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# Association of Cholesterol and Superoxide Dismutase in Patients with Polycystic Ovary Syndrome

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***Abstract:***

***Background:*** Polycystic ovary syndrome (PCOS) is a hormonal condition affecting 4-20% of women globally. It causes abnormal androgen production, obesity, irregular menstrual cycles, and increases the risk of diabetes. This study aims to investigate the relationship between SOD activity and cholesterol levels in PCOS women of different ages and blood types.

***Methodology:*** It is original research, in which we draw blood samples from disease and control patients, and after blood grouping and centrifugation we performed the SOD activity with an ELISA kit and then we performed the lipid profiling with the help of Beckman coulter which ensure the level of cholesterol, HDL and triglyceride in disease patients which vary in every age and blood group.

***Results:*** The results show that the SOD value is higher in the age group 26-30 and blood group B+ve, whereas in lipid profiling it also varies in both age and blood group. The value of triglyceride was found higher in blood group A-ve and showed hype in the age group 36-40. The level of cholesterol was found to be higher in blood group A-ve and increased with the age group of 26-30 years. The value of HDL was found higher in the age group 21-25 with blood group O-ve.

***Conclusion:*** This study revealed consistently high levels of cholesterol and HDL across all age and blood groups, while triglyceride and SOD levels exhibited inconsistent elevation among different groups. The study's primary limitation is its small sample size, preventing generalizability. To address this, it's recommended that research focus on diverse risk factors influencing cholesterol, HDL, and SOD levels. This approach can lead to improved variable control within the local population, enabling the development of effective strategies for mitigating these elevations and reducing associated health risks.

***Keywords:*** Polycystic Ovary Syndrome, PCOS, Cholesterol, Triglyceride, SOD, HDL, Blood Groups.
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## Introduction

Dr. Stein and Leventhal 1935 first published a case series of almost 7 women with menstrual disturbance diagnosed with enlarged ovaries. Then the suggested names Stein-Leventhal syndrome, sclerocystic ovary syndrome, and polycystic ovary disease were replaced by the term Polycystic Ovary Syndrome PCOS in the 1990s. (1) Polycystic ovary syndrome is a rapidly common endocrinological condition in women, which affects 8% to 13% of reproductive-aged women. (2)

Recent studies show that PCOS facing young and non-obese women have a magnified risk of prediabetes (preDM), gestational diabetes and diabetes mellitus type 2 as compared to the weight and age-matched women without PCOS. Multifactorial nature environmental factors such as high carbohydrate consumption, poor fetal growth, prenatal androgen exposure, and acquired obesity interact with genetic origins and are involved in PCOS pathogenesis. (5)

PCOS is a serious life-threatening condition and is

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found to be a major concern for women's health. 50-80% are obese women among the ratio of women suffering from PCOS, with impaired glucose tolerance 30-35% are reported, and almost 8-10% are found as diabetic or have a family diabetes history. (6) All over the world Polycystic ovary syndrome prevalence is about 5% to 10% which is alarming and tends to increase day by day. (7) It creates complexity and challenges in its management and diagnosis because symptoms and diagnosis change with age variation. (2)

Women with polycystic ovary syndrome (PCOS) are at an increased risk of developing abnormal lipid levels, such as elevated cholesterol and triglycerides. Up to 70 percent of women with PCOS experience some degree of elevated cholesterol and/or triglyceride levels, which highlights the significant association between PCOS and dyslipidemia. Elevated cholesterol and triglycerides are risk factors for cardiovascular disease, and women with PCOS are at higher risk of developing cardiovascular complications compared to those without the condition. (8) Treating cholesterol is much like treating PCOS, Cholesterol levels can indeed be managed through lifestyle changes and, in some cases, medication. The primary goal is to lower LDL cholesterol levels (the "bad" cholesterol) and increase HDL cholesterol levels (the "good" cholesterol). If lifestyle changes are not sufficient, doctors may prescribe cholesterol-lowering medications, such as statins, to reduce LDL cholesterol levels. (9)

Superoxide dismutase (SOD) is an essential enzyme that plays a crucial role in neutralizing superoxide radicals in the body. Superoxide radicals ($O_2^-$) are a type of reactive oxygen species (ROS) produced as byproducts of various metabolic processes. If left unchecked, superoxide radicals can cause oxidative damage to cells and contribute to oxidative stress, which has been associated with several health conditions, including Polycystic Ovarian Syndrome (PCOS). SOD catalyzes the conversion of superoxide radicals into hydrogen peroxide ($H_2O_2$) and molecular oxygen ($O_2$). Hydrogen peroxide, though still a reactive molecule, is less harmful than superoxide radicals. (10) This imbalance in the antioxidant defense system may contribute to the increased oxidative damage observed in PCOS, potentially affecting various aspects of reproductive health, including fertility. Superoxide dismutase (SOD) is a critical antioxidant enzyme that plays a crucial role in neutralizing superoxide radicals and maintaining redox balance in cells. As a result, alterations in the expression level, concentration, and activity of SOD can have significant implications for various pathological conditions, including cancer, cardiovascular diseases, neurodegenerative disorders, and metabolic diseases, among others. Here's how changes in SOD status can impact these conditions. (11)

Polycystic Ovary Syndrome (PCOS) is a complex and common endocrine disorder that affects reproductive-aged women. Although much research has been conducted, there are still promising areas of investigation that can contribute to a deeper understanding and improved management of PCOS. (12) This review aims to highlight the significant impact of PCOS on psychological health and well-being. The evidence on various psychological symptoms experienced by PCOS women and the effectiveness of different therapeutic approaches will be critically analyzed. By identifying gaps in current knowledge and providing insights into potential treatment modalities, this review can contribute to better management and support for women living with PCOS. (13) Future research directions both physical and psychological aspects of PCOS as well as the correlation between Blood and age group of PCOS women after dealing with SOD. This will help to find out the prevalence according to age and blood group and how cholesterol in PCOS patients associate with SOD.

## Methodology

The study includes females who were in the reproductive age group (20-45), To assess the SOD activity about ABO blood type following the methodology of 14. Briefly, with the consent of patients to engage in the study in an ethical manner sample of 50 normal and 50 PCOS patients was drawn from the pathology lab at Jinnah Hospital Lahore and public laboratory. For sample collection, we use needles, tubes, tube holders, iodine swabs, gloves, tourniquets, and needle disposal boxes. For blood collection I assemble equipment, Identify and prepare the patient, Select the site, Perform hand hygiene and put on gloves, Disinfect

the entry site, Take blood, Fill the laboratory sample tubes, Dispose of the needle in the correct order.

The blood group of all the samples was found out by the slide method using monoclonal- antisera (Anti-A, Anti-B, Anti-D) identified anti-sera related blood based on agglutination principle. The ABO blood group antigens are O-linked glycoproteins that determine either antigen A or B is present when the cell surface of red blood cells is exposed to the terminal sugar residues. Individuals with blood group A have antigen A and anti-B antibodies in serum. However, individuals with blood group B have antigens B and anti-A antibodies. Individuals with AB blood group have both antigens (A and B) present with no antibodies. While individuals with blood group O have both antibodies (A and B) and neither antigen in their serum. The antibodies interact with the loops of transmembrane proteins (Rh antigens) when exposed to the surface of red blood cells. The presence of their respective antibody. After finding out the blood groups of all samples both control and disease samples were centrifuged at 5000 RPM for five minutes. After centrifugation, the supernatant (plasma) of all samples was transferred into 1.5 ml Eppendorf tubes and stored plasma at -80 centigrade for later use.

The SOD ELISA kit is a research-use-only assay designed for the quantitative measurement of human Superoxide Dismutase (SOD) levels.15. After the completion of blood grouping and preservation of the plasma, ELISA performed using the superoxide dismutase colorimetric Kit, followed the SOD Kit instructions and prepared different solution which includes four steps;

The samples were diluted with a 20:100 ratio, 20 microliter of sample, and 100 of buffer used for this step.

Reconstitute superoxide dismutase by adding assay buffer 250 microliter. Added 75 microliters of assay buffer into first well by using a micropipette and mixed the solution 2-3 times into the well after mixing, took 75 microliters from first well and added into second well again mixed 2-3 times (with the help of micropipette using micro tips ) and again took 75 microliters from second well added into third well repeated the above procedure till the next four wells, (taken first seven wells as standard.

For 1x substrate preparation added 2 ml substrate diluent and 70 microliter substrate concentrate. Added this prepared solution 50 microliters into appropriate control and disease samples, also in standard wells.

For this 1X xanthine oxidase prepare step solution prepared by adding 1.5 ml Xanthine oxidase buffer and 60 microliter Xanthine oxidase concentrate. The 25 microliter xanthine solution was added to control and disease samples well, and 50 microliters were also added to the first seven standard wells. After adding Xanthine oxidase leave the wells for 20 minutes at room temperature. The Xanthine oxidase in the presence of oxygen converts the colorless substrate into yellow colored product. Read the absorbance of colored product at 450 nm

(The increase in the level of SOD in the samples causes the reduction in yellow product and a decrease in the superoxide concentration). Generate a standard curve and read the activity of unknown samples and standard curve. The standard curve range of SOD is 0.06 U/mL-4.00 U/mL (superoxide dismutase (SOD) colorimetric Activity kit, 2020).

In this study, a Beckman Coulter AU analyzer was employed to assess cholesterol concentrations in human serum samples using system reagents.16 The reagents were prepared for immediate use, and the analyses encompassed serum and hemolysis-free plasma samples, including those treated with either EDTA or heparin. The cholesterol measurement procedure exhibited linearity within the range of 25 to 700 mg/dL, demonstrating its suitability for accurate quantification of cholesterol levels in the given samples.

After following serum and EDTA or heparinized plasma samples, a quantitative assessment of HDL cholesterol was performed using Beckman Coulter AU system reagents in human serum. Notably, this method eliminated the need for manual calculations, as results were automatically generated in mg/dL at 37 ° C and printed.

The HDL cholesterol measurements demonstrated a

linear correlation spanning from 2.5 to 200.0 mg/dL, thereby establishing the method's reliability across this range.

For each specimen, we used fasted serum samples free from hemolysis and free clots (* 12 h). Glycerol was not detected in any of the equipment used for sample collection and storage. Triglyceride levels are linearly related to blood lipid levels from 10 to 1000 mg/dL. Beckman Coulter's AU analyzers were used to perform the measurements using the Triglyceride Reagent. Triglyceride risk classification was determined as follows ranges: <150 mg/dL normal; 150–199 mg/dL borderline high; 200–499 mg/dL high; and ≥500 mg/dL very high; 48–352 mg/dL.

## Result

To study the association of the blood group with the activity of SOD in PCOS patients, blood was drawn from the females diagnosed with PCOS. The relation of oxidant stress with the production of antioxidants (SOD) was determined in control and PCOS patients, every result point is a mean of at least three determinations. The results show the relation between the age and blood group of PCOS patients with the calculated levels of SOD.

## Determination of SOD in various Age Groups

The mean age of the control was 24 and the control mean of SOD was calculated as 5.76 mg/dl. Females in the age group between 26-30 years are more likely to develop PCOS. The calculated SOD value of age group 26-30 years was 2.79 mg/dl, followed by age group 15-20 years (2.35 mg/dl), age group 31-35 years (2.23 mg/dl), age group 21-25 (1.56 mg/dl), age group 36-40 years (0.9 mg/dl) (Figure 1).

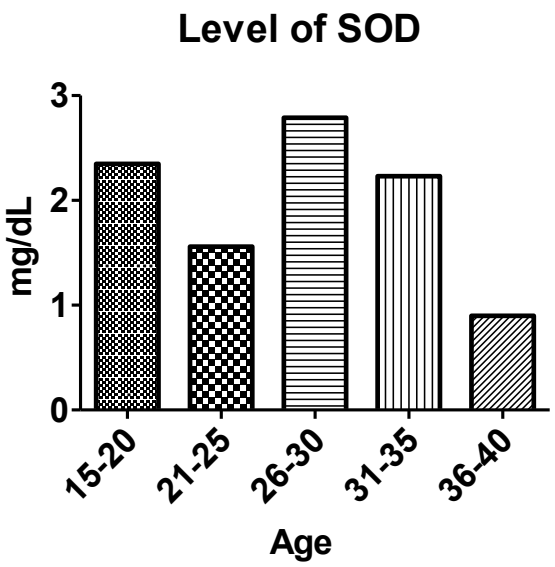



Figure 1: Determination of levels of SOD in various age group. Here categories of age groups in years are mentioned at the x-axis, whereas, their levels of SOD in mg/dL are mentioned at the y-axis. SOD represents sodium oxide dismutase.

The co-relation of blood group with SOD in PCOS patients and control blood group B positive with calculated mean value of SOD (3.72 mg/dl) have highest risk of developing PCOS followed by blood group O negative (2.45), blood group A positive (1.73 mg/dl), blood group O positive (1.67 mg/dl), blood group AB positive (1.52 mg/dl) and blood group A negative (0.23 mg/dl). One of the nested case-control studies which contained 50 PCOS and 50 control samples measured the different parameters of antioxidative indicators and also checked the lipid patterns. SOD levels were found to be increased in women with PCOS.

Endothelial dysfunction in PCOS patients was found to be linked to oxidative stress in a group of young, non-obese PCOS patients (Figure 2).

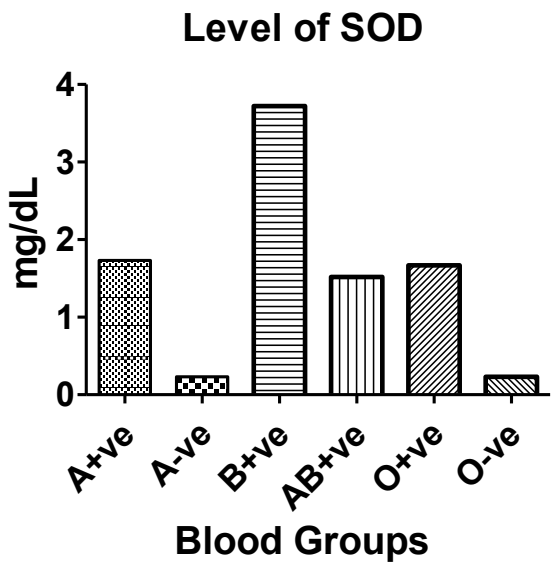



Figure 2: Resolution of the level of SOD in various blood groups. In the graph categories of blood group are expressed at the x-axis, while the level of SOD in mg/dl is shown at the y-axis.

**Triglyceride results:**

Student T-test was performed on the difference between the triglyceride and two parameters of PCOS women with age and blood group. For statistical analysis, $p<0.05$ was used and was taken as statistically significant. Association of triglyceride level in PCOS patients with blood group system. The mean control of triglyceride was estimated at 149.27mg/dl. It shows that the level of triglyceride was highest in A negative blood group (701.83mg/dl) as compared to O positive (278.03mg/dl), A positive( 274.15mg/dl) B positive (150.19mg/dl), O negative (173.62mg/dl), AB positive (173.87mg/dl) and B positive (150.19mg/dl) blood group systems (Figure 3).

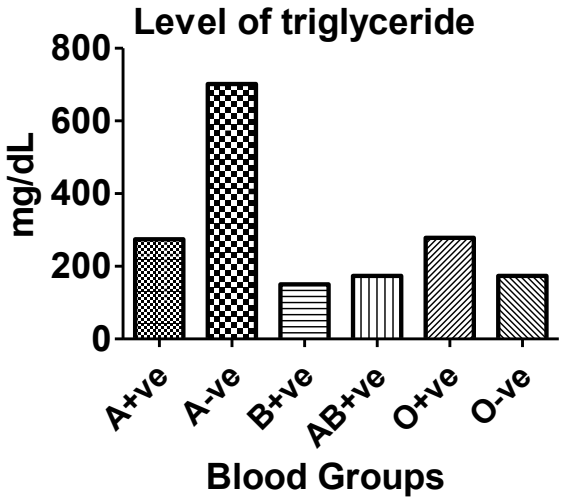



**Figure 3:** Find out levels of Triglyceride in various blood groups. Here categories of blood groups are mentioned at the x-axis, whereas, levels of triglyceride in mg/dL are mentioned at the y-axis.

Association of triglyceride level in PCOS patients with the age group. The mean control of triglyceride was estimated at 149.27mg/dl with a mean age of 24. According to calculated results, the concentration of triglyceride was highest between the 36-40 age group (406.07mg/dl) as compared to 26-30 (244.48mg/dl), 21-25(237.71mg/dl), 15-20(209.64mg/dl) and 31-35 (125.08mg/dl) age groups. Triglyceride levels related to blood group in females having PCOS have been determined. Each data point represents the mean of three readings. Triglyceride levels related to age group in females having PCOS have been determined (Figure 4).

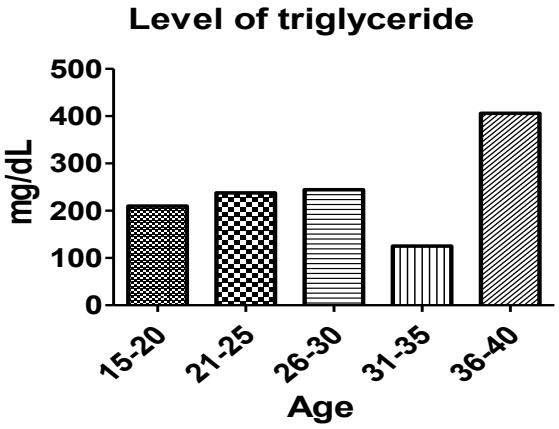
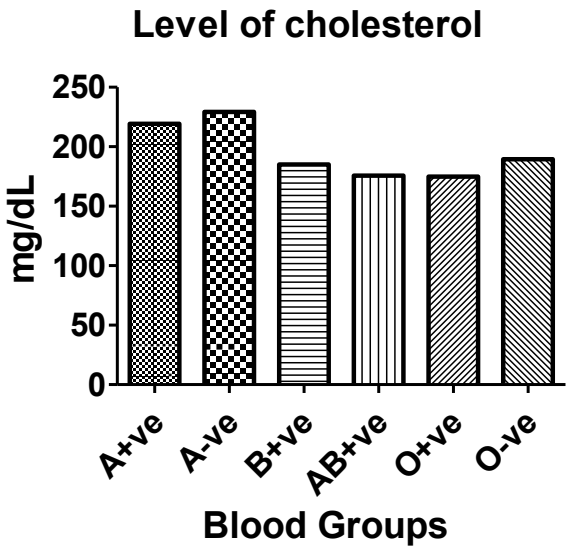



**Figure 4:** Determination of level of triglyceride in various age groups. Categories of age groups in years are expressed at the x-axis, while, their levels of triglyceride in mg/dL are written at the y-axis.

**Cholesterol results:**

Patients with blood group A negative, have the highest mean value of Cholesterol at 229.25mg/dl followed by A positive blood group with 219.37 mg/dl, O negative blood group with 189.43mg/dl, B positive blood group with 185.07mg/dl with, blood group AB positive with 175.81mg/dl and O positive with 174.93mg/dl (Figure 5).

**Figure 5:** Find out levels of Cholesterol in various blood groups. Categories of blood groups are written on the x-axis, whereas, while levels of cholesterol in mg/dL are mentioned on the y-axis.




Association of Cholesterol in PCOS patients with the age group. The control mean of Cholesterol was calculated as 152.43 with a mean age of 24. The highest age group observed with cholesterol was 26-30 years and the mean cholesterol value was estimated at 197.39, In the age group 21-25 calculated cholesterol was 187.02 and 183.57 in the age group 31-35, 177.005 36-40, 165.002 in the age group 15-20. The studies show that PCOS women who are non-obese have a higher level of lipoprotein and then matched control 15.3mg/dl versus 9.1 mg/dl and one-third of patients of PCOS had elevated levels (Figure 6).

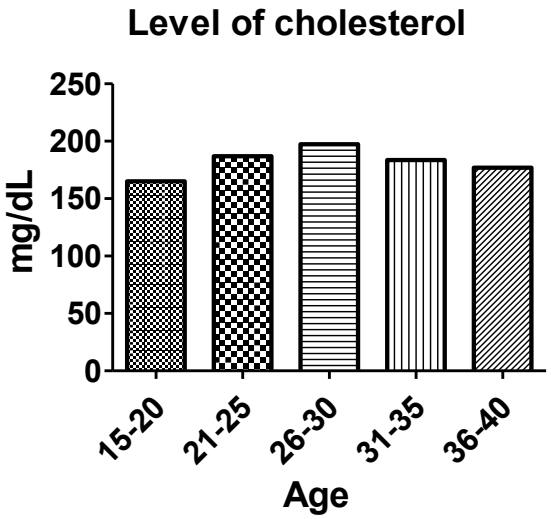



**Figure 6:** Expression of levels of Cholesterol in various age groups. Here categories of age groups in years are mentioned at the x-axis, while, levels of cholesterol in mg/dL are mentioned at the y-axis.

**HDL results:**

Association of HDL level in PCOS patients with age group. The mean control of HDL was estimated at 38.662mg\dl. According to calculated results the concentration of HDL was highest between the 21-25 age group (51.286mg\dl) as compared to 26-30 (46.565mg\dl),15-20 (47mg\dl),36-40 (36.925mg\dl) and 31-35 (0.855mg\dl) age groups. (Figure 7).

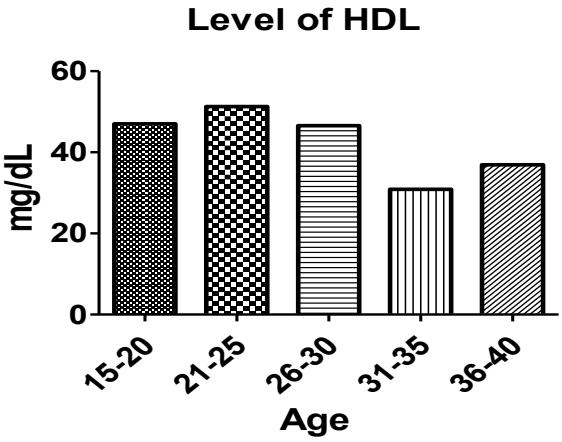



**Figure 7:** The finding of levels of HDL in various age groups. The categories of age groups in years are mentioned on the x-axis, while, their levels of HDL in mg/dL are written on the y-axis.

Patients with blood group O negative have the highest mean value of HDL (62.21mg\dl), followed by blood group O positive (53.725mg\dl), blood group A positive (46.852mg\dl), blood group B positive (43.99mg\dl), blood group AB positive (35.39mg\dl) and blood group A negative (21.43mg\dl) (Figure 8).

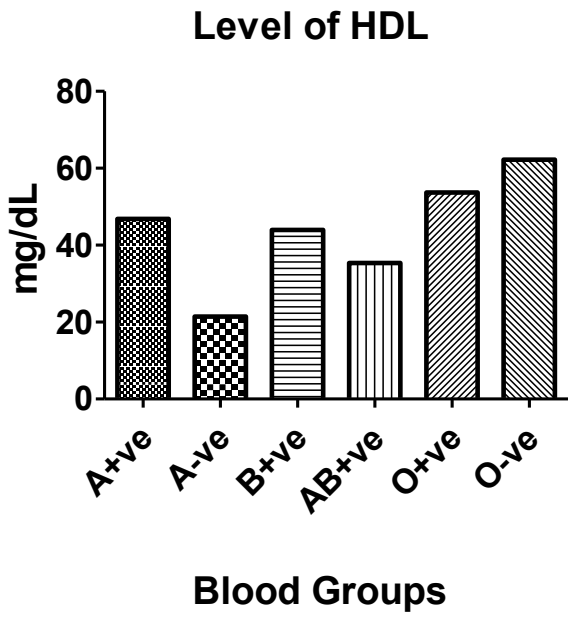



**Figure 8:** Find out levels of HDL in various blood groups. Here categories of blood groups are mentioned at the x-axis, while levels of HDL in mg/dL are mentioned at the y-axis.

**Discussion:**

Superoxide Dismutase (SOD) activity in human blood, specifically looking at the differences between females and the effect of age on SOD activity. The study found that females exhibited higher SOD activity compared to males, with a statistical significance indicated by the p-value being less than 0.001. Overall, the study's findings indicate that there are significant gender differences in SOD activity, with females showing higher levels. Additionally, SOD activity decreases with age, starting around 28 years old, and this decrease is not uniform across all age groups. (17) These findings suggest potential age-related changes in the body's ability to counteract oxidative stress, which could have implications for various health outcomes that are in agreement with the findings of this study possibly due to the adoption of a Western lifestyle, or priorities they set for daily life. There is a relationship between ABO blood groups and oxidative stress in patients with essential hypertension. Blood groups A, B, and AB are associated with increased plasma lipid peroxide levels, which might experience rapid depletion of red blood cell glutathione potentially due to altered scavenging enzyme activity. (18) These findings could have implications for understanding the underlying mechanisms of oxidative stress in hypertensive patients and may contribute to the development of targeted interventions for managing hypertension-related complications that are quite similar to the mentioned findings.

Individuals with the B antigen (specifically B positive or AB blood types) had higher triglyceride levels compared to subjects who did not possess this antigen. (19) This finding indicates an association between the presence of the B antigen and elevated triglycerides that are not in line with available work and findings which is much needed for further work in different regions of the world for better results availability.

The triglyceride levels reach their peak values between the ages of 40 and 50, after which they may experience a slight decline in triglyceride concentration. (20)

Triglyceride levels are particularly higher in women who are using estrogen-based medications, such as hormonal contraceptives or hormone replacement therapy. These trends are in agreement with the findings of this study which highlight the influence of age and gender, as well as hormonal factors, on triglyceride concentrations in the bloodstream. Hormonal changes that occur throughout a woman's life, such as puberty, pregnancy, and menopause, can impact lipid metabolism, including triglyceride levels.

Blood group A was found to have higher levels of both cholesterol and triglycerides. (21) This suggests that individuals with blood type A might be at a greater risk of elevated lipid levels, which are associated with health concerns. These are in line with our results. This implies that hormonal changes associated with menopause might interact with blood groups and influence lipid levels.

Cholesterol levels, including both total cholesterol and its various components like low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), can start to rise around the age of 20,22 is a similar trend and prove all the results true. During early adulthood, especially after the age of 20, there can be changes in lifestyle, dietary habits, and metabolism that impact cholesterol levels. For instance, poor dietary choices, lack of physical activity, smoking, and excessive alcohol consumption.

The study found that HDL-C levels were positively associated with age. In other words, as individuals get older, their HDL-C levels tend to increase.23 This statement is in opposite trend with our results as analysis related to high-density lipoprotein cholesterol (HDL-C) levels, age, and various factors that might influence this relationship.

Individuals with blood group AB appear to have higher levels of HDL-C. HDL-C is often referred to as "good cholesterol" because it plays a role in removing excess cholesterol from the bloodstream. (24) This does not match our results.

## Conclusion:

Overall, it was found that cholesterol and HDL were high in all age and blood groups, whereas triglyceride and SOD were inconsistently raised in various groups. This indicates that all these factors are not dependent on age or blood groups, but instead elevated due to some anonymous reasons, which need to be explored for better understanding. The major limitation of this study is the low sample size due to which we cannot generalize the results, but it is suggested to work on various risk factors responsible for the rise of cholesterol, HDL, and SOD. It will help to control the variables in the population of this region and aid in developing some strategies to mitigate it and reduce morbidities.

## Author's Contribution:

Muhammad Sarwar worked on designing and conception of the study. Iqra Ramzan performed all experiments and contributed to the writing of the manuscript. Lazqa arranged blood samples and helped in experimentation. Ayesha Umar Choudhary analyzed the data, proofread the final manuscript and input her expert opinion in finalizing the draft.

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**Data Availability:** Data will be available and provided on request to the corresponding author.

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