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RESEARCH ARTICLE

TOTAL PROTEIN, ALBUMIN AND SERUM PROTEIN ELECTROPHORESIS PATTERN OF ALCOHOLICS IN IKERE-EKITI, EKITI STATE

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Abstract: Plasma proteins such as albumin are indispensable in maintaining numerous essential roles of living cells. This objective of this study was to determine the total serum protein, albumin and serum protein electrophoresis pattern of alcoholics. One hundred samples comprising sixty (60) subjects (alcohol users) and forty controls (non-alcohol users) were recruited for this study. Total protein and albumin were determined using spectrophotometric methods and protein electrophoresis respectively. The results were presented in tables and chat as mean ± standard deviation. Statistical analysis was done using one way analysis of variance (ANOVA) and Student's t-test using IBM SPSS version 24.0. A p-values of <0.05 was considered significant. The results obtained showed that the mean value of total protein in subjects and control was 50.22±8.44 g/L and 74.38±8.12 g/L, albumin was 30.89±5.05 g/L and 45.13±5.18 g/L, globulin was 19.33±6.11 g/L and 29.25±5.36 g/L, while albumin/globulin ratio was 1.6 and 1.54 respectively. Statistically, total protein, albumin and globulin were significantly lower (p<0.05) in subjects compared with control group. This study concludes that alcohol consumption significantly reduced the level of total protein, albumin and globulins. The combination of these parameters in combinations may be a useful indicator for identification and determination of severity of alcoholic liver diseases. Regular screening exercise of alcoholics should be carried out to detect individuals at risk of hepatic dysfunction.

KEYWORDS: Alcoholics, Plasma proteins, Protein Electrophoresis, Total protein, Albumin

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INTRODUCTION:

Alcohol is an organic compound that has a carbon atom bonded to the hydroxyl functional group (-OH). Alcohol is absorbed from both the stomach and the small intestine, but the latter absorbs it more quickly [1]. Although the rate of absorption varies, alcohol is exposed to the same concentration as blood in the fluids, cardiac tissues, brain, and muscle. When ingested on an empty stomach, ethanol is absorbed very quickly. Depending on the type, drinks often contain 20–30% ethyl alcohol. Because the liver receives blood from the stomach and small intestine via the portal vein, its percentage of ethyl alcohol is higher. Only 2-5% is lost unchanged through urine and breath, with the majority of its elimination occurring through hepatic metabolism [2].

Total protein is a clinical chemistry parameter that indicates the level of protein in serum. Most plasma proteins are made in bone marrow, lymph nodes, spleen, and liver plasma cells [3]. Serum contains a wide range of proteins such as albumin and several globulins. Although each of these proteins can be analyzed separately, total protein estimation is a quick and affordable analysis that does not segregate between the different protein types [4]. The liver produces albumin, the most prevalent protein in serum, which serves as a transport protein for a variety of compounds such as bilirubin, enzymes, drugs, hormones and more. Additionally, it preserves the vascular space's fluid volume [5]. Changes in serum albumin levels are linked to a number of clinical disorders [6]. Of the total serum protein content, the globulins make up a substantially smaller portion. The four components of globulins are labeled α_1, α_2, β and γ [7]. In clinical medicine, electrophoresis, including protein electrophoresis, is frequently used to help diagnose a variety of clinical illnesses, including acute and chronic inflammations, monoclonal gammopathies, paraproteinemia's, hemoglobinopathies, nephropathy, liver ailments, etc. [8].

In Nigeria, drinking alcohol is a prominent aspect of adult life and has a significant impact on social, religious, political, and economic interactions [9]. Alcohol is consumed at almost all ceremonies such as weddings, funerals and festivals. Its consumption is widespread in Nigerian society, both in the country's rural and urban areas [10]. Hepatocellular carcinoma, alcohol liver disorders and liver fibrosis is significantly increased by chronic alcohol us [11]. Almost all of the body's major organs, including the thyroid, adrenal gland, liver, pancreas, and liver, become dysfunctional as a result of chronic alcohol use [12]. Furthermore, alcohol may hasten oxidative stress either directly or indirectly, which could enhance modification of biological structures and tissue damage [13].

According to reports, heavy drinking can lead to malnutrition due to potential alterations in intestinal absorption mechanisms and the dysfunction of several organs, including the liver and pancreas [14]. It is unclear how ethanol affects serum protein electrophoresis in humans as a sign of hepatic alterations in alcoholics, despite the vast literature on the hepatic effects of ethanol [15-16]. Alcohol consumption appears to have increased recently among women, men, and adolescents of both sexes, which has raised serious public health concerns with increased potential of possible organ damage. Therefore, this study was carried out to determine the total serum protein, albumin and serum protein electrophoresis pattern of alcoholics in Ikere, Ekiti State.

MATERIAL AND METHODS:

Study area

This study was carried out in Ikere. Ikere is the second most populous and principal city of Ekiti State, Nigeria. The area lies between latitudes 70 30' North of the equator and longitudes 50 14' East of the Greenwich meridian. The city has an area of 262 km^2 and population density of 778.3/km^2 [17].

Study design

A cross sectional study design was employed in this study. Alcoholics between the ages of 15 and 40 years comprising of both males and females were recruited for this study.

Sample size

The sample size was calculated using the formula: $N= z^2pq / d^2$

Where; N= the desired sample size; z = is a constant given as 1.96 which corresponds to the 95% confidence level; p = expected prevalence (4.5%); q = 1.0 – p; d = acceptable error (5%)

$$N = \frac{(1.96)^2 \times 0.045 \times (1 - 0.045)}{(0.05)^2}$$

$$N = \frac{(1.96)^2 \times 0.045 \times 0.955}{(0.05)^2}$$

$$N = \frac{3.8416 \times 0.045 \times 0.955}{0.0025}$$

$$N = 66.03 \approx 66$$

Study population
The population of this study consists of one hundred (100) individuals comprising of sixty (60) subjects (alcohol users) and forty (40) non-alcohol users (controls) in Ikere, Ekiti State.

Ethical approval
Ethical approval for this study was obtained from the Health Research Ethics Committee, Bamidele Olumilua University of Education, College and Technology Ikere, Ekiti State, Nigeria. Informed consent was sought from each subject who participated in the study before the collection of sample.

Inclusion criteria
Apparently healthy males and females with history of alcohol intake, not currently undergoing any medication who gave their consent were included in this study.

Exclusion criteria
Individuals currently undergoing medications and those who did not give their consent were excluded from the study.

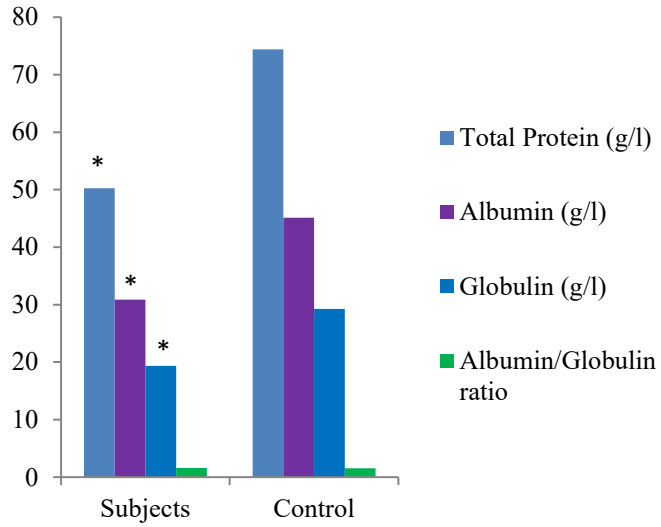
Sample collection
From each participant, about 3mL of blood was collected into plain bottles by veinpuncture. They were labeled and allowed to clot. The serum was separated by centrifugation. The serum was carefully withdrawn into a pre-labeled tube. Specimens not tested immediately were stored at -20^0C. The serum samples were used to determine the total protein, albumin and protein electrophoresis.

Analytical methods
Total Protein was determined using Biuret method.
Principle:
Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex. The intensity of color is directly proportional to the total protein concentration in the specimen. It is determined by measuring the increase in absorbance at 530 - 570 nm.
Albumin was determined using Bromocresol Green (BCG) method.
Principle:
The measurement of serum albumin is based on its quantitative binding to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Protein Electrophoresis was determined using electrophoresis technique.
Principle:
The separation of proteins by electrophoresis is based on the fact that charged molecules usually migrate through a matrix/medium upon application of an electrical field. The rate at which proteins move in an electric field is determined by a number of factors of the electrophoretic system and the nature of proteins itself. Some factors to mention are the strength of the electric field, temperature of the system, pH of the ions, concentration of buffer etc. Proteins vary in their size and shape and have the charges determined by the dissociation contents of their amino acids. Smaller proteins usually migrate faster, and larger proteins take a longer time. This physical property of proteins is exploited for its separation by employing the electrophoretic technique.

Statistical analysis
All results were presented as mean ± standard deviation using tables and charts. Statistical analysis was done using one way analysis of variance (ANOVA) and Student's t-test using IBM SPSS version 24.0 (IBM Corp., Armon, NY, USA). A p-values of <0.05 was considered significant.

RESULTS:



*Values are significantly lower at $p>0.05$

Figure 1: Total protein, albumin and globulin of the subjects and control

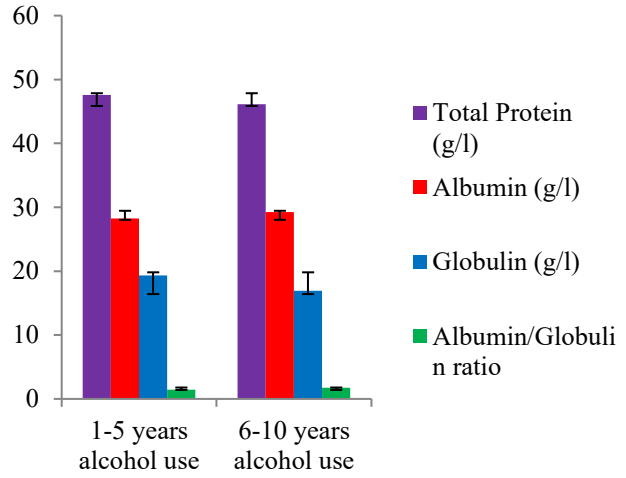
Figure. 1 showed the mean values of total protein, albumin and globulin of the subjects (alcohol users) and control (non-alcohol users). The results obtained showed that the mean value of total protein in subjects and control was 50.22±8.44 g/L and 74.38±8.12 g/L, albumin was 30.89±5.05 g/L and 45.13±5.18 g/L, globulin was 19.33±6.11 g/L and 29.25±5.36 g/L, while albumin/globulin ratio was 1.6 and 1.54 respectively. Statistically, total protein, albumin and globulin were significantly lower ($p<0.05$) in subjects compared with control group.

Table 1: Total protein, albumin and globulin of Subjects with respect to gender

Parameters	Male Control (Mean ± SD)	Male Subjects (Mean ± SD)	Female Control (Mean ± SD)	Female Subjects (Mean ± SD)	p-value
Total Protein (g/l)	73.25±6.25[a]	49.29±6.14[b]	74.84±8.61[a]	50.29±5.45[b]	0.000*
Albumin (g/l)	44.36±3.66[a]	30.26±4.18[b]	43.71±4.34[a]	30.66±3.58[b]	0.000*
Globulin (g/l)	28.89±3.96[a]	19.03±4.88[b]	31.13±5.00[a]	19.63±4.69[b]	0.000*
A/G ratio	1.54±0.93	1.59±0.86	1.40±0.87	1.56±0.76	0.641

*Values in a row with different superscript is significant at p<0.05.

Table 1 showed the total protein, albumin and globulin of the subjects with respect to gender. From the results obtained, the mean values of total protein, albumin and globulin were significantly lower ($p<0.05$) in male (49.29±6.14, 30.26±4.18 and 19.03±4.88 g/L) and female subjects (50.29±5.45, 30.66±3.58 and 19.63±4.69 g/L) compared to male (73.25±6.25, 44.36±3.66 and 28.89±3.96 g/L) and female control (74.84±8.61, 43.71±4.34 and 31.13±5.00 g/L) respectively. On the other hand, total protein, albumin and globulin was non-significantly higher ($p>0.05$) in female subjects in comparison with male subjects.



*Value is statistically significant at $p<0.05$

Figure 2: Total protein, albumin and globulin of subjects with respect to duration of alcohol use

Figure. 2 showed the total protein, albumin and globulin of alcohol users with respect to duration of alcohol use. The results obtained showed that the mean value of total protein among alcohol users for 1 to 5 years and alcohol users for 6 to 10 years was 47.56±8.14 g/L and 46.15±6.45 g/L, albumin was 28.24±5.06 g/L and 29.24±6.11 g/L, globulin was 19.32±16.91 g/L and 16.91±4.68 g/L, while albumin/globulin ratio was 1.46±1.11 and 1.73±1.31 respectively. Total protein, albumin and globin was lower in alcohol users of 6-10 years compared with alcohol users of 1-5 years, however, only globulin was significantly lower ($p<0.05$).

Table 2 showed the serum protein electrophoresis profile of subjects and control. The results obtained showed the mean value of albumin in

non-alcohol users, alcohol users and chronic alcoholics was 45.13±5.18, 31.44±4.35 and 24.62±2.61, α_1 globulin was 17.36±6.41, 14.14±5.89 and 6.85±1.04, α_2 globulin was 12.66±4.42, 11.26±1.32 and 5.32±1.56, β_1 globulin was 5.22±1.41, 6.15±1.42 and 7.04±1.89, β_2 globulin was 5.98±2.60, 7.04±1.23 and 11.21±2.21, while γ globulin was 16.11±2.36, 24.01±3.21 and 21.69±4.51 respectively. Albumin, α_1 and α_2 globulin was significantly higher (p<0.05) in non-alcohol users compared with chronic alcoholics, while β_2 and γ was significantly lower (p<0.05) in non-alcohol users compared with chronic alcoholics.

Table 2: Serum protein electrophoresis profile of subjects and control

	Non-Alcohol users (Mean ± SD)	Acute Alcoholics (Mean ± SD)	Chronic Alcoholics (Mean ± SD)	p-value
Albumin	45.13±5.18[a]	31.44±4.35[b]	24.62±2.61[c]	0.000*
α_1 globulin	17.36±6.41[a]	14.14±5.89[a]	6.85±1.04[b]	0.001*
α_2 globulin	12.66±4.42[a]	11.26±1.32[a]	5.32±1.56[b]	0.002*
β_1 globulin	5.22±1.41[a]	6.15±1.42[a]	7.04±1.89[a]	0.137
β_2 globulin	5.98±2.60[a]	7.04±1.23[a]	11.21±2.21[b]	0.000*
γ globulin	16.11±2.36[a]	24.01±3.21[b]	21.69±4.51[b]	0.041*

*Values in a row with different superscript is significant at p<0.05

Figure. 3 showed the stained and processed cellulose acetate paper showing serum protein electrophoretic separation. From the figure 1 to 4 are chronic alcohol users, 5 and 6 are non-alcohol users (control), while 7 to 10 are acute alcohol users.

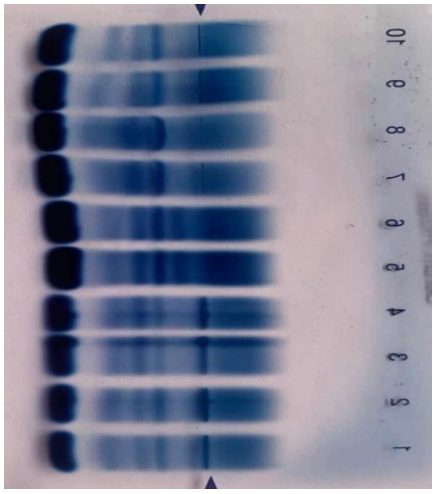


Figure 3: Stained and processed cellulose acetate paper showing serum protein electrophoretic separation

DISCUSSION:

This study aimed to determine the total serum protein, albumin and serum protein electrophoresis pattern in alcoholics in Ikere-Ekiti. The results of this study showed that total protein, albumin and globulin was significantly lower (p<0.05) in subjects (alcohol users) in comparison with control group. This finding might be explained by the fact that acute alcohol exposure decreases albumin. The participants' dietary state could also contribute to the drop in serum albumin levels [18-19]. A possible target for the production of an adduct by the alcohol metabolite acetaldehyde is albumin. The amount of serum globulin can increase as a result of this albumin or other protein adducts stimulating the production of immunoglobulins [20]. Serum albumin concentrations can fall after renal losses in nephrotic syndrome and after losses via the gastrointestinal tract in protein-losing enteropathies, in addition to decreased synthesis or hepatic insufficiency [21]. The result of this study is in agreement with report of previous authors who reported significant lower values of total protein, albumin and globulin among alcoholics subjects compared with control groups [18-19,22-23]. Progressive hypoalbuminemia is one of the symptoms of chronic alcoholic liver damage [19-20].

In addition, the study by Torruellas et al.[24] on liver-function tests in known alcohol users reported that total proteins, albumin and the albumin-globulin ratio were the most frequently abnormal tests and had significantly lower values in this group, while serum albumin and globulin levels in a group of non-alcohol users (control) were within normal ranges. In another study, serum proteins and albumin levels in alcoholics were considerably lower than in controls [23]. In their investigation, Queremel and Jialal[25] found that the presence of excessive urobilin, a high initial mucoprotein level, and low levels of total-serum protein and albumin were the most often seen abnormal tests in alcoholics. Chronic and excessive alcohol consumption produces a wide spectrum of hepatic lesions, the most characteristic of which are steatosis, hepatitis, and fibrosis/cirrhosis [26]. The pattern of abnormal liver-function test results reported in a series of cases seems to make it pretty clear that chronic alcohol users frequently experience liver-function abnormalities.

In this study, male alcoholics compared to male controls and female alcoholics compared to female controls both had significantly decreased levels of total protein, albumin, and globulin (p<0.05). In contrast, there was no statistically significant difference (p>0.05) between the total protein, albumin, and globulin levels of male and female alcoholics as well as male and female controls, although female subjects had higher values than male subjects. However, the lack of a statistically significant gender difference in this investigation has no recognized clinical implications. Data indicate that alcohol use may prevent the production of proteins, especially in heavy or frequent drinkers [26]. Heavy or moderate alcohol use, especially in females, may influence the transport and binding of substances by albumin such as bilirubin [27]. By interacting non-covalently with albumin, bilirubin is delivered to the liver. There is a strong link between excessive alcohol use and liver damage. Alcohol intake has been associated with more than 60 illnesses and affects almost every organ in the body [28]. This finding concurs with that of who found that women had considerably greater amounts of total protein and albumin than men [28-30]. In another population research, it was reported that male plasma proteins were higher than female plasma proteins [27,31].

The total protein, albumin and globin levels of the alcohol users in this study did not alter significantly (p>0.05) with respect to the duration of alcohol use. This may be explained by the fact that albumin makes up more than 50% of the total plasma protein biologically, and that variations in albumin concentrations frequently cause changes in plasma protein levels [27,32]. It is sufficient to mention that, among the analyzed respondents, both plasma protein and albumin levels exhibited identical findings, particularly with regard to the time period of alcohol use by prior investigations [27,31,33]. In a different study, the albumin level was discovered to be significantly lower in all the examined groups when compared to the normal group, even though only the heavy alcoholic group showed a significant rise in globulin levels when compared to the other groups [34].

The findings of this study demonstrated that compared to chronic alcoholics, α_2 globulin was significantly higher (p<0.05) in non-alcohol users and alcohol users, while β_2 was significantly lower (p<0.05) in non-alcohol users and alcohol users. When non-alcohol users and acute alcohol users were compared to chronic alcoholics, there was no significant difference n albumin, α_1, β_1 and γ respectively (p>0.05). Progressive hypoalbuminemia is a characteristic of chronic alcoholic liver disease [35]. Chronic alcohol consumption lowers plasma proteins levels and causes intrahepatic protein export-type buildup. Acetaldehyde appears to be the mediator for these effects [27]. This result is consistent with earlier investigations. This fining is in agreement with previous studies [27,36-37].

Following high resolution two-dimensional electrophoresis and isoelectric focusing-multiple gel piece electrophoresis, changes associated with alcohol in human serum protein patterns have been detected using silver staining [38]. In the serum of acute alcoholics and chronic alcoholics the concentration of α_1-acid glycorportein was consistently increased and in a high proportion of sera elevated levels of IgA, α_1-antichymotrypsin, haptoglobins and apo A-I lipoprotein were observed. Increased levels of additional unidentified polypeptides were also detected. Silver staining used in conjunction with high resolution two-dimensional electrophoresis and isoelectric focusing-multiple gel piece electrophoresis has been used to identify alcohol-related alterations in human serum protein patterns [38]. The quantity of 1-acid glycoprotein was consistently higher in the serum of alcoholics and chronic alcoholics with liver disease, and enhanced levels of IgA, 1-antichymotrypsin, haptoglobins, and apo A-I lipoprotein were also found in significant proportions of the sera. Additional

unidentified polypeptides with increased concentrations were also found.

The above observation is in line with other research [38-40]. When diagnosing patients with chronic liver illness, serum protein electrophoresis is a helpful technique, particularly in cases of liver cirrhosis and chronic active hepatitis [39]. If a biopsy is not planned, it can be argued that liver cirrhosis exists. Albumin and α_2-globulin levels in these patients are uniformly lower. When liver cirrhosis is present, the γ-fraction is frequently polyclonal, and the typical dip between the β- and γ-band may not be present (β-γ bridging) [38,40].

CONCLUSION:

This study came to the conclusion that alcohol use considerably decreased the levels of total protein, albumin and globulin. The lowered levels of total protein, albumin and globulin were more pronounced in female alcoholics and the subject's length of consumption had no significant impact on their levels of total protein, albumin or globulin. Numerous alterations in cellular processes and the oxidant-antioxidant system are linked to alcohol intake. Combining all of these variables may provide a valuable indicator for identifying and evaluating the severity of alcoholic liver disorders. To identify alcoholics who may be at risk of hepatic impairment, regular screening exercises should be conducted.

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