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Editorial

Cutting Corners: Dilemma of shortcuts in Research Paradigm

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Massive increase in economic challenges, high inflation, political instability, social unrest, and unemployment in the last few years has enhanced "Brain Drain" in Pakistan. Unfortunately educated, skilled, and competent individuals leave the country to seek favorable and high salaried job opportunities across the world. Top on the list are doctors who leave the country for two reasons: one is post-graduate training and residency and secondly for better career and mone-tary incentives.[1] One of the basic parameters required to be eligible for jobs in western world e.g., USA, UK etc. is "Research", as it is believed to enhance problem solving and critical thinking skills of an individual as well as improve the knowledge of scientific methods.[1] Addition of research experience to a profile helps the admissions committees in selection in highly competitive programs. Research experience reveals dedication of individuals in their fields. Preference is given to those who have participated in clinical or laboratory research, have publications to their list and understand research methodology and data analysis.[2]

To meet this criterion, medical students and fresh graduates look for shortcuts to add research to their portfolio. Challenges of learning academics and clinical skills burden these youngsters along with limited time and finances.[3] They seek help from senior colleagues to be added to some ongoing project. Some of them want to learn the steps involved and be a part of the whole process but others have a wish to be added as an author to some publication without substantive contribution to work. This type of gift authorship can distort the credit of the authors and leads to ethical concerns, such as fairness and honesty. Academic and professional organizations discourage such authorship and label these as unethical. To be an author one should have intellectual contribution to the study design, analysis, interpretation, writing and review of the paper.

Research in science is not additional, it's absolute and without inquisitive minds the field of medicine will stand still. Knowledge of research enables the doctors to evaluate their practice and polishes them to advance their discipline. The ability to question, evaluate and then test and apply different approaches in different scenarios are essential to be a critical thinker and life-long learner.[4] The essence of research is lost when shortcuts are sought by the undergraduates or fresh graduates. Except the few who participate in different steps like concept, data collection, analysis and writeup, others are participating in false practices. To tackle this issue, it is crucial to focus on the following inquiry: "What motivates intellectually capable individuals to seek out expedient solutions?" Deficient knowledge and exposure, curriculum overload, internet inexperience, an uncooperative community, difficulty in selecting a topic, and limitation of financial resources along with mentors in the field, all fit to the answer.[3] Instead of blaming undergraduate and fresh graduate doctors, a constructive approach is required. Senior health professionals and higher authorities in the government should develop the infrastructure to conduct research. The areas that need to be addressed include significant resources including financial support, equipment, and experienced researchers. Another important pivotal point is developing research culture. Students should be encouraged at undergraduate level to be engaged in research and this can be achieved by arranging mentorship programs, workshops and establishing research centers and promoting collaboration among institutes and universities. Research and publication of scientific papers during

undergraduate studies is a basic pillar that all medical students should learn to develop from the beginning of their careers, beyond the enrichment of their resumes as future physicians. This will develop the substantial skills and competencies that allow them to respond in the most effective way to the needs of global health.[5] The responsibility lies on senior health professionals, higher authorities e.g., health ministry and ministry of education, Higher education commission, Pakistan Medical and Dental Council etc. to include research as an essential part of the curriculum, promote the environment and encourage a culture of high quality research.

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Original Article

Comparative Immune Profiles: Platelet-to-Lymphocyte and Neutrophil-to-Lymphocyte Ratios in Preeclampsia versus Healthy Women

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Abstract

Background: Pre-eclampsia is a hypertensive disorder linked with immune dysregulation and systemic inflammation in pregnancy. An elevated neutrophil count alongside a relatively stable lymphocyte count leads to an increased Neutrophil-to-Lymphocytic Ratio (NLR), serving as a parameter of widespread systemic inflammation. Platelet to lymphocyte ratio (PLR) is a also used as a hematological marker that reflects the balance between platelets and lymphocytes. A higher NLR and PLR may reflect increased endothelial activation and damage, contributing to the pathogenesis of preeclampsia.

Objective: The objective of the present study was to compare the Neutrophil to lymphocytic ratio (NLR as well as platelet-to-lymphocytic ratio (PLR) between women affected by preeclampsia (PE) and those with uncomplicated pregnancies.

Methods: It was a cross-sectional comparative study. Fifty-five registered preeclamptic patients were enrolled as cases along with fifty-five healthy pregnant women. Total leukocyte counts, including neutrophils and lymphocytes as well as platelet count, were calculated using an automated hematology analyzer, and the Neutrophil-to-Lymphocyte Ratio (NLR) as well as Platelet to Lymphocyte ratio were subsequently calculated.

Results: Higher value of TLC and neutrophils were recorded in pre-eclampsia. The normotensive women had higher platelet as well as lymphocyte count. NLR was significantly high in the preeclampsia contrary to PLR ratio that showed a higher value in normotensive pregnancies.

Conclusions: In conclusion, our study highlights significant alterations in the immune-inflammatory profile of patients with preeclampsia compared to controls. The observed high neutrophil-to-lymphocyte ratio (NLR) and low platelet-to-lymphocyte ratio (PLR) suggest a distinct systemic inflammatory response associated with preeclampsia.

Keywords: Preeclampsia, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

Introduction

Pre-eclampsia, is a hypertensive disorder affecting about 2-8% of pregnancies worldwide, characterized by elevated blood pressure and proteinuria after 20 weeks of gestation and is linked with notable fetal and maternal health complications and fatalities.[1] Preeclampsia is linked with immune dysregulation, endothelial dysfunction, and systemic inflammation, all of which contribute to its pathogenesis.[2] The dysfunction of the immune system plays a crucial role in the pathophysiology of preeclampsia. In a typical pregnancy, there's a modified innate immune response characterized by heightened inflammation and active complement system. This response is heightened even more in pregnancies complicated by preeclampsia.[3] Preeclampsia is

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widely regarded as a two-stage disorder, with the initial phase implicating defective trophoblast invasion and failure in the remodeling of spiral arteries, which are fundamental steps in its pathogenesis.[4] Consequently, this leads to compromised uteroplacental blood flow, resulting in hypoxia. Oxidative stress within the placenta ensues, prompting the release of various factors including proinflammatory cytokines, antiangiogenic agents, exosomes, and cell-free fetal DNA, can enter the maternal circulation.[5] These factors contribute to disrupted endothelial regulation with marked increase in permeability of the vessels. Both neutrophils and platelets can exacerbate endothelial dysfunction. Increased production of superoxide and decreased nitrite release, leading to the activation and modulation of neutrophils, are linked to widespread endothelial dysfunction in preeclampsia. Preeclampsia appears to be characterized by heightened levels of inflammation compared to uncomplicated pregnancies, with preeclamptic patients exhibiting an increase in leukocyte count, primarily driven by elevated neutrophil levels and decreased lymphocyte levels.[6,7] Neutrophils release pro-inflammatory cytokines and reactive oxygen species, while platelets release various soluble and adhesion molecules, promoting interactions with endothelial cells. This cascade of events has sparked considerable interest in the exploration of various biomarkers. It is vital to promptly identify and precisely predict preeclampsia (PE) for optimal management and better outcomes for affected women and their offspring. An elevated neutrophil count alongside a relatively stable lymphocyte count leads to an increased Neutrophil-to-Lymphocytic Ratio (NLR), serving as a parameter of widespread systemic inflammation.[7] Platelet to lymphocyte ratio (PLR) is a hematological marker that reflects the balance between platelets, which are involved in clotting and inflammation, and lymphocytes, a pivotal component of the body's immune system is a type of white blood cell essential for mounting an effective defense against pathogens. The platelet to lymphocytic ratio (PLR) as an alternative marker has arisen as a significant indicator in evaluating widespread generalized inflammation and immune response in a range of autoimmune and cardiovascular conditions.[8] A higher NLR and PLR may reflect increased endothelial activation and damage, contributing to the pathogenesis of preeclampsia. Although certain studies have proposed these ratios as a potential indicator for evaluating inflammation and immune response in preeclampsia (PE), the research outcomes remain inconclusive.[9,10,11] The objective of the present study was to compare the Neutrophil to lymphocytic ratio (NLR as well as platelet-to-lymphocytic ratio (PLR) between women affected by preeclampsia (PE) and those with uncomplicated pregnancies.

Methods

It was a cross-sectional comparative study. The place of the study was the Physiology department at Federal Postgraduate Medical Institute, Shaikh Zayed Hospital, Lahore, in collaboration with two other tertiary care hospitals in Lahore, spanning from March 2012 to April 2012, following approval from the respective Ethical Review Boards of those institutions. The study adhered to all ethical standards set by the Institutional Ethical Review and complied with the principles outlined in the 1975 Helsinki Declaration, which was revised in 2000. All the participants were briefed about the study and included in the study after their consent and written informed consent. To calculate the sample size, the formula for two population means, provided by the World Health Organization (WHO) calculator was used[12] and fifty-five registered preeclamptic patients were enrolled as cases, meeting criteria of a more than 140 mmHg of systolic and more than 90mmHg of diastolic blood pressure on multiple occasions, six hours apart, along with proteinuria of a minimum 300 mg in urine in 24-hour. Fifty-five healthy pregnant women with normal blood pressure, paired based on age and gestational age, with normal Body Mass Index, were incorporated as controls through a convenience sampling technique. Subjects suffering from chronic diseases such as diabetes mellitus, pregestational hypertension, renal disease, multi-fetal pregnancy, liver disease, history of smoking, or previous history of preeclampsia were omitted from the study. Blood pressure measurements were documented, maternal venous blood samples were collected in EDTA-coated vacutainers. Total leukocyte counts, including neutrophils and lymphocytes as well as platelet count, were calculated using an automated hematology analyzer, and the Neutrophil-to-Lymphocyte Ratio (NLR) as well as Platelet to Lymphocyte ratio were subsequently calculated.

SPSS 16 was used to analyze the Data. Shapiro wilk test of normality was applied to check the distribution of the data. Mean ± SD was given for all those parameters that showed normal distribution. Independent samples T test was used to compare the values between preeclamptic and normotensive pregnancies. A p value < 0.05 was considered statistically significant.

Results

Out of the total 110 patients, 55 presented with pree-

Table I: *Demographic and medical characteristics of healthy and preeclamptic pregnancies*

Variable	Group		p Value
	Normotensive n = 55	Preeclampsia n = 55	
Maternal age (years)	25.83± 4.8	27.32 ± 4.3	<0.001*
Gestational age(weeks)	37.8 ± 1.36	36.51 ± 2.0	0.03
BMI	22.80 ± 3.0	23.76 ±1.2	<0.001*
Systolic blood pressure(mmHg)	111.27 ± 7.4	147.81 ± 8.0	<0.001*
Diastolic blood pressure(mmHg)	72.00 ± 6.2	99.63 ± 5.6	<0.001*

n = number of study participants, p value < 0.05 considered significant shown with hysteric*, Independent samples t test applied to calculate p value.

clampsia while the remaining 55 exhibited normal healthy pregnancies. The maternal age as well as gestational age of the participants at the time of sample collection was not different statistically as shown in Table I. Hematological parameters including Total leukocyte count (TLC), Neutrophil count, Lymphocyte count and platelet count demonstrated a notable variance in outcomes between the case group and controls with higher value of TLC, neutrophils, and NLR in preeclampsia (Table II). The normotensive women had higher platelet as well as lymphocyte count. NLR was significantly high in the preeclampsia contrary to PLR ratio that showed a higher value in normotensive pregnancies (Figure I).

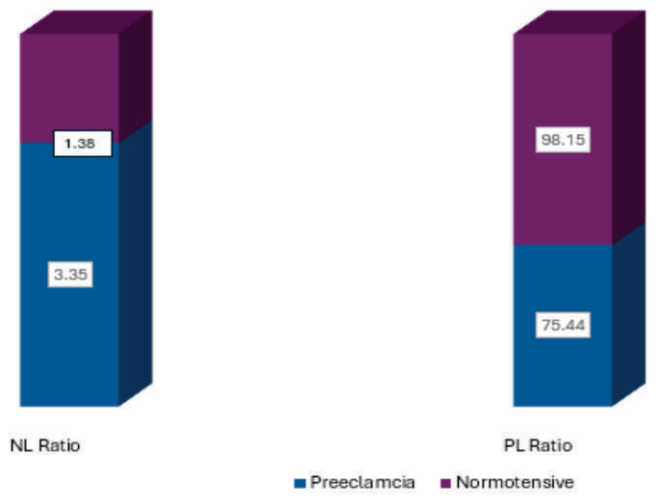

Figure I: *NL Ratio and PL Ratio in preeclampsia and normotensive pregnancies.*

Table II: *Hematological characteristics of normal and preeclamptic pregnancies*

Variable	Group		p Value*
	Normotensive	Preeclampsia	
Total Leukocyte count	7072 ± 1620	9890 ± 1458	< 0.001
Neutrophil count×10^3µL	5.32 ± 0.36	6.88 ± 0.60	< 0.001
Lymphocyte count×10^3cu.mm	3.88 ± 0.40	2.14 ± 0.45	< 0.001
Platelet count×10^3cu.mm	377 ± 34.0	159.22±36.7	< 0.001

p value < 0.05 considered significant shown with hysteric*, Independent samples t test used to calculate p value.

NL Ratio: Neutrophil to lymphocytic ratio, PL Ratio: Platelet to lymphocytic ratio, a p value of < 0.05 considered significant, calculated by independent samples t test.

Discussion

The findings of our current study highlight a significant increase in total leukocyte number and neutrophil-to-lymphocytic ratio (NLR) among patients of preeclampsia compared to those with healthy pregnancies. The findings align with several studies reinforcing the significance of persistent and heightened systemic inflammation in the development of endothelial dysfunction in preeclampsia with similar alterations in hematological parameters.[6,7] Preeclampsia's etiology is multifaceted, with the most prevalent theory revolving around inadequate placental development, resulting in hypoxia and the release of proinflammatory cytokines. Additionally, there's evidence indicating immune maladaptation in preeclampsia. High total leukocyte count and elevated neutrophil levels play pivotal role in the development of preeclampsia, reflecting underlying systemic inflammation and endothelial dysfunction.[13] An elevated neutrophil count alongside a relatively stable lymphocyte count leads to an increased Neutrophil-to-Lymphocytic Ratio (NLR), serving as a parameter of widespread systemic inflammation. In line with our research, Oylumlu et al. illustrated that elevated value of the neutrophil-to-lymphocytic ratio (NLR) was independently linked with patients of preeclampsia (PE) compared to healthy pregnancies.[14]

Neutrophils, as key mediators of the inflammatory response, contribute to the release of pro-inflammatory mediators and reactive oxygen species, accelerating endothelial injury and vasoconstriction.[15] This inflammatory cascade leads to impaired placental perfusion, endothelial dysfunction, and the characteristic hypertension and proteinuria seen in preeclampsia. Additio-

nally, neutrophils may exacerbate oxidative stress and damage to vascular endothelium, further perpetuating the pathophysiological processes underlying this hypertensive disorder of pregnancy.

Additionally, we observed a notable decrease in platelet count in individuals with pre-eclampsia and this observation aligns with the well-established association between PE and platelet dysfunction.[16] In preeclampsia, low platelet count, or thrombocytopenia, can result from several interconnected mechanisms. One primary cause is endothelial dysfunction and microangiopathy, leading to platelet activation, consumption, and subsequent sequestration within damaged vessels.[17] Additionally, abnormal placental development in preeclampsia can trigger an increase in the levels of anti-angiogenic factors like soluble fms-like tyrosine kinase-1 (sFlt-1), which contribute to platelet aggregation and consumption. Moreover, the systemic inflammation characteristic of preeclampsia can lead to increased platelet destruction and impaired production in the bone marrow.[18] Ultimately, thrombocytopenia in preeclampsia contributes to the risk of coagulopathy, hemorrhage, and adverse maternal and fetal outcomes. However, it is crucial to pay attention to the presence of studies with contrasting findings. Some investigations have reported no significant differences in total leukocyte count, NLR, or platelet count between preeclamptic and normotensive pregnancies.[19] These conflicting results may be attributed to variations in study populations, sample sizes, diagnostic criteria for preeclampsia, and methodologies used for hematological assessments. The current study reported a lower platelet to lymphocyte ratio (PLR) in pre-eclampsia in accordance with previous research,[20] shedding light on potential biomarkers and underlying mechanisms of this complex disorder. Platelets and lymphocytes are integral components of the immune system, and alterations in their ratio may reflect systemic inflammation and immune dysregulation, both of which are implicated in the pathophysiology of preeclampsia. The elevated PLR observed in preeclampsia suggests a possible imbalance between pro-inflammatory and anti-inflammatory responses.[21] Platelets play a crucial role not only in hemostasis but also in inflammatory processes, where they release various mediators that can exacerbate endothelial dysfunction and promote vascular inflammation. Meanwhile, lymphocytes are key regulators of immune responses, and alterations in their levels could signify immune system dysregulation, further contributing to the inflammatory milieu observed in preeclampsia.[22]

Activated platelets release a variety of soluble and adhesion molecules that initiate interactions among platelets, leukocytes, and endothelial cells. Previous data indicate a significant contribution of platelets to the pathogenesis of preeclampsia.[23] Elevated ratio of Neutrophil to Lymphocytes as well as Platelet to Lymphocyte suggest a disturbance between inflammatory (neutrophils and platelets) and anti-inflammatory (lymphocytes) components of the immune system. This imbalance may contribute to the inflammatory cascade observed in preeclampsia, leading to endothelial dysfunction and hypertension.[24] The implications of a higher PLR in preeclampsia help in understanding the relationship between PLR and preeclampsia could potentially lead to the progression of new treatment approaches targeting immune dysregulation and inflammation in affected individuals. The identification of a higher PLR in preeclampsia highlights the intricate interplay between inflammation, immune dysregulation, and vascular dysfunction in the pathogenesis of this condition. Furthermore, while this study adds knowledge to the existing literature and supports the fact that there is a link between altered hematological parameters and preeclampsia, it is imperative to acknowledge the necessity for additional research to clarify the underlying mechanisms and clinical implications of these hematological changes. Longitudinal studies with larger sample sizes and multicenter collaborations may provide more comprehensive insights into the hematological alterations associated with pre-eclampsia and their implications for maternal and fetal health outcomes.

Conclusion

In conclusion, our study highlights significant alterations in the immune-inflammatory profile of patients with preeclampsia compared to controls. The observed high neutrophil-to-lymphocyte ratio (NLR) and low platelet-to-lymphocyte ratio (PLR) suggest a distinct systemic inflammatory response associated with preeclampsia. The alteration in neutrophil-to-lymphocyte ratio (NLR) and low platelet-to-lymphocyte ratio (PLR) may appear earlier in the course of disease far before the classic cascade of PE begins. Hence these findings underscore the potential utility of NLR and PLR as biomarkers for the early identification and monitoring of preeclampsia. This will improve both perinatal morbidity and mortality. Further research is warranted to elucidate the under-

lying mechanisms driving these immune alterations and their implications for the pathogenesis and management of preeclampsia.

Conflict of Interest: None

Funding Disclosure: None

Ethical Consideration: The study was approved by the ethical review board. Informed written consent was obtained from the participants, and the confidentiality of their data was clearly explained.

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Authors Contribution:

All the authors contributed equally in accordance with ICMJE guidelines.

MR: Study design and concept, Acquisition of data and final approval

MZ: Data Collection, analysis and final revision

BI: Data Collection, statistical analysis and final revision

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Original Article

Lipid Profile and Ankle Brachial Index in Obese Male Subjects with Obstructive Sleep Apnea: Cross sectional analytical study

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Abstract

Background: Obstructive sleep apnea (OSA) and dyslipidemia are common medical disorders that independently increase vascular morbidity and mortality. Currently, there is no conclusive data indicating that Obstructive Sleep Apnea serves as a risk factor for disrupted lipid profiles and subclinical atherosclerosis, as assessed by ankle-brachial index (ABI).

Objective: The objective of this cross-sectional study was to assess lipid profiles, blood sugar levels, and ankle brachial index in obese male participants with OSA and to compare them with obese individuals lacking OSA.

Methods: In the present study, 64 obese males with BMI > 25kg/m2 were included between ages 20 - 45 years. Subjects having acute or chronic inflammatory conditions were excluded. Obstructive sleep apnea (OSA) diagnosis involved two subjective assessment tools, the Berlin and STOP BANG questionnaires, followed by overnight portable pulse oximetry. The study participants were partitioned into two distinct groups, 32 with OSA and 32 without OSA. Following an overnight fast lasting between 10 to 12 hours, blood samples were collected. Fasting blood glucose and lipid profiles were then analyzed using a spectrophotometer. ABI was measured by using a Doppler ultrasound device. The data was collected and then entered SPSS version 22 and analyzed. The study utilized both Independent Samples t-test as well as Mann-Whitney U test to analyze and compare the quantitative variables across the two groups.

Results: Comparison of ABI between the study groups showed a non-significant difference p-value= 0.435. Fasting blood sugar levels showed insignificant difference (p-value=0.778) among the study groups. Comparison between the groups showed a nonsignificant difference in triglyceride levels (p=0.413) cholesterol (p-value=0.523), HDL (p-value=0.190), and LDL (p-value=0.888).

Conclusion: Normal lipid profile and normal ABI indicate the absence of detectable atherosclerosis in young apparently healthy OSA subjects without known comorbidities.

Keywords: Obstructive sleep apnea, Ankle-brachial index, Dyslipidemia

Introduction

Obstructive sleep apnea (OSA) manifests as the partial or complete collapse of the upper airway during sleep, leading to decreased intrathoracic pressure, intermittent hypoxia, and disrupted sleep patterns.[1] This condition is notably prevalent among obese males.[2] Moreover, despite being frequently underdiagnosed, OSA is notably linked to elevated rates of cardiovascular morbidity and mortality.[3] The connection between OSA and cardiovascular risk stems from several key mechanisms: repeated intermittent hypoxia, stimulation of the

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central nervous system, and fluctuations in intrathoracic pressure. These processes trigger oxidative stress, inflammation, disruptions in vasomotor function, and heightened sympathetic activity. Ultimately, they contribute to the development of atherosclerosis in the blood vessels.[4] Dyslipidemia stands out as a significant risk factor for cardiovascular diseases and is notably prevalent in OSA.[5] Moreover there is an increased risk for atherosclerosis in male patients with severe OSA.[6] The Ankle Brachial Index (ABI) reflects the ratio between ankle and brachial systolic blood pressure, serving as a valuable marker for subclinical atherosclerosis. ABI values below 0.9 indicate the presence of peripheral arterial disease (PAD) and serve as a robust predictor of cardiovascular events.[7] ABI serves as a valuable tool for evaluating both the risk of atherosclerosis and the extent of coronary involvement in suspected patients.[8] While obstructive sleep apnea is linked to cardiovascular diseases, the precise underlying mechanism remains incompletely understood. OSA is considered a potential atherogenic factor that contributes to arterial wall damage. Middle-aged individuals with OSA often exhibit early indicators of atherosclerosis.[9] Considering elements of the metabolic syndrome, certain studies have observed elevated triglyceride levels[10,11] and diminished high-density lipoproteins (HDL) levels in individuals having obstructive sleep apnea.[12] Conversely, other investigations have failed to establish a significant correlation between obstructive sleep apnea and dyslipidemia.[13,14] It is worth noting that most of these researches were not explicitly tailored to evaluate lipid profile. Hence, further evidence is required to draw definitive conclusions in this regard. Enhanced comprehension of the distinct associations between obstructive sleep apnea, metabolic syndrome, and insulin resistance is crucial for the development of tailored therapeutic interventions aimed at mitigating the elevated cardiometabolic risks observed in OSA patients. The objective of the current, cross-sectional study was to measure the lipid profile, blood sugar and ankle brachial index in obese male subjects having obstructive sleep apnea and compare them with obese subjects without OSA.

Methods

This cross-sectional study was conducted at Postgraduate Medical College (PGMI), Lahore, after obtaining ethical approval from the Institutional Review Board (IRB) during the years 2014-2015. The study enrolled employees and students of PGMI who met the inclusion and exclusion criteria. Sixty-four obese apparently healthy male subjects within the age of 20-45 years, with a value of BMI more than 25 kg/m2, were recruited after providing written informed consent.

The screening for obstructive sleep apnea (OSA) utilized two questionnaire proformas, namely the Berlin questionnaire and STOP-BANG questionnaire. Overnight pulse oximetry was done with portable pulse oximetry on all subjects, regardless of their risk level based on the questionnaires. Subjects were classified into the group of individuals with obstructive sleep apnea if they exhibited ≥4 percent oxygen desaturation index (ODI) with 5 to 15 events in an hour.[15] A portable pulse oximeter (Spirodoc pulsox) was utilized for the assessment. Prior to bedtime, the device was connected to the patients. The pulse oximeter sensor was placed on a fingertip, and the device was fastened to the chest. The next day, the researcher collected the device. The data, saved in the device's memory, was then transferred to a computer through a specialized interface. Afterwards, the data underwent analysis using specific software (Winspiro PRO 5.6.0; Medical International Research). The pulse oximeter captured parameters including heart rate, minimum and baseline oxygen saturation (SpO2), and Oxygen Desaturation Index (ODI). A diagnostic criterion of Oxygen Desaturation Index ≥4% (Chung et al., 2012), with 5-15 events per hour (ODI≥4%), was employed. Oxygen Desaturation Index (ODI) refers to the cumulative count of oxyhemoglobin desaturations of ≥4% from the initial baseline, measured per hour of recording. SpO2 is the arterial oxygen saturation level, with minimum SpO2 is the minimum SpO2 level during period of analysis while baseline SpO2 is the initially mean SpO2 level in the first 3 minutes of the recording. Delta index is the index of SpO2 fluctuation calculated in intervals of 12 seconds and pulse rate variation index is the variation of pulse frequency by hour of analysis. Using sterilized technique, blood sample was drawn from vein following a 10-12 hour fasting period. The sample was dispensed into a yellow top serum vial. The blood was centrifuged at a speed of 3000 revolutions per minute (rpm) for 10 minutes. Following centrifugation, the serum was extracted and preserved at -40°C until needed for future use.

Blood glucose levels were measured using the

enzymatic colorimetric method with a kit manufactured by Fortress Diagnostic, United Kingdom. Total cholesterol in the serum was measured using the enzymatic colorimetric method with a kit also manufactured by Fortress Diagnostic, United Kingdom. Triglyceride levels in the serum were determined using the GPO-POD enzymatic colorimetric method, with a kit manufactured by Analyticon Biotechnologies AG, Germany. HDL cholesterol levels in the serum were determined using the precipitation method, with a kit manufactured by Analyticon Biotechnologies AG, Germany. LDL cholesterol levels in the serum were measured using a kit from Analyticon Biotechnologies AG, Germany.

To measure the Ankle Brachial Index, an ordinary sphygmomanometer and a Doppler ultrasound device equipped with an 8-MHz probe (Hi dop, Bistos) were employed. Blood pressure readings of the upper and lower limbs were taken after a 30-minute resting period. The subject's blood pressure was measured in the supine position. Blood pressure cuffs were placed over each brachial artery for brachial blood pressure and above each malleolus for ankle blood pressure. The cuffs were inflated to 20 mmHg above the systolic pressure and then deflated at a rate of 2 mm/sec. Systolic blood pressure measurements were taken in the right and left brachial arteries, dorsalis pedis, and posterior tibial arteries.[16]

The data was recorded and analyzed using IBM-SPSS (Statistical Package for the Social Sciences), version 22. Distribution of the data was assessed using the Kolmogorov-Smirnov test. For normally distributed variables, mean ± standard deviation was presented, while median with interquartile range was utilized for variables with non-normal distribution. Qualitative variables were presented as frequencies and percentages.

Mann-Whitney U test was employed to compare quantitative variables with non-normal distribution between the two groups and an independent samples t-test was utilized for normally distributed quantitative variables. Statistical significance was defined as a p-value less than 0.05.

Results

In this study, 64 subjects were classified into two groups. In group I, there were 32 obese males diagnosed with obstructive sleep apnea, while in group II 32 obese males without obstructive sleep apnea were included. Median IQR of age for Group I showed a value of 31.00 (28.25-35.00) years for age (Median IQR) and for group II it was 30.50(24.25-32.00) with no statistically significant difference. Median IQR of ABI of study population was 1.08 (1.00 – 1.16). In group I median IQR of ABI was 1.08 (1.00 – 1.16) and in group II was 1.09 (1.01 – 1.16) and the comparison of the study groups show a insignificant difference p= 0.435 (Table II). In group I median IQR of fasting blood glucose(mg/dl) was 89.75 (71.48 – 109.25) and in group II it was 86.15 (72.66 – 109.68). Fasting blood sugar level showed no significant difference (p= 0.778) between the study groups as shown in Table II. In group I the median IQR of triglycerides(mg/dl) was 183.50 (133.25 – 272.25) and in group II was 168.00 (126.75 – 268.00) as shown in Table I. Comparison between the groups showed a non-significant difference of p= 0.413. In group I mean ± SD of cholesterol (mg/dl) was 179.72±33.36 and in group II was 173.47±43.81. Comparison of cholesterol showed no significant difference p=0.523 (Table I).

Table I: *Comparison of lipid profile across the two groups*

Variables	Group-I (Obese subjects with OSA) n=32	Group-II (Obese subjects without OSA) n=32	*p value*
HDL (mg/dl) [b]	39.2±6.7[d]	41.5±7.2[d]	0.190
Cholesterol (mg/dl) [b]	179.7±33.3[d]	173.4±43.8d	0.523
Triglycerides (mg/dl)[a]	183.5 (133.2 – 272.2) [c]	168.0 (126.7 – 268.0) [c]	0.413
LDL (mg/dl) [a]	85.0 (67.7 – 135.9) [c]	88.2 (67.4 – 108.7) [c]	0.888

OSA: Obstructive sleep apnea, HDL: High density Lipoprotein, LDL: Low density lipoprotein,
Values presented as median (IQR)c and mean ± SD d
a Compared using Mann- Whitney U test
b Compared using Independent Sample "t-test"
$p < 0.05$ considered significant
HDL indicates High Density Lipoproteins and LDL for Low Density Lipoproteins

In group I mean ± SD of HDL (mg/dl) was 39.22±6.76 and in group II was 41.53 ±7.20 as shown in Table I. Comparison of HDL (mg/dl) between the study groups showed p =0.190. In group I the median IQR of LDL (mg/dl) was 85.00 (67.75 – 135.95) and in group II was 88.20 (67.45 – 108.75). No significant difference was found in the comparison between the two groups p value

Table II: *Comparative analysis of fasting blood sugar and ankle brachial index across the two groups*

Variables	Group I (Obese subjects with OSA) n=32	Group II (Obese subjects without OSA) n=32	*p-value*
Blood Sugar Fasting (mg/dl) [a]	89.7 (71.4 – 109.2) [c]	86.1 (72.6 – 109.6) [c]	0.778
ABI [a]	1.08 (1.00 – 1.16) [c]	1.09 (1.01 – 1.16) [c]	0.435

Values are given as median (IQR)c
a Comparison by Mann-Whitney U test
p < 0.05 considered significant
ABI: Ankle Brachial Index

0.888 (Table I).

Discussion

Increasing evidence suggests that OSA is associated with dyslipidemia due to intermittent hypoxia, which triggers lipid peroxidation and disrupts the sympathetic system.[17] The present study has not shown any significant difference of triglycerides (p= 0.413), HDL (p= 0.190), LDL (p =0.888), cholesterol (p= 0.523), and fasting blood glucose (p= 0.778) between the two groups i.e., obese subjects diagnosed with OSA and obese subjects not having OSA. Our findings align with the study by Karkinski et al. (2017), which similarly reported no significant differences in lipid blood levels among obese subjects with and without OSA.[18] Previously prevalence rate of metabolic syndrome in patients who were not obese has been reported up to 22.5% in studies investigating the relationship between metabolic syndrome and individuals of normal weight, overweight, and obesity.[19] Likewise, Sharma et al., 2007 conducted a comparison among three patient groups: 40 patients with obesity and OSA, 40 obese subjects who were OSA-negative, and 40 subjects with normal-weight and no OSA. They discovered no variance in metabolic status among the subjects with obesity and OSA and obese subjects with no OSA. The findings of multivariate analysis in their study revealed obesity as the primary factor influencing metabolic abnormalities in this cohort and these results support the current study.[20]

The present study has reported a nonsignificant difference (p= 0.435) of Ankle Brachial Index (ABI). A research study conducted on C57BL/6J mice examined the effects of intermittent air exposure and Chronic Intermittent Hypoxia (CIH) on mice fed either a regular diet or a high cholesterol diet. The findings revealed that mice exposed to both CIH and a high cholesterol diet displayed elevated levels of total cholesterol, very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) compared to mice fed only a high cholesterol diet. This suggests that chronic intermittent hypoxia, when combined with a high cholesterol diet, contributes to dyslipidemia and potentially accelerates the development of atherosclerosis.[21] Furthermore, it's important to note that Ankle Brachial Index, a screening test for detecting subclinical atherosclerosis, may not be sufficiently sensitive in identifying early-stage atherosclerosis in asymptomatic middle-aged individuals.[22] Given that our study focused on young (aged 20-45 years) and healthy subjects, ABI may not be the most effective screening tool for detecting vascular changes in this population. Additionally, Steiropoulos et al. investigated the presence of early atherosclerotic lesions in newly diagnosed obstructive sleep apnea (OSA) patients without known comorbidities using Transcranial Doppler ultrasound, Common Carotid Artery Intima Media Thickness (CCA-IMT), and Ankle Brachial Index measurements. Their study concluded that OSA patients without recognized comorbidities exhibited only a minimal increase in CCA-IMT, and there was no other evidence of significant vascular disease present.[23] The lack of differences in metabolic parameters among obese individuals with OSA and individuals without OSA, challenges the conventional perception of the relationship and link among OSA and metabolic health. One possible explanation for our findings could be the heterogeneity of OSA severity and its effects on metabolic outcomes. It is plausible that the metabolic impact of OSA may vary depending on factors such as the degree of nocturnal hypoxia, sleep fragmentation, and individual metabolic susceptibility.

Furthermore, our study underscores the complexity of the interplay between OSA and metabolic health. While OSA is commonly associated with metabolic disturbances, it is essential to recognize that obesity itself is a significant risk factor for dyslipidemia, insulin resistance, and cardiovascular disease. Thus, the metabolic abnormalities observed in obese individuals may be predominantly driven by obesity-related factors rather than OSA alone.

Conclusion

Normal lipid profile and normal ABI indicate absence of detectable atherosclerosis in young apparently healthy OSA subjects without known comorbidities.

Conflict of Interest: None

Funding Disclosure: None

Ethical Consideration: The study was approved by the ethical review board. Informed written consent was obtained from the participants, and the confidentiality of their data was clearly explained.

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Authors Contribution

All the authors contributed equally in accordance with ICMJE guidelines.

SK: Conception of idea & design, acquisition, analysis & interpretation of data, drafting the article, critical review, final approval of the manuscript

HH: Acquisition of data, drafting the article, critical review, final approval of the manuscript

RI: Analysis & interpretation of data, drafting the article, critical review, final approval of the manuscript

MS: Conception of idea & design, analysis & interpretation of data, final approval of the manuscript

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Original Article

Comparison of Serum Vitamin D Levels Between Asthmatics and Healthy Individuals: A Cross-Sectional Study

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Abstract

Background: Serum vitamin D3 plays a pivotal function not solely in managing mineral homeostasis but also in modulating allergic reactions, cancer and autoimmune diseases. It manages over 200 genes, thus governing cell replication, apoptosis and differentiation. Deficiency of Vitamin D3 in asthmatics is significantly correlated with increased airway sensitivity, impaired pulmonary functions, reduced management of asthma and insensitivity to corticosteroids.

Objective: This research aimed to determine and compare serum levels of vitamin D3, counts of inflammatory cell, and lung function indices between asthmatics and healthy subjects in Lahore.

Methods: This was an observational and cross-sectional study executed at the University of Health Sciences, Lahore, over a 1-year span, involving 60 asthmatic patients as sample and 30 healthy controls. Following a desired clinical assessment, pulmonary function evaluation was conducted using a spirometer, and blood samples were collected for the analysis of inflammatory cell counts and serum levels of vitamin D3. All data were inputted and examined utilizing SPSS version 22. The Kruskal-Wallis test was employed to compare differences in group medians. For Pairwise comparison of groups Mann Whitney U test was applied. A p-value of < 0.05 was considered statistically significant.

Results: The serum vitamin D3 levels were significantly lower among asthmatics compared to controls. Additionally, all participants, including normal controls, exhibited severe vitamin D3 deficiency. Moreover, asthmatic subjects showed a significant decrease in lung function parameters.

Conclusion: The vitamin D3 levels were significantly lower in asthmatics compared to the controls.

Key words: Vitamin D3, Asthma, Lung function test.

Introduction

Bronchial asthma presents as an airway disorder marked by inflammation and hyperresponsiveness of the airway mucosa, resulting in intermittent exacerbations and periods of remission accompanied by symptoms such as cough, dyspnea, and chest tightness.[1,2] As per the 2004 Global Initiative for Asthma (GINA) report, the prevalence of asthma in Pakistan stands at 4.3%.[3]

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Prominent triggers include upper respiratory tract infections, food allergies, exposure to dust, a familial predisposition to allergic conditions, residing in urban areas, and premature cessation of breastfeeding.[4,5]

Inflammation mediated through eosinophils and helper T (Th) cells is pivotal for causing swelling in the mucosal, submucosal, and adventitial layers during airway inflammation, as well as for remodeling the epithelial and subepithelial regions.[6,7] Eosinophil levels in the blood act as an indicator of the severity and marker of lung inflammation.[8] T helper lymphocytes (CD4 cells) can differentiate into Th1 cells, which produce INF-γ, or Th2 cells, responsible for generating Interleukin 4 and 5.

The cytokines from Th2 cells attract more eosinophils and promote increased production of IgE, contributing to delayed allergic responses. Conversely, INF-γ from Th1 cells stimulates higher production of IgG, helping to alleviate delayed allergic reactions.[9]

The initiation and maintenance of immune responses are facilitated by antigen-presenting cells (APCs), which can be directly inhibited by vitamin D3.[10] This vitamin acts as an immune modifier by interacting with its receptor (VDR) on various cells, including T cells and activated B cells.[11] The immune modulating effect may shift the cytokine equilibrium generated by the Th1 and Th2 lymphocytes towards predominance of Th2.[12] However, studies on T cells from human cord evidenced that both Th1 and Th2 lymphocytic cells can be inhibited by vitamin D3.[13] Therefore, these immune-modulating effects of the vitamin D3 on these lymphocytes of Th origin in asthma might depend upon the duration of exposure period to this vitamin.

Furthermore, vitamin D3 plays a critical role in mitigating chronic infections (as tuberculosis) and upper airway infections (UAIs) may be influenza or the common cold, which frequently trigger asthma, particularly in regions like Pakistan.[14] Additionally, vitamin D3 impedes the airway smooth muscle growth, thereby the process of remodeling. Although bronchial smooth muscles in asthmatic as well as in healthy airways tend to proliferate in response to Platelet-Derived Growth Factor (PDGF), the active form of vitamin D3 (calcitriol), counteracts this phenomenon.[15] This remodeling contributes to the bronchial wall thickening, a significant contributor to asthma exacerbations.[16]

The purpose of this study was to measure and compare the serum levels of the vitamin D3 and blood inflammatory cell count in asthmatic individuals and in control group.

Methods

It was a comparative study executed at the Department of Physiology and Cell Biology in the University of Health Sciences Lahore (UHS) and at Gulab Devi Hospital from February 2011 till January 2012. The study approval was by the Ethics review committee of UHS Lahore.

A total number of 60 asthmatics from Gulab Devi Chest Hospital Lahore were included and were segregated into the three groups as: 20 mild asthmatics, 20 moderate asthmatics and 20 severe asthmatics in accordance with the National Heart Lung and Blood Institute (NHLBI) recommendations. A total of 30 age matched healthy individuals were enrolled for the purpose of comparison.[17] Purposive sampling was done.

Desired history was undertaken and clinical assessment was performed after written informed consent. Patients having chronic obstructive airway bronchitis, acute or long term tuberculosis, emphysematous lung problems or any other cardiovascular, endocrinological or some other systemic illness; patients with the vitamin D3 deficiency features such as unexplained muscular pains, bone aches or obvious deformities; parasitic infestation evidence in the last 1 year and those on long term medicines including corticosteroids and supplementation with calcium and vitamin D3 were excluded from the study. Spirometry was performed by using the digital spirometer, Spiro lab II. Forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), ratio between FEV1/FVC and maximum voluntary ventilation were performed. Blood samples were obtained by aseptic techniques and stored in separate aliquots. Serum vitamin D3 levels were assessed by using ELISA and white blood cell count was performed by using the 5-part differential Hematology analyzer.

For statistical conclusion, the software SPSS version 22 was used. Data was presented as median with IQR and Kruskal Wallis statistics was used to analyse group means. For Pairwise comparison of groups Mann Whitney U test was applied. A p-value of < 0.05 was considered statistically significant.

Results

The median (IQR) age, anthropometric indices including height in cm, weight in Kg and BMI in Kg/m^2 of the

Table I: *Baseline anthropometric parameters of the study subjects.*

	N S	M A	Mod A	S A
Parameter	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Age (in years)	22 (18.25-22.75)	19.5 (16.2-22)	20.5 (18.2-22)	18.5 (16.2-22)
Height (cm)	169.5 (157-175)	155 (150-165)	155 (150-167)	156 (152-160)
Weight (kg)	57 (50-71.7)	49.5 (43-55)	47.5 (43.2-58.7)	45.5 (42.5-49.7)
BMI (kg/m^2)	21.95 (18.7-23.7)	20.2 (17.4-23.6)	20.56 (18.4-22.7)	18.7 (17.3-20.1)

A (asthma), M (mild), Mod (moderate), N (normal), S (severe)

study subjects is given in table I. The comparison of spirometry values between the normal subjects and the three groups of asthmatic subjects is presented in table II. It was shown that FEV1, FVC, ratio of FEV1/FVC and MVV were all lower in asthmatics as compared to healthy subjects. A comparison of blood parameters such as serum vitamin D3 levels and white blood cells counts is presented in table IV which shows that serum vitamin D3 levels were significantly less in the asthmatics than in the healthy ones. Conversely, the white blood cells were significantly higher in the asthmatics than in the normal ones.

Table II: *Comparison of lung function tests among the study groups.*

Indices	N A	M A	Mod A	S A	*p*-value
FEV_1 (in liters)	3.35 (2.78-3.62)	2.32 (2.0-2.6)	1.75 (1.4-2.06)	1.15 (0.96-1.17)	0.001*
FVC (liters)	3.66 (3.04-4.18)	2.72 (2.4-3.4)	2.46 (2.07-2.76)	1.71 (1.35-2.2)	0.001*
FEV_1 to FVC (%)	88.48 (85.28-93.9)	81.03 (76.8-90.3)	74.25 (67.6-82.1)	71.73 (56.5-75.1)	0.001*
MVV (liters/min)	126.2 (103.8-144)	77.45 (71.9-94.4)	68.7 (56.7-82.7)	41.9 (35.3-51.4)	0.001*

**p-value if < 0.05 is of significance. Data is summarized as median (& IQR).*
Kruskal Wallis stats was employed to determine the difference of continuous indices
A (asthma), M (mild), Mod (moderate), N (normal), S (severe)

Table III: *Comparison of lung function tests pairwise*

Variables	NS. vs SA.	*p*-value	N S. vs Mod A.	*p*-value	SA. vs Mod A.	*p*-value
FEV_1 (liters)	3.35 vs 1.15	0.002*	3.35 vs 1.75	0.002*	1.15 vs 1.75	0.02*
FVC (liters)	3.66 vs 1.71	0.001*	3.66 vs 2.46	0.003*	1.71 vs 2.46	0.04*
FEV_1/ FVC (%)	88.48 vs 71.73	0.001*	88.48 vs 74.25	0.001*	71.73 vs 74.25	0.02*
MVV (liters/min)	126.2 vs 41.9	0.001*	126.2 vs 68.7	0.002*	41.9 vs 68.7	0.002*

**p-value if < 0.05 is of significance. Data is summarized as median (& IQR).*
Mann Whitney Test was employed to determine the difference of continuous indices
A (asthma), M (mild), Mod (moderate), N (normal), S (severe)

Discussion

The current study investigated serum levels of vitamin D3 and asthma severity markers in both normal individuals and those with asthma. It also assessed pulmonary function through tests, inflammatory cell counts, and anthropometric measurements. The findings revealed notable disparities in vitamin D3 levels across the study cohorts. Specifically, among normal subjects, the median vitamin D3 level was 13.48 ng/ml. Mehmood and companions (2009) described deficiency of vitamin D3 as

Table IV: *Comparison of blood parameters among the study groups*

Variables	N S	M A	Mod A	S A	*p*-value
Total leukocytic count ($x10^3/mm^3$)	8.24(7.2-8.7)	8.86(7.6-10.6)	9.04(7.9-10.5)	12(8.9-12.09)	0.002*
Eosinophilic count ($x10^3/mm^3$)	0.30(0.08-0.56	0.56(0.45-0.68)	1.35(0.42-1.4)	1.2(0.5-1.9)	0.001*
Lymphocytic count ($x10^3/mm^3$)	2.42(1.6-2.6)	2.28(2.1-3.05)	3.20(2.24-3.3)	2.90(2.2-3.1)	0.003*
Levels of Vitamin D_3 in serum (ng/ml)	13.48(8.8-21.9)	7.21(4.5-9.6)	9.51(6.2-12.5)	6.65(4.51-8.59)	0.001*

**p-value if < 0.05 is of significance. Data is summarized as median (& IQR).*
Kruskal Wallis stats was employed to determine the difference of continuous indices
A (asthma), M (mild), Mod (moderate), N (normal), S (severe)

Table V: *Comparison of blood parameters pairwise*

Variables expressed in median	NS. vs SA.	*p*-value	N S. vs Mod A.	*p*-value	SA. vs Mod A.	*p*-value
Total leukocytic count ($x10^3/mm^3$)	8.24 vs 12	0.001*	8.24 vs 9.04	0.032*	12 vs 9.04	0.001*
Eosinophilic count ($x10^3/mm^3$)	0.3 vs 1.2	0.001*	0.3 vs 1.35	0.001*	1.2 vs 1.35	0.04*
Lymphocytic count ($x10^3/mm^3$)	2.42 vs 2.9	0.021*	2.42 vs 3.20	0.001*	2.9 vs 3.20	0.25
Levels of vitamin D_3 in serum (ng/ml)	13.48 vs 6.65	0.001*	13.48 vs 9.51	0.002*	6.65 vs 9.51	0.002*

**p-value if < 0.05 is of significance. Data is summarized as median (& IQR).*
Mann Whitney Test was employed to determine the difference of continuous indices
A (asthma), M (mild), Mod (moderate), N (normal), S (severe)

levels below 20 ng/ml in serum, indicating that the study healthy population group exhibited this deficiency.[18] Individuals with mild asthma showed a median vitamin D3 level of 7.21 ng/ml, those with moderate asthma had median of 9.51 and those with severe asthma had a level of 6.65 + 2.84 ng/ml. The measures differed significantly from each other.

According to Karim et al.'s classification from 2011, levels of vitamin D3 less than 10 ng/ml are considered severely deficient, which can lead to various health complications.[19] This cut off is supported by several studies conducted in Lahore. For instance, Haque et al. found that 66% of healthy population had vitamin D3 measure less than 20 ng/ml.[20] In this study, there is a significant difference in vitamin D3 levels among asthmatics, and levels were as low as 6.65 ng/ml in severely asthmatic group. Restricted data exists regarding vitamin D3 deficiency in the healthy Pakistani population, and no study has specifically evaluated serum vitamin D3 levels in asthmatics until now.[19] To our knowledge, this study is the first of its kind among asthmatics aged 12 to 25 years.

The pulmonary function parameters analyzed in our study exhibited significant variations among the different groups examined, encompassing FEV1, FVC, the FEV1/FVC ratio, and MVV. These outcomes are consistent with earlier findings by Ayub and companions in 1997, suggesting that lung volumes even in healthy adults are comparatively lower than those in similar European population.[21] The median values for FEV1, FVC, and the FEV1/FVC ratio in our study align closely with those reported by Memon et al. in 2007, although their study encompassed individuals aged 15 to 65 years.[22] However, our results diverge from those presented by Khan and Saadia in 2006, who observed higher mean values for FEV1 (2.38 liters), FVC (3.35 liters), and FEV1/FVC ratio (70%) in subjects aged 19.5-30 years.[23] In our study, these parameters were notably lower in the asthmatic group. Additionally, our findings contrast with the values documented by Williams et al. in 1978, who noted lower FEV1 (2.28 liters) and FVC (2.76 liters) values in adult nonsmoker females from Lahore aged 21.8 years in classical literature.[24]

Conclusion

A marked insufficiency of serum vitamin D3 is evident in both asthmatic patients and in the control group of healthy individuals. Asthmatic participants demonstrate a decline in lung function test outcomes, while there is a rise in the number of inflammatory leukocytes, which correlates with the severity of the disease.

Conflict of Interest: *None*

Funding Source: *None*

Ethical Consideration: The study was approved by the ethical review board. Informed written consent was obtained from the participants, and the confidentiality of their data was clearly explained.

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Authors Contribution

All the authors contributed equally in accordance with ICMJE guidelines.

HSK: Conception of idea & design, acquisition, analysis & interpretation of data, drafting the article, critical review, final approval of the manuscript

HMW: Acquisition of data, drafting the article, critical review, final approval of the manuscript

AS: Analysis & interpretation of data, drafting the article, critical review, final approval of the manuscript

FA: interpretation of data, final approval of the manuscript

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Original Article

Virtual Screening of Novel Non-Lactam Inhibitors Against CTX-M-15 β-Lactamase for Enhancement and Remodeling of Antibiotic Efficiency

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Abstract

Background: The CTX-M-15 are beta-lactamases that break down practically all the antibiotics that belong to the beta-lactam group resulting in antibiotic resistance in bacteria. Several beta-lactam inhibitors can be used in combination with different cephalosporin antibiotics to treat infections caused by microbes that produce the CTX-M-15 enzyme.

Objective: This study aims to use a multi-step virtual screening strategy to screen three new non—lactam inhibitors against CTX-M-15.

Methods: Study was designed and conducted in the Department of Biotechnology at the University of Central Punjab. A multi-step virtual screening strategy was used to screen three new non— lactam inhibitors against CTX-M-15. The CTX-M-15 binding sites were explored to determine the possible target sites of the inhibitors. Compounds from the E-LEA3D were subjected to virtual screening, and their performance was evaluated based on the binding energies and various other factors. Using Auto Dock Vina, the docking complexes were formed and visualized in Pymol where their RMSD values and binding energies were compared.

Results: The best results were found in the case of Isoproterenol as it inhibited the lactamase activity of CTX-M 15 by forming a stable docked complex. The molecular docking simulations using Autodock Vina revealed favorable binding interactions, indicating the ability of Isoproterenol to bind to the active site.

Conclusion: The lactamase activity of CTX-M 15 was inhibited by Isoproterenol as they form a stable docked complex. Designing inhibitors against the CTX-M-15 type β-lactamase represents a promising avenue for combating drug-resistant bacteria.

Keywords: Beta-Lactamase, Pymol, Virtual Screening, Drug Resistance, Antibiotics

Introduction

The introduction of antibiotics in this world was one the greatest achievements of all time. The Beta-lactam group was the main reason that first ever antibiotic penicillin G worked against pathogenic bacteria and the identification of the beta-lactam group gave rise to the production of all today the antibiotics we use such as cephalosporin, carbapenems, and monobactams. However, the excess use of these antibiotics has led to resistance in bacteria against the beta-lactam group by the production of the enzyme beta-lactamase. CTX-M 15 beta-lactamase is of one the enzyme contributing to multi-drug resistance against antibiotics.[1,2]

The mechanism of action of beta-lactamase is the hydrolysis of the beta-lactam ring causing antibiotics to cease

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to work. To overcome the issue, much research is being done to design inhibitors against beta beta-lactamase enzyme to produce a novel antibiotic that can be effective against resistant bacterial strains. As a result, techniques for identifying new anti-lactamase drugs with modes of operation are urgently required. Furthermore, virtual screening technologies like molecular docking and molecular dynamics simulations have made it easier to screen huge drug libraries for potential inhibitors. High-throughput screening has also played a crucial role in identifying novel inhibitors by screening diverse chemical libraries. Generally, β-lactamase inhibitors work in two ways; they might bind as the substrate forming unfavorable interactions like acyl-enzymes or may inactivate the enzyme permanently by undergoing secondary reactions with the enzyme at the active site. For instance, NDM-1 has a flexible hydrolysis mechanism, making the inhibition very difficult. Certain covalent inhibitors have been found to have high potency, yet their lack of specificity and high toxicity renders them inappropriate for use as adjuvants.[2,3]

A virtual screening approach is being used to screen novel β-lactam inhibitors against CTX-M-15. Bioinformatics and experimental approaches are also being utilized to establish an understanding of the binding of CTX-M-15 with inhibitors. RSCB Protein Data Bank can be used as a source for the crystal structure of CTX-M-15. Various computational tools were used for different processes, such as Discovery Studio 2.5 was used to remove the water molecules and add hydrogen atoms to the enzyme, the conformation of the receptor-ligand complex was predicted by Patch Dock and Autodock Vina in molecular docking, docking validation was carried out using iMods.[4]

A potential inhibitor for CTX-M-15 type beta-lactamases which was found due to the virtual screening approach is Isoproterenol. Further, molecular docking was performed, and following that post molecular docking analysis or molecular dynamic simulation to study the interaction between the protein-ligand complex. Binding affinity, RMSD value, hydrogen bonds, and salt bridges were analyzed and the best interaction between the inhibitor and enzyme was chosen based on these scores. The potential efficacy of the inhibitor Isoproterenol against CTX-M-15 carrying bacterial strains to inhibit b-lactamases was validated.[4]

The newly designed inhibitor against CTX-M-15 beta-lactamase through in- silico approaches show promising results overcoming antibiotic resistance. Furthermore, new strategies and mechanisms can be utilized to develop such inhibitors against beta-lactamase that exhibits higher potency, selectivity, and kinetic properties. The structural activity relationship can be enhanced in designing new drugs that displays efficiency against antibiotic drug-resistant bacterial strains.[5]

This research was designed to employ a multi-step virtual screening approach to identify novel non-lactam inhibitors against CTX-M-15, a β-lactamase known for conferring antibiotic resistance in bacteria.

Methods

First, the crystal structure of the natural CTX-M-15 extended-spectrum beta-lactamase enzyme (PDB: 4HBT) was obtained from the RCSB PDB Protein Data Bank (https://www.rcsb.org). By using the Discovery Studio Visualizer. 6 (Studio, 2009), All water molecules and ligands were removed, and polar hydrogen atoms were introduced. The binding sites were determined using residue 1 Å (Angstrom) surrounding the crystal structure.

To identify the possible ligand compounds virtual screening was performed using e-LEA3D (https://chemoinfo.ipmc.cnrs.fr) – drug design server.[7] The de novo drug design and virtual screening tool was used to design new ligands to optimize the interaction between the ligand and enzyme. All the screening compounds are molecules that fall within Lipinski's rule of five i.e., molecular mass < 500 Da, hydrogen bond donors ≤ 5, hydrogen bond acceptors ≤10, octanol–-water partition coefficient log P (Clog P) ≤ 5.

To predict the conformation of ligand-receptor complex, Autodock Vina[8,9] and PatchDock[10] (http://bioinfo3d.cs.tau.ac.il/PatchDock/) were used. Next, conformation having the best fitness scores were was extracted from each docking clusters. The exhaustiveness setting in each docking was set to 8 and the simulation box (number of points in x, y, and z dimensions) was set to 40× 40×40. Autodock Vina was used to prepare pdbqt files of protein and ligand. Polar hydrogen atoms were added to create a charge for efficient binding and their binding affinity was calculated using the scoring function of the tool Autodock Vina. Pymol (https://pymol.org/2/) was used to visualize the docking complexes.

Post-docking analysis or molecule dynamic simulations

were performed to check the stability of the ligand within the enzyme's active site. For the molecular dynamic simulation studies, iMods[11] was used. IMods (https://imods.iqfr.csic.es/) is a tool that performs Normal Mode Analysis (NMA). Along with iMods, to study the molecular interaction between protein-ligand complex Protein - Ligand Interaction Profiler (PLIP) (https://plip-tool.biotec. tu-dresden.de/plip-web/plip/index) was used.

Results

The best results were found in the case of Isoproterenol as it inhibited the lactamase activity of CTX-M 15 by forming a stable docked complex. The molecular docking simulations using Autodock Vina revealed favorable binding interactions, indicating the ability of Isoproterenol to bind to the active site.

The virtual screening of FDA-approved drugs was performed to find the appropriate inhibitor that would form the docking complex with the prior mentioned molecule to inhibit its activity. Compounds from the E-LEA3D were subjected to virtual screening, and their performance was evaluated based on the binding energies and various other factors. Using Auto Dock Vina, the docking complexes were formed and visualized in Pymol where their RMSD values and binding energies were compared. Among the available options, the best results were found in the case of Isoproterenol, which can be used as a potential inhibitor for CTX-M-15 type beta-lactamases (Fig I, II). The hydrolytic activity was seen no more hence a promising future against antibiotic resistance. As a result, the suggested putative inhibitor might be employed as lead molecules for future therapeutic candidates against bacteria that produce beta-lactamases.

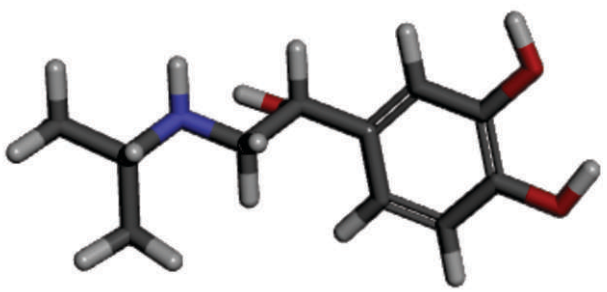
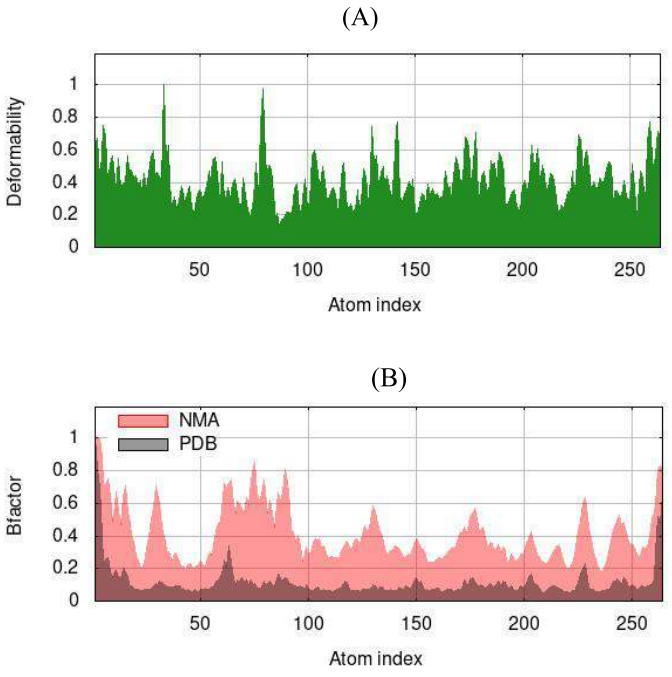


Figure I: *Structure of Isoproterenol used as an inhibitor for beta-lactamase CTX-M-15 found through virtual screening using Le3D*

Figure II: *Docked complex between CTX-M-15 and Isoproterenol visualized in Pymol*

Molecular Dynamic (MD) simulations involve computational techniques to check various parameters. For this purpose, iMods (Internal coordinates normal mode analysis server) was used as it is the best free online tool available that helps in Normal Mode Analysis (NMA). Patch Dock was used for online docking between the enzyme and inhibitor. The complex was selected based on score. The selected complex from Patch Dock was saved in .pdb format and then uploaded on iMods for simulation studies. Figure III shows the MD Simulation of the docked complex formed between CTX-M-15 and Isoproterenol. The detailed results are mentioned



below in figure III.

The molecular interactions in a protein-ligand complex

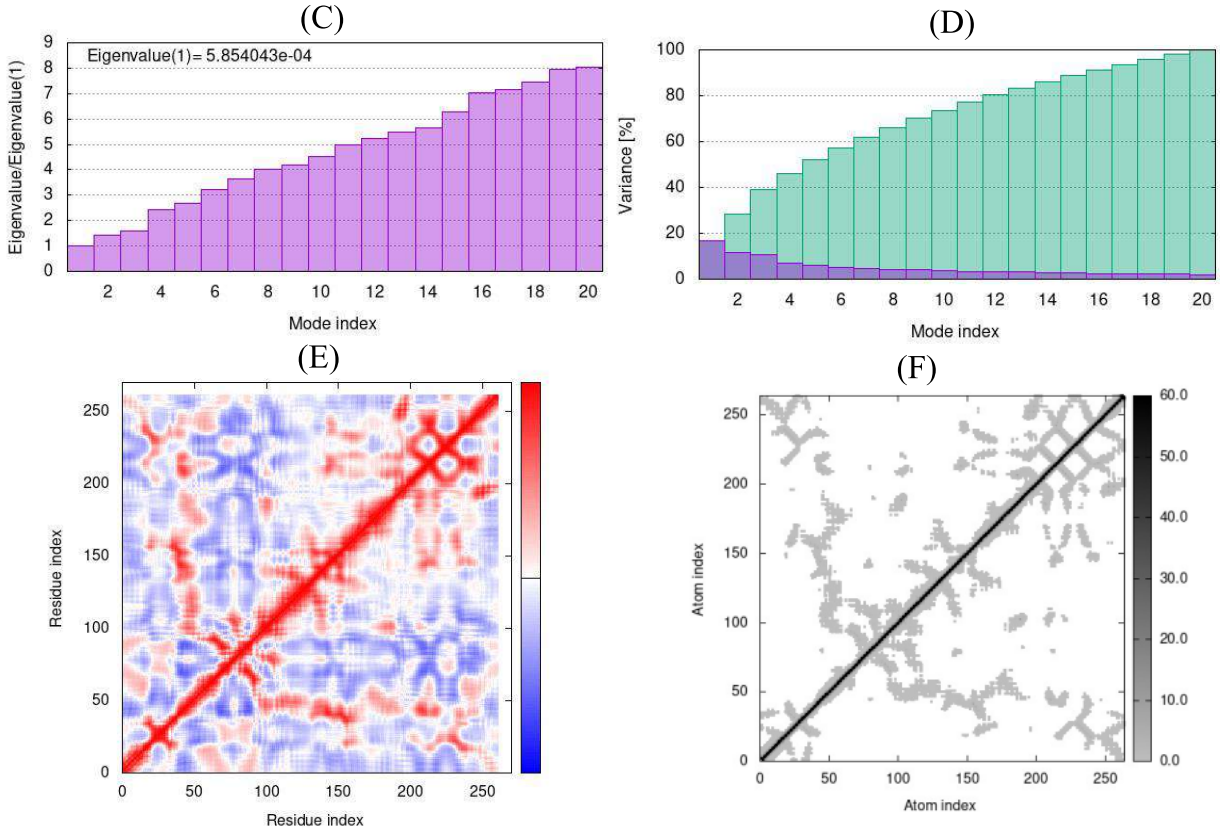
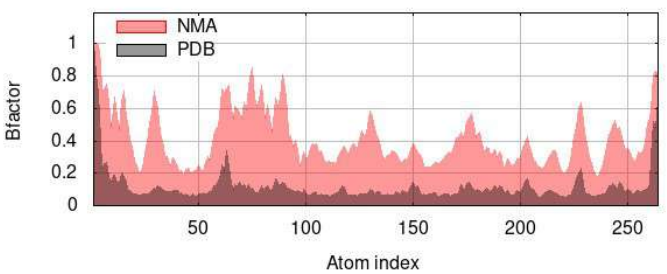


Figure III: *MD Simulations by IMODs*

A) B-factor/Mobility of the docked complex formed between CTX-M-15 and Isoproterenol B) A comparison between the already present PDB docked complex with no B-factor and the docked complex under study. C) Eigenvalue of the docked complex. D) %Variance of the docked complex between CTX-M-15 and Isoproterenol. E) Covariance matric of the docked complex - uncorrelated (white) correlated (red) or anti-correlated (blue) motions. F) Elastic network model of the docked complex - darker color means stiffer spring



can be studied through Interaction Profile Generation using tools like Protein Protein-Ligand Interaction Profiler (PLIP). PLIP generates various small molecule interactions and sulphate sulfate interactions. Only two of those interactions; small molecule interaction (EDO-A-308) and sulphate sulfate (SO4-A-301) are presented respectively. Figure IV represents the visual representation of the docked complex analyzed by PLIP. While figure V provides the Visual representation of SO4-A-301 (sulfate interactions) - hydrogen bonds (dark blue rods), water bridges (light blue rods), and salt bridges (yellow dotted line)

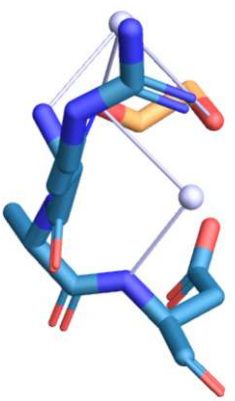


Figure IV: *Visual representation of EDO-A-308 (small molecule interactions) hydrogen bonds (dark blue rods) and water bridges (light blue rods)*

Table I: *Hydrogen Bonds*

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Sidechain	Donor Atom	Acceptor Atom
1	62A	ALA	1.93	2.89	167.09		283 [Nam]	2058 [O3]

Table II: *Water Bridges*

Index	Residue	AA	Dist. A-W	Dist. D-W	Donor Angle	Water Angle	Donor Atom	Acceptor Atom	Water Atom
1	61A	ARG	3.20	2.85	165.78	108.66	272 [Nam]	2058 [O3]	2303
2	61A	ARG	3.97	2.85	165.78	81.44	272 [Nam]	2056 [O3]	2303
3	63A	ASP	3.59	2.92	154.34	104.59	288 [Nam]	2058 [O3]	2186

Table III: *Hydrogen Bonds*

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Donor Atom	Acceptor Atom
1	70A	SER	2.73	3.29	117.48	351 [O3]	2026 [O3]
2	130A	SER	1.82	2.74	157.69	815 [O3]	2026 [O3]
3	237A	SER	3.52	4.04	114.62	1612 [Nam]	2026 [O3]

Table IV: *Water Bridges*

Index	Residue	AA	Dist. A-W	Dist. D-W	Donor Angle	Water Angle	Donor Atom	Acceptor Atom	Water Atom
1	70A	SER	3.17	3.12	144.22	108.58	2026 [O3]	351 [O3]	2246
2	70A	SER	4.06	2.99	139.64	77.56	346 [Nam]	2025 [O3]	2166
3	130A	SER	2.88	3.12	144.22	73.81	2026 [O3]	813 [O2]	2246
4	236A	GLY	3.45	2.80	147.00	102.56	2025 [O3]	1611 [O2]	2139

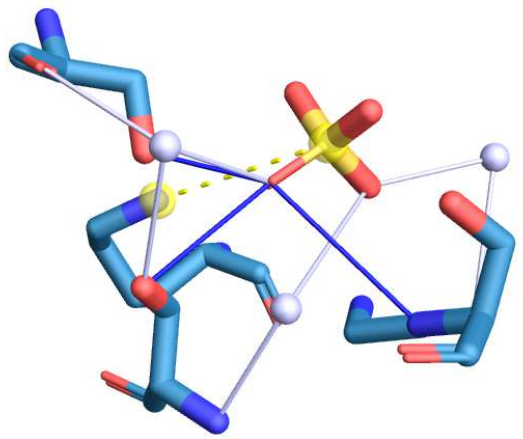


Figure V: *Visual representation of SO4-A-301 (sulfate interactions) - hydrogen bonds (dark blue rods), water bridges (light blue rods), and salt bridges (yellow dotted line)*

Table V: *Salt Bridges*

Index	Residue	AA	Distance	Ligand Group	Ligand Atoms
1	234A	LYS	4.75	Sulfate	2022, 2022

Binding Affinity of CTX-M-15 With Isoproterenol

The binding affinities were found by docking through Autodock vina. The structure with the highest negative value (smallest value) was chosen for further analysis in Imods.

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.5	0.000	0.000
2	-6.4	1.426	2.206
3	-6.3	2.610	3.995
4	-6.3	2.351	3.339
5	-6.1	1.850	2.186
6	-5.3	3.001	4.559
7	-5.1	25.455	27.072
8	-5.1	25.125	26.220
9	-5.1	25.314	26.367

Figure VI: *Binding affinities of the docking complexes formed between CTX-M-15 and Isoproterenol*

The first RMSD (i.b. and u.b.values) should be zero in case of a successful docking.

Discussion

The emergence and spread of antibiotic-resistant bacteria pose a significant threat to global public health. The pace of discovering new antibiotics is sluggish, necessitating innovative approaches for effective treatment of bacterial infections. One promising strategy involves designing drug combinations to mitigate the emergence of antibiotic resistance. By formulating combinations where resistance to one drug coincides with increased susceptibility to another, we can potentially thwart the development of resistance and enhance treatment efficacy. CTX-M 15 β-lactamase, because of its hydrolytic activity, plays a critical role in producing resistance to β-lactam antibiotics. Bacteria carrying the CTX-M-15 gene exhibit significant resistance to β-lactam antibiotics, such as penicillin and cephalosporins.[12] In this study, the potential inhibitory activity of Isoproterenol was explored to inactivate CTX-M 15 β-lactamase, as the previously available inhibitors and their residual binding are no longer effective.[4]

Isoproterenol, a non-selective beta-adrenergic agonist, is proposed as a CTX-M-15 β-lactamase inhibitor. It has several medicinal applications. It is often used to treat bradycardia and heart block by raising heart rate and cardiac output, as well as a bronchodilator. These characteristics, together with its current clinical application, show its potential as a dual-purpose therapeutic drug.

The use of Isoproterenol as an inhibitor against CTX-M 15 β-lactamase is a novel approach that has not been extensively investigated. Isoproterenol docking with CTX-M 15 β-lactamase shows that different residues bind with the inhibitor and have different affinities. Ser70 is known to form hydrogen bonds with the inhibitor whereas Ser273 and Ser130 form a stable docked complex by establishing hydrogen bonds.[13] Different bioinformatics sites and tools have been utilized to check the potential of docking between CTX-M 15 β-lactamase and the inhibitor. As for ligand and inhibitor's crystal structure retrieval RCSB PDB Protein Data Bank and e-LEA3D – drug design server have been used respectively.[7] Autodock Vina was used for docking and iMods for the analysis. The NMA graph is far higher than the PDB graph showing that the complex is stable with a binding affinity of -6.5.[14,15]

The CTX-M-15 enzyme, belonging to the CTX-M-1 subgroup, is widely prevalent among Gram-negative pathogens. In the current study, molecular docking simulations using Autodock Vina revealed favorable binding interactions, indicating the ability of Isoproterenol to bind to the active site. The lactamase activity of CTX-M 15 was inhibited by Isoproterenol as they form a stable docked complex. The hydrolytic activity was seen no more hence a promising future against antibiotic resistance.[16]

Based on the results obtained, further experimental studies should be conducted to validate the inhibitory activity of Isoproterenol against CTX-M 15 β-lactamase. Additionally, understanding the structural basis of the interaction between the inhibitor and CTX-M-15 β-lactamase can aid in the design of more effective compounds. Overall, the development of inhibitors against CTX-M-15 β-lactamase represents a promising strategy for combating drug-resistant bacteria and provides hope for the future in the fight against antibiotic resistance.[17] Future studies should focus upon inhibitor development by different mechanisms which that involve irreversible inhibition, competitive inhibition, and metallo-beta-lactamase inhibition. Strategies to enhance inhibitor potency, selectivity, and pharmacokinetic properties must be explored, including structure-activity relationship studies and prodrug approaches to increase the effectiveness of the drug.[18] In vitro assays, such as enzyme inhibition assays, can provide quantitative data on the inhibitory potency of Isoproterenol. Moreover, in vivo studies, such as animal models or clinical trials, are crucial to assess the effectiveness and safety of Isoproterenol as an inhibitor.[19,20]

Conclusion

Based on these findings, the study concluded that Isoproterenol offers a high possibility of binding to and strongly inhibiting the lactamase activity of CTX-M-15, as supported by the molecular docking experiment aided by AutoDock Vina. The favorable binding interactions supported the ranked mapping of Isoproterenol with the active site of the enzyme. Thus, the data obtained are indicative of the fact that attempts to develop inhibitors against CTX-M-15 β-lactamase should be considered as one of the relevant approaches to addressing the issue of increasing bacterial resistance to antibiotics. More development in any such inhibitor could contribute greatly to the continued fight against drug-resistant infections.

Conflict of Interest: None

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Ethical Consideration: The study was approved by the ethical review board. Informed written consent was obtained from the participants, and the confidentiality of their data was clearly explained.

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Authors Contribution

All the authors contributed equally in accordance with ICMJE guidelines.

A: Conception of idea & design, acquisition, analy-sis & interpretation of data, final approval of the manu-script

MS: Acquisition of data, drafting the article, analysis and reporting, final approval of the manuscript

AR: Analysis & interpretation of data, drafting the article, critical review

AR: Conception of idea & design, interpretation of data, results writeup, final approval of the manuscript

RA: Acquisition of data, drafting the article, analysis and reporting, final approval of the manuscript

SMI: Analysis & interpretation of data, drafting the article, critical review

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Original Article

Hepatitis B Awareness Among Medical Students And Their Vaccination Status

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Abstract

Background: Hepatitis B infection is one of the most prevalent health issues globally. Health workers are more vulnerable to this infection because they come in contact with blood and other body fluids.

Objective: The present study was aimed to assess Hepatitis B awareness and vaccination status among medical students.

Methods: It was a descriptive cross-sectional study conducted by department of Community Medicine, Lahore Medical & Dental College. The duration of study was three months. A Structured questionnaire was used for data collection. It was divided in three sections. The first part was related to background information, second part consisted of knowledge about Hepatitis B virus (HBV) infection and third part consisted of questions regarding vaccination status.

Results: Among the respondents 50% were males and 50% females. According to 85% of the students, blood transfusion is a risk factor for contracting HBV. Jaundice, a symptom of hepatitis B, was reported by 77% of the students. 76% agreed that HBV infection can lead to cirrhosis, while 57% mentioned liver cancer as a possible outcome. A majority of respondents (97%) agreed that hepatitis B is preventable, and 92% of students stated that vaccination can protect against the infection. Regarding vaccination status, 53% of students were vaccinated, with 79% of them being fully vaccinated.

Conclusion: Medical students were well aware about hepatitis B infection. As health professionals are vulnerable to develop hepatitis B infection, every medical college should make policies regarding HBV screening as well as vaccination of medical students and medical faculty.

Key words: Hepatitis B awareness, Vaccination status, Medical students.

Introduction

Hepatitis B infection is one of the most prevalent health issues globally. This infection leads to liver cirrhosis and liver cancer. World Health Organization reported annually 600,000 peoples infected with HB virus which ultimately, proved fatal.[1]

The main modes of transmission hepatitis B infection are intravenous drug use, sexual intercourse, vertical transmission from mother to child and contact with infected blood and body fluids.[2,3] According to a survey, health care worker especially (96%) doctors were more prone to have hepatitis B infection while general population was less likely to contract such infection.[4] The most common source of transmitting hepatitis B infection in health worker is via needle stick injury; the ratio of these cases is 66,000 per annum.[5]

In medical setting health workers are more vulnerable to this infection because they come in contact with blood and other body fluids.[6,7] To save health professionals from this dangerous infection WHO recommended professionals working in medical setting should be vacci-

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nated against hepatitis B.[8] Fortunately, hepatitis B vaccine is 95% effective and can prevent from the chronic consequences of this disease.[9]

Vaccinated individuals are less likely to get these infections while unvaccinated are 30% more at risk of transmission of this infection.[10] Medical students during their clinical course work deal with patients, blood, injections and surgical instruments, being inexperienced they are at a higher risk to acquire blood borne infections like hepatitis so they must have knowledge about the precautionary methods to prevent such kind of infections.[11]

Medical students must have knowledge regarding hepatitis B infection because they have to work in hospital setting and deal with all kind of patients. So, it is very important to educate medical students about the harmful effects of this infection and also importance of Hepatitis B vaccination. The present study aimed to assess the Hepatitis B awareness and vaccination status among medical students.

Methods

A descriptive cross sectional study was conducted in Lahore Medical and Dental College (LMDC), Lahore. The study was approved by the Institutional Review Board of Lahore Medical & Dental College. Duration of study was three months from May to July 2022. The data was collected after getting verbal consent from students. The Study population was fourth year MBBS class. The participants of study were 138 fourth year MBBS students. A Convenient sampling method was used.

A structured questionnaire was used to collect data from respondents. There were 3 sections of the questionnaire. The first part included background variables like gender (male or female), Pre-medical qualification (FSc, A levels or American Board) and occupation of mother and father (Doctor or non-Doctor). The second section consisted of questions relating to Hepatitis B awareness and the last part was related to the vaccination status of the respondents.

Data was entered, cleaned and analyzed using Statistical Package for Social Sciences (SPSS), version 20. Data was presented as tables. Frequencies and percentages were calculated.

Results

Among 138 study participants 50% were male, pre-entry qualification of 82% were FSc and 17 % was A-Level (Table I). Fathers of 17% of students and mothers of 9% were doctors.

Table I: *Background information of fourth year MBBS students (n= 138)*

Background Information		n	%
Gender	Male	69	50.0
	Female	69	50.0
Pre-Entry Qualification	F.Sc	114	82.6
	A-Level	23	16.7
	American Board	1	0.7
Fathers' occupation	Doctor	24	17.4
	Non-Doctor	114	82.6
Mothers' occupation	Doctor	12	8.7
	Non-Doctor	126	91.3

As documented in Table II, around 70% of the medical students were aware Of hepatitis B infection, 96% knew that it was caused by a virus. The findings showed that 54% students perceived health professionals as more vulnerable group of having Hepatitis B infection, 69% believe people with multiple sex partners and 59% reported illicit drug users were high risk groups. Majority of students (85%) blamed blood transfusion as the main risk factor. The methods of spreading this infection were quoted by the study respondents as blood transfusion by 86%, use of contaminated needles by 58% and unprotected sex by 50%. Regarding main symptoms of Hepatitis B 51% mentioned loss of appe-

Table II: *Students' awareness about hepatitis B*

Awareness about Hepatitis B		n	%
Type of disease	Non Communicable	42	30.4
	Infectious	96	69.6
Causative agent	Bacteria	6	4.3
	Virus	132	95.7
High risk group	Health professionals	75	54.3
	Having multiple sex partners	95	68.8
	Homosexuals	44	31.9
	Illicit drug users	81	58.7
	Barbers	43	31.2
Risk factors	Smoking	7	5.1
	Alcohol abuse	20	14.5
	Piercing & Tattoos	57	41.3
	Blood transfusion	118	85.5
	Dental visits	43	31.2
	Consuming contaminated food	17	12.3
	Drinking contaminated water	25	18.1

Mode of infection spread	Transfusion of blood	119	86.2
	Un protected sex	69	50.0
	Vertical transmission from mother to her baby	51	37.0
	Use of contaminated needles	80	58.0
Signs & Symptoms	Fever	64	46.4
	Loss of appetite	71	51.4
	Nausea	52	37.7
	Vomiting	51	37.0
	Jaundice	104	24.6
Complications	Hepatitis	26	18.8
	Cirrhosis	105	76.1
	Liver cancer	79	57.2
	Kidney disease	26	2.2
Preventable disease	Yes	134	97.1
	No	4	2.9
Preventable by vaccination	Yes	127	92.0
	No	11	8.0

tite, 46% mentioned fever, 37% reported nausea and vomiting. 76% reported liver cirrhosis and 57% reported liver cancer as main complications. 92% medical students perceived that vaccination is a great protection against HB infection.

Table III shows that around 53% respondents were vaccinated, out of these, 79% were fully vaccinated. The main reason given for not being vaccinated by study participants was that they never considered it important. Around 11% of students did not respond to this question (Table III).

Table III: *Vaccination status of the respondents (n=138)*

Awareness about Hepatitis B		**N**	**%**
Hepatitis B vaccination done	Yes	72	52.9
	No	66	47.1
Fully vaccinated receiving doses at 0, 4 & 16 weeks (N = 72)	Yes	57	79.2
	No	15	20.8
Reasons for not being vaccinated against Hepatitis B (N = 66)	Lack of motivation	4	6.1
	Not needed	5	7.6
	Never thought of vaccinatio	49	74.2
	Fear of injection	1	1.5
	No response	7	10.6

Discussion

This present study showed awareness level of medical students regarding hepatitis B infection. The findings of present study showed that 96% students reported virus is causative agent of hepatitis B infection, similar finding were also reported by 94.% respondents in another study.[12] Medical students perceived the most vulnerable groups for Hepatitis B infection to be health professionals (54%), other studies also showed that majority of their respondents agreed that health professionals were at great risk.[13] The findings of other studies corroborated with present study that hepatitis B infection is transmitted through body fluid and infected blood.[14-16] Inpresent study 85% of the respondents considered blamed blood transfusion as the main risk factor of spreading hepatitis B infection. In another study 97% participants also claim blood donation as a mode of transmission.[9] In agreements with the present study the other studies also highlighted that unsafe sex with multiple partner can transmit Hepatitis B virus.[17] The main symptoms of Hepatitis B mentioned by students were loss of appetite (51%), fever (46%), nausea and vomiting (37%). In other studies participants reported different symptoms such as: fever (42%), loss of appetite (25.7%) and nausea and vomiting: (28%).[18] Liver cirrhosis (76%) and liver cancer (57%) disease were reported as result of hepatitis B infection. In another study 83.1% students reported that Liver cirrhosis is symptom of hepatitis B and liver cancer was reported by (69%) while other studies also showed similar results.[19,20] Majority of respondents (98%) agreed that hepatitis B is preventable and vaccination can protect from this disease (92%). In current study, 53% of students were vaccinated but the vaccination status of students of another study was less (40%) than the present study.[21] A study related to vaccination status represent half of the students were vaccinated which was also less than the present study.[22] More awareness must be generated among medical students and the general population about vaccination against Hepatitis B in vulnerable groups. It should be made compulsory for all students entering medical colleges to get fully vaccinated against Hepatitis B. As the current study was conducted only in 4th year MBBS class of a medical college, therefore it lacks generalizability.

Conclusion

Medical students had good knowledge about hepatitis B infection but their vaccination status was not satisfactory.

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Ethical Consideration: The study was approved by the ethical review board. Informed written consent was obtained from the participants, and the confidentiality of their data was clearly explained.

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Authors Contribution

All the authors contributed equally in accordance with ICMJE guidelines.

RR: Conception of idea & design, acquisition, analysis & interpretation of data, drafting the article, critical review, final approval of the manuscript

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About the Journal

About the Journal

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Authors are invited to submit articles for publication, reporting original work in Medicine, Dentistry, Public Health, Health administration, Biological Sciences, Medical education, and Allied Health Sciences. Other regular features within the journal include systematic review articles, case reports, short communication, and letters to the editor. Editorial articles are by invitation only. However, those received and found to be of an outstanding nature will be considered for publication.

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- Final approval of the version to be published; AND
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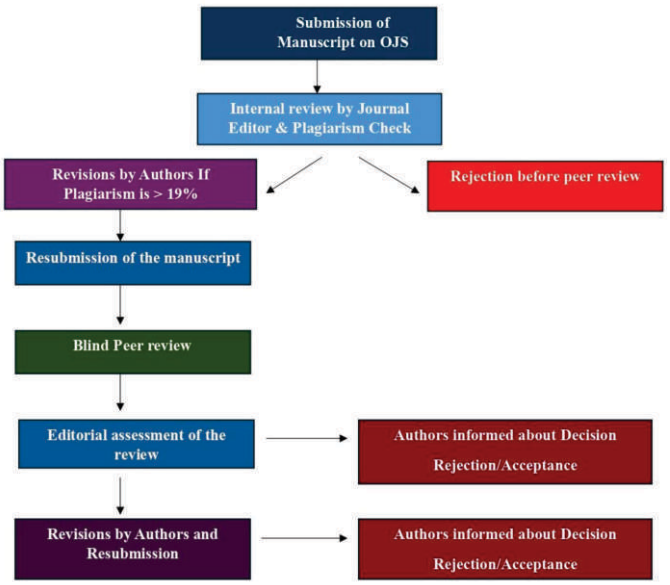

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