THE EFFECT OF ABO BLOOD GROUP CLASSIFCATIONS ON BIOCHEMICAL MARKERS OF HEALTHY ADULTS

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ABSTRACT

This study was aimed at evaluating the effect of ABO blood groups on liver function and lipid profile. A total of seventy (n=70) adult of both sexes were recruited into the study. All participants were assumed healthy provided they have not been on medication for a period of two weeks prior to their recruitment. Biochemical markers assessed includes liver function tests such as; albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT). Lipid profile such as cholesterol, triglyceride and low-density lipoprotein (LDL). Blood group O has the highest frequency of 42 %, while AB has the lowest frequency of 12.9 %. A significant positive correlation was seen between weight and BMI also a negative correlation was seen between total cholesterol and BMI. There was no significant difference in the levels of ALT and triglyceride. In the blood group A, results of the liver function test reveals that the activity of AST and albumin decreased significantly for the liver function test among the blood groups ($p< 0.05$). The level of blood total cholesterol increased significantly ($p< 0.05$) in blood group B; but decreased significantly in blood group A and AB ($p< 0.05$) among the blood groups for the lipid profile assay. These assays show significant differences within the ABO blood groups; this may be an indicator of health status with respect to ABO blood group based on the knowledge of what we know of polymorphisms, that individuals belonging to the different blood groups are not equally viable.

Keywords: Biochemical markers, ABO blood groups and healthy adult.

1. INTRODUCTION

The ABH histo-blood group antigens are a set of polymorphic and inherited glycoconjugate structures that are expressed on the cell surface of human erythrocyte (Amajadi, 2015). These blood groups are discovered by an Austrian biologist Landsteiner in 1901 (Landsteiner and Wiener, 1940). Later on, in 1939, Rhesus blood groups were discovered by Landsteiner and Wiener in 1940. Since 1901, more than 20 distinct blood group systems have been identified but the ABO and Rhesus blood groups remain clinically the most important (Rashaduz et al., 2015). The ABO and Rh blood groups are the most important blood groups so far (Eru et al.,2014). ABO blood group system is divided into four blood types on the basis of presence or absence of A and B surface antigens. The blood groups are A, B, O and AB. The frequency of four main ABO blood groups varies in the population throughout the world. ABO blood group system derives its importance from the fact that A and B are strongly antigenic and anti A and anti B (Rashaduz et al., 2015).

Despite their discovery in 1900, the critical role of ABO blood groups in transfusion medicine, and their apparent link to multiple diseases, the association of blood groups with mortality. Therefore, we decided to examine the hypothesis that blood groups are associated with overall and cause-specific mortality. Mortality and cancer in relation to ABO blood (Etemadi et al., 2015). There is a dearth of information on the ABO and Rhesus blood group distribution in Benue State North central region of Nigeria. The Major tribes in Benue State include Tiv, Idoma, Igede, and Jukuns however there are some other tribes that are few in number that live in the State, these include: Yourba, Hausa and Igbo.

2. MATERIALS AND METHODS

Subjects inclusion criteria

Adult males and females between the ages of 18 to 45 years old, who have not been on any medication in the past two weeks prior to blood collection and willingly gave consent to be part of the research work were included in the work.

Subjects exclusion criteria

Adults above the age of 45 years, children and teenagers were excluded from the research. Adults within the research age categories who are either on medication or unwilling to participate in the research were also excluded from the study.

Participants' blood collection

Blood samples were obtained by venipuncture using vacutainers with heparin as anticoagulant. The blood samples were properly handled and retained in ice to prevent lysis. The samples were refrigerated and immediately transported to the laboratory. The vacuum blood gathering tube was instantly centrifuged at 3000rpm for 10 minutes at room temperature. The sample plasma was aliquoted into labeled Eppendorf tubes and stored at -80^{0}C. The plasma was used to quantify biochemical, liver function test and lipid markers in the blood.

Lipid Profile Tests

Cholesterol

Principle

The cholesterol esters of the sample are hydrolyzed by cholesterol esterase. 4-Cholesten- 3-one and H2O2 are then formed from the released free cholesterol by cholesterol oxidase. A measurable red quinoneimine derivative, that has an absorbance at 500 nm, is formed from hydrogen peroxide (H2O2) and 4-amino-antipyrine in the presence of phenol and peroxidase.

$$\text{Cholesteryl ester} + H_2O \xrightarrow{\text{Cholesteryl ester hydrolase}} \text{cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone monoimino)-phenazone} + 4\,H_2O$$

Elevated levels of cholesterol increase the risk for coronary heart disease (CHD). Cholesterol is measured to help assess the patient's risk status and to follow the progress of patient's treatment to lower serum cholesterol concentrations. Desirable cholesterol levels are considered to be those below 200 mg/dL in adults and below 170 mg/dL in children.

Reagents compositions

- Cholesterol buffer solution (Cholesterol kit, 100 mL) - phenol 132 mg, NaH2PO4 0.78g, NaH2PO4 0.71 g.
- Enzyme reagent (Cholesterol kit, 100 mL dilution) - cholesterol oxidase 12 unit, cholesterol esterase 3.5 unit, peroxidase 6700 unit, 4-aminoantipyrine 17.0 mg/dL
- Cholesterol standard solution (Cholesterol kit) - 300mg/dL

Procedure

- For blank preparation 1.5ml of the enzyme solution and 0.01ml of distilled water was prepared
- For standard preparation 1.5ml of the enzyme solution and 0.01ml of the standard solution was prepared
- Sample preparation contained the mixture of 1.5ml of the enzyme solution and 0.01ml of the serum solution

Calculation:

Absorbance of Sample

$$\frac{\text{Cholesterol Level (mg/dl) X 300}}{\text{Absorbance of Standard}}$$

Triglyceride

Principle
Triglycerides Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm. The reaction sequence is as follows:

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O2 \xrightarrow{\text{Glycerophosphate oxidase}} \text{dihydroxyacetone phosphate} + H2O2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone-monoimino)-phenazone} + 2H_2O + HCl.$$

High levels of serum triglycerides help mark conditions that are associated with increased risk for CHD and peripheral atherosclerosis. High triglycerides are associated with increased risk for CAD in patients with other risk factors, such as low HDL-cholesterol, some patient groups with elevated apolipoprotein B concentrations, and patients with forms of LDL that may be particularly atherogenic. Desirable fasting triglyceride levels are considered to be those below 200 mg/dL, and are further categorized as Borderline, 200-400 mg/dL; High, 400-1,000 mg/dL; and Very High (> 1000 mg/dL). Very high triglycerides can result in pancreatitis and should be promptly evaluated and treated. Triglycerides are also measured because the value is used to calculate low density lipoprotein (LDL)-cholesterol concentrations.

Procedure

1. Reagent blank was prepared by pipetting 1000µ into a test tube
2. Standard was prepared by adding10µ to 1000µ.
3. Sample was prepared by adding 10µ of serum to 1000 µ of the reagent.
4. The preparations were Mixed, incubate for 10 minutes at 20-25oC.
5. The absorbance of the sample (Asample) and standard (Astandard) was measured against the reagent blank at within 60 minutes.

Low Density Lipo-Protein (Ldl)

Principle
Low density lipoprotein in the sample precipitate with polyvinyl sulphate. Their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation. The cholesterol is spectrophotometrically measured by means of coupled reactions described below.

$$\text{Cholesterol esters} + H2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acid.}$$

LDL-cholesterol
Most of the circulating cholesterol is found in three major lipoprotein fractions: very low-density lipoproteins (VLDL), LDL and HDL.

[Total chol] = [VLDL-chol] + [LDL-chol] + [HDL-chol]

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

[LDL-chol] = [total chol] - [HDL-chol] - [TG]/5

Where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dL. LDL carries most of the circulating cholesterol in man and when elevated contributes to the development of coronary atherosclerosis. LDL-cholesterol is measured to assess risk for CHD and to follow the progress of patients being treated to lower LDL-cholesterol concentrations. Desirable levels of LDL-chol are those below 130 mg/dL in.

Procedure**: A**

1. The following was Pipette into labelled centrifuge tubes; 100µ of sample and 100µ of the cholesterol kit (polyvinyl sulphate 3 g/L, polyethyleneglycol 3 g/L).
2. The mixture was mixed thoroughly and let stand for 15 minutes at room temperature
3. It was centrifuge at a minimum of 4000 r.p.m for 15 minutes.
4. Then the supernatant was carefully collected

Procedure**: B**

1. The reagent blank was 20µ of distilled water
2. In other labelled tubes; 20µ was pipetted.

- The total mixture was mixed thoroughly and incubate the test tubes for 30 minutes at room temperature (16-25ºC).
- The absorbance of the standard and sample was read at 500nm against the blank.

Calculations

$$\frac{\text{A Sample}}{\text{A standard}} \times \text{C sample dilution factor} \times \text{Sample dilution factor} = \text{Cholesterol supernatant}$$

LDL Cholesterol = total cholesterol – cholesterol in supernatant

Liver Function Test

Alanine Aminotransferase (Alt)

Principle

Summary Of Test Principle And Clinical Relevance

In this reaction, an enzymatic rate method is used to measure ALT activity in serum. In this reaction, ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD. The system monitors the rate of change in absorbance at 340 nm over a fixed-time interval. The rate of change in absorbance is directly proportional to the ALT activity in the sample.

Alanine Aminotransferase (ALT), also known as serum glutamic-pyruvic transaminase (SGPT), is a pyridoxal-phosphate-dependent enzyme that catalyzes the reversible transfer of an amino group from alanine to □-ketoglutarate, generating pyruvate and glutamate. ALT is found primarily in liver and serum, but occurs in other tissues as well. Hepatocellular injury often results in an increase of serum ALT levels and serum ALT levels can be used as a marker for liver injury. The ALT Activity Assay Kit provides a simple and direct procedure for measuring ALT activity in a variety of biological samples. ALT activity is determined by a coupled enzyme assay, which results in a colorimetric (570nm)/fluorometric (λex = 535/λem = 587 nm) product, proportional to the pyruvate generated. One unit of ALT is defined as the amount of enzyme that generates 1.0 □mole of pyruvate per minute at 37ºC.

Procedure

To label test tubes were 0.05ml of the sample and 3.00ml of the reagent pipetted

- The reaction Mixture, was read at initial absorbance and start timer simultaneously. Read again after 1, 2 and 3 min.

Aspartate Aminotransferase (AST)

Principle

This reaction uses enzymatic rate method to measure the AST activity in serum or plasma. In the reaction, the AST catalyzes the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with the concurrent oxidation of NADH to NAD.

Procedure

To label test tubes were 0.05ml of the sample and 3.00ml of the reagent pipetted

- The reaction Mixture, was read at initial absorbance and start timer simultaneously. Read again after 1, 2 and 3 min.

Albumin

Albumin is a protein that is formed in the liver. Approximately 50-60 % of total protein is albumin, the rest is globulin. The albumin helps to maintain normal distribution of water in the body (colloidal osmotic pressure), and also helps in the transport of blood constituents such as ions, pigments, bilirubin, hormones, enzymes, and drugs. The ratio of albumin to globulin is an important indicator of certain disease states. Albumin level is decreased in several liver diseases, malabsorption, diarrhea, eclampsia and nephrosis. The level is increased in dehydration.

Clinical Significance

Albumin is the most abundant serum protein representing 55-65% of the total protein. It is synthesized in the liver and has a half-life of 2 to 3 weeks. The main biological functions of albumin are to maintain the water balance in serum and plasma and to transport and store a wide variety of ligands e.g. fatty acids, calcium, bilirubin and hormones such as thyroxine. Albumin also provides an endogenous source of amino acids. Hypoalbuminaemia is associated with the following conditions: analbuminaemia; impaired albumin synthesis in the liver; liver disease; malnutrition or malabsorption; generalised shock; burns or dermatitis; kidney disease and intestinal disease. Hyperalbuminaemia has little diagnostic relevance except, perhaps in dehydration.

Principle

At pH=4.2, albumin bind with bromocresol green (BCG) to produce a blue-green complex. The change in absorbance at 628 nm correlates with the concentration of albumin.

Procedure

1. The following were pipetted into labelled tube 0.01 and 3.00ml of working reagent. This represents the blank
2. To another test tube was added 0.01ml of the standard reagent. This represents the standard
3. To another test tube was 0.01 ml of serum and 3.00ml of reagent added.

Calculation

Albumin Concentration (g/l or g/dl)

$$= \frac{\text{A sample x Concentration of standard}}{\text{A standard}}$$

3. STATISTICAL ANALYSIS

Data are presented as mean ± standard error of mean (S.E.M). Statistically significant differences in mean values were tested by one-way analysis of variance (ANOVA). The data were analyzed using Statistical Package of Social Sciences 22.0.0.0 (SPSS Inc. 2014) and Microsoft excel 2017 version. The differences were considered significant when $p<0.005$.

4. RESULTS

Effect of anthropometric parameters on Abo blood groups

Participants enrolled in this study were young adult from age 18-45 years old. The characteristics of studied groups informed from the questionnaire were presented in table (Table 1). There was no significant difference between and within the groups with respect of their height, weight and the Body mass index (BMI).

Table 1: Effect of blood group classification on anthropometric parameters.

BLOOD GROUP	FREQUENCY OF OCCURENCE	PERCENTAGE FREQUENCY (%)	WEIGHT (KG)	HEIGHT (M)	BMI (K/M^2)
O	30	42	67.43±1.5	1.67±0.1	25.39±1.5
A	11	15	74.09±5.4	1.67±0.1	27.14±2.0
B	20	28	70.62±3.4	1.64±0.1	27.38±2.0
AB	8	15	65.12±8.0	1.58±0.1	28.65±7.1

The values are expressed as mean ± standard error of mean (SEM).

Effect of liver function tests on Abo blood groups

The table 2, represents the results of the liver function test. in this table AST and albumin test show significant difference among the ABO blood group, but that of Alanine transaminase (ALT) shows no significant difference for all the groups with respect to the blood types.

TABLE 2: Effect of blood group classification on liver function markers

GROUPS	O	A	B	AB
FREQUENCY	30	11	20	11
AST (U/L)	50.24 ± 5.5^a	11.81 ± 2.0^b	26.60 ± 4.3^b	18.25 ± 4.8^b
ALT (U/L)	19.93 ± 3.5	12.72 ± 5.8	23.95 ± 6.8	33.25 ± 12.0
ALBUMIN (G/L)	10.23 ± 1.2^a	6.15 ± 1.4^b	4.30 ± 0.5^b	4.37 ± 1.5^b

The values are expressed as mean ± standard error of mean (SEM) of the liver function test as indicated in the table 2, AST means aspartate aminotransferase and ALT means alanine transaminase; albumin test show significant difference among the ABO blood group. Superscript a '^{a}' and b '^{b}' indicates values that are significantly ($p<0.05$) different from each other.

Effects of lipid profile assays on abo blood groups

In the table 3, it was represented here that the cholesterol assay shows a significant difference among the ABO blood groups. Also, low density lipo-protein also shows significant difference among the blood groups. There is no significant difference among the ABO blood group for triglyceride assay.

TABLE 3: Effect of blood group classification on lipid profile

	O	A	B	AB
FREQUENCY	30	11	20	11
CHOLESTEROL (mg/dl)	301.53 ± 6.9	244.11 ± 42.0^a	349.20 ± 21.5^b	350.20 ± 40.1^b

TRIGLYCERIDE (mg/dl)	216.67 ± 19.7	238.43 ± 45.9	240.57 ± 69.4	171.17 ± 9.2
LDL (mg/dl)	272.63 ± 13.5^{a}	201.18 ± 27.4^{b}	313.45 ± 25.1^{a}	202.00 ± 46.5^{b}

The values are expressed as mean ± standard error of mean (SEM). Superscript a '[a]' and b '[b]' indicates values that are significantly ($p<0.05$) different from each other.

5. DISCUSSION

Reports on the ABO blood groups have proven a lot about the implication of the different blood types. ABO blood group system, a well-known genetic risk factor, has clinically been demonstrated to be linked with thrombotic vascular diseases (Zhou et al., 2016). There is also abundant evidence that blood groups play a role in the susceptibility or resistance to various infectious and non-infectious diseases (Mourant et al., 1978). Some examples of associations between ABO blood group and disease are; haemolytic disease of the newborn, leukaemia, cancer, acquired B resulting from bacterial infection, and leucocyte adhesion deficiency type II (Geoff, 2002). However, the relationship between ABO blood group; liver function and lipid profile are still controversial and this is worthy to be assessed scientifically and strictly. In this study Blood group O has the highest frequency of 42 %, with female as 22 % and male as 20 % while AB has the lowest frequency of 12.9 %, with female as 7 % and male 5.7 %. Predominance of O and A blood group followed by B groups were observed (Roy et al., 2015). It was also observed in the course of this study that the blood group O is the commonest and blood group AB the rarest of the ABO blood group types. Blood group O is common, Blood group AB is the least common (Claudia, 2014). Roy et al., (2015) also corroborates this fact by showing in his work that O blood group was most common followed by A, B and AB.

The present study was conducted to satisfy two objectives: Assess the effect of ABO blood groups on lipid profile of healthy individuals, assess the effect of ABO blood groups on liver function markers of healthy individuals, and assess the effect of ABO blood groups on human anthropometry. There was no significant difference in the levels of ALT and triglyceride. This indicates that blood group doesn't confer levels or cannot be used as a determinant for these biochemical parameters.

Abo Blood Group Relationship Between Anthropometric Data Among The Participants

This study, anticipated to explore any possible association between levels of laboratory findings of renal patients and blood groups. There was no significant difference between and within the groups as regards the anthropometric data which are the height, weight and the Body mass index (BMI). However, these results are inconsistent with some of the previous studies. In 2014 Maninder showed in a research work that participants with blood group O were heavier (64.78kg) followed by females with blood group AB (62.21kg), blood group A (60.88 kg) and then blood group B (60.21kg). Those with blood groups B or AB tended to be slightly lesser in number than those with blood A. Subjects with the B allele were either shorter or of equal height to group A (Kark et al.,2008). There was no association noted between BMI and ABO blood group (Elham, et al., 2012). This research of Elham, corroborates the result of this study.

Abo Blood Group Relationship With Liver Enzymes Activity Among The Participants

The findings of this study are in line with the work of Hisham and Hind (2010). Hisham and Hind (2010), reported that there was no significant relationship between the activities of ALT amongst ABO blood groups in Cohort of healthy blood donors and volunteers from Saudi Arabia. Although the reason was not been addressed (Hisham and Hind, 2010). The activity of AST and albumin decreased significantly for the liver function test among the blood groups ($p< 0.05$) in blood group A. This result shows that ABO blood group variations may have an influence on some liver enzymes activity of serum and also that the plasma concentration of these enzymes can be useful to access the function of the liver viability and its disease conditions Atsaboghena et al. (2013). The liver is the largest solid organ and plays a major role in metabolism with numerous functions in the human body. ALT and AST are critical enzymes in the biological processes. The synthesized ALT and stored AST changes in serum levels have become diagnostic tools and markers for assessing the liver function. Reports have suggested that their levels increase in different hepatic injuries, such as hepatitis and cirrhosis induced by alcohol, drugs, viruses, and also under oxidative stress. (Shu-Lin et al., 2016).

Serum aminotransferases are sensitive markers of hepatocellular injury (Tinsay et al., 2014). Liver function tests (LFT) are a helpful screening tool, which are an effective modality to detect hepatic dysfunction (Thapa and Anuj, 2007).

Liver and kidney are exposed to a lot of oxidant substances that are both from exogen and endogen sources (Ragıp et al., 2008). In the year 2015 Samar et al., showed that Albumin level was at peak B blood group renal patients. Serum aminotransferase levels ALT and AST are two of the most useful measures of liver cell injury. Lesser degrees of ALT level elevation may occasionally be seen in skeletal muscle injury or even after vigorous exercise. Oxidative stress and inflammation could also lead to damaged liver cells (Shu-Lin et al., 2016). However, recent studies have shown that the upper limit threshold of ALT level should be lowered because people who have slightly raised ALT levels that are within the upper limit of normal (35-40 IU/L) are at an increased risk of mortality from liver disease. The two most common forms of autoimmune liver disease are autoimmune chronic hepatitis. Ninety percent of those with each disorder are women. Autoimmune hepatitis (AIH) is characterized by very high serum aminotransferase (ALT and AST) levels autoimmune hepatitis (AIH) is characterized by very high serum aminotransferase (ALT and AST) levels (Arvind, 2014).

Abo Blood Group Relationship with Lipid Profile Among The Participants

It is well established that ABO blood group is associated with cholesterol metabolism. (Li et al., 2015) The relationships between ABO blood groups and cholesterol levels have been established but differ in many studies (Gali et al., 2009). The level of blood total cholesterol increased significantly ($p< 0.05$) in blood group B; but decreased significantly in blood group A and AB ($p< 0.05$) among the blood groups for the lipid profile assay. High serum cholesterol is strongly implicated in the development of cardio vascular diseases. In this study cholesterol assay shows a significant difference among the ABO blood groups. Gali et al., 2009 showed in their work that there is a level of significance between serum cholesterol blood groups. Also, low density lipo-protein also shows significant difference among the blood groups. There is no significant difference among the ABO blood group for triglyceride assay. This was inconsistent with the work of Contiero et al., 1994. In the population studied we did not find any association between total, HDL- and LDL-cholesterol and ABO blood groups while triglyceride levels were higher in individuals with B antigen (B + AB) than in subjects without this antigen. Environmental factors could be more important than genetic factors in the arousal of cardiovascular diseases (Contiero et al., 1994). Individuals with blood group A seem more liable to develop atherosclerosis than those with blood group "non-A". Horby et al., (1989)

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This present study shows the evidence of links between the ABO blood group and the liver viability as regards the liver function test and lipid profile. It is been deduced in this research work that there are no relationship or effect of blood group on human anthropometry. Further experimental studies are also needed to unravel the molecular mechanisms linking ABO blood type, to the various maladies that has been unraveled in this study. ABO blood typing may hence become part of a multifaceted strategy for cancer risk assessment. There are no previous studies that has investigated the variations of biochemical tests by blood groups to compare our findings with, and accordingly this study presents a new piece of knowledge that may participate in future studies.

6.2 Recommendations

- Further research should be carried out to help establish these realized intriguing relationships that actually exist between the ABO blood group and the various markers assayed for.
- The following up of the individuals used as subjects for this research as regards the findings is important.
- Notwithstanding, this research indicates the need for more specific definition of liver viability and biochemical biomarkers of healthy individuals among adults that are young and this will lead ultimately to take more accurate diagnosis.
- This research work gives a new piece of knowledge which can part in future researches.

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