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RESEARCH ARTICLE

CHRONIC LIVER DISEASE: DISTRIBUTION AND BIOMARKERS IMPACTS IN EDO STATE

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ABSTRACT: Aim - This study aimed to determine the distribution of chronic liver disease, associated risk factors, and its impact on gamma-glutamyl transferase (GGT) activity and alpha-fetoprotein (AFP) levels in Edo State. Method: Sixty participants were enrolled, comprising 30 diagnosed with liver disease and 30 healthy controls. Patients were recruited from Edo Central Hospital, Benin City, while controls were from the general population. Demographic data were collected via questionnaires, and GGT activity and AFP levels were measured using standard methods. Result - Results revealed significantly higher GGT (81.43 ± 23.94 ng/ml) and AFP (105.97 ± 191.44 ng/ml) levels in patients compared to controls (22.76 ± 10.01 ng/ml and 6.40 ± 23.43 ng/ml, respectively). Most patients were aged 40–59 years (50%), predominantly female, married, and employed. A minority (6.7%) reported a family history of liver disease. Conclusion- Elevated GGT and AFP levels in patients indicate possible cellular destruction due to inflammation, which is a significant indication of liver ailment. Public education on healthy lifestyles and routine medical checks is recommended to mitigate liver disease prevalence and impact.

Keywords: alpha fetoprotein, gamma glutamyl transferase, biomarkers, chronic hepatitis, Edo State

INTRODUCTION:

The liver is a critical organ in the human body responsible for an array of functions that help support metabolism, immunity, digestion, detoxification, and vitamin storage, among other functions. It comprises around 2% of an adult's body weight. The liver is unique due to its dual blood supply from the portal vein (approximately 75%) and the hepatic artery (approximately 25%). The primary function of the liver is to control the flow and safety of substances absorbed from the digestive system before distribution of these substances to the systemic circulatory system [1].

Liver disease is the major cause of death every year. Approximately about 29 million people suffer from a chronic liver condition [2]. Chronic liver disease is one of the leading causes of public health burden in the Western world, and globally the burden has increased by 10.3% in the recent years [3][4]. Alcoholic liver disease (ALD), hepatitis C virus (HCV), and NAFLD are thought be the leading causes of chronic liver disease worldwide [3]. Hepatitis involves liver inflammation caused by viruses, toxic substances, autoimmune diseases, or inherited conditions.

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Liver cirrhosis, characterized by fibrous tissue formation, results from hepatocyte destruction caused by viral hepatitis, alcohol, or toxins [5]. Hemochromatosis, an inherited disorder, leads to iron accumulation and long-term liver damage. Benign tumors like adenoma, angioma, and focal nodular hyperplasia are also linked to liver conditions [5].

Gamma-glutamyl transferase (GGT) is produced by Kupffer cells, macrophages, and bile duct endothelial cells in healthy individuals. Serum GGT activity increases significantly in hepatocellular carcinoma (HCC), and combining GGT with AFP testing improves sensitivity but reduces specificity [6]. Elevated GGT activity is observed across all liver diseases, with peak levels in biliary obstruction.

Alpha-fetoprotein (AFP) is a protein primarily produced by the liver in fetuses, with levels declining post-birth [7]. Elevated AFP levels indicate liver damage, chronic diseases like hepatitis and cirrhosis, or certain cancers. AFP serves as a tumor marker, particularly for HCC and hepatoblastoma [9]. AFP binds to metabolites like bilirubin and fatty acids. For HCC detection, AFP thresholds exceed 400 U/L, with specificity ranging from 20–60%. AFP-L3, a biomarker exclusive to HCC, is produced by HCC cells and aids in early detection [10]. Estimation of duo of GGT and AFP levels among the patients with chronic liver disease will undoubtedly confirm their relevance in the diagnosis of chronic liver disease in Edo state, which is in most cases rarely included as part of batteries of routine tests.

Although serum biomarkers for chronic liver disease have been studied in Edo State and Nigeria, no research has focused on disease distribution and its impact on GGT and AFP levels. This study bridges that gap, emphasizing the importance of these biomarkers in understanding chronic liver diseases and their diagnostic relevance.

MATERIALS AND METHODS

Study Design and Participants

This study was conducted at the Edo Central Hospital, in Benin City, Edo State, Nigeria. It was a case control study. A total of sixty participants enrolled for this study. Thirty of which were diagnosed with different types of liver disease, including; alcohol liver disease (6), chronic hepatitis B (16), chronic hepatitis C (6), and liver cirrhosis (2), while thirty (30) subjects were healthy controls who have no history of any form of liver disease. The test participants were recruited among the patients attending the Edo Central Hospital, Benin City, while the controls were recruited within Benin City. Participants were selected using a convenient sampling method and meeting specific inclusion criteria. The inclusion criteria were adult male and female above 18years who are diagnosed with all forms of chronic liver disease, while the study excluded anyone who had received hepatitis vaccine or suffering from any underlying ailment. Informed consent was obtained from all the participants' prior enrollment for this study. Pains and gains of the study, and the confidentiality of the data obtained and final results were well explained to the participants both in English and local dialects. Ethical approval was obtained from the Research and Ethics Committee of the Ministry of Health, Benin City, Edo state.

Questionnaire / Ethical Consideration

The questionnaire comprised inquires carefully designed to retrieve information, they include; age, gender, state of origin, occupation, marital status, family history of liver disease, underlying disease condition, type of liver disease, degree/extent of smoking and alcohol consumption, treatment regimen, current medication and level of education. Ethical approval with reference number HA.737/5/T[1]/008 was obtained from the Health Research Ethics Committee, Ministry of Health, Benin City, Edo State to carry out this study.

Sample Collection / Preparation

Five (5) milliliters of venous blood was collected from the participants using sterile syringe and needle, and dispensed into plain containers under aseptic condition. The sample was allowed to clot. The clot was then dislodged and centrifuged at 5000rpm for 5 minutes to separate the serum from the clot. The serum was then dispensed into another clean dry plain container and used to determine the gamma glutamyl transferase activity and alpha fetoprotein level of the subjects. All samples were stored at -20^{0}C prior analysis.

Laboratory Investigations

Determination of Gamma Glutamyl Transferase Level

Gamma glutamyl transferase activity of the samples was assessed using spectrophotometric method. The reagent was sought and got commercially from Fortress Diagnostics (product code BXC0361) and used according to the manufacturer's instructions [11].

Determination of Alpha Fetoprotein Level

Alpha fetoprotein was level was determined from the participants' sample with a commercially purchased Enzyme Linked Immunosorbent Assay (ELISA) kit from Calibiotech (Catalog number: AF237T) [12].

Data Analysis

Data were analyzed for normality using Kolmogorov Smirnov Z. Descriptive data were expressed as mean and standard deviation for continuous variables and as percentages for categorical variables. Comparative analysis between two groups was done using independent sample t-test, while one-way analysis of variance involving the LSD post hoc multiple comparison test was used for multiple (>2) groups. Statistical significance was set at $P \leq 0.05$. All statistics were performed using SPSS for windows (version 20.0).

RESULTS:

Table 1 shows the socio-demographic characteristics of the study population. The study compared socio-demographic characteristics, lifestyle factors, and clinical profiles of 30 patients living with liver diseases and 30 healthy controls. The mean age of patients (46.90 ± 15.0 years) was significantly higher ($p < 0.001$) than the control group (29.43 ± 9.47 years). Most control subjects were aged 20-39 years (80%), male (66.7%), single (80%), unemployed (56.7%), and predominantly from Edo state (63.3%). In contrast, patients were primarily aged 40-59 years (50%), female (60%), married (76.7%), employed (80%), and from other Nigerian states (40%).

Table 1. Socio-Demographic Characteristics of the Study Population

Characteristics	Control Group (n = 30) Mean ± SD or n (%)	Patients Living with Liver Disease (n = 30) Mean ± SD or n (%)
Age (years), Mean ± SD	29.43 ± 9.47	46.90 ± 15.0*
	24 (80.0)	10 (33.3)
20-39 years	6 (20.0)	15 (50.0)
40-59 years	0 (0)	5 (16.7)
≥60 years		
Sex		
Males	20 (66.7)	12 (40.0)
Females	10 (33.3)	18 (60.0)
Marital Status		
Married	6 (20.0)	23 (76.7)
Single	24 (80.0)	7 (23.3)
Occupation		
Employed	13 (43.3)	24 (80.0)
Unemployed	17 (56.7)	3 (10.0)
Retired	0 (0)	3 (10.0)
State of Origin		
Edo State	19 (63.3)	11 (36.7)
Delta State	7 (23.3)	7 (23.3)
Other States	4 (13.4)	12 (40.0)

Table 2 shows Some selected life-styles of the study population. Lifestyle data showed that 90% of the control group and 70% of the patients did not smoke. Among smokers, most patients consumed two sticks per day (55.6%). Alcohol consumption was higher among patients (83.3%) than controls (63.3%). Among alcohol consumers, 68% of controls drank one bottle daily. Physical activity was minimal, with 83.3% of patients and 33% of controls reporting no exercise.

Table 2. Selected Life-Style Profile of the Study Population

Life-style	Control Group (n = 30) N (%)	Patients Living with Liver Disease (n = 30) N (%)
Smoking Habit		
No	27 (90.0)	21 (70.0)
Yes	3 (10.0)	9 (30.0)
Extent of Smoking (n, 3 & 9)	1 (33.3)	4 (44.4)
One stick/day	1 (33.3)	5 (55.6)
2 sticks/day	1 (33.3)	0 (0)
Ten sticks/day		
Alcohol Consumption		
No	11 (36.7)	5 (16.7)
Yes	19 (63.3)	25 (83.3)
Extent of Alcohol Consumption (n, 19 & 25)	10 (52.6)	17 (68.0)
1 bottle of alcoholic	2 (10.5)	7 (28.0)
beverage	4 (21.0)	0 (0)
2 bottles	1 (5.3)	1 (4.0)
3 bottles	1 (5.3)	0 (0)
4 bottles	1 (5.3)	0 (0)
5 bottles		
7 bottles		
Exercise Status		
None	10 (33.3)	25 (83.3)
Once a week	3 (10.0)	3 (10.0)
2 times a week	2 (6.7)	1 (3.3)
3 times a week	4 (13.3)	1 (3.3)
5 times a week	1 (3.3)	0 (0)
Daily	10 (33.3)	0 (0)

Table 3 shows some of the clinical characteristics of the patients living with liver diseases. Clinical characteristics indicated that hepatitis B was the most common liver disease (53.3%), followed by hepatitis C (20%), hepatitis A (20%), and cirrhosis (6.7%). Half of the patients were on medication, with the most common drugs being Tenofovir (40%) and Lamivudine (20%). Among those on medication, 60% had been treated for less than one month, and 40% for a month or longer.

Table 3. Clinical Characteristics of the Patients Living with Liver Diseases (n = 30)

Characteristics	Frequency	Percentage
Type of Liver Disease		
Hepatitis A	6	20.0
Hepatitis B	16	53.3
Hepatitis C	6	20.0
Liver Cirrhosis	2	6.7
Family History of Liver Disease	28	93.3
No	2	6.7
Yes		
Ongoing Medications		
No	15	50.0
Yes	15	50.0
Type of Medication (n = 15)	2	13.3
DDA Tablets	2	13.3
Entecavir	3	20.0
Lamivudine	6	40.0
Tenofovir	2	13.3
Ursodiol		
Duration of Medication (n = 15)	9	60.0
< *One Month*	6	40.0
≥*One Month*		

Table 4 shows the mean serum activity of GGT and concentration of AFP compared between patients living with liver diseases and their healthy control. Significant biochemical differences were observed between groups. Patients had significantly higher mean serum gamma-glutamyl transferase (GGT) activity (81.43 ± 23.94 vs. 22.76 ± 10.01, $p < 0.001$) and alpha-fetoprotein (AFP) levels (105.97 ± 191.44 vs. 6.40 ± 23.43, $p < 0.01$) compared to controls. However, analysis of variance (ANOVA) showed no significant differences in GGT ($p = 0.293$) and AFP ($p = 0.670$) levels among the different liver disease types.

Table 4. The Mean Serum Activity of GGT and Concentration of AFP Compared between Patients Living with Liver Diseases and their Healthy Control

Variables	Control (n = 30) Mean ± SD (*Range*)	Patients with Liver Diseases (n = 30) Mean ± SD (*Range*)	t-statistics	P – Value
GGT	22.76 ± 10.01 (*10 - 47*)	81.43 ± 23.94 (*0.54-130.40*)	-12.38	<0.001
AFP	6.40 ± 23.43 (*36 - 134*)	105.97 ± 191.44 (*1.07-666.80*)	-2.82	0.006

Data is expressed as mean ± standard deviation and range

The mean serum activity of GGT and concentration of AFP compared among different types of liver diseases are shown in Table 5. Further analysis revealed no significant variations in GGT and AFP levels based on patient characteristics, including age, sex, smoking, alcohol consumption, family history of liver diseases, exercise habits, or medication status. Additionally, no differences were observed between patients on medication for less than one month and those on medication for one month or longer.

Table 5. The Mean Serum Activity of GGT and Concentration of AFP Compared among Different Types of Liver Diseases

Type of Liver Disease	Number of Patients	GGT Level	AFP Level
Hepatitis A	6	69.16 ± 6.99	63.91 ± 127.98
Hepatitis B	16	87.06 ± 27.45	122.11 ± 226.58
Hepatitis C	6	73.83 ± 24.02	61.56 ± 91.59
Liver Cirrhosis	2	96.0 ± 2.82	236.24 ± 329.30
Statistics (ANOVA)		*P = 0.293*	*P = 0.670*

Table 6 shows the mean activity of GGT and concentration of AFP of patients living with liver diseases compared according to some of their characteristics. Data indicated no significant differences in mean activity of GGT and concentration of AFP of subjects when compared among age groups; between sexes; between smokers and non-smokers; between those who drink alcohol and those who don't; between those who have family history of liver diseases and those who don't have; between those who exercise and those who don't; between those who are undergoing medications and those who are not; and among those who are not on medication and those who have been on medications for a period of <1 month and ≥1 month

Table 6. The Mean Activity of GGT and Concentration of AFP of Patients Living with Liver Diseases Compared according to Some of their Characteristics

Variables	N	GGT Level (Mean ± SD; *P – Value)*	AFP Level (Mean ± SD; *P – Value)*
Age			

20-39 years	10	76.90 ±	106.41 ±
40-59 years	15	27.45	182.91
≥60 years	5	83.40 ±	96.15 ±
		25.31	205.39
		84.60 ±	134.54 ±
		11.61	203.49
		P = 0.773	*P = 0.932*
Sex			
Males	12	88.66 ±	101.90 ±
Females	18	23.38	193.56
		76.61 ±	108.68 ±
		23.71	195.60
		P = 0.181	*P = 0.926*
Smoking Habit			
No	21	81.09 ±	78.47 ±
Yes	9	22.49	152.10
		82.22 ±	170.13 ±
		28.48	261.55
		P = 0.908	*P = 0.236*
Alcohol			
Consumption	5	73.40 ± 7.73	49.57 ±
No	25	83.04 ±	79.45
Yes		25.81	117.25 ±
		P = 0.421	206.01
			P = 0.480
Family History			
of Liver Disease	28	81.78 ±	101.56 ±
No	2	24.76	193.02
Yes		76.50 ± 3.53	167.65 ±
		P = 0.769	220.96
			P = 0.645
Exercise Status			
No	25	81.44 ±	119.70 ±
Yes	5	24.77	205.79
		81.40 ±	36.92 ±
		21.70	66.79
		P = 0.997	*P = 0.386*
Ongoing			
Medication	15	75.20 ±	87.60 ±
No	15	22.82	155.10
Yes		87.66 ±	124.33 ±
		24.14	226.14
		P = 0.157	*P = 0.608*
Duration of			
Medication	15	75.20 ±	87.60 ±
None	9	22.82	155.10
<1 month	6	84.11 ±	129.12 ±
≥1 Month		24.87	253.66
		93.0 ± 24.20	117.15 ±
		P = 0.292	200.35
			P = 0.873

DISCUSSION:

Globally, chronic liver disease is one of the leading causes of death and is associated with a sedentary lifestyle, high alcohol consumption, and obesity. Chronic liver disease is defined as a progressive deterioration of liver functions, including the production of clotting factors, removal of toxic metabolic products, and bile excretion for at least 6 months [13]. The most common causes of chronic liver disease are viral infections, alcohol consumption, toxins, and nonalcoholic fatty liver disease (NAFLD). Of these, NAFLD has become the most common liver disorder worldwide, reaching a prevalence of 60% and 24% in patients with chronic liver disease and the general population, respectively [14]. This study aimed to determine the distribution of chronic liver disease, associated risk factors, and its impact on gamma-glutamyl transferase (GGT) activity and alpha-fetoprotein (AFP) levels

These findings highlight the demographic and clinical distinctions between patients and controls, emphasizing the impact of hepatitis B, lifestyle factors such as alcohol consumption, and the significant elevation of GGT and AFP in liver disease patients. However, the lack of significant differences in GGT and AFP levels among liver disease types and patient subgroups suggests uniformity in biomarker expression across diverse conditions.

In this study, the mean age of the patients living with liver diseases is 46.90 ± 15.0 years. Previous workers found the same pattern of presentation of chronic liver disease according to age. For instance, the retrospective analysis of a diverse cohort of 2,017 patients with a clinical diagnosis of cirrhosis, Sajja et al. [15] found the average presentation age of liver cirrhosis, as a result of alcoholic liver disease to be 51

± 10 years, which was significantly greater than that of HCV ($p < 0.001$) and less than that of cryptogenic/NAFLD/NASH ($p < 0.001$), and nearly 90% of these patients (n=823/921) was over 40. Similarly, the average age at presentation of cirrhosis due to HCV was 50 ± 9, and again the majority of these patients (n=646/719) was over 40. The average age for presentation of cirrhosis due to cryptogenic/NAFLD/NASH causes was 60 ± 12, significantly greater than that for HCV ($p < 0.001$). The incidence among this middle age group may be attributed to many factors including lifestyles, occupation and sedentary lifestyle especially among women in this study.

In this study, a greater proportion of the afflicted patients were females (60%). This finding disagrees with some workers ; Non-alcoholic Fatty Liver Disease Study Group [18 - 19]. They all reported that women folks are less likely to suffer most chronic liver disease, with approximately 55–70% of cases occurring in men.

Approximately 71 million people worldwide (1.0%) have chronic HCV, with a prevalence of 1.0% in the United States, 1.5% to 1.8% in Europe, 1.0% in Africa, and 0.5% to 0.7% in Asia [20 - 23]. The distribution pattern of liver disease in this study indicated as hepatitis A 6 (20%), hepatitis B 16 (53.3%), hepatitis C 6 (20%) and liver cirrhosis 2 (6.7%). Comparing these results with other workers, the results agrees with other workers [24], they found the distribution of hepatitis B and hepatitis C to be in the range of 2 to 20% and 0.5% to 15% respectively. When reviewing the prevalence of chronic liver disease in Nigeria in comparism with Europe, in the study of Nwokediuko *et al.* 2013 [25], they worked on pattern of liver disease admissions in a Nigerian tertiary hospital, they found the prevalence of liver cirrhosis to be 20.4% and hepatitis B virus infection (49.4%) respectively. These findings disagreed with our work. The high increase of hepatitis B infection may be due to non-participation in hepatitis B vaccination in some part of the country due to cultural and religion beliefs and the fewer sample size.

Majority 25(83.3%), of the people living with liver disease in this study, disclosed in their questionnaire that they indulged in alcohol consumption. The extent of drinking per day ranged from one bottle to four bottles (1 bottle 17 (68%), 2 bottles 7 (28%), 3 bottles 0(0%), 4 bottles (4%)) respectively. According to Roerecke *et al.* [26] there was no increased risk of liver disease for occasional drinkers. Also, drinking of one bottle per day when compared to long-term abstainers increased risk for liver cirrhosis in women, but not in men. They also found out that the risk in women drinker is higher and consistent when related to men folks. However, drinking ≥5 drinks per day according to their findings was associated with a substantially increased risk in both women (RR = 12.44, 95% CI: 6.65 – 23.27 for 5–6 drinks, and RR = 24.58, 95% CI: 14.77 – 40.90 for ≥7 drinks) and men (RR = 3.80, 95% CI: 0.85 – 17.02, and RR = 6.93, 95% CI: 1.07 – 44.99, respectively). In this study, none of the participants drink more than 4 bottles per day which is insignificant.

According to Rutledge and Asgharpour [27], there is a growing body of evidence that demonstrates that cigarette smoking is associated with increased progression and severity of liver disease, particularly fibrosis and liver cancer. Among the patients that smoke (n = 9), in this study, a greater proportion (55.6%) take 2 sticks/day, none was diagnosed for fibrosis or liver cancer, although, inference cannot be conclusively drawn because of the duration of this study. Nwokediuko et al. 2013[25] found ingestion of herbs and roots (45.5%) and cigarette smoking (30.1%) to be among the factors that responsible for the development of chronic liver disease.

Other evidence-based study from the North central of Nigeria attributed prevalence and distribution of chronic liver disease especially hepatitis B and C to some cultural practices such as drinking from same cup, tribal marks or the practice of circumcision [25], [28]; [29 - 30]. In a cross-sectional study carried out by Joo et

al. [31] in South Korea, they found association between sitting time and non-alcoholic fatty liver disease. This fact was obvious in this study, as majority (83.3%) of the patients do not exercise at all.

In this study, there is significantly higher ($p < 0.001$) mean GGT activity in patients living with liver diseases (81.43 ± 23.94) compared with the control group (22.76 ± 10.01). Many workers have reported higher activity of gamma-glutamyl transferase in chronic liver disease. Silva et al. recorded higher activity in patients with chronic hepatitis C virus infection [32]. Zhang et al. [33] and Teschke et al. [34] reported association between alcoholic liver disease and increased gamma-glutamyltransferase activities in serum and liver.

Similarly, the mean AFP level was significantly higher ($p < 0.01$) among the patients living with liver diseases (105.97 ± 191.44) compared with the control (6.40 ± 23.43). Wang et al. [35] found increase in alpha fetoproprotein in nonalcoholic steatohepatitis. Dabeva et al. [36] also found elevated AFP during hepatocyte injury, repair, and regeneration. Tai et al. in their study, reported high level of alpha fetoprotein in chronic hepatitis C [37].

Limitations

This study suffered limitations in the area of sample size. Many participants enrolled for this study at the beginning, however, majority of them opted out before the completion citing reasons such as personal, religion and cultural. Also, majority refused to volunteered personal information based on the same reasons.

CONCLUSION:

In this study, chronic hepatitis infection recorded the highest distribution among the patients living with liver disease. The level of alpha fetoprotein and activity of gamma glutamyltransferase were significantly increased among the chronic liver patients. Efforts should be made by the concerned government agencies such as the publicity arm of Ministry of Health (MOH) in the state, and its counterpart department at the local government, National Agency for Food Drug Administration Commission (NAFDAC), as well as tradition institutions, to advocate for regular medical check. They should also educate the masses the benefits of regular exercise, and to embrace vaccination, especially in the areas where vaccine awareness is low. Also to be advocated for, is moderate healthy lifestyles and improved hygienic practices during circumcision and incision as they serve as avenue where infections are transferred. Early report of ailment is encouraged for early detection, management and treatment.

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Authors Contributions

BIGA, MOI and were responsible for the conceptualization of the study. The methodology was developed by FOO and GEO. Samples and Data analysis was performed by FOO. The literature review was prepared by BIGA, MOI, GUO, FOO, GEO and AOO. All authors contributed to the review and editing of the manuscript. BIGA provided supervision throughout the study. All authors contributed to funding of this research. All authors read and approved the final version of the manuscript.

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