

SJMLS - 9(2) - 025**Comparative Analysis of Ferritin Levels and Liver Enzyme Activity in Liver Disease Patients within Benin City**

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Abstract

Liver disease encompasses a spectrum of hepatic pathologies, ranging from minor disturbances in liver function to severe conditions such as cirrhosis and hepatocellular carcinoma. The aim of this study was to assess the serum concentrations of ferritin and liver enzymes (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) in patients diagnosed with liver disease in Benin City, Edo State. A cohort of eighty-eight (88) individuals, consisting of forty-four (44) subjects diagnosed with various forms of liver disease and forty-four (44) healthy controls devoid of liver pathology history, were recruited. Blood samples were collected via standard venipuncture technique, and subsequent analysis included measurement of ferritin levels using Enzyme Linked Immunosorbent Assay (ELISA) and determination of AST and ALT activities via colorimetric method. Predominantly, male participants accounted for 52.3% of the cohort, with females comprising 47.7%. Elevated levels of AST and ALT were evident in liver disease patients compared to controls, whereas ferritin levels displayed no significant disparity between the control group (112.24±11.92) and liver disease patients (100.03±19.00) (p>0.05). The findings underscore heightened levels of ALT and AST in individuals afflicted with liver disease compared to healthy controls, thus emphasizing the potential utility of these biomarkers in liver disease diagnosis and management.

Keywords: Liver disease, Ferritin, Liver biomarkers

Introduction

The liver is the second largest organ in the human body and performs over 5,000 different physiological tasks, such as eliminating toxins from the blood, breaking down food into nutrients, regulating hormone levels, fending off infections and disease, healing from wounds, and metabolising iron, cholesterol, and glucose to maintain proper levels (Gao *et al.*, 2008). Liver disorders are on the rise because most individuals don't think about their livers until something goes wrong (Anvith and Pragna, 2015). According to Scalone *et al.* (2015), liver illnesses are a major global health concern that placed a heavy strain on both individuals and healthcare systems. They can be inherited or brought on by a number of liver-damaging conditions, such as alcoholism, viral hepatitis, non-alcoholic fatty liver disease (NAFLD), and other hereditary and environmental factors (Asrani *et al.*, 2019).

Hepatitis, or inflammation of the liver, is caused by viruses (hepatitis A, B, C, D, and E), toxins, alcohol misuse, or autoimmune diseases (Sivakrishnan and Pharm, 2019). Chronic drinking and hepatitis are two of the many liver disorders and conditions that can lead to liver cirrhosis, an advanced stage of liver scarring. It's possible that symptoms won't show up until after serious harm has been done, which could result in consequences such as portal hypertension, ascites, and hepatic encephalopathy (Gines *et al.*, 2021). Fat buildup in the liver causes Non-Alcoholic Fatty Liver Disease (NAFLD), which can range from simple fatty liver to non-alcoholic steatohepatitis (NASH), which causes inflammation and cell destruction. It is linked to

obesity, insulin resistance, and metabolic syndrome and is frequently asymptomatic (Liu *et al.*, 2010). A genetic condition known as haemochromatosis results in the body accumulating iron, which damages the liver over time (Pietrangelo, 2007). Numerous clinical studies that evaluate the presence, absence, increase, or reduction of usual enzymes, metabolites, or chemicals associated with regular activities can be used to verify proper liver function. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and ferritin are a few of these enzymes or metabolites that can be utilized to evaluate liver function (Lee *et al.*, 2012).

The intracellular protein ferritin is in charge of storing iron and is essential for maintaining iron homeostasis. Dysregulation of iron metabolism can result in iron overload in the liver, despite ferritin's critical role in iron storage (Zhang *et al.*, 2021). Chronic liver disorders and hereditary haemochromatosis are two conditions that can throw off the equilibrium and lead to excessive iron buildup in hepatocytes. This iron overload may be indicated by elevated ferritin levels (Pietrangelo, 2007). Inflammation is a common feature of liver illnesses, and inflammation sets off the release of pro-inflammatory cytokines. Hepatocytes can collect ferritin as part of the acute phase response in response to inflammatory signals. Thus, in a variety of liver diseases, elevated ferritin levels in the liver may act as a biomarker of ongoing inflammation (Del Campo *et al.*, 2018). The bloodstream may be exposed to intracellular substances, such as ferritin, as a result of hepatocellular damage. Accordingly, serum ferritin levels can act as a stand-in marker for hepatocellular damage, giving medical professionals important insights into the degree and course of liver illnesses (Tirnitz-Parker *et al.*, 2013). According to some recent studies, serum ferritin is linked to both hepatic inflammation and hepatic iron accumulation, it may be used as a marker to monitor the development of hepatic fibrosis in non-alcoholic fatty liver disease (NAFLD) patients. Researchers found that patients with non-alcoholic fatty liver disease (NAFLD) have increased blood ferritin levels, which may be related to hepatocyte damage and insulin resistance (Yoneda *et al.*, 2010; Manousou *et al.*, 2011). Monitoring ferritin levels in serum and liver tissues can help in the early detection and management of liver illnesses because ferritin is not only a passive marker but also a possible diagnostic tool due to its response to inflammatory and iron-related changes (Nielsen *et al.*, 2000). Two enzymes important in liver function are

aspartate aminotransferase (AST) and alanine aminotransferase (ALT). They are frequently quantified in order to track liver function. Both AST and ALT are released into the bloodstream when the liver cells are damaged or inflamed (Lee *et al.*, 2012). ALT is more specific to the liver, whereas AST can be seen in many other tissues, such as the liver, heart, muscles, and kidneys (Huang *et al.*, 2006). Accordingly, liver diseases such cirrhosis, non-alcoholic fatty liver disease (NAFLD), and hepatitis are frequently linked to elevated ALT levels (Wong *et al.*, 2009). When determining the prognosis of liver diseases, serial monitoring of AST and ALT levels is essential. A level drop can indicate improved liver health and positive responses to treatment, but a persistent rise might indicate continued liver damage. To the best of our knowledge, there is a noticeable gap in locally conducted research specifically investigating the simultaneous assessment of ferritin and liver enzyme levels in patients with liver diseases in Benin City. Therefore, the aim of this study was to determine the levels of ferritin and some liver enzymes in patients with liver disease in Benin City, Edo State.

Materials and Methods

Study Area

This hospital-based case-control research was conducted at the University of Benin Teaching Hospital in Benin City, Edo state, Nigeria between January – April 2024. University of Benin Teaching Hospital is located at Egor Local Government Area in Benin City. It is one of the major federal tertiary medical institutions in South – South, Nigeria. Patients from different part of the state attend various clinics at University of Benin Teaching Hospital.

Study Population

Forty-four (44) adults with confirmed diagnosis of liver disease in Benin City were enrolled for this study. Only subjects who gave informed consent and met the inclusion criteria were enrolled in this study. Equal number of healthy participants was also recruited as control for this study.

Ethical Approval/ Questionnaire

Approval for this research was sought and got from the ethics committee of the University of Benin Teaching Hospital, Benin city, Edo State, Nigeria with registration number NHREC – UBTH – HREC/21/12/2022B. Informed consent

was also obtained from all the participants. A well-structured questionnaire was administered to collect data and other socio-demographic information of each participant.

Sample Collection and Analysis

Under aseptic conditions, about 5 millimeters of venous blood samples were obtained from the ante-cubital vein of each subject using a sterile needle and syringe. This was then dispensed into a clean dry plain container. The sample was left undisturbed for a few minutes to clot and centrifuged at 4000rpm for about 5 minutes to separate serum from the clot. The serum was then dispensed into another clean and dry plain container and stored at -20°C prior analysis.

Estimation of Ferritin Concentration

Concentration of ferritin was determined using the Calbiotech Ferritin ELISA test kit (Catalog Number: FR248T). Each patient's sample was analyzed for ferritin according to manufacturer's instructions.

Estimation of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)

ALT and AST concentrations were determined using the enzyme activity assay. The reagents (Randox) were commercially purchased from Randox Limited United Kingdom.

Statistical Analysis

Data obtained from this research was presented and analyzed using statistical package for social sciences (SPSS) version 21.0 (IBM Inc. USA). Independent sample t- test was used to compare means and results were expressed in means \pm standard error of mean. Differences were considered to be statistically significant when the p value obtained is < 0.05 .

Results

Table 1 shows the sociodemographic characteristics of participants diagnosed with liver disease. This study revealed a predominantly male representation, comprising 52.3% of the participants, while females accounted for 47.7%. Age distribution among participants varied, with 34.1% falling within the 20-35 age bracket, 27.3% between 36-50 years, 29.5% aged 51-65 years, and 9.1% over 65 years old. In terms of marital status, the majority of participants were married,

constituting 70.5% of the subject, while the remaining 29.5% were unmarried.

Table 2 shows the lifestyle characteristics of participants diagnosed with liver disease. Among the participants, none reported smoking, indicating a prevalence of 0.00%. The majority of participants (86.4%) reported never consuming alcohol, while a smaller proportion (13.6%) reported occasional alcohol consumption. Regarding medication usage, the majority of participants (88.6%) reported being on medication, while a smaller percentage (11.4%) reported not being on any medication. In terms of physical activity levels, participants exhibited varied patterns. Approximately 20.5% engaged in intense physical activity, 29.5% in light physical activity, another 29.5% in moderate physical activity, and the remaining 20.5% were sedentary. All participants reported following a balanced diet, with none reporting an unbalanced diet.

Table 3 shows the distribution of liver disease among the participants. The most prevalent liver disease observed among participants was Hepatitis, accounting for the majority at 56.8% ($n = 25$). Following Hepatitis, Fatty Liver Disease was also notable, representing 15.9% of cases ($n = 7$). Hepatocellular Carcinoma and Hepatomegaly were each reported in 9.1% of cases ($n = 4$), while Alcoholic Liver Disease, Cirrhosis, Jaundice, and cases categorized as Unknown were each observed in 2.3% of cases ($n = 1$).

Table 4 shows the mean comparison of liver function parameters between the control group and participants diagnosed with liver disease. Among the liver function parameters examined, significant differences were observed in AST (Aspartate aminotransferase) and ALT (Alanine aminotransferase) levels between the control group and participants with liver disease. Participants with liver disease (27.93 ± 3.57) showed significantly elevated levels of AST compared to the control group (9.66 ± 0.63) ($t = -5.039$, $p = 0.001$). ALT levels were significantly higher in participants with liver disease (17.00 ± 2.08) compared to the control group (4.64 ± 0.21) ($t = -5.925$, $p = 0.001$). However, no significant difference was observed in ferritin levels between the control group (112.24 ± 11.92) and participants with liver disease (100.03 ± 19.00) ($t = 0.545$, $p = 0.587$).

Table 1: Sociodemographic Characteristics of Participants with Liver Disease.

Variable	Category	Frequency	Percentage (%)
Gender	Male	23	52.3
	Female	21	47.7
Age (Years)	20-35	15	34.1
	36-50	12	27.3
	51-65	13	29.5
	>65	04	9.1
	Marital Status	Married	31
	Unmarried	13	29.5

Table 2: Lifestyle Characteristics of Participants with Liver Disease.

Variable	Category	Frequency	Percentage (%)
Smoking	Yes	0	0.00
	No	44	100.0
Alcohol consumption	Never	38	86.4
	Occasionally	6	13.6
On Medication	Yes	39	88.6
	No	5	11.4
Physical Activity	Intense	9	20.5
	Light	13	29.5
	Moderate	13	29.5
	Sedentary	9	20.5
Diet	Balanced	44	100.0
	Unbalanced	0	0.0

Table 3. Distribution of Liver disease

Liver Disease	Frequency	Percentage
Alcoholic Liver Disease	1	2.3
Hepatocellular Carcinoma	4	9.1
Cirrhosis	1	2.3
Fatty Liver Disease	7	15.9
Hepatitis	25	56.8
Hepatomegaly	4	9.1
Jaundice	1	2.3
Unknown	1	2.3
Total	44	100

Table 4. Mean comparison of Liver Function Parameter of Control and Participants with Liver Disease

Parameters	Control (n=44)	Exposed (n=44)	T	P-value
Ferritin	112.24±11.92	100.03±19.00	0.545	0.587
AST	9.66±0.63	27.93±3.57	-5.039	0.001
ALT	4.64±0.21	17.00±2.08	-5.925	0.001

Values are shown in Mean±SEM, p<0.05 is considered significant, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase.

Discussion

Accurate evaluation of liver function is essential for the diagnosis and treatment of various illnesses. When it comes to assessing liver health, serum ferritin levels and liver enzymes like aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are often employed biochemical markers. These markers have attracted significant attention because they have the potential to represent the state of the liver (Woreta, 2014). Ferritin, a crucial protein involved in the process of iron metabolism, has been identified as a promising biomarker for liver disorders. High levels of ferritin in the blood have been linked to liver inflammation, fibrosis, and excessive accumulation of iron, all of which are frequently observed in liver diseases. In addition, it has been proposed that ferritin levels are associated with the severity and progression of diseases, which makes it a potentially useful tool for evaluating liver health (Milic *et al.*, 2016). In this study, the mean level of ferritin among the participants is normal. There is no significant value between the control group and the participants. This result disagrees with Chen *et al.* (2022) who reported associated ferritin in advanced liver fibrosis treatment - naive autoimmune hepatitis in 38 of 97 patients. Oikonomou *et al.* (2017) also reported that high ferritin level is associated with patients with decompensated liver cirrhosis.

AST and ALT are hepatocyte-specific enzymes, predominantly located in the liver's major functioning cells. Their entry into the bloodstream takes place when there is injury or damage to liver cells. Elevated serum levels of AST and ALT are reliable markers of hepatocellular injury and are commonly employed in the diagnosis and monitoring of liver disorders (Gowda *et al.*, 2009). It is worth mentioning that although both enzymes are increased in liver damage, AST is also present in other tissues such as the heart and skeletal muscles, whereas ALT is more exclusive to the liver (Ozer *et al.*, 2008). In this study, the level of AST is elevated among the patients with liver diseases. This observation is compatible with hepatic injury, as AST is principally located in hepatocytes and is released into the bloodstream following liver cell destruction. The observed increase in AST levels reflects the severity of liver pathology in this cohort and shows the usefulness of AST measures in detecting liver disorders. This finding agrees with prior research done by Sheth *et al.* (1998) who showed an increase in AST in patients with hepatic cirrhosis with an increase in AST/ALT ratio. Karim

et al. (2015) similarly reported an increase in AST in patients with chronic liver disease. These data reveal higher AST level in patients with liver illness, reinforcing the significance of AST as a valid biomarker for liver failure.

In this study, the gender breakdown indicated 52.3% male participants and 47.7% female participants. One possible cause for this distribution could be related to the frequency of liver illness among genders, as previous research has suggested variances in the susceptibility of males and females to liver diseases (Lonardo *et al.*, 2019). Results from this study agrees with the findings of Clark (2006), they recorded that males tend to have higher rates of liver disease due to factors such as higher alcohol intake and increased prevalence of hepatitis B and C infections. Of the 44 sufferers of liver disease in this study, none indicated to be a smoker, which collaborate medical advice that smoking cessation is helpful for liver health, since smoking can exacerbate liver damage and raise the risk of liver disease development (Harris *et al.*, 2016), while 38(86.4%) never took alcohol, 6(13.6%) agreed to take alcohol occasionally (Table 2). This finding is consistent with advice for people with liver illness to abstain from alcohol totally or limit intake significantly to prevent worsening of liver damage (EASL, 2012). This result may also have been seen to indicate a likely adherence to abstinence or low-risk alcohol intake guidelines, which is critical for individuals with liver disease to prevent further liver damage.

The bulk of participants come between the 20-35 age group (34.1%), followed by the 36-50 age range (27.3%), 51-65 age range (29.5%), and those beyond 65 years old (9.1%). This distribution could be due to the cumulative influence of risk factors for liver disease over time, such as alcohol intake, obesity, and exposure to hepatitis viruses (Pimpin *et al.*, 2018) (Table 1).

The most prevalent liver condition recorded among the participants is hepatitis, accounting for 56.8% of cases. Hepatitis comprises different types, including viral hepatitis (such as hepatitis B and C) and non-viral hepatitis, and it is a prominent cause of liver disease globally (Williams, 2006). The high frequency of hepatitis in this study reflects the large impact of viral infections on liver health in the studied population (Stanaway *et al.*, 2016). Fatty liver disease is the second most prevalent ailment reported, constituting 15.9% of cases. This disorder, defined by the accumulation of fat in the liver, is typically connected with

obesity, metabolic syndrome, and excessive alcohol intake (Almeda *et al.*, 2009). Hepatocellular carcinoma, a primary liver cancer, accounts for 9.1% of cases. This is problematic as hepatocellular carcinoma often originates in the context of underlying liver disease, such as cirrhosis or chronic viral hepatitis, showing extensive liver damage in a fraction of participants. Cirrhosis, hepatomegaly, and jaundice are observed less often, each contributing roughly 2-3% of cases. These disorders are commonly signs of severe liver disease and may suggest considerable liver damage and functioning. Alcoholic liver disease accounts for just 2.3% of cases in our study, which may represent cultural or behavioural differences in alcohol intake habits in the study population (Table 3). Globally, this result disagrees with Moon *et al.*, 2020, he and his coworkers attributed the cause of chronic liver disease to NAFLD (60%), HBV (29%), HCV (9%), and ALD (2%). However, the prevalence of cirrhosis is put at 833/100,000 (range 447-1100) in European countries, but data on cirrhosis prevalence in other areas, particularly resource-limited settings, are sparse (Byass *et al.*, 2014; Pimpin *et al.*, 2018).

Similarly, from the findings of this study, ALT is significantly elevated. ALT, like AST, is largely found in hepatocytes, and its release into the bloodstream suggests hepatic damage (Singh *et al.*, 2011). The higher ALT levels detected in our study reflect continued liver damage and also the severity of liver disease in this population. This data is consistent with recent research by Huang *et al.* (2006), indicating higher ALT levels in patients with liver pathology, further supporting the application of ALT as a marker for liver injury and functioning. Similarly, a study by Andy and Keffee (2003) has also connected an increase in ALT with liver illness specifically nonalcoholic fatty liver disease. According to them, it could be an accurate predictor of the disease prevalence. Nyblom *et al.* (2004), similarly observed an increase in AST and ALT in patients with severe alcoholic liver disease. Also, Giannini *et al.* (2002) recommended increase in AST/ALT ratio as a predictive usefulness and model for end stage liver disease in individuals with viral liver cirrhosis. No significant difference in ferritin levels was seen between patients with liver disease and the control group (Table 4.4). Ferritin, a protein involved in iron metabolism, has been proposed as a possible biomarker for liver illnesses due to its relationship with liver inflammation and fibrosis

(Milic *et al.*, 2016). However, the lack of significant variation in ferritin levels shows that ferritin may not be as sensitive or specific as AST and ALT in diagnosing liver pathology in this particular cohort. This conclusion contradicts other researchers that have revealed higher ferritin levels in patients with distinct form of liver disease. A Japanese study involving 86 individuals with histopathologically confirmed non-alcoholic steatohepatitis (NAFLD) also revealed higher ferritin levels in patients with NAFLD (Yoneda *et al.*, 2010). Ferritin levels were shown to largely vary in NAFLD patients based on the stage of fibrosis, rising in early to moderate fibrosis and falling in cirrhosis. In a more recent study, Kim *et al.* (2018) discovered a favourable correlation between postmenopausal women's blood ferritin levels and NAFLD. Additionally, compared to middle-aged women, Sabrina *et al.* (2017) revealed that the blood iron to ferritin ratio accurately predicted a decreased risk of severe hepatic steatosis in young adult women.

Overall, our research shows how important it is to monitor AST and ALT in Benin City for evaluating liver health and identifying liver disorders. These results highlight the necessity for ongoing research into biomarkers for precise diagnosis and treatment of liver illnesses and add to the body of knowledge already available on liver function measures.

Conclusion

The investigation delineated notable elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels among individuals diagnosed with liver disease, indicative of pronounced hepatocellular injury and underscoring the clinical significance of these enzymes as pivotal biomarkers for assessing liver dysfunction severity. Conversely, the study discerned no statistically significant variance in ferritin levels between liver disease participants and the control cohort, implying that ferritin may not exhibit equivalent sensitivity or specificity as AST and ALT in discerning hepatic pathology within this demographic.

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Authors Contributions

ECI and BIGA conceived and designed the study. The project's execution and manuscript revision were done by ECI, MOO, FOO, UCO and UIA. All the authors participated in writing and revision of manuscripts. The authors committed to taking responsibility for every part of the work, having reviewed and approved the completed document.

Conflicts of Interest

None Reported

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