ORIGINAL ARTICLE

UNRAVELING CORONARY ARTERY DISEASE VULNERABILITY IN PATIENTS WITH ABO GENE POLYMORPHISM: A CASE-CONTROL STUDY IN PAKISTAN PERSPECTIVE

Saadia Saad[1], Syed Tousif Ahmed[2], Ambrina Khatoon[2], Jawaid Ansari[2], Asaad Javaid Mirza[1]

[1]Baqai Medical University, Karachi, Pakistan, [2]Ziauddin University, Karachi, Pakistan

Objectives: This study was aimed to explore the association of ABO gene variants with coronary artery disease.

Methodology: This was a case-control study. Cases and controls were individuals with greater than 50% and less than 30% stenosis, respectively. One hundred thirty-eight samples were obtained, with 69 cases and 69 controls. The operator completed a proforma regarding demographics, medical history, and blood group. The bench work included blood typing, followed by blood genotyping by DNA extraction, PCR, and then Sanger's sequencing. The single nucleotide polymorphisms (SNPs) selected for the ABO gene were rs8176746 and rs8176719.

Results: The results show that 68.1% of the participants were male cases, compared to 49.27% controls. The frequency of patients belonging to the old age group (60-75 years) was 65.21% in cases and 34.78% in controls. A+ was the most prevalent group in cases, with 33.33%, followed by 24.6% B+, 23.2% O+, and 4.3% AB+. Patients with Rh-ve groups were rare. On the contrary, 33.33% of patients were O+ in controls. A chi-square test showed that the A+ blood group was significantly associated with CAD, having a p-value of 0.01. Although blood genotypes did not show a significant p-value, the odds of having genotype AA was 1.35 times higher in cases compared to the controls.

Conclusion: This study shows that the A+ group is significantly associated with CAD. The data obtained through Sanger's sequencing determined the genetic variants of blood groups, but no statistically significant association was found between them and CAD.

Keywords: ABO blood groups, ABO genotypes, coronary artery disease

Citation: Saad S, Ahmed ST, Khatoon A, Ansari J, Mirza AJ. Unraveling Coronary Artery Disease Vulnerability in Patients with ABO Gene Polymorphism: A Case-Control Study in Pakistan Perspective. Pak Heart J. 2023;56(03):205-210. DOI: https://doi.org/10.47144/phj.v56i3.2601

INTRODUCTION

The ABO system is classified into four groups, A, B, AB, and O, depending upon the blood group antigen on the red blood cells. The blood group antigens are A and/or B, and/or H antigens. They are present not only on RBCs but also on WBCs, platelets, endothelium, and body fluids, such as saliva, sweat, breast milk, amniotic fluid, and gastric secretions.[1] Any alteration at the gene level, such as single nucleotide polymorphisms (SNPs), deletions, insertions, aversions, or alternative splicing, can cause nucleotide change and ultimately result in the formation of a modified blood group antigen or may cause altered antigenic expression.[2] These antigens hold multiple functions like channels, adhesion molecules, enzymes, and receptors for ligands, viruses, bacteria, and parasites. The association of these antigens in the pathogenesis of different diseases is yet to be explored. Still, in literature, the antigens acting as adhesion molecules play a part in human diseases and normal red cell development.[3]

Over the past few decades, various researchers have extensively investigated the genetic makeup of the ABO blood group system to find out any correlation between blood groups and the pathogenesis of various systemic ailments. Many of them have successfully found a definite link between them. Therefore, the clinical importance of these blood groups has increased far ahead and is not merely restricted to blood transfusion sciences. There is accumulating evidence that blood groups play a significant role in the pathogenicity of numerous human disorders, including infectious diseases, psychiatric diseases, neoplastic diseases, and cardiovascular diseases.[4]

Cardiovascular diseases are a group of disorders of the heart and blood vessels. Coronary artery diseases (CAD) and stroke have the highest prevalence worldwide among these disorders. CAD is an atherosclerotic disease that causes inflammation and narrowing of coronary arteries and is represented as stable or unstable angina, myocardial infarction, or a sudden cardiac arrest leading to death.[5] They are one of the leading causes of death globally.[6] Of around 16 million deaths due to non-communicable diseases, 82% are in low-middle-income countries, and 37% are related to CVDs.[7] In South Asia, deaths from CAD have augmented by 87.8% in the last decade, and the death toll is anticipated to intensify further by at least half a percent in the coming years.[8] It is alarming to note that according to the findings of a study, the disease is particularly increasing in young Asians.[9]

Therefore, abundant research has been done on the disease itself and its associated risk factors to reduce the disease's burden. Some established and modifiable risk factors are smoking, obesity, hypertension, diabetes, and hypercholesterolemia. These are also predictable factors for successful aging and a healthy life.[10] Besides these traditional risk factors, a positive family history acts as a crucial risk factor in the pathogenicity of CAD. This gives rise to premature coronary artery diseases in offspring and acts as a major predictor of CAD, not only because of the inherited susceptibility genes but also because of the shared lifestyles, which include diet and sedentary routine that may exaggerate the predisposition to cardiac events.[11] Multiple genome-wide studies have been conducted in this regard and have linked over 60 genetic loci to CAD.[12] One such locus is the ABO gene located at chromosome 9q34.[13] Globally, the genetic variants of the ABO gene have already been identified and linked to CAD.

However, there is a scarcity of literature involving studies on the native population. A recent study from Pakistan has identified ABO genotypes using the RFLP technique and has linked the same to CAD.[14] It necessitates conducting more studies to come to a probable conclusion about the Pakistani population. Therefore, this study aimed to investigate genetic variants of ABO and to look for their association with CAD using a more advanced Sanger's technique, which has not been used in Pakistan until now.

METHODOLOGY

Study Group: This is a case-control study. The individuals admitted to the cardiology ward of Ziauddin Hospital for angiography were recruited for the study. Angiography was performed on patients with unstable angina, aortic stenosis, atypical chest pain, abnormal heart stress test, or who were diagnosed with a heart attack. The individuals having greater than 50% stenosis on angiography in any of the three coronary vessels were included in the cases. The controls comprised individuals with less than 30% stenosis on the angiograph. The samples were collected from March 2022 to August 2022, and a non-probability purposive sampling technique was employed. The participants who belonged to the age range of 25-75 years were included. The study was approved by the Ethical Review Committee of Ziauddin University (Ref # 5230322SAPHY). All the patients were informed about the study, and written consent was acquired. The sample size calculated was 138, i.e., 69 cases, and 69 controls, i.e., the ratio of cases to controls was 1:1. Sample size estimation was done through the website openepi. The odds ratio (4.66) was taken out from a previous article to calculate sample size at a 5% level of significance and 80% power.[15]

The subjects were inquired about their demographics, medical history, and blood groups to fill up the proforma, which was done by the operator. Their angiography reports were taken to note the degree of stenosis and the number of coronary vessels diseased. Those with chronic liver or kidney disease or any malignancy were excluded from the study.

Blood Sampling and Typing: A trained phlebotomist drew 4.0 ml of venous blood from the participants using all aseptic measures to control the chances of infection and cross-infection. Of this 4.0 ml, 2 ml was taken in the red top vacutainer to detect the blood group, whereas the other 2 ml was drawn in the purple top (EDTA) vacutainer for DNA extraction. The blood was stored at -20^{o}C. Blood typing was done by forward and reverse grouping.

Genotyping of ABO Gene: In order to determine the genetic variants of the individual blood groups through Sanger's sequencing, 18 samples were selected out of 69 from cases and similarly from controls. As sequencing is an expensive procedure and this was a self-funded project, all of the samples were not sent for sequencing; representative samples from each blood group, i.e., A, B, and O, were matched between cases and controls and then sent for sequencing. In this way, three samples from blood group A, three from blood group B, and three from O were chosen from cases, and similarly, matched samples were chosen for controls. The blood samples were centrifuged for genotyping, and the buffy coat was separated. The spin column method for genomic DNA extraction was performed using a Qiagen Kit according to the research protocol. Each DNA sample was quantified

by using a spectrophotometer at 260 nm and 280 nm (A260/280). 2 SNPs were selected as ABO gene variants. These SNPs were the most commonly explored ABO gene variants in the literature. These were *rs8176746* (G>A/T) and *rs8176719* (-/C). The SNP *rs8176746* differentiates between blood groups A and B, whereas *rs8176719* tags for the O blood group. Amplification of the ABO gene was carried out by conventional PCR using a DreamTaq Green PCR Master Mix. Primers for restriction sites were obtained commercially for annealing purposes. The optimization of the annealing temperature of primers was done, and the best temperature for annealing was found to be 60˚C. Amplification products were then observed using agarose gel electrophoresis. After purification, these samples were sent for Sanger's sequencing. For alignment and trimming of sequences, Mega X software was used, and polymorphic sites were then analyzed. All statistical analyses were performed using SPSS 25. All categorical variables are expressed in frequency and percentages. A chi-square test was employed to show the association of each variable with CAD. The odds ratio was evaluated to compare the risk of CAD between cases and controls. A p-value of <0.05 was considered statistically significant.

RESULTS

Study subjects' characteristics: A total of 138 patients were enrolled in the study, of which 69 were cases, and 69 were controls (see Table 1). The data shows that 68.11% of the individuals were males in cases, whereas 49.27% were males in controls. Females accounted for 31.88% of the cases; females were 50.72% in controls. The chi-square test showed a statistically significant p-value of 0.025. Most (65.2%) of the patients in cases belonged to old age ranging from 60-75 years, whereas in controls, subjects were majorly (55%) middle-aged aged 44-60. A p-value of 0.001 was calculated for the association between age and CAD. Family history of CAD plays an important role in assessing the risk of CAD in the offspring. The same results showed that 79.7% of the cases had a positive family history of CAD.

On the contrary, in controls, the positive history accounted for merely 46.37%. The p-value was <0.001, which depicts a highly significant statistical value. While assessing the comorbid, hypertension showed a significant p-value of 0.007 with CAD, whereas diabetes showed a non-significant p-value of 0.864.

Table 1: Study Subjects' Characteristics

Variables	Cases (n=69)	Controls (n=69)	P-value
	n (%)	n (%)	
Gender			
Male	47(68.11%)	34(49.27%)	0.025
Female	22(31.88%)	35(50.72%)	
Age Range (years)			
25-44	1(1.44%)	7(10.14%)	0.001
44-60	23(33.33%)	38(55.07%)	
60-75	45(65.21%)	24(34.78%)	
BMI (kg/m²)			
<18.5	0 (0.0%)	1(1.44%)	0.798
18.5-24.9	36(52.17%)	35(50.72%)	
25.0-29.9	12(17.39%)	12(17.39%)	
30.0-40	21(30.43%)	21(30.43%)	
Family History of CAD	55(79.7%)	32(46.37%)	<0.001
Smoking Status			
Current smoker	12(17.39%)	16(23.18%)	0.603
Ex-smoker	12(17.39%)	9(13.04%)	
Never smoked	45(65.21%)	44(63.76%)	
Hypertension	53(76.81%)	38(55.07%)	0.007
Diabetes Mellitus	38(55.07%)	39(56.52%)	0.864

BMI=body mass index, CAD=coronary artery diseases

Blood Groups, Genotypes, and CAD: The results showed that the distribution of the A+ blood group in cases was 33.3%, which was the most prevalent, followed by 24.6% from B+, 23.2% from O+, 4.3% from AB+, and the Rh-ve were very few. However, in controls, the most prevalent group was O+, accounting for 33.3% of the individuals, followed by B+ (29%), A+ (15.9%) and AB+ (7.2%). When the Chi-square test was employed for the individual association of each blood group with CAD, only the A+ blood group showed a statistically significant p-value of 0.01 (see Table 2).

Table 2: Association of Blood Groups with CAD

Blood groups	Cases (N=69)	Controls (N=69)	Odds ratio [95% CI]	P-value
A+	23 (33.3%)	11 (15.9%)	2.64 [1.16-5.96]	0.010
B+	17 (24.6%)	20 (29%)	0.80 [0.37-1.70]	0.560
O+	16 (23.2%)	23 (33.3%)	0.60 [0.28-1.27]	0.180
AB+	3 (4.3%)	5 (7.2%)	0.58 [0.13-2.53]	0.470
AB-	2 (2.9%)	1 (1.4%)	2.02 [0.17-22.92]	0.560
B-	3 (4.3%)	3 (4.3%)	1.00 [0.10-5.13]	>0.999
O-	5 (7.2%)	3 (4.3%)	1.71 [0.39-7.49]	0.470
A-	0 (0%)	3 (4.3%)	0.13 [0.006-2.69]	0.190

CI=confidence interval

The odds ratio suggested that the odds of having the blood group A+ is 2.64 times higher in cases compared

to the controls. However, none of the other blood groups showed a significant p-value.

For genotype determination, gel images were visualized (see Figure 1), and Sanger's sequences were then analyzed. The predicted genotypes were AA, AO, BB, BO, and OO. Most of the genotypes were similar in frequency in both cases and controls, except for AA and AO genotypes. The frequency of AA genotype in cases was 27.7%, whereas in controls, the frequency was 22.2%. Likewise, 5.5% of individuals had AO genotype in cases; however, in controls, the frequency was found to be 11.1%. None of the genotypes produced a statistically significant p-value. However, the odds ratio showed that the odds of having genotype AA were 1.35 times higher in cases than in the controls (see Table 3).

Table 3: Association of Blood Genotypes with CAD

Genotypes	Cases (n=18)	Controls (n=18)	Odds Ratio [95% CI]	P-value
AA	5 (27.77%)	4 (22.22%)	**1.35 [**0.29-6.13**]**	0.700
AO	1 (5.55%)	2 (11.11%)	0.47 [0.038-5.70]	0.550
BB	4 (22.22%)	4 (22.22%)	1.00 [0.20-4.81]	>0.999
BO	2 (11.11%)	2 (11.10%)	1.00 [0.125-7.99]	>0.999
OO	6 (33.33%)	6 (33.33%)	1.00 [0.25-3.99]	>0.999

CI=confidence interval

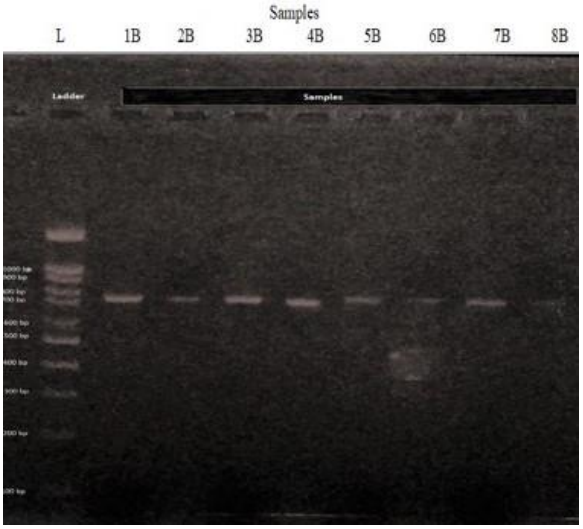

Figure 1: Agarose gel electrophoresis of ABO gene amplification using primer; rs8176746; Product size = 734

Sample# 1B,2B,3B,4B= Cases
Sample# 5B,6B,7B,8B= Controls

DISCUSSION

This study was planned to investigate the vulnerability of blood genotypes to CAD in a subset of the Pakistani population. The results revealed a highly significant association between positive family history and CAD. Numerous studies are in line with this result.[16,17] A study concludes that a positive family history increases the odds of having CAD in the offspring by approximately 2-fold, independent of conventional risk factors, indicating the influence of genetic factors in the vulnerability of the disease.[18] Male gender, increasing age, and hypertension are some known risk factors of CAD, which can be represented by a significant p-value in this study. On the contrary, surprisingly, smoking and diabetes did not show a significant association in this study. However, they also act as strong risk factors for CAD. Another study showed similar results of having no significant association between diabetes and CAD. It showed lower renin levels in diabetic patients that were seen to play a protective part against CAD.[19]

Blood groups and their genetic variants are an unmodifiable risk factor that has been gaining popularity over the last two to three decades. This study showed a higher preponderance of A+ blood groups towards CAD than other blood groups with a significant p-value, whereas blood group O+ was higher in patients without CAD. Most of the studies align with these results, whereas some depict contrasting results. Specific antigens on RBCs, platelets, or endothelial cells determine the ABO blood group. A and B antigens produce the A and B phenotypes, respectively, whereas the absence of these antigens results in the O phenotype. The ABH antigen has a glycosyltransferase activity, whereas the O blood group lacks this enzymatic activity. According to some research, this glycosyltransferase activity in blood group A modulates platelet glycoproteins and glycolipids, which in turn results in disturbance of platelet activation and aggregation, causing platelet-driven thrombosis.[20] When genetic variants of ABO were explored in association with CAD, none of the genotypes showed a significant p-value. However, the odds ratio suggested that the AA genotype was approximately 1.4 times more prevalent in CAD cases than the controls.

A recent UK-based study in the last quarter of 2020 found that the ABO blood group locus regulates the ABH antigen expression on platelets and von Willebrand factor in healthy individuals. AA genotypes were seen as the major determinant of high expression phenotype. Consequently, antigen expression on vWF was significantly higher in high

expression traits. According to the researchers, further studies are recommended on the issue to determine whether this inter-individual variation in ABO expression on platelets and VWF has a role in causing CVDs.[21] Lack of association in this study could probably be because of a smaller number of samples that were sent for sequencing. Although plenty of research favors the AA genotype as a risk factor for CAD, some contrasting studies are also found in the literature. PK Chawla et al., in their study of association, found the BB genotype to develop CAD with a 5-fold increased risk than any other genotype. In contrast, the O1O1 genotype was found to be atheroprotective.[15] Another recent study from Pakistan presented similar results in which the odds of acute MI were approximately three times higher in the BB genotype than others. Although no association between ABO phenotypes and acute MI was found.[14] The notable issue with the study was that the researchers had enrolled apparently healthy individuals as controls with no evident history of previous CAD. However, asymptomatic CAD could be silently present. This restriction might have affected the results of the mentioned study. In order to overcome this limitation, individuals who had healthy coronaries as per angiography (the most reliable tool for detecting CAD) were recruited as controls in this study.

The study has multiple strengths as it is the first-of-its-kind study on the Pakistani population that has determined blood genotypes using Sanger's sequencing. This study would contribute to the existing body of scientific knowledge in the field of cardiovascular genetics of the subcontinent, potentially uncovering unique genetic associations or variations specific to the region. Discovering specific genetic markers or variants associated with CAD within the Pakistani population can aid in the development of genetic risk assessment tools. These tools can be utilized to identify individuals with increased genetic susceptibility to CAD, facilitating proactive measures such as lifestyle modifications and pharmacological interventions to reduce disease burden. Moreover, according to WHO, every ethnicity should have its own normative data for developing targeted prevention and treatment strategies.

This study holds numerous limitations. The data was collected from a single center, and the majority of the ethnicities from Pakistan could not be included. It would have been better if the data was collected from multiple centers so that the majority of the ethnicities could be included in the study, which could have given a better idea of whether the association of blood groups with CAD was ethnicity-dependent. The sample size chosen for sequencing was relatively small due to financial constraints. A comparatively bigger sample size is required to present a reliable association between two variables. Some blood parameters like cholesterol level, D-dimers, serum soluble intercellular adhesion molecule-1 (sICAM-1), bleeding time, and clotting time could not be recorded because of the absence of data in the clinical records of the patients.

CONCLUSION

The results of this study show that the A+ blood group is significantly associated with CAD. The odds of blood group A+ were 2.64 times higher in cases than controls. The data obtained through the sequencing technique determined the different genetic variants of blood groups, but no statistically significant association was found between them and CAD. However, a larger sample size would be required to rule out a significant association in the Pakistani population.

Further research is warranted to investigate additional genetic markers, environmental factors, and lifestyle variables that may contribute to CAD risk within the Pakistani population. Understanding the complex interplay between genetics, lifestyle, and environment is crucial for comprehensive risk assessment, prevention strategies, and personalized management of CAD.

AUTHORS' CONTRIBUTION

SS, STA, and AK: Concept and design, data acquisition, interpretation, drafting, final approval, and agree to be accountable for all aspects of the work. JA, and AJM: Data acquisition, interpretation, drafting, final approval and agree to be accountable for all aspects of the work.

Conflict of interest: Authors declared no conflict of interest.

REFERENCES

1. Ewald DR, Sumner SC. Blood type biochemistry and human disease. Wiley Interdiscip Rev Syst Biol Med. 2016;8(6):517-35.
2. Denomme GA. Molecular basis of blood group expression. Transfus Apher Sci. 2011;44(1):53-63.
3. Abegaz SB. Human ABO Blood Groups and Their Associations with Different Diseases. Biomed Res Int. 2021;2021:6629060.
4. Franchini M, Lippi GJBm. The intriguing relationship between the ABO blood group, cardiovascular disease, and cancer. BMC Med. 2015;13(1):1-3.
5. Álvarez-Álvarez MM, Zanetti D, Carreras-Torres R, Moral P, Athanasiadis G. A survey of sub-Saharan gene flow into the Mediterranean at risk loci for coronary artery disease. Eur J Hum Genet. 2017;25(4):472-6.
6. Tessler J, Bordoni B. Cardiac Rehabilitation. In: StatPearls. Treasure Island (FL): StatPearls Publishing; June 4, 2023.

7. Barolia R, Sayani AH. Risk factors of cardiovascular disease and its recommendations in Pakistani context. J Pak Med Assoc. 2017;67(11):1723-9.
8. Tan ST, Scott W, Panoulas V, Sehmi J, Zhang W, Scott J, et al. Coronary heart disease in Indian Asians. Glob Cardiol Sci Pract. 2014;2014(1):13-23.
9. Saleem M, Durrani AK, Adeeb M, Siddique AR. Psychosocial risk factors of cardiovascular disease in Pakistani adolescents and young adults: A Systematic Review. J Pak Med Assoc. 2020;70(9):1601-4.
10. Yaghooti-Khorasani M, Ghazizadeh H, Bijari M, Mohammadi-Bajgiran M, Oladi MR, Zare-Feizabadi R, et al. Evaluation of ABO blood group in subjects with CVD risk factors in a population sample from northeastern Iran. Diabetes Metab Syndr. 2020;14(6):1689-95.
11. Kulkarni P. Family History of Coronary Artery Disease as an Additional Risk Factor Associated with Coronary Artery Disease: A Descriptive Observational Study. JCTCD. 2015; 2(1):1-3.
12. Khera AV, Kathiresan S. Genetics of coronary artery disease: discovery, biology and clinical translation. Nat Rev Genet. 2017;18(6):331-44.
13. Hamrefors V. Common genetic risk factors for coronary artery disease: new opportunities for prevention? Clin Physiol Funct Imaging. 2017;37(3):243-54.
14. Yousuf FA, Azam I, Tareen AK, Kazmi KA, Muhammad JS, Iqbal MP. Association of the BB genotype of the ABO gene with the risk of acute myocardial infarction in hospital-based study. Pak J Med Sci. 2023;39(1):133-8.
15. Chawla PK, PC RR, Deshpande AS, Ashavaid TF. Genetic variants of Abo blood group and coronary artery disease. J Cardiovasc Med Cardiol. 2020;7(2):104-9.
16. Otaki Y, Gransar H, Berman DS, Cheng VY, Dey D, Lin FY, et al. Impact of Family History of Coronary Artery Disease in Young Individuals (from the CONFIRM Registry). Am J Cardiol. 2013;111(8):1081-6.
17. Timmerman N, de Kleijn DPV, de Borst GJ, den Ruijter HM, Asselbergs FW, Pasterkamp G, et al. Family history and polygenic risk of cardiovascular disease: Independent factors associated with secondary cardiovascular events in patients undergoing carotid endarterectomy. Atherosclerosis. 2020;307:121-9.
18. Safarova MS, Bailey KR, Kullo IJ. Association of a Family History of Coronary Heart Disease with Initiation of Statin Therapy in Individuals at Intermediate Risk: Post Hoc Analysis of a Randomized Clinical Trial. JAMA Cardiol. 2016;1(3):364-6.
19. Ferrannini G, Manca ML, Magnoni M, Andreotti F, Andreini D, Latini R, et al. Coronary artery disease and Type 2 Diabetes: A Proteomic Study. Diabetes Care. 2020;43(4):843-51.
20. Zhong M, Zhang H, Reilly JP, Chrisitie JD, Ishihara M, Kumagai T, et al. ABO Blood Group as a Model for Platelet Glycan Modification in Arterial Thrombosis. Arterioscler Thromb Vasc Biol. 2015;35(7):1570-8.
21. O'Donghaile D, Jenkins PV, McGrath RT, Preston L, Field SP, Ward SE, et al. Expresser phenotype determines ABO(H) blood group antigen loading on platelets and von Willebrand factor. Sci Rep. 2020;10(1):18366.

Address for Correspondence:

Dr. Saadia Saad, MPhil Scholar, Baqai Medical University, Karachi, Pakistan.
Email: saadia.12606@zu.edu.pk