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Lipid profile pattern and different abo blood groups of apparently healthy students in college of health technology, calabar, cross river state

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Abstract

This study was done to see whether the possibility of using blood groups as predictive factors for diseases associated with metabolism of lipids. Lipid profile was tested in one hundred (100) apparently healthy students in College of Health Technology, Calabar consisting of forty (40) males and sixty (60) females of different ABO blood groups aged between 18 and 30 years. Lipid profile parameters were analyzed according to enzymatic assay using a commercial kit from Randox laboratories, United Kingdom and Calculation using Friedewald's equation. Monoclonal ABO blood grouping reagent by Rapid Labs Limited, United Kingdom was used to determine the blood groups. Of these, 22 were blood group A, 17 were blood group B, 4 were blood group AB and 57 were blood group O. Total cholesterol (5.28±0.89mmol/L), triglycerides (2.45±0.66mmol/L) and very low density lipoprotein (1.15±0.30mmol/L) were highest in blood group AB while high density lipoprotein (1.70±0.51mmol/L) were lowest compared to other blood groups. Blood group A had the highest level of low density lipoprotein (1.99±0.33mmol/L) compared to other blood groups. Blood group O showed the highest level of high density lipoprotein (2.95±1.74mmol/L) compared to other blood groups. The study showed that blood group AB might have a higher tendency for diseases associated with lipid metabolism while blood group O may have a lower risk for such diseases. Other risk factors such as family history and environmental factors should also be considered in assessing diseases associated with lipid metabolism.

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Introduction

In biology and biochemistry, lipids are bio-molecules that are soluble in non-polar solvents. They consist of saponifiable lipids, such as glycerides (fats and oils) and phospholipids, as well as non-saponifiable lipids, principally steroids (IUPAC. Compendium of Chemical Terminology, 1997). Lipids are small hydrophobic molecules that carry out a multitude of crucial roles; they act as structural elements in biological membranes, they store energy and they function as signalling molecules in cellular response pathways. (Subramaniam *et al.*, 2011). Lipid profile is measured for cardiovascular risk prediction and has now become almost a routine test.

The test includes five basic parameters: total cholesterol (TC), high density lipoprotein- cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), triglycerides (TG) and very low density lipoprotein-cholesterol (VLDL-C) (Campose *et al.*, 2005). Although lipids are highly essential, abnormal levels contribute to the progression of atherosclerosis. (Durrington, 2003). The abnormalities in lipids can be accessed via lipid profile panel which is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids such as cholesterol and triglycerides (Usoro *et al.*, 2006).

The ABO and Rh blood groups are among the most clinically significant blood group systems (Seeley *et al.*, 1998). Karl Landsteiner first described the ABO blood group in 1900, and it marked the beginning of blood banking and transfusion medicine (Ali *et al.*, 2005). The classification of blood groups into type A, B, AB and O in ABO system as well as Rh (D) positive and Rh (D) negative in Rhesus blood group system is based on the presence or absence of group specific inherited antigenic substances on the surface of the red blood cells.

The antigens may be proteins, carbohydrates, glycoprotein's, glycolipids depending on the blood group system. The two significant blood group systems were discovered during early experiments with blood transfusions; the ABO group in 1901 and the Rhesus group in 1939 (Landsteiner and Weiner, 1940). In the ABO blood group, individuals are classified into four major blood groups, A, B, AB and O, according to the presence of the antigens and agglutinins. Type A blood has type A antigens, type B blood has type B antigens, type AB blood has both types of antigens, and type O blood has neither A nor B antigens. In addition, plasma from type A blood contains type B antibodies, which act against type B antigens, whereas plasma from type B blood contains type A antibodies, which act against type A antigens. Type AB has neither type of antibody and type O blood has both A and B antibodies (Seeley *et al,* 1998). Several studies have elucidated the role of blood groups as predisposing factors for diseases such as gastric cancer (Wang *et al.*, 2012), peptic ulcer (Edgren *et al.,* 2010), pancreatic cancer (Risch *et al.*, 2013), breast cancer (Flavarjani *et al.*, 2014), and upper urinary tract cancer (Kvist *et al.*, 1988), ovarian cancer (Gates *et al*, 2011), bladder cancer (Nakata *et al.*,1995) and pulmonary diseases (Kauffmann *et al.*, 1996). Of these, studies on association of blood groups and cardiovascular diseases (CVD) have been of great significance. During the last few decades, some reports have suggested that ABO blood groups are associated with risk of ischemic heart diseases and developing severe manifestation of atherosclerosis (Meade *et al.*, 1994). Certain studies have reported that A blood group are predisposed to cardiovascular disease and that type O blood group is a protective anti-artherogenic factors (Stakisaitis *et al.,* 2002). It is quite evident that certain blood groups have certain diseases more commonly than others. Thus, this study was designed to investigate the relationship between ABO blood groups and lipid profiles of apparently healthy students in College of Health Technology, Calabar, Cross River State.

Materials and methods

Study area

This study was conducted in College of Health Technology, Calabar in Calabar Municipality, a Local Government Area in Cross River State. Calabar Municipality is located between latitude 04 15' and 5 North and longitude 8 25' East. Calabar

Municipality is bounded to the North by Odukpani Local Government Area and in the North East by the Kwa River. Its Southern shores are bounded by the Calabar River and Calabar South Local Government Area. Apart from Calabar Municipality being the headquarters of the Southern senatorial district. There are ten wards in Calabar Municipality.

Two ethnic groups from the indigenous population of this area; these are the Quas and the Efik. However, because of its cosmopolitan status, there abound people from all parts of the state and Nigeria in the area. By virtue of the location of Calabar Municipality along the waterfront, the Efik embraced Western culture and carried out successful trade occupy the bulk of the hinterland of Calabar where farmers, hunters, traders, and blacksmiths are found. (http://kekerete.tripod.com/CRSG/calmun.html).

Calabar Municipality had an area of 142km² and a population of 179,392 at the 2006 census. The postal code of the area is 540(Post Offices- with map of LGA. NIPOST, 2012)

Study population

This study utilized a simple random sampling procedure to recruit one hundred (100) students, comprising forty (40) males and sixty (60) females. Subjects recruited were students of College of Health Technology, Calabar aged between 18 and 30 years.

Inclusion and exclusion criteria

Only apparently healthy subjects within the age range 18 and 30 years were recruited for this study. Subjects with pregnancy, DM, history of smoking and any other visible ailments were excluded from this study.

Ethical considerations

Ethical approval for the study was obtained from Department of Medical Laboratory Science on behalf of Students Affairs Department of College of Health Technology, Calabar. All the students who met the inclusion criteria for the study were recruited after obtaining verbal consent and confidentiality of the participant's results ensured.

Sample collection and analysis

Two specimen bottles were used for each subject. Anticoagulant bottles containing tripotassium EDTA (k3 EDTA) for blood grouping test and plain container for lipid profile assay. After overnight fasting of about 10-12 hours, 5 ml of blood was collected aseptically by vein puncture from all the subjects between 8–10 am into already labelled bottles, without undue pressure to either the arm or the plunger of the syringe. The samples in the K3 EDTA anticoagulant bottles were tested immediately for blood group using commercially prepared anti-sera (anti-A, anti-B, anti-AB and anti-D). The blood samples in the plain containers were allowed to clot and centrifuged at 4000rpm for 10 minutes. The serum was separated into another clean dry plain containers and stored frozen until analysis was done at room temperature.

Biochemical determination of lipid profile: This was measured by enzymatic spectrophotometric method using a commercial kit by Randox Laboratories, United Kingdom.

Determination of serum total cholesterol: The enzymatic procedure for total cholesterol determination in serum based upon the Trinder (1969) method was used. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Determination of serum high density lipoprotein-cholesterol: The method of Lopes-Virella *et al.* (1977) for the determination of high-densitycholesterol in serum was employed. Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined.

Enzymatic Determination of serum triglycerides: The spectrophotometric method of Tietz (1995) was employed. The triglycerides are determined after

enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Determination of serum low density lipoprotein cholesterol: The Friedewald *et al.* (1972) equation was used to calculate the LDL-cholesterol in mmol/L. LDL-cholesterol in serum was calculated, using the result obtained from estimation of total cholesterol: LDL=total cholesterol – {HDL+(TG/2.2)}.

Determination of serum very low density lipoprotein cholesterol: This was estimated as ½ of triglyceride (TG) in mmol/L (VLDL= TG/2.2)

ABO blood grouping: Quality monoclonal blood grouping reagents manufactured by Rapid Labs Limited, United Kingdom were used. ABO and Rh blood groupings were carried out by standard tile techniques (Lewis *et al.*, 2011) with appropriate positive and negative controls using one drop of whole blood mixed with one drop of appropriate antisera and rocked gently for agglutination.

Data analysis

This was carried out using Statistical Package for the Social Sciences (SPSS) version 22 and the results were calculated to determine the mean, standard deviations and percentages of the measured parameters. The single factor analysis of variance (ANOVA) was used to calculate the difference in mean of each lipid profile fractions between different ABO blood groups and results were considered statistically significant at $p<0.05$.

Results

The results of the distribution of ABO blood groups in all subjects and Lipid Profile Levels are shown in the Tables 1 to 3.

Table 1 shows the percentage distribution of ABO blood groups in the studied population. Out of the one hundred (100) students recruited for the study, fifty- seven (57%) of the subject were of blood group O, twenty-two (22%) were of blood group A, seventeen (17%) were of blood group B, and four (4%) were of blood group AB.

Table 1. Percentage distribution of ABO blood groups in the studied population.

Blood groups	No. Of observations	Precentage (%)
O	57	57
A	22	22
B	17	17
AB	4	4
TOTAL	100	100

Table 2 shows the percentage distribution of ABO blood group based on gender. It indicated that 38% were males while 62% were females. Among the males tested, 9% were of blood group A, 6% were of blood group B, 1% were of blood group AB while 22% were of blood group O. Of all the females tested 13% were of blood group A, 11% were of blood group B, 3% were of blood group AB, while 35% were of blood group O.

Table 2. Percentage distribution of ABO blood groups of population by gender.

Gender	A	B	AB	O	Total
Male	9(9%)	6(%)	1(1%)	22(22%)	38(38%)
Female	13(13%)	11(11%)	3(3%)	35(35%)	62(62%)
Total	22(22%)	17(17%)	4(4%)	57(57%)	100(100%)

Table 3 shows the mean serum lipid profile among the different ABO blood groups of apparently healthy students in College of Health Technology, Calabar. The mean serum total cholesterol was higher in blood group AB when compared to blood group B, blood group A and blood group O and was statistically significant. There was a higher mean serum triglyceride level in blood group AB when compared to blood group O, blood group B, and blood group A and was statistically significant. Also, there was a higher mean serum very low density lipoprotein level in blood group AB compared to blood group A, blood group B, and blood group O and was statistically significant. Mean serum high density lipoprotein was higher in blood group O when compared to blood group A, blood group B, and blood group AB.

However, the difference was statistically not significant. The mean serum low density lipoprotein was higher in blood group A when compared to blood group B, blood group O and blood group AB and was statistically significant.

Table 3. Lipid profile pattern of different ABO blood groups among apparently healthy students in College of Health Technology, Calabar.

Blood groups	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)
A(n=22)	4.56±1.39	1.44±0.57	2.00±0.92	1.99±0.33	0.65±0.28
B(n=17)	4.68±1.19	1.63±0.52	1.96±0.64	1.91±1.22	0.73±0.30
AB(n=4)	5.28±0.89	2.45±0.66	1.70±0.51	0.93±0.31	1.15±0.30
O(n=57)	4.14±1.23	1.65±0.62	2.95±1.74	1.69±1.19	0.75±0.29
F-Value	3.133	4.221	0.283	3.231	1.543
P-value	0.029	0.007	0.838	0.026	0.208
Prob.level	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05
Comment	S	S	NS	S	S

Mean ± standard deviation, S = significant, NS = non-significant, n = number.

Discussion

Several literatures have been reported that the relationship between ABO blood groups and the development of cardiovascular disease is still very unclear (Alireza *et al.*, 2006; Biancari *et al.*, 2002).

Hypercholesterolemia is usually considered a cardiovascular risk which could predispose to the development of ischemic heart disease (Grover *et al.*, 1995). Also low levels of high density lipoprotein cholesterol, elevated low density lipoprotein cholesterol level and high levels of triglycerides are recognized risk factors of cardiovascular diseases (Grover *et al.* 1995).

The study showed that total cholesterol (TC), triglycerides (TG) and very low density lipoprotein-cholesterol (VLDL-C) were significant higher (P<0.05) in blood group AB compared to other groups. It was also observed in the same group that HDL-C was remarkably reduced though was not significant (P>0.05). This result is in agreement with the findings of Airhomwanbor *et al.*, 2018, where they showed that blood group AB subjects in Ekpoma have higher level of TC, TG and lower level of HDL-C though were not significant. This also agrees with the findings of Meade *et al.*, 1994, where they showed higher incidence of ischemic heart diseases in patients with blood group phenotype AB as compared with blood group O, A or B. Similar findings have been reported by Girgla *et al.*, 2011 and Farah *et al.*, 2017, where they showed a significant relation of phenotype AB with serum lipid parameters in North Indian population and Saudi Arabian respectively. On the contrary, Biswas *et al.*, 2013 in a Bengalis Asian-Indian population showed that blood group AB in healthy controls had a decreased risk of coronary heart disease (CHD), while the O blood group is more frequent in CHD patients. The higher concentrations

of HDL-C in subjects of blood group AB seemed to contribute to the protective role of CHD events in subjects of controls group. Blood group O associated with lower HDL-C level, smoking habits, and family history significantly increase the risk of CHD. Since high levels of serum cholesterol and low high density lipoprotein cholesterol are known to be major risk factors in the development of cardiovascular disease (CVD), these results indicate that people with blood group AB may be at a higher risk of developing these diseases.

The fact that blood group A had the highest level of LDL-C while blood group AB had the highest TC, TG and lowest HDL indicate that both blood groups A and AB might be predisposed to diseases associated with lipid disorders. This agrees with the earlier work by Iheanacho *et al.*, 2018, in Aba which showed that blood group A had the highest LDL-C levels compared to other blood groups. However, this contradicts the study by Ureme *et al.*, 2018 in Oweri metropolis which reported that subjects with blood group O have a higher level of LDL-C and lower HDL-C compared to non-O groups.

This also disagree with previous report by Anvari *et al.*, 2009 in Iran who demonstrated that the prevalence of coronary heart disease in blood group O is higher than other ABO blood groups due to an elevated level of LDL-C in O blood group. The mechanism underlining such blood type differences in association with lipid profile levels is still unclear. However, it may be attributed to a distinct ABO genetic association pattern with plasma lipids (Carpeggiani *et al.*, 2010).

The fact that transfer of a specific oligosaccharide residue to H antigen by glycosyl transferases does not occur in group O, leaving blood group O individuals with unconverted H-substance (Yamamoto *et al.*, 1990) may be a key role. Moreover, given that ABO glycotransferase is associated with cholesterol metabolism (Li *et al.* 2014) and much broader impact on atherosclerotic-CVD (Zhang *et al.*, 2012), CVDs may not only be conferred on non-O blood group as suggested by previous studies.

The study also revealed that HDL-C was higher in blood O compared to other blood groups though was not significant ($p>0.05$). Similar findings have been reported by Iheanacho *et al.*, 2018, where they showed that blood group O had high level of HDL-C compared to others. This was attributed to the deficiency of glycotransferase enzyme that encodes the blood group O phenotype which was previously proposed to protect against myocardial infarctions. However, there are many factors that play major role to produce clinical cardiovascular disease example is soluble adhesion factors like E-Selection which promote arterial inflammation in the presence of clotting factor such as factor VII (Anti-haemophilic factor). As opposed to this study, Ureme *et al.*, 2018 showed that subject with blood group O have a higher level of LDL-C and lower HDL-C compared to non-O blood groups (A,B, and AB). They suggested that subjects with O blood group may have higher tendency for diseases associated with lipid metabolism than non-O blood group types. The results obtained from this study shows that subjects of blood group AB may be genetically predisposed to cardiovascular diseases than subjects with other blood groups. Also the subjects with blood group O may have a protective anti-artherogenic factor due to increased HDL-C content found in their blood.

Conclusion

The study shows that among different ABO blood groups, subjects with blood group AB are associated with increased levels of TC and TG and also have reduced levels of HDL-C as compared to other blood groups. Subjects with blood group O have elevated levels of HDL-C compared to other blood groups. This suggests that subjects with blood group AB may be most at risk for developing diseases associated with lipid metabolism while blood group O subjects may have a lower risk for such diseases. Other risk factors such as family history and environmental factors should also be considered as they may play a major role to produce clinical cardiovascular diseases. Therefore, further studies with a much larger sample

size should be carried out to confirm the findings.

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