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ESTIMATION OF GAMMA-GLUTAMYL TRANSFERASE AND ALKALINE PHOSPHATASE ACTIVITIES AMONG ALCOHOLICS IN ABAKALIKI

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ABSTRACT

The present study was designed to evaluate the plasma GGT and ALP levels in alcoholics in Abakaliki using a cross-sectional laboratory-based design involving eighty (80) respondents of fifty alcoholics and thirty non-alcoholics. A self-structured questionnaire was administered to both groups to collate data on participant's demographics. GGT and ALP were analyzed calorimetrically, using Randox and Spectrum reagents respectively and according to the manufacturer's recommendations. Data generated from the study were analyzed using SPSS version 23. The results, showed significant (r = -0.298, p =0.007) increase in GGT in alcoholics (6.78±3.919) compared to non-alcoholics (4.33±3.698) while no significant (r = 0.185, p = 0.185) change was observed in ALP values in alcoholics (97.22±38.94) compared to non-alcoholics (113.53±47.791). GGT was not significantly correlated with age (r = 0.002, p =0.991) and BMI (r = 0.051, p = 0.724). Also, ALP was not significantly correlated with age (r = -0.232, p = 0.105) and BMI (r = -0.043, p = 0.765). In conclusion, GGT measurement may be a suitable screening test in assessing liver involvement in alcoholics.

Key words: gamma-glutamyl transferase, alkaline phosphatase, alcoholics.

INTRODUCTION

Increased alcohol availability, production, importation and consumption has been reported across all age groups in Nigeria [Lasebikan and Ola, 2016]. Among the social activities in Nigeria, alcohol consumption is widely considered [Lasebikan et al 2018]. In 2016, the Nigeria statistics bureau (NBS) reported that at least N208 billion was expended by Nigerians on alcohol alone [Adebowale, 2019]. According to the report, this sum was more than the budget of Ondo State for that year. More also, a total sum of N74.4 billion, N44 billion, N37 billion and N30 billion was reported to be expended on alcohol in the South South, South East, South West, and North-Central zones of the country respectively [Adebowale, 2019].

However, the risk factor for illness, disability, and mortality has been linked to alcohol consumption [Rehm, 2011]. According to World Health Organization, alcohol consumption has been linked with about 3 million deaths each year, globally as well as to the disabilities and poor health of millions of people [WHO, 2022]. The global burden of disease due to harmful use of alcohol stands at 5.1% [WHO, 2022]. Higher rates of alcohol related death and hospitalization are seen among disadvantaged and vulnerable populations. According to International Agency for Research on Cancer (IARC) as cited by Adebowale (2019), for every 10 cancer cases in Nigeria, one can be traced to alcohol and the overall cases of cancers attributed to alcohol stood at 4.7 percent.

Currently, the role of liver enzymes in independently predicting alcoholism is attracting attention of researchers. Alcohol is known to be metabolized in the liver through the action of many enzymes and pathways. The liver, however, as the largest organ of the body, is the major site of most metabolic processes and a secretory hub of many biochemical substances including enzymes. The degree of change in the activities of these enzymes in serum is dependent on the level of damage to the liver. Alcohol consumption however, has been reported to cause liver dysfunction and initiate liver disease [Corrao et al. 1999]. A few studies have examined the relationship between alcohol and liver enzymes by measuring liver function such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP) [Robinson and Whitehead, 1989; Whitehead et al 1996]. At the liver and biliary epithelial cells are situated the GGT and ALP, thus they are sensitive markers of diseases of the hepatobiliary system. Elevated levels of GGT are associated with increased risk of disease and all-cause mortality. GGT is a marker of oxidative stress and its serum level is associated with inflammatory markers [Bo et al 2005], except in the presence of heavy drinking [Wannamethee and Shaper, 2010]. Aside cholestatic liver diseases, raised serum levels of ALP are associated with bone diseases [Koehler et al 2014].

Patel and O'Gorman had postulated in 1975 that GGT estimation may play an important role in early detection of liver damage resulting from long-term effects of alcohol consumption even before clinical signs have appeared [Patel and O'Gorman, 1975]. Studies had also, reported significant changes in serum ALP and GGT levels by chronic alcohol consumption [Patel and O'Gorman, 1975; Nishimura and Teschke, 1982; Sripanidkulchai et al 2004; Das and Vasudevan 2005]. Moreover, the high incidence of liver related disease conditions and deaths in Nigeria might be related to alcohol consumption as a risk factor [Andersen and Osler 2004; WHO 2018; Adebowale, 2019]. The present study thus, focused on measuring the levels of GGT and ALP in alcoholics, to provide baseline data on liver involvement in alcoholism.

MATERIAL AND METHODS

Study Area

This was a cross-sectional laboratory-based study carried out within the city of Abakaliki, the capital of Ebonyi State. Ebonyi state has 13 local government areas and is a southeastern state in

Nigeria with a population of 915,438 as at year 2019. The predominant inhabitants of Abakaliki are Christians and of the Igbo ethnic group.

Study participants

The study comprised 80 adult male subjects who were divided into two groups: a control group of 30 non-alcoholics (individuals who denied habitual alcohol consumption) and case group of alcoholics who randomly visits drinking joints at Abakaliki city. They were randomly selected without any prior notification. All participants were males and 18 years and above. Blood samples were collected from both groups for GGT and ALP investigation. Both groups were negative for hepatitis B and C virus serology. The study started with an informal visit to major drinking joints/beer parlors within Abakaliki to obtain an unofficial permission from the pub landlords to recruit their customers for the study. This was followed with a second visit at night, during which the customers present were addressed individually and their blood specimens collected after agreeing and signing the informed consent. The demographic information of the participants was obtained through a semi-structured questionnaire. Participants in the case group were included if they 1. agreed to have been taken up to 3 or more bottles of alcohol in a week 2. report not on any treatment for liver diseases/ailments and 3. Are not infected with Hepatitis B or C. Exclusion criteria included subjects 1. who take less than 3 bottles of alcohol per week, 2. Who are on treatment for liver related ailments 3. Positive for Hepatitis B or C. The approval for the study was got from Ebonyi State University research and ethics committee Abakaliki.

Data Collection

A semi-structured questionnaire was administered to both groups and each participants self-reported their lifestyle and medical history including marital status, age, behavioral activities (exercise, smoking and alcohol consumption). The body mass index (BMI) was calculated from the weight (measured to nearest 0.1kg) and height (measured to nearest 0.1cm) of the participants. Alcohol intake was defined as the intake of at least three bottles of an alcoholic beverage per week. Participants were classified into current smokers, ex-smokers and non-smokers depending on the last time they smoked cigarette. Using a standardized procedure, trained medical staff members carried out the medical examinations. A non-fasting antecubital vein blood samples were collected from each subject and frozen immediately at −20°C for two weeks before analysis. Serum levels of γ-glutamyl transferase (GGT), and ALP levels were measured via enzymatic methods using manufacturer's recommendations as described by Randox diagnostic kits (Randox Laboratories Ltd. United Kingdom) and Spectrum diagnostic laboratory limited respectively and read using Erba Chem 7 semi-automatic clinical chemistry analyzer (Erba Diagnostics Mannheim, Germany). The laboratory screening of the blood specimens for HBsAg and HCV and subsequent analysis of GGT and ALP was carried out at the Alex Ekwueme Federal University Teaching Hospital Abakaliki from 10^{th} – 13th April 2022.

GGT determination

The procedure for GGT determination used for this study was based on Randox Gamma GT colorimetric assay as described by Randox Laboratory limited.

Materials/Equipments

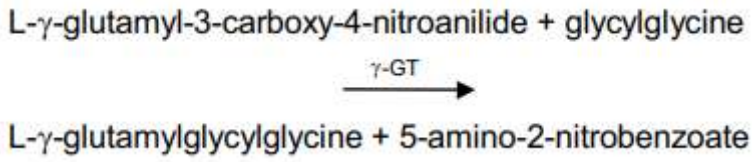
The materials used for the analysis included Randox GGT kit which contains buffer/Glycylglycine Tris buffer (100 mmol/l, pH 8.25), glycylglycine (100 mmol/l), substrate (L-γ-glutamyl-3-carboxy-4-nitroanilide 2.9 mmol/l). Others are 100 ul and 1 ml pipette, timer, testubes, test tube racks, water bath to maintain temperature at 37°C, Spectrophotometer (Erba Chem 7 analyser) with wavelength capability of 410 nm.

Sample preparation

The blood samples were collected from the ante-cubital veins of participants in heparin containers after which they were centrifuged at 3000 revolution per minute using a buckit centrifuge in the laboratory. The non-haemolysed plasma was immediately frozen (−20°C) for 7 days prior to analysis.

Principle of the test

L-B-glutamyl-3-carboxy-4-nitroanilide which acts as enzyme substrate is converted to 5-amino-2-nitrobenzoate in the presence of glycylglycine in the reagents and γ-GT in the sample. The 5-amino-2-nitrobenzoate formed was measured at 405nm.



Test procedure

The vial of substrate was first reconstituted with 3.0 ml of buffer/glycylglycine and used immediately as working reagent. 0.10 ml of the sample and 1.00 ml of the working reagent were added into a cuvette, the solution was mixed thoroughly and read in a spectrophotometer at 405 nm wavelength as described in the manual.

Calculation

The formula: unit per liter (U/L) = 1158 x ΔA 405 nm/min was used to calculate the GGT activity

The quantitative range using this methodology is 11-50 U/l at 37^0C for men. GGT level higher than 50U/L was considered elevated for men.

ALP determination

The procedure for ALP determination used for this study was based on Spectrum diagnostics alkaline phosphatase colorimetric method as described by Spectrum diagnostic laboratory limited.

Materials/Equipments

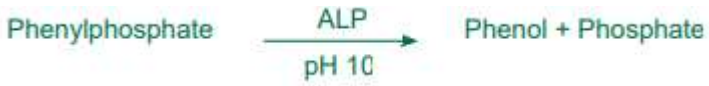
Reagent 1 (R1 Buffer) pH 10 comprised of disodium phenylphosphate (5.0 mmol/L), carbonate-bicarbonate buffer (50 mmol/L). Reagent 2 (R2 Standard) comprised of phenol (Equal to 20 kind and king U). Reagent 3 known as R3 blocking reagent comprised of 250 mmol/L of sodium arsenate, 60 mmol/L 4-aminoantipyrine, 150 mmol/L potassium ferricyanide, and buffer of pH 10. Reagent 4 known as R4 color reagent was made of 150 mmol/L of potassium ferricyanide. Others are 50 ul and 1 ml pipette, timer, testubes, test tube racks, water bath to maintain temperature at

37°C, Spectrophotometer (Erba Chem 7 analyser) with wavelength capability of 510 nm.

Sample collection and preparation

The blood samples were collected from the ante-cubital veins of participants in heparin containers after which they were centrifuged at 3000 revolution per minute using a buckit centrifuge in the laboratory. The non-haemolysed plasma was immediately frozen (−20°C) for 7 days prior to analysis.

Principle of the test



From the equation above, phenyl phosphate in the presence of ALP enzyme is converted to phenol and phosphate. In the presence of potassium ferricyanide and 4-aminoantipyrine the phenol liberated was measured at 510nm.

Test procedure

The procedure for the test was as described in Spectrum diagnostic leaflet.

Calculation

The formula. $\frac{\text{OD serum sample} - \text{OD serum blank}}{\text{OD Standard}} \times n$ was used in calculating the ALP activity.

The quantitative range for adults using this methodology is 21 - 92 U/L. ALP level higher than 92 U/L was considered elevated for men.

Statistical analyses

Descriptive statistics was used to perform baseline analyses. Assessment of significance of differences in distribution of categorical and continuous data was done using unpaired student's t-tests (means). $P<0.05$ was chosen as the level of significance in the study. Relationship between Age, BMI, alcohol intake and liver enzymes was tested using Pierson correlation.

RESULT AND DISCUSSION

Data regarding biochemical evaluation of alcoholics and non-alcoholics (control) are presented in Tables 1, 2, and 3.

Table 1: Socio-demographic characteristics of respondents

	Alcoholics (% or mean±SD)	Control/non alcoholics (% or mean±SD)	All subjects (% or mean±SD)
	N=50	N=30	N=80

Age (years)	1.84±0.738	1.23±0.43	NA
18-25	30.0	76.7	47.5
26-35	60.0	23.3	46.3
36-45	8.0	0.0	5.0
>56	2.0	0.0	1.3
BMI (kg/m^2)	25.23±4.949	25.83±2.768	NA
State of Origin			
Abia	6.0	6.7	6.3
Anambra	2.0	10	5
Ebonyi	70.0	56.7	65
Enugu	6.0	13.3	8.8
Imo	12.0	6.7	10
Kogi	4.0	6.7	5
Marital status			
Married	16.0	3.3	11.3
Single	84.0	96.7	88.8
Education			
Educated	86.0	96.7	90.0

Not-educated	14.0	3.3	10.0

SD; Standard deviation, %; percentage.

From Table 1, the mean age of alcoholic group (1.84±0.738) is higher than those of non-alcoholic/control group (1.23±0.43), Majority of respondents are within the age of 18-25 years. There is no much difference in the BMI of both groups. Majority of subjects are single (88.8%), educated (90.0%) and from Ebonyi state (65.0%).

Table 2: Mean and standard deviation and p-value of all the parameters between alcoholic and non-alcoholic

Parameter	non-alcoholic (n=30) (mean±SD)	alcoholic (n=50) (mean±SD)	r-value	p-value
ALP(IU/l)	113.53±47.791	97.22±38.94	0.185	0.1
GGT(IU/l)	4.33±3.698	6.78±3.919	-.298**	0.007

*SD- Standard deviation, p-value <0.05 is significant, ** correlation is significant at the 0.01 level (2-tailed).*

From Table 2, the ALP activity between non-alcoholics and alcoholics is insignificant. However, GGT value was higher among the alcoholics compared to non-alcoholics and the increase was statistically significant.

Table 3: Relationship between Age and the observed serum levels of the parameters in alcoholic

Parameter	alcoholic (n=50) (mean±SD)	r-value	p - value
Age			
ALP(IU/l)	97.22±38.94	-0.232	0.105
GGT(IU/l)	6.78±3.919	0.002	0.991

BMI			
ALP(IU/l)	97.22±38.94	0.02	0.89
GGT(IU/l)	6.78±3.919	-0.043	0.765

SD- Standard deviation, p-value <0.05 is significant

Table 3 shows that the levels of ALP and GGT in alcoholics were not significantly ($P > 0.05$) correlated with their age and BMI.

DISCUSSION

In the present study as shown in Table 2, GGT level was significantly ($p < 0.05$) higher in alcoholics compared to the non-alcoholics. This finding was similar to that reported in many other similar studies [Patel and O'Gorman 1975; Horner et al 1979; Robinson and Whitehead 1989; Lutz et al 1992; Martins and Borges 1993; Isichei et al 1994; Sripanidkulchai et al 2004; Das and Vasudevan 2005; Jang et al 2012; Torkadi et al 2014]. Patel and O'Gorman who investigated serum enzyme levels in alcoholism reported that among all the enzymes assayed, GGT had the highest (48%) raised activity in serum [Patel and O'Gorman 1975]. 88.1% and 61.4% pathological serum values of GGT were seen among alcoholics in Jos, Nigeria, and in Castrop-Rauxel, Germany respectively [Isichei et al 1994]. Lutz and Bausbach in 1992 reported that 55.6% of alcoholics had pathological GGT values.

Similarly, in a study that investigated dynamic changes of serum GGT in chronic alcoholism, out of 155 chronic alcoholics who were undergoing detoxification treatment, GGT was reported to be elevated in 29% of the subjects [Horner et al 1979]. Similarly, in a population-based study in Northern Thailand, serum GGT of alcohol drinkers was significantly higher than that of non-drinkers [Sripanidkulchai et al 2004]. Das and Vasudevan in 2005 reported significant high GGT values in alcoholics. Patients with alcoholic liver disease (ALD) was observed to have elevated GGT activity compared to non-ALD [Torkadi et al 2014]. According to Robinson and Whitehead, 'those who regularly consume more than 6 units of alcohol per day are 5·3 times more likely to have a raised GGT level than those who are teetotal or drink only socially' [Robinson and Whitehead 1989]. When factors such as alcohol consumption, cigarette smoking, exercise level and obesity were taking into account, the highest probability for a raised GGT was associated with heavy drinking followed by Obesity [Robinson and Whitehead, 1989].

The ALP values observed in non-alcoholics compared to alcoholics were of no statistical significant ($P > 0.05$) (Table 2). In early 80's ALP was reported to increase in a less intensive manner than GGT in ALD [Lai et al 1982]. Also, a population-based study in Northern Thailand observed no difference in serum ALP of alcohol drinkers compared to non-drinkers [Sripanidkulchai et al 2004]. In 1975, another study reported only 5% rise in ALP in alcoholics [Patel and O'Gorman 1975]. Interestingly, dissimilar observations were reported in most other studies [Horner et al 1979;

Martins and Borges 1993; Das and Vasudevan 2005; Jang et al 2012; Koehler et al 2014]. Firstly, Horner et al. reported 16% increase in ALP activities among 155 chronic alcoholics undergoing detoxification treatment. Secondly, moderately increased ALP was observed in alcoholics in comparison to healthy controls as well as in heavy drinkers in comparison to moderate drinkers in a previous study [Das and Vasudevan, 2005]. According to Das and Vasudevan, ALP was raised to a record high in 91% of heavy drinkers and 42% in moderate drinkers. Thirdly, elevated levels of ALP was also seen in ALD compared to non ALD [Torkadi et al 2014]. Explaining the reason behind the less intensive manner ALP is raised in ALD compared to GGT, an earlier study opined that this could mean that the effect of alcohol on the liver is to liberate a large amount of GGT in the serum, disproportionate to the amount of ALP [Lai et al 1982].

Our study shows that the levels of ALP and GGT in alcoholics did not significantly correlate with age and BMI (Table 3). This finding is dissimilar to reports by other studies. A previous study on serum GGT and ALP of people in Khon Kaen, the Northeastern Thailand observed that ALP but not GGT values in both sexes were clearly age dependent [Sripanidkulchai et al 2004]. According to the study, increase in age was accompanied with increase in ALP values in males, while in females, ALP decreases as age increases. The association between BMI and GGT in alcoholics was also observed in a similar study [Wannamethee and Shaper, 2010]. Heavy alcoholics were reported to have low BMI in a previous study [Das and Vasudevan, 2005]. The study explained that the reduced BMI could be due to fat mass reduction in these patients. A fall in body weight and BMI had been adjudged the best clinical indicator of apparently continuing alcohol abuse [World et al 1984]. Lower body weights could be a result of reduced adipose tissue in these patients. In chronic alcoholics, loss of adipose tissue in chronic alcoholics who continue to drink has been reported to be due to simultaneous inadequate nutritional intake [Das and Vasudevan, 2005].

According to Lutz and Bausbach, most of the alcoholics with raised enzyme levels were 30-49 years old (45%), however in our study they were 26-35 years (Table 1). The age range is almost similar and represent the active adult age group. Among the ages of 15 to 49 years, the World Health Organization observed that the leading risk factor for mortality and disability is alcohol [WHO, 2022].

The overall changes in serum levels of GGT and ALP in the present study may be a result of alcohol induced pyridoxine deficiency and hepatic mitochondrial injury. The nexus between alcohol vis a viz liver enzymes has been well documented [Selinger et al 1982; Das and Vasudevan, 2005; Breitling et al 2009; Bruha et al 2012; Jang et al 2012; Torkadi et al 2014]. Moreover, since increased level of GGT reported in the present study is not associated with an increase in ALP, one may be tempted to attribute the increase in GGT to external sources than the liver. In another study, the high sensitivity of serum GGT than serum ALP in association with alcohol drinking had been explained to be due to the ability of alcohol and its metabolites to increase serum GGT by its hepatotoxic or by reducing the antioxidant levels, which is the promoter for hepatocirrhosis [Sripanidkulchai et al 2004]. However, further studies with a larger sample size that will adjust the confounding effect of age, sex, body height, weight, BMI, cigarette smoking, coffee or tea drinking, physical activity, and presence/absence of co morbidities are needed to confirm our finding. Studies have shown that these factors may affect the association of alcohol and liver enzymes [Robinson and Whitehead 1989; Sripanidkulchai et al 2004; Breitling et al 2009; Wannamethee and Shaper 2010; Jang et al 2012; Koehler et al 2014]. Higher levels of ALP are found in the liver, bone and bile ducts. In addition to its hepatic origin, GGT as a microsomal enzyme is also known to be produced in tissues like intestine, pancreas, renal tubules and biliary epithelium. GGT has been

implicated in alcohol use by keeping intracellular glutathione at adequate levels, thus protecting cells from oxidative stress resulting during metabolism [Whitfield, 2001]. Alcohol can elicit GGT increase either by liver cell damage, hepatic microsomal enzyme induction [Patel and O'Gorman 1975; Das and Vasudevan 2005] or alcoholic pancreatic damage [Wu et al 1976].

CONCLUSION

GGT activity was observed to be increased in alcoholics compared to non-alcoholics. No significant changes were observed in ALP activity in the both groups. The GGT and ALP levels were observed not to significantly correlate with the age and BMI of alcoholics. Thus, in screening or monitoring of liver involvement in alcoholics, GGT may be a more useful test. However, a large population-based study may be needed to confirm our findings.

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