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Enzymes as markers of liver damage in apparently healthy alcohol drinkers resident in Vom community

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Abstract

This study was conducted to investigate the effect of alcohol consumption on the liver of apparently healthy human subjects resident in Vom and its environs. Blood samples were collected from 120 subjects and serum level of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) were estimated by IFCC kinetic method using available commercial Reagent Kit (DIALAB Scientific Laboratories, Austria) and colorimetric end point method. All the enzymes, ALT, AST and ALP assayed were significantly higher ($p<0.05$) in alcoholics than non-alcoholics. The frequency distribution shows that greater percentage of alcoholics have above normal levels of all liver enzymes assayed while greater percentage of non-alcoholics have normal levels of the liver enzymes, the age group (41- 50 years) has greatest percentage of high levels of the enzymes and greater percentage of men have high levels of the enzymes than women. Therefore, the study suggests that excessive consumption of alcohol predisposes humans to the risk of developing alcoholic liver disease and men are at greater risk than women in Vom and its environs.

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Introduction

The liver is one of the most important organs in the body due to it's numerous physiological and biochemical functions. It is the largest organ in the body and weighs between 1.2 to 1.5kg (Burtis and Ashwood, 2001). As the key organ of metabolism and excretion, the liver has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents; thus it is subjected to variety of diseases and disorders (Rajesh *et al.,* 2009). Hepatobiliary damage describes the disease conditions which interfere with the functions of cells, tissues or structures of the liver and the obstruction to bile flow (Vasudevan and Sreekumari, 2007)

Hepatocyte injury is common worldwide (Dufour *et al.,* 2000), mainly caused by toxic chemicals, excessive consumption of alcohol, Viral/Bacterial infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages (Dianzani *et al.,* 1991). Diseases of the liver could be fatal and life threatening, so there is increased emphasis on prevention. Currently, more attention is being placed on developing screening tests for detection of early, asymptomatic disease of the liver, as early diagnosis is vital to the effective management of the disease.

Biochemical tests are important in diagnosis and monitoring of liver diseases. These tests are usually referred to as "Liver function tests" (LFTs). Liver function tests are several laboratory tests conducted to investigate the functionality of the liver. They are the most widely performed biochemical tests in the laboratory (Vasudevan and Sreekumari, 2007). The tests commonly measure liver enzymes activities, which identify liver cells damage, and hence serve as markers of liver damage. The enzyme tests include but not limited to determination of the relative increases in serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP) beyond normal levels. Although ALT activity is more specific for liver disease (Achliya *et al.,* 2003), it may be useful to measure the activities of both aminotransferases.

The symptoms of liver diseases may be acute or chronic. Chronic liver damage is much more common than acute. The incidence of chronic liver disease is two times higher in men than in women, and may range from mild to severe, depending on the type of liver disease present (Dufour *et al.,* 2000). An estimated 80% of individuals with acute viral hepatitis are never diagnosed clinically, although some may be detected by increased aminotransferases in the face of non-specific or absent clinical symptom. AST and ALT activities are seldom >10 times the upper reference limit in liver diseases other than acute hepatic injury. Alkaline phosphatase is more than three times the upper reference limit in < 10% of cases of acute hepatic injury (Ellis *et al.,* 1978)

Thus, the present study was initiated to investigate the enzyme levels of a well population of alcohol consumers in Vom and its environs, in order to assess the effect of alcohol on the functionality of their livers. It also appears there is paucity of information on the prevalence of liver diseases in Vom, Plateau State and the whole of Nigeria.

Materials and methods

A total of 120 subjects were randomly selected from a well population of human beings all resident in Vom and its environs, within Jos South Local Government Area of Plateau State, Nigeria. They were both males and females between the ages of 20 and 60 years. The study was conducted with the consent and understanding of all the subjects used. A questionnaire form was used to obtain relevant health information and demographic data, so sick people were not used for the study. Fasting venous blood samples were obtained by venepuncture from the

ante-cubital vein using new sterile disposable syringes and needles, after initial sterilization of the cubital fossa with a cotton wool soaked in 70% alcohol. A 5ml blood was collected from each subject and transferred into clean and dry tubes, then allowed for proper retraction and clotting for 15 minutes and centrifuged at 3000 revolution per minute (rpm) for 10 minutes. The serum was separated into clean and dry specimen bottles using clean Pasteur pipette for each specimen. All the serum samples were frozen at -200c until they were analyzed.

Biochemical assays

Estimation of Aspartate aminotransferase: AST activity in Serum specimen was assayed colorimetrically by modified IFCC kinetic method (Reitman and Frankel, 1957) using available commercial reagent kit, supplied by DIALAB scientific laboratories, Austria. Into clean labeled test tubes, for test and blank, were added 1000µL of the working reagent and the tubes were incubated for approximately 5 minutes at 370c. Then 100µL of sample was added to the sample tubes, mixed and absorbance was read at 340nm. The initial absorbance was read against air after 1 minute and a timer was started and absorbance read again after exactly 1, 2 and 3 minutes against reagent blank and the activity was calculated.

Estimation of Alanine aminotransferase (ALT): ALT activity in serum specimen was assayed colorimetrically by modified IFCC kinetic method similar to AST (Reitman and Frankel, 1957) using available commercial reagent kit, supplied by DIALAB scientific laboratories, Austria.

Estimation of Alkaline phosphatase (ALP): ALP activity in blood was assayed by colorimetric end point method (Moss and Henderson, 1999). Into clean labeled test tube was placed 0.5ml of Alkaline phosphatase substrate and incubated for 3 minutes at 370C. At timed intervals, 0.05ml of standard and samples were added to their respective test tubes. The contents of the tubes were mixed gently and incubated for exactly 10 minutes at 370C, while 0.05ml of deionized water was used for the blank. Then 2.5ml of ALP colour developer was added to each tube, mixed and absorbance read spectrophotometrically at 590nm and ALP activity was calculated.

Statistical analysis

All data were expressed as mean± standard deviation (SD). The results were analyzed by Analysis of Variance (ANOVA) using the statistical package for social sciences (SPSS) for windows.

Results

Data obtained from biochemical assays were analyzed and presented in tables; values expressed as mean ± SD and percentage(%) frequency distributions.

Table 1 presents the mean serum levels of AST, ALT and ALP in alcoholics and non-alcoholics and shows that the values of all these enzymes are significantly higher ($p < 0.05$) in alcoholic than non-alcoholics. The frequency distribution of enzyme levels shows that a greater percentage of subjects who are alcoholics have high (above normal) levels of all liver enzymes assayed while greater percentage of non-alcoholics have normal level of liver enzymes(Table 2). The frequency distribution of enzyme levels across age groups shows that age group (41- 50 years) has the greatest percentage of above normal levels of all liver enzymes ALT, AST and ALP (Table 3). The frequency distribution of serum levels of enzymes in males and females shows that a greater percentage of men have higher than normal levels of the enzymes ALT, AST and ALP than women (Table 4).

Discussion

Serum enzymes are the most commonly used and sensitive biochemical markers for the assessment of hepatocellular injury and its resultant liver disease.

Table 1. Serum levels of AST, ALT and ALP in alcoholics and non-alcoholics.

Enzymes activity	Alcoholics		Non-alcoholics	
	n	Mean ± SD	n	Mean ± SD
AST(IU/L)	51	27.93 ± 17.74*	69	22.96 ± 11.03
ALT(IU/L)	51	32.89 ± 27.54*	69	22.98 ± 14.41
ALP(IU/L)	51	18.18 ± 9.46*	69	14.55 ± 5.21

n - represents the number of subjects in each group. The values with asterisk (*) are significantly different from the control at p value less than 0.05. Samples were analyzed in duplicates.

Table 2. Frequency distribution of serum levels of enzymes among alcoholics and non-alcoholics.

Study group	Enzyme activity (IU/L)		
	ALT n (%)	AST n (%)	ALP n (%)
Alcoholics			
Normal	43 (35.8)	38 (31.7)	48 (40.0)
High	8 (6.7)	13 (10.8)	3 (2.5)
Total	51 (42.5)	51 (42.5)	51 (42.5)
Non-alcoholics			
Normal	64 (53.3)	62 (51.7)	69 (57.5)
High	5 (4.2)	7 (5.8)	0 (0)
Total	69 (57.5)	69 (57.5)	69 (57.5)

n represents the number of subjects; % represents the percentage distribution.

The enzymes most commonly used are the aminotransferases (ALT and AST), alkaline phosphatase (ALP) and Glutamyltranspeptidase (GGT). Abnormal increase in aminotransferases especially ALT reflect liver cell damage (hepatotoxicity), whereas ALP is more specific for cholestasis-hepatobiliary damage (Nduka, 1997; Mayne, 1998). ALT and AST are found in the cytoplasm and mitochondria of liver cells in high concentrations but low in blood (Aliyu *et al.,* 2007), however increased activities of these enzymes in serum are due to increased membrane permeability and leakage into the blood circulation when hepatocytes are injured (Benjamin, 1978).

Alcohol is a toxin that is harmful to the liver and alcoholic liver disease-particularly cirrhosis - is one of the leading causes of alcohol-related death.

Table 3. Frequency distribution of serum levels of the enzymes activities across age range.

Age range	Enzyme activity (IU/L) ALT n(%)		AST n(%)		ALP n(%)	
	Normal	High	Normal	High	Normal	High
20-30	19 (15.8)	2 (1.7)	20 (16.7)	1 (0.8)	21 (17.5)	0 (0)
31-40	50 (41.7)	4 (3.3)	47 (39.2)	7 (5.8)	53(44.2)	1.0(0.8)
41-50	28 (23.3)	7 (5.8)	25 (20.8)	10(8.3)	33(27.5)	2.0(1.7)
51-60	10 (8.3)	0 (0.0)	8 (6.7)	2 (1.7)	10 (8.3)	0 (0)
Total	107(89.2)	13(10.8)	100(83.3)	20(16.7)	117(97.5)	3(2.5)

n represents the number of subjects. % represents the percentage distribution.

Table 4. Frequency distribution of serum levels of the enzymes activities in males and females.

Sex	Enzyme activity (IU/L) ALT n (%)	AST n (%)	ALP n (%)
Normal levels			
Male	61 (50.8)	57 (47.5)	68 (56.7)
Female	46 (38.3)	43 (35.8)	49 (40.8)
Total	107 (89.2)	100 (83.3)	117 (97.5)
High levels			
Male	9 (7.5)	13 (10.8)	2 (1.7)
Female	4 (3.3)	7 (5.8)	1 (0.8)
Total	13 (10.8)	20 (16.7)	3 (2.5)

n represents the number of subjects. % represents the percentage distribution.

The significant elevation of all the serum enzymes in alcohol drinkers in our study may be attributed to excessive alcohol consumption. These are early indicators of liver disease, as the liver might be experiencing gradual damage unknown to the subjects. This is in agreement with O'shea *et al.,* (2010) who reported that excessive alcohol consumption is associated with fatty liver, and if persistent, it can lead to alcoholic steatohepatitis, liver fibrosis and cirrhosis. Alcohol may be one of a number of factors causing injury, and the specific role of alcohol alone may be difficult to assess in a patient

with multi-factorial liver disease. However, a number of laboratory abnormalities, including elevated serum aminotransferases, have been reported in patients with alcoholic liver injury, and used to diagnose alcoholic liver disease (Nalpas *et al.,* 1986). Alcohol has been shown to affect hepatocytes, by inducing reactive oxygen radical production, mitochondrial damage and steatosis. Chronic alcohol could also deplete intra-cellular glutathione stores, which predisposes to increased reactive oxygen species (ROS) production and increased susceptibility to hepatocyte death (Nunez *et al.,* 2001).

The percentage distributions of alcohol consumers (ALT- 6.7%, AST- 10.8%, ALP- 2.5%) with enzyme activities higher than normal values are greater than that of non-alcohol consumers (ALT- 4.2%, AST- 5.8%, ALP- 0), while the percentage distributions (ALT-35.8%, AST- 31.7%, ALP- 40%) of alcohol consumers with normal values of enzyme activities are lower than that of non-alcoholics (ALT- 53.3%, AST- 51.7%, ALP-57.5%). This may be due to the fact that alcohol increases the levels of enzymes in blood, an early indicator of liver injury; and might imply that alcoholics are at greater risk of developing liver damage than non-alcoholics. This is in agreement with earlier findings. One epidemiological study has estimated that for every 1-litre increase in per capita alcohol consumption (independent of type of beverage), there was a 14% increase in cirrhosis in men and 8% increase in cirrhosis in women (Corrao *et al.,* 1997). In 2003, about 44% of all deaths from liver disease among adult Americans were attributed to alcohol (Yoon and Yi, 2006).

The increasing nature of percentage distribution of high levels of serum enzymes across the age groups up to a maximum value in age group (41- 50 years) may be due to the fact that the effect of alcohol on the liver is age-dependent, as there is a prolonged effect of alcohol intake on the liver as the subject grows older while continually drinking alcohol. In addition, other factors that could play roles might be the efficiency of the liver depreciating due to the natural aging process and economic factor, as those subjects in the 41- 50 years age bracket might have more money to spend on purchasing alcohol than the lower age groups, while there might be reduction in alcohol intake in the age group 51 - 60 years, due to some factors that are yet unknown. Mann *et al.* (2003) reported that cirrhosis mortality rates due to alcohol consumption range considerably among age groups – they are very low among young people but increase substantially in middle age. Infact, cirrhosis is the fourth leading cause of death in American people of ages 45 - 54 years. The greater percentage distribution of men having high levels of serum enzymes than women might be due to the fact that more men consume alcohol than women in the community. Although it was earlier reported that women have higher risk of developing cirrhosis due to alcohol consumption than men (Hezode *et al.,* 2003) and several studies have shown differing blood alcohol levels in women versus men, after consumption of equal amounts of alcohol (Baraona *et al.,* 2001), but the quantity of alcohol consumed by men in our study area is greater than the women.

The results of this study therefore suggest that excessive consumption of alcohol predisposes humans to the risk of developing liver disease and men above 40 years of age are at greatest risk of developing alcoholic liver injuries. Thus, excessive consumption of alcohol should be discouraged among men and routine diagnostic laboratory tests should be encouraged.

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