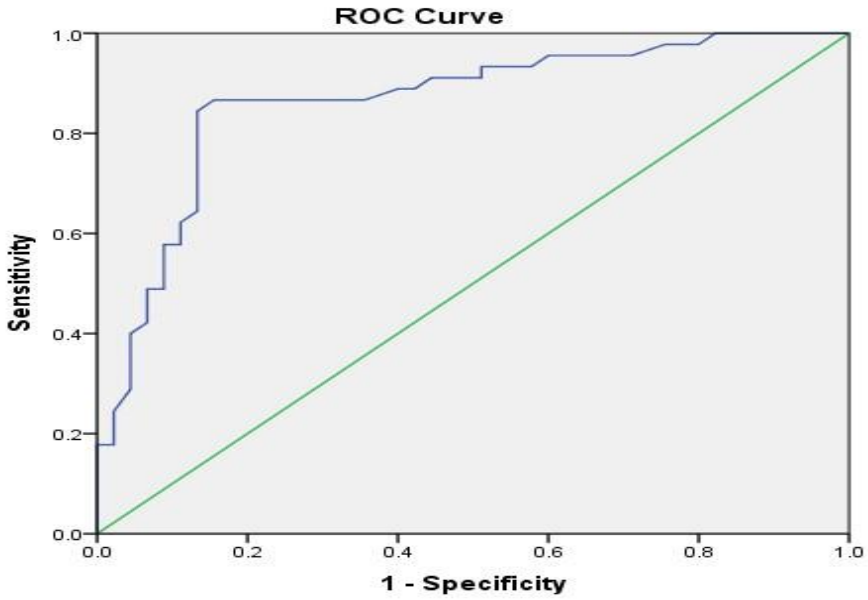

Figure-2: ROC curve for the diagnosis of Diabetes Mellitus using Plasma fcDNA concentrations

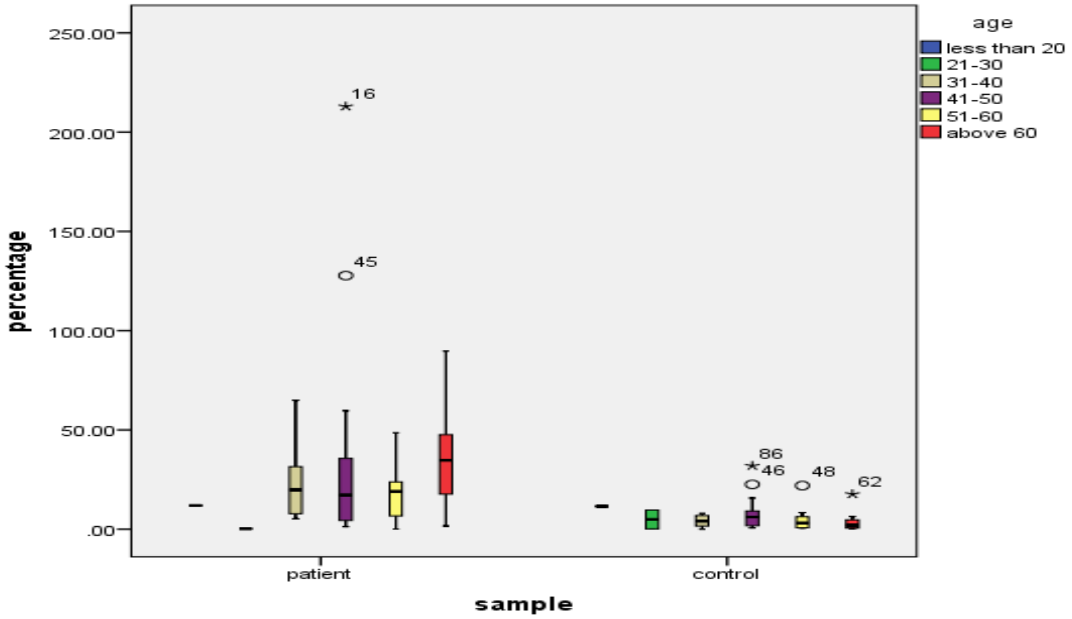

Figure-3. A) Correlation of fcDNA level in ng/mL with age in patients and healthy controls

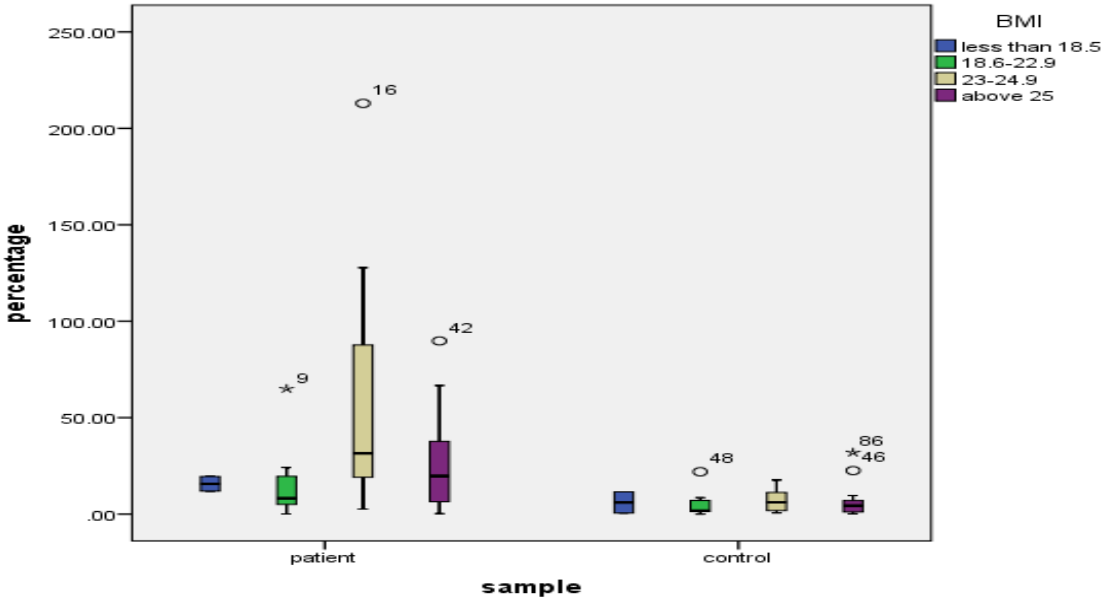

Figure-3. B) Correlation of fcDNA levels in ng/ml with BMI in patients and healthy controls.

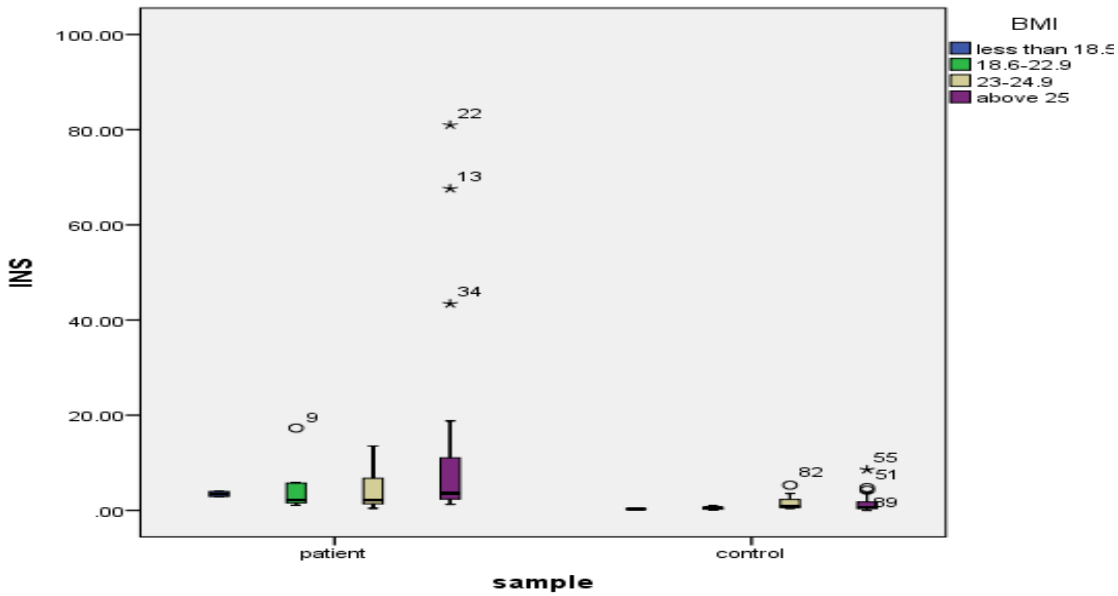

Figure-4: A) Correlation of INS DNA fragments levels in ng/ml with age in healthy controls and patients.

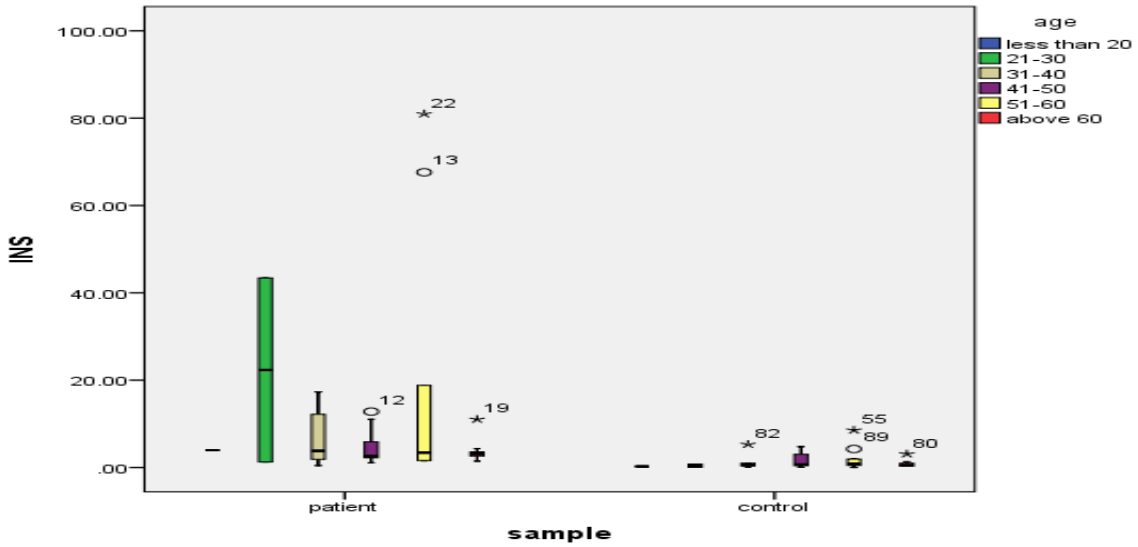

Figure-4: B) Correlation of INS DNA fragments levels in ng/ml with BMI in patients and controls

DISCUSSION

Diabetes is a growing health issue, and early diagnosis is key to managing it. Cell-free DNA based biomarkers are playing an increasingly important role in the early detection of diabetes through liquid biopsy. With quantification and methylation status analysis of cell-free DNA, it is possible to identify the genetic markers associated with diabetes in a patient's blood sample. This can help doctors detect the disease before it becomes more serious and provide more personalized treatments for each patient.[20] It is found that fcDNA levels in those with Type 2 Diabetes (T2DM) were significantly higher than those without, regardless of if they had any complications or not. This suggests that fcDNA can be a useful tool for personalizing the management of T2DM patients. Accordingly, fcDNA levels are positively correlated with adverse metabolic outcomes in T2DM patients with or without complications, suggesting that it is

appropriate for use as a biomarker for monitoring the effect of therapeutic interventions on reducing cardiovascular events in these patients. Different approaches have been employed for the quantitative analysis of fcDNA but the quantitative real time PCR assay has been the most reliable and promising due to its high specificity and sensitivity. We used Quantitative real-time PCR as a more reliable tool due to high specificity and sensitivity to determine the circulating copies of demethylated INS. The ROC curve was used to determine the sensitivity and specificity of the assay used for diagnosis purposes. In the plasma of patients, a relatively higher amount of free circulating DNA was observed as compared to the normal healthy person. Normal values of the ROC curve in this assay are indicated in the figure-2.

We chose to stick with the established methods of using serum samples for fcDNA isolation in our study since it is a popular approach for liquid biopsy. This gave us the ability to generate results that could be compared with other previous experiments. Moreover, we have successfully managed to reproduce quality results using relatively small serum volumes - something that has been a challenge in previous experiments with similar assays, indeed, the cancer field where these assays are most highly advanced is in many cases moving toward sample volumes an order of magnitude larger than those used here. Our findings from direct & indirect measurements showed that the quantity of the sample used was enough to carry out further analysis. Methylation is a cell-specific phenomenon, with genetic material being unmethylated in certain tissues. This profile will be preserved in the release of cfDNA from those tissues, making it possible to identify β-cells through this marker.

CONCLUSION

In our study, a four-fold higher concentration of fcDNA has been found in diabetic patients as compared to the control. A significant correlation of fcDNA was observed with body mass index. As the BMI increases, the amount of fcDNA also increases in diabetes mellitus. Similarly, a significant correlation of fcDNA was observed with age as age grows the level of fcDNA also elevated. So, the methylation-specific PCR assay for the INS gene can be helpful to detect beta cell death in diabetes and may provide an understanding of the progression of the disease.

Conflict of Interest

The author declares that there is no conflict of interest

AUTHOR'S CONTRIBUTION

ZK: Conceptualization and formal analysis
ZA: Data collection, formal analysis, methodology
NMA: Methodology writing
FA: Data collection and drafting of the article
ZK: Design of study and revision of the article
SF: Critical revision of the article

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Original Article

TO DETERMINE THE FREQUENCY OF CLOSTRIDIUM DIFFICILE INFECTION IN ANTIBIOTIC ASSOCIATED DIARRHEA

Attique Abou Bakr[1], Naeem Aslam[2], Mamoona Ghias[3], Imran Mehfooz[4]

ABSTRACT

Background: Antibiotic associated diarrhea (AAD) is characterized by loose, watery stools, three or more times a day after intake of antibiotics. It is important to exclude all other possible causes of diarrhea before labeling it AAD. To determine the frequency of clostridium difficile infection in antibiotic associated diarrhea.

Material and Methods: It was a cross sectional study done in department of Gastroenterology, outpatient clinic, and ward, AIMC/ Jinnah Hospital, Lahore from 23 May 2019 to 23 November 2020. 234 patients fulfilling the selection criteria were enrolled in the study from the outpatient department and ward of Gastroenterology, Jinnah Hospital Lahore. Informed verbal consent was obtained and patients were subjected to focused history and physical examination as well as their demographic details were recorded. Antibiotics taken by the patient were documented. The antibiotic was withdrawn and the patient was treated per hospital protocol. A stool culture was sent for all patients to see CDI. The patient was labeled as CDI positive if stool culture is positive. All this information was recorded on a predesigned annexure.

Results: Out of 234 cases of antibiotic induced diarrhea; the Mean Age was 55 ±13 years (minimum was 27 and maximum was 66 years) 56% of cases were male and 44% cases were female, 28.6% of cases had age < 50 years and 71.4% cases had age ≥ 50 years, 54.7% cases used Beta-Lactam antibiotics and 45.3% cases used non- Beta-Lactam antibiotics, 68.4% cases had Bristol grade 6 and 31.6% cases had Bristol grade 7. Out of 234 cases of antibiotic associated diarrhea; 43.6% cases had CD infection and 56.4% cases had No CD infection. Stratification 0f CDI was done with regards to age groups, Gender, type of antibiotic used and Bristol grade; p-value was found to be 0.35, 0.41, 0.4 and 0.03 respectively.

Conclusion: The majority of patients with antibiotic induced diarrhea were male, had older age, used Beta-lactam antibiotics, had Bristol grade 6 and had a non-CD infection. Regarding CD infection; it was more common in older age, female gender and Beta-lactam antibiotic users; but associations were not significant. However, CD infection was more common in antibiotic induced diarrhea of Bristol grade 7 and the association was significant.

Key Words: Antibiotic, Diarrhea, Clostridium, Infection

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INTRODUCTION

Antibiotic associated diarrhea (AAD) is characterized by loose, watery stools, three or more times a day after intake of antibiotics.[1] It is important to exclude all other possible causes of diarrhea before labeling it AAD. It occurs as a result of an overgrowth of some pathogenic intestinal organisms or due to a reduced concentration of fecal flora. Almost all antibiotics are implicated in the

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pathogenesis of AAD especially cephalosporins, penicillins aminoglycoside, fluoroquinolones and clindamycin. Simultaneous intake of multiple antibiotics increased duration of antibiotic treatment, history of diarrhea after antibiotics, and age of more than 65 years are important risk factors for AAD.[2,3]

Clostridium difficile (CD) is a spore-forming gram-positive anaerobic bacteria. It is normal commensal in 1-3% of normal adults. Above mentioned antibiotics may cause CD to overgrow and produce toxins A and B resulting in clostridium difficile infection (CDI). It is responsible for approximately 20% of cases of AAD. CDI causes inflammation of bowel mucosa which in turn causes diarrhea. CDI may range from simple diarrhea to life threatening condition called pseudomembranous colitis. With the increasing use of antibiotics, CDI is becoming one of the leading causes of hospitalization and deaths[1] and the severity of CDI has dramatically increased over the last decade.[2]

CDI prevalence varies worldwide and is estimated to be 0.9-2% in the general population, 1% in the European population and 3% in the Asian population.[3] In a study conducted in Iran, CDI was found to be positive in 21% of patients with diarrhea.[4] In Chinese studies, CD was isolated in 8.7%-30% of stool samples.[4,5] A study conducted in Karachi showed that CD was positive in 29.18% of AAD.[6]

The rationale for this study was that data on CDI incidence from Pakistan is scarce. Furthermore, in a country like Pakistan, there is depressed nutritional and immunological status and injudicious use of antibiotics which pose a threat of a very high prevalence of CDI. There is global emergence of resistant strains of CDI, its frequency and strains in our country would be different from the western world. So by knowing the frequency, the physician can predict the existence of the disease in a patient presenting with diarrhea after antibiotic use. So, the patients can be diagnosed at early stages by applying screening tests and definite treatment can be started to halt the disease progression. This may reduce morbidity and mortality associated with this grave but treatable disease.

MATERIAL AND METHODS

This was a cross-sectional, descriptive study that was done in OPD and ward of Gastroenterology, Jinnah Hospital Lahore. The duration of the study was 18 months from 23 May 2019 to 23 November 2020. The sample size was calculated by using the WHO calculator. The sample size came to be 234 by keeping the confidence interval at 95%, relative precision at 20%, 5% margin of error and anticipated CDI incidence at 29.18%.[7] Sampling technique was Non-probability consecutive sampling. The patient either male or female and between the ages of 16-70 years included due to any cause in the hospital who developed diarrhea after at least three days of antibiotics(even a single dose of antibiotic). There should not be a history of diarrhea in the last two weeks before starting antibiotics. Exclusion criteria involved the presence of other causes of chronic diarrhea like IBS, IBD, malabsorption syndrome, neoplasms, celiac disease, thyrotoxicosis assessed by history and clinical examination and patients with unstable cardiopulmonary, neurological, or psychiatric disease assessed by history, examination, clinical record and laboratory tests TFTs, Celiac screen and endoscopy.

Two hundred and thirty-four medical outpatients who fulfilled inclusion criteria were enrolled at Jinnah Hospital Lahore after written informed consent and subjected to focused history and physical examination as well as their demographic details were recorded. Antibiotics taken by the patient were documented. The antibiotic was withdrawn and the patient was treated per hospital protocol. A stool culture was sent for all patients to see CDI. The patient was labeled as CDI positive if stool culture is positive. All data were entered in the same proforma.

Statistical analysis was done using SPSS version 22. Frequency and percentage were

calculated for qualitative data like gender and presence of CDI. Mean + standard deviation was calculated for quantitative data like age. Data was stratified for age and gender type of antibiotics used. Post-stratification chi-square was applied to see any effect modifier by considering p value<0.05 as significant.

RESULTS

This study was conducted on 234 cases. The mean age was 55 ±13 years (minimum was 27 and maximum was 66 years). Out of 234 cases of antibiotic induced diarrhea; 56% cases were male and 44% cases were female, 28.6% cases had age < 50 years and 71.4% cases had age ≥ 50 years, 54.7% cases used Beta-Lactam antibiotics and 45.3% cases used non- Beta-Lactam antibiotics, 68.4% cases had Bristol grade 6 and 31.6% cases had Bristol grade 7. Stratification 0f CDI was done with regards to age groups, Gender, type of antibiotic used and Bristol grade; p-value was found to be 0.35, 0.41, 0.4 and 0.03 respectively.

Table-1: Distributions of the variable with frequency and percentage

Variable		Frequency	Percentage
Age group	Less than 50 years	67	28.6
	Equal or more than 50 years	167	71.4
Gender	Male	131	56
	Female	103	44
Antibiotic type	Beta Lactum	128	54.7
	Other	106	45.3
Bristol grade	06	160	68.4
	07	74	31.6
CDI	Yes	102	43.6
	No	132	56.4

Table-2: Stratification of variables with regards to CDI (n = 234)

Variable		CDI		p-value
		Yes	No	
Age group	Less than 50 years	26	41	0.35
	Equal or more than 50 years	76	91	
Gender	Male	54	77	0.41
	Female	48	55	
Antibiotic type	Beta Lactum	59	69	0.4
	Other	43	63	
Bristol score	06	62	98	0.03
	07	40	34	

DISCUSSION

Depending on the specific drug, the host's defense, and exposure to infections, AAD commonly affects 5–35% of patients taking antibiotics. The alteration of the normal microbiota, which leads to pathogen overgrowth or metabolic abnormalities, may play a role in the etiology of AAD. The first step in treating AAD is early diagnosis, which is then followed by efficient therapy and the implementation of control measures. Clostridium difficile infection (CDI) is most frequently contracted in conjunction with the use of antibiotics, which leads to the disturbance of the normal colonic microbiota. After Ingestion, C. difficile spores vegetate, grow, and exude toxins that cause CDI and in most instances, cause diarrhea and pseudomembranous colitis (PMC).

Since 2000, rates and severity of CDI significantly rose in the Western population. In 2005, Hospitals in Montreal, Quebec, reported four times higher rates than the baseline year of 1997, with mortality rising from 1.5% to 6.9%. An epidemic strain, also known as NAP1/BI/027, has been detected in North America, Europe, and Asia. This organism has the potential to produce 23 times more toxin A and toxin B as a control strain in vitro,[3] third toxin (binary toxin

CDT), and has high-level resistance to fluoroquinolones.[3] Another strain that also possesses binary toxin and is linked to high mortality in humans is toxin type V, ribotype 078 and it will continue to be implicated in outbreaks. The frequency of the NAP1/BI/027 strain in Europe has declined over the past five years.[7] However, there is no report of a decline in CDI rates or NAP1/BI/027 incidence in other parts of the world; the latter strain continues to account for 25–35% of all CDIs.

The most frequent symptom of C. difficile is diarrhea. Stools are typically watery or mucoid, soft and unformed, and have a distinct odor. They are not usually bloody. Fever, abdominal pain, and leukocytosis are the other clinical characteristics. The diagnosis of CDI is commonly missed when adynamic ileus causes a halt of stool passage. Such patients are at a higher risk of developing sepsis and toxic megacolon from C. difficile infection.[8-10]

CDI has a recurrence rate of 15%-30%. Relapse or reinfections with a different strain are both examples of recurrences. Susceptibility to recurrence is probably due to the ongoing disturbance of the fecal flora by antibiotics.[11-15] This study was conducted on 234 cases. The majority of patients with antibiotic-associated diarrhea were men, older than the average age, beta-lactam users, bristol grade 6 patients, and those with non-CD infections. Although there was a higher prevalence of CD infection among older people, women, and those who used beta-lactam antibiotics, these correlations were not statistically significant. However, there was a substantial correlation between CD infection and antibiotic-induced diarrhea in Bristol grade 7. The results of this study support our objectives. A large-scale population-based study is required for achieving significant results, which can be generalized.

CONCLUSION

Our study highlights that a considerable number of AAD cases were found to have CDI. Early detection of CDI is of paramount importance, as with appropriate measures we can reduce morbidity and mortality. The following inferences can be drawn from the study: 1. C. difficile-associated disease is a growing problem in nosocomial and community settings. 2. Most culprit drugs were found to be Beta-lactam antibiotics. 3. Clinical suspicion is more important because stool assays for diagnosing CDI are not widely available. Hospitalized patients receiving antibiotics are at greater risk of acquiring CDI. 4. Infection control procedures can offer a potential improvement in outcome and can cause a significant reduction in public health-related expenditures.

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Conflict of interest: None

AUTHOR'S CONTRIBUTION

AAB: Concieved idea, main researcher and supervisor
NA: Data collection and data analysis
MG: Critical review
IM: Proof reading and manuscript writing

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Review Article

AN OVERVIEW: ROLE OF PHYTOCHEMICALS IN THE PROPHYLAXIS OF MIGRAINE

Syeda Mah-E-Noor Zahra[1], Mahnoor Fatima[2], Babar Ahmad[3], Ibrahim Sohail[4]

Abstract

Migraine is one of the most painful and debilitating conditions encountered by 22.7% of people in Pakistan, thus many therapeutic strategies are being used to prevent and treat the symptoms and underlying pathology. The role of phytochemicals in the prevention of migraine attacks has been studied at different research centers around the world for many years. It has been found that intake of food as well as phytochemicals containing antioxidants such as flavonoids, polyphenols and alkaloids are involved in the reduction of migraine episode frequency. The current literature supports the use of these plant derived substances therefore if proven useful in other research including animal studies and human trials, can be given as an alternative or along with other prophylactic medical treatments so that better results can be yielded.

Key Words: Migraine, Headache, Serotonin, Vasodilation

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INTRODUCTION

The word "Migraine" is derived from "*Hemikrania*" a Greek word meaning 'pain in half of the head' as it is associated with a headache involving usually one side of the head.[1] It is considered a neurological as well as neurovascular disorder[2] and is characterized by recurrent headaches in which the pain is pulsating in nature[3], with moderate to severe intensity lasting from at least an hour to three days.[4]

The exact underlying mechanism is unknown but many theories exist regarding different factors involved in the pathophysiology of migraine and its signs and symptom.[5] Studies suggest that sensory nerves that surround blood vessels of the head and neck perceive pain when vasodilation occurs. The stretching of vascular smooth muscle along with pulsations from blood pumping cause the typical "throbbing" headache.[6]

Many systemic inflammatory diseases are linked with the predisposition of migraine attacks such as inflammatory bowel disease, multiple sclerosis and rheumatic diseases etc.[7-9] High cytokine levels in the blood indicate the presence of inflammation in the body during migraine episodes.[10,11] Studies support the involvement of other factors e.g., impaired cerebral glucose metabolism. Many studies link migraine attacks with low levels of the neurotransmitter serotonin and high levels of adenosine respectively.[12] Similarly, mitochondrial disability, where an imbalance occurs between the supply and demand of energy is also thought to be involved. The underlying oxidative stress may contribute towards disease susceptibility.[13,14]

One reason can be increased excitability in the cerebral cortex region and increased activity of pain neurons located in the trigeminal nucleus.[15] Another culprit involved is suggested to be Calcitonin Gene-Related Peptide (CGRP), a neuropeptide that

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causes vasodilation thus leading to neurogenic inflammation.[16]

Genetics play a big role in the predisposition of migraine as the studies reveal most of the patients with migraine tendencies have a family history of migraine attacks.[17] Mental and physical stress are frequently related to migraine headaches.[18] Hunger, sleep disturbances, hypertension along with hormonal factors such as menstruation, menarche and menopause, use of oral contraceptive pills as well as pregnancy are also involved in the onset of pain.[19]

Studies show a correlation between migraine with certain diets such as foods containing tyramine and monosodium glutamate (MSG) therefore chocolates, cheese and alcoholic as well as carbonated drinks can be considered culprits behind the disease.[20] Environmental aspects such as bright lights, loud noises, smoke, humidity, sudden change in temperature or extreme weather are also thought to be linked with the triggering of attacks.[21]

The diagnosis of a migraine is mainly based on signs and symptoms.[22] Most of the people affected have an aura which is a transient period of visual disturbance indicating that the headache will occur soon. Headaches occur along with the feeling of heaviness and numbness in the upper limb and face on the same side.[23] Nausea and vomiting also occur as well as sensitivity to light, smell or sound.[24] If a migraine episode lasts longer than 3 days, it is termed "Status Migrainosus".[25] Other conditions like cluster headaches and meningitis can mimic the symptoms.[26]

Lifestyle modifications including a healthy diet and nutritional supplements significantly improve the symptoms of migraine. The incorporation of magnesium supplementation has shown positive results.[27] Alterations in behavior and habits such as healthy routines and smoking cessation greatly reduce the frequency of migraine attacks.[28] Acupuncture, massage and physiotherapy have been reported to help.[29]

If the condition persists, medication can be started such as beta-blockers, calcium channel blockers, angiotensin system inhibitors, antidepressants, anti-psychotics and anti-epileptic agents.[30] In an acute attack, nonsteroidal anti-inflammatory drugs (NSAIDs), opioid analgesics or acetaminophen along with caffeine and aspirin are given for pain relief.[31] Antiemetics can also be given to subside the gastrointestinal effects.[32]

Triptans are the mainstay treatment of an acute attack of migraine.[33] The use of ergotamine is discouraged according to many researches.[34] In extreme cases, anesthetics can be given to combat the intense and resistant pain.[35] The main focus is to reduce the frequency in the first place thus prophylactic treatment is given to reduce not only the intensity of pain in upcoming migraine episodes but also to prevent the onset of attack to begin with.[36] But many adverse effects may be encountered with this drug.[37] Therefore, plant-derived substances studied that can be used as dietary supplements.[38]

DISCUSSION

Phytochemicals being alternatives to conventional therapy can be used for both acute and preventive treatment[39] as they have been reported to exert antioxidant effects. Their anti-inflammatory mechanisms and neuroprotective actions may also participate in their effectiveness.[40]

Correlation between dietary intake of phytochemicals containing active ingredients such as flavonoids and tannins etc. and prophylaxis of migraine has been established in many studies including recent researches.[41] Polyphenols one of them has shown to lower the severity of migraine by reducing oxidative stress.[42] A herb known as feverfew (*Tanacetum parthenium*) has also been reported to exert neuroprotective effects by inhibiting the release of serotonin from platelets and histamine.[43,44] Extracts of *Salix alba* showed a reduction in nitrite levels and neurotoxic stimuli induced serotonin turnover.[45] *Calotropis gigantea Linn*

expressed its effectiveness by interacting with dopamine and serotonin receptors whereas *Sargassum cristae folium* which contains alkaloids increases serotonin levels thus improving the symptoms of migraine.[46,47] Cannabinoids, containing terpene have analgesic and anti-inflammatory effects as suggested by researches.[48,49] Evidence regarding the benefits of Ginkgolide B in migraine prophylaxis is also present in different studies which suggest it to have glutamate modulatory and anti-platelet activity.[50]

CONCLUSION

The positive actions of these phytochemicals in the prophylaxis and treatment of migraine are supported by research data therefore detailed human trials can be conducted to determine the efficacy of the phytochemicals in migraine treatment especially prevention of acute attacks. Adverse effects can be assessed using animal models first. These thorough studies can be proven beneficial for patients especially in reducing the frequency and intensity of migraine episodes.

AUTHOR'S CONTRIBUTION

SMNZ: Conception of work, Acquisition of data and supervision
MF: Substantial contribution in design
BA: Drafting article reviewing of article
IS: Reference writing and reviewing of article

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Case Report

KAWASAKI'S DISEASE

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ABSTRACT

Kawasaki Disease is a medium vessel vasculitis. It occurs commonly in children under 5 years of age and has a propensity of affecting the coronary arteries. The etiology and the pathogenesis of this disease are not known yet, but the major contributors are genetic factors, immune reactions and infections. The diagnosis of Kawasaki disease is based on clinical manifestations that appear on a temporal sequence. This disease is self-limiting in most cases, but it can lead to severe complications such as coronary artery aneurysms or thrombo-embolic events in up to 25% of the cases. Once the disease has been acquired prompt recognition and early treatment can be a lifesaver for the patients. Intravenous immune globulin (IVIG) and aspirin remained the treatment of choice for such patients.

Key Words: Coronary aneurysms, Lymph node, Vasculitis

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INTRODUCTION

Kawasaki disease (KD) is also known as "Mucocutaneous lymph node syndrome" as one of its clinical presentations is the swelling of lymph nodes and mucous membranes inside the mouth, nose, eyes, and throat. The incidence of KD in children under five years of age is reported in the United States as 25/100,000 children and has varied to approximately 250/100,000 children in Japan.

Kawasaki disease presents with acute febrile illness associated with systemic vasculitis. It affects young children mostly under 5 years of age and can be complicated by the development of, coronary artery aneurysms and other long-term cardiovascular sequels. An increased risk of developing Kawasaki disease is observed in Asian male children under 5 years of age.

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Japanese and Korean population has shown a high incidence of this disease as compared with global statistics. Seasonal increases in incidence have been observed in winter in North America.

CASE DESCRIPTION

A 6-year-old girl who was previously healthy presented in the Pediatric OPD of Farooq Hospital on 27th May, 22 with a 7 days history of high-grade fever and a 2-day history of arthralgia.

On the second day of her illness, she had left-sided cervical lymphadenopathy. A day after, a maculopapular rash appeared on her neck and trunk while sparing her extremities, which spontaneously resolved within two days. In addition, the patient had a bilateral conjunctival infection and dried, cracked lips, and swollen red tongue. During this period, she was given antibiotics, antivirals, and antipyretics; however, her symptoms were not resolved. The arthralgias were initiated in her ankle joint and then advanced in an ascending manner involving all the large and small joints impeding her ability to move. There was no significant history of dyspnea, orthopnea, peroxymal norturnal dyspnea

(PND), chest pain, or palpitations. She was referred to us for the evaluation of prolonged fever. Upon examination, the child was irritable, but alert, weighing 25kg and height of 124 cm (both above the 90th centile). Her vitals were in the normal range. She had bilateral congestion of the eyes without any discharge (conjunctivitis), along with dried lips and swollen tongue (strawberry tongue). The cervical node of 1.5 x 1.5 cm was also palpable unilaterally. A maculopapular eruption of rash on the neck and trunk was also observed. There were no positive findings of edema and erythema of the hand and feet and no perineal and periungual desquamation in this child.

On systemic review, no visceromegaly was observed, bowel sounds were audible, first and second Heart sounds were audible with no added sounds, her chest was bilaterally clear, and reflexes were elicited.

Her Laboratory investigations further revealed normochromic normocytic anemia with leukocytosis and neutrophilia. Her ESR and CRP levels were raised to 100min/1st hour and 288.5 mg/L respectively. The autoimmune antibodies came negative. Other labs were also insignificant. Upon arrival, the ECG showed sinus tachycardia that subsequently disappeared. Her echocardiography indicated an aneurysm in the left coronary artery.

After admitting to the hospital, she was treated with IVIG along with aspirin and clopidogrel for 2 days and after 48 hours the patient was clinically improved with major declines in her ESR and CRP levels i.e., 98min/1st hand 75mg/L respectively. Under observation for 5 days her examination findings became insignificant and she was discharged on aspirin and clopidogrel with the follow-up of repeat baselines and echocardiography.

DISCUSSION

Kawasaki disease (KD) is a multisystem febrile vasculitic disease of children described for the first time in 1974 by Kawasaki et al.[1] Along with rheumatic carditis Kawasaki disease is the leading cause of acquired cardiac disease in children.[2] A lot of geographical, seasonal, and ethnic variation is seen in KD.[3] Thus, several research articles suggest different underlying etiologies like viruses, genetic predispositions, and immune variations.[4] Till today autoimmune basis of KD has the most strong evidence for underlying etiological factors[5] supported by a high level of eosinophil and Interleukin5 at the site of lesions.

The major persistent impact of KD is on the heart of children in the form of coronary artery lesions (CAL). It ranges from fistula and dilatation to aneurysm formation in coronaries. Lesions may be transient for 6-8 weeks to permanent in nature depending upon the timing and appropriateness of treatment offered to the baby.[6]

Diagnosis of KD is still today is clinical one as suggested by the Japan circulation society (JCS) and the American heart society (AHA). Fever lasting more than 5 days is essential for diagnosis while conjunctivitis, cervical lymphadenopathy, oral mucosal lesions, and edema of hands and feet or peeling rash are other requisite for diagnosis.[7] In some patients, all 5 criteria are not fulfilled but the urgency of treatment demands labeling of diagnosis to avoid the impending complication of CAL. Such patients are labeled as Atypical KD or Incomplete KD.[8] AHA in such patients also recommends support of supplementary laboratory criteria for establishing the diagnosis. This includes serum albumin, platelet count, ALT, urine white cells, and anemia.[9] Fulfilling all essential 5 criteria is also not necessary if coronary changes are detectable at the time of admission.[10]

Our patient M age 6 years admitted with a high fever of 7 days and difficulty in moving about at the time of admission. She had eye congestion and cervical lymphadenopathy. Because of missing essential criteria help of echocardiography was taken which revealed aneurysmal dilatation of the left coronary artery. 100 mm ESR and 288 mg/L CRP supplementary supported for diagnosis. The introduction of treatment IVIG and aspirin

not only relieved her fever and body aches and pains but an indicator of inflammation also returned to normal.

Treatment of KD is debated regularly by different guidelines regarding the choice of drugs, time of therapy, and duration of therapy.[11] IVIG is the mainstay of treatment to avoid CAL. Earlier immunoglobulin (2gm/kg) was given to lessen the chance of developing coronary complications. Aspirin is very effective in subsiding inflammation and fever (70-100 mg/kg/day). Duration of aspirin therapy is variable but the dose should be reduced to a minimum as the fever and inflammatory markers reduce to normal.

CONCLUSION

Kawasaki disease is common in Asian Population. It affects male gender and children under five years of age.

AUTHOR'S CONTRIBUTION

NA: Critical review
MBA: Introduction writing
WZ: Discussion writing
MA: Introduction writing
ASA: Supervision and conception of idea

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