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The Evolving Face of Diabetes

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"Diabetes is a remarkable affliction, not very frequent among men… for the patients never stop making water, but the flow is incessant, as if from the opening of aqueducts… The patient is short-lived, if the constitution of the disease be completely established; for the melting is rapid, the death speedy. Moreover, life is disgusting and painful; thirst, unquenchable; excessive drinking, which, however, is dispro- portionate to the large quantity of urine, for more urine is passed; and one cannot stop them either from drinking or making water. Or if for a time they abstain from drinking, their mouth becomes parched and their body dry; the viscera seems as if scorched up; they are affected with nausea, restlessness, and a burning thirst; and at no distant term they expire. They thirst, as if scorched up with fire …, and the emaciation is dreadful; nor does any great portion of the drink get into the system, and many parts of the flesh pass out along with the urine"[1]

Aretaeus of Cappadoccia 130 BC

Although recognised for millennia, it is likely that the cachectic, polyuric and wasting form of diabetes described by ancient physicians was type 1 diabetes, in extremis. In the modern times, the overwhelming majority of diabetes is type 2 diabetes, with global numbers in hundreds of millions. Pakistan, with a diabetic population of 33 million, is now ranked third among the countries with the highest number of patients with diabetes in the world.[2]

Formal accounts of type 2 diabetes are hard to find in historical texts, probably because for most part of history, this form of diabetes, with its gradual onset and measured pace, wasn't considered a disease. The term "diabete gras" or fatty diabetes, was coined by Lancereaux,[3] a French pathologist, in 1880, but this did not become a clinically recognised entity until the first half of the 20th century. One of the earliest clinical accounts of what we today know as type 2 diabetes can be credited to Elliot Joslin, in his 1916 monograph "The Treatment Of Diabetes Mellitus With Observations Upon The Disease Based Upon Thirteen Hundred Cases" which he described the diet and lifestyle changes which are even today the core principles of management.[4] Even then, the diagnosis of diabetes was very different from today, and indeed a large number of people who are diagnosed with diabetes today would be considered normoglycaemic by the standards of mid-20th century. Diabetes, for the vast majority of physicians, was only about raised blood glucose, which left untreated, led to complications in kidneys, eyes and nerves. The primary treatment target was glycaemic control, and the main drugs in the diabetes armoury were insulin, and insulin secretagogues like sulfonylureas. The role of insulin resistance in the pathogenesis of type 2 diabetes gained recognition in the latter half of the 20th century,[5] leading to the development of drugs which targeted insulin resistance. This led to the development of metformin in the 70's, and the thiozolidinediones in the late 90's, drugs which addressed the core problem of insulin resistance, rather than merely treating the symptom, vis hyperglycaemia. Around the turn of the century, came the appreciation that type 2 diabetes is not a disease which occurs in isolation, but usually overlaps with central obesity, hypertension and coronary heart disease. This led to the concept of the "syndrome X", later renamed the metabolic syndrome, a co-existence of cardiovascular risk factors including central obesity, hypertension, dyslipidaemia and hyperglycaemia.[6] Diabetes was now a disease of the cardiologist as much as that of an endocrinologist. More and more previously distinct conditions are now coming within the ambit of diabetes, making it truly a cross-disciplinary disease. A striking example is our new understanding of the link between type 2

diabetes, non-alcoholic fatty liver disease (NAFLD) and polycystic ovary syndrome (PCOS), which were previously the sovereign domain of endocrinologists, hepatologists and gynaecologists separately. It is now recognised that the single theme which underpins all three is insulin resistance, and the manifestation of either multiple cysts in the ovaries or hepatic steatosis, is but a consequence of this underlying pathology. This has led to the American Diabetes Association recent recommendation for screening for hepatic fibrosis in all patients with type 2 diabetes[7]. Indeed, it is increasingly being recognised that the nomenclature of these conditions needs to be revised to reflect the critical role of diabetes and obesity in their pathogenesis. Nonalcoholic fatty liver disease has now been renamed metabolic dysfunction-associated steatotic liver disease (MASLD),[8] and it is proposed that PCOS should be renamed to multisystem reproductive metabolic syndrome.[9,10]

This paradigm shift in how we approach these diseases is also reflected in the current treatment strategies, with antidiabetic medicines standing shoulder to shoulder with the conventional system focused therapies. Furthermore, this has also led to an emphasis on developing diabetes drugs with advantages beyond mere glucose lowering. Medicines like sulfonylureas, meglitinides, and to some extent, dipeptidyl peptidase inhibitors, which have primarily glycaemic roles, are now being increasingly replaced with SGLT2 inhibitors and GLP1 agonists, which have shown to be potent agents with benefits which encompass not only diabetes but also chronic kidney disease, NAFLD and heart failure.

It is certain that the face of diabetes will keep on evolving, but one thing is becoming clear: diabetes is not just raised sugar in the blood. Our new understanding pictures diabetes as a continuum, with a considerable part of its journey undertaken before overt hyperglycaemia. A new nomenclature has been proposed for type 2 diabetes: dysglycaemia based chronic disease, or DBCD, with four stages. In this classification, Stage 1 is only marked by insulin resistance, manifesting as clinical stigmata including central obesity and acanthosis nigricans, and measurable by tests such as hyperinsulinaemic euglycaemic clamp or HOMA-IR. Stage 2 refers to non-diabetic range hyperglycaemia, presently known as prediabetes. Stage 3 marks what we currently call diabetes, with diabetic range values of fasting and post glucose hyperglycaemia. Stage 4 is the stage of vascular complications of diabetes.[11]

In conclusion, our growing understanding of diabetes as a multifarious condition with labyrinthine interactions with other metabolic disorders has completely transformed our management strategies. Two thousand years after Aretaeus first described diabetes in such graphic detail, we can truly say that diabetes is not merely a state of hyperglycaemia with osmotic symptoms. It is a more fundamental derangement in the body's milieu, with insulin resistance at its core, and like a hydra, it has many faces, and many tentacles which reach into every organ system in the body. The only way one can manage such a multifaceted condition is by adopting a multidisciplinary approach to target all its protean manifestations.

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Effect of Caffeine Consumption on Sleep Quality of Health Professionals of Multan

Bushra Shaheen,[1] Noaman Ishaq,[2] Nausheen Ata,[3] Hafiz Muhammad Imran Aziz,[4] Sadia Javaid,[5] Shahzad Gul[6]

Abstract

Objective: Objective of this study was to evaluate effects of caffeine intake on sleep quality of health professionals of Multan

Material and Method: This study was accompanied in pharmacology department of Bakhtawar Amin Medical and Dental College. Total 300 participants were equally divided in 03 groups: low caffeinated, moderate caffeinated and high caffeinated groups using caffeine intake questionnaire. Their sleep was evaluated using Pittsburgh sleep quality index. Data was entered on SPSS and scrutinized using chi square and post hoc tuckey tests.

Results: Mean of sum Pittsburgh sleep quality score of low caffeinated, moderate caffeinated and severe caffeinated was 10±3.184, 27±3.373, and 37.93±3.383 respectively. Intergroup comparison revealed with a p value of <0.01 all the time that depicts there are significant difference of sleep quality in all three groups.

Conclusion: Health professional that had no or low caffeine intake had better sleep as compared to professionals with moderate and severe caffeine daily intake. Similarly Moderate caffeine intake professionals had better sleep quality as compared to one who had daily high caffeine intake habits.

Key words: Caffeine intake, Health professional, Sleep quality

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Introduction

Caffeine, chemically known as 1,3,7-trimethylxanthine, is the most consumed psychoactive substance in the world. Mostly, it is obtained from cocao plant. Human body degrades caffeine into metabolites by specific hepatic enzyme system labelled as "Cyp P450". It has a half-life of about 2-10 hours in adult. The approximate global daily consumption of caffeine is 76mg. Caffeine containing products like tea, coffee, energy drinks as well as chocolates are consumed variably at national as well as global levels.[1] Data suggests that doctors and medical students have to exert extra effort beyond their mental threshold and physical health to cope with exams stress as well as excel in their career. Doctors and surgeons usually use caffeine to reduce fatigue, enhance alertness, improved performance and long-term memory.[2] But prolonged use of highly caffeinated products leads to addiction and intoxication.

Multiple clinical and experimental observations advocate adverse effects of caffeine consumption behavior on sleep pattern like marked reduction of duration of sleeping period, increase frequency of awakening during sleep period.[3] So, it is the need of time to study the relation between sleeping behavior and caffeine consumption in humans. Also estimate the safety window for caffeine consumption. A peaceful sleep is a blessing of God and is necessary for maintaining health at optimal condition. An adult human must take a sleep for not less than seven hours on daily basis.[4]

In addition to the recommended sleep period the quality of sleep should also be considered. Quality of sleep is measured by "parameters like latency to sleep initiation, sleep maintenance and feeling fresh and relaxed upon awakening".[5] Caffeine is found stimulatory in nature

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when its action is observed on neuronal tissue like central nervous system. Caffeine effect is associated with its interaction with adenosine receptor. Caffeine blocks the binding site on A2A adenosine receptor A2A.[6] There are many neurotransmitters that play key role in sleep, like melatonin, orexin and dopamine. Melatonin is a sleep inducer released by pineal gland under darkness or at night by retina while orexin is a peptide that remains at high levels at day times. Drug developers play with these physiological agents by enhancing or antagonizing their effects to produce a new drug like ramelteon and suvorexant. Neurotransmitter like dopamine production and release pattern are influenced by adenosine. Rise in dopamine levels (in corpus striatum) are associated with awakening and alertness of brain and vice versa for sleep. Caffeine increases dopamine receptors availability in the striatum.[7] Adenosine acts as an autonomous regulator of circadian clockwork by suppressing retino-hypothalamic tract activity in suprachiasmatic nucleus. Suprachiasmatic nucleus is a primary circadian pacemaker which is responsible for fluctuating sleep depth. Caffeine is an antagonist at binding site of adenosine, it produces a hype in neuronal cells of suprachiasmatic nucleus.[8,9] People consume caffeine is the form of black tea and coffee. Person consumes caffeine in between 195 to 390 mg per day remains mentally alert and free from sleep deprived symptoms. However, when levels of caffeine cross the threshold of 400 mg it produces palpitations, tachycardia and also change the sleeping behavior.[10] Quality of sleep is drastically decline after excessive quantity of caffeine is consumed in one shot specially when going to bed.[11,12] On the other no such worse impact on sleep quality has been observed in habitual heavy caffeine consumers specially during day time.

This study is designed to evaluate the relation between quality of sleep and caffeine consumed by personals of health department.

Material and Methods

This comparative transverse study was conducted in pharmacology department of Bakhtawar Amin medical and Dental college. Approval certificate was taken from ethical review committee of Bakhtawar amin medical and dental college (letter no.1476-23/E.C/BAMD&C). Study duration was 04 month (Aug-Dec 2023). Sample size was determined by evaluating research work on caffeine consumption in different regions.[13,14] A total 300 health professional that are working in multan city, between age group of 25-55 years and had a BMI of 18.5-24.9, were part of this study. Health professional with nicotine addiction or any psychiatric ailments were not part of this study. They were randomly divided in three group (n=100 in each group). Group I was included person with low caffeine intake (60 ± 30 mg/kg) mg/day while group II and group III were classified as moderate (145± 25 mg/kg) and high caffeine (350 ± 140 mg/day) intake groups. consumption had been estimated by average daily consumption of tea, coffee, carbonated drinks and chocolates by using Caffeine consumption questionnaire.[15] sleep quality of participants were evaluated by using Pittsburgh sleep quality index (PSQI), a standard questionnaire to evaluate sleep quantity and quality.[16,17] Data was evaluated using IBM SPSS version 23. PSQI was quantitative parameter that was analyzed through chi square test followed by post hoc tuckey test. The differences between two annotations were considered statistically noteworthy if the p value was identical or less than 0.05 ($p \leq 0.05$).

Results

Mean Age of group I, II and III was 35±0.25, 37±0.50 and 37±0.33 respectively. BMI of group I was 22.94 while BMI of group II and III was 22.56 and 23.70. when Pittsburgh sleep quality index was evaluated, mean of sum of low caffeinated was lowest with a value of 10±3.184. This value was quite low than 27±3.373, a value of mean of moderate caffeinated group. Group III, highly caffeinated group mean score was as highest as 37.93±3.383. Graphical representation of mean of all three groups is as displayed in diagram 01. Intergroup comparison of sleep quality index depicted p value of < 0.01 all the time that found a gross difference of sleep quality with increasing caffeine intake and this indicates that with the increase in the consumption of caffeine, PSQI scores also increase.

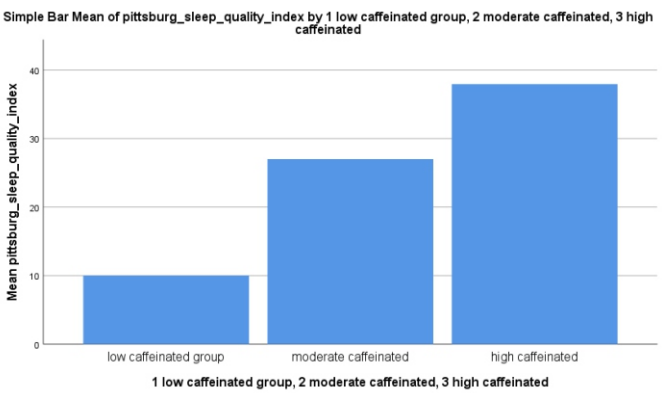

Figure 1: *Graphical representation of mean Pittsburgh sleep quality index score*

Discussion

Table 1: *Intergroup comparison*

Groups in comparison	P value
I and II	< 0.01
I and III	< 0.01
II and III	< 0.01

Statistics of this study revealed striking high intake of caffeine i.e. above ninety percent, among the health professionals of different disciplines of various hospitals and medical colleges of Multan. It also supports the statistics of regular consumption of caffeine among medical professionals. This observation is in accordance with a study performed in different institute.[18] Another exploration work based on survey conducted. showed intake of caffeine among participants were more than ninety one percent. [19]

PSQI scoring system was introduced to in this study and tried to discover the relation between PSQI scores and quantity of caffeine intake. Participants with high caffeine intake showed high PSQI score in comparison to participants with moderate to low caffeine intake. PSQI score for participants with moderate caffeine intake were much high in comparison to low caffeine intake participants. Similar observations were claimed by multiple teams of researchers in 2022.[20,21] In present study, rise in quantity of caffeine intake relates with surge in value of PSQI score. It also narrated intake of caffeine adverse the quality of sleep pattern among participants like latency to sleep and awaked midnight from a sleep. Even animal model designed to observe the relation between quantity of caffeine consumed and sleep quality showed similar relation in results.[22] Similar observations were shared in multiple surveys and studies conducted in different institutes.[23] Multiple mechanisms at molecular level are associated with caffeine that alters sleep patterns among participants. Caffeine pharmacologically antagonizes the adenosine leads to rise in activity of dopamine in striatum. Caffeine also possesses inhibitory effect on retino-hypothalamic tract that is associated with excitatory effect on suprachiasmatic nucleus.[24,25]

Above discussion shows a direct proportional relation between quantity of caffeine consumption and quality of sleep in participants. A multi-center study in future would provide a much better and bigger picture.

Conclusion

Sleep quality of health professional has direct influence of amount of caffeine they consume. Health professional that no or little caffeine user had better sleep as compared to professionals with moderate and severe caffeine consumption. Likewise Moderate caffeine intake professionals had better sleep quality as compared to one who had daily high caffeine intake routines.

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Authors Contribution

BS, NI: Conceptualization of Project
BS, NI: Data Collection
NA, SJ: Literature Search
HMIA, SG: Statistical Analysis
SG, SJ: Drafting, Revision
NA, HMIA: Writing of Manuscript

Perception of Medical Students Regarding Gender Discrimination in Their Learning Environment

Mahnoor Jathol,[1] Irem Rasheed,[2] Ameelia Sadaqat,[3] Amal Rana,[4] Rameen Arshad[5], Waleed Ahmad[6], Sadaf Saddiq[7]

Abstract

Objective: To evaluate perception of gender discrimination among students in medical colleges.

Methods and Materials: A descriptive study was conducted among 316 students in one public and one private medical college of Lahore from December 2023 to March 2024 through a self-administered questionnaire. Associations were determined between background variables and gender discrimination responses using chi-square test of independence with p-value <0.05.SPSS version 22 was used for data analysis.

Results: In our study female students voiced higher rates of dissatisfaction with their medical college experiencing unequal treatment in both lectures (p = 0.001) and in wards (p = 0.007).Female students perceived that there is disadvantage associated with having a child during education (p = 0.038) and expressed greater concerns about parenting during medical studies (p = 0.038). They reported getting lesser opportunities for advancement like applying for doctoral thesis as compared to male students. Both genders felt that females are given preferences in getting scholarships.

Conclusion: Gender disparities persist in medical college experiences, with female students reporting higher dissatisfaction, fewer opportunities, and unequal treatment. Initiatives are needed to address these disparities and promote gender equality in medical education.

Keywords: Gender discrimination, Gender inequality, Gender disparity.

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Introduction

Discrimination based on gender is a common civil rights violation that has many forms. Achieving gender equality is the number 5 goal of the United Nations' 17 sustainable development goals to be fulfilled by year 2030.[1]

Gender disparities continue to exist in various societies around the world and medical field is no exception. It is crucial to understand how individuals perceive and react to them, especially young people. Research has shown that gender-based abuse, denigration, and disrespectful attitude have a detrimental effect on students' and junior professionals' overall satisfaction with the educational experience and thus create an impact on their future careers. Mistreatment has been shown to have negative effects on learners, including post-traumatic stress disorder, depression, low self-esteem, burnout, and decisions on what specialty to pursue.[2-4]

Furthermore, even seeing the mistreatment of other pupils has a negative effect. Medical students report a greater prevalence of gender discrimination and sexual harassment compared to other students and academic staff. Men still hold greater positions of decision-making authority at medical institutions, despite the fact that women make up the majority of medical students.[5]

Dependence on supervisors among students is a known

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risk factor for gender discrimination and sexual harassment and makes students more susceptible to abuse at medical schools. Both sexes are impacted by these factors, but women in particular. One pathway for enhancing chances of resolution and preventing recurrences is to figure out how to increase students' reporting of these instances. A recent systematic review of studies from other countries, the majority of which were conducted in the United States, Canada, Pakistan, and the United Kingdom, revealed that between 49 and 68 percent of all medical students had experienced some form of GD while in medical school, with female students being more affected than their male counterparts. Male students primarily describe such events during their obstetrics and gynaecology training, whereas female students report such encounters across a range of specialties like orthopaedic surgery.[6,7]

The students painted a picture of a clinical setting where men and women were expected to behave differently and have distinct goals for their families and careers. Although it was supposed that female students would work hard, be conscientious, and be responsible, it was also believed that they would be insecure and put a lot of pressure on themselves. They thought that particular specialties were discouraged because it was frequently assumed that they would be the ones to care for the family and children. Men and women all throughout the world decide to oppose gender inequity and bias. These colleagues and collaborators address a wide range of civil action areas, from food security and sexual rights to economic development, political involvement, and health care. The findings of the study will not only contribute to the existing literature on gender studies but also provide vital insight that can help institutions to improve gender related policies and promote gender equality.

Materials and Methods

Our study was descriptive cross-sectional study which was conducted in one public and one private medical college of Lahore from December 2023 to March 2024. All medical students registered in third, fourth and final year MBBS classes of selected medical colleges. A sample size of 316 students were calculated with conve-nient sampling technique. A self-designed questionnaire was used and its validity and reliability was determined by a sample run.

After the approval of synopsis, the subjects who gave consent were included in the research. The questionnaire was composed of two sections. The first section contained background information of medical students and second section contained questions to assess their perception regarding gender discrimination. SPSS version 22 was used for data analysis. Data is presented as tables and graphs. Frequencies and percentages were given for categorical variables. Associations were determined between background variables and gender discrimination responses using chi-square test of independence. P value of less than 0.05 was considered significant. Verbal consent was taken from study participants ensuring confidentiality. IRB\ERC approval was obtained from Lahore medical and dental college before start of study. Permissions from other institutions were also taken. Confidentiality and anonymity was not breached at any stage. Moreover names of institutions are not to be mentioned.

Results

The study delved into the demography of medical students concerning gender discrimination in the learning environment, revealing a balanced distribution between male (47.5%) and female (52.5%) participants. These students, with an average age of 21.50 years and predominantly from urban backgrounds (88.3%), represented various stages of their medical education, with the majority in their 3rd (47.5%), 4th and 5th (34.2%) year. While approximately half of the participants were day scholars (51.3%), the rest resided on campus. Eighty eight percent students belonged to urban background.

Table 1: *Demography Of Medical Students Regarding Gender Discrimination In Learning Environment*

Parameters	N	%
Gender		
Male	150	47.5
Female	166	52.5
Age (years)		21.50±1.82 (18-27) years
Class		
2nd year	1	0.3
3rd year	150	47.5
4th year	57	18.0
5th year	108	34.2
Day Scholar		
Yes	162	51.3
No	154	48.7
Background		
Rural	48	11.7
Urban	361	88.3

In analyzing gender disparities within the medical college experiences, notable differences emerged. Female students voiced higher rates of dissatisfaction with their medical college experience compared to males (p = 0.034). However, perceptions of gender equality within the college did not significantly differ between male and female students (p = 0.670). Nonetheless, concerning opportunities for advancement, females reported significantly fewer perceived opportunities compared to their male counterparts (p=0.002). Similarly, female students perceived that there is disadvantage associated with having a child during education (p = 0.038) and expressed greater concerns about parenting during medical studies (p = 0.038).

Table 2: *Gender Disparities in Medical College Experiences: A Comparative Analysis*

	Gender		
	Male	**Female**	**p-value**
Student Satisfaction at Medical College			
Very dissatisfied	19	21	.034
Dissatisfied	27	18	
Neutral	40	62	
Satisfied	38	50	
Very satisfied	26	15	
Gender Equality in Medical College			
Yes	85(26.8%)	98(31%)	.670
No	65(20.5%)	68(21.5%)	
Opportunities for Advancement Based on Gender			
Male	8 (2.5%)	6 (1.8)	.002
Female	76 (24%)	117(37%)	?
Not Sure	27 (8.5%)	24 (7.5%)	
Disadvantages of Having a Child During Education			
Yes	88(27.8%)	92(29.1%)	.038
No	45(14.2%)	26(8.2%)	
Concerns About Parenting During Medical Studies			
Never	88	92	
Rarely	45	26	.038
Observations of Unequal Treatment in lectures			
Never	55(17.4%)	87(27.5%)	.001
Rarely	39(12.3%)	50(15.8%)	
Often	56(17.7%)	19(6%)	
Observations of Unequal Treatment on Wards			
Never	57(18%)	94(29.7%)	.007
Rarely	35(11%)	29(9.1%)	
Often	58(18.3%)	43(13.6%)	
Perception of Gender-Based Poor Treatment			
Yes	53(16.7%)	34(10.7%)	.003
No	97(30.6%)	132(41.7%)	
Ease of Applying for Doctoral Theses by Gender			
Male	74(23.4%)	135(42.7%)	<.0001
Female	76(24%)	31(9.8%)	
Preference in Scholarship Applications			
Male	52(16.4%)	111(35.1%)	<.0001
Female	98(31%)	55(17.4%)	

Furthermore, female students reported experiencing unequal treatment in both lectures (p = 0.001) and in wards (p = 0.007) more frequently than males. Interestingly, while male students perceived experiencing gender-based poor treatment more frequently (p = 0.003), they found it significantly easier to apply for doctoral theses compared to females (p < 0.0001). Additionally, both male and female students displayed significant differences in being preferred for scholarships (p < 0.0001).Both genders felt that females are given preferences.

Discussion

Total no. of students in our study was 316 out of that 52.5% were females and 47.5% were males. Mean age was 21.5±1.82(18-27 years). In our study 40.7% of medical students were satisfied, 23.4 were dissatisfied as a student in their college while 32.2% were neutral. Out of satisfied students 20.2% were male students and 20.5% were females. In Suez canal university the overall mean score of DREEM was also more positive than negative, with females perceiving the educational environment more positive than males.[8] A total of 57.8% students believed that there is gender equality in their institute and 42.2% thought otherwise. Out of 57.8% students who perceived their institute's environment to promote gender equality 26.8% were males and 31% were females. While the majority of both male and female students believed their medical college promotes gender equality, the difference is not statistically significant (p-value 0.670). In Brazil a cross-sectional study on gender-discrimination showed females disproportionately affected (77% vs 22% of men).[9] Presents a stark difference from our study group.

Another study conducted in Pakistani medical colleges, it was found that 30.8% of medical students have even faced gender based violence.[10]

In terms of better opportunities for advancement, 24% male students think that females are privileged and 37% females perceived that females are given more opportunities. A study conducted in Jordan showed female medical students were more likely to perceive that their gender (t(634) = 3.58, p < 0.001) and people's perception of their gender (t(634) = 4.25, p < 0.001) are barriers to

their career advancement.[11] Interestingly out of female students 70.4% believe that they have an advantage in terms of career opportunities but when it comes to their male counterparts such level of satisfaction is not seen. Only 5.3% male students think they receive better opportunities based on their gender.

It is a general perception that having a child during career building years is a disadvantage specially to females in underdeveloped country like ours. Majority (56.9%) of our students thought the same. Out of these 27.8% (88) were males and 29.1% (92) were females. Female students felt that having a child gives them disadvantage during medical education with a statistically significant difference (p-value 0.038). A study conducted in Toronto, Canada interviewed 23 females and 11 males, students highlighted that childcare during residency is a substantial source of stress to balance with medical career due to unpredictable schedules and financial burden too.[12] Out of 316 students 75 students thought that there is unequal treatment based on gender in their college lectures, out of these 6% were females and 17.7% were males. While 27.5% of females and 17.4% of males perceived that there is no discrimination with a p-value of 0.001. When it comes to the clinical side/wards 58% males mentioned unequal treatment in wards whereas only 43% females reported unequal behaviour. Out of 316 students 57 male students and 94 female students said they never face any discriminatory behaviour in wards with a p-value 0.007 which is statistically significant. A study in England taking large majority of students contrary to our study reported teaching staff privilege male students by focusing on their clinical teaching and giving direct comments based on stereotypical assumptions of females having families later in life. Female students are labelled as nurses by patients.[13] Male students are more likely to experience gender-based poor treatment, with a statistically significant difference (p-value 0.003).

According to our study 81.3% of female students think that males get more opportunity for postgraduation/ doctoral thesis and 51.7% of male students % males think that females have an advantage over males in this regard. If we consider all students 42.7% females and 23.4% males are of the opinion that males have an edge when it comes to be chosen for doctoral thesis/ research projects and this highly statistically significant (p-value <0.001). In a study of data reveal large gender inequality in obtaining a doctoral degree with a cum laude distinction with 6.57% of all PhD male students compared to 3.68% of all female PhD students.[14]

In our study 35.1% females had the perception that males are preferred when it comes to awarding scholarships and 16.4% males think the same (p-value <0.0001). Out of females with 66.8% females thought males are preferred while nominating for scholarships and interestingly 65% of male students think that females are preferred over male students. In comparison A study of Leiden university showed that the number of male students applying for scholarships is more as well as the number of male students finally receiving the grant is also higher. among students applied for LISF student grant has 2 findings i-e males apply more than females, second it was investigated whether the success rate of receiving a LISF grant differed between male and female applicants, by performing a binomial GLMM with gender ASA fixed factor and the decision as dependent variable, with year added as a random factor (Sd = 0.501). As expected, the likelihood of receiving a grant was somewhat higher for male applicants than for female applicants (b Gender = −0.176, z = −1.96, p = 0.049).[15]

Conclusion

Overall, these findings underscore the existence of gender disparities in various facets of medical college experiences, from opportunities for advancement to concerns about parenting and perceptions of unequal treatment. Addressing these discrepancies is imperative for fostering a more equitable and supportive learning environment within medical colleges.

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Authors Contribution

AS: Conceptualization of Project
AR, RA, WA: Data Collection
MJ, AS, IR: Literature Search
SS, IR, MJ: Statistical Analysis
MS: Drafting, Revision
MJ: Writing of Manuscript

Assessing the Level of Digital Health Literacy in Health Care Professionals in a Tertiary Care Hospital

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Abstract

Objectives: This study aimed to assess the digital health literacy of the healthcare workers in a tertiary care hospital.

Material and Methods: The study was conducted in Mayo Hospital, Lahore for duration of six months. It was a cross-sectional study. Healthcare practitioners, including medical doctors, nursing staff and other allied health professionals were included in the study. Following the ethical approval & informed consent, data was collected using a standardized pre-designed questionnaire the Digital health literacy instrument (DHLI). Statistical analysis was done using the SPSS 26.

Results: A total of 285 healthcare workers participated in the study. 69.1% were doctors, 30.9% were nurses and allied health professionals. Healthcare workers had desirable levels of skills in various domains of Digital health literacy instrument (DHLI) including navigation skills, protecting privacy, operational skills, information search and adding content. Scores were comparable between the physicians and the allied health professionals.

Conclusions: With the changing global environment and wide availability of online healthcare related information and applications, it is important to have the necessary skills to avail these opportunities. The results from our study show the health care professionals have good literacy related to digital health resources. A desirable level of DHL can help the health care workers to improve their patient care, communication skills and health literacy of their patients.

Keywords: Digital health literacy, health care providers, operational skills, protecting privacy, information searching.

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Introduction

The role of Digitization in health care is growing steadily in the last few decades. The global health environment is rapidly changing, and a lot of health-related information is available online. To make use of these opportunities doctors should have the necessary knowledge and skills related to these digital resources; these skills are called digital health literacy (DHL). Digital health literacy refers to the capability of individuals to read, understand, and use digital technologies and online health information effectively, to make their health related decisions.[1] In today's technology-driven world, where the internet and digital tools play a significant role in healthcare, digital health literacy is becoming increasingly important. It encompasses skills such as evaluating the credibility of health websites, navigating online health resources, utilizing health apps, and understanding electronic health records. Developing digital health literacy empowers individuals to take charge of

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their health, communicate effectively with healthcare providers, and make informed decisions about medical treatments and interventions. It is a cost-effective solution for emerging health challenges, especially in low- and middle-income countries.

Different instruments have been developed to assess the level of digital health literacy e.g., Digital health literacy instrument and e-health literacy instrument (e-HEALS). Results from previous studies show an ade-quate level of knowledge and information regarding DHL among different groups, i.e., students, patients, and healthcare workers.[4,5,7] Different factors influence the level of digital health literacy in a population. These include age, gender, and ethnicity. It has also been found to be positively associated with the level of education.[3,4]

A study conducted in various universities in Lahore showed that digital health literacy was related to the level of usage, educational status, and level of skill.[4]

Digital health literacy among healthcare professionals is paramount in the modern healthcare system. As technology continues to transform the way medical information is accessed and shared, healthcare professionals need to be adept at using these digital tools effectively. Desirable levels of digital health literacy in health care professionals can help in patient management and also influence the digital health literacy level of the patients. A study conducted in Iran using a digital health literacy instrument (DHLI) showed that healthcare professionals had adequate skills in various domains of DHL including navigational and operational skills and protecting the privacy of the patients.[5] There are various barriers to the digital heath literacy including inefficient skills in users, online scams, unconfirmed resources of information and power crises in low-income countries such as Pakistan.[6] There have been very few studies related to the digital healthcare related literacy in different population groups in Pakistan so far.

Materials and Methods

This cross-sectional study was conducted at Mayo Hospital, Lahore and involved healthcare professionals, including medical doctors and allied health practitioners. Non-probability sampling technique was used to recruit the participants. All the participants who were health care providers, were working at Mayo Hospital, Lahore at the time of study and gave consent for participation were included in the study. Data was collected after the ethical approval from the institutional review board (vide letter no 112/RC/KEMU, dated 28-2-2023) for a period of six months (1-03-2023 to 31-08-2023). Informed consent was taken from participants and data was collected using a standardized questionnaire known as the Digital Health Literacy Instrument (DHLI) to assess digital literacy levels.[2] Permission to use the questionnaire was obtained from the author of the questionnaire via email. Demographic information including the gender, age, specialty and level of education were collected from the participants. Questions related to frequency of internet usage, device preference and reason for use of internet were included in the questionnaire as well. The second part was comprised of 21 questions covering seven main categories of the digital health literacy instrument. All items were scored on a 4-point likert-type scale. The categories include operational skills, information searching, navigation skills, evaluating reliability, determining relevance, adding self-gene-rated health content, and protecting privacy. Descriptive analysis was done using the SPSS version 26. Mean scores were calculated for all the categories of DHLI. Mean score of different groups was compared using the Independent Sample T-test.

Results

350 questionnaires were circulated among the hospital staff of Mayo Hospital. Out of 350 questionnaires, 292 were filled and returned by the study participants, and 7 incomplete forms were rejected. After removing the incomplete forms a total of 285 participants were included in the study. 197(69%) were doctors and 88(30.9%) were nurses and allied health professionals. 125 (43%) were male and 160 (56.1%) were females. The mean age of respondents was 30.49 years(S.D= 6.88). the table 1 shows the demographic characteristics of all the participants. Table 2 shows the pattern of internet usage among the participants. 80% of the participants reported using the internet 'almost every day'. Only one participant reported having 'almost never' used the internet services. Mobile Phone was found to be the most used device, followed by laptop, whereas public devices such as computers were least preferred by the participants. Most of the participants (53.1%) self-rated their internet skills as 'Good'. Various health-related reasons to use the internet were also assessed. Information searching for health and various disorders was the most reported reason for internet usage. Descriptive statistics of different categories of DHLI are shown in table 3. The Inde-

pendent T-test, used to compare the DHLI mean scores, showed significant statistical difference (p value <0.05) between the two genders for one variable only i.e. the mean score for 'determining data relevance' was higher in males. The T-test showed no significant statistical difference (p value<0.05) between the two participant groups(doctors and nurses/allied health professionals). The ANOVA test suggested statistical significance (p value<0.05) between the mean DHLI scores of few variables and level of education and work experience. The result was significant for 'adding content' and level of education, and 'adding content' and 'navigational skills' and years of work experience.

Discussion

A high level of digital health literacy enables healthcare professionals to provide accurate information to patients, collaborate efficiently with colleagues, make well-informed decisions, and support the integration of technology into clinical practice. This study aims to assess the level of digital health literacy in doctors and paramedical staff.[8]

The results of our study show that nearly all healthcare

Table 1: *Demographic features of study participants:*

Category		number of participants (n)	Percentage (%)
Gender	Female	160	56.1%
	Male	125	43.9%
Age (years)	30.49 years (S.D=6.88)		
Work Experience	Less than 5 years	147	51.6%
	5-10 years	89	31.2%
	11-15 years	22	7.7%
	16-20 years	19	6.7%
	More than 20 years	8	2.8%
Designation	Doctors	197	69.1%
	Nurses and allied health professionals	88	30.9%
Specialty	Medicine and allied	161	56.5%
	Surgery and allied	86	30.2%
	Basic sciences	38	13.3%
Level of education	Diploma	45	15.8
	Bachelors	25	8.8%
	Masters or higher	16	5.6%
	MBBS	90	31.6%
	Post graduate qualification	107	37.5%
	others	2	0.7%

Table 2: *Internet usage among the participants.*

Category	N (number of participants)	Percentage
Frequency of Internet use		
(Almost) every day	230	80.7%
Several days a week	37	13%
About once a week	17	6%
(Almost) never	1	0.3%
Means of Internet access		
Mobile phone	263	92.3%
Laptop	69	24.2%
Computer device at home	7	2.5%
Tablet	13	4.6%
Work related computer	7	2.5%
Public computer	1	0.4%
Self-rated Internet skills		
Excellent	45	15.8%
Good	152	53.3%
Average	75	26.3%
Reasonable	10	3.5%
Poor	3	1.1%
Number of respondents who have ever used the Internet to…		
Information search on health and illness	281	98.6%
Appointment scheduling with a health care provider	144	50.5%
Read/post a health-related review on internet	200	70.2%
Use any health-related application	195	68.4%
Query about their healthcare provider	117	41.1%
Monitoring symptoms of disease	191	67%
Sharing personal health related information	127	44.6%
Accessing your own medical record.	94	33%
Take an online self-management course	83	29.1%
Post a message on a health related forum or social media platform.	112	39.3%

Table 3: *Mean of different variables of DHLI questionnaire.*

Variables	Percentage of total score	Mean ±S.D	Interpretation
Protecting Privacy	83.80	10.0526 ± 1.73	Very Desirable
Operational Skills	65.6%	7.8702 ± 2.09	Desirable
Navigational skills	74%	8.8912 ± 2.76	Desirable
Adding Content	73%	8.7614 ± 2.08	Desirable
Determining Data Relevance	77.9%	9.3474 ± 1.97	Desirable
Evaluating Data	73.5%	8.8314 ± 2.18	Desirable
Information Searching	81%	9.7263 ± 2.41	Very Desirable

professionals rely on digital resources for health-related information daily or several times a week. This aligns with earlier research; for instance, a study conducted in the UK demonstrated that approximately 81% of primary healthcare personnel utilize electronic health resources.[9] Similarly, a survey in Ethiopia found that 60% of healthcare professionals exhibited a high level of e-health literacy.[10,11] Since more than 90% of our country's population has access to mobile phones, it was the most used device for health information search in our study.[12,13]

Scores on the Digital health literacy instrument (DHLI) showed that healthcare professionals have desirable or very desirable scores on all the domains of the instrument. It is a known fact that health literacy is related to the education level of the individuals.[6,14] Since healthcare professionals such as nurses and doctors, have a college degree or higher, they are expected to have good health literacy as well. Moreover, easy access to online health resources may also contribute to a better digital health literacy as well. A study conducted in Iran showed that physicians have higher scores on DHLI compared to allied health professionals (nurses and other paramedical staff).[15,16] Our study, however, showed comparable scores on DHLI between medical doctors and allied health professionals.

In digital health care, ensuring the privacy and security of data is not only a legal and ethical obligation but also vital for maintaining patient trust, maintaining successful doctor-patient relationships, and promoting the widespread adoption of digital health solutions.[17,18] HCPs in our study have a desirable score on 'protecting privacy' on the DHLI. Operational and Navigational skill item scores also showed desirable levels among the health care professionals. As the trends are shifting more and more towards digital healthcare, having strong operational and navigational skills among healthcare professionals is crucial for improving patient care, optimizing management plans, and staying up to date with technological developments.[14,19] The skills to search the online sources efficiently, evaluate their credibility and find relevant information empower the health care professionals to stay informed about health conditions, treatment options, and preventive measures, enabling them to make decisions about their health and the health of their patients in the digital age. Scores on 'Information search' showed a 'very desirable level' of digital health literacy among the health care professionals. The variables 'Evaluating Data, ' 'adding content', and 'determining data relevance' were also scored in the desirable range by our study participants. This study had some limitations. Being a cross-sectional study, it does not help establish causal relationships based on the results. Furthermore, the research was confined to a single healthcare facility and relied on convenience sampling for participant recruitment which limits the generalizability of our results. Additionally, digital health literacy was assessed using a self-administered scale instead of an objective assessment tool. Nevertheless, despite these limitations, the study recruited a substantial number of participants and gathered data through a standardized questionnaire.

Conclusion

Our findings suggest that healthcare professionals including doctors and allied health professionals have a high level of digital health literacy, possibly due to their higher educational level and frequent utilization of the internet for e-health resources.

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FN: Conceptualization of Project
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Validity and Reliability of 'Objective Structured Assessment of Technical Skills' (OSATS) for Vaginal Delivery

Dr. Jaweria Faisal

Abstract

Objective: To assess validity and reliability metrics of objective structured assessment of technical skills (OSATS) for assessing the competence of technical skills of vaginal delivery

Materials and Methods: Co-relational reliability study, the validation study was conducted at the Department of Obstetrics and Gynaecology of Akhtar Saeed Medical College and Farooq Hospital Rawalpindi from April to December 2023. The study comprised nine candidates at different training levels (LHV, staff, postgraduate trainees & senior registrars), who were evaluated on OSATS by 8 evaluators over 54 NVD observations. Cronbach's alpha and intraclass correlation coefficient (ICC) were used to estimate reliability metrics of OSATS scores. The construct validity; that OSATS can measure and reveal the underlying construct, was determined by three shreds of evidence; evidence of content, response process and internal structure, and evidence of improvement in performance scores as the procedure-specific experience accrues with OSATS using linear mixed model and evidence of the discriminatory power of the tool between various levels of the training program, with one-way ANOVA.

Results: The OSATS tool demonstrated good reliability metrics with Cronbach's alpha of 0.897 and good inter-rater reliability calculated by intraclass correlation coefficient as 0.801. OSATS was validated as examinee scores improved showing gradual progression on performance curves with succeeding OSATS encounters. Validation also yielded that the tool can differentiate among different training levels with a p-value of 0.019

Conclusion: The OSATS tool was found to be valid and reliable for assessing the competence of technical skills of vaginal delivery.

Keywords: Cronbach's alpha, Validity, OSATS, Reliability, Workplace-based assessment.

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Introduction

Acquisition of technical procedural capabilities must ensue parallel to the attainment of knowledge and professionalism. Assessment drives learning.[1] In competency-based education, workplace-based assessment (WPBA) has gained importance as it demonstrates that professionals are making appropriate progress through a structured program of specialty training.[2,3] Objective Structured Assessment of Technical Skills (OSATS) is a WPBA, where series of standardized procedural tasks are measured.[4]

The OSATS was first introduced by surgical educators at the University of Toronto for formative assessment.[5] Since then OSATS, has demonstrated high validity and reliability metrics in technical skills assessment.[6,7,8] OSATS is being used, by the Royal College of Obstetrics and Gynaecology for various assessments but OSATS for normal vaginal delivery is not available anywhere. One study has been done for OSATS for vertex delivery at the University Hospital of Nice but that form hasn't

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been acquired by accreditation bodies.[9]

Mostly, normal vaginal delivery (NVD) is learned by the master-apprenticeship model, by observation and assisting, gradually becoming competent to perform it independently; and assessed subjectively by looking in Logbooks. Surgical and allied Disciplines have historically lacked objective assessment for technical skills of learners.[4] Whenever assessment is largely subjective and base heavily on preceptor ratings, it carries low reliability.[2] To ensure the technical proficiency of trainees on NVD, we crucially need an assessment tool that is capable of providing objective scores to evaluate this predefined competency and ratify its proper training, before doctors are allowed to perform it unsupervised.[9] OSATS permits objective structured assessment of technical skills on a global rating scale, but teachers are also not sensitized with this new techniques.[3,10]

In my research, modified Delphi's method was used to design OSATS tool, a set of 20 items of six responses with descriptors that could measure the underlying construct, the educational achievement, that candidates are competent to perform safe normal vaginal delivery. The rationale of this study was to establish its scores validity and reliability metrics so that a standardized objective tool would be available for enhancement of training. It may be incorporated in curriculum of midwives, and doctors for formative assessment and revalidation of NVD skill to improve patient care

Patients and Methods

After taking approval from Akhtar Saeed Medical College and Farooq Hospital Rawalpindi Institutional Review Board Ref. No. RAC&IRB-05/04/2023, the study was conducted at the Department of Obstetrics and Gynaecology. Inclusion criteria: All candidates; LHV, staff, postgraduate trainees (PG) & senior registrars (SR), who were already conducting NVD in low risk mothers, were briefed about the study proposal so that they could give an informed consent to participate. A total number of 54 patients, who had signed the inpatient consent form, were included in the study. Sampling techniques was non probability convenience sampling. Any delivery in high-risk woman with comorbidities was excluded. All candidates were pre trained on NVD skill by using OSATS that was freely available in labour ward. The candidates selected the time of WPBA with OSATS with assessors provided they both agreed. For data collection, while conducting the delivery, each candidates was observed by two assessors simultaneously. They both rated the performance of candidates on NVD skill with OSATS form on rating scale, independent of each other. All assessors were voluntary pre-trained consultants. This study was not duration limited, it finished when all candidates had taken six OSATS. The immediate feedback covered strength and suggestions for improvement. Detailed psychometric analysis was done to assess OSATS construct validity and reliability. Cronbach's alpha, was calculated from average (Mode) of the 6 response of each candidate's OSATS score. The standard error of measurement to create confidence bands around observed scores was used to indicate the precision of measurement. For estimating inter-rater reliability, Intraclass correlation coefficient was used. To establish the caseload, all consecutive OSATS of each candidate were numbered. OSATS scores has capacity to reveal and prove the underlying construct, the construct validity; was determined by three evidences. Evidence of content, response process and internal structure. Evidence of improvement in performance scores as the NVD experience increased with OSATS. To see progression, learning curves for each candidate was mapped by plotting her OSATS scores against the total procedure-specific caseload, during total time till all OSATS are signed off, using Linear mixed model. Evidence of the discriminatory power of the tool between various levels of the training program, using one-way ANOVA with level of training program as the independent variable and the examination scores, performance, as the dependent variables. All scores were entered in Statistical Package for Social Sciences (SPSS 26) datasheet for analysis.

Results

Nine candidates were evaluated by 8 evaluators over 54 NVD observations with OSATS. Cronbach's alpha based on standardized Items (n= 20) was calculated as 0.897. Intraclass Correlation Coefficient came as 0.801 (0.737-0.861) as the random error in the responses are very low, the 95% confidence bands of each item shows a very close difference. All items correlated positively with each other. The mean ratings on each item ranged from 3.09 to 3.74 with 'documentation' getting the least and 'positions the patient' being awarded the highest mean score.**(Table-I)** As validity evidence, performance curves showed gradual progression as candidate's accrued experience with OSATS on specific skill. Candidates learned, removed their mistakes, gradually performed better and scored highest in their last attempt. (Figure 1). The result showed that senior regis-

trars (SR) took a very high OSATS score as compared to the others with a p value 0.019. (Table II). Although the average OSATS score of all candidates differ slightly, p value 0.126, but their pair to pair comparison showed that senior registrars were way better than staff, LHV and post graduate trainee with significant P value 0.003

Table 1: *Descriptive item analysis of OSATS questionnaire*

No.	Items	Mean	Confidence interval	
			Lower	Upper
1	Patient doctor communication	3.50 ± 1.34	3.13	3.87
2	Positions the patient	3.74 ± 1.26	3.40	4.09
3	Infection control	3.31 ± 1.40	2.93	3.70
4	Bladder emptying	3.30 ± 1.27	2.95	3.64
5	Fetal heart monitoring	3.46 ± 1.28	3.11	3.81
6	Lignocaine perineal infiltratic	3.43 ± 1.42	3.04	3.81
7	Medio-lateral episiotomy	3.46 ± 1.41	3.08	3.85
8	Perineal phase management	3.61 ± 1.32	3.25	3.97
9	Positions hands on head	3.57 ± 1.16	3.26	3.89
10	Control extension	3.26 ± 1.31	2.90	3.62
11	Uterotonic	3.52 ± 1.30	3.16	3.87
12	Cord cutting	3.57 ± 1.42	3.19	3.96
13	Placental separation	3.61 ± 1.37	3.24	3.98
14	Controlled cord traction	3.13 ± 1.29	2.78	3.48
15	Placental inspection	3.46 ±1.31	3.10	3.82
16	Trauma evaluation & repair	3.37 ± 1.09	3.07	3.67
17	Handling of instruments/ knots	3.11 ± 1.28	2.76	3.46
18	Hemostasis and tissue respect	3.28 ± 1.12	2.97	3.58
19	Count completed	3.26 ± 1.14	2.95	3.57
20	Documentation	3.09 ± 1.19	2.77	3.42

Table 2: *Comparison of OSATS score with respect of candidate and training program*

Characteristic	Category	Mean ± Standard deviation	P value
Candidate	LHV	56.50 ± 18.13	0.126
	SR	82.50 ± 29.08	
	SR	83.33 ± 22.55	
	PG 3	74.50 ± 26.49	
	PG 3	74.33 ± 23.95	
	PG 2	59.50 ± 18.36	
	PG 2	63.67 ± 23.69	
	Staff	49.33 ± 16.00	
	LHV	68.83 ± 16.46	
Training program	Staff	49.33 ± 16.00	0.019
	LHV	62.67 ± 17.72	
	PG	68.00 ± 22.75	
	SR	82.92 ± 24.81	

Table 3: *Post ANOVA comparison of OSATS score with respect of training programs*

Candidate 1	Candidate 2	Difference of mean between candidate 1 and candidate 2	P value
Senior Registrar (SR)	Staff	33.58	0.003*
	LHV	20.25	0.026*
	PG	14.92	0.057
Post-Graduate	Staff	18.67	0.065
	LHV	5.33	0.489
LHV	Staff	13.33	0.224

(Table III). This shows that instrument is valid and can discriminate well between candidates of varying learning levels.

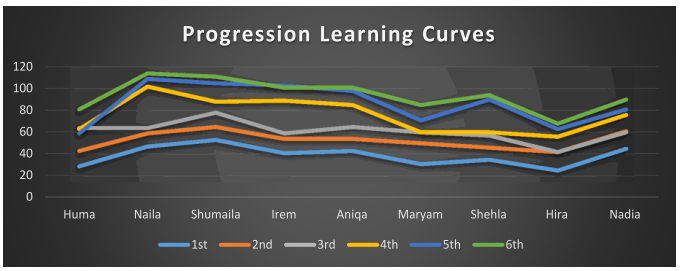

Figure 1: *Progression learning curve.*

Discussion

An assessment tool is a set of items that can measure the underlying construct.[11] Reliability is prerequisite of validity and refers to true variance in the ability of the students. The goal of psychometric analysis is to estimate and minimize, the error variance (E), so that the observed score X is a good measure of the true score.[12] Reliability coefficients, Cronbach's alpha estimate measurement error in assessment data, '0' indicate no reliability and '1' indicate no measurement error with perfect reliability.[12, 13] The Cronbach's alpha of our questionnaire was sufficiently high (0.897). Likewise Carsuzaa F et al., reported Cronbach's alpha 0.9 in a validation study of French version of OSATS.[14] If assessment scores are not reproducible with great level of certainty, the accurate interpretation of the test scores becomes questionable and validity evidence to support or refute the assessment is compromised. A study by Fouillen KJ et al., for validation of OSATS, established its construct validity with good internal consistency and inter-rater reliability, its finding are comparable to my findings.[15] Reliability also depends on number of items, sample size, observations, rating scale, objectivity and standardization.[16] In my study, for standardi-

zation and objectivity, every candidate was tested on the same skill of NVD as WPBA, with the same items, OSATS, and by the pre-trained examiner according to the same criteria of rating scale with descriptors and scoring rubric.

The largest threat to the reproducibility is rater inconsistency. Intraclass correlation coefficient (ICC) determines if items can be rated reliably by different raters.[17] The ICC '0' indicates no reliability among raters and '1' means perfect reliability. My study showed good rater consistency with ICC score (0.801- 0.988). This compares well to a research carried out by Maybodi FR et al., on applicability of OSATS (ICC = 0.99), where no significant difference was found between the checklist scores of the two raters.[18] In a validation study of OSATS by Schmidt MW et al., excellent inter-rater reliability by ICC was calculated as (0.923–0.924, $p < 0.001$).[6]

Construct validity that tool can measure which it intends to measure, is the whole validity, it requires different sources of evidence; content, response process and internal structure. Content validity was ensured by obtaining assessors agreement for OSATS items. This tool is representative of our demographic needs and represent curricula of doctors and staff, as the skill of normal vaginal delivery is taught to them by going through the four training levels; observation, to independent performance. The internal structure of the OSATS demonstrated good evidence of homogeneity in the items with a positive correlation. This OSATS validation study showed good discrimination between performances of candidate on NVD skill at different stages of training. Highest scores were taken by senior most candidates, indicating that OSATS based decisions were accurate. Likewise, Ramazani F et al., argued that increasing scores on OSATS correlate well with level of training.[19] Results also depicted a significant improvement in every candidate's performance on OSATS with increasing procedural experience giving another evidence of construct validity. This is consistent with reports from other OSATS researches stating that an increased experience and training, leads to better competency.[19,20]. Validity evidence of this study correlate well with many international studies where OSATS is considered the best tool.[21] Navrazhina K et al., reported significant correlation between trainee's scores and year of training. They also reported increasing scores on rating scales ($P = .003$) and checklist ($P = .04$) with more advanced procedural training with OSATS.[22] In another study by Chavescampos ME et al., for the construct validation, OSATS scores showed the capacity to differentiate the performance between experts and beginners as in my research.[23] Response process: For, fit for purpose assessment tool construction, its domain, purpose, and construct was defined. For OSATS instrument generation modified Delphi's model was used. With given reliability and validity evidences, OSATS, seems to be an effective instrument that generated objective scores for assessing the competence of technical skill of vaginal delivery as in international studies.[24] This study is also comparable to local study conducted in Services Institute of Medical Sciences, where OSATS was validated for formative assessment in Gynaecology.[25] Timely and constructive feedback is essence of workplace based assessments (OSATS) which promote reflective practice.[26] This study was limited to single hospital which may be reflected as strength as it limits extraneous elements which may affect the evaluator and candidate's performance assessment on the other side, limited number of observed candidates barred a comprehensive analysis.

Conclusion

With these evidences, OSATS was found to be valid and reliable for assessing the competence of technical skills of vaginal delivery in WPBA but for its acceptance as summative assessment further validation and time is required.

Conflict of Interest: None

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Authors Contribution

JF: Conceptualization of Project
JF: Data Collection
JF: Literature Search
JF: Statistical Analysis
JF: Drafting, Revision
JF: Writing of Manuscript

Association between Oxidative Stress, and Risk of Development of Cardiovascular Disease in Diabetic Patients

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Abstract

Objective: A cross sectional study was carried out to find the association between oxidative stress, and risk of the development of cardiovascular disease in diabetic patients

Material and Methods: Study included 60 patients and 30 controls with age 20-45 years. About 30 diabetic patients with duration of diabetes>five year (Group A) and 30 cases of type 2 diabetics with duration of diabetes<one year (Group B) were included. Another 30 subjects with no history of any disease were recruited as controls (Group C). Blood sample of both subjectsand controls was drawn to estimate the levels of serum malondialdehyde, serum catalase, fasting blood sugar, serum catalase, serum cholesterol and serum triglyceride using standard kits.

Results: Male/female ratio was 1:2&1:3 in groups A & B respectively. Majority of middle-aged diabetic of both groups were smokers, professional and having moderately active lifestyle. Nearly all patients were non-obese with positive family history of diabetes. Mean levels of fasting blood sugar, serum MDA, serum cholesterol was high in Group A compared to Group B and controls. The mean level of serum triglycerides was high in Group B compared to Group A& controls. The levels of serum catalase were low in both A & B compared to group C. According to analysis of variance, significant difference was observed in values of fasting blood sugar, serum MDA, serum catalase, serum cholesterol and triglycerides between groupsA, B & C.

Conclusion: Reduced level of catalase and increased values of malondialdehyde cause imbalance of oxidative stress that may increase the risk of cardiovascular disease in both new and known diabetic patients.

Keywords: Oxidative stress, diabetes, cardiovascular disease.

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Introduction:

Diabetes mellitus is an assemblage of metabolic ailments explained primarily by increased blood sugar levels ensuing from impairments in secretion or function of insulin or by the combination of both. Chronic raised levels of blood sugar results in various systemic problems including renal and a vascular diseases, retinopathy etc.[1,2] It has been shown that the start of diabetes type II is associated with metabolic risk factors related with cardiovascular issues like dyslipidemia, high blood pressure, and prothrombotic factors.[3]

The incidence of diabetes is increasing worldwide due to urbanization, population growth, obesity and aging. In Asian countries, the prevalence of diabetes is high in young and middle age people4.In Pakistan, the incidence of diabetes has been estimated tobe 14.62%. The risk factors associated with diabetes, as identified in Pakistani population, include advancing age, the number of diabetic family members, obesity and hypertension.[5]

Hyperglycemia is known to induce oxidative stress-mediated injury of cells. Elevated glucose levels in the blood can increase glucose uptake by cells, leading to an

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increase in the production of reactive oxygen species (ROS). Hyperglycemia-encouraged oxidative stress causes dysfunction of epithelial cells that play the main role in development of micro/macro-vascular ailments. Hyperglycemia also activates formation of diacylglycerol which activates the enzyme protein kinase C & NADPH-oxidase, which in turn, causes the synthesis of reactive oxygen species and increases oxidative stress in ailment of diabetes6. The basic mechanism of such cellular insult is a disparity between free radical formation and cell's ability to eliminate them. Increased free radical production and decreased scavenging by the antioxidant system results in the synthesis of malondialdehyde (MDA) and may affect the function of vascular, renal and retinal tissues in addition of islet of cells. To mitigate oxidative stress in the body, non-enzymatic (ascorbate, tocopherol etc.) and enzymatic antioxidants (catalase, glutathione peroxidase, superoxide dismutase-1 etc.), play important roles. Reduction in their levels increases predisposition to oxidative damage and may result in cardiovascular problems and other complications.[7]

Weak antioxidant defense systems, unresponsive to high production of ROS, cause oxidative stress and are unable to remove this high level of ROS. This accumulation of free radicals leads to increased production of malondialdehyde (MDA) and synthesis of conjugated form of diene which are cytotoxic/mutagenic and may cause bad effect of oxidation on the usual function of vascular, retinal and kidney issues in addition to harmful impact on pancreatic islet cells.[8]

Raised oxidative stress or decreased antioxidant capacity could potentially be the root cause of the complications observed in diabetic patients. Cardiovascular disease is the main reason of death in diabetic patients, which in many situations, appears to be affected by oxidative stress. ROS negatively modulates calcium handling of myocardium leading to the development of arrhythmia, and increasing cardiac remodeling by influencing hypertrophic signals and apoptosis. ROS also promotes the formation of atherosclerotic plaque[9]. Amongst these, catalase is an important antioxidant enzyme and MDA is an oxidative damage product and their imbalance may have a role in the development of oxidative stress induced cardiovascular complications in diabetic patients[10]. Lipids are found to be one of the sole victims of ROS. Changes in the metabolism of lipids (cholesterol and triglycerides), in diabetic patients, suggests that per oxidative injury maybe responsible for the development of diabetic complications including myocardial infarction[11]. Serum malondialdehyde is directly and serum catalase inversely related to oxidative stress and development of cardiovascular disease in diabetic patients. Cardiometabolic issues, including Type-2 diabetes, are growing apprehension worldwide. The incidence of diabetes is rising due to high population growth, aging, urbanization, and obesity. Oxidative stress plays pivotal role in progression and development of diabetes and its complications including cardiovascular disease. The prognostic significance of oxidative stress biomarkers is still poorly understood. Proper knowledge of oxidative imbalance is based on high values of malondialdehyde and reduced values of catalase that may help to understand complications of diabetes. Proper therapies may help to reduce impact of oxidative stress that may be useful to lessen diabetic complications.

Materials and Methods

Stdy included 60 patients and 30 controls. Duration of study was October 2022 to November 2023. Study was carried out at CMH Lahore Medical College & Institute of Dentistry, Lahore The study was approved by the ethics committees and written consent was taken from each patient according to the principles of the Declaration of Helsinki. Participants were recruited from the general population of Lahore city using non-probability purposive sampling. Patients with age 20-45 years, with uncontrolled diabetes (HbA1c>6.5% along with FBS >126 mg/dl) were included in the study. Subjects with type 1 diabetes, BMI>27 Kg/m^2, diagnosed hypertension, usage of anti-oxidant, anaemia were excluded from the study. A total of 30 type 2 diabetes mellitus patients with duration of diabetes > five year (Group A) and 30 cases of type 2 diabetics with duration of diabetes<one year (Group B) were included in the study. Group A taken as known diabetic and group B as newly diabetics. Another 30 subjects with no history of any disease were recruited as controls (Group C). Sample size was calculated using the sample size calculator software available online (https:// riskcalc.org/samplesize) for continuous outcomes at confidence level of 95% and power of 80%. The sample size was calculated using the formula:

$$n_1 = \left(1 + \frac{1}{k}\right)\sigma^2\left(\frac{z_{1-\alpha/2} + z_{1-\beta}}{\mu_0 - \mu_1}\right)^2 ; n_0 = kn_1$$

where μ_0 and μ_1 are the means of the end-points in group one and group two, n_1 is the sample size in group one,

n_2 is the sample size in group two, $k = n_1/n_2$ is the ratio of two sample sizes, and σ^2 is the variance of the two samples (assumed common). After a 12-hour fast, BMI of all subjects wasassessed. Blood sample of both subjects and controls was drawn to estimate the level of serum malondialdehyde, serum catalase, fasting blood sugar, serum catalase, serum cholesterol and serum triglyceride using standard kits. Fasting blood sugar was estimated by Auto Analyzer using glucose oxidase kit method. Serum Catalase was estimated using hydrogen peroxide. Serum Malondialdehyde will be measured by using thiobarbituric acid method. A study-specific questionnaire was used to collect data. Data was analyzed by SPSS 23.Qualitative variables were expressed as frequency and relative frequency. Quantitative variables were expressed as mean ± Standard deviation, Study variables (subject to normal distribution) were compared by One-way Anova. $P<0.05$ was considered as significant.

Results

Demographic characteristics of diabetic group (A & B) and controls showed that in Group A (diabetes with>5 yrs.), male /female ratio was 1:2. Mean age of majority was<40 years followed by middle age (41-50 years) and age>50 years. In this group 60% were former smokers and 40% were regular smoker. All diabetics of this age groups were professionally active. Their lifestyle was based on their exercise and walking. It is observed that active lifestyle was observed in majority (33-40%) and about 27% were formally active. Family history was observed in nearly all diabetics with BMI<25 Kg/m^2. **(Table-1)**. Demographic characteristics of diabetic group (A & B) and controls showed that in Group B (diabetes<1 yrs.), male /female ratio was 1:3. Mean age of majority was<40 years followed by age>50 years and middle age (41-50 years). In this group majority was former/non-smokers. All diabetics of this age group were professionally active. Their lifestyle was based on exercise and walking. Active lifestyle was observed in majority (60%) and about 23% were formerly active and 16% had no physical activity. Family history was observed in nearly all diabetics with BMI<25 Kg/m^2 **(Table-1)**. Mean levels of fasting blood sugar, serum MDA, serum cholesterol was high in Group A compared to Group B and controls. Whereas the mean level of serum triglycerides was high in Group B compared to Group A& controls. On the other hand the level of serum catalase was low in both A & B compared to Group C **(Table-2)**. Analysis of variance (one way) of biochemical parameters was carried out between groups and within groups of diabetic subjects (Group A & B) and controls. According to analysis significant ($P<0.001$) difference was observed in values of fasting blood sugar, serum MDA, serum catalase, serum cholesterol and triglycerides between groups **(Table-3)**.

Table 1: *Demographic Characteristics of Diabetic Groups (A&B) and Controls Presented by Frequency and Percentages.*

Variables	Diabetic Group A (>5yrs)	Diabetic Group B (<1 yrs.)	Controls (Group C)
Gender (n%)			
Male	10 (33.33%)	07 (23.33%)	14 (46.66%)
Female	20 (66.66%)	23 (76.66%)	16 (53.33%)
Age distribution			
<40 years	15 (50%)	22 (73.33%)	20 (66.66%)
41-50 yrs	10 (33.33%)	02 (6.66%)	04 (13.33%)
>50 yrs	05 (16.66%)	06 (20%)	01(3.33%)
Smoking			
Never /Former smokers	18 (60%)	25 (83.33%)	23 (76.66%)
Regular smoker	12 (40%)	05 (16.66%)	07 (23.33%)
Profession (n%)			
Employ	16 (53.33%)	23 (76.66%)	28 (93.33%)
Others	14(46.66%)	07 (23.33%)	02(6.66%)
Life Style/physical activity (n%)			
Never /Rare	08 (26.66%)	05 (16.66%)	26 (86.66%)
1-2 times weekly	12 (40%)	18 (60%)	02(6.66%)
3-6 times weekly	10 (33.33%)	07 (23.33%)	02 (6.66%)
Family history of diabetes			
No	01 (3.33%)	28 (93.33%)	30 (100%)
Yes	29 (96.67%)	02 (6.66%)	-
Obesity(BMI= 30- 34 Kg/m^2)			
Yes	01 (96.67%)	01 (96.67%)	-
NO	29 (3.33%)	29 (3.33%)	30 (100%)

Table 2: *Mean and Standard Deviation in Diabetics Groups (A & B) and Controls*

Parameters	Group A	Group B	Controls
Fasting blood sugar (mg/dl)	202.16±34.20	186.55±28.21	97.67±13.95
Serum MDA (mMol)	1.41±0.6	1.33± 0.44	0.88±0.27
Serum Catalase (mg/dl)	1.83±0.22	1.95±0.16	5.4±0.48
Serum cholesterol (mg/dl)	257.4±78.71	212.60±18.28	173.3±35.76
Serum Triglycerides(mg/dl)	286.6±115.85	290.1±97.36	137.6±35.76

Table 3: *Analysis of Variance (One Way) of Biochemical Parameters In Groups (Group A &B) and Controls*

Serum Malondialdehyde				
Sources	**Sum Squares**	**Mean Square**	**F-Statistic**	**P-value**
Between Groups	2.86	1.43	6.63	0.002
Within Groups	12.10	0.21		
Serum Catalase				
Between Groups	9.8	4.9	133.99	0.00
Within Groups	2.2	0.03		
Serum Cholesterol				
Between Groups	37609.80	18804.9	7.48	0.002
Within Groups	67875.32	2513.90		
Serum Triglycerides				
Between Groups	151565	75782.5	9.40	0.00
Within Groups	217601.6	8059.31		
Fasting blood sugar				
Between Groups	199615.87	99807.9	66.17	0.000
Within Groups	90498.60	1508.31		

Discussion

Male/female ratio showed that femalesare more prone to develop diabetes than males. The incidence of diabetes was high with a period less than one year as compared to duration of diabetes>5.0 years. A study also found increased incidence of type 2 in females. The reason may be diversities in environment and lifestyle with difference in economic status. In addition, sex steroid hormones may have a significant effect on metabolism giving energy, composition of body, functions related with vascular system. Thus, imbalance of hormones may increase the risk of cardiovascular issues especially in diabetic females.[12] However, a survey that included eleven studies comprising 96,581 diabetic patients found that males were more prone to develop diabetes than females.[13]

According to our study majority of middle-aged diabetic of both groups were smokers, professionals and having moderately active lifestyle. Nearly all patients were non-obese with positive family history of diabetes. A study also found that incidence of diabetes is more in middle-aged and the risk due to ageing was increased in these diabetics. It is demonstrated that type 2 diabetes may worsen the process of ageing and gradually predisposediabetics to complications like retinopathy, renal impairment, and cardiovascular issues. It is proposed that type 2 diabetes encourages immature senescence in different cells like endothelial cells, β-cells, and cardiomyocytes due to high BMI,inactive lifestyle and positive family history which may increase the risk of progression of cardiovascular issues even with less duration of diabetes.[14]

Relationship between normal/low BMI was less studied. It is thought non-obese diabetic havea changed genetic vulnerability to diabetes and is related to severe & progressive diabetes. A study was conductedon 5339 obese and lean type 2 diabetics in China to find out the association of type 2 diabetes withgenetic variants. Results showed that the genotype risk score was more related with the risk or issue for slim type 2 diabetics than for obese ones. Alsodys function of beta cells and low levels of insulin was noted in non-obese diabetics.[15] A review of study reported that incidence of type 2 diabetes was usually higher in less developed countries and the highest increase in incidence of diabetes is assumed in coming lifetime. Conversely, pathological/ physiological characteristics and issues, that increase the risk of developing type 2 diabetes in non-obese individuals is much discussed.[16]

A study of about 15.40% regular smokers, 4.80% former smokers and 79.80% non-smokers in which patients were middle-aged diabetics was carried out. It is found that regular /heavy smokers showed a higher risk of developing cardiovascular issues as compared to former smokers and non-smokers.The exact mechanism of impact of smoking on diabetes is not known. It is proposed that smoking along with diabetes may help to develop cardiovascular issues via increased oxidative stress and injury of endothelial cells resulting in the formation of plaques of atheroma.[17]

One of the risk factors i.e. sedentary/in-activelifestyle and increased complications of diabetes is widely studied. It is proposed that regular exercise and walk boosts the antioxidant defense of the body. However, exercise without guidance engulfs defenses causing damage due to free radicals. Thus individual who only exercise vigorously on weekends only may experience more harm than benefit. It is proposed that injuries due to moderate to high-intensity exercise with short-duration causes increased production of ROS that damages many tissues of body like skeletal muscle.[18] A data based on about 1990 diabetic and 1930 non-diabetics was conducted on Spanish people. Study tried to compare relationship of health to quality of life (physical &mental) and limitation of activities in both groups and found its impact on cardiovascular issues. It is concluded that low quality of life may increase the risk of cardiovascular problems, even in diabetics.[19]

Mean levels of fasting blood sugar, serum MDA, serum

cholesterol was high in diabetics with duration of diabetes >5 years compared to diabetics with duration <one year. Dyslipidemia is known in type 2 diabetes affecting 73% to 86% patients characterized by hypertriglyceridemia (HTG) and hyperglycemia. HTG appears due to increased synthesis, low clearance with known dyslipidemia in diabetics[20]. We agreed with a study, carried out in 60 diabetics. Among these 30 diabetics are newly diagnosed and 30 were known. Their blood levels of fasting blood sugar, cholesterol, triglycerides and MDA was estimated. Study found high values of fasting blood sugar, cholesterol, triglycerides and MDA in diabetics of large duration compared to recent diabetics. It is suggested that duration of diabetes is independently related with raised values of lipid peroxidation due to chronic oxidative stress due to excess of free radicals. These free radicals cause complications of diabetes including cardiomyopathy, neuropathy and retinopathy[21]. According to studies the key risk issue for process of dyslipidemia in diabetics are aging, hypertension, inactive lifestyle, high BMI and prolonged duration of diabetes.[22,23]

We observed, however, increased values of serum triglycerides in newly diagnosed diabetics compared to known diabetics. Most of the studies found high levels of serum triglycerides in known diabetics compared to newly diagnosed. It is proposed that high levels of triglycerides are related to micro-vascular complications due to endothelial dysfunction and lipid peroxidation. It is suggested that estimation of triglyceride may be helpful to monitor the metabolic position in clinical situations.[24]

Low levels of serum catalase was observed in both new and known diabetic. first_page

Study observed low levels of catalase in both new and known diabetics. A study tried to elucidate the association of diabetes with antioxidant defense. Study included 102 middle aged diabetics and investigated the levels of superoxide dismutase, catalase and status of vitamin D and A. An inverse activity of catalase and superoxide dismutase was related with risk of type 2 diabetes. The probable influences are lifestyle, triglyceride and cholesterol that may mediate the link of superoxide dismutase and catalase with risk of type 2 diabetes[24]. Number of studies proposed the association of low value of catalase and risk of developing the type 2 diabetes. One of the studies proposed that low catalase causes failure of function of pancreatic beta cells and damage by reactive oxygen species. Besides, hyperglycemia is one of the factors to down regulate the expression of catalase.[25]

Conclusion

Reduced levels of catalase and increased values of malondialdehyde cause imbalance of oxidative stress that may increase the risk of cardiovascular disease in both new and known diabetic patients. However, more studies are needed to find the gender specific risk issues, intensity/duration of exercise, emphasis on oxidative/anti-oxidative enzyme, dyslipidemia and reason of non-obese diabetes. Sex-dimorphouspatho-physiological devices of type 2 diabetes and its complications may help to additional personalized care of diabetes in coming years and give more perception in gender related risk issues.

Conflict of interest : None

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Authors Contribution

HA: Conceptualization of Project
SIAS: Data Collection
RK: Literature Search
HA: Statistical Analysis
SIAS: Drafting, Revision
RK: Writing of Manuscript

Depression in Caregivers of Patients with Bipolar Disorder

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Abstract

Objective: Bipolar disorder is a chronic illness which affects psychological health of caregiver of the patients. The aim of the study was to determine frequency of depression in caregivers of patients of bipolar disorder and to determine the contributing factors leading to depressive disorder among caregiver of bipolar affective disorder.

Material and Methods: Correlational cross-sectional research design was used for the current study. Convenient sampling technique was used and data from (N=220) caregivers both males and females, aged more than 18 years accompanying the patients coming in OPD of psychiatry department Sir Ganga Ram Hospital Lahore Pakistan were gathered. Through convenient, non-purposive sampling technique data from (N=220) caregivers both males and females, aged more than 18 years accompanying the patients coming in OPD of psychiatry department of Sir Ganga Ram Hospital, Lahore Pakistan were taken. Hospital Anxiety and Depression scale (HAD) was administered for data collection. Descriptive and inferential statistics were used for data analysis.

Results: A score of 8 and above indicated presence of depression. Two hundred and twenty caregivers were included in study and out of them 58 caregivers (26%) had depression.

Conclusion: A difficult emotional environment, anxieties, and everyday challenges can be detrimental to an individual's physical and mental well-being. The ways in which patient traits, caregiver qualities, and support systems interact to ultimately determine the amount of care that caregivers must provide.

Keywords: Depression, bipolar, care-giver, HAD scale.

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Introduction

Bipolar disorder is a chronic mental illness causing extreme shifts in mood and energy levels, from manic episodes of heightened excitement to depressive episodes of deep sadness or hopelessness.[1] Bipolar disorder is a persistent sickness related with seriously debilitating symptoms that can significantly affect the both patients and their caregivers and can have long-lasting unfavorable consequences for the patients' psychological and physical, instructive, and interpersonal relations.[2] Bipolar disorder classified into three types: Bipolar type I, Bipolar type II, and Bipolar disorder not otherwise specified (NOS).[3] Bipolar type I involves manic episodes followed by hypomanic or major depressive episodes. Bipolar type II includes hypomanic and major depressive episodes. Bipolar Disorder NOS encompasses symptoms not meeting criteria for Bipolar type I or type II.[3] For Bipolar type I and Bipolar type II, the overall lifetime prevalence was 1.06% and 1.57% respectively showing Bipolar type II disorder to be more prevalent.[4] These conditions, though not meeting full diagnostic criteria, still significantly impact functioning. Recognition of bipolar disorder as sixth leading cause of disability adjusted life years by the World Health Organization in people of working age (15 to 44 years)

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underscores the need for effective diagnosis and support.[5]

Caregivers are the people who join in or offer types of assistance to a patient. It very well may be a spouse, parent, or it can be an individual who has the most successive contact with the patient and can be reached by treatment staff in the event of a crisis.[6] Formal caregiving involves paid assistance, often professional or institutional, while informal caregiving refers to unpaid care provided by individuals or families to relatives, friends, or neighbors.[7] Depression is likely to be high among family caregivers in the form of hopelessness, helplessness, and emotional distress.[8] Clinicians hardly pay any attention to the needs of caregivers.[9] Reports suggest that caregivers experience depressive symptoms at twice the rate of non-caregivers. These individuals fulfill a crucial role in providing support to family members who are ill, incapacitated, or disabled 10 Up to 90% of people with mental disorders live with relatives. The effected person is dependent on the caregiver for emotional support, personal care and practical help. These demands can result in stress to caregivers.[11] Care givers negative experiences not only affect their own health but are also important because their involvement is essential for optimal treatment of patients.[12] Close family members of people with bipolar disorder often bear the burden of emotional anguish, depression, and high rates of mental health care utilization.[2,13] It has been advised that caregiver personal health, older age, female gender and spending more time with the patient are risk factor for caregivers' depression.[14,15] Parents reports high level of depressive symptoms than spouses and more in patients with history of suicidal attempt or ideation.[15]

Many of the previous studies done in Pakistan either focus on depression in patient secondary to a neuropsychiatric illness themselves or focus on caregivers on depression, schizophrenia, and dementia etc. or don't differentiate mental illness separately.[16,17] The objective of current study was to determine frequency of depression in caregivers of patients of bipolar disorder and to determine the contributing factors leading to depressive disorder among caregiver of bipolar disorder.

Material and Methods

Correlational research design was used for the current study. Convenient sampling technique was used and data from (N=220) caregivers both males and females, aged more than 18 years accompanying the patients coming in OPD of psychiatry department Sir Ganga Ram Hospital Lahore Pakistan were gathered. Caregivers who live with the patient, in the same environment, for at least twelve months and was involved directly in giving care to the patient were included. Caregivers who have psychiatric disorder and have any history of psychoactive drug abuse were excluded from the study. Consent was taken from the caregivers, confidentiality and time for finishing the questionnaire was specified to them. Hospital Anxiety and Depression scale (HAD) was utilized for evaluation.[18] There are two questionnaires on it: one for depression and one for anxiety. Seven of the items in even serial i.e. 2, 4,6,8,10,12 and 14 related to depression and items in odd numbers are related to anxiety i.e 1,3,5,7,9,11 and 13. Each item on the scale meant that a person can score from 0 to 21. A cut off of 8/21 was required for the identification of depression. Cronbach's alpha of the scale was .80 which showed good internal reliability. Two hundred and twenty caregivers accompanying the patients coming in outpatient department were included. Written informed consent was taken from the caregivers. Each caregiver was interviewed and then marked down. Additionally, Urdu version of Hospital Anxiety Depression Scale was applied on caregivers to measure the frequency of depression. All data was entered into SPSS version 26 for further analysis. Quantitative data was presented by mean and standard deviation. Qualitative data was presented by frequency and percentages. Data was stratified for gender, duration of disease and poor compliance in patients to address the effect modifiers. Chi-square test was used for post-stratification.

Table 1: *Frequency distribution of caregivers of bipolar patients according to Demographics characteristics (n=220)*

Demographic Characteristics	No. of Caregivers	%
Age (years)		
21-30	22	10
31-40	99	45
41-50	77	35
>51	22	10
Mean ± SD	39.31 ± 8.12 years	
Gender		
Males	90	45
Females	121	55
Relationship with Patients		
Child	88	40
Parents	55	25
Siblings	22	10
Spouse	55	25

Results

A total of 220 caregivers of bipolar affective disorder meeting the inclusion criteria were selected from in-patient department. A detailed interview was carried, and all the answers were recorded on the pre-designed Performa. The presence or absence of depression in care givers of bipolar disorder patients was noted.

Results of table 1 revealed that when caregivers of bi-polar patients were distributed according to different age groups, there were 22 caregivers who were in the age group of 21-30 years making 10% of study group. In age group of 31-40 years, there were 99 caregivers (45%) while 41-50 years age group contained 77 care-givers (35%). There were 22 (10%) caregivers in age group of >51 years. Mean age was 39.31 + 8.12 years in study population. Results showed that 121 caregivers were females (55%) and 90 caregivers were males (45%). The results also indicated the distribution of caregivers according to the relationship with patients. There were 88 children (50%), 55 parents (25%), 22 siblings (10%) and 55(25%) spouses.

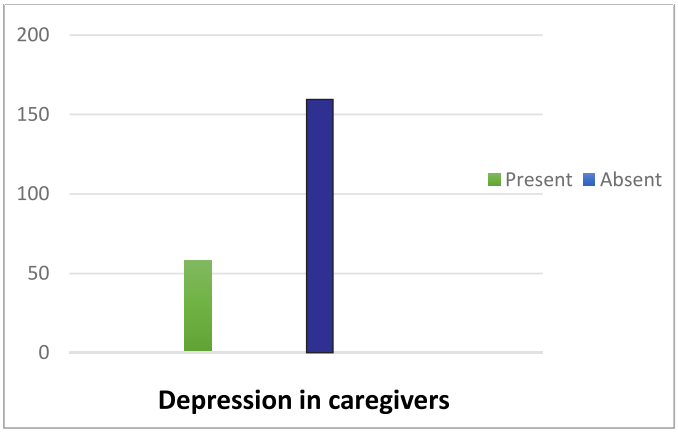

Fig-1: *Frequency of depression in caregivers of bipolar disorder patients (N=220).*

Figure 1 shows distribution of cases according to pre-sence or absence of depression in the caregivers of bipolar patients during their inpatient stay at the hospital. It can be seen that the recent study, 58 caregivers (26%) had HAD scores of more than 8, confirming the diagnosis of depression while 162 (74%) caregivers had no depre-ssion at all. **Fig-2** indicates the stratification of depressed care-givers according to gender, duration of the disease and compliance of the patients respectfully. It can be noted that the gender and duration of disease had significant effect (p value <0.05) on the presence of depression whereas compliance of patient had no clinically signifi-cant effect (p value >0.05).

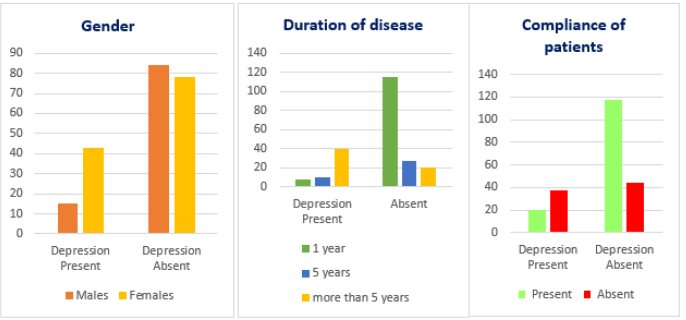

Fig-2: *Depressed caregivers according to Gender, Duration of Disease and Compliance of Patients (N=220)*

Discussion

The effects of bipolar disorder on functioning have been the subject of several research, but the effects on the families of those who are affected have received far less attention. In this research, 26% of caregivers of bipolar patients reported depression. This is consis-tent with recent research findings from Pakistan, which showed that about 29.6% of bipolar patients' caregivers had depression.[19] In a study examining the relationship between the mental health and mental burden of care-givers of patients with chronic mental disorders, as well as the relationship between these variables and social support and coping strategies, the findings indicated that mental health is associated with a lower frequency of positive symptoms and social support, while mental burden is associated with a higher frequency of negative symptoms.[20] The present study found that the source of burden for caregivers in both groups was more objective than subjective. This could be attributed to inadequate social supports, such as long-term and short-term hos-pitalization services, rehabilitation, outpatients, or per-manent patient care. Additionally, the presence of a mental patient may have an impact on the gender and income of the caregiver's family. These results are in line with the demands of caregivers for bipolar illness patients who have severe symptoms and who have to bear a heavier load.

The relationship between the study group's stigma and the caregiver's female gender could potentially be ex-plained by elevated social role expectations. Of the care-givers in the current study, 45% were men and 55% were women. In addition to caring for the bipolar patient and handling home duties, women are more frequently required to socialize with people outside the family. As

a result, family members may experience increased stigma if their female spouse or kid does not fulfill this social role requirement.[15,21] Conversely, because certain behaviors like risk-taking and interpersonal aggression are more socially acceptable in men, female caregivers who take on or restart these responsibilities may face greater discrimination than their male counterparts.[21] Numerous studies have looked at the relationship between carers' physical and mental health and caring for a bipolar patient. The majority of research found a correlation between greater levels of psychological stress and depression and caregiving.[13,15] According to longitudinal studies, caregivers' depression levels rose when they assumed a caregiving duty.[8] It is yet unclear how caregivers' psychological health may be affected over time by stepping into and out of the caring role in addition to other roles like marriage, parenthood, and work. Contextual factors, such as gender, race/ethnicity, and age, have also been found to account for variations in the mental and physical well-being of caregivers, in addition to methodological disparities.[22] According to a meta-analysis on the relationship between caregiver gender and health, female caregivers were shown to be less physically healthy and to have a greater likelihood of depressive symptoms than male caregivers.[23] Understanding factors like expressed emotion and caregiver stress across cultures is vital for managing relapse and enhancing quality of life. High expressed emotion at home raises the relapse risk for bipolar individuals, leading to blame and pressure. Family therapy breaks this cycle. Recognizing stigma around mental illness within families is key for improving patients' and caregivers' quality of life.

Conclusion

Mental illnesses like bipolar disorder disrupt daily life and harm family well-being. Stressful environments and caregiver burdens worsen physical and mental health. Family dynamics are crucial in conditions like bipolar disorder. The process of providing care involves the patient and an individual responsible for their long-term care. In conditions like schizophrenia and bipolar disorder, long-term care is necessary, leading to caregiver burden. Despite increasing research on caregiver distress, nega-tive outcomes, lack of support, and interventions to alle-viate caregiver stress, there is a gap in understanding the mental distress of caregivers and the complexities of their caregiving experiences. The interplay among patient features, caregiver traits, and support mecha-nisms all of which ultimately influence the caregivers' burden of care has received very little research atten-tion. Enhancing the psycho educational supportive program for family caregivers for people with bipolar disorder can help improve depression outcomes in these caregivers by reducing their burden and improving their ability to handle pressure from caring for their patients. The creation of counseling services to enhance adaptability, personal satisfaction, and versatility for caregivers for individuals with psychiatric instability, including bipolar disorder. Furthermore, in order to develop effective treatment and preventative strategies, additional qualitative studies on the burden and coping mechanisms of caregivers for people with bipolar disorder is required.

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Authors Contribution

SB:	Conceptualization of Project
SB, FAK:	Data Collection
SB, FAK:	Literature Search
FAK, ST:	Statistical Analysis
SB, MA:	Drafting, Revision
FAK, ST, MA:	Writing of Manuscript

Assessment of Telehealth Efficacy in Delivering Preventive Oral Healthcare: A Study Examining Patient and Caregiver Perspectives in Pakistan

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Abstract

Objective: This study aimed to explore the perspectives of patients and consultants regarding a telehealth-based preventive oral healthcare program at the pediatric dentistry department of a private tertiary care dental hospital in Pakistan

Material and Methods: An anonymous questionnaire was given to parents and guardians, and a focus group with clinicians was conducted to gather data. To summarize the data, descriptive statistics, chi-square tests, and theme analysis were utilized.

Results: Forty-five parents/guardians and six clinicians participated in the study. The kids mean age was 4 years (SD = 3.1 years), and most of the parents were between 30- and 39-years age group (56.4%). The telehealth service demonstrated high satisfaction (78.1%) and acceptability (61.41%). Notably, native Urdu-speaking were much more likely to agree that telehealth was a good way to provide preventive. Parents who reported difficulty in accessing traditional dental visits were predominantly rated telehealth high in terms of usefulness, technical quality, and satisfaction. Key themes from the consultants focus group was that it was advantageous for patients with special needs or residing in rural areas, reduced burden on families, and an increased emphasis on prevention.

Conclusions: The study findings underscore the continued benefits of integrating telehealth into the provision of preventive oral health

Keywords: Telemedicine, Oral Health, Preventive Health Services, Patient Perspective, Caregiver Attitudes, Dental Care, Pakistan

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Introduction

Telehealth, which involves using technology to deliver healthcare services remotely, has extended beyond medicine to various fields, including dentistry.[1,2] Initially, telehealth dentistry primarily focused on providing remote diagnosis and consultations. The aim was to improve healthcare in rural areas, ensure timely access to care, reduce waiting times for appointments, and address social and geographical inequalities.[3] Telehealth has notably enhanced communication between general and specialist dental practitioners, playing a key role in large-scale dental screening programs. This has resulted in significant cost savings compared to traditional methods in remote and urban areas, ultimately enhancing access and care for underserved populations.[4]

The utilization of remote dental services, has emerged as a powerful tool in the field of dentistry. Tele dentistry enhances the training of professionals by providing

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access to continuous education and specialized consultations, which are particularly beneficial in underserved areas.[3] Additionally, it improves the effectiveness of primary healthcare by enabling early diagnosis and intervention, thus reducing the need for advanced treatments. Furthermore, tele dentistry has been shown to enhance the quality of oral healthcare services by facilitating regular monitoring and follow-up care, leading to better patient outcomes.[4] Successful integration of telehealth into oral healthcare requires training oral health professionals in digital media usage, modifying workflows, and bolstering management support. Resource management challenges identified in previous studies may hinder access to telehealth tools, thereby reducing their effectiveness in maintaining care quality and oral health indicators.[5]

A study found that patients and caregivers, especially those having trouble getting dental appointments, were highly satisfied and found the service acceptable.[6] Another study observed fewer referrals to specialized care and improvements in how oral health teams approach patient care. A study in 2021, pointed out that teledentistry has the potential to decrease plaque and gum inflammation levels, as well as the occurrence of white spot lesions.[7] A study conducted in 2012, highlighted the importance of telehealth in reaching at-risk and underserved communities, underscoring its potential for improving preventive oral healthcare.[8] Maintaining oral health is essential for overall well-being, and access to dental care plays a crucial role in promoting good oral hygiene.[9]

Despite its importance, numerous barriers hinder a significant portion of the population from accessing necessary dental care, contributing to a higher prevalence of oral diseases. These barriers include the limited availability of oral health services, geographic constraints, financial limitations, and transportation issues. Providing universal dental care is essential to reduce disparities in oral health.[10]

In recent years, telehealth has emerged as a promising solution to overcome these barriers and improve access to preventive oral healthcare. By leveraging technology, telehealth can reach underserved populations, bridging the gap in oral healthcare access. Patients can receive preventive oral healthcare without physical visits to dental practices, receiving virtual consultations, educational sessions, and even screenings and assessments. Moreover, telehealth enables early intervention and timely treatment of oral health issues, leading to improved outcomes and reduced healthcare costs in the long term.[11]

Despite the growing recognition of telehealth's potential, there remains limited literature on the benefits and practicality of virtual or "online" tele dentistry in providing preventive dental care, such as dental screening and dietary counselling, particularly for paediatric populations.[12] This study aims to assess the experiences, satisfaction levels, and feasibility perspectives of patients/guardians and providers participating in a prevention-focused telehealth program at the pediatric dentistry department of a dental hospital.

Material and Methods:

The study was conducted in the Department of Paediatric Dentistry at a dental college in Multan, Pakistan. The Zoom videoconferencing platform was used for synchronous telehealth. Parents and guardians got a WhatsApp message with information about the appointment and a link to join the virtual lesson. The one-click link lets anyone with an internet-connected gadget join the session. During sessions, families could use the video interface to discuss their main dental issues and share photos through the chatbox, which let clinicians assess them in real time. After a careful look at dietary habits and a caries risk assessment, consultants made and showed personalised care plans for each patient that were tailored to their specific needs. This included how to brush teeth, live demos using a typodont model, an evaluation of current brushing techniques, a prescription for a certain toothpaste, suggestions for oral hygiene aids, dietary advice, and ideas for tooth brushing games, videos, or resources. Resources and important messages were shown on the Zoom share screen and WhatsApp to everyone at the end of the meeting. Parents and guardians who were able to take the online poll had used Zoom before and did not need an interpreter.

The parent/guardian questionnaire was an online survey with 30 questions based on the Telehealth Satisfaction Questionnaire (TSQ). The questions looked at technical quality, communication impact, ease of use, comfort, accessibility, perceived usefulness, effectiveness, intention to use or reuse, and overall satisfaction with video or audio consultations in the home. A facilitator guide was used by the clinicians' focus group to talk about the telehealth process's satisfaction, ease of use, usefulness, and efficiency, as well as communication skills, parental views, problems, and possible solutions. The hour-long focus group was led by an external co-investigator, and

the session was recorded and transcribed.

The Institutional Review Board (IRB) at Bakhtawar Amin Medical & Dental College in Multan, Pakistan, granted approval for the study with IRB Num (236/23/COD). Informed consent was obtained online from participants, and written in person consent was obtained from doctors. An information sheet was provided beforehand to all participants, outlining the purpose, duration, and other details of the study. Participants were assured that their data would be kept confidential and only used for research purposes. To analyse questionnaire data, descriptive statistics were used to look at the distribution, and survey answers were used to create thematic groups. Chi-square tests and analyses of variance looked at how factors are rela-ted to each other. Coding was done manually. The Ritchie and Spenser framework was used for the analysis.

Results

A total of 45 guardians participated in the study, which was conducted from June 2023 to October 2023. A significant portion of the parents (46.6%) were from the 30-39-year-old age group, and the majority had between 3 and 4 children (53.4%). A substantial majority (71.2%) reported speaking Urdu as their primary language at home, indicating a diverse cultural background among the participants. Travelling difficulty from appointments to their children's dental appointments was reported by a significant majority (64.5%) of the parents (Table 1). The children of the surveyed parents showed a mean age distribution, with a considerable number (60%) aged between 4 and 8 years. The data revealed that a vast majority of children (80%) did not report dental pain, and a notable 91.1% did not report dental swelling, indicating a prevalence of dental issues among the pediatric population surveyed. However, a large number of children (64.9%) had dental decay, emphasizing the importance of comprehensive dental care. A significant majority (64.5%) of respondents expressed that traveling to dental appointments posed challenges, highlighting the logistical barriers families face when accessing pediatric dental care. Some participants preferred traditional, face-to-face consultations for learning essential aspects of dental health, as indicated by a low score (2.6, SD = 1.01). With regard to parental demographics, a diverse range of languages spoken at home was noted, with Urdu being the predominant language (71.2%), followed by English (24.4%), Punjabi (2.2%), and Saraki (2.2%). This diversity underscores the importance of culturally sensitive healthcare provision.

In terms of travel difficulty, a clear majority (64.5%) agreed or strongly agreed that travelling to dental appointments was challenging, underscoring the logistical barriers faced by families in accessing paediatric dental care.

A low score (2.6, SD = 1.01) on the effectiveness of learning oral hygiene skills or healthy diet habits via telehealth compared to in-person visits indicates a subset of participants might prefer traditional, face-to-face learning for these essential aspects of dental health. This points to an opportunity for enhancing how educational content is delivered and engaged with in a telehealth setting. The overall quality of the telehealth experience, as indicated by a score of 14.8 out of 25, suggests that technical aspects and the user experience need significant improvements. The feedback highlights the need for better audio/visual clarity (2.5/5) and a more user-friendly system (2.6/5), which are critical for a successful telehealth encounter.

Communication scores generally reflect a positive experience, with participants finding it relatively easy

Parent	*n* (%)	Child	*n* (%)
Age (years)		**Age (years)**	
20–29	6 (13.4)	0–3	8 (17.7)
30–39	21 (46.6)	4–8	27 (60)
40–49	18 (40)	9–12	10(22.3)
Parents' language		**Dental pain (Self-reported)**	
English	11 (24.4)	Yes	9 (20)
Urdu	32 (71.2)	No	36 (80)
Punjabi	1 (2.2)		
Saraki	1 (2.2)	**Swelling (Self-reported)**	
Pashto	0 (0)	Yes	4 (8.9)
Other	0 (0)	No	41 (911)
Number of children		**Dental decay (Self-reported)**	
1–2	16 (35.5)	Yes	19 (64.9)
3–4	24 (53.4)	No	26 (35.1)
>5	5 (11.1)		
Travelling Difficulties			
Strongly disagree/ disagree	5 (11.1)		
Neither	11 (24.4)		
Strongly agree/agree	29 (64.5)		

to converse with their healthcare provider (4.1/5). However, the lower scores regarding the provider's understanding of the child's health condition (2.1/5) reveal a crucial area for enhancement in ensuring effective and empathetic communication within the telehealth platform. Satisfaction levels with telehealth services are indicative of their acceptance and perceived benefits, especially in gaining valuable skills for maintaining dental health (3.1/5), dietary improvements (3.6/5), and the confidence to apply these skills effectively (3.1/5). Yet, the overall satisfaction score (21.7/35) signals a cautious optimism among participants, suggesting that while telehealth is valued for its accessibility and potential, there's a considerable scope for enriching the user experience to meet and exceed user expectations fully. The willingness to reuse telehealth services (3.1/5) and the comfort level with this mode of communication (3.1/5) show a general positivity towards telehealth as a healthcare delivery method.

Summary components	Survey question	Mean score (SD)
Usefulness (score = 1 6.4/25)	Telehealth saves me time traveling to the hospital or a specialist clinic	4.1 (0.66)
	I obtain better access to health-care services via telehealth	3.2 (0.71)
	I do not need assistance while using the system	3.1 (0.47)
	I think the health-care provided through telehealth is similar to what would be provided in the clinic	3.4 (1.03)
	I think that consulting in person would have been a better way to learn how to take care of my teeth or eat well.	2.6 (1.01)
Quality (score = 14.8/25)	The tele dental method is simple and easy to comprehend.	2.6 (0.47)
	I could see and hear my consultant as if we were face to face.	2.5 (0.51)
	I felt like I got enough attention during the tele dental program.	3.5 (0.78)
	I can easily talk to my health-care provider through telehealth	4.1 (0.69)
	The health-care provider is able to understand my child's health-care condition	2.1 (0.81)
Satisfaction (score = 21.7/35)	Overall, I am satisfied with the quality of service being provided via telehealth	2.1 (0.75)
	I find telehealth an acceptable way to receive health-care services	2.6 (1.08)
	I feel comfortable communicating with my health-care provider through telehealth	3.1 (0.62)
	I will use tele dentistry program in future too	3.1 (0.85)
	I have learnt essentials methods to help keep my kids' teeth healthy	3.1 (0.62)
	I now have useful skills to help me improve the food my child eats.	3.6 (0.51)
	I am sure that I can use these skills in real life.	3.1 (0.57)

The analysis of telehealth services based on the child's age and the number of children in a family showed that differences in satisfaction, usefulness, and quality of these services were small and statistically insignificant. For children under 4 years, the differences in satisfaction (0.67), usefulness (1.16), and quality (0.84) were not significant enough to suggest that the child's age impacts parents' perceptions of telehealth services. Similarly, families with fewer than 3 children experienced only slightly higher satisfaction, usefulness, and quality compared to those with 3 or more children, but again, these differences were not statistically meaningful. Essentially, whether considering the age of the child or the number of children in a family, these factors do not significantly alter how parents view the effectiveness and quality of telehealth services.

		Summary component factors (*p*-values)					
		Satisfaction		Usefulness		Quality	
Independent *t*-test results		Mean difference (SE)	*p*-Value	Mean difference (SE)	*p*-Value	Mean difference (SE)	*p*-Value
Child's age	<4 years	0.67 (1.27)	0.61	1.16 (1.65)	0.068	0.84 (1.23)	0.63
	≥4 years						
Number of children	<3	0.71 (1.06)	0.466	1.03 (1.62)	0.563	1.14 (1.09)	0.127
	≥3						

Qualitative Analysis

Telehealth sessions were highlighted for patients with transportation difficulties, emphasising their potential advantages. The analysis also highlighted benefits for paediatric patients with anxiety or unique needs like sensory difficulties, developmental delay, or autism disorder. Consultants were of the view that they could effectively involve anxious and introverted patients in oral hygiene education by conducting the sessions in the patients' familiar home environment. These sessions helped reduce sensitivity to the dental environment, preparing patients for future in-person treatment. Telemedicine improves dental care accessibility, especially in underserved rural areas with limited dental specialists. Patients expressed satisfaction with remote consultations, eliminating the need for travel and consulting with specialists. The telemedicine programme alleviated the burden on families associated with in-person visits. Overall, the relaxed environment facilitated the exchange of oral health preventive information.

As in surgery, during documentation, clinicians have to engage the child and give them instructions to sit still or limit movement. From the clinician's perspective, the primary advantages of a telehealth service are improved communication and connections, as well as a greater focus on prevention as compared to in-person appointments. Clinicians perceived that the method of communication resulted in increased parental involvement during the session. The consultations were frequently demonstrated to incorporate counselling for siblings as well, fostering bonds with all members of the family and offering comprehensive family-centred care.

“Guardians must be present with the child, holding the phone or device during telehealth consultations. It entails a dialogue between the parent, the child, and the healthcare provider. Often, clinicians exhibit oral hygiene methods to the child while the parents are engrossed in their phones, hence improving the cooperative nature of telehealth consultations.”

“Parents often exhibited higher levels of involvement and openness in discussing personal matters, including social and stress-related concerns. This provided a fresh perspective to better understand the families' domestic circumstances, ultimately facilitating the establishment of strong connections and the customisation of patient-centred care.”

Technical obstacles encompassed inadequate internet connectivity among parents or the use of outdated devices, resulting in delays or interruptions in video transmission. Considerable time and effort were often spent instructing parents on installing and using the app before appointments.

Clinicians also observed that patients exhibited a lesser degree of seriousness towards the telehealth service compared to in-person appointments, resulting in higher rates of non-attendance compared to face-to-face appointments.

Due to the lack of reliable internet connections and access to technology, not all patients, especially those in rural or low-income areas, were able to participate in these consultations."

"Certain patients preferred in-person interactions because they felt disconnected from the care provided through teledental programmes."

Discussion

This study was conducted at the Pediatric Dentistry Department of a private dental hospital in Multan, Pakistan. The survey found that parents were generally satisfied with the telehealth services. Overall, parents were willing to utilize the service again, appreciating the convenience and ease of communication it provided. The benefits of telehealth were particularly significant for families facing transportation issues. Additionally, parents who spoke English or Urdu and were highly literate were more likely to accept the service. A 2021 systematic review found that tele dentistry procedures significantly reduce plaque, gingival indices, and white spot lesions.[13]

These constraints highlight the need to ensure equal access to tele dentistry services for all patients to improve its reach and efficacy in dental treatment. Barriers such as limited literacy, lack of technological knowledge, slow internet access, and a preference for in-person care can significantly impact the implementation of tele dentistry. Limited literacy and technological knowledge can prevent patients from effectively using telehealth platforms, while slow internet access can hinder the quality of virtual consultations. Additionally, a desire for in-person care may reduce the acceptance of tele dentistry among certain patient groups. Addressing these issues is crucial for boosting tele dentistry’s efficacy and expanding its reach in dental care.[14]

There are strong reasons to continue implementing telehealth, especially in primary preventive dentistry, beyond the initial impact of the COVID-19 pandemic and related

restrictions. Telehealth holds promise for addressing the oral health needs of rural and remote populations, where access to healthcare services is often limited, if provided with good internet and digital literacy skills, especially in developing countries like Pakistan. Telehealth services can enhance access to dental care by connecting patients with providers in the comfort of their homes. A study in developing countries found that dentistry can enhance access to and delivery of oral health care at a lower cost. Several private digital platforms in Pakistan currently offer these services, improving access to dental care for individuals who lack easy access to traditional in-person services.[15,16]

The study highlighted the challenges families encountered when travelling long distances to access tertiary dental care services, underscoring the importance of telehealth in addressing these accessibility issues. Feedback from parents underscored the difficulties associated with attending multiple appointments. Consequently, the findings supported the permanent incorporation of tele dentistry program for initial screening, consultations, and paperwork with positive feedback already evident. This adjustment not only enhanced efficiency but also contributed to an improvement in overall patient satisfaction, reflecting the positive impact of integrating telehealth services. Additionally, it allowed for greater flexibility in scheduling and reduced wait times for patients.[17,18]

Interestingly, current research revealed a disparity in the satisfaction and feasibility ratings of the telehealth service between families speaking English or Urdu and those with high literacy rates. This highlights the importance of considering cultural and language barriers when implementing telehealth services to ensure equitable access and satisfaction among all patient populations. Language and cultural challenges can significantly impact the viability and patient satisfaction of telehealth services. To guarantee fair access for all patients, it is crucial to implement measures such as providing interpreters or translators for non-English and non-Urdu-speaking patients. Additionally, ensuring that telehealth platforms are user-friendly and accessible to individuals with varying levels of technological literacy is essential. Moreover, cultural competency training should be provided to all staff members. The findings indicate that children with impairments and/or unique needs, who frequently experience dental problem, can significantly benefit from continued tele dentistry utilisation, given the availability of suitable infrastructure and support.[19,20]

Furthermore, telehealth could help the dental system save money and time. According to previous study, low-cost cellphone preventive services can save a lot of money by lowering the caries rate in kids from low-income families.[21,22] While telehealth offers flexibility and is well-received by patients, families, and clinicians, challenges such as appointment cancellations and non-attendance persist. To address this issue, the hospital should implement SMS reminders for telehealth appointments. However, the practicalities and cost-effectiveness of this option warrant further assessment.

Although personalised preventive advice via telehealth is beneficial, research suggests that face-to-face appointments are more effective in reducing plaque levels and cavities, especially in educating patients on proper oral hygiene practices and fluoride techniques. Therefore, It is important to carefully think about the role of telehealth as a useful addition to regular dental visits in addition to in traditional in person consultations for preventative care.

Conclusion

Parents of paediatric patients and clinicians alike rated preventive dental telehealth visits satisfactory. The benefits of the programme were more pronounced for individuals having residence far away from hospital, those that have special needs related to healthcare, whose primary language was Urdu, and had a good internet connection. Clinicians reported improved communication with families, increased parental involvement, and enhanced comfort for children at home. Disadvantages included the excessive time and effort needed to fix technological problems, bad internet connections, and more missed appointments.

Even with these problems, the results show that preventive oral health services delivered through virtual platform are still useful as an addition to in-person care, especially for patients with specific needs. Future research should include larger and more diverse patient populations, incorporating interpreters as needed, to further explore how telehealth services can overall impact the healthcare quality. This knowledge can inform improvement and enhancement in telehealth services, consultant training, and the development of best practices.

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Authors Contribution

HJ: Conceptualization of Project
HP: Data Collection
IE: Literature Search
HA: Statistical Analysis
SUK: Drafting, Revision
WH: Writing of Manuscript

Appraisal of Adequate Nutritional Knowledge and Related Sociodemographic Factors among Cancer Patients

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Abstract

Objective: To assess nutritional knowledge among cancer patients.

Material and Method: It was a cross sectional descriptive study conducted at Ittefaq hospital, affiliated with Sharif Medical and Dental College, Lahore from 6th November,2021 to 6th May,2022. 192 cancer patients aged 15-70 years were selected by non-probability convenient sampling technique.

Results: The mean age of sample population was 43.46±15.257 years. Out of 192 participants, 56.3% of participants were females. About 25% of participants were illiterate. Majority of participants had monthly family income ranging from 25,000 to 50,000 rupees. Participants were undergoing various treatment modalities with the highest percentage (88%) of patients receiving chemotherapy. About 78% of participants had adequate nutritional knowledge. Participants had poor knowledge regarding diet disease relationship, role of snacking and amount of fat in egg. We found statistically significant association between nutritional knowledge and education, monthly income, and treatment modality.

Conclusion: In the study, more than two thirds of participants had adequate nutritional knowledge. Treatment modality, monthly income, and education level were observed as major determinants of nutrition-related knowledge.

Keywords: cancer, malnutrition, treatment, knowledge

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Introduction

Diet plays pivotal role in maintenance of health and prevention of diseases by providing various nutrients to our body required for normal functioning.[1] Cancer cases are increasing day by day worldwide becoming one of the main public health issues. This increase in incidence rates is due to increase in environmental carcinogens, unhealthy dietary habits and sedentary lifestyles.[2,3]

Malnutrition and cancer are deeply interrelated. Cancer and its aggressive treatment both cause huge effects on health and nutrition, leading to cancer cachexia. The complicated etiology of malnutrition in cancer patients is influenced by the location and type of tumor, stage of the disease, side effects of the aggressive therapies, socioeconomic status, lack of nutritional knowledge, inadequate nutritional therapy, number and interest of caretakers, financial stress as well as lack of medical staff awareness.[4] Muthike CW, et al found out that nutritional practices among cancer patients revolve around various factors which are inter-related. These include lack of diet-disease knowledge, patient's dietary preferences, poor choice of food quality, improper hygiene, ignorant cultural practices, myths and superstitions, metabolic state impacted from cancer, impact

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of chemo-radiation, nausea and taste alterations associated with treatment, poor management of symptoms, limited role of appetite-enhancers, difficult timely navigation through health system, financial and psychiatric influences of cancer, poverty of interest to seek nutritional advice and unavailability of qualified nutritionist.[3]

Many studies have suggested that a healthy diet containing rich amount of vegetables, fruits, whole grains and legumes and less amount of red meat is very helpful to help body fight against cancer. Such diet provides various vitamins, minerals and other protective substances which are beneficial against cancer. American Institute of Cancer Research (AICR) recommends healthy eating and maintaining healthy weight to reduce risk of new and recurrent cancers[5] as obesity is one of the important risk factors for various carcinomas including breast and prostate carcinomas.[2,6] Patients undergoing cancer treatment should also have ample knowledge regarding food safety because immune system is compromised in some treatments which increases risk of food-borne infections.[7] As there is no nutritional knowledge study done in cancer patients previously in Pakistan, this study will also help to find the sociodemographic determinants associated with nutritional knowledge in these patients so that dietary modifications would be implemented in future to improve nutritional status which will in turn produce better survival and quality of life.

Material and Method

It was a cross sectional descriptive study conducted at Ittefaq hospital, affiliated with Sharif Medical and Dental College, Lahore from 6th November, 2021 to 6th May, 2022. After taking permission from Ethical Review Committee(No SMDC/SMRC 155-20 Dated: 19-01-2021), a sample of 192 cancer patients aged 15-70 years of both genders with any type of cancer presented in outdoor or indoor departments, was selected by non-probability convenient sampling technique. Patients who were unconscious and who did not give consent were excluded. Data was collected by three researchers and recorded on semi-structured questionnaire containing 18 questions in knowledge section. A score of ≥ 9 (50%) was considered adequate. Data collected was entered and analyzed using the SPSS version 21. Mean ± standard deviation was calculated for the quantitative variables like age. Frequency and percentages were calculated for categorical variables like gender, occu-pation, education and nutritional knowledge. Effect modifiers like age, gender, education, employment, family size, diagnosis and treatment modality were addressed through stratification. Post stratification, data was analyzed by using Chi-square test taking p-value ≤0.05 as significant.

Results

The mean age of the sample population was 43.46 years ±15.257 years standard deviation. 56.3% of participants included in the study were females while 43.7% were males. The most common cancer among females was breast carcinoma while the most common cancer among males was prostate carcinoma. This study showed that out of 192 participants, 150 (78.1%) participants scored ≥9 out of 18, which is indicative of adequate nutritional knowledge. 42 (21.9%) had inadequate nutritional knowledge as they scored less than 9 out of 18 (Table 1). Stratification was done for categorical variables like age, gender, education, marital status, employment status, family size,

Table 1: *Frequency distribution of respondents according to the adequacy of nutritional knowledge*

Nutritional knowledge	Frequency	Percentage
Adequate (≥9/18)	150	78.1
Inadequate (<9/18)	42	21.9
Total	192	100

Table 2: *Association of adequacy of nutritional knowledge with stratified data on basis of various sociodemographic factors*

Sociodemographic characteristic of respondents		Nutritional knowledge		Total	p-value
		Adequate	Inadequate		
Education status	Illiterate	17	31	48	0.001
	Primary/middle	44	09	53	
	Matric/intermediate	61	02	63	
	Graduation &above	28	0	28	
	Total	150	42	192	
Monthly family income (in rupees)	Less than 25000	25	14	39	0.01
	25000-50000	56	16	72	
	51000-75000	31	10	41	
	Above 75000	38	2	40	
	Total	150	42	192	
Treatment modality	Chemotherapy	137	32	169	0.04
	Radiotherapy	6	5	11	
	Surgery	6	5	11	
	Palliative	1	0	1	
	Total	150	42	192	

monthly family income, diagnosis, and treatment modality. Chi-square test was applied, and our results revealed that there was significant asso-ciation ($p \leq 0.05$) between level of knowledge of respon-dents and their educational status, monthly family income, and treatment modality (Table 2). There was no significant association found between adequate nutritional knowledge and age, gender, marital status, employment status, family size, or diagnosis. Majority of participants 92.7% and 62.5% were not aware of the diet-disease relationship and importance of snacking during cancer respectively while only 37.5% of participants knew the correct frequency of food intake by cancer patients, i-e: 3 meals and 2 snacks.

Discussion

Nutrition plays an important role in prevention or causation of most cancer. Moreover, malnutrition during cancer can lead to adverse treatment outcomes leading to poor prognosis. This study showed that certain sociodemographic characteristics of population greatly affect the participants' responses and their level of knowledge. The study included 192 participants from various age groups. The minimum age of respondent in our study was 17 years while the maximum age was 70 years. The mean age of our sample was 43.46 years±15.257 years. Almost 62 % of participants in this study belonged to middle age group, ranging from 31 years to 60 years. Majority (58%) of participants of study conducted by Muthike CW also belonged to middle age group.[3] The relatively early onset of cancer is mainly due to various nutritional and lifestyle changes, leading to obesity which in turn promote the cancer development.[8] The study participants were divided based on marital status with 135 married, 37 unmarried, 2 divorced and 18 widowed participants. There was no association found between marital status and nutritional knowledge in our study. This contrasts with the findings of study conducted by Parmenter K et al. in England where there was a significant difference of nutritional knowledge among married and unmarried, widowed or divorced groups.[9] A highly significant association was found between education status and nutritional knowledge (p value 0.001). This result is in accordance with a study conducted by Muthike CW who also found strong association between nutritional knowledge and education status.[3] We found statistically significant difference among nutritional knowledge of people belonging to various income groups (p value 0.01). This finding is in accordance with the study conducted on patients of breast carcinoma by Patella MN et al. to assess the nutritional knowledge where education status and social status have been strongly associated with nutritional knowledge of patients.[10] Participants of the study were receiving different treatment modalities including chemotherapy, radiotherapy, surgery, and palliative care. Patients receiving chemotherapy were in the majority with 88% while 5.7%, 5.7% and 0.5% of participants were receiving radiotherapy, surgery and palliative care respectively. Majority (67%) of participants of study conducted by Muthike CW were also receiving chemotherapy.[3] It was concluded in this study that nutritional knowledge of participants varied with the type of treatment modality (p value 0.04)

Mean nutritional knowledge score of the study came out to be 11.53±2.705 (64%). The maximum knowledge score was 18/18 while minimum score was 5/18. 78% of participants in this study had adequate nutritional knowledge while 22% of participants had inadequate knowledge. This study results are close to the findings of Patella MN et al. who found that 60% of breast cancer patients had adequate nutritional knowledge.[10] Similarly, Nambala E et al. also found 60% of patients with non-communicable diseases in their study had sufficient nutritional knowledge.[11] Regarding knowledge about relation of inadequate nutrition with any disease, majority (92.7%) of participants were not aware of this relationship. Similarly, Muthike CW also found very poor knowledge score in diet disease relationship section.[3] Majority (62.5%) of participants thought that snacking is not required while only 37.5% of participants knew that snacking plays an important role in maintaining health of cancer patients. American Cancer Society recommends having snacks in addition to meals during a day. During cancer treatment, energy requirement of human body is increased, and snacking can help to provide needed energy and maintain healthy weight.[12] Moreover, it is also proven that fruits and vegetables taken as snacks decrease the mortality rates in cancer patients.[13] Only 37.5% of participants knew the correct frequency of food intake by cancer patients, i-e: 3 meals and 2 snacks. Enough number of participants (40.6%) responded to have only 3 meals per day while 18.2 % of participants answered to have 2 meals and 3 snacks daily. AICR recommends at least 5 servings per day, 3 meals and 2 snacks.[5]

This study was conducted in one center only and nutri-

tional practices were not evaluated in this study. So, further research is recommended.

Conclusion

More than two-thirds (78.1%) of our study participants had adequate nutritional knowledge. The worst scores were found in diet-disease relationship, the role of snacking, and required frequency of food intake. Education, income status, and treatment modality of participants showed statistically significant association with nutritional knowledge.

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Authors Contribution

AR, MSI: Conceptualization of Project
AR, MAS, LA: Data Collection
AR,AIB: Literature Search
AR, MAS, MSI: Statistical Analysis
LA, QA: Drafting, Revision
AR, AIB: Writing of Manuscript

Frequency of Anemia in Type-I & type-II diabetes Mellitus Patients of Akhtar Saeed Trust Hospital

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Abstract

Objective: This study aimed to estimate the frequency of anemia and its association with type-I and type-II diabetes mellitus.

Material & Methods: In this cross-sectional study, the patients presented with type-I and type-II diabetes mellitus were enrolled. Samples were collected from the outdoor and indoor patients of Akhter Saeed Trust Hospital Lahore for a one-year duration. HbA1c was performed to confirm the control of diabetes mellitus, and CBC showed the presence of anemia. This study was conducted on patients of both genders and all age groups. Based on age, history, and therapy, type I and type II diabetes mellitus were classified. A chi-square test was performed to assess any significant correlation between diabetes mellitus and anemia in all age groups and both genders.

Results: This study was performed on 237 patients, 66.85 were female, and 33.2% were diabetic males. Among all diabetic patients, only 26.58 % were without anemia, and the remaining 73.42% developed any state of anemia among mild, moderate, or severe. The p-values show a significant association (p=0.001) between diabetes and anemia. It was also found that patients with good control of diabetes show good management of anemia in both types of diabetes, and with poor control, anemia development chances are high.

Conclusion: Anemia is associated with both type-I and type-II diabetes mellitus. The good control of diabetes mellitus is necessary for the management of anemia.

Keywords: Type-I diabetes mellitus, type-II diabetes mellitus, anemia, diabetic control

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Introduction

Diabetes mellitus (DM) refers to elevated blood sugar levels during periods of fasting or after meals. Sustained high blood sugar is a defining feature of DM, accompanied by damage and impaired function in organs such as the kidneys, nerves, heart, and blood vessels.[1] In 2011, the International Diabetes Federation reported that 366 million people worldwide were affected by DM, a number projected to increase to 552 million by 2030. The World Health Organization, in agreement with the American Diabetes Association and the Canadian Diabetes Association, established diagnostic criteria for DM in 2006. These criteria encompass a random plasma glucose level of 200 mg/dL (11.1 mmol/L), a 2-hour plasma glucose level of 200 mg/dL (11.1 mmol/L) following a 75 g glucose load, or a fasting plasma glucose level of 126 mg/dL (7.0 mmol/L) observed on two or more occasions.[2]

Anemia, a condition characterized by a deficiency in red blood cells or hemoglobin, can be intricately linked to both type-I and type-II DM. While the mechanisms and impacts might differ, the presence of anemia can

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further complicate the management and overall health of individuals with either type of diabetes. Type-I DM is an autoimmune disorder where the body's immune system attacks and destroys the insulin-producing cells in the pancreas. This results in a lack of insulin, which is essential for glucose uptake by cells.[3]

The association between type-I DM and anemia often revolves around a few key factors. The same autoimmune process that targets insulin-producing cells can also affect other cells and functions within the body. In some cases, it can reduce the production of red blood cells, resulting in anemia. People with type I DM might face challenges in managing their diet due to insulin dependence. This can sometimes lead to inadequate intake of essential nutrients like iron, vitamin B12, and folate, which are crucial for red blood cell production.[4] Such nutritional deficiencies can contribute to anemia. While kidney complications are more commonly associated with type II DM, individuals with type I DM can also experience kidney dysfunction. Kidney impairment can affect the production of erythropoietin, a hormone that stimulates red blood cell production. Reduced erythropoietin levels can lead to anemia.[5]

Type II DM is characterized by insulin resistance, where the body's cells become less responsive to insulin. This leads to elevated blood sugar levels. Anemia's association with type II DM is influenced by several factors. Many individuals with type II DM experience chronic low-grade inflammation. This inflammation can interfere with the body's ability to produce red blood cells, contributing to anemia.[6] Kidney complications are more prevalent in type II DM. The kidneys play a significant role in producing erythropoietin, which stimulates the bone marrow to create red blood cells. When kidney function is impaired, erythropoietin production decreases, leading to anemia. Some medications used to manage type II DM, such as metformin, can affect nutrient absorption and utilization. Prolonged use of these medications might contribute to nutritional deficiencies, further exacerbating anemia. Uncontrolled blood sugar levels can impact the health of blood vessels, leading to reduced oxygen supply to tissues and exacerbating anemia-related symptoms like fatigue and weakness.[7] Managing anemia in individuals with diabetes requires a comprehensive approach that addresses the specific factors at play. Regular monitoring of complete blood count (CBC), iron, vitamin B12, and folate levels is essential. Nutritional counseling, supplementation when necessary, and close collaboration with healthcare providers are crucial components of managing anemia in the context of diabetes.[8] In conclusion, anemia's association with both type-I and type-II DM highlights the complexity of these conditions. While the underlying mechanisms and risk factors might differ, anemia can significantly impact individuals with DM's health and quality of life. Understanding these connections and implementing appropriate management strategies can help mitigate the effects of anemia and contribute to more effective diabetes care.[9,10]

Given the high prevalence of DM in our region and the well-established association between DM and anemia, our study was designed with a dual purpose. We aimed to assess the frequency of anemia among diabetic patients while investigating the intricate relationship between these two conditions. Additionally, we sought to determine how anemia correlates with diabetic patients' varying levels of glycemic control, distinguishing between good and poor management. Our study's implications are far-reaching. By raising awareness about the importance of regular anemia screenings and proper management, we aim to empower diabetic patients to take charge of their health. Moreover, our findings shed light on the potential risk of anemia in well-controlled diabetic patients, as well as those struggling with their condition. Ultimately, this research contributes to a more comprehensive understanding of the intersection between diabetes and anemia. It equips healthcare providers and patients alike with essential insights, encouraging improved care practices. As the study outcomes emerge, we anticipate its pivotal role in driving awareness, refining healthcare strategies, and enhancing the overall well-being of individuals dealing with diabetes and its potential complications, including anemia.

Materials & Methods

The cross-sectional study was conducted in Akhter Saeed Trust Hospital Lahore from January 2022 to January 2023. All diabetic patients of the said duration were part of this study, and non-diabetic patients were excluded. 237 samples were collected from diabetic patients receiving therapy to control their diabetes. Patients with type-I and type-II DM were part of this study. Blood samples were collected in the ethylenediamine tetraacetic acid (EDTA) vacutainer to perform a CBC and hemoglobin A1C (HBA1c). CBC was performed on Sysmex XP-100. HbA1c was performed on an automated analyzer Audicom AC-600 based on ion exchange chromatography. A peripheral smear was performed on

all the samples to confirm the red blood cell morphology which was microcytic, macrocytic, or normocytic. The morphology was assessed to correlate it with hemoglobin concentration which was calculated previously using the hematology analyzer. The anemia (severe, moderate, or mild) was confirmed on the basis of hemoglobin concentration and correlation with RBC morphology. Patients with hemoglobin less than 7 g/dl were considered severe anemic, between 7-10 g/dl moderate, and more than 10 g/dl concluded as mild anemic. Patients with HBA1c of more than 5.5% were included in this study. Patient HBA1c values less than 7.0% were considered good control. If HbA1c was more significant than 7.0%, it showed poor control of diabetes. Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were also noted as evidence of anemia. Patients of both genders (male and female) of all ages were part of the study. Demographics, anemic, and diabetic status were noted in the already-designed data collection performance. To analyze the collected data, Statistical Package for the Social Sciences (SPSS) version 25.0 was used. Data was distributed into different age groups and genders. The frequency of type-I and type-II DM patients were also identified. A chi-square test was performed to assess the association between the different stages of anemia and type-I and type-II DM. The p-values were also calculated to assess the anemia and diabetic control.

Results

A total of 237 samples were included in the study, 66.2% (n=157) were females, and 33.8% (n=80) were males. All diabetic patients were divided into four age groups, patients with ages less than and equal to 18 years, 19-40 years, 41-60 years, and > 60 years. It was found that 06(2.53%), 45(18.98%), 128(54.00%), and 58 (24.47%) of patients were included in the first, second, third, and fourth age groups respectively. Table 1 shows the frequency of anemia among different age groups.

Table 1: *Mild, moderate, and severe anemia in different age groups*

Age groups n (%)	Mild anemia	Moderate anemia	Severe anemia	No anemia
≤ 18 Years	03 (50.00%)	0%	0%	03 (50.00%)
19-40 Years	10 (22.22%)	23 (51.11%)	09 (20.00%)	03 (6.66%)
41-60 Years	27 (21.09%)	54 (42.18%)	32 (25.00%)	15 (11.71%)
>60 Years	15 (25.86%)	27 (46.55%)	10 (17.24%)	06 (10.34%)

In both genders (male and female), 19 (23.75%) of males develop mild, 30 (37.50%) moderate, and 11 (13.75%) severe anemia. Similarly, among females, 32 (20.38%), 71 (45.22%), and 11 (7.00%) developed mild, moderate, and severe anemia. The overall frequency of mild, moderate and severe anemia were 51 (21.51%), 101 (42.61%), and 22 (9.28%) respectively. Table 2 shows the percentiles of mild, moderate, and severe anemia in both genders. The frequency of type-I and type-II diabetes mellitus was 12.23% (n=29), and 87.76% (n=208) respectively. In Table 3 percentile of mild, moderate, and severe anemia is reported in type-I and type-II diabetic patients, showing that 09 (31.03%) and 71 (45.22%) of patients developed moderate anemia respectively, which is the highest among all. Table 4 represents anemia in diabetic patients with good or poor control. The chi-square test showed a significant association (p=0.001) between anemia, and control status of diabetes.

Table 2: *Percentages of mild, moderate, and severe anemia in males and females*

Types of anemia	Male	Female
Mild	19 (23.75%)	32 (20.38%)
Moderate	30 (37.50%)	71 (45.22%)
Severe	11 (13.75%)	11 (7.00%)
No Anemia	20 (25.00%)	43 (27.38%)

Table 3: *Mild, moderate, and severe anemia in type-I & type-II diabetes mellitus*

Types of anemia	Type-I diabetes mellitus n=29	Type-II diabetes mellitus n=208
Mild	06 (20.68%)	52 (25.00%)
Moderate	09 (31.03%)	97 (46.63%)
Severe	03 (10.34%)	49 (23.55%)
No Anemia	11 (37.93%)	10 (4.80%)

Table 4: *Association of anemic patients with good and poor diabetic control*

Types of anemia	Good control	Poor control	Chi-square P-value
Mild	49 (96.07%)	02 (3.92%)	
Moderate	25 (24.75%)	76 (75.24%)	0.001*
Severe	0%	22 (100.0%)	

*P<0.05 is considered significant.

Discussion

In our study, a total of 237 DM patients with all age groups were included, and they were also assessed for excellent and poor diabetic control and association with the development of anemia. There was a significant relationship between anemia and control of DM. It was assessed that patients with type-I and type-II DM are more likely to develop anemia, especially when diabetes is under poor management. Anemia is a common complication in type-II DM patients, impacting diabetes-related complications. However, little is known about anemia prevalence and factors in type-II DM patients in specific regions like Debre Berhan Referral Hospital in North-East Ethiopia. This study addresses the gap by assessing anemia prevalence and determinants among 249 type-II DM patients at Debre Berhan Referral Hospital. The findings reveal a 20.1% anemia prevalence, associating anemia with factors including age (>60 years), poor glycemic control, reduced eGFR, longer diabetes duration (>10 years), and diabetic complications. These insights underscore the importance of anemia screening, tailored interventions, and improved patient care within the type-II DM population, leading to better outcomes.[8]

The objective of another study was to ascertain the prevalence of anemia among individuals with type-II DM patients and evaluate its correlation with gender, age, and glycemic control. The study encompassed patients attending the outpatient diabetic department of Amiri Hospital (Al-Asimah Capital area) from January 1, 2016, to December 31, 2017. Patients were categorized based on glycemic status and gender. The assessment involved analyzing both HbA1C values and hemoglobin levels. Anemia was defined by hemoglobin levels < 13.0 g/dL for men and < 12.0 g/dL for women. The results indicated a significantly higher prevalence of anemia in diabetic females (38.5%) compared to diabetic males (21.6%). Similarly, poorly controlled diabetics exhibited a higher prevalence of anemia (33.46%) compared to those with well-controlled glycemic status (27.9%) (P= <0.05). Notably, patients with anemia had an average age of 60.69 ± 0.198 years, whereas patients without anemia had an average age of 54.07 ± 0.121 years, revealing an increased risk of anemia with advancing age.[11]

A total of 5999 participants older than 40 years old from the general community were enrolled for the study. A total of 1414 participants were diagnosed with DM. To check for anemia, hemoglobin levels were measured. 12.3% of people had anemia (Hemoglobin: 12g/dl in women and 13g/dl in males). Anemia prevalence was higher in women than men between the ages of 40 and 49 years. The risk of developing diabetic retinopathy was twice higher in males with anemia than in women.[12] Although type II DM affects most study participants, anemia is a frequent complication. The prevalence and factors influencing anemia in type-I DM outpatients were examined. CBC was taken in addition to routine testing in patients with type-I DM at the Royal North Shore Hospital (n =135), the Royal Prince Alfred Hospital (n =42), and the Austin Medical Centre (n =135) in Sydney, Australia. 01 in 07 patients (14%) experienced anemia, which is more than six times more common in those with functioning kidneys. More than half (52%) of patients with macroalbuminuria developed anemia, compared to 24% of patients with microalbuminuria and less than 8% of patients with normoalbuminuria. Compared to diabetic patients with adequate renal function, diabetic patients with renal impairment had anemia more than six times as frequently. Anemia, a significant underappreciated burden, is frequently seen in type I DM patients.[13] The hundred children of six to seventeen years of age with type-I DM were included. Based on their clinical symptoms and eventually the discovery of islet cell autoantibodies, it was determined that all individuals had type-I DM. The patients were divided into four groups according to how long they had the disease: those with newly diagnosed type-I DM, those with the condition for one to three years, those with the condition for four to six years, and those with the condition for more than six years. The following parameters have been determined: red blood cells, hemoglobin, HbA1c, hematocrit, MCV, MCH, MCHC, and serum iron content. The levels of hematocrit, hemoglobin, red blood cells, and MCV are significantly lower in children when compared to children who have had type-I DM for a longer period. Statistical analysis showed inverse associations between the MCH concentration and children who have had type-I DM for more than a year.[14] In this study, it was found that patients with poor diabetic control are more prone to developing anemia and vice versa in patients with good control of diabetes. The cross-sectional study provides a snapshot of data at a specific point in time. It cannot establish causality or show how variables change over time. A larger sample size may provide more resilient results. Future studies may add risk factors and can be conducted for a longer period.

Conclusion

Diabetic patients of both types (I & II) are more likely to develop anemia in all age groups, especially when diabetes is not under control. The association between anemia and DM is not dependent on gender. Both genders have an equal chance of facing anemia due to many underlying factors.

Conflict of interest *None*

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Authors Contribution

AW, AFK: Conceptualization of Project
RA: Data Collection
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Frequency of Enteric Fever Among Children Presenting with Acute Febrile Illness

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Abstract

Objective: To determine the prevalence of enteric fever in children with acute febrile illness.

Material and Method: From June 1st, 2023 to December 31st, 2023, researchers from the Department of Paediatrics at Aziz Bhatti Shaheed Teaching Hospital in Gujrat gathered data in a cross-sectional study. The study comprised a total of 150 children who were experiencing an acute febrile illness. Capillary and venous blood samples totaling around 5 ml were taken from individuals in a sterile manner. Enteric fever was considered positive when blood culture for S. Typhi (Wilson and Blair bismuth sulphite agar showed jet black colony with a metallic sheen). Data were entered and analyzed with statistical analysis program v25. We stratified by age, gender, duration of fever, and body mass index to examine the impact of these factors on the development of enteric fever. The Chi-square test was used for post-sorting the data. When the p-value was less than 0.05, it was determined to be significant.

Results: The frequency distribution results of gender showed that 66(44.0%) were male and 84(56.0%) were female patients in our study. The mean age of patients in our study was 6.58±3.11 year with minimum value was 1 years and maximum value was 12 years. In our study, the frequency distribution results of enteric fever showed that, 34(22.7%) had enteric fever.

Conclusion: Acute fevers often result from typhoid. Acute fever is often caused by typhoid because of improper hygiene.

Key words: Acute Febrile Illness, Enteric Fever.

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Introduction

Salmonella enterica subsp. Entericaserovar Typhi is the causative agent of typhoid fever. Because of the widespread lack of infrastructure to provide clean water and toilets in poor communities. In 2010, it was predicted that there were 12 million cases of typhoid fever and 130,000 fatalities worldwide. Pakistan has one of the highest burden rates in the world, with more than 100 new cases per 100,000 people each year. The incidence of laboratory confirmed typhoid and paratyphoid infections in India was estimated to be 9.7 and 0.9%, respectively, in a recent systematic review and meta-analysis.[1-3]

Typhoid is still quite common, but there have been fewer confirmed cases in recent years. Blood or bone marrow culture confirmation is necessary for a final diagnosis of typhoid fever. Due to low levels of bacteremia and previous antibiotic usage, blood culture has a number of drawbacks, including the volume of blood needed. Particularly in resource-poor nations where laboratory diagnostic facilities are inadequate, the detection of bacterial febrile diseases presents a significant difficulty.[4-6]

Clinical differentiation between malaria and other bacterial febrile infections can be challenging since their symptoms overlap significantly. The similarity in clinical presentation between diverse bacterial febrile infections

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makes laboratory detection of these diseases challenging outside of emergency situations. A study by Chipwaza B, et al. showed that frequency of enteric fever was 10.3% among children presenting with acute febrile illness. Another study by Sandhya T, et al. has shown that frequency of enteric fever was 32.40% among children presenting with acute febrile illness.[7-8]

Limited data is available on this subject in Pakistan. Moreover results of different studies in different populations have shown variability in results, so these results cannot be generalized on all populations due to this variability. Therefore, it is my intention to assess the prevalence of enteric fever in children with acute febrile illness. A verified diagnosis is necessary for a conclusive diagnosis of typhoid fever. Patients with typhoid fever can be better managed with antibiotic treatment if the disease is identified and diagnosed quickly and accurately.

Material And Method

The cross-sectional study was conducted at Department of Pediatrics, Aziz Bhatti Shaheed Teaching Hospital, Gujrat from June 1st, 2023 to December 31st, 2023. Children having ages between 1-12 years of either gender and presenting with acute febrile illness were included in this study. Children with H/o tuberculosis on medical record, H/o malaria on medical record and H/o diarrhea on medical record (during last 2 weeks) were excluded from study. Sample size was 150 which was calculated by using WHO sample size calculator with margin of error as 5%, 95% confidence level and anticipated frequency of enteric fever as 10.3%.[7]

After receiving approval from the hospital's ethics council, the research enrolled a total of 150 children from the paediatrics department at Aziz Bhatti Shaheed Teaching Hospital in Gujrat. Each parent gave their agreement after being assured of the study's confidentiality and the lack of harm to their child. Capillary and venous blood samples totaling around 5 ml were taken from individuals in a sterile manner. After that, the collected sample was sent to same hospital laboratory. Enteric fever was considered positive when blood culture for S. Typhi (Wilson and Blair bismuth sulphite agar showed jet black colony with a metallic sheen).

Acute febrile illness was defined as when children presented with temperature >101°F (by thermometer) for last 4 days. Enteric fever was defined as when positive blood culture for S. Typhi (Wilson and Blair bismuth sulphite agar showed jet black colony with a metallic sheen) by laboratory test. Data were entered and analyzed with statistical analysis program v25.0. Quantitative factors such as age, duration of fever, and weight were provided as means and standard deviations. For qualitative factors such as gender and enteric fever, frequencies and percentages were calculated. We stratified by age, gender, duration of fever, and body mass index to examine the impact of these factors on the development of enteric fever. The Chi-square test was used for post-sorting the data. When the p-value was less than 0.05, it was determined to be significant.

Results

The frequency distribution results of gender showed that 66(44.0%) were male and 84 (56.0%) were female patients in our study. Female patients were more than male patients. The mean age of patients in our study was 6.58±3.11 year with minimum value of 1 years and maximum value of 12 years. In our study 84(56.0%) patients were from 1-6 years age groups and 66(44.0%) patients were from 7-12 years age group. Most of the patients were from 1-6 years age group. The mean weight of patients in our study was 19.74±9.33 kg, the minimum value was 6 kg and maximum value was 36 kg. The frequency distribution results of weight showed that 99(66.0%) patients were from <25 kg weight group and 51(34.0%) were from >25 kg weight group. The mean value of duration of fever of patients in our study was

Table 1: *Frequency distribution of different variables*

Gender	Frequency	Percent
Male	66	44.0
Female	84	56.0
Total	150	100.0
Age groups		
1-6 years	84	56.0
7-12 years	66	44.0
Total	150	100.0
Weight of child		
[illegible]	99	66.0
>25 kg	51	34.0
Total	150	100.0
Duration of fever		
≤3 days	73	48.7
>3 days	77	51.3
Total	150	100.0
Enteric fever		
Yes	34	22.7
No	116	77.3
Total	150	100.0

3.49±1.16 days with minimum value of 2 days and maximum value of 5 days. In our study the duration of fever (days) results showed that 73(48.7%) patients had <3 days of duration of fever and 77(51.3%) had >3 days duration of fever. In our study, the frequency distribution results of enteric fever showed that, 34(22.7%) had enteric fever. By stratification of enteric fever with respect to different variables, it was found that, both genders, age groups, weight groups and duration of fever have equal chances to have enteric fever (p>0.05).

Table 2: *Stratification of enteric fever with respect to different variables*

Variables		Enteric fever		p-value
		Yes	No	
Gender	Male	18(27.3%)	48(72.7%)	0.232
	Female	16(19.0%)	68(81.0%)	
Age groups	1-6 years	24(28.6%)	60(71.4%)	0.051
	7-12 years	10(15.2%)	56(84.8%)	
Weight	≤25 kg	24(24.2%)	75(75.8%)	0.521
	>25 kg	10(19.6%)	41(80.4%)	
Duration of fever	≤3 days	15(20.5%)	58(79.5%)	0.546
	>3 days	19(24.7%)	58(75.3%)	

Discussion

Infections with typhoid fever remain a serious public health concern. About 17 million people are infected with typhoid fever each year, and more than 600,000 die as a result. The annual typhoid case count in England and Wales is between 150 and 200. Africa and Latin America account for the bulk of new cases and fatalities. In the United Kingdom, the incidence of typhoid fever is among the lowest in the world, with just around one case for every one million people.[9] Antibiotic resistance has been an issue since at least 1950; by 1989, it has been documented in several countries, most notably in Asia and the Middle East. Disease outbreaks induced by resistant strains have recently occurred in India and Pakistan. Rainfall, moderate temperatures, and stagnant waterways offer environments perfect for mosquito larva, making tropical regions like Sub-Saharan Africa particularly vulnerable to the disease. Nearly a quarter of all infant fatalities in Africa can be attributed to cerebral malaria and anaemia.[9-10] In 2010, there were likely 219 million confirmed cases of malaria, according to the World Health Organisation. Young children are particularly vulnerable to the health consequences of malaria. It's a key cause of poverty and a barrier to progress in the economy. Clinical differentiation between malaria and other bacterial febrile infections can be challenging since their symptoms overlap significantly. The similarity in clinical presentation between diverse bacterial febrile infections makes laboratory detection of these diseases challenging outside of emergency situations.[10] A study by Chipwaza B, et al. showed that frequency of enteric fever was 10.3% among children presenting with acute febrile illness.[7] Another study by Sandhya T, et al. has shown that frequency of enteric fever was 32.40% among children presenting with acute febrile illness.[8] This research indicates that paediatric instances of typhoid fever are more prevalent than previously thought. Typhoid fever was shown to be more prevalent among children ages 5-9, which is in line with previous findings from Nigeria and Pakistan.[11-12] Since these are school-aged youngsters, it is reasonable to assume that they are consuming dangerous locally-made chilled beverages and ice creams at school. Breiman et al. found the highest prevalence of typhoid fever in children ages 2–4 and 5–9, which runs counter to our own research.[13] In addition, similar to findings from studies conducted in Pemba, Zanzibar, and Nigeria, females were more likely to contract typhoid than males.[14-16]

Conclusion

Acute fevers often result from typhoid. Acute fever is often caused by typhoid because of improper hygiene.

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Authors Contribution

MS: Conceptualization of Project
AM: Data Collection
AZ: Literature Search
AH: Statistical Analysis
MUK: Drafting, Revision
L: Writing of Manuscript

Correlation of High-Density Lipoproteins and Low-Density Lipoproteins in Polycystic Ovary Syndrome (PCOS) Patients

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Abstract

Objective: To investigate the correlation of high-density lipoproteins and low-density lipoprotein with poly cystic ovary syndrome patients (PCOS).

Materials and Methods: Data from 80 females were collected in a descriptive cross-sectional study. Participants were categorized into two groups normal healthy individuals and PCOS patients aged between of 18-45 years. Serum HDL and LDL levels were measured to assess liver function.

Results: The results revealed that most PCOS patients were between 26 and 35 years old. PCOS patients exhibited significantly reduced HDL levels (40.30 mg/dL). A correlation between HDL and LDL values in PCOS patients was observed. The Mean ± SD of High-Density lipoproteins (mg/dL) in healthy patients was found to be 50.52 ± 28.297and in PCOS was 40.30 ± 8.549 The mean ± SD of Low-Density Lipoproteins (mg/dL) was found to be 106.50 ± 30.750 in healthy patients and 119.98 ± 27.776 in PCOS patients. In normal healthy individuals and PCOS patients the p- value of < 0.001 in HDL and <0.235 in LDL was observed.

Conclusion: In conclusion, the current study highlighted a substantial link between HDL levels in healthy and PCOS patients. It also demonstrated that no significant change in LDL levels of PCOS and healthy patients. After comparing the high density and low-density lipoprotein these findings enhance our understanding of PCOS-related complications, enabling earlier and more accurate diagnoses.

Keywords: Polycystic Ovary Syndrome (PCOS), High-Density Lipoprotein (LDL), Low-Density Lipoprotein (LDL).

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disease in females (Lovwers and Laven, 2020).[1] The criteria by the National Institute of Health (NIH) were used to see the PCOS prevalence, it was shown to range from 4% to 6.6% among unselected reproductive-age women residing in the South-east of the United States. PCOS prevalence estimates range from 2% and 26% (Deswal et al., 2020).[2] Numerous pathophysiological abnormalities, including insulin resistance, hyperinsulinemia, and dyslipidemia, are seen in hyperandrogenic PCOS patients. Ultrasound examination of women suffering from polycystic ovary syndrome shows, polycystic ovaries. Insulin resistance is a key component of polycystic ovary syndrome (Zehravi et al., 2021).[3]

PCOS was present in 19.58% of the individuals. Approximately 1 in 5 females with Type 2 diabetes have PCOS. This percentage is much greater than the anticipated 1.14% to 11.04% PCOS prevalence among all female

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adolescents (Cioana et al., 2022).[4] Anovulation also found in dyslipidemic PCOS patients, alteration in lipid profile leads to infertility (liu et al., 2019).[5]

The link between the anthropometric characteristics of PCOS-afflicted women. They claimed that PCOS is the utmost prevalent endocrine disorder in female of fertility age, is related to reproductive and metabolic problems. According to recent studies, women with PCOS show early signs of developing an abnormal cardiovascular risk profile. Although these abnormalities differ depending on the culture, women with PCOS typically exhibited abnormal lipid profile patterns. Anthropometry was a straightforward and well-liked study method for identifying metabolic risk in PCOS-affected individuals. They looked at the anthropometry and lipid profiles of 86 married PCOS women between the ages of 18 and 35 applying Pearsons correlation of coefficient. The outcomes revealed that more than 80% PCOS-affected females had irregular anthropometric measurements, and more than 70% of these females also had dyslipidemia, like decrease HDL cholesterol and increased triglyceride and the low-density lipoproteins levels. Thus, it was determined that the most significant anthropometric factors associated with abnormal lipid profile in women of South Indian having PCOS were BMI & waist circumference. (Himabindu et al., 2017).[6]

According to research, specific nutrients such vitamins, minerals, and vitamin-like compounds should be included in the therapy of PCOS. Therefore, it was suggested that a vitamin or mineral shortage may be the root of PCOS. The nutritional supplements affect the oxidative stress caused by PCOS. Oxidative stress shapes the pathophysiology of PCOS. They concluded that antioxidant biomarkers and oxidative stress can effectively alter women's risk for cardiovascular events and PCOS severity. By reducing the impact of oxidative stress on PCOS, Simple dietary supplements could lessen these dangers. Supplemental nutrients like vitamins, minerals, probiotics, and other food additives can dramatically reduce PCOS symptoms. (Dubey et al., 2021).[7]

Treatment of PCOS may include lifestyle changes, weight loss by daily diet restriction and exercise must be included in daily life, recommended by health care providers. Medications, to regulate periods, clinician recommended hormonal therapy and in case of diabetes metformin and thiazolidinediones are widely used (Marinkovic et al; 2021)8. Statin drugs used to treat dyslipidemia and sodium-glucose co-transporter 2 (SGLT-2) inhibitors could be useful for PCOS patients due to beneficial cardiovascular and glycemic effects, which is an important issue in PCOS females (zhang et al; 2022).[9]

When compared to control subjects, women with PCOS had higher mean TGs and VLDL readings, but decreased HDL values. HDL is a diverse sub-particle class of lipoproteins known as High-density Lipoproteins includes a heterogeneous class of lipoproteins. Because lipid and protein ratios vary, these subclasses have different compositions and functions. The classification for these lipoproteins is founded on either density or composition (Wang et al., 2018).[10] Because high-density lipoprotein is the strongest antiatherogenic high-density lipoprotein subtype, research have pointed out a clear co-relation between lower levels of this lipoprotein and ischemic heart disease, even in individuals who were not obese (zhang et al; 2022)[9]. Independent of BMI, PCOS in early adulthood is linked to a higher long-term risk of diabetes and dyslipidemia. (Wang et al., 2018).[10]

There are five different types of lipoproteins are chylomicrons, high density lipoprotein, low density lipoprotein and very low-density lipoprotein. All these types of lipoproteins classify according to their composition and density. Lipoproteins are one of the complex particles in body, function of these lipoproteins are transport of lipids like cholesterol, triglycerides and phospholipids between the cells. As the name show high density lipoprotein have the height density of lipoprotein, composed of highest portion of proteins to lipids. Research shown a strong inverse association between HDL cholesterol and risk of atherosclerosis (bailey et al., 2022).[11] HDL is termed as good cholesterol, major role of HDL is reverse cholesterol transport, it removes excess cholesterol and carries cholesterol from peripheral tissues and back to liver through blood. The liver flushes it from the body. HDL reduces the adhesion molecules expression in endothelial cells and inhibits recruitment of monocytes into arterial wall so, it reduces the risk of heart disease and stroke. HDL also plays a protective role in case of injury. Evidence has shown that genetic, environment, lifestyle, obesity and dietary habits impair HDL function. (kosmas et l., 2018).[12]

LDL particles are composed of phospholipid, unesterified form of cholesterol, fatty acid ester of cholesterol, hydrophobic apo-B protein is also embedded in LDL membrane help in binding of LDL particle to specific receptors present on cell surface. Defects in function of LDL receptor can cause hypercholesterolemia an

autosomal dominant disorder known as familial hypercholesterolemia. LDL receptor consists of 839 amino acids and with single chain glycoprotein. LDL receptors found in liver and in other tissues (Hevonoja et al., 2023).[13] The objectives of this research were to estimate different lipoprotein mainly HDL and LDL in polycystic ovary syndrome patients to determine that PCOS patients have prevalence of decrease HDL levels. Decrease levels of HDL leads to multiple complications like heart attack, stroke, peripheral artery disease due to atherosclerosis.

This research has a profound impact on medical practice, patient care, and public health. It addresses various aspects of the condition, leading to improved diagnosis, treatment, and overall well-being of women with PCOS. As research continues, it will undoubtedly bring further insights and advancements, leading to better healthcare practices and improved quality of life for those who suffer from PCOS.

Material and Methods

It was a descriptive cross-sectional study. The study location was the Department of Biochemistry, NUR International University and the outpatient Department of Obstetrics and Gynaecology, Fatima Memorial Hospital Lahore. A convenient Sampling Technique was used. In this type of sampling, selected only those samples that were easily available, easy to approach, and agreed to participate in the research. The Sample size consisted of 80 female individuals. Those females included healthy female participants and PCOS females. A total of 80 patients were divided into two groups that were Group A and Group B. Each group contained 40 female participants, aged 18 – 45 years. Group A was a control group with healthy females. Exclusion criteria of group A was Patients having PCOS, Diabetic patients, kidney and liver cirrhosis patients. Group B contained female participants suffering from PCOS. Exclusion criteria for Group B was patients having diabetes, kidney and Liver liver disease. A convenient Sampling Technique was used. A structured research Performa recorded all the lab investigations of the serum Lipid levels including HDL and LDL levels of both groups. Ethical Approval was obtained from the IRB department of NUR International University and Fatima Memorial Hospital. Informed consent was obtained from each participant after explaining to them the whole procedure and the reason for conducting the study. Only those subjects who submitted their consent were included in this study.

A structured research Performa recorded all the lab investigations of the serum Lipid levels including HDL and LDL. The measurements taken were then compared to the reference normal values of HDL > 50 mg/, and LDL < 130 mg/ dL (Rashidi, 2018).[14]

Properly trained and fully equipped technologists obtained a blood sample from each patient. The blood sample of each patient was collected by a standard aseptic method. Sample vials were marked properly with the patient's name and case number. Blood was withdrawn in 12 hours fasting and centrifuge it for 15 minutes at 3500x g to separate Serum and stored at -80C (butler et al., 2023).[15] lipid profile was measured by enzymatic colorimetric method with Arena BioScien kits according to the manufacturer's instructions by BioCor Microlab-300 (Germany) semi-automatic chemistry analyzer. All the collected data was entered and analysed statistically using IBM SPSS Statistics Software. The quantitative variable, age was presented as groups in percentages. Shapiro-Wilk test was used to evaluate normality. An independent sample t-test was performed on the groups to check significance where the p-value was taken as > 0.05 and results were presented as mean and SD.

Results

Study enrolled total 80 female participants aged 18-45 years. HDL and LDL levels were measured in participants, out of total 80 participants, 40 participants include in group A i.e. healthy females and 40 in group B having diagnosed PCOS patients. Out of 40 participants in Group A (control group), the ages of 4 were 18-25 years, 15 were 26-35 years, and 21 were 36-45 years. In Group B out of 40 PCOS patients, the ages of 6 were 18-25 years, 19 were 26-35 years, and 15 were 36-45 years.

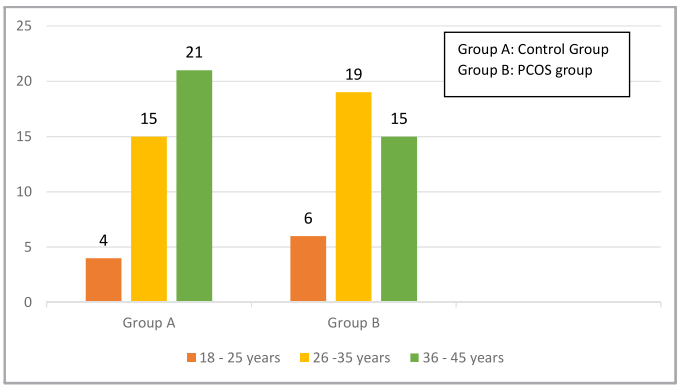

Fig-1: *Age of participants in Control (Group A) and in PCOS (Group B).*

All the selected samples of participants were inves-

tigated for lipid profile. Taking the reference values of HDL and LDL calculate Mean ± SD. Values explain in table 1.

Table 1: *High-Density Lipoprotein and Low-Density Lipoprotein of the Participants in Group A and B*

Groups	High-Density Lipoprotein mg/dL (Mean ± SD)	Low-Density Lipoprotein mg/dL (Mean ± SD)
Group A (Control Group)	50.52 ± 28.297	106.50 ± 30.750
Group B (PCOS Group)	40.30 ± 8.549	119.98 ± 27.776

After calculating Mean ± SD of HDL and LDL, correlate values between the groups in table 2.

Table 2: *Correlation of High-Density Lipoprotein and Low-Density Lipoprotein in Group A and Group B*

Group	High-density lipoprotein mg/dL	Mean ± Std. Deviation	Sig. (p-value)
A	>= 50	1.80 ± .410	< 0.001[a]
B	< 50	1.40 ± .494	
Group	Low-density lipoprotein mg/dL	Mean ± Std. Deviation	Sig. (p-value)
B	>= 130	1.40 ± .500	0.235[a]
A	< 130	1.55 ± .503	

Values are mean ± S.D. of carefully conducted independent sample t -test. values within a column show the level of significance ($p < 0.05$) observed at a 95% confidence interval (CI).

Discussion

Women of reproductive age are frequently affected by hormonal disorders, such as polycystic ovarian syndrome (PCOS). Their distinctive features include numerous small ovarian cysts, irregular menstrual periods, and hormonal irregularities (de Arajo et al., 2018)[16]. In the globe, one in 15 women suffer from this complicated endocrine condition (Bou Nemer et al., 2018)[17]. Most studies show that 35–50% of PCOS-afflicted women are overweight (BMI of 25 kg/m^2) or obese (BMI of 27 kg/m^2) (Hart et al., 2004).[18]

All laboratory assessments of the serum lipid level including HDL and LDL of groups were documented by structured research using Performa. Using IBM SPSS Statistics Software, all obtained data were in putted and statistically analysed, a separate t-test with a p-value of 0.05 or above was run, and the findings were shown as mean and standard deviation. Descriptive analysis was performed on the HDL and LDL, in the control group and PCOS group.

Taking the Reference value of HDL is >40 mg/dL for Men and >50 mg/dL for Women (Information et al., 2017). Current study contains women patients, so an independent Sample t-test was performed to correlate HDL in the Group A that is control group and in patients with PCOS. There were significant differences as a p-value of <0.001 in scores for no PCOS in the control group and patients with PCOS. The magnitude of the mean ± SD (1.80±0.410, 95% CL) to Mean±SD (1.40 ± 0.494, 95% CL) was very small. Thus, it shows that HDL and PCOS are significantly correlated in PCOS patients.

Taking the Reference value of LDL is 130 mg/dL (Information et al., 2017) (Rashidi, 2018)[14]. An independent Sample t-test was performed to correlate LDL in control group without PCOS and patients having PCOS. There were no significant differences as a p-value of 0.235 in scores for Group A, that is healthy patients (group A) and the patients having PCOS. The magnitude of the mean ± SD (1.40 ± 0.500, 95% CL) to the Mean ± SD (1.55 ± 0.503, 95% CL) wasn't very small. Thus, it shows that LDL and PCOS are not significantly correlated in PCOS patients. According to a recent study, PCOS-afflicted women typically experience moderate hypercholesterolemia (Liu et al., 2019)[5]. It can be concluded that HDL levels decrease in dyslipidemia in patients with Polycystic Ovary Disease. When it comes to their lipid profiles, women with PCOS have lower HDL levels (Kim & Choi, 2013).[19]

Wild et al. recently published a meta-analysis of lipid levels in worldwide cross-sectional studies of women with PCOS (mostly conducted in women from Europe and America). Women with PCOS had TG levels 26 mg/dL (95% CL, 17 to 35) higher and HDL-C levels 6 mg/dL (95% CI, 4 to 9) lower than controls (Wild et al., 2011).[20] The scores for individuals with PCOS and those without PCOS in the Group A or control group differed significantly, with a p-value of 0.235. There was very little difference between the Mean±SD (1.40± 0.500, 95% CL) and the Mean ± SD (1.55 ± 0.503, 95% CL). As a result, it demonstrates that in PCOS patients, LDL and PCOS do not significantly correlate.

A prospective cross-sectional study was conducted between July 2014 and December 2016 to investigate the relationship between the hormone profile, lipid profile, clinical profile, and free testosterone index in PCOS subjects. 76 PCOS-afflicted women were included in the study, and they were split into two groups based on

whether they had hyperandrogenism or not. They claimed that not all patients exhibit hyperandrogenism, the main symptom of PCOS. Hormonal and metabolic problems both have an impact on the hyperandrogenic phenotype in PCOS patients. They concluded that FGS was not associated with hyperandrogenism in PCOS patients but that triglycerides, testosterone, and SHBG were (Hestiantoro et al., 2019).[21] Furthermore, increased levels of triglyceride-glucose, triglyceride/HDL, and total cholesterol/HDL ratios were found to be substantially linked with IR in Iranian women with PCOS (Kheirollahi et al., 2020).[22]

According to the metabolic syndrome criteria given in the WHO in 1999, the following conditions must also be present in these individuals glucose intolerance, insulin resistance and type 2 diabetes mellitus. Raised plasma triglycerides levels (>150 mg/dL) and low HDL cholesterol (35 mg/dL in men and 39 mg/dL in women), elevated arterial compression (>140/90 mmHg), If you have obesity (waist to hip ratio >0.9 for men and >0.8 for women) and a BMI >30 kg/m^2, or if you have micro-albuminuria (urinary albumin elimination rate 20 g/min or albumin-creatinine ratio 30 mg/g) and have central obesity (Ovalle, 2002).[23]

Connection to endocrine and metabolic parameters was the topic of a cross-sectional study that involved 52 females having PCOS and oligomenorrhea. They claimed that women with polycystic ovarian syndrome (PCOS) display an aberrant lipoprotein profile, defined by increased plasma triglyceride concentrations, mildly higher low-density lipoprotein (LDL) and lowered high-density lipoprotein (HDL).

A prospective study was reported between July 2014 and December 2016 to investigate the relationship between the hormone levels, lipid profile and testosterone levels in PCOS patients. 76 PCOS-afflicted women were involved in the research and split into two groups according to whether they had hyperandrogenism. They claimed that not all patients exhibited hyperandrogenism, which is the main symptom of PCOS. Both hormonal and metabolic problems affect the hyperandrogenic phenotype in patients with PCOS. They concluded that FGS was not associated with hyperandrogenism in PCOS patients, but that triglycerides were (Hestiantoro et al., 2019)[21]. Cardiovascular diseases are one of the leading causes of death in worldwide. Studies reveal that there is a clear corelation between low levels of high-density lipoprotein concentration with coronary heart disease (kosmas et al.,2018).[12] It was crucial to monitor cardiovascular risk variables and inflammation in PCOS-afflicted women. As the CRP and IL-6 levels in these women were much greater, this indicated pro-atherogenic inflammation, which was linked to a higher risk of cardiovascular disease. The findings proposed that inflammation is a probable therapeutic target in reducing cardiac disease risk in female having PCOS (González et al., 2009).[24]

The utmost widespread endocrine disorder among female of fertile age is polycystic ovarian syndrome (PCOS) and it is also a contributes to persistent anovulation. Peripheral insulin resistance, hyperinsulinemia, and centripetal fat distribution are key components of metabolic disturbances (siddiq et al. 2005)[25]

Conclusion

We have concluded that there are abnormal serum lipid levels, in Polycystic ovary syndrome Patients. Our findings indicated that serum High-density lipoproteins were lower in the patients of polycystic ovary syndrome compared to healthy participants. Low HDL cholesterol is a major risk factor for cardiovascular disease. However, the levels of Low-density lipoproteins remained within the normal range. These findings after comparing the high den-sity and low-density lipoprotein these findings enhance our understanding of PCOS-related complications like cardiovascular disease and enabling earlier and more accurate diagnoses.

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Authors Contribution

A, AA: Conceptualization of Project
A, FK: Data Collection
AM: Literature Search
SAJ: Statistical Analysis
AA: Drafting, Revision
A, SA: Writing of Manuscript

Acceptability of Sayana Press in Postnatal Patients – An Experience at Tertiary Care Hospital

Iqra Matloob, Madeeha Rashid, Mawrah Mughal, Qamar Mand, Kiren Khurshid Malik

Abstract

Objective: To ascertain the acceptability of Sayana Press in postnatal patients of Gynae of Tertiary Care Lahore.

Material and Methods: A cross-sectional quantitative research was conducted from 01-07-2022 to 31-12-2022 (6months). One hundred and six postnatal patients of Gynae Unit I, of Services Hospital Lahore, were selected after informed consent and ethical approval from IRB. Socio demographic and clinical data like age, parity, socioeconomic status, educational status and occupation were noted. Then subcutaneous Sayana Press injection was given and each patient was followed for 3 months at which the acceptability was noted in each woman as described in operational definition. A pre designed proforma was used to collect data.

Results: The mean age of the participants was determined to be 29.5+3.39 years, with 42.5% falling into the 31–45-year age group and the remaining 57.5% falling into the 15–30-year age group. Out of 106 females, 32.1% (n=34) were poor, 53.8% (n=57) were middle and 14.2% (n=15) were in upper class. Out of 106 females, 4.7% (n=5) were illiterate, 8.5% (n=9) were primary, 38.1% (n=41) were middle, 31.1% (n=33) were matric and 17.0% (18) were graduate. Out of 106 females, 57.8% (n=61) were housewife, 0.9% (n=1) were filed worker and 41.5% (n=44) were working woman. Out of 106 females, 23.6% (n=25) lived in rural area and 76.4% (n=81) lived in urban areas. Out of 106 females, 78.3% (n=83) accepted Sayana press.

Conclusion: We concluded that Sayana press is acceptable and convenient among most study participants.

Keywords: Sayana press, Acceptability, Injectable

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Introduction:

Many countries are currently facing the rapid population growth crisis. More than 95 per cent of the world's population lives in developing countries. One significant public health issue facing developing countries is the high population burden.[1] Therefore, the voluntary control of fertility is of utmost importance to the modern society. Family planning services and products are available at many public and private facilities. Male condoms and pills, along with injectable are among the most popular modern contraceptive method currently in use.[2] However, injectable contraceptives are more difficult to access because a trained healthcare worker must administer it with a needle and syringe. Contraceptive choices are also influenced by individual preferences, societal standards, gender preferences, women's education, whether they live in an urban or rural area, and if family planning is seen as acceptable.[3] Health professionals working in facilities usually give injectable contraceptives, while community-based initiatives have been around since the 1970s in many nations.[4] A possible chance to increase access to injectables outside of clinic

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settings and towards home and self-injection is presented by the launch of Sayana® Press, a three-month injectable formulation of depot medroxyprogesterone acetate (DMPA).[5] Sayana Press is a subcutaneous formulation that comes in a single dose inside the UnijectTM injection device, in contrast to injectable contraceptives that are currently available on the market. Because the simple disposable single-use syringe makes it unnecessary to quantify dosages or give intramuscular injections, women may be able to administer the drug on their own at home.[6] In a study, acceptance rate of Sayana® Press was found to be 84%.[7] In another study, this rate was found to be 98%.[9] There is international data available, however very fewer local studies are carried out till date. An effective birth control regimen must be followed for 16–20 years of a woman's approximately 25 childbearing years in order to prevent the need for an abortion.[9] According to a study about 85% of women get pregnant after unprotected intercourse.[10] It is stated that if contraception is used consistently for avoiding unwanted pregnancy, maternal deaths would decline by 25–35%.[11-12] A survey in India suggested that abortions are responsible for 10–20% of all maternal deaths.[13]

The World Health Organisation (WHO) released a comprehensive list of medical requirements for 14 different forms of birth control in 2009.[14] The failure of any method is defined as percentage of users who get pregnant despite of its use in first year; the lower the failure rate, the efficient is the method. With ideal usage first-year failure rate of injectable progestin is 0.2% while using casually first failure rate is 6%.[14] When birth control methods used ideally, the failure rates stay below 1%, while with typical use, they fall within the range of 7% to 9%.[10,15,16]

Progestins and estrogen both block the hypothalamic-pituitary axis in CHCs.[17] Progestins work to prevent conception by thickening the cervical mucus and suppressing ovulation. It results in endometrial atrophy as well. By suppressing FSH and preventing the formation of an ovarian follicle, estrogens prevent pregnancy.[17] Reduced bleeding intervals (40–50%), irregular bleeding, breast soreness, mood fluctuations, and headaches are the side effects of CHCs. Additionally, they help treat polycystic ovarian syndrome and are linked to a lower risk of colon, endometrial, and ovarian cancer.[17] These are recognized as non-contraceptive benefits of these techniques.

Progesterone only methods are available as pills, injections, implants, and intrauterine devices (IUDs). It poses less risk of VTE than CHC.[18] As compared to CHC, POPs use lower dosages of first-generation progestins. The side effects of POPs are unscheduled bleeding.[19] Patients should be prescribed with a non-hormonal method while taking certain medications, including antituberculosis, the antiretroviral, and certain anticonvulsants.[20] They can also use IUDs that also provide additional benefits; however, side effect profile is the same. These include DMPA, Implanon, Nexplanon, Mirena and Kyleena. There is a decline in unintended pregnancies and is attributed to an increase in the use of LARC.[21] As Sayana press is easily administered by the personal and does not require repeated doses, to look for its acceptance a study was carried out in Services hospital as no local studies are available.

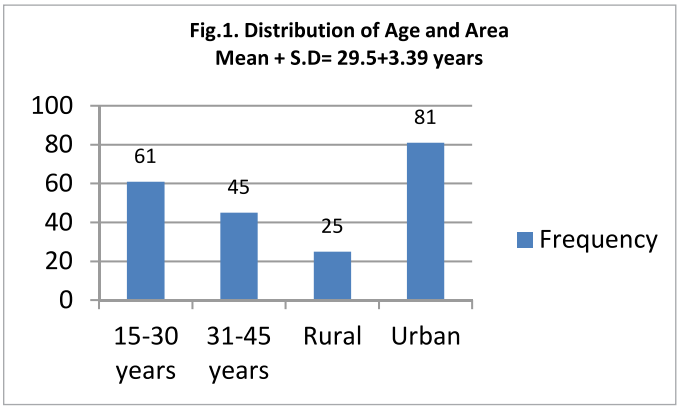
Materials and Methods

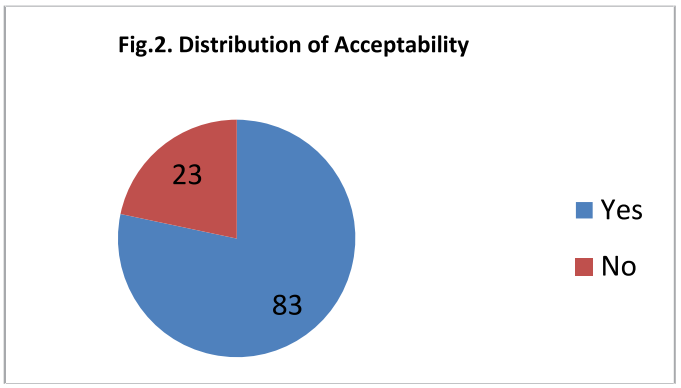
After approval from IRB, and taking informed consent, quantitative cross-sectional research consisting of total 106 postnatal patients of Gynae Unit I, of Services Hospital Lahore, were conducted for duration of 6 months from July to December 22. Socio demographic and clinical data like age, parity, socioeconomic status, educational status and occupation were noted. Then subcutaneous Sayana Press injection was given and each patient was followed for 3 months at which the acceptability was noted in each woman. All this data was noted in a pre-designed proforma. Data was analysed using SPSS 25. Age was presented as mean and standard deviation. Frequency & percentages were calculated for socioeconomic status, educational status, occupation, residential area, parity and acceptability (yes/no). Stratification was done for age, parity, socioeconomic status, educational status, occupation and place of living. Post-stratification chi square test was applied and p-value ≤ 0.05 was taken as significant

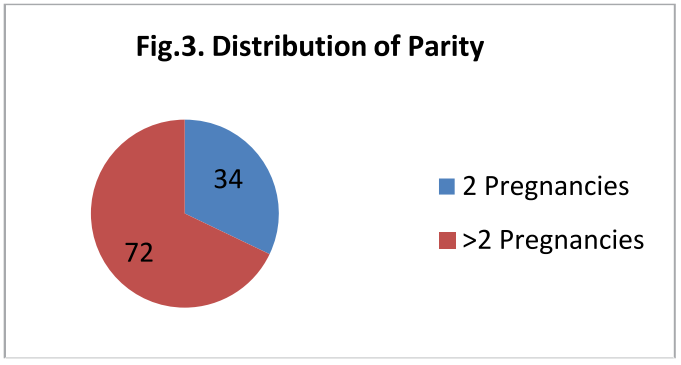
Results

106 postnatal patients fulfilling inclusion criteria were selected to analyse the frequency of acceptability of Sayana press. After the patients' ages were distributed, it was found that, of the 106 females, 47.5% (n = 61) belonged to the 15–30 age group and 42.5% (n = 45) to the 31–45 age group. The mean age was determined to be 29.5 + 3.39 years. (Fig.1) Distribution of socioeconomic status was done which showed that out of 106 females, 32.1% (n=34) were poor, 53.8% (n=57) were middle and 14.2% (n=15) were in upper class. Distribution of educational status was done which showed

that out of 106 females, 4.7% (n=5) were illiterate, 8.5% (n=9) were primary, 38.1% (n=41) were middle, 31.1% (n=33) were matric and 17.0% (18) were graduate. Distribution of place of living was done which showed that out of 106 females, 23.6% (n=25) lived in rural area and 76.4% (n=81) lived in urban areas. (Fig.1) The study showed that 83 (78.3%) accepted Sayana press and 23(21.6%) denied using it. (Fig.2) Results revealed that 72(67.9%) of the selected population had parity of more than 2 and 34 (32%) had a parity of less than 2. (Fig.3).







Discussion

2011 saw the introduction of Sayana® Press, a potentially beneficial new solution that might improve contraception acceptance, particularly at every level among nations with limited resources.[22] The product is a subcutaneous version of medroxyprogesterone acetate (DMPA-IM), an intramuscular depot injectable contraception that is supplied in prefilled UnijectTM injection systems.[23] Sayana® Press has the capacity to notably enhance contraceptive utilization for women seeking a discreet, reversible, and efficient method globally.[24] The straightforward usage of Sayana® Press opens up the prospect of transitioning responsibilities from clinically trained medical worker to local distributor and facilitates self-injection.[25] A WHO consultation panel acknowledged that lay health professionals can utilize a "compact, auto-disposable device" such as UnijectTM.[26] In current research, out of 106 females,78.3% accepted Sayana press. In comparison to study done by Bertrand et.al. 8435 women in total accepted a form of contraception from the DBCs: 26% accepted Cycle Beads, 23% chose Sayana® Press, and 52% accepted pills.[2] This study showed that individuals who chose Sayana® Press, had not used any family planning method, had a high degree of acceptability, which is compatible with initial pilot studies of Sayana® Press in other Sub-Saharan African countries. In our study the acceptability to Sayana press was higher. The approach was seen by supporters as distinct, efficient, and simple to use. The worries with Sayana® Press were not unique to this method; rather, they were similar to worries about other methods (anxiety of adverse effects, like return to fertility, efficacy, and protection) same as in our study.[26] A study by Cover et al. comprised 380 healthy adult females who gave their consent to test self-injection and to use injectable contraception. 98% of the 380 self-injectors demonstrated competency after training. Following that, 5 women stopped taking medication, and 7 women lost follow-up, leaving 368 participants for the follow-up study. Of them, 88% demonstrated proficiency at the time of instruction and three months afterward.[8] In our study the competence was not measured though. The limitations of the study were that it was confined to one hospital and multicenter research would probably teel a broader view of the response by society. These are the data of women presenting to hospital; however, such options should be provided to women that are unable to come to hospital or use distant facilities.

Conclusion

In current study, we determine the frequency of acceptability of Sayana press in postnatal patients of gynae unit 1 of services hospital, Lahore. We found that out of 106 females, 78.3% (n=83) accepted Sayana press. Therefore we concluded that Sayana press is practicle

and acceptable among most study participants in the study.

Conflict of Interest: **None**

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Authors Contribution

IM: Conceptualization of Project
MR: Data Collection
MR: Literature Search
MM: Statistical Analysis
QM: Drafting, Revision
KK: Writing of Manuscript

Knowledge, Attitude and Practices Regarding Hepatitis C Among Adolescents in Multan, Pakistan

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Abstract

Objective: To assess the knowledge, attitudes and practices of adolescents regarding Hepatitis C infection in Multan.

Material and Methods: From August 5, 2019, to February 5, 2020, an analytical cross-sectional study was undertaken in both private and government educational institutions in Multan. A sample of 278 participants was determined, maintaining a 95% confidence internal. The data was collected using a structured questionnaire with systematic random sampling. Data was analyzed through Chi-square test with a significance level of $p<0.05$ using SPSS version 22. Results: Among the total of 278 students, 167 (60.1%) were males, and 111 (39.9%) were females. The mean age of the participants was 14.8 ± 2.2 years. Out of these 278 students, 185 (66.5%) were enrolled in classes up to the 10th level, while 93 (33.5%) were pursuing education in higher classes. A positive history of contact with Hepatitis C patients was reported by 28 (10.1%) students, injection therapy by 37 (13.3%), and ear/nose piercing by 111 (39.9%). No instances of a history of surgical procedures, blood transfusions, or tattooing were identified in this study. Adequate knowledge was observed in 64 (23%), a positive attitude in 18 (6.5%), and good practices were demonstrated by 14 (5%) of these students.

Conclusion: This study revealed that adolescents exhibited deficiencies in knowledge, attitude, and practices. The study identified a significant association between poor practices, negative attitudes, and variables such as gender, age, type of school, occupation, literacy, contact with patients, and ear/nose piercing (p-value<0.05).

Keywords: Knowledge, attitude, adolescents

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Introduction

Hepatitis C virus infection continues to pose a notable obstacle to public health, as its worldwide occurrence is approximated at 2.5% of the population.[1] Worldwide, an estimated 185 million people have been infected, with 130-150 million chronically affected.[2,3] leading to an annual death toll of 70,000 from liver-related diseases.[4] Lack of awareness, inadequate blood screening, nosocomial transmission, and insufficient treatments contribute to the escalating Hepatitis C infection in many developing countries.[5] In Pakistan, the virus presents a significant healthcare concern, with an estimated prevalence of 6.7% and a viremic prevalence of 5.8% among adults. This places Pakistan as the second-highest country worldwide in terms of viremic infection.[6]

Hepatitis C infection often becomes chronic, potentially resulting in severe complications such as liver scarring, cirrhosis, liver failure, and hepatocellular carcinoma. Transmission occurs through unsterilized needles, con-

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taminated blood transfusions, surgical and dental procedures, intravenous drug usage, vertical mother-to-infant transmission, and practices like tattooing.[7] Hepatitis, which involves inflammation of the liver, is primarily triggered by the Hepatitis B virus (HBV) and Hepatitis C virus (HCV). This condition represents a significant worldwide health challenge, further aggravated by inadequate healthcare infrastructure, economic hurdles, and limited public understanding of infectious diseases.[8]

Pakistan, categorized in the "Intermediate zone" by WHO for viral hepatitis prevalence, encounters the spread of the disease through bloodborne, sexual, and mother-to-child transmission pathways.[9] In 50% to 80% of instances, HCV infection progresses to chronic hepatitis, exhibiting a greater likelihood of long-term carriage than hepatitis B.[10] In developing nations such as Pakistan, several activities facilitate the spread of HCV. These include blood transfusions, reusing needles for tattoos, ear and nose piercings, recycling syringes, drug consumption, shaving by barbers, and inadequate sterilization of dental and surgical tools. At present, no vaccine exists to prevent infection by HCV.[11]

Adolescents, who experience substantial psychological and social shifts as they move from childhood to adulthood, are especially susceptible to hazards like drug use, smoking, and unprotected sexual activities. These behaviors increase their exposure to infectious diseases, including Hepatitis C.[12] This study aims to assess adolescents' awareness and attitudes toward Hepatitis C, emphasizing the urgent need for heightened public awareness in Pakistan, particularly among youth. Comprehensive health education campaigns, awareness programs in schools, and government initiatives addressing surveillance, prevention, care, and treatment are crucial to alleviate the burden of Hepatitis C infection.

Material and Methods

An analytical cross-sectional study was carried out in Multan, encompassing private and government schools and colleges, from August 5, 2019, to February 5, 2020. The sample size was determined using the open epi sample size calculator, considering a 32.2% positive attitude derived from prior studies, with a 95% confidence interval and a 5% margin of error. Data collection utilized a systematic random sampling technique and a pre-tested structured questionnaire. Participants, aged 10-18 years, were selected from both private and government educational institutions, excluding those who did not provide consent.

The institute's ethical review committee granted approval for the study, ensuring that data gathering adhered to the standards of privacy and confidentiality. Permission from the principals of the respective schools and colleges was secured before initiating the research. Each participant provided their informed consent. Data, encompassing both quantitative variables like age and qualitative variables such as gender, history of blood transfusion, surgical procedures, contact with Hepatitis C patients, injection usage, and history of tattooing and ear piercing, were entered and analyzed using SPSS version 22.

For quantitative variables, the mean and standard deviation were computed, whereas qualitative variables were summarized using frequencies and percentages. To control for effect modifiers like age, gender, student class, school type (private vs. government), parents' education, family income, parents' occupation, history of blood transfusion, surgical procedures, contact with Hepatitis C patients, injection usage, and history of tattooing and ear piercing, stratification was employed.

Table 1: *Socio-demographic profile of respondents*

Gender	**Frequency (n=278)**	**Percentage**
Male	167	60.1
Female	111	39.9
Age groups	**Frequency**	**Percentage**
Up to 15 Years	175	62.9
More than 15 Years	103	37.1
Type of institution	**Frequency**	**Percentage**
Private	112	40.3
Government	166	59.7
Family Income	**Frequency**	**Percentage**
Less than upto Rs. 50000	175	62.9
More than Rs. 50000	103	37.1
Occupation	**Frequency**	**Percentage**
Job holder	113	40.6
Self business	119	42.8
Farmer	46	16.5
Level of education of participants	**Frequency**	**Percentage**
Up to 10th	185	66.5
Above	93	33.5
Literacy among parents of participants	**Frequency**	**Percentage**
Illiterate	92	33.1
Literate	186	66.9

Subsequent to stratification, chi-square tests were conducted, establishing the level of significance at a p-value of less than or equal to 0.05. Adolescent: Individual whose age is between 10-18 years. Knowledge assessment was conducted through a questionnaire, with scores below 11 classified as poor and scores of 11 or higher considered as indica-tive of adequate knowledge regarding hepatitis C. Attitude: Attitude evaluation was performed using a scale within the questionnaire, categorizing attitudes as positive for scores above 4 and negative for scores of 4 or below.

Practices were assessed using scale for the questions asked about practices which classified prac-tices as good with score >5, adequate with score 5 and poor for score less than 5.

Results

This study included a total of 278 respondents who met the inclusion criteria established for our research. Mean age of students was 14.81 ± 2.20 years. (T**able No. 1).**

Table 2: *Stratification of knowledge regarding Hepatitis with confounders*

Effect Modifiers		Knowledge		Total	P – Value
		Yes	No		
Gender	Male	28	139	**167**	**0.003***
	Female	36	75	**111**	
Age groups (in years)	Up to 15	7	168	**175**	**0.001***
	Above 15	55	48	**103**	
Type of school	Private	46	66	**112**	**0.000***
	Government	18	148	**166**	
Father's occupation of respondents	Job	28	85	**113**	**0.004***
	Business	18	101	**119**	
	Farming	18	28	**46**	
Level of education of participants	Up to Metric	9	176	**185**	**0.000***
	Above Metric	55	38	**93**	
History of contact with patient	Yes	18	10	**28**	**0.000***
	No	46	204	**250**	
Injection therapy	Yes	8	29	**37**	**0.000***
	No	64	177	**241**	
History of ear/ nose piercing	Yes	36	75	**111**	**0.003***
	No	28	139	**167**	

*P- value ≤ 0.05 was taken as statistically significant

A significant association (p-value<0.05) was found when knowledge of participants was stratified with regards to gender, age, type of institution, father's occupation, level of education, history of contact with HCV patient and history of injection and ear / nose piercing. **(Table No.2).** A significant association (p-value<0.05) was found when attitudes and practices of respondents were stra-tified with regards to gender, age, type of institution, father's occupation, parent's education, history of contact with HCV patient and history of injection and ear / nose piercing.

Table 3: *Stratification of attitude and practices regarding Hepatitis with confounders*

Effect Modifiers		Practice		Total	P – Value
		Yes	No		
Gender	Male	7	160	**167**	**0.000***
	Female	14	97	**111**	
Age groups (in years)	Up to 15	5	170	**175**	**0.001***
	>15	14	89	**103**	
Type of school	Private	14	98	**112**	**0.001***
	Government	16	150	**166**	
Father's occupation of participants	Job	14	99	**113**	**0.000***
	Business	9	110	**119**	
	Farming	7	39	**46**	
Parent's literacy	Illiterate	14	78	**92**	**0.001***
	Literate	5	181	**186**	
Level of education of participants	Up to Metric	12	173	**185**	**0.000***
	Above Metric	14	79	**93**	
History of contact with patient	Yes	8	20	**28**	**0.001***
	No	6	244	**250**	
History of ear/ nose piercing	Yes	14	97	**111**	**0.001***
	No	6	161	**167**	

*P- value ≤0.05 was taken as statistically significant

Discussion

Among the 278 participants, 167 (60.1%) were identified as male, while 111 (39.9%) were female patients. Significantly, research carried out in Faisalabad, Pakistan by Iqbal et al. revealed a predominance of female participants at 59.4%, a result that diverges from our own observations.[13] This variance can be attributed to the fact that their study was conducted among medical students, and the majority of medical students in our society are female. In contrast, research conducted in Malaysia by

Ahmed et al. found a majority of male participants, accounting for 59%, which closely corresponds with the outcomes of our study.[14] Positive history of contact with Hepatitis C patients was noted in 28(10.1%), injection therapy in 37 (13.3%), and ear/nose piercing in 111 (39.9%). No instances of a history of surgical procedures, blood transfusion, or tattooing were found in our study. In contrast, a study in Iran revealed that 27% of respondents were unaware that HCV could be transmitted between individuals, while 72.1% were aware of needle-sharing as a transmission route.[15] Additionally, only 21.5% and 19.4% knew about sexual intercourse and mother-to-baby transmission, respectively. Another study in Faisalabad, Pakistan, reported a 19% family history of hepatitis, aligning with our study results.[13]

Among the total participants, 64 (23.0%) exhibited adequate knowledge, 18 (6.5%) had a positive attitude, and 14 (5.0%) demonstrated good practices. A study conducted in Abbottabad reported that 62% of respondents had poor knowledge, while 38% possessed adequate knowledge of hepatitis C. Regarding attitudes, 67.8% of total participants displayed a negative attitude, with 32.2% exhibiting a positive attitude towards hepatitis C. In terms of practices, 66.2% showed negative practices, while 33.75% maintained good practices, which closely mirrors our study results.[16] Similarly, a study in Malaysia by Ahmed et al. also indicated poor and average levels of knowledge among students, aligning with our findings.[14] Another study in Iran found a low level of knowledge regarding Hepatitis C among nurses, consistent with our study results.[17] Additionally, a study in Egypt revealed that 68.2% of participants lacked sufficient knowledge about HCV disease, and 86.4% exhibited negative practices. Notably, statistically significant improvements in knowledge and practices were observed after the application of an educational program.[18]

A survey involving 340 dental students in Brazil revealed that over half (54%) exhibited a high level of knowledge, and 97.7% displayed positive attitudes towards Hepatitis C (HCV). Notably, the students' advanced year of study (last year) ($P < 0.001$) and the type of university (federal) ($P = 0.049$) significantly influenced their high knowledge levels about HCV. Additionally, a positive attitude toward HCV-infected patients was notably influenced by age ($P=0.004$) and male gender ($P=0.022$).[19] These findings are in contrast with our study, possibly reflecting the higher educational and awareness levels in Brazil.

In an Indian study involving students, it was observed that a majority (72.5%) were aware of the Hepatitis C vaccine but possessed limited knowledge about the mode of infection spread, preventive measures, associated complications, and the availability status of the vaccine against Hepatitis C virus.[20] These results align with our study, considering the proximity and similar developmental status of India as our neighboring country.

In a study targeting medical students in Dammam, it was found that around 75% demonstrated a lack of understanding concerning HCV transmission, with fewer than 25% showing a moderate level of awareness. More than half of the students possessed an adequate knowledge of HCV screening, prevention, and treatment. A significant majority were well-informed about the clinical manifestations and complications associated with HCV.[21]

Conclusion

Our study revealed inadequacies in knowledge, attitude, and practices among adolescents. Subpar knowledge, negative attitudes, and inadequate practices were notably associated with gender, age, school type, father's occupation, parental literacy, student's education level, and history of contact with hepatitis patients, as well as ear/nose piercing ($p\text{-value}<0.05$). A considerable 39.9% of the total respondents had a history of ear/nose piercing, while 13.3% reported a history of injection therapy.

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Conflict of Interest: None

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Authors Contribution

SS, IQ: Conceptualization of Project
AW: Data Collection
HK: Literature Search
MF: Statistical Analysis
AS: Drafting, Revision
SS, IQ: Writing of Manuscript

Frequency of Urinary Tract Infection in Diabetic Patients Using Dapaglifzlozin

Ch Adnan Ahmed Ather,[1] Rashid Iqbal,[2] Syed Muhammad Naeem Afzal,[3] Sohail Bashir Sulehria,[4] Qurat ul Ain,[5] Sibgha Kanwal[6]

Abstract

Objective: To assess the frequency of urinary tract infection in diabetic patient using dapagliflozin

Material and Method: This descriptive case series conducted on180 patients, from 30-70 years with type 2 DM fulfilling selection criteria. Demographic information was also recorded. Then patients were prescribed 10 mg dapagliflozin for 24 weeks. Then patients were followed-up in OPD for 24 weeks. After 24 weeks, urine sample was taken and sent to the laboratory of the hospital. Reports were assessed and if bacterium detected in urine sample, then urinary tract infection was labeled. Patients with urinary tract infection were managed as per hospital protocol.

Results: In our study, of 180 cases, mean age was calculated as 49.95+5.35 years. Gender distribution shows that 110(61.1%) cases were male and 70(38.9%) cases were females. Frequency of urinary tract infection in diabetic patient using Dapagliflozin was recorded in 11(6.1%) of the patients. The data stratified for age, BMI, HbA1c, and smoking, to control effect modifiers came to be significant at $p < 0.05$

Conclusion: We concluded, that frequency of urinary tract infection is not higher in diabetic patient using dapagliflozin. Patients with increase age, prolonged duration of diabetes, family history of UTI, uncontrolled diabetes and female gender are at risk for developing UTI.

Keywords: Urinary tract infection, Diabetic, Dapagliflozin

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Introduction

Metabolic disorder, known as type 2 diabetes affects millions worldwide.[1] Adults with diabetes currently number 463 million, but by 2030 and 2045, that number is expected to rise to 578 and 700 million, respectively. 90% of all forms of diabetes are Type 2.[2] Diabetes can result in life-threatening complications or even early death. Common complications of diabetes include cardiovascular disease, renal disease, retinopathy, diabetic foot, and neuropathy.[3,4]

Moreover, urinary tract infections (UTIs) are more common in diabetics. An estimated 150 million people are anticipated to get this infection annually. Bladder dysfunction, low immunity, and glycosuria all of which are thought to be risk factors. Diabetics are likely to get UTI, which have poorer consequences.[5,6] Moreover, resistant microorganisms are more frequently found. Pyelonephritis, consequence of urinary tract infections in diabetics that can lead to organ damage and even death.[7] SGLT2i, or sodium-glucose cotransporter-2 inhibitors, are drugs that treat type 2 diabetes by acting on kidneys. In kidneys, glucose is reabsorbed in the loop of Henle's proximal tubule. The SGLT2 protein, is in charge of this reabsorption. SGLT2i medications such as canagliflozin, dapagliflozin, and empagliflozin act at this site.[8] It was concluded in one meta-analysis that, dapagliflozin 5 mg raised the chance of overall adversities, whereas dapagliflozin 10 mg had lower risk of UTI than its 5 mg counterpart.[9] Patients with diabetes who get UTIs have trouble controlling their blood sugar,

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which makes blood sugar monitoring more necessary, lowers quality of life, and puts financial burden on the patient. Different research indicated that diabetes patients on 10 mg of dapagliflozin had 0.09% prevalence of urinary tract infections.[10] Prescriber information does not regard it as a precaution prior to prescribing. Further investigation is required to determine whether SGLT2 inhibitor use in these patients increases the risk of urinary tract infection beyond attributable risk to either of these risk factors in isolation.[11] So, we have conducted this study so that we can get local evidence and we can plan screening of diabetic patients using dapagliflozin on regular intervals for urinary tract infection during follow-up and routine examination in order to improve management protocols and prevent UTI and provide improved quality of life to patients.

Materials and Methods

This descriptive case series was conducted at Department of Medicine, Govt. Teaching Hospital Shahdra, Lahore over a period of six months. The study included 180 patients, selected using non-probability consecutive sampling, with 95% confidence level and 3% margin of error, considering an expected percentage of urinary tract infection of 4.3% in diabetic patients treated with dapagliflozin. Inclusion criteria involved patients aged 30-70 years of both genders diagnosed with diabetes, while exclusion criteria included patients currently positive for urinary tract infection, those with serum creatinine >1.2mg/dl, and pregnant females. Data collection involved enrolling eligible patients from the hospital's outpatient department, obtaining informed consent, and recording demographic and medical information. Patients were prescribed 10 mg dapagliflozin for 24 weeks and followed up in outpatient department. After 24 weeks, urine samples were collected, and if bacteria were detected, urinary tract infection was labeled, and patients were managed according to hospital protocol.

Data analysis was performed using SPSS version 26. Quantitative variables were presented as mean and standard deviation, while qualitative variables were presented as frequency and percentage. Data was stratified by various factors to control for effect modifiers, and chi-square tests were applied to compare urinary tract infection rates in stratified groups, with a p-value ≤ 0.05 considered significant.

Results

A total of 180 cases fulfilling the selection criteria were enrolled to assess the frequency of urinary tract infection in diabetic patient using dapagliflozin. Table 1 shown below is displaying qualitative variables of the study population. Age distribution shows that 87(48.3%) were between 30-50 years of age whereas 93(51.7%) cases were between 51-70 years of age. Gender distribution shows that 110(61.1%) cases were male and 70 (38.9%) were females. Frequency of smoking in the cases was recorded in 46(25.6%) whereas 134(74.4%) were nonsmokers. Frequency of family history of UTI was recorded in 35(19.4%) cases whereas 145(80.6%) had no history of UTI. Frequency of frequency of urinary tract infection in diabetic patient using Dapagliflozin was recorded in 11(6.1%) of the patients whereas 169 (93.9%) cases had no findings of the UTI. Table 2 is showing quantitative variables of study population, mean duration of diabetes mellitus was calculated as 8.28+ 3.54 years, mean BMI 30.67+2.37. and mean of HbA1c calculated was 9.7+2.37. frequency of UTI noted high among females (3.9%), age group 51-70 years (6.1%), prolonged duration of DM > 5year (6.1%), family history of UTI (6.1%), high BMI > 30mg/kg^2 (3.9%), smoking (4.4%) and uncontrolled DM HbA1c >7% (3.9%) as compared to their counterparts. The data stratified for age, BMI, HbA1c, and smoking, to control effect modifiers came to be significant at $p < 0.05$, as shown in table 3.

Table 1: *Analysis of Qualitative Variables*

PARAMETERS		Frequency (n=180)	Percentage
Age	30-50	87	48.3%
	51-70	93	51.7%
Gender	Male	110	61.1%
	Female	70	38.9%
Smoking	Yes	46	25.6%
	No	134	74.4%
Family history of UTI	Yes	35	19.4%
	No	145	80.6%
UTI	Yes	11	6.1%
	No	169	93.9%

Table 2: *Analysis of Quantitative variables*

PARAMETERS	MEAN (n=180)	SD
Duration of DM (years)	8.28	3.54
BMI Kg/m^2	30.67	2.37
HbA1c (%)	9.7	2.37

Table 3: *Data stratification using chi square*

CHARECTERISTICS		UTI		P value
		YES	NO	
Age (years)	18-50	0	87(48.4%)	0.001
	51-70	11 (6.1%)	82 (45.5%)	
Gender	Male	4 (2.2%)	106 (58.9%)	0.08
	Female	7 (3.9%)	63 (35%)	
Duration Of DM (Years)	Upto 5	0	35 (19.4%)	0.086
	>5 Y	11 (6.1%)	134(74.5%)	
HbA1C (%)	Upto 7	4 (2.2%)	114 (63.3%)	0.000
	>7	7 (3.9%)	55 (30.6)	
BMI (kg/m^2)	Upto 30	4 (2.2%)	75 (41.7%)	0.000
	>30	7 (3.9%)	94 (52.2%)	
Smoking	Yes	8 (4.4%)	46 (25.6%)	0.035
	No	3 (1.6%)	123 (68.4%)	
Family History	Yes	11 (6.1%)	35 (19.4%)	0.086
	No	0	134 (74.5%)	

Discussion

SGLT2 inhibitor, dapagliflozin has demonstrated encouraging outcomes in treatment of diabetes; yet, questions have been raised about its possible link to urinary tract infections. Clinical decision-making and patient care require an understanding of the prevalence and contributing variables to UTTIs in diabetes patients using dapagliflozin. Our study has observed the incidence of UTIs in our research group, taking into account a range of clinical and demographic variables.

In our study of 180 cases, mean age and BMI was calculated as 49.95+5.35 years and BMI 30.67+2.37. Gender distribution shows that more male patients 61.1% versus 38.9% females. Similar demographics were seen previously, one study found mean age of type 2DM was 45.1 years in males and 45.0 years in females and there were more male patients 52.5% as compared to females 47.5%.[12] Previous study discovered that BMI of 20–21 kg/m^2 was associated with increased chance of developing DM.[13]

Frequency of urinary tract infection in diabetic patient using Dapagliflozin was recorded in 11(6.1%) of the patients. And occurrence is high among those with prolonged duration of DM, females, uncontrolled DM, increase age, and family history of UTI. Similar, results were documented in one previous review that 5.3% of diabetic individuals using dapagliflozin at doses of 5 or 10 mg had UTIs. Compared to males, women were more impacted (76.2%; $p < 0.05$) and patients over 50 years old (85.7%) had the highest prevalence of UTIs.[14] Higher doses increase the amount of glucose excreted in urine, which might possibly raise the risk of UTIs by creating ideal habitat for bacterial growth. The findings of one recent review confirmed this rise in risk. Since there was no statistically significant correlation between UTIs and dapagliflozin dosage strength.[14] In contrast to all, one study found higher incidence of UTI in SGLT2i group (33.49%), as compared to non-SGLT2 inhibitor (11.72%) and found old age and female gender as risk factors. However, there was no significant difference in UTI between the dapagliflozin and empagliflozin (34.00% and 33.03%, respectively) and with SGLT2 inhibitors risk of UTI increased by 3.70.[15]

Another review, has studied different doses, and UTI frequency was recorded 3.6%, 5.7%, 4.3%, and 3.7% of cases for dapagliflozin 2.5 mg, 5 mg, 10 mg, and placebo, respectively. It was also seen that, UTIs were the infrequent (0.3%) cause of treatment cessations and majority of infections that were identified were mild to severe and were treated with conventional antibiotics.[16] While another study found that frequency of urinary tract infection was 0.09% in diabetics using 10 mg dapagliflozin.[10] It was reported earlier that, UTIs are more common in people with type 2 diabetes who use dapagliflozin, particularly if the medication is taken for more than 24 weeks and at a dosage of 10 mg per day, $p < 0.0001$.[17] Genital tract infections are linked to a higher risk while using SGLT-2 inhibitors however, UTIs are not.[18]

Limitations of this study include, potential biases due to non-probability consecutive sampling, absence of comparison group, and single centered study limiting the generalizability of the findings.

Cocnlusion:

It is concluded that, type 2 diabetics taking dapagliflozin 10mg are at low risk of developing UTI. Patients with increase age, prolonged duration of diabetes, family history of UTI and uncontrolled diabetes and female gender are at risk for developing UTI.

Conflict of Interest: None

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Authors Contribution

CAAA: Conceptualization of Project
RI: Data Collection
SMNA: Literature Search
SBS: Statistical Analysis
QUA: Drafting, Revision
SK: Writing of Manuscript

Unveiling the Antioxidant Potential of Carica Papaya Leaf Extract in Ovalbumin-Induced Asthma

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Abstract

Objective: The main objectives of this study were to determine the antioxidant potential of Carica papaya leaf extract (PLE) in a mouse model of bronchial asthma by measuring superoxide dismutase SOD, Catalase, Glutathione peroxidase, and Malondialdehyde (MDA) levels in bronchial tissues.

Materials & Methods: Male albino mice weighing 20-25g were randomly divided into three groups. Group 1 (control group) was treated with 1% phosphate-buffered saline (PBS). Groups 2 and 3 were sensitized with ovalbumin intraperitoneally (I.P) on days 0 and 14. Animals of these group were subsequently challenged with ovalbumin intranasally from day 21 to 27. The animals of Group 3 were treated with Carica papaya leaf extract (PLE) orally at 100mg/kg body weight on days 21-27. After sacrifice, the lung tissue of animals was isolated and stored at -80 C for measuring superoxide dismutase (SOD), Catalase, malonaldehyde (MDA), and Glutathione peroxidase (GPx).

Result: The administration of Carica papaya leaf extract resulted in a significant decrease of MDA levels in treatment group as compared to diseased group (7.9±0.38 Vs 8.06±0.2 nmol/mg protein). Treatment with PLE also increased activities of antioxidant enzymes such as glutathione peroxidase (115.3±2.7 Vs 106.±3.6 u/mg protein) catalase (0.16±0.01 Vs 0.14±0.01Ku/mg protein) and Superoxide dismutase SOD (24.1±0.35 Vs 21.7±0.54 u/mg protein) in the treatment group as compared to diseased group $p \leq 0.05$.

Conclusion: Carica papaya leaf extract demonstrates promising antioxidant properties in the context of ovalbumin-induced asthma, offering a novel avenue for natural-based therapeutics for respiratory conditions.

Keywords: Carica papaya leaf extract, catalase, glutathione peroxidase (Gpx), Malondialdehyde (MDA) oxidative stress, ovalbumin (OVA), phosphate-buffered saline (PBS), superoxide dismutase (SOD).

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Introduction

Asthma is a chronic respiratory disease that affects individuals of all ages. It is often characterized by episodes of wheezing, coughing, chest tightness, and dyspnoea.[1] Approximately 339 million people suffer from asthma around the globe, with prevalence rates continuing to rise in urban areas particularly. This escalating burden requires the urgent need for effective therapeutic interventions that can reduce symptoms, improve lung function, and enhance the overall quality of life in asthmatic patients.[2]

The pathogenesis of allergic asthma involves a complex interplay between environmental factors and immune dysregulation directed by genetic predisposition.[3] The development of airway inflammation involves various immune cells, including eosinophils, mast cells, T lym-

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phocytes, and dendritic cells.[4] Production of ROS also plays an important role in accentuating airway inflammation. These reactive oxygen species include superoxide anion (O2•−), hydrogen peroxide (H2O2), and hydroxyl ions (•OH). These highly reactive molecules can cause oxidative stress and ultimate damage to cellular components including lipids, proteins, and DNA.[5]

Multiple stimuli such as allergens, pollutants, and respiratory viruses can lead to the development of oxidative stress. ROS upregulates nuclear factor kappa B (NfkB) which stimulates the production of nitric oxide and prostaglandins. As a result, inflammatory cascade in airways involving recruitment and activation of eosinophils and neutrophils occurs.[6] The release of pro-inflammatory mediators, cytokines, and chemokines perpetuates a cycle of inflammation, causing tissue damage in bronchial tissues of asthmatic patients.[7] Airway hyperresponsiveness and remodeling can also happen as a result of oxidative stress. Multiple studies have shown that reactive oxygen species can induce structural changes in airways such as goblet cell hyperplasia, subepithelial fibrosis and hypertrophy of bronchial smooth muscles. These pathological processes are responsible for airway obstruction and exacerbation in symptoms subsequently.[8]

Conventional treatment of bronchial asthma involves the use of bronchodilators and anti-inflammatory medications to control symptoms and reduce bronchial inflammation. However, these pharmacological treatments have their own side effects.[9] Alternatively, there is increasing interest in exploring complementary approaches for asthma management. Natural herbs and plants possessing antioxidant properties have been tried in past. Research in biomedical sciences have identified multiple bioactive compounds having antiasthmatic effects owing to their antioxidant properties.[10]

Carica papaya, commonly known as papaya, is one of the emergent tropical fruit-bearing plant which has been cultivated in many parts of the world. Various parts of the papaya plant including the leaves, seeds, and latex, have been utilized in traditional medicinal system for decades. Papaya fruit has been used as digestive, carminative, expectorant and sedative agent. The seeds of the unripe fruit have been used as anti dysentery and abortifacient.[11]

Carica papaya leaves extract (PLE) has been found as a rich source of bioactive compounds such as flavonoids, phenols, carotenoids and vitamins. This extract displays multiple pharmacological properties including its antioxidant, anti-inflammatory, antiviral, and immunomodulatory activities.[12]

In recent years, Carica papaya leaf extract has attracted considerable attention due to its antioxidant potential. Studies have shown that Carica papaya displayed hepatoprotective, neuroprotective and nephroprotective role in various animal models via its antioxidant activity.[13] Given the supportive evidence of role of oxidative stress in asthma pathophysiology, there is a need for exploring the therapeutic potential of natural antioxidants, such as Carica papaya leaf extract, in ameliorating oxidative damage in asthmatic individuals.

The aim of present study is to investigate the antioxidant potential of Carica papaya leaf extract in ovalbumin-induced asthma. By harnessing the therapeutic potential of PLE, we endeavor to contribute to the growing body of knowledge on natural antioxidants and their potential applications in respiratory health and disease.

Materials & Methods

It was an experimental study conducted at Hide here text from editor for a duration of 6 months from July 2015- January 2016. All the experimental procedures were carried out after approval by Review board and Ethical Committee of the institute (Ref No. UHS/ education/126-14/874). Six to eight weeks old male BALB/c mice (20-25 grams) were obtained from the animal house of University of Health Sciences Lahore. The animals were kept under standard laboratory conditions i.e., 12 hours of light-dark cycle and 22-25°C. Animals were given free access to standard food and water.[14]

Carica papaya leaves were collected locally and shade-dried. powdered form was yielded in pestle and mortar (400g total). This sample was extracted with 2 liters of ethanol by cold maceration. At 50°C, the extracted material was filtered and allowed to evaporate in a water bath. There were 20 grams of semi-solid extract produced. After that, the extract was refrigerated at 4°C for further use.[15]

Fifteen albino mice were equally divided into following three groups (n= 5 per group) by sample random sampling technique. Group 1 served as control group (received PBS 1ml/kg) while Group 2 was diseased group which was sensitized and challenged with Ovalbumin (OVA). Group 3 was treatment group which was given Papaya leaf extract (PLE).[15]

Asthma was induced using the established ovalbumin

(OVA) sensitization and challenge protocol. Briefly, the control group received PBS, 1ml/kg body weight (I.P) on Days 0 and 14 and subsequently given challenge with PBS on Days 21 to 27 while animal in groups 2 and 3 were intraperitoneally (I.P) sensitized with 20 µg of OVA emulsified in alum (2 mg) on Days 0 and 14. All animals of group 2 & 3 were challenged with aerosolized 1% OVA for 30 minutes daily on Days 21 to 27.[16] Mice in Groups 3 received daily oral administration of PLE (100 mg/kg body weight) throughout the challenge period (Days 21 to 27). The control group and OVA-challenged group (Group 2) received PBS orally (Fig. 1).

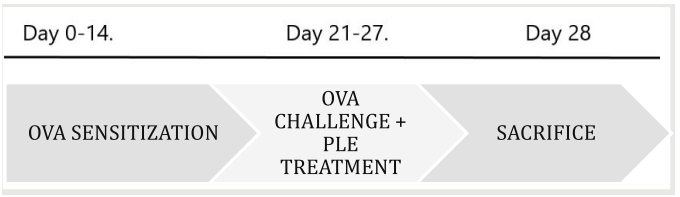

Fig 1. *Experimental protocol of asthma induction and treatment in albino mice*

On Day 28, all mice were euthanized by giving light ether anaesthesia. Lung tissues were collected immediately, washed with saline and stored at -80°C until further analysis.[16]

1% Lung tissue homogenates (w/v) were prepared using PBS. The homogenates were then centrifuged at 600 rpm for 10 minutes to obtain the clear supernatant for enzymatic assays. The activity of SOD, catalase (CAT) and Glutathione peroxidase (GPx) enzymes in the lung tissue homogenates was measured according to the kits protocol. The level of MDA in the lung tissue homogenates was determined using the thiobarbituric acid reactive substances (TBARS) assay. The absorbance was measured by a spectrophotometer at specific wavelengths for each assay. Protein concentration in the homogenates was evaluated using the Bradford assay.[17,18]

Data was interpreted as mean ± standard deviation (SD). Statistical analysis was carried out by using SPSS version 22. One-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test was applied to compare differences among the groups. P value less than or equal to 0.05 was considered statistically significant.[19]

Results

Determination of superoxide dismutase (SOD Activity): Our study demonstrated that superoxide dismutase (SOD) activity in U/mg protein) in diseased group was decreased significantly (21.7 ± 0.54) as compared to control (25.9 ± 0.4) and treatment group (24.1 ± 0.35) respectively (P ≤ 0.05). Fig 2, Table 1.

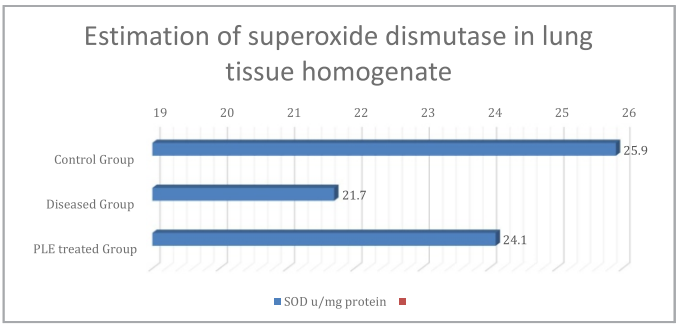

Fig. 2. *Evaluation of Antioxidant enzyme (SOD) in mouse model of allergic airway inflammation*

2. Evaluation of Catalase Activity: This research reflected a typical raised level of catalase activity in lung tissue in control and PLE group (0.17 ± 0.02 and 0.16 ± 0.01) respectively, in comparison to asthmatic mice of group 2, having significant decreased catalase level (0.14 ± 0.01) Ku/mg protein (P ≤ 0.05). Fig 3, Table 1.

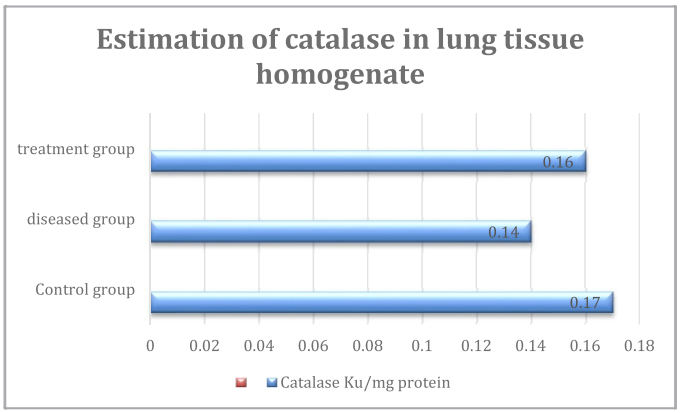

Fig. 3: *Evaluation of Antioxidant enzyme Catalase (CAT) in mouse model of allergic airway inflammation*

Our study indi-cates a significant decrease in glutathione peroxidase in diseased group as compared to control group (120.5±2.4 Vs 106.1±3.6 u/mg protein). However, treatment with PLE significantly increased GPx level in lung homogenate to 115.3±2.7 (p ≤ 0.05). **(Fig-4, Table-1)** Estimation of Malondialdehyde (MDA) Level: Regarding MDA level, our research indicated a signi-ficant decrease in MDA levels of treatment group (8.06±0.2 nmol/mg protein) as compared to diseased group (10.7±0.79), suggesting reduced oxidative stress (P ≤ 0.05). These findings of treatment group were also com-parable with

control group (8.06 ± 0.2 Vs 7.9 ± 0.38) Fig 4, Table 1.

Fig. 4 *Evaluation of Antioxidant enzyme (GPx) and lipid peroxidation marker (MDA) in mouse model of allergic airway inflammation*

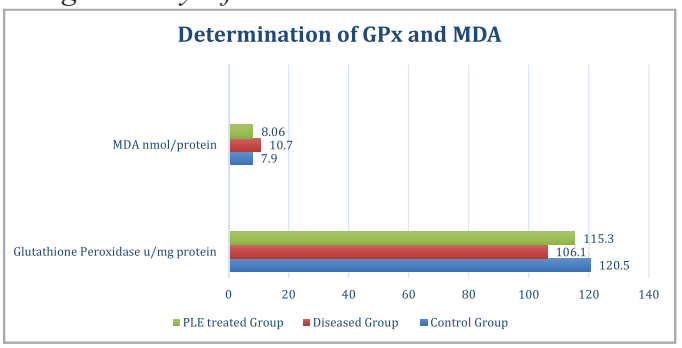

Table 1: *Depicting effect of PLE on SOD, Catalase, GPx and MDA level in lung tissue homogenates of mice.*

Parameters	Control Group (Mean±SD)	Diseased Group (Mean±SD)	PLE treated Group (Mean±SD)
SOD (U/mg protein)	25.9 ± 0.4	21.7 ± 0.54	24.1 ± 0.35
Catalase (kU/mg protein)	0.17± 0.02	0.14 ± 0.01	0.16 ± 0.01
Glutathione Peroxidase (U/mg protein)	120.5 ± 2.4	106.1± 3.6	115.3 ± 2.7
MDA (nmol/mg protein)	7.9 ± 0.38	10.7 ± 0.79	8.06 ± 0.2

Discussion

Asthma is a prevalent chronic inflammatory airway disease. Airway hyperresponsiveness, inflammation, and mucus production are underlying mechanisms involved in asthma pathogenesis. An increasing body of research indicates that oxidative stress is a major factor in the onset of asthma.[1] During asthmatic attacks, inflammatory cells generate excessive amounts of reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide. These ROS can ultimately damage bronchial cells and contribute to airway inflammation and hyperresponsiveness.[4,5] Carica papaya is a native plant which possess many biological activities proven by scientific researches in the past. Numerous bioactive substances found in Carica papaya leaves, including rutin, papain, chymopapain, cystatin, ascorbic acid, α-tocopherol, ρ-coumaric acid, and caffeic acid, might be involved in the plant's anti-oxidant activity.[12]

The present study explored the antioxidant potential of Carica papaya leaf extract (PLE) in a murine model of asthma by evaluating the activity of antioxidant enzymes and the marker of oxidative stress (SOD, catalase, glutathione peroxidase and MDA). We confirmed that PLE administration enhances antioxidant enzyme levels along with a moderate decrease in lipid peroxidation marker in lung tissue homogenates. These findings are consistent with a research conducted by Nisa et al., whereby the antioxidant activity of papaya leaf extract was verified by the DPPH and FRAP test.[20]

Our findings are also aligned with previous studies that have documented the antioxidant properties of PLE. In vitro studies have shown that PLE can scavenge free radicals and inhibit lipid peroxidation. A previous study by Salla et al., proved antioxidant and apoptotic activity of papaya peel extract in HepG2 cells via increasing catalase, SOD and glutathione peroxidase activity.[21] Additionally, research in animal models of other inflammatory conditions have also demonstrated PLE's ability to enhance antioxidant enzyme activity and reduce oxidative stress markers.

The precise mechanisms by which PLE exerts its antioxidant effects are not fully comprehended, still several pathways have been proposed in this context. PLE is rich in various antioxidant compounds which can directly scavenge free radicals, preventing them from damaging cells. Additionally, PLE may upregulate the level of antioxidant enzymes like SOD, catalase, and glutathione peroxidase, further bolstering the body's defense against oxidative stress. In our previous study, PLE exhibited it's anti-inflammatory activity in murine model of asthma by downregulating IL-4, IL-5, TNF alpha, Eotaxin and NFkb.[15] Modulation of NFkb by papaya leaf extract may lead to attenuation of oxidative stress in bronchial tissues. The current study's findings add to the growing body of knowledge suggesting that targeting oxidative stress could be a promising strategy for asthma management. PLE, with its demonstrated antioxidant potential, might offer a complementary approach to traditional asthma medications. While this study provides encouraging results, some limitations need to be considered. Firstly, the investigation was conducted in a murine model, secondly, the optimal dosage and formulation of PLE for therapeutic use in asthma require exploration. It is therefore suggested to translate these findings to humans through clinical trials.

Conclusion

This study demonstrates the potential of PLE as an

antioxidant agent in an animal model of asthma. The observed increase of antioxidant enzyme activity and decrease of MDA levels suggest its ability to mitigate oxidative stress, a crucial factor in asthma pathogenesis. Future research is warranted to explore the clinical applications of PLE and its role in asthma management.

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Authors Contribution

AI: Conceptualization of Project
SR, SI: Data Collection
: Literature Search
SM, SI: Statistical Analysis
SI: Drafting, Revision
SR, MSM: Writing of Manuscript

Comparison of Outcome of Autologous Platelet Rich Plasma Membrane Plus Dartos Flap Versus Simple Dartos Flap In Hypospadias Surgery

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Abstract

Objective: To compare the outcome of application of autologous platelet-rich plasma membrane layer and dartos fascial flap versus only dartos fascial flap in mid and distal penile hypospadias surgery.

Material and Methods: This randomized controlled trial (NCT06275646) was conducted at Pediatric General surgical department, the Children's hospital and the University of Child health, Lahore for the period of 12 months. Non probability, purposive sampling was used for data collection and randomization was done to assign the treatment. Total of 220 patients fulfilling the selection criteria were admitted after taking an informed consent. Cases were randomly divided into two groups A and B using random generator of Excel program. In group-A, dartos flap and Platelet Rich Plasma(PRP) membrane layer was applied and in group-B, only a preputial dartos fascial flap was applied.

Results: The mean age of all cases was 5.86±3.10 years, while the mean age in group-A and group-B was 5.78±3.13 years and 5.95±3.08 respectively, with non-significant p-value > 0.05. The mean hospital stay was statistically same in group-A (6.67±2.80 days) as compared to group-B (7.34±2.79 days), p-value 0.08 (i.e. > 0.05). The mean time for urethral stent removal was statistically same in group-A (9.31±3.08 days) as compared to group-B (9.81±2.81 days), p-value 0.21 (i.e. >0.05). In group- A and group-B, 32(29.1%) cases and 43(39.1%) cases had complications respectively (infection, urethrocutaneous fistula, glans dehiscence, Meatal stenosis). The complications rate was statistically same in both groups, p-value > 0.05.

Conclusion: It is concluded that patients who received dartos flap and PRP membrane coverage had less incidence of urethrocutaneous fistula as compared to cases received a preputial dartos flap only. Other complications were statistically same in both study groups.

Keywords: Mid penile hypospadias, distal penile hypospadias, surgery, dartos flap and PRP membrane, preputial dartos flap, complications.

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Introduction

Hypospadias is most common congenital condition of phallus in which the opening of urethra present on the ventral aspect of phallus anywhere proximal to its actual location.[1] The incidence of hypospadias ranged from 0.6/10,000 births to 464/10,000 births[1]. Patients with hypospadias are usually referred for surgery during early childhood or infancy.[2]

Hypospadias repair surgery has been progressed steadily over recent years.[3] However, the results of hypospadias repair are still mostly unfavorable with reported complications rate as high as approximately 50% or above.[1,4] The commonest complications following hypospadias surgery usually accompany with urethral fistulas, followed by stenosis of meatus, stricture of urethra, formation

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of urethral diverticulum, dehiscence of glans, and cosmetically unfavorable outcomes necessitating redo surgeries.[5]

Modern surgical interventions claim that it is now accessible to make a functionally and cosmetically acceptable phallus. Assessment of outcomes include: rate of complications (such as wound infection, glans dehiscence, Urethrocutaneous fistula (UCF) and meatal stenosis), cosmetic penile appearance, functional ability (urination, sexual intercourse), and psychological issues like improvement in value of life and psychosexual life of patient.

Various types of techniques for urethroplasty has been evolved for example simple tubularization of the urethral plate, giving incision in midline of urethral plate and formation of Tubularized incised plate (TIP), use of adjacent preputial skin flaps as covering layer, different types of skin grafts, grafts from buccal mucosa, and mobilized flaps enriched with vascular supply (mostly involving the inner preputial skin).[6] Methods of providing vascularized tissue coverage to neo-urethra include application of dartos fascia, tunica vaginalis, de-epithelized skin, and corpus spongiosum.[7] Recently it is reported that platelet rich plasma membrane has the capability to minimize post-operative complications occurring after repair of hypospadias, especially post-operative infection & urethrocutaneous fistula formation by improving wound healing with help of platelets themselves and platelet derived growth factors present in Platelet Rich Plasma (PRP). In this study, UCF occurred in 10% of patients and infection in 5% of patients using PRP material as compared to patients in which repair was done without the PRP, in which fistula rate was 25% and infection rate was 35%.

Another study reported that UCF developed in 2(6%) in total 33 cases when autologous PRP membrane was used as an alternative covering layer in repair of hypospadias. No complications were reported in relation to the sampling of blood. No statistically significant difference was observed between study and the control group (undertaking the same surgery, but with different mean). Another comparative study showed complications and outcome rate of application of PRP layer versus dartos flap layer only during primary surgery of distal hypospadias. They reported 12 (13.3%) complications in group A (in which TIP urethroplasty done with PRP membrane was applied), and 24 (26.7%) patients had complications in group B (dartos flap only as second layer). Urethrocutaneous fistula after primary Snodgrass repair was seen in 9(10%) patients in A group, and in 12(13.3%) patients in B group. Partial or complete dehiscence of glans seen in 1.1% of patients in A group, and in 4.4% of patients in B group. In A group no patient had a superficial wound infection, as compared to B group in which 6.7% of patients had wound infection. One patient (1.1%) of urethral stricture and meatal stenosis was observed in both groups, both were conservatively managed by regular dilatation of meatus and urethra weekly for 2 to 3 months. [8]

Material & Methods

This randomized control trial (NCT06275646) was conducted at Pediatric Surgery Department, The Children's Hospital and University of Child Health Sciences, Lahore. After approval from the ethical review committee of the hospital, patients (Age 6months to12 years) with mid, distal penile or sub coronal hypospadias having minimal chordae (less than 20) were included in the study. Patients having proximal hypospadias, moderate to severe chordae, previously operated cases, a preoperative androgens treatment (for example in penoscrotal hypospadias and small sized phallus) were excluded from study. The study was carried out between 1st September 2021 to 30th August 2022.

A total of 220 patients (110 in each group) fulfilling the selection criteria were admitted after taking an informed consent. Patient's demographic data includes name, age and contact details were obtained. Patients were divided randomly into two groups, A and B using balloting method. In group-A, a dartos flap and PRP layer was applied and in group B patients, only the preputial dartos flap layer was applied. Whereas PRP membrane was prepared at the time of surgery, a 2.5 × 1.5cm PRP layer was made from 8-10 cc of whole blood of patient with doubled centrifugation of sample for about 15-20 minutes at 2500 -3000 RPM. In the operation theatre, 2 to 3 drops of calcium chlorate added in coagulum, and incubation of sample was done for 2-3 minutes at 37°C until a bright red to whitish layer was formed at the middle of tube. The centrifuged blood was then separated into three layers, depending on its density: the red blood cells in bottom layer; the PRP membrane, (approximately 1-1.5ml) enriched with white blood cells and platelets in middle layer; and the platelet-poor plasma in top layer. After extracting from the tube, the clot was separated from the RBCs, and gently pressed between two saline soaked gauzes to form a thin membrane. All patients were operated through the Snodgrass technique with a suitable sized catheter (NG8–10Fr). Neo-urethra was formed by continuous layer with absorb-

able suture sized 7/0 or 6/0, followed by second covering layer with dartos fascia by interrupted absorbable sutures. After that a thin PRP layer was applied between dartos and skin. Closure was done with absorbable suture followed by antiseptic dressing. The dressing changed on the fourth postoperative day and wound examined. The catheter was kept in placed for 10–12 days for urine drainage, and after removing the catheter, wound and stream of urine was observed in patients of both groups. Surgical team of same level did all surgical procedures to reduce the risk of biasness.

Data was entered and analyzed through SPSS -24. Quantitative variable like age was presented as mean ± S.D. The qualitative data like gender and complications such as wound infection, glans dehiscence, urethrocutaneous fistula and meatal stenosis was presented as percentage and frequency. Chi-square test was applied to compare complication such as (wound infection, glans dehiscence, urethrocutaneous fistula and meatal stenosis) in both study groups. P value equal to or less than 0.05 was considered as significant.

Results

The mean age of all cases was 5.86 ± 3.10 years, while the mean age in group-A and group-B was 5.78 ± 3.13 years and 5.95 ± 3.08 respectively, with nonsignificant p-value > 0.05. The mean hospital stay was statistically same in group A (6.67 ± 2.80 days) as compared to group-B (7.34 ± 2.79 days), p-value 0.08 (i.e. > 0.05). The mean time for urethral stent removal was statistically same in group-A (9.31 ± 3.08 days) as compared to group-B (9.81 ± 2.81 days), p-value 0.21 (i.e. > 0.05).

Table 1: *Comparison of Complications in both study groups*

		Study groups		Total
		Group-A	Group-B	
Complications	*Yes*	32(29.1%)	43(39.1%)	75(34.1%)
	No	78(70.9%)	67(60.9%)	145(65.9%)
Total		110(100%)	110(100%)	220(100%)

Chi-square = 2.45, p-value = 0.118 (significant)

Table 2: *Comparison of Wound infection at 2 week, 4 weeks, 12 weeks in both study groups*

Wound infection		Study groups		Chi-square	p-value
		Group-A	Group-B		
At 2 weeks	Yes	15(13.6%)	18(16.4%)	19.04	0.015
	No	95(86.4%)	92(83.6%)		
At 4 weeks	Yes	3(2.7%)	3(2.7%)	0.00	1
	No	107(97.3%)	107(97.3%)		
At 12 weeks	Yes	0(0%)	0(0%)	--	--
	No	110(100%)	110(100%)		

Chi-square = 2.45, p-value = 0.118 (significant)

Table 3: *Comparison of Glans Dehiscence, Urethrocutaneous Fistula and Meatal stenosis at 2nd weeks*

Types of complications		Study groups		Chi-square	p-value
		Group-A	Group-B		
Glans Dehiscence	*Yes*	3(2.7%)	5(4.5%)	0.519	0.471
	No	107(97.3%)	105(95.5%)		
Urethrocutaneous Fistula	*Yes*	24(21.8%)	38(34.5%)	4.40	0.036
	No	86(78.2%)	72(65.5%)		
Meatal stenosis	*Yes*	1(0.9%)	2(1.8%)	0.56	0.5
	No	109(99.1%)	108(98.2%)		

Discussion

In general, postoperative urinary diversion, wound care, and dressings have helped to ease postoperative care without compromising outcomes of surgery. Traditionally, a variety of urinary diversion methods has been used in hypospadias repair to maintain patency of neourethra and to keep urine away from wound thereby reducing the possible complications.[9] In their study, mean age at operation was 2.45 years (range: 1–4 years). One more study was done in 180 boys (age range 12–65 months).[8] In current study, cases of 2-12 years were taken, with mean age of 5.86 ± 3.10 years.

Alternatively, some reports also suggest that urethroplasty without stenting excludes the problems of bladder spasm and discomfort during removal of stent without increasing rate of complications.[10] In current study in group-A and group-B, 32(29.1%) cases and 43(39.1%) cases had complications respectively, the complications rate was statistically same in both groups, with p-value greater than 0.05. In our study till 2nd week of follow up 3(2.7%) cases in group-A and 5(4.5%) cases in group-B had Glans dehiscence, with no statistical difference, p-value was > 0.05.

Recently a study was done on 180 boys (ranging from 12–65 months) in which they were divided randomly into two groups. In A group, TIP urethroplasty done with PRP layer and in B group, dartos fascial flap was used. They reported that there was statistically significant difference in the complications rate between both groups. A total of 36(20%) patients had complications in both groups. The authors reported that 12 (13.3%) patients had complications in A group as compared to B group in which 24 (26.7%) patients had complications. They concluded that PRP membrane might be used as substitute layer for repair of distal hypospadias, particularly when a healthy layer is absent.[8] While in current study

we found similar complications rate, overall infection rate was same but according to Southampton criteria Group-A was relatively better. Another study reported that the Platelet rich fibrin patch can be an efficient and safe covering method in repair of hypospadias. This procedure can be used as an additional approach to cover the neo-urethra for hypospadias repair, especially when the healthy tissue is not available as coverage layer. This, in turn can reduce the incidence of post-operative complications.[11] In current study, in group-A and group-B there were 1(0.9%) and 2(1.8%) cases who had meatal stenosis, p-value > 0.05. These findings are similar to another study that reported that only one case (1.1%) of urethral stricture and meatal stenosis was reported in each group, all of which were conservatively managed by regular weekly dilatation of meatus and urethra for two to three months.[8]

The commonest complication occurring after repair of hypospadias is urethrocutaneous fistula (UCF) with an incidence fluctuating from 4 to 28%. One of the most important procedures used to reduce the possibility of fistula formation is application of an intermediate layer between the neo-urethra and the skin. Dartos flap and PRP coverage are among several techniques which are gaining fame.

In study done in 2019, in which 180 patients were divided into two groups randomly. In A group TIP urethroplasty done with PRP layer and in B group dartos flap was used. Glans dehiscence occurred in 1(1.1%) patient in A group, and 4(4.4%) patients in B group. Urethrocutaneous fistula was reported in 9(10%) patients in A group, and 12 (13.3%) patients in B group. No case of superficial wound infection reported in group A, compared with group B in which six patients had wound infection (Mahmoud et al., 2019).[8] A study was planned by Guinot and colleagues to evaluate the, safety, feasibility, and efficacy of autologous PRF membrane in hypospadias surgery. They reported that UCF occured in 2/33(6.1%) patients when autologous PRF membrane used as coverage in hypospadias surgery. The median follow-up of patients was 8 months (range from, 6–18 months). It was a descriptive study with no comparison.[10]

A local study reported that among 88 cases treated with autologous platelet rich plasma patch, urethrocutaneous fistula was developed in 11.36% of the cases. In our study, the UCF rate was 21.8% and 34.5% in group A and B respectively with p-value < 0.05.

In current study, in group-A and group-B there were 1(0.9%) and 2(1.8%) cases who had meatal stenosis, p-value > 0.05. These findings are similar to another study that reported that only one case (1.1%) of urethral stricture and meatal stenosis was recorded in both groups.[8]

In a recent study comparison of autologous PRP material plus dartos flap vs only dartos flap in mid penile hypospadias surgery was compared. In patient group using PRP, infection occurred in 5% and fistula in 10% of patients. While in group without using PRP infection occurred in 35% and fistula in 25% of patients.[5] In our study, infection occurred in 13.6% and fistula in 21.8% of patients with using PRP membrane, while in group without using PRP, infection occurred in 16.4% and fistula in 34.5% of patients.

Conclusion

It is concluded that patients who received dartos flap and PRP membrane coverage had less incidence of urethrocutaneous fistula as compared to cases who received a preputial dartos flap only. Other complications were statistically same in both study groups. Hence in future dartos flap and PRP membrane coverage should be encouraged to apply for mid penile and distal hypospadias surgery to achieve better outcomes.

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AR, MA, M AA, AI:	Statistical Analysis
AR, MA, M AA, AI :	ImranDrafting, Revision
AR, MA, M AA, AI:	Writing of Manuscript

Significance of Hematological and Biochemical Parameters in Diagnosed Patients of Dengue infection

Atiqa Arshad,[1] Omair Farooq,[2] Zainab Yousaf,[3] Asim Mumtaz,[4] Eazaz Khan,[5] Ana Fayyaz[6]

Abstract

Objective: The study aimed to assess the variation of Total leukocyte count (TLC), Hematocrit (HCT), platelets, serum albumin, and alanine transaminase (ALT) among patients with different severities of dengue infections.

Materials & Method: It was a cross-sectional study conducted during five months (1st June to 31st October) of the year 2023. Patients admitted to the dengue ward and diagnosed with dengue viral syndrome, dengue hemorrhagic fever, and dengue shock syndrome were included. About three to five milliliters of blood was collected for hematological and biochemical parameters like TLC, HCT, platelets, serum albumin, and ALT. The results were retrieved through an automated hematology and chemistry analyzer. The data was analyzed through SPSS version 25.0.

Results: Out of 134 patients, 52.2% were males and 47.8% were females. The mean age of patients was 44.22+17.87 years. There were 70.9%, 20.1%, and 9.0% of patients who presented with dengue viral syndrome, dengue hemorrhagic fever, and dengue shock syndrome respectively. Among total subjects, 69 (51.49%) patients had leukopenia, 68 (50.74%) patients had increased HCT level, 103 (76.86%) patients had thrombocytopenia, 09 (6.71%) patients had decreased level of serum albumin, and 122 (91.04%) patients have increased ALT levels. A statistically significant association was found between length hospital stay (days) with TLC (0.002) and dengue infections (DHF, DVS, and DSS) with platelets (0.012). All other parameters showed non-significant rise or fall.

Conclusion: Hematological parameters such as thrombocytopenia and leukopenia vary according to the severity of dengue infection. However, no statistically significant difference was found except TLC. Biochemical changes such as elevated ALT, and decreased serum albumin could serve as predictors of dengue complications but further studies are required to confirm the finding.

Keywords: Dengue viral syndrome (DVS), dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS), hematological changes, biochemical changes.

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Introduction

Dengue is a mosquito-borne tropical disease caused by an arbovirus transmitted by the Aedes aegypti mosquito and affects millions of people around the world. Most cases of dengue viral syndrome (DVS) are mild and self-limiting.[1] In some instances, the disease can progress to severe dengue as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).[2] Four serotypes of dengue virus are antigenically distinct namely "DENV-1, DENV-2, DENV-3, and DENV-4". A fifth serotype (DENV-5) was detected through genetic sequence analysis in Sarawak state of Malaysia in October 2013.[3]

DVS tends to be more prevalent among adults and teenagers. Its manifestation can vary, ranging from a mild

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fever to a more debilitating illness. The severe form is marked by a sudden onset of high fever, intense headache, retro-orbital pain, muscle and joint aches along rash, mainly experienced during the initial phase of fever.[2,4] During the critical stage, the skin might display a flushed appearance accompanied by a petechial rash, usually appearing as the fever subsides, typically between days three to seven. This phase is linked to capillary leakage and bleeding. Some cases can develop into DSS, involving abnormalities in clotting, increased plasma leakage, and raised vascular fragility. An increase in capillary permeability results in fluid loss leading to hypovolemic shock and failure of multiple organs.[5]

For diagnosis of DVS, clinical presentation, laboratory tests, and patient's medical history are required. Accurate and timely diagnosis is crucial for providing appropriate medical care and managing DVS effectively.[6] Laboratory tests are essential to confirm the diagnosis and determine the severity of dengue infection. Currently, the serological test is used to confirm the diagnosis of dengue infection such as the detection of the dengue NS1 antigen (sensitivity 76% and specificity 98%) by the ELISA method.[7]

The progression of the disease results in hematological and biochemical abnormalities. They could be used to reduce mortality and morbidity by early detection of the problems and the introduction of efficient management techniques. Patients with severe dengue infection may be effectively identified with plasma leakage by using a combination of hematological and biochemical indicators, such as HCT, albumin concentration, platelet count, and aspartate aminotransferase (AST).[8] Dengue infection is now increasingly linked to hepatic involvement of variable severity.[9] Abnormal values of liver function tests are frequently observed in dengue infection. In dengue-infected patients, increased ALT and reduced serum albumin were observed.[10,11] These parameters may be utilized to anticipate the start of the leaking phase in advance. Therefore, the present study aimed to identify the significance of hematological and biochemical parameters among patients with different severities of dengue infection.

Materials & Methods

This cross-sectional study was conducted in Farooq Hospital Westwood Lahore during five months (1st June to 31st October) of the year 2023. Patients admitted to the dengue ward and diagnosed with DVS, DHF, and DSS according to WHO conventional classification of dengue[12] were included. The diagnosis was made by disease specialists and patient record was maintained in the hospital data software.[4] Patients who were on long-term medication with underlying chronic disease and those with negative dengue NS-1 antigen tests were excluded. The relevant information of patients was collected using a structured data collection proforma with informed written consent (for children, a guardian consent was obtained). On admission, about three to five milliliters of blood was separately collected in ethylenediamine tetraacetic acid (EDTA) vacutainer for TLC, HCT, and platelets and in clotted vacutainer for biochemical investigations (ALT and serum albumin). Complete blood count to check hematological parameters (TLC, HCT, and platelets) was performed on Mindray BC-5000 while ALT and serum albumin were estimated through Selectra Pro-M using the photoelectric colorimetric principle. The collected data was analyzed through Statistical Package for the Social Sciences (SPSS) version 25.0. For quantitative variables, data was presented as mean + SD. Qualitative variables were presented as frequency and percentages. The multivariate analysis was used to find the estimated marginal means and association between study variables.

Results

A total of 134 patients were included in this study, 70 (52.2%) were males and 64 (47.8%) were females. The range of age was 03 years to 86 years. The mean age of patients was 44.22+17.87 years. According to the clinical criteria, 95 patients (70.9%) had DVS, 27 patients (20.1%) had DHF, and 12 patients (9.0%) had DSS. The mean age of DSS (46.50+14.93) patients was higher than DHF (46.04+12.62), and DVS (43.42+19.48) patients (statistically non-significant). The mean duration of hospital stay of these patients was 4.38+1.85 days.

The normal range for hematological parameters was: TLC (4.00-11.00 ×109/L), HCT (36.00-46.00 %), and platelets (150.00-450.00×109/L). In total, 69 (51.49%) patients had leukopenia, 68 (50.74%) patients had increased HCT levels and 103 (76.86%) patients have thrombocytopenia.

The highest mean value of TLC (14.50 + 2.23) was observed in DVS patients with 11 days of hospital stay, while the lowest mean value of TLC (2.85+1.57) was observed with 09 days of hospital stay. In DHF, the highest mean value of TLC (5.56+0.91) was observed in patients with 03 days of hospital stay, and the lowest mean value (2.20+2.23) with 07 days of hospital stay.

The highest and lowest mean values of TLC were (5.26 + 0.99), and (4.20+1.28) were observed in DSS patients with 04 days and 03 days of hospital stay respectively. Statistically significant distinct variations in TLC patterns are evident among different cases of DVS, DHF, and DSS (Figure 1).

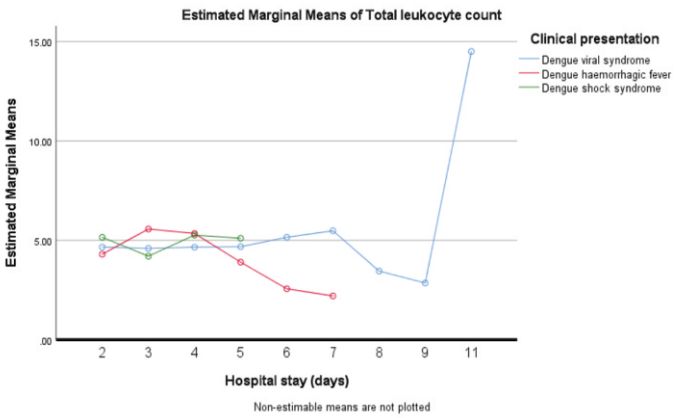

Figure 1: *Pattern of Total Leukocyte Count Estimated Marginal Means in Dengue Infections*

The highest mean value of HCT (42.92+2.04) was observed in DVS patients with 08 days of hospital stay, while the lowest mean value of HCT (36.50+4.09) was observed with 09 days of hospital stay. In DHF, the highest mean value of HCT (41.95+2.19) was observed in patients with 04 days of hospital stay, and the lowest mean value (37.23+3.34) with 02 days of hospital stay. The highest and lowest mean values of HCT were (45.90 +5.79), and (36.12+2.59) were observed in DSS patients with 05 days and 04 days of hospital stay respectively but was statistically not significant. The pattern of HCT in DVS, DHF, and DSS patients is given (Figure 2).

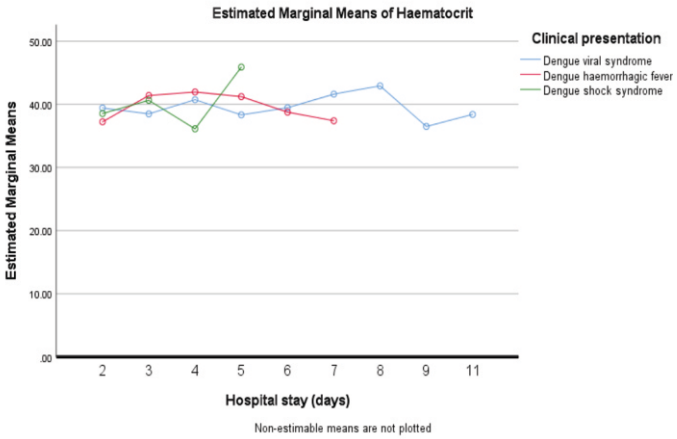

Figure 2: *Pattern of hematocrit estimated marginal mean values in dengue infections*

In DVS patients, statistically significant highest mean value of platelets (146.40+26.86) was observed with 07 days of hospital stay, while the lowest mean value of platelets (35.00+59.67) was observed with 11 days of hospital stay. In DHF, the highest mean value of platelets (115.0+26.68) was observed in patients with 05 days of hospital stay, and the lowest mean value (25.00 +59.67) with 07 days of hospital stay. The highest mean values (80.00+59.67) and lowest mean values (47.50 + 42.19) of platelets were observed in DSS patients with 05 and 02 days of hospital stay respectively. The pattern of platelets in DVS, DHF, and DSS patients is given (Figure 3).

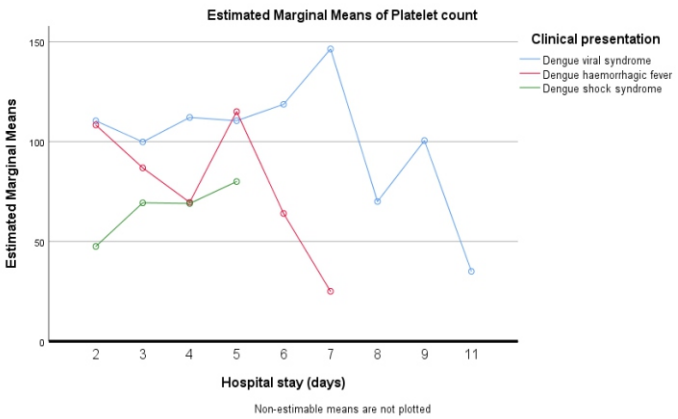

Figure 3: *Pattern of Platelet estimated marginal mean values in dengue infections*

The normal ranges for biochemical parameters were: serum albumin (3.50-5.20 g/dL) and ALT (less than 41 U/L). In this study, out of 134 patients, 09 (6.71%) have decreased levels of serum albumin, and 122 (91.04%) have increased ALT levels. The mean values of serum albumin and ALT were also observed during the clinical course of DVS, DHF and DSS. The highest mean value of serum albumin (4.23+0.07) was observed in DVS patients with 04 days of hospital stay, while the lowest mean value of serum albumin (3.50+0.31) was observed with 11 days of hospital stay. In DHF, the highest mean value of serum albumin (4.40+0.18) was observed in patients with 02 days of hospital stay, and the lowest mean value (3.70+0.13) with 03 days of hospital stay. The highest and lowest mean values of serum albumin were (4.50+0.31), and (3.90+0.22) were observed in DSS patients with 05 days and 02 days of hospital stay respectively. All these findings were statistically non-significant. The pattern of serum albumin showed a marked difference between DVS, DHF, and DSS (Figure 4).

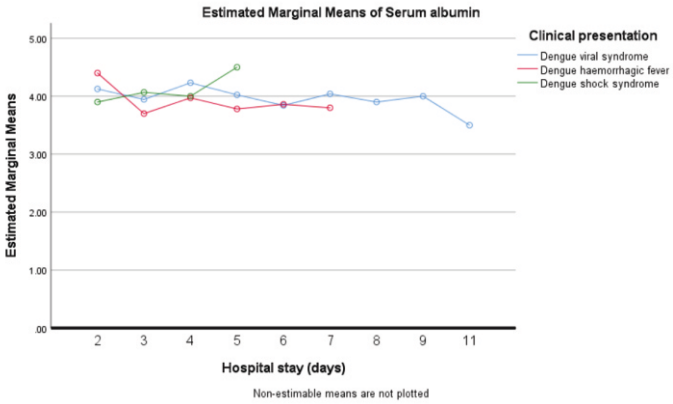

Figure 4: *Pattern of serum albumin marginal mean values in dengue infections*

The highest mean value of ALT (207.50+58.50) was observed in DVS patients with 09 days of hospital stay, while the lowest mean value of ALT (64.0+82.73) was observed with 11 days of hospital stay. In DHF, the highest mean value of ALT (233.0+36.99) was observed in patients with 05 days of hospital stay, and the lowest mean value (68.33+47.76) with 02 days of hospital stay. The highest and lowest mean values of ALT were (165.0 + 58.50), and (53.66+47.76) were observed in DSS patients with 03 days and 02 days of hospital stay respectively. All these findings were statistically non-significant. The pattern of ALT showed a marked difference between DVS, DHF, and DSS (Figure 5).

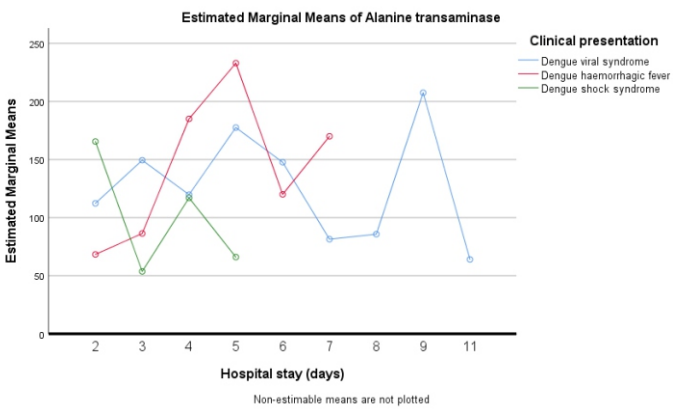

Figure 5: Comparison of alanine transaminase means values in dengue infections

The association between study variables was determined by multivariate analysis. A statistically significant association was found among hospital stay (days) with TLC (0.002) and clinical presentation of dengue infection with platelets (0.012). The other variables were found statistically non-significant. The p-value of <0.05 was considered statistically significant.

Table 1: *Observed association between study variables*

Factors	Dependent variables	Mean square	p-value	Observed power
Hospital stay (days)	Total leukocyte count	16.21	0.002*	0.964
	Platelet count	3178.38	0.525	0.398
	Hematocrit	20.06	0.778	0.265
	Serum albumin	0.14	0.186	0.628
	Alanine transaminase	7653.15	0.356	0.498
Clinical presentation of dengue infection (DVS, DHF, and DSS)	Total leukocyte count	5.54	0.332	0.242
	Platelet count	16337.70	0.012*	0.767
	Hematocrit	0.71	0.979	0.053
	Serum albumin	0.11	0.333	0.241
	Alanine transaminase	6373.24	0.397	0.208

Statistical test: Multivariate analysis
*Significant p-value

Discussion

Differentiating dengue from other viral infections may be challenging due to the absence of specific early clinical features, besides Polymerase Chain Reaction (PCR) or NS1 antigen testing within the first 48 hours, which offers a greater likelihood of positive results. However, these tests cannot differentiate among those progressing to DVS, DHF, and DSS. The DHF and DSS are characterized by significant plasma leakage, resulting from various host factors emerging in the later stages of the illness. Changes in blood parameters during dengue infection could serve as predictive indicators for individuals at higher risk of plasma leakage and aid in the early detection of these complications. This approach enables clinicians to potentially identify prior development of DHF and DSS, facilitating effective patient management and reducing morbidity and mortality[9]. Previous studies have extensively presented the detailed clinical spectrum of dengue virus infection[13-15]. Our study, however, aimed to assess how TLC, HCT, platelets, serum albumin, and ALT vary among patients with different severities of dengue.

This study showed the raised incidence of dengue infection among males compared to females. In Pakistan, the male population typically spends extended periods outdoors due to employment, resulting in greater exposure to mosquitoes than females. Additionally, women in Pakistan tend to cover themselves more with clothing as compared to men. These study findings align with

previous research by Gandhi and Shetty[16] and Asghar et al.[17] The prevalence of dengue cases was notably higher among the age group of 11-50 years compared to the elderly (50-70 years of age). This elevated frequency within the younger age bracket can be attributed to the larger population size in this demographic and their increased exposure to mosquitoes, mainly due to their active engagement in outdoor activities. Consistent findings from other studies on dengue patients also endorsed this observation, highlighting an increased intensity of dengue within the younger age groups in contrast to the older population.[18,19] In this study, 70.9% of patients presented with mild dengue infection (DVS), and 29.1% of patients presented with severe dengue infections (DHF and DSS). The incidence of classic dengue fever of 70% was also found in other studies.[20,21] Our study showed that the mean duration of hospital stay was 4.38+1.85 days and this finding is consistent with another study which showed the mean duration of hospital stay (3.43 ± 2.085) days.[22]

In this study, thrombocytopenia, leukopenia, and high HCT were found in 76.86%, 51.49%, and 50.74% of the patients respectively. This finding is in agreement with a previous study showing 80% thrombocytopenia.[4] Another study from India has documented thrombocytopenia, leucopenia, and high HCT in dengue infection.[25]

The increase in HCT levels among dengue patients is often linked to plasma leakage caused by increased vascular permeability. While a 20% rise in HCT has been considered as a diagnostic threshold previously,[23] this study demonstrated less increase in HCT than expected. A similar observation of a lower-than-expected rise in HCT levels has been documented previously. This suggests the necessity for revised recommendations regarding HCT elevation for diagnosing dengue. The commonly observed leukopenia and thrombocytopenia in dengue patients result from bone marrow suppression and the binding of dengue antigens to platelets[24]. This study showed notable variations in TLC with severity of dengue infection but thrombocytopenia was not found significant in association with severity. This finding is in agreement with the previous study conducted in 2004.[26]

Our study revealed that 91.04% of dengue-infected patients have high ALT levels. These findings were consistent with a previous study conducted in Lahore, Pakistan.[24] In dengue patients, there was a consistent pattern of low serum albumin levels, with the severity of the disease. The lowest levels were recorded in cases of DSS. This decline in albumin levels is linked to increased plasma leakage and increased vascular permeability seen in DSS. Consistent findings of decreased serum albumin levels were also noted in other studies conducted in India.[20]

Conclusion

Based on the above findings, it is evident that hematological parameters like leukopenia (decreased total leukocyte count) vary with severity of disease (Hospital stay). Thrombocytopenia, increased HCT and biochemical parameters like increased ALT and decreased albumin may serve as a screening tool for early therapeutic response, however additional studies are required. Patients exhibiting compromised parameters should receive additional care to prevent the onset of complications.

Conflict of Interest: *None*

Source of Funding: *None*

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Authors Contribution

OF: Conceptualization of Project
EK: Data Collection
AF: Literature Search
ZY: Statistical Analysis
AA: Drafting, Revision
AM: Writing of Manuscript

Prevalence And Determinants of Missed Childhood Vaccination Among Infants Between 0-23 Months At THQ Shakargarh, Pakistan

M.Tariq,[1] Azhar Yaqoob,[2] Rahat Khan,[3] Uzma Malik[4]

Abstract

Objective: To determine the prevalence and the risk factors of missed immunization among infants between 0-23 months at Shakar Garh, Pakistan.

Material and Method: This is a cross-sectional descriptive-analytical study. The sample of 334 participants was selected through stratified random from the area of Shakarghar. The immunization data was collected through the parents or guardians of the children. The information regarding demographic variables and risk factors was collected through a self-developed questionnaire. The data was entered into SPSS for analysis. Descriptive analysis, Chi-square, and regression analysis were applied to the data.

Results: Our findings showed that the adjusted prevalence of missed immunization is 32%. The prevalence of missed immunization decreased with good knowledge about immunization. A multivariate logistic regression analysis indicated that parents' education, income level, access to TV, radio, and internet, distance from a health care facility, the attitude of health workers, and rural lifestyle were risk factors for missed immunization, $p<0.05$.

Conclusion: Nearly one-quarter of infants in the study area were missed their immunization. Parents' education, income, access to technology, and the attitude of health workers are all important factors in determining partial and un-immunization. So in countries like Pakistan, the health system should focus on increasing health workers' communication skills to reduce vaccine-preventable diseases, especially in low-resource areas.

Keywords: immunization, missed, partial, complete, risk, factor

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Introduction

Immunization is the procedure whereby a individual is ended protected or resilient to an transferable illness, classically by the administration of a vaccine.[1] Childhood immunization is one of the most important public health measures for reducing deaths among children under age of five. As a result, WHO started the Expanded Programme on Immunization (EPI) in May 1974 to combat vaccine preventable diseases (VPD).[2] However, VPD such as diphtheria, tuberculosis, tetanus, pertussis, polio, and measles occurred internationally due to incomplete and non-immunization of children, which still accounts 8.8 million deaths per year in children under 5 years. World Health Organization (WHO) lays tremendous importance on lifelong vaccination. It is the key value of Sustainable Development Goals. The WHO also established Global Vaccine Action Plan in 2012. The strategy intends to avoid millions of deaths globally by raising immunization rates and availability of vaccine by the year 2020. One of its essential aspects is to eliminate polio from the world completely.[3] Vaccines are complex substances that elicit the immunogenic response when given to humans. The first vaccine was produced

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by Edward Jenner in 1796 for the elimination of smallpox. In 1980 smallpox was declared eradicated worldwide.[4] Due to vaccination, immune complex is formed which is a complex network of organs, cells and proteins that defends the body against infection& protecting the body's own cells. The immune system keeps a record of every germ (microbe) it has ever defeated so it can recognize and destroy the microbe quickly. There are two type of immunity, active& passive. Naturally occurring immunity& induced immunity are two different things. A person's vaccination is considered artificial active immunity, whereas passive immunity occurs when antibodies are transferred. On the other hand, natural immunity results in active immunity against infection and passive immunity from the mother's antibodies to the infants.[5]

A child's required vaccines should be administered by the time they turn one, and the shots should be documented on the vaccination card which is handed over to the parents. There are 12 VPD that children are covered. These vaccinations include; polio, TB; whooping cough; diphtheria; tetanus; hepatitis B, measles; Haemophilus; Hepatitis C; and Rota. UNEP's goal is that "every kid" must be vaccinated against the target diseases.[6]

In Pakistan EPI programme was launched in 1978. According to this schedule children were vaccinated against polio, diphtheria, pertussis, tuberculosis, and measles. Later, by the provision of development associates, a numeral of novel vaccines e.g. hepatitis B, haemophilus influenzae type b (Hib) and pneumococcal vaccine (PCV10) were familiarized in 2002, 2009 and 2012, and injectable polio vaccine in 2015. The EPI aims also to protect mothers and newborn against tetanus. The programme also added rotavirus vaccine in 2017, which will prevent the children against diarrhoea.[7] In 1983, the WHO's Expanded Program on Immunization (EPI) Global Advisory Group (GAVI) advised that children be immunized at every opportunity.[8]

Table 1: *Pakistan's EPI vaccination schedule*[9]

Disease	Causative agent	Vaccine	Doses	Age of administration
Childhood TB	Bacteria	BCG	1	Soon after birth
Hepatitis B	Virus	Hep B birth dose	1	Soon after birth
Poliomyelitis	Virus	OPV	4	OPV0:soon after birth OPV1:6 weeks OPV2:10 weeks OPV3:14 weeks
		IPV	I II	IPV-I:14 weeks IPV-II:9 Months
Diphtheria	Bacteria	Pentavalent vaccine (DTP + Hep B + Hib)	3	Penta1:6 weeks Penta2:10 weeks Penta3:14 weeks
Tetanus	Bacteria			
Pertussis	Bacteria			
Hepatitis B	Virus			
Hib pneumonia and meningitis	Bacteria			
Measles and rubella	Virus	Measles and rubella (MR)	2	MR.1:9 months MR.2:15months
Diarrhea due to rotavirus	Virus	Rotavirus	2	Rota1:6 weeks Rota2:10 weeks
Typhoid	Bacteria	Typhoid conjugated vaccine(TCV)	1	9, Months

In 2016, the province of Punjab had eliminated neonatal & maternal tetanus completely. According to the Pakistan Demographic and Health Survey (2012-2013) and Pakistan Social and Living Standards Measurement Survey (2014-2015) EPI coverage is 65 % to 88%. Pakistan ranks third in the world regarding the percentage of children who have not been vaccinated or are under-vaccinated. In report 2015 by WHO stated that over 4 million Pakistani newborns missed their third DTP3 dosage.[10]

VPD account for most baby and children's deaths in low & middle incomes countries. Pakistan is one of them. In countries like Pakistan, several factors influence the vaccination, which are.[11]

1. **Distance/travel conditions/access:** Distance, travel conditions and access to vaccination facilities play significant role in vaccination rates. A study conducted in Nigeria, Kenya & Liberia in which found that distance/access and travel led to 43% decrease in vaccination, while in Nigeria, Kenya and Liberia it was 30%. [12]
2. **Poor health staff motivation, performance/ competence and attitudes:** All these factors led to decrease vaccination.In Uganda 13% mothers treated rudely.[12]
3. **Lack of resources/logistics:** Vaccine shortages, problems with the cold chain &vaccine unavailability all led to partial immunity. Vaccine shortages are typically caused by a lack of funds, storage space, or poor ordering and delivery procedures.[13]
4. **Lack of parental knowledge concerning which children, when, where:** Numerous studies assume that parents would vaccinate their children if they had a comprehensive understanding of vaccine-preventable diseases& the immunization schedule.[14]

5. **Fear of side effects:** Several myths& misunderstandings like sickness of kids, child older than 12 months and the belief that underweight children should not be vaccinated due to the side effects of vaccine. Vaccination to sick child most common erroneous contraindication in numerous studies (e.g., in Kenya, Nigeria, and Pakistan).[15]
6. **Conflicting priorities:** e.g Drive large distances, Long wait for vaccinations, weddings and funerals led to miss their child's vaccinations. The postpartum period is a time when many families refuse to allow their newborns to be vaccinated.[16]

In research published in 2016, 7.73% children never immunized in Pakistan under the age of 5years. This data was taken from 2001–2002 Household Integrated Economic Survey& it was 87.4% in rural areas.[17] In another study conducted in Islamabad in 2016, it was noted that different variables are responsible for partial vaccination in Pakistani. All provinces have different immunization rates. KPK province (including FATA) had a coverage rate 38%, whereas Baluchistan had below 16%. According to reports, immunization rates are highest in Punjab and urban areas. People who don't get their children vaccinated report having a bad experience (24.7 percent) with healthcare facility staff, mistrust (34.1 percent), reporting absence of healthcare facility staff (14.1 percent), fearing the use of dirty syringes (55.3 percent), long periods of waiting (64.7 percent),[18]

In 2019 research was conducted in Sindh, Pakistan, in which factors were examined that influence the timeliness of childhood vaccination. In this research 2013-2014, MCH Program indicator Surveys were used. The participants were 1143. The research showed that 20.8% children were fully immunized on time according to the schedule& it also showed that the immunizations was not time which was varied from measles vaccines to BCG from 2.3% to 89.3 respectively.[19]

Pakistan has a significant vaccine coverage problem. This disparity can potentially be life-threatening to the general public's health. In Pakistan, for children aged 0–23 months, the current study utilized an internationally standardized instrument to determine the prevalence and variables related to missing immunizations. The results of this study will serve as a starting point for developing and executing a facility-level quality improvement program to address the issue of missing vaccinations that have been found thus far. The result of the study can improve immunization service delivery and the gaps in the existing literature by adding valuable knowledge.

Materials and Methods

Cross-sectional descriptive study was conducted at Tehsil Head Quarter (THQ) Hospital Shakar Garh district Narowal from July, 2021 to March 2022. The sample was 334, with the age of 0-23 months. The children above 23 months and who are suffering from chronic illness were excluded, The sample size was calculated using the prevalence of missed childhood vaccination as 31% taken with 5% as margin of error and 95% confidence interval by this formula

$$n = \frac{(Z^2 1 - \propto/2(1-P)}{d^2}$$

Results

Table 1 explains the frequency, percentage, means, and standard deviation of the demographic variables. Table 2 illustrates the chi-square and One-way ANOVA results to compare the groups based on immunization groups. The result was presented as a number (%) for the multiple comparisons, and the Wilcoxon-rank test was applied to check the hypothesis significance.

Discussion

A vaccination schedule must be adhered in order to

Table 2: *Frequency, Percentage, Mean, Standard Deviation of the study variables (N=334)*

Variable	Categories	F(%)	M(SD)
Age (months)	0-6	32(9.6)	18(1.91)
	7-12	134(40.1)	
	13-18	102(30.5)	
	19-23	66(19.8)	
Gender	Male	167(50)	1.5(0.51)
	Female	167(50)	
Child immunization	Unimmunized	46(14))	1.08(.83)
	Fully immunized	228(86)	
	Partial immunized	60(18)	
The nearness of the health facility	<5km	131(39.8)	1.60(.49)
	>5Km	201(60.2)	
Socio-economic status	Lower	134(40)	2.14(.79)
	Middle	116(34.7)	
	Higher	84(25.1)	
Residential area	Urban	201(60.2)	1.62(.45)
	Rural	133(39.8)	

Note: F=frequency, %= percentage, M=Mean, SD=Standard Deviation

Table 2: *Characteristic of subjects according to immunization status (N=334)*

Variables	Categories	FI (228)	MI (106)	p-value
Childbirth	Hospital	228(100)	56(53)	.00
	Home	0	50(47)	.01
Immunization card	No	0	71(66)	.02
	Yes	228(100)	34(44)	0.03
Received vaccines	At birth	228(100)	51(48)	
	6 weeks	228(100)	48(45)	0.01
	10 weeks	228(100)	45(42)	0.01
	14 weeks	228(100)	39(37)	0.01
	9months	228(100)	37(34)	0.00
	15months	228(100)	32(30)	0.02
	23month	228(100)	28(26)	0.01
BCG marks	Yes	228(100)	53(50)	.01
	No	0	53(50)	.00
Why child miss their vaccine	Did not know about vaccine when due		5(4)	0.05
	Did not know where to get the vaccination		-	
	Nobody informed me		-	
	I have no time		20(18)	0.03
	I don't believe in its effectiveness		1(0.09)	
	Against religious belief		10(9)	0.07
	Child was sick		60(57)	0.01
	Don't trust on government			
	The staff was not cooperative		10(9)	0.02
	The staff did not know how to work			0.03
Did you ever refuse about vaccinate the child	Yes		66(62)	.00
	No	228(100)	40(38)	.02
Refusing reason	Vaccine out of stock		15(37)	.01
	Queue was long		15(37)	.01
	Non-cooperative staff		10(9)	.04
The health worker asked for the card	Yes	190(83)	36(34)	0.00
	No	38(17)	72(68)	
Health worker attitude	Poor	30(13)	40(38)	.01
	Average	150(65)	70(66)	.00
	Good	48(19)	20(19)	.02

Note: FI=Fully Immunization, MI= Missed Immunization, p<0.05

avoid illness. The primary focus of the study was on infants under the age of 23 months. At birth, 270 (83%) of the infants were vaccinated on time, which may be due to the required immunization of newborns before they were released from the hospital. Missed vaccinations accounted 17% of all cases in this investigation.[20] Findings of this study were similar to studies from Minjar-Shenkora district (34.4%),55 Gondar city (24.3%), and Addis Ababa (17.7 percent). But the present figure was greater than those from Ethiopia's Debre Markoss and Ghana's Techiman Municipalities, which reported 8.3% and 5.2%, respectively.[21] The gap may be due to children in the current research were from rural areas, whereas in the previous studies, children were from urban areas. The lack of health services in rural areas has been reported to discourage vaccine uptake. The challenges faced in getting to healthcare facilities are important hurdles to completing child immunization. The same thing has been found in Nigeria. It was more likely that mothers in rural regions were impacted by social beliefs, considerable distance & health care facilities, lack of vaccine information lead to inadequate vaccination &non-compliance,[22] The study conducted in Jaddah Saudia Arabia in 2019 it was found that 59.3% lack of vaccination was due to sick child & 21.3% were due to long travelling. In another study conducted in 2017 at King Abdulaziz University Hospital in Jeddah found that the most prevalent cause of delayed vaccination was difficulty getting an appointment (30 %).[23]

In study conducted in Mansoura district Egypt noted that no caregivers refused to immunize the children& 10% delayed immunization was due to deficient information regarding the child may get a disease due to non vaccination 72%, vaccine are necessary to protect the child 91%& vaccine are safe 85%. Uptake of vaccination services is dependent not only on the quality of these services but also on other factors including attitude of the parents & healthcare workers.[24]

Conclusion

The study indicated that incomplete vaccination rates remain above the acceptable threshold. According to mothers, the immunization schedule has been postponed/omitted due to maternal social involvement and illness in the mother and/or child. Because of this, extensive health education and action on the identified fundamental issues are necessary to promote full immunization. Expanding ANC and educating parents on vaccination schedules and the dangers of incomplete immunizations are two of our top recommendations.

Conflict of Interest *None*

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Authors Contribution

MT: Conceptualization of Project
A: Data Collection
A: Literature Search
MT: Statistical Analysis
MT: Drafting, Revision
R: Writing of Manuscript

Incidence of Rotavirus and Efficacy of Enterococcus Faecium SF68 in Infantile Diarrhea

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Abstract

Objective: To know the incidence of diarrhea due to Rotavirus in infants and to evaluate the efficacy of probiotic E. faecium SF68 in acute infantile diarrhea.

Material and Methods: It was a randomized controlled clinical trial Reg. No 01/159/16 conducted in Children Hospital Lahore. 70 infants suffering from acute diarrhea were divided randomly into two groups. Infants of control group received routine treatment for diarrhea while in infants of experimental group also received probiotic Enterococcus faecium SF68 in addition to routine treatment for diarrhea. Stool samples of all infants were sent for Rotavirus testing.

Results: Stool samples of 19 out of 70 infants were positive for Rotavirus. Probiotic decreased frequency of diarrhea in infants of experimental group.

Conclusion: In this trial, 27% infants were suffering from acute Rotavirus gastroenteritis. Enterococcus faecium SF68 decreased the severity of diarrhea in infants.

Keywords: Infants, Rotavirus diarrhea, Probiotics, Enterococcus faecium Sf68.

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Introduction

Diarrhea is the significant health problem worldwide leading to hospitalization and death in infants and young children in third world countries. According to WHO, diarrhea is the fecal discharge having frequent and watery stool which occurs due to infection of small bowel resulting in loss of electrolytes and fluids.[1] Worldwide, 525,000 children below five years of age die because of diarrheal disease annually. This shows 8% of all deaths, so diarrhea is the important reason of mortality in young children.[2] Most cases of diarrhoeal diseases do not require laboratory investigation as they are diagnosed clinically. Presentation having acute watery and non-bloody diarrhoea shows a viral aetiology, whereas diarrhoea having blood in stool and high-grade fever shows bacterial infection.[3] Rotavirus and E. coli are one of the main causative agents of diarrhea in young children in developing countries. Globally, Rotavirus being main cause of diarrhea in infants leads to significant mortality in children below five years. In addition to E. coli and Rotavirus, other enteropathogens which cause diarrhea in young children include Campylobacter, Vibrio chloera, Salmonella and Shigella species[4]. Rotavirus was recorded to be responsible for about 215,000 deaths in children under five years of age in 2013. Due to high mortality in children, vaccination for Rotavirus is important public health strategy.[5] The focus for treatment of diarrhea is prevention as well as hydration therapy and to promote good nutritional status.

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.Children having mild dehydration should be given oral rehydration salts (ORS) and those who are not able to take fluids or children suffering from severe diarrhea should be given intravenous (IV) fluids. Children suffering from acute diarrhea should also be given zinc supplements.[6] Probiotics are microorganisms which are beneficial to the host by colonizing in the human body. They promote reproduction and growth of beneficial intestinal flora and provide immunity against pathogenic bacteria.[7] Enterococcus faecium SF68 is a probiotic initially launched in Austria and Switzerland. It is indicated to be used in acute gastroenteritis in adults as well as in children and for treatment of diarrhea associated with antibiotics. However, previous trials already done on SF68 were not of good standard having poor allocation concealment and even no blinding in few trials. Analysis on the role of SF68 in diarrhea especially in infants cannot be made.[8]

Materials and Methods

The study was done in Children Hospital Lahore from July 2016 to October 2016. The study was conducted according to the principles of World Medical Association. The trial was reviewed and approved by Ethical Committee of Children Hospital Lahore. After taking consent from the parents, infants were enrolled in the study. It was a randomized controlled Reg. No 01/159/16, single blinded, clinical trial and study followed Consort guidelines. The age of infants in the study was selected from six months to twelve months. The infants having acute diarrhea were enrolled in the trial. According to WHO, "Acute diarrhea is defined as the passage of three or more loose or liquid stools per day and not lasting more than 14 days." The infants suffering from typhoid fever, chronic disease or having bloody diarrhea were excluded from the study. In this study, non-probability purposive sampling was done. The sample size was calculated according to previous study[9]. In previous study, sample size was 32. We enrolled 35 infants in each group. The infants were divided in to two groups according to randomization technique. The random number list was generated by the computer. The randomization was done by a person who was not associated with the study. The person who did randomization also prepared the sealed envelopes which contained the management plan of the infants. The parents of infants received sealed envelopes in accordance with randomization. The infants were put in control and experimental groups after opening of envelopes, so management plan was concealed from researcher until beginning of intervention. After enrollment, stool sample was sent for microbiological testing of Rotavirus. Total 70 infants were enrolled in the study and each group had 35 infants. Infants of control group or group A were given routine or standard management for diarrhea. Standard treatment of diarrhea included ORS, Zn supplements and IV fluids in case of severe dehydration. Infants of experimental group or group B received probiotic E. faecium SF68 in addition to standard treatment for diarrhea. E. faecium SF68 was in capsule form. The contents of capsule were mixed with water for feeding. The probiotic was given twice daily for five days. Feeding was continued in infants of both groups with soft diet. Frequency of stool was recorded in both groups for five days. As Rotavirus is the important causative agent of diarrhea in infants, so stool samples were sent for testing of Rotavirus. Stool samples were stored at 2 to 24 8 C° in lab grade refrigerator. ProSpecT ROTA VIRUS KIT (OXOID) was used for detection of Rotavirus antigen. Sample was processed as follows:

1. Dilution of sample was done with 1 ml of diluent adding to 2-3 drops of liquid stool or pea size stool in case of semiliquid stool in a glass tube.
2. It was mixed well in gyromixer. Sample was kept for 10 min in test tube stand.
3. Sample was the centrifuged at 400 rpm for 5 min & Supernatant was taken.
4. Then we performed the Eliza test.

The data was processed by SPSS 20. Stool frequency was presented in mean and SD. Qualitative variables were shown as proportions and frequencies. For analyzing the significance between two groups ANOVA was used. For pairwise comparison among two groups, post hoc Tukey's test was applied.

Results

This trial was carried out in Children Hospital Lahore. All the infants enrolled in the study had moderate to severe dehydration and they required IV fluids. Clinical findings related to dehydration and some other important findings are shown in **Table-1**. Samples of stool were taken from all infants and sent for Rotavirus detection. Out of 70 samples, 19 were found to be positive for Rotavirus. The number of samples positive for Rotavirus in each group was shown in **Table-2**. Diarrheal frequency was noted by observing number of stools infants were passing in a day. It was observed for

Table 1: *Clinical findings in control group and experimental group*

Signs	Group A (control)	Group B (SF68)
Cold clammy skin n (%)	17 (49%)	12 (34%)
Dry mucous membrane n (%)	33 (94%)	34 (97%)
No tears n (%)	12 (34%)	7 (20%)
Pallor n (%)	21 (60%)	11 (31%)
Throat congestion n (%)	1 (2.9%)	0 (0%)
Oral thrush n (%)	1 (2.9%)	0 (0%)
Enlarged tonsilsn (%)	1 (2.9%)	0 (0%)

Table 2: *Number of stool samples positive for Rotavirus in group A and group B*

Groups	Stool for *Rotavirus*	
	Positive	Negative
Group A (control) n (%)	10 (29%)	25 (71%)
Group B (SF68) n (%)	9 (26%)	26 (74%)

period of five days. **Table-3** shows the mean number of stools ± SD of control group and experimental group. ANOVA showed diffe-rence between group means was significant from day 1 to day 5.

Discussion

The study was carried out in Children Hospital Lahore. The age group of infants in the study was from 6 months to 12 months. In infants, Rotavirus is an important causative agent of diarrhea having significant global impact on childhood hospitalization and death[10]. In Africa and Asia Rotavirus is responsible for 49% of all deaths due to diarrhea. The incidence of diarrhea due to Rotavirus is similar in poor and developed countries but the morbidities are higher in low-income versus rich population. This may occur due to lack of health facilities as well as presence of other conditions like malnutrition[11]. In this study, 70 infants were enrolled and randomly divided into two groups. Stool samples of all infants were sent for microbiological testing of Rotavirus. Out of 70 samples of stool, 19 were found to be positive for Rotavirus. In this trial 27% infants were suffering from Rotavirus. Stool samples of 10 infants of group A (29%) and 9 infants of group B (26%) were found to be Rotavirus positive. All the infants had moderate to severe dehydration and IV fluids were given to them. Rotavirus surveillance which was conducted during 2015-2016, analyzed 3446 children less than five years hospitalized due to diarrheal illness and found 802 (23.2%) positive on ELISA[12]. Previous study suggested that prevalence of Rotavirus decreased with increase in age. This is due to the fact that infants have higher exposure to contaminated materials as they put everything into their mouths.[5] Standard management of diarrhea was given to the infants of group A and infants of group B were also provided with probiotics in addition to standard management of diarrhea. Stool frequency was recorded in both groups for five days. Probiotics are supplements or food that contain beneficial microbes like yeast and bacteria which colonize the small intestine and provide health benefits. Whether the probiotic bacteria can be used in the management of intestinal disorders, the various researches in this regard are still going on.[13] Their use is encouraged to support healthy gastrointestinal system and to boost the immune system. Currently, the use of probiotics via different food products is in great demand. Probiotics are known as functional food. Functional food will be defined as food which resembles traditional food but they have known physiological benefits[14]. Previous studies explored therapeutic effect of probiotics on acute diarrhea as they helped in decreasing stool frequency and duration of diarrhea. However, good quality researches are still required in clinical settings to evaluate role of probitics[7]. Enterococcus faecium SF68 is a member of lactic acid bacteria (LAB), which is used as a probiotic in food supplements and pharmaceutical preparations in animals and humans[15]. Previous trials regarding use of E. faecium SF68 in humans are not sufficient. One study recently published showed beneficial effect of E. faecium SF68 in reducing the duration of diarrhea and length of hospital stay in infants[16]. In one trial E. faecium SF68 decreased the duration of diarrhea in non-Rotavirus cases in infants, however E. faecium SF68 probiotic did not significantly decrease duration of diarr-

Table 3: *Number of stools after treatment in control group and experimental group*

Groups	Day 1	Day 2	Day 3	Day 4	Day 5	p-value
Group A(control) n=35	10.89±2.94	9.17±2.93	7.46±3.16	6.26±3.00	4.63±2.50	
Group B(SF68) n=35	7.97±3.11	5.03±3.08	3.66±2.66	2.86±2.53	2.26±2.34	<0.001

hea in Rotavirus positive cases in infants[17].

In this clinical trial, infants of group B had reduced stool output than infants of group A having p-value less than 0.001 from day 1 to day 5. Stool frequency was reduced significantly in infants of group B. Infants in group B received E. faecium SF68 twice daily for five days. E. faecium SF68 capsule contains 75 million live micro-organisms and its contents were mixed with water for feeding the infants. In animal studies E. faecium SF68 promoted expression of pro-inflammatory cytokines like TNF-α, IL-17A, IL-22 and thus improved immune responses against pathogens. In one study, E. faecium SF68 increased production of NO by phagocytic cells of pigs thus it modulates innate immunity against pathogens[18]. It can be considered as a good probiotic due to its immunomodulatory effects.

Conclusion

In this randomized controlled trial, 27% of infants were suffering from Rotavirus gastroenteritis. Probiotic E. faecium SF68 reduced the severity of acute diarrhea in infants.

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Authors Contribution

FAK: Conceptualization of Project
ZI: Data Collection
FYB: Literature Search
NY: Statistical Analysis
FP: Drafting, Revision
STZ: Writing of Manuscript

Influence of Serum Uric Acid on Mean Arterial Pressure in Females in Local Population

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Abstract

Objective: To find out the relationship and effect of hyperuricemia on mean arterial pressure in local population.

Material and Methods: The Study design was Cross sectional and the setting was in Lady Aitchison hospital Lahore. Duration of this this was March 2017- August 2017. Written informed consent was taken prior to data collection. Detailed history was asked. Mean arterial pressure was calculated by measuring systolic and diastolic blood pressures and putting them in the formula: MAP = diastolic pressure + 1/3 (pulse pressure). Uric acid was measured by uricase method after taking 1 ml venous blood under aseptic measures.

Result: A total of 60 patients with increased serum uric acid levels, with Mean Arterial Pressure ranging between 83.33 and 126.67 were considered. We use simple linear regression model and see the effect of Uric Acid on Mean Arterial Pressure. The model shows that for 1 mg/dl increase in Uric Acid, Mean Arterial Pressure increases by 14.89 at 5% level of significance. 72.74% of the variation in the Mean Arterial Pressure can be defined using the Uric Acid. Conclusion: Positive relationship has been seen between hyperuricemia and mean arterial pressure.

Keywords: Hyperuricemia, systolic blood pressure, diastolic blood pressure (DP), pulse pressure (PP) mean arterial pressure (MAP), hypertension.

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Introduction

Serum uric acid is the end product of purine metabolism.[1] Uric acid is produced both by exogenous and endogenous sources. Exogenous sources depend upon dietary intake of animal proteins, high fructose intake, alcohol consumption etc. while endogenous source is from liver, muscles, intestines, vascular endothelium and kidneys.[2] Its concentration in the serum is an important parameter regarding human well being. Normal level of uric acid in males is between 3.5 and 7.0 mg/dl and in females, between 2.5 and 5.7 mg/dl.[3] This level may be increased either due to increased production by liver or decreased excretion by intestines or kidneys. Clinical condition in which the level exceeds the normal range is called hyperuricemia, the prevalence of which is being increased gradually over the last 40 years. It has reached up to 21% in general population of United States and even double in South East Asia (52%).[4]

Hyperuricemia imparts great burden on health as documented by various researches which have confirmed direct or indirect correlation of hyperuricemia with a number of other diseases like gout, diabetes mellitus, hypertension, kidney diseases, and heart failure.[5] Besides, it is also a source of vascular diseases as it produces reactive oxygen species.

Hypertension, commonly known as high blood pressure, is a rapidly growing public health issue. According to 2017 guideline, a patient is considered to be hypertensive

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if his systolic BP ≥ 130 mmHg or a diastolic BP ≥ 806. It is also called silent killer as it is considered as one of the leading risk factors for various cardiovascular diseases, kidney disorders, brain and other diseases.[7] Globally prevalence of hypertension came out to be 31 - 56 %.[8] The ranking of hypertension has changed from fourth in 1990 to second and first in men and women respectively by 2017. It shares the mortality rate of millions of patients in the world. Worldwide around 7.5 million (12.8%) of the total deaths per year occur due to hypertension.[9] The number will reach 1.56 billion adults by 2025.

Mean arterial pressure is the average calculated blood pressure in one cardiac cycle, and is calculated by the formula: MAP = DP + 1/3(PP).[10] Normal value of MAP is considered between 70 and 100 mmHg. It has been observed through researches that, to predict adverse events of hypertension, high systolic blood pressure is more significant in patients of age greater than 50 years and high diastolic blood pressure in hypertensive individuals of age lesser than 50 years. So, probably due to different importance allocated to systolic and diastolic blood pressures, mean arterial pressure is considered to be used both for the purpose of diagnosis and statistical analysis of blood pressure as compared to systolic and diastolic blood pressure separately and hence as a better alternative predictive tool for assessment of adverse outcomes of hypertension.[11]

In addition to the above-mentioned causes of hyperuricemia, the subjects who have elevated levels of triglycerides, low-density lipoprotein, total cholesterol, apolipoprotein-B levels, ratio of triglycerides to high-density lipoprotein cholesterol in their serum are at more risk for its development. Moreover, a 4-fold or greater prevalence of hyperuricemia has been noted in patients with uncontrolled blood pressure[5] This is due to under excretion of uric acid through kidneys rather than its over-production. So, a bidirectional relationship is considered between hyperuricemia and hypertension.

Most of the studies done so far paid little attention to see the relationship of uric acid level and mean arterial pressure. So, this cross-sectional study has been conducted to assess the relationship of uric acid level on mean arterial pressure in local population of Lahore.

Materials And Methods

This cross sectional study was carried out after approval from hospital and ethical committee. 60 females (age ranging between 55 to 70 years) who fulfilled the criteria were taken from outpatient department of Lady Aitchison hospital Lahore. Written informed consent was taken prior to data collection. By using 5% level of significance, 95% confidence level, 90% power of test for expected percentage of uric acid level, participants were enrolled. Detailed history was taken from the patients. Participants' age and blood pressure were recorded. Serum uric acid between 2.5 and 5.7 mg/dl was considered as normal. Whereas, systolic blood pressure <130 mmHg and diastolic blood pressure <80 mmHg was taken as normal and mean arterial pressure was calculated by the above mentioned formula in each individual.

Blood sampling was done under aseptic measures. Centrifugation was done and serum samples were measured for serum uric acid by uricase method. Cups were numbered and stored in freezer at -70°C. Data was analyzed by R version 4.2.1 (2022-06-23 ucrt) – "Funny-Looking Kid". Pearson correlation coefficient was used to identify the strength of linear relationship between uric acid level and mean arterial pressure. p-value ≤ 0.05 was taken as significant.

Result

A total of 60 patients with increased serum uric acid levels along with mean arterial Pressure, ranging between 83.33 and 126.67 were considered. Furthermore, using a simple linear regression model, the effect of uric acid on mean arterial pressure was computed.

Firstly, to check the model assumptions, it was found that the distribution of the mean arterial pressure of patients in Normal using QQ Plot shown in Fig 1. Secondly, using pearson correlation coefficient it was found that there is a strong positive linear relationship between the two at 5% level of significance as shown in the Table 1. Fig 2 signifies that the computed model is homogeneous i.e., Variance of residuals are the same across the different values of the predictor. The model shows that for every one unit increase in uric acid (mg/dl), mean arterial pressure increases by 14.89 on average

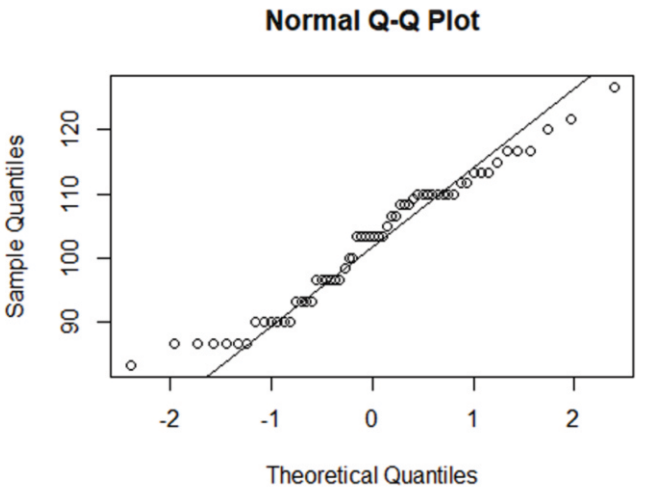
Table 1: *Correlation Coefficient*

Estimate	Statistic	p.value	parameter	conf. low	conf. high	method	alternative
0.8555772	12.58681	< 0.0001	58	0.7686415	0.9114807	Pearson's product-moment correlation	two. sided

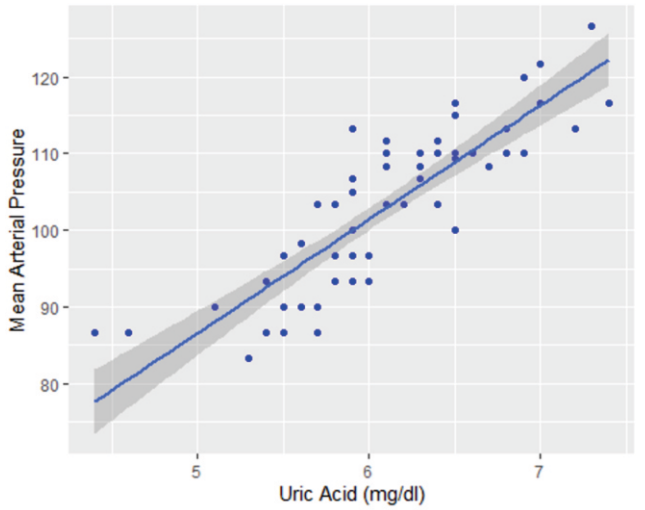
while keeping the other effects constant at 5% level of significance, shown in Table 2. Adjusted R-squared was found to be 0.7274 which means 72.74% of the variation in the mean arterial pressure can be defined using the uric acid.

Table 2: *Regression Coefficient*

	Estimate	Std. Error	t value	Pr (>|t|)
(Intercept)	12.12819	7.211900	1.681691	0.0980062
Uric Acid (mg/dl)	14.88595	1.182663	12.586811	0.0000000



Fitted Model



Discussion

Relationship between hypertension and hyperuricemia, being bidirectional, has long remained interested for the researchers. According to a cross-sectional study, an increase of 1mg/dL serum uric acid shares 20% rise in prevalence of hypertension in a general population of east Asia.[12] The possible pathophysiology behind this is, increased levels of uric acid cause renal artery disease, upregulation of renin-angiotensin-aldosterone system, oxidative stress, systemic inflammation and endothelial dysfunction.[2,13] On the other hand, hypertension may cause hyperuricemia and this may be attributed to renal under functioning due to high systolic and diastolic blood pressures.[4]

This cross-sectional study, which is consisted of 60 females with hyperuricemia, shows a strong positive linear relationship between uric acid and mean arterial pressure. Our results further demonstrate that for every one unit increase in serum uric acid (mg/dl), mean arterial pressure increases by 14.89 on average. In addition to it 72.74% of the variation in the mean arterial pressure is attributed to uric acid.

A study conducted in Japan documented odds ratio about 1.2 for every 1 mg/dl increase in serum uric acid. Males with hyperuricemia reported a greater risk of incident hypertension, as I mg/dl increase in serum uric acid caused 9% increase in the incident hypertension risk. Another study observed progressive increase in both systolic and diastolic blood pressures across the serum uric acid tertile, whereas confounders like age, gender, glomerular filtration rate and basal metabolic rate were kept adjusted.[14]

Results of most of the previous studies are consistent with our study. A cross sectional study from National Health and Nutrition Examination Survey (NHANES) observed a linear relationship between uric acid and blood pressure (both systolic and diastolic) in the group taking antihypertensives. Ning Ding et al. noticed a gradual decrease in systolic and diastolic blood pressures by increasing uric acid whereas the relationship between them was seem to be U shaped in the non-treatment group.[15]

In 2020, Jianga Y by cross lagged approach, observed a temporal relationship between hyperuricemia and hypertension and documented that hyperuricemia precedes hypertension.[16] A similar kind of statement has also been documented in a study that was done on 11488 subjects in 2022 by Xue Tian et al. According to them, increased initial serum uric acid as well as gradual rise over a period of time, both can predict the progression of hypertension from prehypertension.[17] Xue Tian and his companions conducted a study including 60,285 participants and concluded that hyperuricemia preceded hypertension, hence a temporal relationship was seen between hyperuricemia and hypertension.[18] It has also been revealed through different publications that progression from normal to low and moderate to high levels of serum uric

acid are associated with worst outcomes of new onset hypertension whereas opposite observations were seen in case of low or normal uric acid levels.[19]

Similar kind of findings have also been observed in childhood demonstrating that increased serum uric acid level in childhood is closely related to persistent childhood hypertension showing a key role played by childhood hyperuricemia in the development of hypertension.[20] Supporting this study, a survey by Korean National Health and Nutrition Examination showed 9.4% prevalence of hyperuricemia associated with high systolic blood pressure in school going subjects aged between 10-18 years.[21]

As far as the effect of urate lowering drugs in the treatment of high blood pressure is concerned, controversial results have been observed. Some of these drugs reduce the systolic blood pressure[22] whereas allopurinol fails to show such results.[23]

Almost all of the above mentioned studies favor our study that increase in serum uric acid results in increase in mean arterial pressure which is considered as a better diagnostic tool of hypertension. Yet, most of the literature commented the change in mean arterial pressure by 1 mg/dl increase in serum uric acid levels in their own areas and lacked information in our population, so our study filled that deficit. So, by keeping in control the uric acid level in serum, systolic and diastolic blood pressures can also be kept within normal level. However, our research focused only female population and thus further researches on both genders and wide range of ages are needed to estimate heterogenous effect.

Conclusion

Present study demonstrates a positive linear relation between serum uric acid and mean arterial pressure. Furthermore, simple linear regression model was used to see the influence of uric acid on mean arterial pressure and found that with 1 mg/dl increase in serum uric acid causes an increase of 14.89 in mean arterial pressure. Moreover, 72.74% of the variation in the mean arterial pressure is being defined by our proposed model.

Conflict of Interest: None

Funding source est: None

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Authors Contribution

RN, FN: Conceptualization of Project
RN: Data Collection
FN: Literature Search
AN: Statistical Analysis
MBM: Drafting, Revision
QM: Writing of Manuscript

Original Article

Antioxidant Potential of Cydonia oblonga in Isoproterenol-Induced Myocardial Infarction in rats

Azka Khan, Asima Ayyub, Maria Qamar, Shahnaz Akhtar, Umair Samee, Fiaz Ahmed

Abstract

Objective: An experimental study was carried out to find the antioxidant potential of methanolic leaf extract of Cydonia oblonga in Isoproterenol-Induced Myocardial Infarction in groups of rats.

Material and Methods: A total of 30 Wistar rats included in the study. Rats were divided into 5 groups comprising 6 animals per group. Group I was given normal saline while Group II was given normal saline followed by isoproterenol. Group III, Group IV and Group V received methanolic extract of C. oblonga 50 mg/kg, 100 mg/kg and Carvedilol 2 mg/kg respectively by oral route followed by isoproterenol. Blood samples were estimated for cardiac biomarkers (Troponin T, CK-MB and LDH). Anti-oxidative stress biomarkers (catalase, superoxide dismutase, glutathione peroxidase, malanodialdehyde) were also estimated using cardiac tissue.

Results: C. oblonga at doses of 50 mg/kg & 100 mg/kg significantly reduced the levels of cardiac markers Troponin T, CK-MB and Lactate dehydrogenase in treatment groups of rats. Significantly high values of anti-oxidants superoxide dismutase, catalase, and glutathione peroxidase were found only at high dose of extract of CO. Significantly decreased level of melanodialdehyde was found in treatment groups.

Conclusion: Study revealed that due to antioxidant characteristics, C. oblonga showed cardioprotective activity against myocardial infarction.

Keywords: C. oblonga, antioxidant, cardio-protective effect

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Introduction

Cardiovascular diseases (CVDs) are the principal cause of early death and disability in human beings with a rise in incidence globally. About 22.20 million cardiovascular disease related deaths are predictable to occur in up to year of 2030.[1,2] The underlying cause of most cardiovascular diseases is atherosclerosis, leading to conditions like heart attacks, strokes due to thromboembolism, vascular damage and arrhythmias.[3] Oxidative stress (OS) occurs when there's an imbalance between oxidative and antioxidative processes, leading to increased oxidation and results in inflammation[2,4] This oxidative stress contributes to cardiac dysfunction, encompassing myocardial infarction, ischemia /reperfusion, heart failure and athero-sclerosis.[5,6] Despite effective cardiac drugs, significant gaps still persist in treating cardiovascular diseases. Medicinal plants, with their safety profiles, offer potential benefits for addressing these issues7'8. The leaf extract of Cydonia oblonga, or quince, contains phenolic content, vitamin E, and fatty acids, while gallic acid, a polyphenolic metabolite, serves as an antioxidant with medicinal applications. CO exhibit

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physiological benefits, potentially supplementing cardiovascular, pulmonary, and immunological defenses.[9]

Due to the reported anti-oxidative and anti-atherosclerotic properties of Cydonia oblonga, the experimental study was designed to find the antioxidant potential of methanolic leaf extract of Cydonia oblonga in Isoproterenol-Induced oxidative stress in groups of rats.

Material and Methods

The study was conducted after IRB approval from UHS Ethical Committee. Simple random sampling technique was used and total 30 male Wistar rats (weighed 225-250 g) were taken and kept in a suitable environment in Experimental Research Laboratory UHS, Lahore at temperature of 22-24°C with 45-65% humidity and 12 h/12 h dark/light cycle. All rats were given rat chow and water ad libitum. The study was performed at the Department of Pharmacology, University of Health Sciences, Lahore.

Isoproterenol (Sigma Aldrich) in the dose 85.0 mg /kg body weight /day was added in saline and was given intraperitoneally10 and Methanolic extract of Cydoniaoblonga (CO): 50 mg/kg body weight and 100 mg/kg body weight were dissolved in saline solution and was orally given11.Carvedilol (Sigma Aldrich) about was dissolved in saline solution and was orally given at dose of 2mg/kg body weight/ day 12.The leaves of CO were taken from Sawat-Pakistan and confirmed by botanist at Punjab university. The extract of CO was made with methanol (2 L). The concentrated mixture was dried and kept at 4°C. Induction of Myocardial Infarction in groups of rats by giving injection of isoproterenol (ISO) with a dose of 85mg/kg/day on 2 days (22nd and 23rd day of experiment)13. 30 Wistar rats were divided into five groups (6 rats in each group). Group I (Negative Control) received orally normal saline 10ml /kg body weight by oral for 23 days. Group II (Diseased Control) received orally normal saline 10 ml / kg body weight for 21 days followed by intraperitoneal injection of isoproterenol 85mg/kg body weight on day 22nd and 23rd. Group III (low dose CO) and Group IV (high Dose CO) received orally leaf extract of C. oblonga 50 and100 mg /per kg body weight for 21 days respectively followed by injection of ISO (85 mg/kg) on 22nd and 23rd days. Group V (Carvedilol) received orally Carvedilol 2mg /kg body weight for 21 days followed by injection of Isoproterenol (85mg/kg body weight) on 22nd and 23rd day. All animals were sacrificed at 24th hour, after the last treatment. The blood was taken via cardiac puncture, centrifuged; serum was separated and stored at -20oC for estimation of cardiac enzymes. The cardiac tissue was rinsed in ice-cold saline and homogenized in phosphate buffer saline (pH 7.4) for estimation of oxidative stress markers. This estimation utilized Elabscience Assay kit. Data

Table 1: *Levels of cardiac enzymes in all groups (n=06) of rats Values are expressed as Mean ± SD*

Parameters	Group I (Negative control)	Group II (Diseased control)	Group III (CO low dose)	Group IV (CO high dose)	Group V (Carvedilol) allopathic
Troponin T (ng/L)	330±65.49	516±45.06[a]	280±48.34[b]	169±68.66[b]	351±65[b]
CK-MB (U/L)	81±61.74	168.3±30.79[a]	112±5.4[b]	69±11.17[b]	93±5.3[b]
LDH (U/L)	85±2.26	180±33.57[a]	165±14.06[b]	66.25±9.2[b]	87.17±14.6[b]

[a] *shows a significant group difference with group I*
[b] *shows a significant group difference with group II*

Table 2: *Variation in levels of antioxidant and oxidants enzyme in study group animals (n=6) and controls Values are expressed as Mean ± SD*

Parameters	Group I (Negative control)	Group II (Diseased control)	Group III (CO low dose)	Group IV (CO high dose)	Group V (Carvedilol)
SOD (U/min/mgprot)	98.17±3.323	58.06±7.242[a]	88.85±6.719[b]	105.9±5.175[b]	97.16±3.056[b]
Catalase (U/mg prot)	66.85±1.89	33.77±12.41[a]	44.72±3.406[b]	54.53±2.905[b]	55.67±3.44[b]
GSH (nmol/g)	31.77±1.206	16.12±3.346[a]	36.32±1.014[b]	47.75±4.151[b]	40.16±4.702[b]
MDA (nmol/mg)	0.6471±0.563	7.88±0.520[a]	4.738±0.576[b]	1.048±0.241[b]	1.951±0.327[b]

[a] *shows a significant group difference with group I*
[b] *shows a significant group difference with group II*

was analyzed by SPSS 21. The variables were expressed as Mean ± Standard deviation. The statistical significance of data was estimated by One-way ANOVA. Mean differences between different groups was carried out by using Post hoc Tukey's test.

$P \leq 0.05$ was taken as significant.

Results

Levels of cardiac marker enzymes (troponin T, CK-MB and LDH) were significantly decreased ($P<0.001$) after taking carvedilol (group 5) as compared to controls (group 2). However, the levels of cardiac enzymes were significantly reduced ($P<0.001$) after taking low and high doses of CO as compared to group 2 and group 5. (diseased controls and carvedilol). This showed that both high and low doses of CO are more effective to reduce the level of cardiac enzymes as compared to allopathic drug carvedilol **(Table1).** Levels of antioxidants (SOD, Catalase and GSH) were found to be significantly increased ($P<0.05$) only at the dose of 100mg/kg body weight. On the other hand, level of oxidant or marker of lipid peroxidation MDA was significantly reduced ($P<0.05$) at the dose of 100 mg/kg only **(Table 2).**

Discussion

Our study concluded that Quince or Cydonia oblonga may reduce the levels of biomarkers (Troponin T, CK-MB & LDH) and have good therapeutic effect in cardiovascular problems. A review study included 12 researches based on cardiovascular effectiveness of quince. Study reported that all parts of quince (leaf, seed, and fruit) may be used to reduce risk factors related to cardiovascular disease. The factors are blood pressure, metabolism of glucose, obesity and level of lipids.[14] An experimental study was carried out in groups of rats. Study was conducted to explore the phytochemical and cardio protective potential of two different extractions. This study found the significant antioxidant and cardioprotective ability of CO observed with a dose of 50 mg phenolic extract of quince. It is evident that by using this dose, CO reduced the level of serum cardiac enzymes CK-MB, LDH &trop T and increased tissue parameters (SOD, CAT &GSH) and decrease MDA. It is concluded that cardio protective activity of the extract of quince may be due to its phyto-constituents that neutralize the cardiotoxicity and help to reinstate the cardiac injury in experimental animal.[15]

We also observed that CO with its high dose significantly increased level of antioxidant enzymes (SOD, Catalase, GSH) and decreased level of oxidant (MDA) in group of rats, suffering with cardiovascular issues. The antioxidant property of extract of CO was examined on cells of Ha CaT using the DCF-DA (fluorescent probe). Study found that production of ROS was markedly decreased by extract of CO. Study also found nontoxic effect of extract of CO on all tested doses and have antioxidant activity via controlling the ROS production in cells.[16] Another study also found that extract of CO showed antioxidant role mainly due to the polyphenols present in extract of CO.[17]

It is thought that polyphenol a constituent of extract of CO is a powerful antioxidant and help to slow down the oxidation of free radicals. The phytochemicals can forage a ROS via different mechanisms, counting the inhibition of different enzymes taking part in production of ROS and protection of antioxidant defenses.[18]

These results indicate higher antioxidant activity for certain parts of quince fruit, namely pulp and peel represent easy sources of natural antioxidants with powerful application in pharmaceutical/ nutritional fields, as therapeutic or preventive agents in cardiovascular and other diseases in which free radicals are frequently produced.

Conclusion

Cardioprotective effect of Cydoniaoblonga was observed (in both low and high doses) by reducing the levels of biomarker enzymes. Although it santioxidant potential was shown only at higher doses. However, further studies are needed to explore the key effects of CO consumption as cardiovascular preventive.

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AK: Conceptualization of Project
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Portal Vein Velocity in Liver Cirrhosis across Child-Pugh Category: A Comparative Analysis

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Abstract

Objective: Determine Child-Pugh category frequency in cirrhotic patients and compare mean portal vein flow velocity across categories A, B, and C.

Material and Methods: This cross-sectional survey study was conducted in the Department of Diagnostic Radiology, Services Hospital, Lahore, and the duration of this study was from June 16, 2021, to December 16, 2021. Seventy cirrhotic patients were enrolled. Child-Pugh category, history, exams, and investigations were performed. Portal vein velocity was measured by Ultrasound Doppler. Data were analyzed using SPSS version 25, applying one-way ANOVA with p-value ≤0.05 considered significant.

Results: Seventy cirrhotic patients (49 males, 21 females) with mean age 40.66±16.61 years and mean BMI 27.15±8.14 kg/m^2 were included. Class-C comprised 42.9%, while Class-A and B were 32.9% and 24.3%, respectively. Significant differences in mean portal vein flow velocity were observed across Child-Pugh categories ($p<0.05$).

Conclusion: Portal vein peak velocity decreases with worsening Child-Pugh category, with reversed flow in Class C cirrhosis.

Keywords: Liver Cirrhosis, Child-Pugh Category, Portal Vein Blood Flow.

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Introduction

Chronic diseases contribute to about 60% of global deaths, with chronic liver disease (CLD) alone causing nearly 2 million deaths annually. CLD marks a stage where the liver parenchyma's regenerative ability is lost due to persistent injurious stimuli, resulting in liver failure. This condition significantly diminishes the quality of life, leading to increased morbidity and premature death. In the USA, CLD-related mortality climbed from the tenth leading cause of death in 2001 to the ninth among males in 2016.[1]

In developing nations like Pakistan, CLD is even more prevalent, ranking as the fifth most common cause of death and the eleventh leading cause of disability.[1-2] The Child–Pugh score, initially introduced by Child and Turcotte, aimed to predict operative risk in patients undergoing portosystemic shunt surgery for variceal bleeding. The original version included parameters like ascites, hepatic encephalopathy (HE), nutritional status, total bilirubin, and albumin.[3]

However, it falls short in measuring the effects of increased intrahepatic resistance associated with cirrhosis. This resistance augments pressure in the portal vein, leading to the opening of various collateral pathways. These hemodynamic events contribute to a progressive decline in portal venous velocity with escalating portal hypertension, resulting in increased frequency and severity of ascites, varices, and portal vein thrombosis.[4] The

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mortality and morbidity in CLD patients are exacerbated, and the Child-Pugh score inadequately captures these effects.[5]

Discrepancies arise between local and international data, with local studies lacking p-values, making it challenging to establish statistical significance. Additionally, the Child-Pugh score in Class-C patients differs between local and international studies. Given the complications associated with liver biopsy, the gold standard for assessing liver fibrosis6, and the failure of the Child-Pugh score to predict outcomes related to portal vein flow velocity, there is a compelling need for a local study. Such a study could determine if mean portal vein flow among different Child-Pugh categories is significantly lower, providing insights into alternative treatment options to reduce complications and mortality in these patients. Defined as hepatitis C-positive patients with a shrunken liver and nodular surface on ultrasound.

Patients with cirrhosis classified into A, B, and C based on Child-Pugh scoring.[7] Measured using B-mode ultrasound, mean portal vein flow velocity is the distance blood covers in cm over one second, presented as the mean.

Materials and Methods

The study, conducted at the Diagnostic Radiology department of Services Hospital Lahore from June 16, 2021, to December 16, 2021, employed a cross-sectional survey design with a non-probability consecutive sampling technique. The sample size of 70 cases was calculated with a 95% confidence interval, 10% margin of error, and an expected class A percentage of 24.3%. Inclusion criteria involved patients of both genders, aged 20-60, with liver cirrhosis per the operational definition, providing written informed consent. Exclusion criteria included individuals with specific medical histories and conditions. Upon approval from the hospital's ethical review commi-ttee, 70 eligible patients at Services Hospital in Lahore were counseled, obtained written consent, and under-went detailed assessments for Child-Pugh classification. History, examination, and investigations were conduc-ted, categorizing patients into classes A, B, and C. Dopp-ler ultrasonography with automated velocity tracing was used for measurements, following a standardized scanning protocol. Portal vein velocity, as per the opera-tional definition, was measured. All data, including demographic details, were recorded in the proforma. Standardized procedures in the hospital lab, with consistent Doppler measurements by the same consultant, aimed to eliminate bias and control confounding variables through exclusion.

Child Pugh score			
Factor	**1 point**	**2 points**	**3 points**
Bilirubin (mg/dL)	< 2	2-3	>3
Serum Albumin (g/dL)	>3.5	2.8-3.5	<2.8
PT INR	<1.7	1.71-2.30	>2.30
Ascites	None	Mild	Moderate to Severe
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)
Severity of cirrhosis			
	Class A	**Class B**	**Class C**
Total points	5-6	7-9	10-15
1-year survival	100%	80%	45%

For data analysis, SPSS v25 was used. Numerical variables (age, BMI, mean portal vein flow velocity) were presented as Mean±S.D. and categorical variables (gender, Child-Pugh class) as frequency and percentage. One-way ANOVA compared mean portal vein flow velocity among Child-Pugh classes A, B, C ($p \leq 0.05$). Stratification was performed by age, BMI, gender, and Child-Pugh class. Post-stratification involved the Chi-square test for Child-Pugh class and one-way ANOVA for portal vein flow, with $p \leq 0.05$ considered statistically significant.

Results

Selected 70 liver cirrhosis patients: 49 males (70.0%), 21 females (30.0%). Mean age: 40.66±16.61 years. Majority, 39 (55.7%), aged 46-60 years; 18 (25.7%) aged 20-30 years; 13 (18.6%) aged 31-45 years. Mean BMI: 27.15±8.14 kg/m². BMI distribution: 40 (57.1%) normal, 25 (35.7%) overweight, 5 (7.1%) obese. Child-Pugh distribution: 30 (42.9%) Class-C, 23 (32.9%) Class-A, 17 (24.3%) Class-B (Table-1). Significant differences in mean portal vein flow velocity were observed among patients with different Child-Pugh classes ($p< 0.05$) (Table-2). Stratification of Child-Pugh class with respect to gender ($p=0.779$), age groups ($p=0.794$), and BMI ($p=0.852$) showed statistically insignificant differences (Table-2). Stratification of mean portal vein flow velocity by various variables revealed statistically insignificant differences with respect to gender ($p=0.990$), age groups ($p=0.362$), and BMI ($p=0.615$) (Table-3).

Table 1: *Characteristics of the study population*

Gender	Frequency	Percent
Male	49	70.0
Female	21	30.0
Total	70	100.0
Age groups	**Frequency**	**Percent**
20-30 years	18	25.7
31-45 years	13	18.6
46-60 years	39	55.7
Total	70	100.0
Body mass index (BMI)	**Frequency**	**Percent**
Normal (18-24.9)	40	57.1
Overweight (25-29.9)	25	35.7
Obese (≥30)	5	7.1
Total	70	100.0
Child-pugh class	**Frequency**	**Percent**
Class-A	23	32.9
Class-B	17	24.3
Class-C	30	42.9
Total	70	100.0

Table 2: *Comparison mean portal vein flow velocity among different Child Pugh classes.*

Portal vein flow velocity (cm/s)	Child-pugh class			p-value
	Class-A	Class-B	Class-C	
N	23	17	30	
Mean	35.35	26.18	14.23	0.001
Std. Deviation	3.725	5.015	2.967	

Gender	Child-pugh class			Total	p-value
	Class-A	Class-B	Class-C		
Male	16	13	20	49	0.779
	32.7%	26.5%	40.8%	100.0%	
Female	7	4	10	21	
	33.3%	19.0%	47.6%	100.0%	
Total	23	17	30	70	
	32.9%	24.3%	42.9%	100.0%	

Age groups	Child-pugh class			Total	p-value
	Class-A	Class-B	Class-C		
20-30 years	7	4	7	18	0.794
	38.9%	22.2%	38.9%	100.0%	
31-45 years	5	4	4	13	
	38.5%	30.8%	30.8%	100.0%	

BMI	Child-pugh class			Total	p-value
	Class-A	Class-B	Class-C		
Normal	15	9	16	40	0.852
	37.5%	22.5%	40.0%	100.0%	
Overweight	7	7	11	25	
	28.0%	28.0%	44.0%	100.0%	
Obese	1	1	3	5	
	20.0%	20.0%	60.0%	100.0%	
Total	23	17	30	70	
	32.9%	24.3%	42.9%	100.0%	

Table 3: *Stratification of mean portal vein flow velocity with respect to different variables*

Gender	Portal vein flow velocity (cm/s)			p-value
	n	Mean	Std. Deviation	
Male	49	24.08	9.661	
Female	21	24.05	10.925	0.990
Age groups	**Portal vein flow velocity (cm/s)**			**p-value**
	n	**Mean**	**Std. Deviation**	
20-30 years	18	24.67	10.857	
31-45 years	13	27.23	11.584	0.362
46-60 years	39	23.74	8.955	
BMI	**Portal vein flow velocity (cm/s)**			**p-value**
	n	**Mean**	**Std. Deviation**	
Normal	40	23.95	10.048	
Overweight	25	25.04	10.454	0.615
Obese	5	20.20	7.190	

Chi square tests applied; p value less than 0.05 considered significant

Anova and chi square tests applied; p value less than 0.05 considered significant

Discussion

Exact assessment of liver damage and deranged hemodynamics is vital for treatment planning, monitoring and prognosis evaluation in CLD. Liver biopsy, a long-standing gold standard, diagnoses and stages liver damage, specifically in patients who are asymptomatic.[8] Liver biopsy carries risks and complications such as morbidity, pain, bleeding, diagnostic inaccuracy, accidental injury to surrounding viscera and variability in both inter-observer and intra-observer, complicating follow-up.[9-11] A 2019 study by Farooq et al. on portal vein flow velocity in different Child-Pugh score classes found velocities of 18.75±1.88 cm/s in class A, 14.25±0.98 cm/s in class B, and 8.15±1.84 cm/s in class C. However, the study did not provide a p-value.[12] Another study by Afif et al. reported velocities of 16.5±3.6 cm/s in class A, 14.2±4.2 cm/s in class B, and 3.7±14.3 cm/s in class C, with a significant p-value of 0.001.[13]

This has prompted the adoption of safer methods to assess chronic liver disease and its complications. Gray-scale and Doppler ultrasound (USG) studies are now integral components of the investigation battery for evaluating CLD patients.[8-11] As liver transplant availability increases globally for liver failure, the importance of Child-Pugh categories as a key prognostic factor in end-stage CLD patients has grown significantly.[9]

Imaging, pathology, and clinical assessments collectively open new therapeutic avenues. Ultrasound plays

a well-established role in chronic liver disease and cirrhosis evaluation.[14-17] Doppler USG is crucial for blood flow analysis.[18-19] Blood flow dynamics in the hepatic artery, hepatic veins, and portal vein are well-documented in previous studies.[17-19] Changes in liver hemodynamics could be substitute parameter for assessment of parenchymal changes in CLD and their adverse effects.[9-10]

Child Pugh stages CLD using encephalopathy, serum bilirubin, prothrombin time, ascites and serum albumin. It includes 3 stages, A, B, and C, indicating escalating severity of CLD.[7] Doppler US reveals portal vein hemodynamic changes in liver cirrhosis, including reduced velocity, loss of pulsatility, and a shift from hepatopetal to hepatofugal flow in advanced cases.[16-19]

The normal portal vein velocity ranges from 16 to 40 cm/sec, but in chronic liver disease, velocities decrease due to rising portal venous pressure and collateral formation. Few studies in Pakistan have explored Doppler ultrasound's role in chronic liver disease assessment. Our study establishes Doppler ultrasonography as valuable for evaluating portal vein hemodynamics in Pakistani liver cirrhosis patients, revealing an association between Doppler findings and Child Pugh categories. Mean portal vein velocity decreases with advancing Child Pugh class, and flow reverses in class C cirrhosis. An Iranian study supports these findings, showing similar associations with Child Pugh categories.[9]

In 2017, Afif et al. investigated maximum velocities in the portal vein, hepatic vein, and hepatic artery, as well as the hepatic artery resistive index in Singaporean liver cirrhosis patients. They observed flattened hepatic vein waveforms correlated with the extent of CLD. CLD patients exhibited markedly increased hepatic vein velocity but markedly decreased portal vein velocity, with progressive decrease in maximum mean portal vein velocity as cirrhotic level increased.[13]

In our study involving 70 cirrhotic patients, we observed a substantial decrease in mean portal vein peak velocity with advanced cirrhosis. The average PVV values were 35.35±3.73 cm/sec in Class A, 26.18±5.01 cm/sec in Class B, and 14.23±2.967 cm/sec in Class C. These findings align with similar results reported in studies by De Gottardi, Afif AM, Zhang H jun, and Tian L.[19-22] Healthy individuals show hepatopetal pattern on Doppler Imaging of the portal vein.[16-19]

In advanced chronic liver disease (CLD), there is a gradual decline in portal venous flow due to heightened resistance and pressure. It manifests as a bidirectional (to-and-fro) flow pattern, suggesting near-stagnation in the portal venous system. As cirrhosis progresses, fibrosis and architectural distortion obstruct hepatic venules and sinusoids. Arterioportal and porto-systemic shunting exacerbate the condition, resulting in hepatofugal (reversed) mean portal vein velocity and a further decreased velocity.[18-19]

Our study results align with international findings, providing valuable insights into a non-invasive and cost-effective assessment method for monitoring the progression and management of CLD in Pakistan.

Conclusion

Doppler ultrasound proves vital in determining the intricate the mean portal vein velocity in CLD patients and tracking its progression. Mean peak portal vein velocity decreases with the increasing severity of Child Pugh category and flow reverses in Child Pugh class C cirrhosis. These findings align with those reported in other studies.

Financial Disclosures None

Conflict of Interest None

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Authors Contribution

I I : Conceptualization of Project

RM : Data Collection

HSA: Literature Search

KM : Statistical Analysis

AR : Drafting, Revision

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Effectiveness of Urinary Kidney Injury Molecule-1 to diagnose Subclinical Acute Kidney Injury induced by Extracorporeal Shock Wave Lithotripsy

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Abstract

Objective: To find the effectiveness of Kidney Injury Molecule-1 (KIM-1) as biomarker in diagnosing Extracorporeal Shock Wave Lithotripsy (ESWL) induced renal damage by taking serum creatinine concentration as gold standard

Materials and Method: In this interventional study, a total of (80) diagnosed patients of nephrolithiasis undergoing ESWL of age ≥ (18) years were enrolled from the Outpatient Department of Mayo Hospital, Lahore. After informed consent, urine and whole blood samples were drawn pre and post ESWL. Samples after centrifugation were analyzed for urinary KIM-1 levels employing sandwich ELISA technique. While serum creatinine levels were measured by colorimetric photometry in the Advanced Research Lab of Biomedical Sciences, King Edward Medical University, Lahore.

Results: Of (80) patients, 53 (66.3%) of them were males and 27 (33.8%) were femaless. Serum creatinine levels were decreased by 0.15 times from the baseline after procedure of ESWL but remained within normal clinical range. Whereas urinary KIM-1 levels were raised 2.04 times than the baseline after ESWL ($p< 0.001$) depicting subclinical acute kidney injury. At a cut off value of >50.9pg/ml, urinary KIM-1 had a 71.25% sensitivity and 46.25% specificity with AUC of 0.699 ($p<0.001$) to predict acute kidney injury after ESWL. A strong positive correlation ($r= 0.62$, $p<0.001$) was found between pre and post ESWL values of KIM-1.

Conclusion: The noninvasive biomarker KIM-1 was observed more effective in our study for diagnosing ESWL–induced subclinical acute kidney injury than serum creatinine.

Keywords: Kidney injury molecule-1, KIM-1, biomarker, extracorporeal shock wave lithotripsy, ESWL, subclinical acute kidney injury, nephrolithiasis, urolithiasis

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Introduction

Nephrolithiasis or urolithiasis is a common condition that affects people all over the world, with rates ranging from (7-13%) in North America, (5–9%) in Europe, and (1–5%) in Asia.[1] Stone management is associated with acute and chronic morbidity and is costly due to high recurrence.[2] Extracorporeal Shock Wave Lithotripsy (ESWL), a less invasive treatment modality is considered the best option for kidney stones smaller than 2.5cm.[3] ESWL carries a lot of hazards in addition to its many benefits. These include inflammation, cavitation, and renal ischemia reperfusion injury.[4] Acute kidney injury (AKI) induced by ESWL is usually measured by serum creatinine concentration but it is raised after few days and is unable to detect subclinical kidney injury.[5] Recently, researchers have investigated multiple ways to determine ESWL–induced subclinical kidney injury and various biomarkers are under investi-

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gation for this purpose. The most recent kidney damage indicator to emerge is Kidney Injury Molecule 1 (KIM-1). KIM-1 is a (38)-kilo Dalton (kDa) glycoprotein. KIM-1 is found in proximal tubular cells and is hypothesized to aid in the clearance of apoptotic and necrotic cells.[6] Due to its high sensitivity and specificity, it was approved for the detection of drug-induced nephrotoxicity in rats. The Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have now approved it for use in safety biomarker panels to help detect kidney tubular injury in phase 1 trials.[7]

Indonesian researchers conducted a systemic review in 2018 and concluded that KIM-1 is useful in determining ESWL induced renal damage. The pre and post value of this biomarker has been used in several studies to gauge the safe time between two lithotripsy sessions.[8] The notion to determine this biomarker's levels in relation to renal injury in our population was sparked by the paucity of data and inconsistent results of a few researches using it. In patients receiving ESWL, the current study intended to assess effectiveness of KIM-1 to diagnose subclinical acute kidney injury induced by ESWL by taking serum creatinine concentration as gold standard.

Materials and Method

In this pre and post interventional study, STARD guidelines were followed to report studies of diagnostic accuracy.[9] In this study 80 patients of age ≥ 18 years undergoing 1st session of ESWL were taken as study subjects from Urology Department of Mayo Hospital, Lahore. All patients had radiopaque unilateral stone(s) but had normal levels of serum creatinine before intervention. Ultrasound KUB was used to determine the size and site of the stone. Non-probability convenient sampling technique was used for sample selection of patients. Patients having history of using nephrotoxic drugs within four weeks before ESWL, hydronephrosis, glomerulonephritis, CKD diabetes mellitus, cardiac disorders, hypertension and inflammatory disorders were excluded from the study.

All guidelines for the use of human biological samples were followed in this research project. The study was approved by the institutional review board of King Edward Medical University (KEMU), Lahore vide letter no.43/RC/KEMU, Dated: 13/01/2020. The subjects gave written, informed consent at the time of recruitment. Demographic and clinical data of the patients were documented by taking the history and scrutiny of existing medical records. Following all aseptic measures two blood samples to measure serum creatinine levels were collected from every patient. 1st sample was drawn before the procedure and 2nd sample was drawn at the 3rd day of intervention. Blood samples were centrifuged at 1000rmp for 10 minutes to separate the serum which was stored at -80˚C until further use. Similarly, two urine samples were collected from every patient, 1st before the procedure and 2nd after 3 hours of ESWL. Urine samples were centrifuged using Labcon disposable 15mL urine centrifuge tubes and the superficial fractions were kept at -80°C until further use.

Creatinine levels in serum were measured by Jaffe's reaction colorimetric photometric method, using Human, creatinine liquicolor test kit having Catalog no: 10051, as it is less vulnerable to interference from Jaffe positive chemicals other than creatinine. The micro lab 300 automatically calculated the concentration of creatinine in a sample.

The levels of KIM-1 were determined in urine by sandwich enzyme-linked immunosorbent assay (ELISA) technique using Human KIM-1 ELISA Kit provided by ELABSCIENCE Biotechnology Inc (Houston, Texas, USA), having catalog no: E-EL-H6029. In the above-mentioned ELISA kit, the manufacturers protocol was followed. At a wavelength of 450 nm, standard curves were plotted on graphs automatically by Accu Skan FC micro-plate reader (Fisher Scientific, Pittsburgh, Pennsylvania, USA) for KIM-1, and results were read from the curves for each sample. Acute kidney injury was defined according to the Risk, Injury, Failure, Loss, End-stage (RIFLE) criteria according to which serum creatinine (SCr) ≥ 1.5, ≥ 2.0 and ≥ 3.0 from the baseline was considered as risk, injury, and failure, respectively.[10]

Data was analyzed using SPSS version 26.0 (SPSS, Chicago, Illinois, USA) and MedCalc Version 22.009 (MedCalc Software, Mariakerke, Belgium). Shapiro-Wilk and Kolmogorov-Smirnov tests were employed to verify that continuous data was normal. Association of levels of urinary KIM-1 and serum creatinine pre and post ESWL was calculated by applying Wilcoxon Signed Ranks Test. We evaluated the sensitivity and specificity for urinary KIM-1 and serum creatinine at various cut-off values. To measure the accuracy of urine KIM-1 and serum creatinine as a marker of subclinical AKI, a receiver operating characteristic (ROC) curve analysis was performed. Spearman correlation analysis was performed to check the relationship of KIM-1 and serum creatinine with each other. A p value of <0.05 was considered statistically significant.

Results

Among 80 patients of urolithiasis, males were (53) (66.3%) and females were (27) (33.8%). Mean age of patients who underwent ESWL was (36.04±11.81) years. Mean size of the stone was (1.37 ± 0.66) cm. In the patients of nephrolithiasis, serum creatinine levels were decreased by (0.15 times) after ESWL, while urinary KIM-1 levels were raised (2.04 times) from the baseline after ESWL. Pre and post ESWL concentrations of serum creatinine and urinary KIM-1 are given in **Table 1**. The ability of serum creatinine and urinary KIM-1 levels to diagnose sub clinical acute kidney injury was assessed using ROC curve analysis. The graphs showing area under the curve (AUC) are given in **Fig-1&2**. The values of the validation criteria for the respective cut-off values of serum creatinine and KIM-1 are given in **Table 2**. A correlation analysis was performed to find the relation-ship of kidney injury biomarkers with each other before and after ESWL. The values of correlation coefficients are given in Table 3. Urinary KIM-1 levels were strongly and positively correlated to each other before and after ESWL.

Table 1: *Levels of serum creatinine and urinary KIM-1 in the patients undergoing ESWL*

Variables	Before ESWL (n = 80)	After ESWL (n = 80)	Wilcoxon Signed Ranks Test
	Median (IQR)	Median (IQR)	p-value
Serum Creatinine (mg/dl)	0.80 (0.70-1.0)	0.70 (0.50-0.80)	<0.001**
Urinary KIM-1 (pg/ml)	78.25 (25.05-329.50)	262.50 (36.68-636.25)	<0.001**

***A p< .001 was considered as highly significant.*

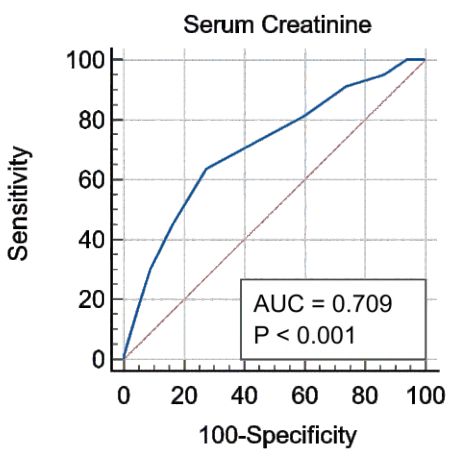

Fig-1. *ROC curve of serum creatinine*

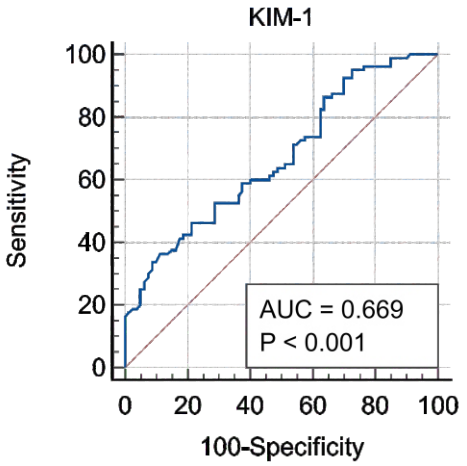

Fig-2: *ROC curve of urinary KIM-1*

Table 2: *Cut-off values and values of validation criteria for serum creatinine and urinary KIM-1*

Variables	Serum Creatinine	KIM-1
Cut-off value	≤0.70	>50.9
Sensitivity %	63.75	71.25
Specificity %	72.50	46.25
Positive Likelihood Ratio %	2.32	1.33
Negative Likelihood Ratio %	0.50	0.62
Positive Predictive Value %	10.9	6.5
Negative Predictive Value %	97.4	96.8

Table 3: *Correlation of kidney injury biomarkers*

	Pre ESWL sCr	Post ESWL sCr	Pre ESWL-KIM-1
Pre ESWL sCr	_____		
Post ESWL sCr	r= 0.23*	_____	
Pre ESWL-KIM-1	r= 0.00	r= 0.02	_____
Post ESWL KIM-1	r= -0.23*	r= 0.05	r= 0.62**

**p<0.05. **p<0.001*

Discussion

In Pakistan, ESWL was initially introduced in 1989 and now it is the most common type of lithotripsy performed for renal stones.[11] Its popularity grew rapidly, thanks in part to the notion that it was completely risk-free. After a decade of clinical ESWL, we now know that this is not the case. ESWL is quite successful at breaking kidney stones, but it can also produce significant renal damage, which can lead to long-term complications that are irreversible. Shock wave lithotripsy is associated with hemorrhage, reperfusion injury and in some cases scarring. All of these factors are responsible for chronic kidney damage.[12] According to a study, patients undergoing ESWL had dose-dependent kidney

fibrosis and this fibrosis was responsible for a partial or complete loss of function in the affected area. Moreover, 10% of ESWL patients may develop a urinary tract infection after treatment.[13] Acute kidney injury is diagnosed by RIFLE criteria according to which increased serum creatinine is used to define risk, injury, and failure of kidney.[10]

To determine the AKI induced by ESWL, we measured already established biomarker serum creatinine concentration. In our study the difference in the levels of creatinine pre and post ESWL was statically significant, but the levels were within normal range [0.70 (0.50-0.80)], which indicated that there was kidney injury but it was subclinical. Moreover, creatinine was decreased by 0.15 times after ESWL rather than increasing. Serum creatinine at a cut off value of ≤ 0.70mg/dl had a 63.75% sensitivity and 72.50% specificity with AUC of 0.709 ($p<0.001$) to predict acute kidney injury after ESWL. However, we classified the kidney injury as subclinical because sCr≤0.70 mg/dl was within the clinically normal range and did not predict any AKI if RIFLE criteria were followed.[10] This verified the fact that serum creatinine was not a good marker for the detection of ESWL-induced renal injury in our clinical setup.

This finding is in line with previous studies in which SCr was considered as a flawed biomarker. A recent review explained the discrepancy in criteria of acute renal damage on the basis of serum creatinine levels.[5] After a significant renal injury, the serum concentration of creatinine may take 24–36 hours to rise and remain normal in subclinical damage. It only increases abruptly if the kidney function decline under 50%. Moreover, the blood levels of sCr also rise nonspecifically in septic shock and muscular dystrophy and many laboratories use diverse methods to measure sCr levels in which there is considerable interference with other colored compounds.[5] Therefore, rather than serum creatinine levels, we pro-posed that urinary KIM-1 levels could be used as a reliable predictor of renal injury. KIM-1 is not present under normal conditions but becomes abundant in injured tubular membrane cells.[6] In our study urinary KIM-1 levels were raised 2.04 times from the baseline after ESWL. Similar results were concluded by a recent review conducted by Indo-nesian investigators., in which KIM-1 was raised by 0.2 times ($p=0.05$) after ESWL in the patients of nephrolithiasis.[8]

In our study, ROC curve analysis concluded that at a cut off value of >50.9pg/ml, urinary KIM-1 levels had a 71.25% sensitivity and 46.25% specificity with AUC of 0.699 ($p<0.001$) to predict acute kidney injury after ESWL. Our results as is comparable to the results of a meta-analysis of 11 studies having 2979 patients. According to which urinary KIM-1 had an estimated sensitivity of 74.0% and specificity of 86.0% to diagnosis AKI. Moreover, the summary receiver operating characteristic curves analysis of their study showed an area under the curve of 0.86(0.83–0.89) for KIM-1 to predict AKI which is in line with our results.[14]

Similar results were shown by a randomized clinical trial in which post ESWL values of KIM-1 were raised as compared to pre ESWL values ($p < 0.0001$). Additionally, after three days from the second session, this biomarker remained considerably higher ($p = 0.027$) and after seven days, it recovered to pre-ESWL values, suggesting that a seven-day gap was necessary between ESWL sessions in order to allow for full recovery of kidney functions.[15] In our study a strong positive correlation ($r= 0.62$, $p<0.001$) between pre and post ESWL values of KIM-1 indicated that urinary KIM-1 can be used to access renal injury after ESWL in place of serum creatinine which has a weak positive correlation ($r = 0.23$, $p<0.05$) before and after ESWL.

Conclusion

Hence, it is recommended that urinary KIM-1 may be used as promising noninvasive biomarker to assess kidney injury in post lithotripsy patients as it rise far before the level of serum creatinine which is used to diagnose kidney damage as of yet. KIM-1 might be used in future as a predictive marker for the detection of sub clinical renal damage at an early stage, before this damage become irreversible. Moreover, more studies with different variables like association of frequency of shock waves with renal damage and association of number of sessions of ESWL with renal damage could be done with the help of this marker. Urinary KIM-1 can be used to reduce morbidity and mortality due to hospital acquired kidney injury and as the rise in urinary KIM-1 concentration open new avenue for the clinicians to opt new interventions and strategies to prevent complications of this procedure.

Conflict of Interest None

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Authors Contribution

RA: Conceptualization of Project
MR: Data Collection
NC, ANA: Literature Search
KQ, ANA: Statistical Analysis
MR, NC, AJG: Drafting, Revision
MR, RA: Writing of Manuscript

Wilms Tumor Different Histology Patterns Observed in Tertiary Care Hospital

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Abstract

Objective: The objective of this descriptive study was to investigate the age distribution, gender prevalence, clinical presentations, histological patterns, tumor staging, and outcomes of WT in a local pediatric population.

Material and Methods: The study conducted over 9 months at Children Hospital, Lahore. The study included 120 patients with WT. Clinical data, age, gender, and staging information were collected. Biopsies were analyzed for histological patterns, and SPSS version 26 was used for statistical analysis.

Results: The study revealed a mean age of 5.79 years, with a slight male predominance (58.3%). Abdominal mass was the predominant clinical presentation (47.5%), and the tri-phasic histological pattern, often with blastemal predominance, was most prevalent (52.5%). The majority of cases were diagnosed at Stage-I (59.2%). No significant associations were found between age, gender, and tumor subtypes.

Conclusion: The findings reveal a predominance of the tri-phasic histological pattern with blastemal predominance, aligning with global trends.

Keywords: Wilms Tumor, Histology Patterns, Children

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Introduction

Wilms' tumor (WT) is the most common kind of kidney tumor in children. It appear as a single nodule, several unilateral lesions, or tumors in both kidneys. Renal tumors account for around 7-8% of all pediatric malignancies in children under 15 years old. Among these tumors, WT, also known as nephroblastoma, is the most common neoplasm.[1] WT is often distinguished by three histological components: blastemal, epithelial, and stromal. Each tumor displays distinct histological appearances due to variable proportions and degrees of maturation.[2,3] Despite the ease of diagnosing classical triphasic WT, a missing component can lead to differentiating diagnoses in small biopsy samples. For epithelial elements, this can mean renal cell carcinoma, metanephric adenoma, or hyperplastic nephrogenic rest; for stromal elements, it can mean clear cell sarcoma of the kidney, mesoblastic nephroma, or synovial sarcoma.[3] Some embryonal "small round blue cell cancers," such neuroblastoma, primitive neuroectodermal tumor/Ewing sarcoma, lymphoma, and desmoplastic small round cell tumor, may be hard to tell apart from pure blastemal-type Wilms tumor.[4,5] Anaplastic transformation of the three main constituents, particularly the blastema, may take place, resulting in the identification of isolated or widespread anaplasia. Aggressive treatment is required for high-risk malignancies, notably Wilms tumor with diffuse anaplasia and Wilms tumor with blastemal predominance after receiving preoperative chemotherapy.[6] Precise classification and determination of the stage of WT are crucial for determining the appropriate therapy after surgery. The recognition and analysis of nephrogenic rests have a significant impact on prognosis and the course of treatment.[7] Distinguishing between WT and nephrogenic rest based

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purely on morphological characteristics might be difficult, highlighting the need of novel molecular genetic techniques. The presence of molecular genetic indicators such as P53 mutation and MYCN dysregulation shows potential for offering more predictive and therapeutic knowledge in the future [8,9].

Developed nations have a survival rate (OS) of over 90% for WT, but low-income countries experience much worse outcomes, with OS ranging from 11% to 50%[10]. Factors that contribute to the low survival rates in low-income countries include patients seeking medical attention at a late stage of their disease, inadequate nutrition, lack of knowledge about the disease, shortage of trained medical staff, and a limited number of healthcare facilities capable of treating pediatric cancers. Additionally, there is a high rate of patients discontinuing their treatment[11,12]. The lack of comprehensive data in Pakistan about the characteristics and behavior of this curable pediatric solid tumor led to the commencement of this descriptive research, which aims to evaluate the demographic factors and outcomes related with Wilms tumor.

Material and Method

It was a descriptive study conducted in the Children hospital, Lahore. The study was completed in 9 months from April 2023 to December 2023. A total of 50 patients of Wilms' tumor of both genders were included in the study. Patients were examined and clinical data was recorded. All detail information regarding age, gender, staging, and outcome was collected from their files and recorded on proformas and then analyzed. Biopsies from fresh nephrectomy specimens and paraffin embedded blocks were collected from Children hospital Lahore. Data was analyzed using SPSS version 26.

Results

The study included a total of 50 participants. The mean age of the study participants was 5.79±4.19 years, indicating a relatively young age group in which Wilms tumor was observed (Table 1). The gender distribution revealed a slightly higher prevalence among male participants, constituting 58.3% of the total cohort, while females accounted for 41.7%. The diverse sources of specimens collected for analysis were detailed, with abdominal mass being the most prevalent (47.5%), followed by renal masses (23.3%), and various other specimen types including core biopsies and mass biopsies. The diagnoses of Wilms tumor were categorized into monophasic, biphasic, tri-phasic, and other subtypes. Most cases fell into the tri-phasic category 52.5%, followed by biphasic (25.8%), and mono-phasic (12.5%). The tumor staging information indicated that many cases were diagnosed at Stage-I (59.2%), followed by Stage-II (38.3%), and a smaller proportion at Stage-III (2.5%). This distribution of tumor stages provides insights into the extent of disease progression at the time of diagnosis. In table 2, the distribution of Wilms tumor cases across different age groups revealed no significant association with tumor subtype (p=0.784). The highest frequency of cases was observed in the 1-5 years age group for all subtypes, followed by the 6-10 years age group. In gender distribution, no significant association was found between Wilms tumor subtypes and gender (p= 0.773). The prevalence of different tumor subtypes was balanced between males and females.

Table 1: *Study Participants Characteristics*

n	50
Age	5.79±4.19
Gender	
Male	70(58.3%)
Female	50(41.7%)
Specimen	
Abdominal Mass	57(47.5%)
Core Biopsy	4(3.3%)
Renal Mass	28(23.3%)
Renal Tumor	3(2.5%)
L-Kidney	1(0.8%)
Diagnosis	
Mono-Phasic Wilms Tumor	15(12.5%)
Biphasic Wilms Tumor	31(25.8%)
Tri-Phasic Wilms Tumor	63(52.5%)
Other	11(9.2%)
Stage	
Stage-I	71(59.2%)
Stage-II	46(38.3%)
Stage-III	3(2.5%)

Table 2: *Association of Wilms Tumor with Age and Gender*

	Mono-phasic	Biphasic	Triphasic	Other	p-value
	15	31	63	11	
<1 Year	0(0%)	3(9.7%)	4(6.3%)	1(9.1%)	0.784
1-5 Years	6(40%)	14(45.2%)	27(42.9%)	6(54.5%)	
6-10 Years	8(53.3%)	9(29%)	20(31.7%)	3(27.3%)	
11-15 Years	1(6.7%)	5(16.1%)	12(19%)	1(9.1%)	
Male	7(46.7%)	19(61.3%)	38(60.3%)	6(54.5%)	0.773
Female	8(53.3%)	12(38.7%)	25(39.7%)	5(45.5%)	

Discussion

Wilms tumor ranks as the third most prevalent pediatric malignancy at Children hospital, Lahore, following Acute Leukemias and Lymphomas. In contrast, on a global scale, it stands as the sixth most common childhood malignancy.[13] Wilms' tumor represents 7.6% of all malignant tumors in children, giving it a significant presence in the field of pediatric oncology. Notably, Wilms' tumor is more common in men, having a boys-to-girls ratio of 4:1. The gender disparity emphasizes a clear inclination of boys towards this specific juvenile kidney carcinoma.[14] Previous studies also show male predominance in Wilms' tumor.[15] In our study, the gender distribution revealed a slightly higher prevalence among male participants, constituting 58.3% of the total cohort, while females accounted for 41.7%. According to Leslie et al. (2017), Wilms tumor is the most prevalent kind of abdominal cancer in children, often occurring between the ages of 3 and 5 years. The average age of the individuals included in our inquiry was 5.79 ±4.19 years, which corresponds to the usual age range at which Wilms tumor is often diagnosed.[16] According to Caldwell et al. (2017), the United States sees an estimated 650 new cases of Wilms tumor each year. Significantly, their research indicates a slightly elevated probability for females to get Wilms tumor in comparison to boys. The age and gender connections highlight the unique epidemiological characteristics of Wilms tumor, offering crucial insights for physicians and researchers involved in the field of pediatric cancer.[17] There is a scarcity of published data in Pakistan concerning Wilms' tumor or nephroblastoma.[18]

The median age of diagnosis for Wilms tumor remains steady at 3 years, as documented in many worldwide research.[19,20] Most patients with Wilms tumor usually develop the condition between the ages of 2 and 5 years, which is a distinct age range for this kind of kidney cancer in children.[21] Wilms tumor is the main kind of kidney cancer seen in children. However, in the age range of 15 to 19 years, renal cell carcinoma becomes the most common type of tumor. The 5-year relative survival rate for this age group is 76%.[22] The prevalence of abdominal mass is the predominant manifestation, which aligns with the results reported in previous research.[23] Our research found that abdominal mass accounted for the highest proportion of specimens (47.5%), followed by renal masses (23.3%). Other specimen categories, such as core biopsies and mass biopsies, were also included. This is consistent with the finding in the research conducted by Pushpa and Duraisamy (2019) that children diagnosed with Wilms' tumor often have a lump in the abdomen.[24] All tumors seen in our investigation were unilateral, and there were no cases of bilateral tumors. The research revealed that the left kidney was the predominant location for tumor development, which aligns with the results of a prior investigation[(25)].

The prognosis for children diagnosed with Wilms' tumor is heavily dependent on the presence of anaplasia. Of all the Wilms' tumors, 43.2% have a triphasic pattern, whereas 46% have a monophasic pattern. The prognosis in children with Wilms' tumor is mostly determined by the histological features and stage of the tumor.[24] In our study, the diagnoses of Wilms tumor were categorized into mono-phasic, biphasic, tri-phasic, and other subtypes. Many cases fell into the tri-phasic category 52.5%, followed by biphasic (25.8%), and mono-phasic (12.5%). The tumor staging information indicated that most cases were diagnosed at Stage-I (59.2%), followed by Stage-II (38.3%), and a smaller proportion at Stage-III (2.5%). This distribution of tumor stages provides insights into the extent of disease progression at the time of diagnosis. In Reddy et al. (2023) study, there were no case limited to stage V was diagnosed. Stage 1 tumors were encountered most frequently in our study. In countries with limited resources like Pakistan, a significant challenge in cancer care is the common problem of late-stage diagnosis, resulting in more advanced and extensive illness.[26] This research emphasizes a clear difference in the timing of diagnosis, demonstrating the substantial influence of late diagnoses in areas with inadequate resources.

Conclusion

The findings reveal a predominance of the tri-phasic histological pattern with blastemal predominance, aligning with global trends. Most patients were found to be limited to stage 1 and of intermediate risk. Wilms' tumor was found more common between the ages of 1 and 10 years with male predominance. The study underscores the significance of early detection, as evidenced by a substantial proportion of Stage-I diagnoses and emphasizes the importance of understanding local characteristics for accurate diagnosis and treatment planning. Molecular genetics can be recommended for future studies, which will be helpful in targeted therapy and diagnosis of other diseases associated with mutations.

Conflict of Interest *None*

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Authors Contribution

BN: Conceptualization of Project
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SZ, SS,A: Statistical Analysis
SZ: Drafting, Revision
ZJ: Writing of Manuscript

Comparison of Retinol Binding Protein 4 (RBP 4) Levels in Pregnant Females with and without Gestational Diabetes Mellitus (GDM)

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Abstract

Objective: To compare the levels of RBP4 in pregnant females with and without GDM.

Materials and Methods: It was a cross sectional study carried out in Pathology Department KEMU/Mayo Hospital and Lady Aitchison Hospital, Lahore after approval by Institutional Review Board. After informed consent, total 64 pregnant females at 24-28 weeks of gestation undergoing OGTT; 32 in group A with GDM and 32 in group B without GDM were enrolled. Under aseptic conditions, 3ml blood was drawn for RBP-4 to be measured by ELISA.

Results: The means ± SD age of females was 25.5±4.3 and 24.4±4.4 years in group A and B respectively. The age, gestational age, parity, previous history of GDM was not significantly different in 2 groups. The median (IQR) values of RBP4 were 37.3 and 33.2ng/dl in group A and B respectively and were significantly different (p =0.021).

Conclusion: Serum RBP4 concentrations are high in females with GDM as compared to those without GDM.

Keywords: RBP4, GDM

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Introduction

Gestational Diabetes Mellitus (GDM) is defined as any degree of glucose intolerance that is first recognized with the onset of pregnancy.[1] This diagnosis does not apply to pregnant women with previously diagnosed diabetes or overt diabetes.[2] The prevalence of GDM is 1% to 28% with higher ratios in Asian women. A high frequency of GDM (11.8%) has been reported in Pakistan.[1,3] A large number of fetal and maternal complications are caused by GDM. Some of the fetal complications are preterm birth, respiratory distress syndrome, excessive birth weight and hypoglycemia whereas the maternal complications are high blood pressure, pre-eclampsia and risk of diabetes in future.[4] The underlying pathophysiology is failure of pancreas to up regulate insulin secretion relative to insulin resistance created by changes in hyperglycemic hormones (like corticotrophin releasing hormone, growth hormone, placental lactogen and progesterone) during pregnancy.[6] An efficient diagnosis and accurate monitoring of diabetic mothers are important to decrease the risk of diabetic complications. There is disagreement between obstetric, medical and endocrine groups about the effective methods of diagnosis of GDM.[6] The oral glucose tolerance test (OGTT) is currently the recommended method and it is performed worldwide for diagnosis of GDM. But the adequate glucose load amount and cut-off values of OGTT are still controversial.[7,8] HbA1c is also used to diagnose GDM but it has lower diagnostic performance in pregnant women due to anemia and

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biphasic changes in its values.[9,10] Many adipokines for example visfetin, chimerin, adiponectin , leptin, tumor necrosis factor-alpha (TNF-alpha) and Retinol binding protein4 (RBP-4) have been involved in causing insulin resistance and their concentrations in plasma have been investigated for the diagnosis of GDM.[11,12] The cross talk between different adipokines and insulin targeted tissues such as skeletal muscles and the liver plays a significant role in pathophysiology of GDM.[13] RBP-4 is mainly synthesized in hepatocytes and adipose tissues. It is responsible for causing insulin resistance by different pathways; it upregulates gluconeogenesis by inducing the expression on liver's gluconeogenic enzyme phosphoenol pyruvate carboxikinase (PEPCK) and it disrupts glucose transport in muscles and adipose tissues. RBP-4 decreases expressions of glucose transporter-4(GLUT-4) in striated muscles and adipose tissues.[14] In this background, this study is planned to compare the levels of RBP4 in pregnant females with and without GDM.

Materials and Methods

It was a cross sectional study carried out in Pathology Department KEMU/Mayo Hospital and Lady Aitchison Hospital, Lahore after approval by Institutional Review Board. Total 64 pregnant females at 24-28 weeks of gestation undergoing OGTT were included; 32 in group A with GDM and 32 in group B without GDM using non-probability convenient sampling. The pregnant females with history of DM before pregnancy, renal dysfunction, hypertension, hepatic dysfunction were excluded. The relevant information of each patient was recorded in study proforma after informed consent. Under aseptic conditions, 2-3ml venous samples for fasting, one and two hours after 75g glucose were collected from each patient in yellow top vacutainer labeled with Patient's name and ID for the analysis of glucose and RBP4.Serum was separated from samples after clotting through centrifugation at 3000rpm. After ensuring the quality control, the glucose estimation was performed on Beckman Coulter- AU 680 chemistry auto analyzer by Hexokinase method. The patients were labeled as GDM and Non GDM on the basis of OGTT results. The remaining serum was stored in Eppendorf cups labeled with patient's ID at -80°C for RBP-4 analysis. ELISA was performed on samples for RBP-4 using kit by Bioassay Technology Laboratory on Diatrone 710 ELISA plate reader in 2 batches. The data analysis was performed by using SPSS-26). Quantitative variables with normal distribution were presented as mean±SD and skewed data as median (IQR). Qualitative variables were presented as frequency and percentage.

Results

The mean ±SD fasting, 1 and 2 hour blood glucose levels after OGTT were 110±12, 186±45, 149± 35 and 82±7, 124±26, 105±22 mg/dL in group A and group B respectively. Independent sample t test was used to compare these levels between 2 groups that showed significant difference (p value< 0.01). The median (IQR) RBP-4 levels were 37.3 (11) and 33.2 (20) ng/dl in females with and without GDM respectively. Mann Whitney U test was applied to compare RBP-4 levels between two groups that showed significant difference (p value= 0.021).

Table 1: *Demographic and Clinical Characteristics of Study subjects:*

Variables	Group A (GDM) n=32	Group B (Non GDM) n=32	p-value
Age (mean±SD) (years)	25.5 ± 4.3	24.4± 4.4	0.303
Gestational Age (weeks)	26.3 ± 1.8	25.9 ± 1.6	0.421
Parity (PG: MG)	9(28%): 23(72%)	11(34%): 21(66%)	0.590
History of GDM	3(9%)	2(6%)	0.641
Family history of DM	19(59%)	9(28%)	***0.012**

- Independent sample t-test used for age & gestational age.
- Chi Square test used for parity, history of GDM, family history of DM.
- p-Value of < 0.05 was taken as statistically significant.

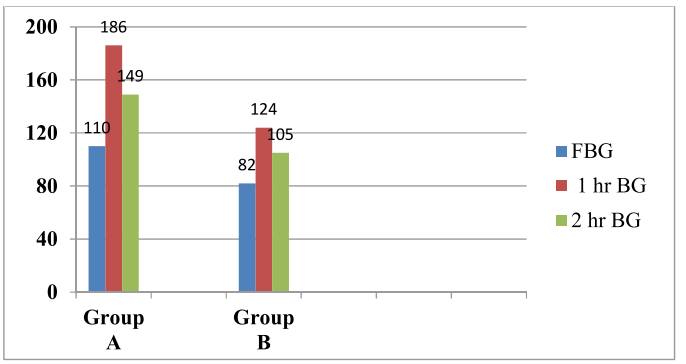

Figure 1: *Comparison of Blood glucose levels in Group A and Group B.*

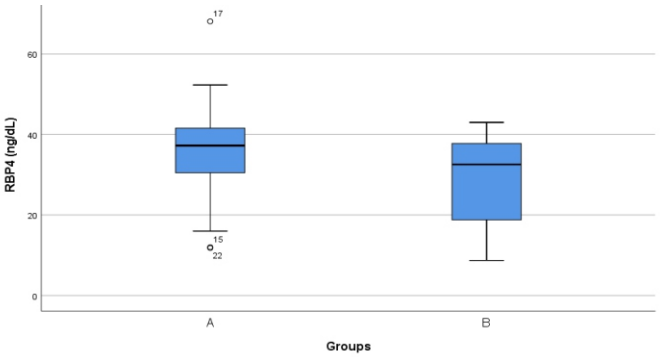

Figure-2: *Comparison of Retinol Binding Protein-4 between Group A and B.*

Discussion

The worldwide prevalence of GDM varies from 1% to 28% and it is 11% in Pakistan.[1,3] Obesity, fatty diet, micronutrients deficiency, advanced maternal age, previous GDM history and history of diabetes in family are some of the risk factors for GDM.[5] The timely diagnosis of GDM is important to reduce maternal and fetal complications The approach to screen GDM varies worldwide.[4] OGTT is used to screen GDM between 24 and 28 gestational weeks in pregnant females. But it has its own limitations like a time-consuming test, needs fasting for at least 8 hours and may result in nausea, vomiting, and headache especially in pregnant women and some participants are unable to complete the test.[2,7] HbA1C is another diagnostic tool but it has some limitations as it is affected by anemia in pregnancy.[9,10] The shortcomings of the existing screening and diagnostic tools raise the need of biomarker that is not affected by conditions mentioned above. Some new biomarkers have been studied for their role in the diagnosis of GDM including adipokines like visfetin, interleukin-6, Leptin, adiponectin , RBP4, Chimerin.[2] RBP4 is one of the adipokine that was investigated for its role in GDM in our study.[2,14] In our study The RBP4 levels were studied in 64 pregnant females; 32 with GDM (group A) and 32 without GDM (group B) diagnosed on the basis of OGTT. The age was comparable between group A and B (p value = 0.303). The findings of our study are in agreement with the study by Chuyao jin. et al and Xiyu Du et al.[14,15] But the results differ from the study of Maghbooli Z et al who showed the age was significant different between study groups (p value=0.001).[16] The frequency of multi parity was 72% for Group A and 66% for Group B and it was comparable to the study by Maghbooli Z et al.[16] The history of GDM was not significantly different between Group A & B (p value= 0.641). This is similar to study done by Fatima S.et al.[17] The findings of our study are not comparable to study done by Xiyu Du at al who showed that previous history of GDM was significant in women with GDM as compared to those without GDM[(14)]. The family history of DM was significantly high in GDM as compared to non GDM (p = 0.01). The findings of our study are in agreement with the study by Fatima S et al.[17] The Fasting blood glucose, blood glucose 1 & 2 hours after 75g glucose was significantly different between group A and B (p value <0.01) in our study. The findings are similar to study by Mengkal Du et al. , Beverly J Tepper et al.[18]

The median (IQR) RBP-4 levels were 37.3 and 33.2ng/dl in GDM and non GDM respectively (p value = 0.02). The results of our study are in accordance with the studies by Xiyu Du et al, Krzysztof C et al, Chiyao Jin et al, Maghbooli Z et al, Mengkai Du et al who showed that RBP4 levels were significantly different between GDM and Non GDM.[14,16,18] The results of our study are not in agreement with the studies of Weerapan K. et al , Asli Yarsi G et al and Khovidhunkit et al that showed no significant difference of RBP4 levels between GDM and Non GDM patients. The difference might be attributed to characteristics of study populations. The study of Weerapan K. et al was performed on Thai women and the difference of ethinicity might be one the reason for this difference.[19,20]

The Role of RBP4 in pathophysiology of GDM is a topic of research worldwide. The relationship of obesity, insulin resistance and DM is due to link between adipokines e.g RBP4 and insulin dependent tissues like liver and skeletal muscles.[13,14,19,20] The relationship of RBP4 levels with GDM was studied in a meta-analyses and it was seen that RBP-4 levels were remarkably high in females with GDM than Non GDM. However, this difference of RBP4 levels beween GDM and non GDM were present particularly in Asian ethnicity. In addition to diagnosis of GDM, RBP4 levels were also found to have a predictive role in GDM. Huag Q-T et al. found that females with GDM had higher values of RBP4 in first trimester than those without GDM.[2,21]

The cut off used to predict GDM varies in different studies. According to a study by Yuan et al. the cut off value of 30.45 µg/mL for RPB4 could diagnose GDM with a sensitivity of 63.6%, specificity of 75% and AUC 0.72 (95% CI 0.64–0.79). Whereas the study by Maghbooli Z et al showed that RBP4 levels equal to 42 µg/ mL could forecast the risk of developing GDM with the sensitivity 0f 75.8%, specificity 65.3%, and p value =

0.001.[16,22] The role of RBP4 in pathophysiology of GDM is also supported by study of Xia sun et al. They studied the effect of Sitagliptin an antagonist of the dipeptidyl peptidase-4 (DPP-4) (an adipokine oversecreted in insulin-resistant obese patients). on the parameters of insulin sensitivity in GDM patients. In addition to increasing insulin sensitivity, reducing fasting blood glucose and insulin levels it also caused marked reduction in RBP-4 levels after 16 weeks of treatment. RBP4 levels reduced from 59.4±16.7 to 42.1±20 (p value=0.023) in the group which was given sitagliptin as compared to the placebo group where it changed from 61.4±17.3 to 57.6±21.8.23

The diagnostic test for GDM in which pregnant females need no fasting and do not require glucose load would not only reduce the nausea and vomiting experienced by pregnant women but also increase compliance of pregnant females for screening of GDM. Moreover, the COVID-19 pandemic has greatly impact the hospital and clinical practices in order to reduce patient undue stay in hospitals and clinics. To perform OGTT in the background of pandemic fear was very challenging. This further emphasizes the importance of a biomarker for GDM that could be practiced in a GP clinic instead of a hospital with minimal stay of female just to get her blood sample drawn. A single blood test would lessen the duration of appointment, help to increase the number of females to be screened and would help to perform test in a non-hospital setting. RBP4 can be a potential biomarker to fulfil the purpose.

Conclusions

RBP4 levels are significantly high in females with GDM as compared to those without GDM. The study had certain limitations that it was performed in a single centre on small scale, the females with GDM were not followed till delivery and postpartum to determine RBP-4 levels and fetal outcome.

Conflict of Interest: **None**

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Authors Contribution

RD: Conceptualization of Project
MS: Data Collection
KJ: Literature Search
MA: Statistical Analysis
NK: Drafting, Revision
AN: Writing of Manuscript

Combined Ultrasonic-Pneumatic Lithotripsy Reduces Operation Time, Hospital Stay in Staghorn Calculi, without Affecting Clearance

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Abstract

Objective: To compared ultrasonic and pneumatic lithotripsy for staghorn renal calculi, focusing on operation time, hospital stay, stone clearance rates, and patient-specific factors like age, gender, BMI, and disease characteristics.

Material and Methods: We retrospectively analyzed staghorn renal calculi patients treated at our institution from 15 March to 15 August 2023. Patients were separated into two groups: Group I (33 patients) received ultrasonic and pneumatic lithotripsy, and Group II (33 patients) received pneumatic alone. Operation time, hospital stay, and stone clearance were key outcomes. Secondary outcomes explored how patient demographics and illness variables affected primary outcomes. Statistical analysis used independent t-tests and chi-square tests, with a significance threshold of $P<0.05$.

Results: The study included 66 patients 33 in Group I and 33 in Group II. Compared to Group II, Group I had considerably shorter mean surgery times and hospital stays across all patient demographics and stone types ($P<0.001$ and $P \leq 0.02$, respectively). Stone clearance rates were similar between groups ($P>0.05$), indicating similar stone-free effectiveness. Despite differences in operation timeframes and hospital stays, patient-specific factors did not affect stone clearance rates between groups.

Conclusion: Patients with staghorn renal calculi benefit from ultrasonic and pneumatic lithotripsy, which reduces operation duration and hospital stay without affecting stone clearing performance. These findings imply that the combination method may improve patient recovery and procedure efficiency. To optimize treatment outcomes, lithotripsy technique selection should also include patient and disease characteristics. Long-term results and cost-effectiveness of various lithotripsy methods need more study.

Keywords: Staghorn renal calculi, Lithotripsy, Ultrasonic lithotripsy, Pneumatic lithotripsy, Operation time, Stone clearance rate

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Introduction

Urology faces a major problem in managing staghorn renal calculi, enormous, complicated branching stones that occupy a big amount of the kidney's collecting system. Due to its mechanical approach of fragmenting stones for extraction or natural evacuation, pneumatic lithotripsy has become a prominent therapeutic option. In search of more efficient and effective treatments, ultrasonic energy and pneumatic lithotripsy have been combined. This combination may reduce treatment duration and improve patient outcomes by improving stone fragmentation. The efficacy of ultrasonic and pneumatic lithotripsy in treating staghorn renal calculi

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has been studied. This comparison aims to determine which technique improves stone-free rates, treatment sessions, patient safety, and recovery. This evaluation is crucial for clinical practise, treatment optimization, and patient quality of life for this onerous condition.

Urology still struggles to treat staghorn renal calculi, huge, branching stones that fill the renal collecting system. These calculi are notorious for their size, complexity, and ability to induce recurrent infections, chronic kidney disease, and renal failure if left untreated.[1] Over time, open surgery has given way to less invasive methods, with pneumatic lithotripsy becoming popular since it physically fragments stones to make them easier to remove.[2] Due to pneumatic lithotripsy's limitations, such as the necessity for several sessions and the risk of remaining fragments, there is growing interest in more effective treatment methods.

Combining ultrasonic energy with pneumatic lithotripsy is a major achievement in this field, combining the benefits of both modalities. Ultrasonic lithotripsy uses high-frequency sound waves to break down stones into tiny bits that can be removed or passed naturally, while pneumatic lithotripsy uses mechanical force to break down larger and harder stones.[3] Research into their comparative efficacy has been spurred by the premise that combining these two techniques could increase stone-free rates, shorten treatment sessions, and reduce complications.

Early comparative studies suggest that combined ultrasonic and pneumatic lithotripsy may improve treatment outcomes for staghorn renal calculi.[4] These studies emphasise the necessity of assessing these treatments' immediate success rates, patient safety, recovery duration, and quality of life.[5] As such, continuing research and clinical studies are vital to finding the most effective and patient-friendly ways to manage this complex ailment, with the objective of improving staghorn renal calculi patients' care. Technological breakthroughs and improved patient outcomes have changed the literature on lithotripsy-managed staghorn renal calculi. Its direct mechanical force in fragmenting big kidney stones has made pneumatic lithotripsy a standard treatment. It has been shown to be effective, however it may require many treatment sessions and leave stone pieces.[6] Ultrasonic lithotripsy uses high-frequency sound waves to shatter stones, giving finer control and perhaps minimising leftover fragments.[7]

Managing staghorn calculi with ultrasonic and pneumatic lithotripsy is recommended. The ultrasonic component disintegrates stone efficiently, while the pneumatic component handles harder, bigger stone particles.[8] Early clinical trials and comparative research suggest that this combination technique may improve stone-free rates, complications, and treatment sessions compared to pneumatic lithotripsy alone.[9]

The literature also stresses the necessity of addressing patient-specific aspects such stone composition, anatomy, and comorbidities when choosing a treatment approach. Some stones work better with ultrasonic energy, whereas others work better with pneumatic force.[10] Optimal outcomes depend on the treating urologist's ability and experience, as well as postoperative care techniques to reduce problems and speed recovery.[11] Finally, ultrasonic and pneumatic lithotripsy may improve staghorn renal calculi therapy, according to the research. It highlights a trend toward individualised treatment that combines technology and clinical knowledge to improve patient outcomes. Further research, particularly bigger randomised controlled trials and long-term follow-up studies, is needed to compare different therapy methods and inform clinical practise.[12]

Material and Method

The methodology section of this study was designed to rigorously evaluate the efficacy of two lithotripsy techniques in managing staghorn renal calculi, assessing various factors such as operation time, hospital stay, stone clearance rate, and the impact of patient demographics (age, gender, BMI) and disease characteristics (duration, type of renal stone) on treatment outcomes. This section outlines the patient selection criteria, treatment modalities, outcome measures, and statistical analyses employed in the study. The study analyzed staghorn renal calculi patients aged 18+ treated at a hospital between March and August 2023. Patients were divided into two groups: those treated with combined ultrasonic and pneumatic lithotripsy techniques and those treated with pneumatic lithotripsy alone.

Group I (Combined Ultrasonic and Pneumatic Lithotripsy): This group underwent a lithotripsy procedure that integrated ultrasonic and pneumatic energies to fragment and remove staghorn calculi. Group II (Pneumatic Lithotripsy Alone): Patients in this group received lithotripsy using solely pneumatic energy for stone fragmentation. Primary outcomes included operation time, hospital stay, and stone clearance rate, while secondary outcomes examined patient demographics and disease

characteristics' impact on primary outcomes. Data from electronic medical records was analyzed to assess post-treatment outcomes, including demographics, disease characteristics, and stone clearance through follow-up imaging studies. The study used descriptive statistics to summarize continuous and categorical variables, with independent t-tests and chi-square tests for comparison. Statistical significance was determined at a p-value of less than 0.05. The study protocol was approved by the institutional review board (IRB) of [Institution Name], ensuring compliance with ethical standards and patient confidentiality. As a retrospective study, patient consent was waived by the IRB, but all patient data were anonymized before analysis.

Results

The study compares two lithotripsy techniques for managing staghorn renal calculi, focusing on operation time, hospital stay, and stone clearance rates. Results show significant differences between the two groups, with combined ultrasonic and pneumatic lithotripsy reducing operation time and hospital stay. However, stone clearance rates showed no significant difference. The study emphasizes the importance of considering individual patient and disease factors when selecting the most effective lithotripsy technique.[20]

The data shows that patients with staghorn renal calculi had an average disease duration of 8.33 months before intervention, with a range of 1 to 23 months. The lithotripsy procedures took an average of 207.90 minutes, reflecting the complexity of managing the condition. The average hospital stay post-operation was 3.96 days, indicating the benefits of lithotripsy as a less invasive approach compared to traditional open surgery. The table 1 presents the outcomes of a study evaluating the efficacy of combined ultrasonic and pneumatic lithotripsy versus pneumatic lithotripsy alone in managing staghorn renal calculi, segmented by age groups (15-49 and 50-70 years). It shows significantly shorter mean operation times for both age groups in Group I (combined therapy) compared to Group II (pneumatic alone), with P-values <0.001 for both age ranges. Similarly, mean hospital stays were significantly shorter in Group I across both age categories, with P-values of 0.02 and 0.04, respectively. However, the stone clearance rates between the groups did not significantly differ within the age groups, indicating similar efficacy in achieving a stone-free state (P-values of 0.81 and 0.08). Overall stone clearance rates showed no significant difference between the groups, with 87.9% in Group I and 81.8% in Group II achieving stone clearance (P=0.49). This indicates that while the combined lithotripsy technique significantly reduces operation time and hospital stay, it does not compromise the efficacy of stone clearance when compared to pneumatic lithotripsy alone.

Table 2 outlines the effects of gender and BMI on the outcomes of lithotripsy for staghorn renal calculi, comparing combined ultrasonic and pneumatic lithotripsy (Group I) with pneumatic lithotripsy alone (Group II). For both males and females, Group I experienced significantly shorter mean operation times (P<0.001 for males, P=0.003 for females) and hospital stays (P< 0.001 for males, P=0.01 for females) compared to Group II, indicating a clear advantage of the combined approach in reducing these parameters. However, stone clearance rates were not significantly different between groups for both genders, suggesting similar efficacy in achieving stone-free outcomes regardless of the lithotripsy technique used. Similarly, when categorized by BMI (normal weight versus overweight to obese), Group I showed significantly reduced operation times and hospital stays across both BMI categories (P<0.001), yet stone clearance rates showed no significant difference, indicating comparable effectiveness in stone removal across BMI classifications. These findings highlight the benefits of combined lithotripsy in reducing operation times and hospital stays without compromising stone clearance efficiency, across different patient demographics.

Table 1: *Impact of Age on Outcomes of Lithotripsy Techniques for Staghorn Renal Calculi Management.*

	Category	Group I	Group II	P-value
Effect of age on mean Operation Time	Age Group 15-49 Years	187.26± 18.95	224.0± 19.2	<0.001
	Age Group 50-70 Years	193.6± 15.44	226.00± 18.42	<0.001
Effect of age on mean Hospital Stay	Age Group 15-49 Years	3.40±0.98	4.46±1.30	0.02
	Age Group 50-70 Years	3.55±1.04	4.44±1.46	0.04
Effect of age on Stone clearance Rate	Age Group 15-49 Years	12±0.853	11±0.633	0.81
	Age Group 50-70 Years	17±0.583	16±0.873	0.08
Stone Clearance	Yes	29 (87.9%)	27 (81.8%)	0.49
	No	4 (12.1%)	6 (18.2%)	

Table 2: *Gender and BMI: Their Influence on Lithotripsy Outcomes for Staghorn Renal Calculi*

	Category	Group I	Group II	P-value
Effect of Gender on Mean Operation Time	Male	186.26±12.783	222.63±11.843	<0.001
	Female	201±3.393	228.42±9.892	0.003
Effect of Gender on Mean Hospital Stay	Male	3.56±0.482	4.31±1.042	<0.001
	Female	3.3±0.872	4.64±1.954	0.01
Effect of Gender on Stone Clearance Rate	Male	21±0.042	16±0.742	0.48
	Female	8±0.984	11±1.045	0.93
Effect of BMI on Mean Operation Time	Normal Weight (BMI: <24.9 kg/m2)	188.78±11.053	228.14±12.322	<0.001
	Overweight to Obese (BMI: >25.0 kg/m2)	192.15±10.492	222.84±9.743	<0.001
Effect of BMI on Mean Hospital Stay	Normal Weight (BMI: <24.9 kg/m2)	3.52±0.743	4±0.492	<0.001
	Overweight to Obese (BMI: >25.0 kg/m2)	3.15±0.742	4.78±0.843	<0.001
Effect of BMI on Stone Clearance Rate	Normal Weight (BMI: <24.9 kg/m2)	12±0.853	10±0.733	0.35
	Overweight to Obese (BMI: >25.0 kg/m2)	17±0.643	2±1.322	1.0

Table 3:

	Category	Group I	Group II	P-value
Effect of Duration ofDisease on Mean Operation Time	Duration of disease <1 years	190±21.854	227.14±15.522	<0.001
	Duration of Disease > 1 years	194.8±9.593	213.6±7.583	<0.001
Effect of Duration ofDisease on Mean Hospital Stay	Duration of disease <1 years	3.53±1.422	4.5±1.422	<0.001
	Duration of Disease > 1 years	3.2±0.733	4.2±0.583	<0.001
the Effect of Duration ofDisease on Stone Clearance Rate	Duration of disease <1 years	24±0.432	24±0.633	0.95
	Duration of Disease > 1 years	5±0.482	3±0.873	0.13
Effect of Type ofRenal Stone on Mean Operation Time	Partial Staghorn Stones	190.07±14.783	225.12±13.583	<0.001
	Complete Staghorn Stones	193.14±9.722	225±11.422	0.006
Effect of Type ofRenal Stone on Mean Hospital Stay	Partial Staghorn Stones	3.5±1.001	4.33±0.733	0.02
	Complete Staghorn Stones	3.42±0.073	4.77±0.754	0.02

Table-3 delves into the impact of disease duration and renal stone type on lithotripsy outcomes, comparing combined ultrasonic and pneumatic lithotripsy (Group I) with pneumatic lithotripsy alone (Group II). It demon-strates that irrespective of the disease's duration (<1 year or >1 year), Group I consistently showed significantly shorter mean operation times (P<0.001 for both durations) and hospital stays (P<0.001 for both durations) than Group II, highlighting the efficiency of the combined approach in accelerating recovery. Interestingly, the stone clearance rate was not significantly influenced by the duration of the disease, indicating that both litho-tripsy methods are comparably effective in removing stones regardless of how long the patient has had the disease. Additionally, when analyzing outcomes based on the type of renal stone (partial versus complete stag-horn stones), Group I again benefited from shorter operation times (P<0.001 for partial staghorn stones, P=0.006 for complete staghorn stones) and hospital stays (P=0.02 for both stone types) compared to Group II. These findings suggest that the advantages of combined ultrasonic and pneumatic lithotripsy in reducing operation times and hospital stays extend across different disease durations and stone types, without compromising the efficacy of stone clearance.

Discussion

The management of staghorn renal calculi through lithotripsy is a complex process due to the variability in stone composition, patient demographics, and the physical characteristics of the stones. A study found that a combined ultrasonic and pneumatic lithotripsy approach was associated with significant improvements in operation time and hospital stay across various patient subgroups. The study also found that both male and female patients in Group I benefited from shorter operation times and hospital stays, regardless of age. However, the stone clearance rate did not show significant differences between treatment groups when stratified by age or gender. BMI's role in influencing lithotripsy out-

comes was also examined, revealing that both normal weight and overweight to obese patients experienced better outcomes in Group I. The study also found that the duration of the disease prior to treatment did not significantly impact the likelihood of achieving stone clearance. The type of renal stone (partial versus complete staghorn stones) also influenced treatment outcomes, emphasizing the need for a tailored approach based on the extent of stone formation within the kidney.

Conflict of interest *None*

Funding Source *None*

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Authors Contribution

FK: Conceptualization of Project
NS: Data Collection
MAS: Literature Search
HBM: Statistical Analysis
HAQ: Drafting, Revision

Evaluation of the Anti-Tubercular Role of Nimbolide in Animal Model of Tuberculous Arthritis

Maham Israr,[1] Sabeen Irshad,[2] Amer Hassan Siddiqui,[3] Mah-e-Noor,[4] Shagufta Nasreen,[5] Rida Ayesha[6]

Abstract

Objective: To evaluate the effect of Nimbolide on tuberculous arthritis in mice.

Material and Method: 24 BALB/c mice were divided by simple random sampling into three groups with 8 animals in each group: normal control, diseased control, and treatment group. Tuberculous arthritis was induced by injecting 10mg of Mycobacterial tuberculosis H37Rv strain in 1 ml of normal saline into the tail vein of diseased control and treatment groups. Nimbolide was administered to the treatment group at the dose of 0.2 mg/kg for 4 weeks from the 5th to the 9th week. Mice were sacrificed under ether anesthesia after 9 weeks.

Results: Nimbolide increased body weight in mice affected by tuberculous arthritis ($p < 0.001$). Nimbolide improved the histopathological changes associated with tuberculous arthritis ($p < 0.001$).

Conclusion: Nimbolide has shown anti-tubercular arthritic activity in mice model of tuberculous arthritis. Nimbolide can be a possible agent for the treatment of tuberculosis and tuberculous arthritis.

Keywords: Nimbolide, Tubercular arthritis

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Introduction

Mycobacterium tuberculosis is the causative agent of Tuberculosis (TB), which is an important cause of death. Almost 10 million people are affected by TB and each year 1.5 million people die from TB worldwide.[1]. TB is more common in low-income countries and it further increases poverty. Pakistan ranks 5th among the countries with a high TB burden.[2] TB mainly targets the lungs, but other extra-pulmonary tissues and organs are also involved. Other sites are the lymph nodes, pleura, urogenital tract, and musculoskeletal system. In the musculoskeletal system, TB mainly affects the spine. Other than the spine, TB may affect any bone or joint.[3] TB causes structural damage and deformity leading to impairment of joints and bones. Treatment options are medical and surgical therapy. After surgical treatment, there are high chances of recurrence, which can be prevented by long-term drug therapy.[4]

There are different drug regimens available for the treatment of TB. However these treatment regimens are becoming ineffective owing to the development of drug resistance[5]. The drugs available for the cure of TB are costly, associated with many side effects, and less efficacious. There is an increasing need to explore medicinal plants as a cure for TB. Medications derived from various plants are used for different diseases in many countries. Plant-based medications are cost-effective and easily available. There is also less chance of resistance associated with medicines obtained from plants. Thus, anti-TB drugs derived from plants can be an alternative to classic anti-TB drugs.[6]

Azadirachta indica commonly known as the Neem tree is found in India, Bangladesh, Pakistan, Nepal, Africa, and other tropics. Neem Leaves, bark, and seeds have shown important anti-inflammatory, anti-bacterial, anti-tubercular, anti-protozoal, anti-malarial, anti-arthritic, insect repellent, anti-cancer and immune-modula-

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tory effects.[7] Neem has been used for TB treatment previously.[8]

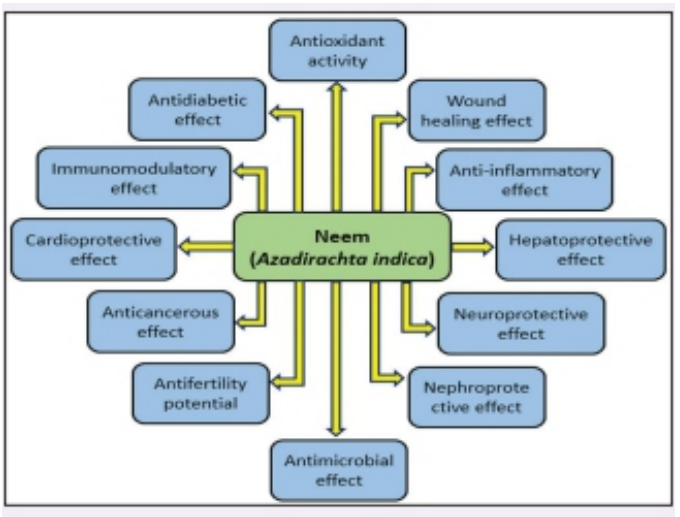

Fig-1: *Picture showing different effects of neem*[9]

Neem is enriched with limonoids. Nimbolide is the limonoid which is isolated from the leaves of neem. It possesses anti-oxidant, anti-cancer, and anti-inflammatory effects.[10,11] It is also shown to have antimicrobial, antimalarial, and anti-HIV activity.[12] Previously, Nimbolide has demonstrated anti-arthritic activity in rheumatoid arthritis induced by freund's adjuvant[13]. This study is designed to look for the anti-tubercular activity of Nimbolide in tubercular arthritis.

Material and Method

It is an experimental study, performed at the Pharmacology Department, Postgraduate Medical Institute (PGMI), Lahore after ethical approval from institutional review board vide letter no. 00-13-A-2023 dated 17-05-2023. The 24 healthy BALB/c mice, 7-9 weeks of age, and weighing 9-12 grams were obtained from the PGMI, Lahore and confined in the animal house of PGMI. The animals were familiarized to 25±2°C temperature, 45-65% humidity, and kept in a light/dark cycle of 12h, under optimal conditions of hygiene. Animals had ad libitum access to rat chow and water. 24 BALB/c mice were divided by simple random sampling into three groups: normal control, diseased control, and treatment group. On day 1, 10mg of Mycobacterial tuberculosis H37Rv strain in 1 ml of normal saline was inoculated into the tail vein of diseased control and treatment groups.[14] 1 ml of saline was injected as a vehicle in the tail vein of the normal control group. Nimbolide was given to the treatment group at the dose of 0.2mg/kg[15] per oral dissolved in 1 ml of 0.1 % DMSO (Dimethyl Sulfoxide) for 4 weeks from the 5th to the 9th week. Mice from the normal and diseased control groups were given 1 ml of 0.1% DMSO per oral from the 5th till the 9th week for 4 weeks. Mice were sacrificed under ether anesthesia after 9 weeks[16]. Each mouse was weighed with a precision balance every week. After sacrificing mice, ankle joints were dislocated and preserved in 10% formalin. Then they were placed in a decalcifying solution for decalcification. Paraffin blocks were made. Then thin tissue slices were cut with the help of a microtome and placed on a glass slide. Slides were stained with hematoxylin and eosin.[3] A histopathologist examined the slides in a blinded manner and looked for inflammation, granuloma formation, and destruction of cartilage. The slides were scored from 0 to 3, where a score of 0 was given when there were no pathological changes. Scores 1 to 3 were given to mild, moderate, and severe changes. To determine statistical significance One-way ANOVA was applied. Post-hoc Tukey's test was applied to determine group mean differences. p-value ≤ 0.05 was reflected as statistically significant.

Results

Tuberculosis is associated with weight loss. Mice in the diseased group exhibited a (p-value <0.001) statistically significant decrease in body weight in comparison to the control group after disease induction to the last day of the study. Mice from the treatment group exhibited a (p-value < 0.001) increase in body weight significantly on days 45 and 60 as compared to the diseased group, as presented in figure no. 2. Tuberculosis is associated with inflammation. There was a significant increase in inflammation in disease control (2.8±0.2) in comparison to normal control as presented in figure no. 7. Mice that were treated with Nimbolide showed a statistically significant reduction in inflammation (1.7 ± 0.4 vs 2.8±0.2) in comparison to mice in the diseased control group as presented in figure no. 3 and 9. It shows the anti-inflammatory potential of Nimbolide and advocates that Nimbolide can be used as an anti-inflammatory agent. Granulomas are a hallmark of TB infection. There was a significant increase in granuloma formation in diseased control (2.7 ± 0.2) in comparison to normal control as presented in figure no. 8. Mice who were given treatment with Nimbolide showed a statistically significant decrease in granuloma formation (1.1± 0.3 vs 2.7 ± 0.2) in comparison to mice in the diseased control group as presented in figure no 4. It shows the anti-tubercular potential of Nimbolide and proposes that Nimbo-

lide can be used as an anti-tubercular agent. Tubercular arthritis is associated with the destruction of articular cartilage. There was a significant destruction of cartilage in diseased control (2.2 ± 0.3) as compared to normal control. Mice who were given treatment with Nimbolide showed a decrease in cartilage destruction which was statistically significant (1.2± 0.4 vs 2.2 ± 0.3) in comparison to mice in the diseased control group as presented in figure no 5. These findings show the anti-arthritic potential of Nimbolide and suggests that Nimbolide can be used as an anti-arthritic agent in tuberculous arthritis.

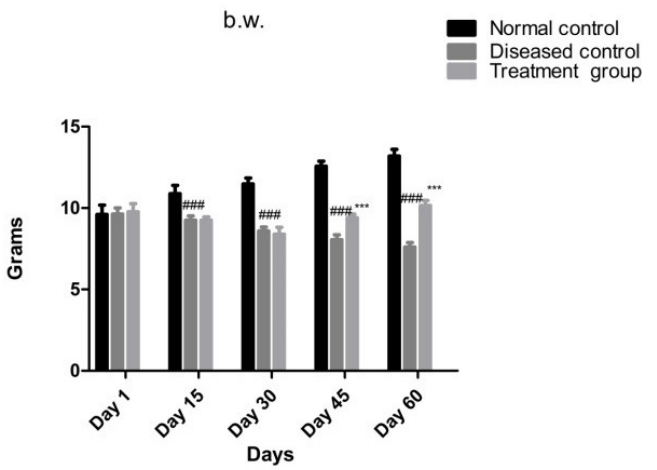

Fig-2: *Representation of the mean ± SD of body weight graphically in all groups (n = 8). ### shows p-value < 0.001 and shows a difference as compared to the control group significantly. *** shows p-value < 0.001 shows a difference as compared to the diseased group significantly.*

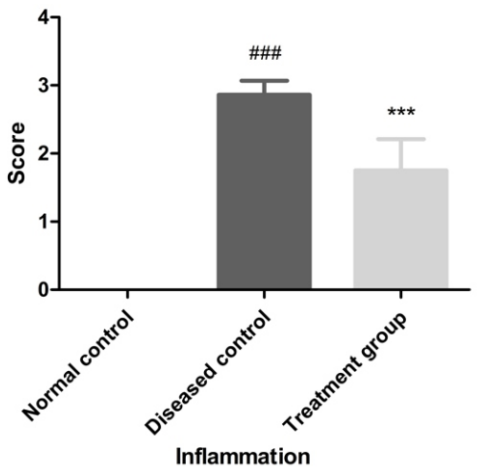

Fig-3: *Representation of the mean±SD of inflammation graphically in all groups (n = 8). ### shows p-value < 0.001 and shows a difference as compared to the control group significantly. *** shows p-value < 0.001 shows a difference as compared to the diseased group significantly.*

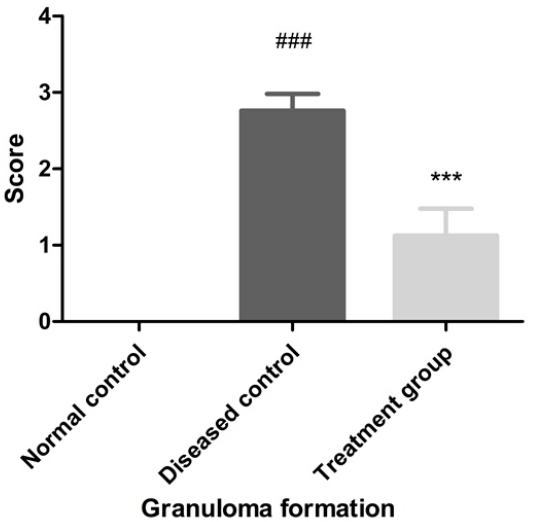

Fig-4: *Representation of the mean±SD of granuloma formation graphically in all groups (n = 8). ### shows p-value <0.001 and shows a difference as compared to the control group significantly. *** shows p-value < 0.001 shows a difference as compared to the diseased group significantly.*

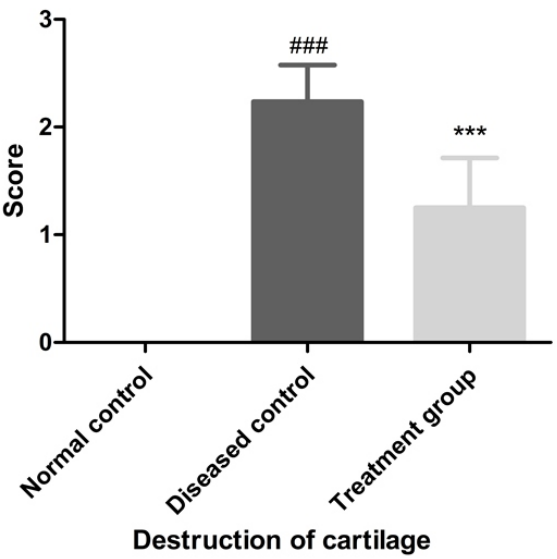

Fig-5: *Representation of the mean±SD of destruction of cartilage graphically in all groups (n = 8). ### shows p-value < 0.001 and shows a difference as compared to the control group significantly. *** shows p-value < 0.001 shows a difference as compared to the diseased group significantly.*

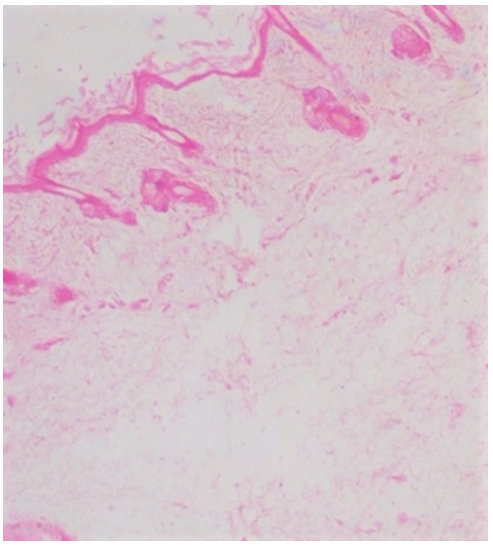

Fig-6: *Photomicrograph (ankle joint) displaying unremarkable inflammation (H & E; 4x) (Normal control)*

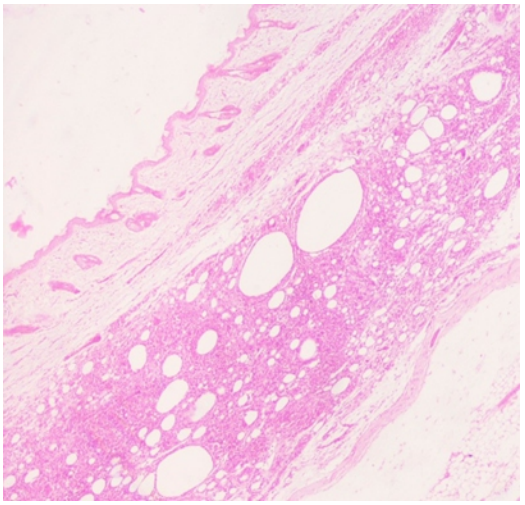

Fig-7: *Photomicrograph (ankle joint) displaying intense inflammation (H & E; 4x) (Diseased control)*

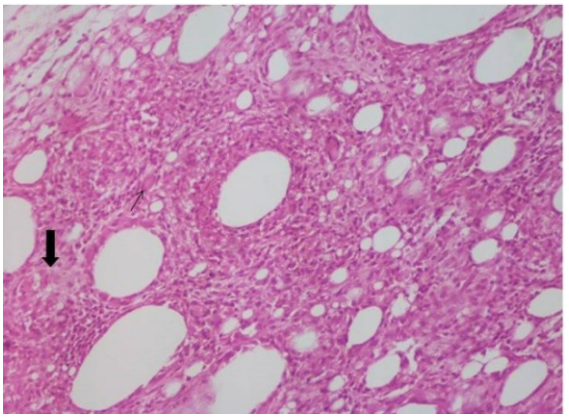

Fig-8: *Photomicrograph (ankle joint) displaying granuloma formation along with giant cell (H & E; 20x) (Diseased control)*

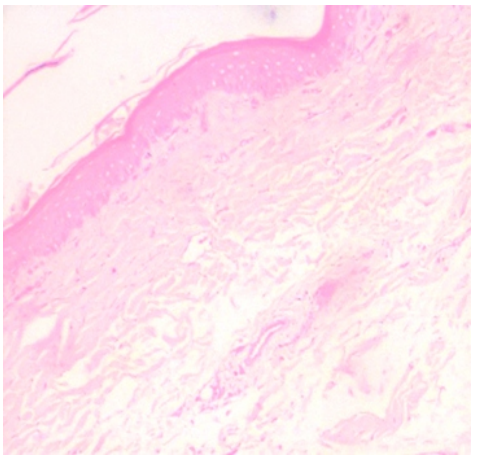

Fig-9: *Photomicrograph (ankle joint) displaying unremarkable inflammation (H & E; 4x) (Treatment group)*

Discussion

Currently different drug regimens are used for the treatment of tuberculosis. However, there is an increased incidence of therapeutic failure due to emergence of anti-tubercular drug resistance.[17] Different medicinal plants are reported to have anti-tubercular potential and can be used as an adjunct drug to standard treatment for TB. Azadirachta indica (Neem) has proven anti-fungal, anti-bacterial, anti-viral, and anti-parasitic properties.[18] In a previous study, neem bark extract cured TB in animals infected with Mycobacterium tuberculosis H37Rv strain and it was non-toxic to the animals.[19] Nimbolide is obtained from neem leaves and it possesses anti-bacterial potential against many resistant micro-organisms.[20] In our present study mice with tuberculous arthritis showed a decrease in body weight while treatment with Nimbolide improved the body weight of mice. It has been reported previously that prolonged inflammation in TB leads to weight loss.[21] It signifies the anti-inflammatory and anti-tubercular roles of Nimbolide which is in line with a previous study where Nimbolide exhibited anti-inflammatory potential.[11] Histopathological examination of mice having tuberculous arthritis showed inflammation, granuloma formation, and cartilage destruction. These findings are in agreement with a previous study where TB infection was linked to inflammatory changes and granuloma formation.[3] Treatment with Nimbolide decreased inflammation and granuloma formation and it also restored the articular cartilage in diseased mice. These findings are in accordance with previous studies, where Nimbolide improved arthritic changes in arthritic rats.[22] These results propose the anti-tubercular and anti-arthritic roles of Nimbolide in tuberculous arthritis.

Conclusion

Nimbolide has shown anti-tuberculous arthritic activity in mice model of tuberculous arthritis. It improved body weight in tuberculous arthritic rats and improved the histopathological changes associated with tuberculous arthritis. Nimbolide has the potential to be used for the treatment tuberculous arthritis.

Conflict of Interest: **None**

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Authors Contribution

MI, SI: Conceptualization of Project
MI: Data Collection
SI: Literature Search
MN: Statistical Analysis
AHS: Drafting, Revision
RA: Writing of Manuscript

Picking The Odd One Out: Cephalic Tetanus – A Case Report From Rural Pakistan

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Abstract

Tetanus is an infectious disease with a high risk of mortality. It is caused by an exotoxin, tetnospasmin, produced by Clostridium tetani, an anaerobic gram positive bacillus. The risk of disease is high in the general population due to the abundance of spores in the environment. There are four clinical types of tetanus, generalized, localized, neonatal and cephalic. Cephalic tetanus is one of the rarest clinical form that presents with trismus and cranial nerve involvement. Cephalic tetanus can be difficult to diagnose since it doesn't often manifest with the typical tetanus symptoms like muscle spasms and stiffness. This case is of a 55 year old male who presented to a tertiary care hospital with symptoms consistent with Bell's palsy for 5 days that progressed to development of trismus and neck muscle stiffness with difficulty of breathing that lead to his referral from rural healthcare setup. The case highlights the importance of detailed history and examination with high grade suspicion to make a clinical diagnosis of cephalic tetanus in a country with epidemiological evidence of disease.

Keywords: Cephalic tetanus, Bell 's palsy, Infectious disease

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Introduction

Tetanus is a disease that causes tonic muscular spasms as it advances, caused by Tetanospasmin, an exotoxin from the anaerobic Clostridium tetani bacteria. There are four clinical types of tetanus, generalized, localized, neonatal and cephalic. Cephalic tetanus is one of the rarest clinical form, it impacts the cranial nerves and accounts for 0.9-3% of cases overall.[1] Cephalic tetanus can be difficult to diagnose since it doesn't often manifest with the typical tetanus symptoms like muscle spasms and stiffness. The combination of trismus with paralysis of one or more cranial nerves is known as cephalic tetanus and the facial nerve is mostly involved.[2] There is a lack of case reports of cephalic tetanus therefore, it is a clinical topic to further explore.

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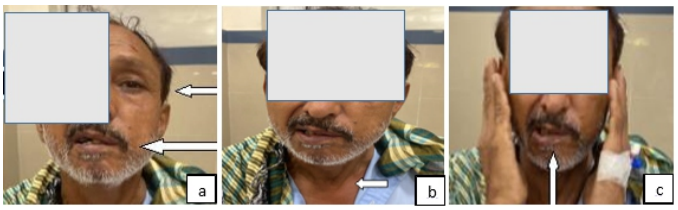
Case Presentation

A non-diabetic, normotensive, nonsmoker 55 years old male presented to the Emergency Department of Holy Family Hospital from a rural area of Pakistan with the history of deviation of angle of mouth to right side and drooling of saliva for last 5 days. He also developed difficulty in opening mouth leading to inability to eat and chew for last two days .He was being managed on lines of Bell's palsy at the periphery. One day before presenting to tertiary care hospital, he developed stiffness of neck muscles with difficulty in breathing and worsening jaw stiffness. He denied any spasm in other parts of body. There was no history of fits or loss of consciousness. Past history revealed that he had a fall while working in the fields about two weeks ago resulting in small lacerations over his knee and face. He took analgesics and local dressing of the wound but did not receive tetanus toxoid booster dose. History for past vaccination status was also negative for any immunization.

On examination he was anxious and agitated, with the following vitals; heart rate 180/min with regular rhythm, blood pressure 120/70 mmHg, and respiratory rate 16/min and oxygen saturation 92% at room air. Mouth

opening was barely one finger across, angle of mouth was deviated to the right side, there was failure to close left eye with loss of nasolabial fold on left side. Facial palsy of lower motor neuron type with House-Brackmann Grade V was observed. He had stiffness of neck muscles (Figure 1 a-c) and difficulty breathing. The rest of the systemic examination was unremarkable. A differential diagnosis of cephalic tetanus was made on clinical grounds with conditions like brain stem stroke and other causes of Bell's palsy that were excluded.



Investigations including baselines blood tests (table 1) and radiological imaging came out to be normal. Renal and liver function tests were also normal. CT scan brain was unremarkable. Nerve conduction study wasn't necessary because of the peripheral character of the facial paralysis, its short duration of evolution, and the absence of traumatic cause to give an idea of the possible interest of surgical exploration.

Test Name	Patient's Value	Reference Value
Hemoglobin (Hb)	16.5g/dl	14-17 g/dl [3]
Total Leucocyte Count (TLC)	10100/µl	4500-11000/µl [3]
Platelet Count	135,000/µl	150,000-350,000/µl [3]
Serum Sodium	144mEq/l	135-146mEq/L [3]
Serum Potassium	4.3mEq/l	3.5-5.0mEq/L [3]
Serum Calcium	9.3mg/dl	9-10.5 mg/dl [3]

Patient was initiated on the treatment for cephalic tetanus; injection tetanus toxoid and tetanus immunoglobulin along with intravenous antibiotics metronidazole and ceftriaxone. Intravenous midazolam was also added. Consent was taken for tracheostomy, but the breathing difficulty and neck muscle stiffness improved with treatment, so it was deferred. Patient was managed in Medical ICU for a week and showed marked improvement in the symptoms and discharged in stable condition.

Discussion

Tetanus is an infectious disease with a high risk of mortality. It is caused by the spores of Clostridium tetani, an anaerobic gram positive bacillus. The risk of disease is high in the general population due to the abundance of spores in the environment, particularly soil, ash, animal excreta and rusty tools. The disease burden has been fairly reduced from the world as a result of effective immunization strategies, however, it is an important public health concern in developing countries due to lack of vaccination.[4] Global incidence has decreased from an estimated 615,000 cases globally in 1990 to about 74,000 in 2019.[5] In the year 2020, tetanus accounted for 0.52% deaths in Pakistan. The age adjusted death rate of 2.15 per 100,000 of population ranks Pakistan 7th in the world.[6]

Cephalic tetanus is a rare form of tetanus that manifests as trismus and cranial nerve involvement. Facial nerve is the most commonly affected cranial nerve. Cephalic tetanus accounts for 1 to 3% of the total number of reported cases of tetanus and has a mortality of 15 to 30%.[7] The mode of transmission is through spore contamination of a craniofacial injury or secondary to otitis media.[8] Cases of cephalic tetanus arising from dental origin have been noted.[9] Rare incidences have been reported where any injury could not been identified making the diagnosis fairly challenging.[10,11] In our case, the portal of entry was through injury on the face as a result of fall in the field which was brought into knowledge through history. Tetanus is a clinical diagnosis which is supported by the epidemiological evidence of disease in the community, therefore thorough history and examination with high grade suspicion should be relied on for initiating the treatment. There is no diagnostic investigation for tetanus, however, serum immunoglobulin levels prior to the administration of anti-tetanus immunoglobulin can be of some help, although the disease has been described in the presence of antibody levels that are considered to be protective.[10] Tetanus can be misdiagnosed as a number of medical emergencies including electrolyte disturbances, seizures, meningitis and stroke, but these conditions usually lack the characteristic features of the disease. However, these may cause a delay in diagnosis that may lead to the progress of the disease to generalized form. In our case, the patient was being managed as a case of Bell's palsy before he developed lock jaw and difficulty in breathing. The differentials were ruled out by a normal brain imaging and electrolyte studies and the presence of trismus and neck muscle stiffness suggesting otherwise. Nerve conduction study was not required due to a peripheral lesion of short course of disease with no surgical cause behind it.[2]

Cases of cephalic tetanus have been reported with only facial nerve involvement of peripheral type. It may also precede the development of trismus like we observed in our case.[2,12] Since cephalic tetanus is a clinical diagnosis, this presentation is a reason in diagnostic delay, which in our patient was observed to a limited extent owing to the timely referral of the patient to a tertiary care facility.

The clinical diagnosis is followed by immediate treatment with tetanus toxoid, anti-tetanus immunoglobulin, antibiotics and symptomatic management using sedatives, oxygen supplementation and in severe cases, tracheostomy. In the case presented, tracheostomy, although planned, was deferred due to good clinical response of the patient to active and passive immunization and antibiotics.

Conclusions

Tetanus is a pure clinical diagnosis, and in cases of generalized tetanus, diagnosing the case may not be challenging for a physician working in Pakistan as it is common due to poor vaccination status. On the other hand, as the presentation of a case of localized or cephalic tetanus might vary, making it challenging to diagnose it early therefore reporting such cases hold great significance to make physicians aware about it for the best interest and safety of the patient.

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