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Effect of alcohol consumption on haemorheology and osmotic fragility of subjects in Nnewi, South-eastern, Nigeria

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Abstract

Background: Alcohol consumption has been known to have physiological, mental, psychological and haematological effects on consumers. **Aim and Objectives:** This is study was aimed at assessing the effects of alcohol consumption on haemorrheology and osmotic fragility of alcoholconsumers in Nnewi metropolis as well as the gender and age variations. **Study Design:** This is a case control study. **Method:** A total of 100 subjects comprising 50 alcohol consumers (35 males and 15 females) and 50 non-alcohol consumers (35 males and 15 females) were recruited. The study subjects were tested for Relative Whole blood viscosity (RWBV), Relative plasma viscosity (RPV), Relative serum viscosity (RSV) and Osmotic fragility (Median Corpuscular Fragility (MCF) after obtaining ethical approval and informed consent of the subjects. RSV, RWBV and RPV were carried out by a modification of the capillary method of Reid and Ugwu (1987). Osmotic fragility was estimated using the method described by Dacie and Lewis (2012). Statistical Package for Social Sciences (SPSS) version 20 software was used for statistical analysis. **Results:** The results showed a significant increase in the mean of RWBV, RPV, RSV and MCF of alcohol consumers when compared with non-alcohol consumers (P<0.05). Similarly, the mean values of RWBV of male alcohol consumers were significantly higher than that of female alcohol consumers. However, there was no significant difference in the mean values of RWBV, RPV, RSV and MCF of alcohol consumers based on different age group (P>0.05). Also the mean values of RPV, RSV and MCF between male and female alcohol consumers showed no significant difference. **Conclusion:** Alcohol consumption results in significant alterations in haemorrheology and osmotic fragility of consumers.

Keywords: Haemorrheology, Osmotic fragility, Alcohol.

INTRODUCTION

An alcoholic beverage is a drink which contains a substantial amount of the psychoactive drug, ethanol (informally called alcohol). It is one of the most widely used recreational drugs in the world and has an important social role in most cultures. People drink to socialize, celebrate and relax. Alcoholic beverages have been produced and consumed by humans since the Neolithic era [1]. High levels of alcohol consumption are associated with an increased risk of alcoholism, malnutrition, chronic pancreatitis, alcoholic liver disease and cancer [2]. In addition, damage to the central nervous system and peripheral nervous system can occur from chronic alcohol abuse. Acute and chronic alcohol consumption causes degeneration in different internal organs and systems of the body and long-term use of alcohol is capable of damaging nearly every organ and system in the body [3]. Alcohol has numerous adverse effects on the various types of blood cells and their functions including generalized suppression of blood cell production and the production of structurally abnormal blood cell precursors that cannot mature into functional cells. Alcohol consumers frequently have defective RBCs that are destroyed prematurely possibly resulting in macrocytosis and anaemia.

Haemorrheology is the study of the flow properties of blood and its element (plasma and cells). It is the science of flow of blood in relation to the pressure, flow, volumes and resistances in blood vessels especially with respect to blood viscosity and red cell deformation in the microcirculation [4]. Proper tissue perfusions can occur only when blood's rheological properties are within certain levels. Alterations of these properties play significant roles in disease processes. Blood viscosity is a measure of the resistance of blood to flow. This biophysical property makes it a critical determinant of friction against the vessel

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walls, the rate of venous return, the work required for the heart to pump blood and how much oxygen is transported to tissues and organs. Blood viscosity is determined by plasma viscosity, haematocrit and mechanical properties (red cell deformability and aggregation) of red blood cells.[5]Although experimental studies on effects of acute alcohol ingestion showed that alcohol ingestion increases haemorrheology,[6] and Hamazaki and Shishido[7]discovered that alcohol ingestion may change haemorrhelogy of consumers, however the mechanisms of the haemorrheological effects of alcohol remains to be elucidated.

Osmotic fragility refers to the degree or proportion of haemolysis that occurs when a sample of RBC's are subjected to osmotic stress by being placed in a hypotonic solution. It is used to measure erythrocyte resistance to haemolysis while being exposed to varying levels of dilution of a saline solution. It is affected by various factors including membrane composition and integrity as well as the sizes or surface area to volume ratios.[8-10] The classic osmotic fragility test, originally described by Parpart*et al*[11] involves a small amount of fresh blood being added to series of solutions with tonicity ranging from 0.1% - 0.9%. Results are then obtained by plotting the percentage of haemolysis against the Sodium Chloride (NaCl) concentrations yielding an osmotic fragility curve. At times it may be expressed as the concentration of NaCl in solution that causes 50% haemolysis of erythrocytes, this is known as the median corpuscular fragility (MCF).It is usually performed to aid with the diagnosis of diseases associated with RBC membrane abnormalities. There are evidence that alcohol and smoking causes membrane deformity and as a result a modification of the osmotic fragility in different cell types.[12]

The study therefore aims at assessing the impact of alcohol on haemorrheological parameters and osmotic fragility of consumers.

MATERIALS AND METHOD

Study Area

The study was carried out in Nnewi metropolis. Nnewi is the second largest city in Anambra State, South Eastern Nigeria. It has an estimated population of over 391, 277. It is made up of four quarters namely; Otolo, Uruagu, Umudim and Nnewichi. The residents are mostly, traders, commercial motorcyclists, health workers and students. It has numerous hotels and drinking joints scattered across the various parts of the town.

Study Design

This is a case control study designed to assess the haemorrheology and osmotic fragility of alcohol consumers in Nnewi metropolis. The subjects were recruited by simple random sampling.

Study population

A total of one hundred adult subjects were recruited randomly for the study, comprising 50 regular alcohol consumers (35 males and 15 females) and 50 non-alcohol consuming age and sex matched subjects (35 males and 15 females).

Ethical consideration

Ethical approval was obtained from the ethics committee of Faculty of Health Sciences and Technology, Nnamdi Azikiwe University and the informed consent of the subjects were obtained before the commencement of the study.

Inclusion and exclusion criteria

Apparently healthy alcohol consumers and non-consumers were included while subjects with any known medical condition, pregnant women and those who withheld their consent were excluded.

Sample collection

Six millilitres of blood was collected from each subjects by standard venepuncture technique and dispensed as follows; 2ml into heparin anticoagulant for Osmotic fragility test and Relative whole blood viscosity (RWBV), three millilitres (3mls) into 0.3ml of 31.3g/l trisodium citrate anticoagulant for relative plasma viscosity (RPV), and 1ml into plain container for Relative serum viscosity (RSV) measurement.

Laboratory analysis

Estimation of Osmotic fragility as described by Parpart et al., [11]

Stock solutions of buffered sodium chloride (NaCl) osmotically equivalent to 100g/l (1.71 mol/l) NaCl, was prepared as follows; NaCl (90g), Na_2HPO_4 (13.65g) and $NaH_2PO_4.2H_2O$ (2.34g) were dissolved in water and the final volume adjusted to 1 litre. In preparing the hypotonic solution for use, a 10g/l solution was made from the 100g/l stock solution by dilution with water. Dilutions equivalent to 9.0, 7.5, 6.5, 6.0, 5.5, 5.0, 4.0, 3.5, 3.0, 2.0 and 1.0 g/l concentrations were made. Five millilitres (5ml) of each of the 11 saline dilutions were delivered into test tubes and 5ml of water delivered to a 12th tube. 50µl of well mixed blood was added to the tubes and mixed immediately by inverting them several times, avoiding foam. The mixtures were left for 30 minutes at room temperature. After which it was mixed again and then centrifuged for 5 minutes at 12000g. The supernatants were used to estimate the amount of lysis in each tube using a spectrophotometer at a wavelength of 540nm. The supernatant from tube one (osmotically equivalent to 9g/l NaCl) was used as blank. A value of 100% lysis was assigned to the reading with the supernatant of tube 12 (water) and the readings from the other tubes were expressed as a percentage of the value of tube 12. The results were then plotted against the NaCl concentrations to get the median corpuscular fragility (MCF).

Determination of RWBV, RPV and RSV

These were carried out by a modification of the capillary viscometry method by Reid and Ugwu.[13]

Statistical analysis

The data obtained were analysed using Statistical package for Social Science (SPSS) version 20(SPSS Inc., Chicago IL, USA). Data were expressed as mean ± SD. The significance of differences in mean values between groups was analysed using t-test, while significance of differences in mean values among different groups was evaluated using one way ANOVA. *P*<0.05 was considered statistically significant.

RESULTS

The mean values of RWBV, RPV, RSV and MCF were significantly higher in alcohol consumers compared to non-consumers (*P*<0.05). (Table 1)

The RWBV in males were significantly higher than those of the females (P<0.05), while there was no significant change in RPV, RSV and MCF of males compared to the females (P>0.05) (Table 2).

There was no statistically significant difference in the parameters when compared among the different age groups (P>0.05). (Table 3).

Table 1: Comparison of parameters between alcohol consuming and non-alcohol consuming subjects

Parameters	Alcohol Consumers (n=50)	Non-Alcohol Consumers (n=50)	*P*-value
RWBV	5.43 ± 1.32	4.01 ± 0.54	0.00*
RPV	2.05 ± 1.08	1.41 ± 0.32	0.00*
RSV	0.82 ± 0.37	0.39 ± 0.20	0.00*
MCF (g/l)	6.07 ± 1.00	4.35 ± 0.78	0.00*

Keys: *Significant P values; RWBV – Relative Whole blood viscosity; RPV - Relative plasma viscosity; RSV – Relative serum viscosity; MCF – Median corpuscular fragility

Table 2: Comparison of parameters between male and female alcohol consumers

Parameters	Male (n=35)	Female (n=15)	*P*-value
RWBV	5.86 ± 1.31	4.41 ± 0.61	0.00*
RPV	2.13 ± 1.18	1.88 ± 0.82	0.47
RSV	0.82 ± 0.39	0.82 ± 0.31	0.98
MCF (g/l)	6.19 ± 0.88	5.78 ± 1.21	0.18

* Significant P values; RWBV – Relative Whole blood viscosity; RPV - Relative plasma viscosity; RSV – Relative serum viscosity; MCF – Median corpuscular fragility

Table 3: Comparison of parameters among alcohol consumers based on age groups

Age Groups (years)	RWBV	RPV	RSV	MCF (g/l)
(A) <25 (n=10)	5.23 ± 1.17	2.35 ± 1.41	0.69 ± 0.32	6.17 ± 1.07
(B) 25-30 (n=19)	5.50 ± 1.56	2.11± 0.92	0.85 ± 0.40	5.96 ± 0.98
(C) 31-35 (n=8)	5.67 ± 1.10	1.62 ± 0.51	0.90 ± 0.32	5.88 ± 1.16
(D) >35 (n=13)	5.33 ± 1.28	1.20 ± 1.29	0.81 ± 0.38	6.27 ± 0.93
F(*P*) value	0.20 (0.90)	0.70 (0.56)	0.56 (0.65)	0.38 (0.77)

Keys: RWBV – Relative Whole blood viscosity; RPV - Relative plasma viscosity; RSV – Relative serum viscosity; MCF – Median corpuscular fragility

DISCUSSION

Blood viscosity is a measure of the resistance of blood to flow. It is determined by water-content and macromolecular components. Our findings shows an increase in Relative whole blood viscosity (RWBV), Relative plasma viscosity (RPV) and Relative serum viscosity (RSV) in alcohol consuming subjects compared to the non-alcohol consuming controls. These are indirect measure of the amount of protein present in the plasma (liquid) part of the blood and may reflect the degree of inflammation present in the body. Increased blood levels of certain proteins such as fibrinogen or immunoglobulins which are increased in inflammation or secreted by some tumours cause the blood viscosity to rise. It can also detect the presence of abnormal paraproteins which can be benign or malignant tumours. The measurement has high stability and accuracy, thus little alterations may be pathologically important. It is noteworthy that because the cellular content of blood has been removed prior to testing, plasma and serum cannot provide accurate insights on the actual flow resistance of a patient's blood sample as the whole blood viscosity. According to Day and James,[14]hyperviscosity syndrome is associated with an increase in serum proteins or cells in conditions such as inflammation, thereby leading to increased blood viscosity. Thus our findings could also be explained by a previous research finding by Reddy *et al*.[15] that excessive drinking can influence metabolism and cause fats to build up in the liver a condition known as fatty liver or steatosis and the excessive fat makes it difficult for the liver to function and encourages inflammation in the liver.

Similarly, series of epidemiological studies had observed a relationship between the relative risk of Cardiovascular disorder and alcohol intake[16] and elevated whole blood viscosity, plasma viscosity and packed cell volume have been strongly linked to primary cardiovascular risk.[17,18] Thus our finding of an increased haemorrheology is expected as the above discoveries means that alcohol consumption leads to increased blood viscosity which could result in cardiovascular disorder. Moreover, our findings of an increase in haemorrheology corresponds with earlier experimental studies[6,7,19]that showed that alcohol ingestion increases haemorrheology.

When erythrocytes are exposed to a hypotonic environment, water enters the cell and causes swelling and eventual lysis. The osmotic fragility test is used to gauge the level of haemolysis in a collected sample of a patient's blood and this is compared to a control sample.[20,21]There was a significant increase in osmotic fragility (MCF) in alcohol consumers. This is in consonance with the findings that alcohol and smoking causes membrane deformity.[12] Similarly excessive alcohol intake causes membrane lesion and leakage of small potassium ion from the cells. The level of membrane lesion increases with increasing alcohol concentration leading to higher osmotic fragility (MCF). This is because Mitochondrial and cellular oxidative stress in chronic alcoholism appears to be major cause of augmented mitochondrial production of superoxide anion ($O2$-) and consequently the production of H_2O_2 triggered by NADH overproduction thereby also increasing permeability.[22]

The form of a normal human red blood cell is a biconcave disc, 8μm in diameter, 2μm thick at the rim and 1μm thick at the centre.[23]These cells are extremely deformable as they progressively elongate under shear stress. The deformability is affected by extrinsic factors such as cell shape and membrane surface area to volume ratio and intrinsic factors such as membrane viscoelastic properties and cytosolic viscosity.[24-26] Deviations from the normal resting biconcave shape, decreased area to volume ratio, higher membrane shear modulus and viscosity or elevated cytoplasmic viscosity tend to reduce deformabilitywhich in turn affects Osmotic fragility.[4]

RWBV was also significantly higher in males than in females. The reason for this is not quite obvious but could be due to normally higher haematocrit values in males as RWBV has been shown to correlate with PCV.[27]

CONCLUSION

There is an altered haemorrheology and osmotic fragility of red cells in alcohol consumers irrespective of age.

Since increased blood viscosity is a known risk factor for cardiovascular disorders, it is therefore recommended that regular alcohol consumers should be encouraged to go for regular cardiovascular examinations to ensure prevention and early detection of cardiovascular diseases. Future studies should incorporate information on the duration, frequency and quantity of alcohol consumption as well as measurement of lipid profile of subjects to correlate alteration in haemorrheology with risk andpredisposition to cardiovascular diseases.

Conflicts of interest

The authors declare that no conflict of interest exists in this research.

Authors contributions

Authors OCO and AOS contributed in the design, conception and data acquisition. Authors OCO, AGI, IMO, and OSI contributed in data analysis and interpretation. All revised and approved of the final manuscript.

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