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Research Article

Assessment of Alpha Fetoprotein and Cancer Antigen-125 in Chronic Liver Diseases

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Abstract

Background and objectives: Alpha-fetoprotein (AFP) is a popular biomarker used for hepatocellular carcinoma, but it has poor sensitivity and specificity for other chronic liver diseases. Many studies suggest Cancer antigen 125 (CA-125) could offer better prospect. This study aimed to assess alpha-fetoprotein and cancer antigen-125 in Chronic Liver disease patients in Jos, Nigeria. *Materials and Methods*: This is a laboratory-based analytical study. A total of 80 patients with confirmed cases of chronic liver disease were recruited from April to July 2024. Enzyme-linked Immunosorbent Assay was used to analyse the blood samples. *Results*: Our data showed that the median levels of AFP and CA 125 varied significantly across the stages of liver disease. In AFP, the median levels were 3.7 ng/mL for hepatitis, 2.9 ng/mL for fibrosis, 5.7 ng/mL for cirrhosis, and 18.8 ng/mL for HCC, with a statistically significant difference across all stages (Kruskal-Wallis $P = 0.049$). Similarly, CA 125 levels increased progressively, with medians of 6.9 U/mL for hepatitis, 9.4 U/mL for fibrosis, 68.5 U/mL for cirrhosis, and 72.4 U/mL for HCC, demonstrating a highly significant difference across CLD stages ($P < 0.005$). The lack of significant impact of AFP in this regression model implies it may not play a major role in determining the progression or severity of CLD. A significant relationship between CA-125 and chronic liver disease suggests that rising CA-125 mirrors disease progression. Moreover, the regression model points to a stronger and significant role for CA-125; this indicator is more critical for assessing the severity of chronic liver diseases. *Conclusion*: Cancer antigen 125 (CA-125) was a more sensitive biomarker than alpha-fetoprotein in detecting chronic liver diseases.

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Keywords

Alfa Fetoprotein, Cancer Antigen-125, Chronic Liver Disease, Alpha-fetoprotein

1. Introduction

Chronic liver disease (CLD) is a serious condition where the liver is damaged over a period of six months or more [1]. This damage disrupts the liver's ability to perform its vital functions, such as producing clotting factors and proteins, removing toxins from the bloodstream, and producing bile for digestion [2]. Chronic liver disease involves destruction of the liver cells and regeneration of abnormal hepatocytes, which could lead to various complications, including fibrosis (scarring), cirrhosis (severe scarring), and even liver cancer [3].

Several factors contribute to chronic liver disease. Some of the most common causes include: Viral infections [4], chronic consumption of Alcohol abuse [5], autoimmune disorders [6], and toxins including cigarette smoke [7]. Other contributing factors to chronic liver disease include haemochromatosis (iron overload) and obesity; imbalanced diets, diabetes, and depression can also play a role [8].

Early diagnosis of chronic liver disease using blood tests could be challenging because symptoms often appear only in the advanced stages [9]. Alpha-fetoprotein (AFP) is a commonly used biomarker for hepatocellular carcinoma (HCC), a severe complication of CLD, according to Heimbach [10]. However, AFP has limitations in sensitivity and specificity for diagnosing CLD severity across various etiologies [11]. Cancer antigen 125 (CA-125) has emerged as a promising biomarker in various liver pathologies; reports suggest CA-125 may offer advantages over AFP in certain liver disease contexts [11]. However, further research is needed to evaluate the potential of CA-125 as a biomarker for chronic liver diseases. Therefore, we aimed to assess alpha-fetoprotein and cancer antigen-125 in Chronic Liver disease patients at the Jos University Teaching Hospital, Nigeria.

2. Materials and Method

2.1. Study Design

This is a laboratory-based analytical study. A total of 80 patients with confirmed cases of chronic liver disease were recruited from April to July 2024.

2.2. Study Area

This study was carried out at the Chemical Pathology Unit, Jos University Teaching Hospital, North Central Nigeria. It is located at latitude $80^{0}24$ ‘North and longitude $80^{0}32$ ‘east, in the North Central geographical zone of Nigeria. The state has an area of 26,899 square kilometres, and an estimated population of 3.5 million people.

2.3. Study Population

For this study, the population comprised confirmed cases of chronic liver disease patients from both the Outpatient Department and Inpatient Department attending Jos University Teaching Hospital (JUTH) within the period of April to July 2024.

2.4. Inclusion and Exclusion Criteria

Inclusion Criteria: Only confirmed cases of chronic liver disease patients within the ages of 18-85years and who are not suffering from any cancer-related case were included in this study.

Exclusion Criteria: Patients who are outside the age bracket of 18-85years and are suffering from any cancer-related case and pregnancy conditions.

2.5. Ethical Consideration

Ethical clearance and approval for the study (JUTH/DSC/REC/127/XXX1/2691) were obtained from the Jos University Teaching Hospital’s research and ethics clearance committee to conduct the research using their patients and facility. And consent/assent from the subjects was obtained, in keeping with the guidelines of the Declaration of Helsinki (1964) and good clinical practice.

2.6. Informed Consent

An informed consent form was developed and shared with all the participants, and they were guided on how to complete the consent form before they were allowed to participate in the study.

Only those who willingly agreed to participate in the study were recruited.

2.7. Sample Size Determination

The sample size was determined based on statistical considerations to ensure the study’s results are representative of the population.

The Fisher formula was used to calculate the adequate sample size for the research.

Formula, $n = \frac{Z^2P(1-P)}{d^2}$

where;

n- The sample size

z- The statistical confidence limit corresponding to 95%, which is =1.96

p- Prevalence of CLD 4.3% [12].

d- Degree of accuracy (precision) =0.043

q- 1-p

Attrition rate=10% of n (sample size)

Final sample size=value of n+10% of the value of n

Sample Size Calculation

n= 1.96^2x0.043 (1-0.043)

0.05^2

n= 3.8416x0.043 (0.957)

0.0025

n= 3.8416x0.041151

0.0025

n= 0.1580856816

0.0025

n= 63.23427264

n≈ 63

Attrition rate = 10% of 63 =6.3≈6

Final sample size =63+6=69

Therefore, 80 case subjects were used for the study.

2.8. Sample Collection and Preparation

A 5mL of blood was been drawn from all eligible individuals at the antecubital vein using sterile disposable vacutainer needles with a suitable holder into clean and properly labelled 7ml plain Clinical Chemistry vacutainer tubes and allowed to clot and retract before centrifuging at 3500 revolution per minute (rpm) for five (5) minutes and the serum was carefully separated into a well labeled cryovials tubes and stored at -20°C until laboratory analysis is carried out.

A structured interviewer-administered questionnaire was used to collect patients' socio-demographic information, while retrospective information was extracted from the folders of patients who met the inclusion criteria. 5mL of blood was drawn from all eligible individuals from the antecubital vein using sterile disposable needles, with a suitable holder, into clean and properly labelled plain vacutainer tubes and allowed to clot and retract before centrifuging at 3500 revolutions per minute (rpm) for five (5) minutes, and the serum was carefully separated into a well-labelled cryovial and stored at -20^0C until laboratory analysis was carried out.

2.9. Sample Analysis

Serum samples were analysed for CA-125 and AFP using the ELISA technique. In this procedure, immobilisation takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CA-125 antibody, as described [8, 10]. Liver function tests of the patients were determined using Agappe Diagnostics kits, Kerala, India, while the CTP of each patient were calculated using the liver function parameters along with the patient's clinical data

2.10. Statistical Analysis

Data were analysed using SPSS version 23. To assess the normality of the AFP and CA-125 distributions, the Kolmogorov-Smirnov test was employed. The test results indicated that AFP had a test statistic of 0.400 (p-value < 0.005), while CA-125 yielded a test statistic of 0.295 (p-value < 0.005), demonstrating that the data generated for both variables did not conform to a normal distribution. As a result, a non-parametric test, specifically the Kruskal-Walli's test, was utilised to compare the biomarkers across different stages of chronic liver disease.

3. Results

The age distribution of the participants ranged from 20 to 85 years, with the majority falling within the 40-49 and 50-59 age groups, each comprising 25% of the sample (20 participants each). This was followed by those aged 30-39, accounting for 22.5% (18 participants). The age group of 60-69 made up 13.8% (11 participants), while the youngest group, aged 20-29, accounted for 6.3% (5 participants). Only 7.5% (6 participants) were aged 70 years or older. The mean age was 48.1 years with a standard deviation of 13.8, and the median age was 47 years, with an interquartile range of 38 to 56 years.

Table 1. *Socio-demographic Characteristics of Study Participants based on age.*

Variable	Frequency	Percent Mean±SD Median (IQR)
Age (years)		
20-29	**5**	**6.3 9.8**
30-39	18	22.5 3.8
40-49	20	25.0 4.5

Variable	Frequency	Percent Mean±SD Median (IQR)
Age (years)		
50-59	20	25.0 5.5
60-69	11	13.8 11.7
≥70	6	7.5 12.8
Total	80	100 48.1±13.8 47(38-56)

Regarding gender, 60% (48 participants) were male, while 40% (32 participants) were female. In terms of marital status, the vast majority of participants, 90% (72 individuals) were married. A smaller percentage, 8.8% (7 participants), were single, and only 1.2% (1 participant) was divorced. Educational attainment varied among the participants. Over half, 53.8% (43 participants), had attained tertiary education. Those with secondary education made up 18.8% (15 participants), while 10% (8 participants) had completed only primary education. A further 17.4% (14 participants) had no formal education.

In terms of occupation, civil servants constituted the largest group at 31.3% (25 participants). Farmers made up 28.8% (23 participants), followed by business people at 22.5% (18 participants). Artisans accounted for 7.5% (6 participants), while retirees and students each represented 5% (4 participants each) of the total sample.

Table 2. *Socio-demographic Characteristics.*

Variable	Frequency	Percent
Sex		
Male	48	60.0
Female	32	40.0
Marital Status		
Married	72	90.0
Single	7	8.8
Divorced	1	1.2
Education		
None formal	14	17.4
Primary	8	10.0
Secondary	15	18.8
Tertiary	43	53.8
Occupation		
Artisan	6	7.5
Business	18	22.5
Civil servant	25	31.3
Farming	23	28.8
Retiree	4	5.0
Student	4	5.0

The risk factors associated with chronic liver disease in the study participants were primarily assessed in terms of smoking, alcohol consumption, and drug use. The majority of participants, 93.7% (75 individuals), reported not smoking, while 6.3% (5 individuals) identified as smokers. In terms of alcohol consumption, 85% (68 participants) reported not drinking alcohol, whereas 15% (12 participants) acknowledged alcohol use. While those that engage in the use of prohibited drugs was the least common risk factor, with only 5% (4 participants) reporting use of prohibited drugs, meanwhile 95% (76 participants) stated they had never used prohibited drugs.

The stages of chronic liver disease among the participants were distributed across four categories as obtained from patients' individual medical records: cirrhosis, fibrosis, hepatocellular carcinoma (HCC), and hepatitis. Fibrosis was the most common stage, affecting 33.7% (27 participants). Cirrhosis was observed in 26.3% (21 participants). Hepatocellular carcinoma (HCC) and hepatitis had equal numbers at 20% each (16 participants each). All 80 participants were accounted for across these stages.

CTP classification of chronic liver disease severity among the participants revealed that 38.8% (31 individuals) had severe disease. Also, 27.5% (22 participants) were classified as having moderate disease, while 33.8% (27 participants) had mild disease.

Table 3. *Clinical characteristics of the study participants.*

Variable	Frequency	Percent
Risk Factors Smoking		
Yes	5	6.3
No	75	93.7
Alcohol		
Yes	12	15.0
No	68	85.0
Prohibited drugs		
Yes	4	5.0
No	76	95.0
Stages		
Hepatitis	21	26.3
Fibrosis	27	33.7
Cirrhosis	16	20.0
HCC	16	20.0
Total	80	100.0
CTP(Class)		
Mild	27	33.8
Moderate	22	27.5
Severe	31	38.8

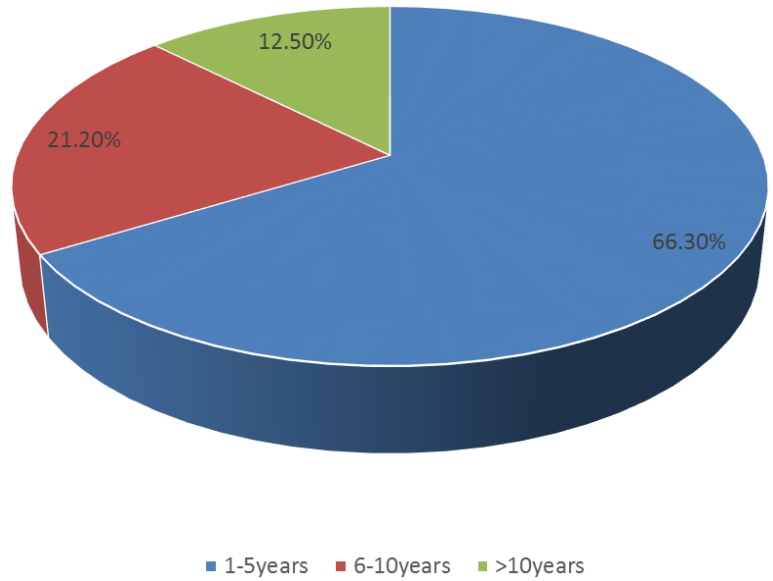


Figure 1. *Showing the clinical characteristics of the study participants based on duration of exposure.*

Table 4. *Showing the socio- demographic characteristics of the study participants.*

Variable	AFP	CA 125
Gender		
Male	1.034±1.88	98.2±1.09
Female	12.76±3.04	39.21±7.22
P-value	0.22	0.19
Marital Status		
Married	72.86±1.60	78.78±1.02
Single	18.38±4.02	41.36±8.60
P-value	0.304	0.289
Educational Status		
Non-Formal	53.70±1.64	67.74±9.58
Primary	1.08±2.21	1.29±7.90
Secondary	1.11±1.74	1.02±1.10
Tertiary	48.58±1.28	57.16±9.79
P-value	0.78	0.04*
Occupation		
Artisans	1.18±1.98	97.92±1.04
Business	47.2±1.39	68.90±8.73
C/Servant	70.50±1.67	52.80±9.14
Farmers	77.61±1.60	99.38±1.09
Herders	5.20±1.23	37.50±7.11
Retiree	83.28±1.59	1.43±1.68
Students	2.60±2.62	6.53±3.01

The mean and median distributions of Alpha-fetoprotein (AFP) and Cancer Antigen 125 (CA125) were reported with notable variability. The mean AFP level was 67.2 ± 153.3, while the median AFP was 4.4, with an interquartile range (IQR) of 2.6 to 22.8. For CA125, the mean level was 74.6 ± 99.7, and the median was 15.6, with an IQR of 7.0 to 145.1.

Table 5. Concentration of AFP and CA-125 in chronic liver disease patients.

Variable	Mean/SD	Median(IQR)
CA-125 (U/ml)	74.6±99.7	15.6(7.0145.1)
AFP(ng/ml)	67.2±153.3	4.4(2.6-22.8)

The median levels of AFP and CA 125 varied significantly across the stages of CLD. For AFP, the median levels were 3.7 ng/mL for hepatitis, 2.9 ng/mL for fibrosis, 5.7 ng/mL for cirrhosis, and 18.8 ng/mL for HCC, with a statistically significant difference across all stages (Kruskal-Wallis P = 0.049). Similarly, CA 125 levels increased progressively, with medians of 6.9 U/mL for hepatitis, 9.4 U/mL for fibrosis, 68.5 U/mL for cirrhosis, and 72.4 U/mL for HCC, demonstrating a highly significant difference across CLD stages (P < 0.005).

In patients with hepatitis, the correlation was negligible, with a Spearman's rho of 0.022 (P = 0.935), indicating no significant relationship. For those with fibrosis, a moderate positive correlation was observed (rho = 0.446, P = 0.020), which was statistically significant. In cirrhosis, a similar positive trend was noted (rho = 0.429), though it was not statistically significant (P = 0.053). Among patients with HCC, the correlation was weaker (rho = 0.359) and not statistically significant (P = 0.172).

Table 6. The relationship between AFP and CA-125.

Variable	AFP	CA125	Rho
Hepatitis	3.7(1.6-14.7)	6.9(5.8-10.3)	0.022(0.935)
Fibrosis	2.9(2.6-4.8)	9.4(6.2-49.6)	0.446(0.020*)
Cirrhosis	5.7(2.7-98.1)	68.5(25.4-207.5)	0.429(0.053)
HCC	18.8(4.4-146.4)	72.4(13.2-202.5)	0.359(0.172)
Kruskal-Wallis *p*-value	0.049*	<0.001*	

For CA 125, median levels increased progressively with worsening CTP staging: 7.3 U/mL (range: 5.9-31.5) in mild cases, 10.8 U/mL (range: 6.9-186.4) in moderate cases and 37.5 U/mL (range: 16.3-164.6) in severe cases, with a statistically significant difference (P <0.001). In contrast, AFP levels did not vary significantly across CTP stages. Median AFP levels were 3.0 ng/mL (range: 2.6-19.2) in mild cases, 3.7 ng/mL (range: 1.6-12.0) in moderate cases and 5.7 ng/mL (range: 2.8-26.0) in severe cases (P = 0.311).

Table 7. Relationship between CA-125, AFP and CTP scoring.

Parameter	CTP			p-value
	Mild	Moderate	Severe	
CA125(Median/IQR)	7.3(5.9-31.5)	10.8(6.9-186.4)	37.5(16.3-164.6)	<0.001*
AFP	3.0(2.6-19.2)	3.7(1.6-12.0)	5.7(2.8-26.0)	0.311

Regression model is CLD=β0 +β1 ·AFP+β2 ·CA-125.
Therefore: CLD=2.207+0.001·AFP+0.002·CA-125.

1) Intercept (β0=2.207): This is the predicted value of CLD when both AFP and CA-125 are 0. (Baseline level of CLD).
2) Coefficient for AFP (β1=0.001): For every 1-unit increase in AFP, the CLD value is expected to increase by 0.001 units, holding CA-125 constant. The p-value is >0.05; the effect of AFP on CLD is not statistically significant.
3) Coefficient for CA-125 (β2=0.002): For every 1-unit increase in CA-125, the CLD value is expected to increase by 0.002 units, holding AFP constant. The p- value is <0.05, meaning that the effect of CA-125 on CLD is statistically significant.

The lack of significant impact of AFP in this regression model implies it may not play a major role in determining the progression or severity of CLD. A significant relationship between CA-125 and CLD suggests that rising CA-125 levels are linked to worsening severity. This might indicate that CA-125 reflects disease progression. Overall, the regression model attributes a stronger and significant role to CA-125, this indicator is likely more critical for assessing the severity of CLD compared to AFP. However, the overall contributions of the predictors are small given the low coefficients.

Table 8. *Regression model for predicting severity of CLD using AFP and CA125.*

Variable	Coefficients		P-value
	β	Std. Error	
Constant	2.207	0.138	0.000
AFP	0.001	0.001	0.196
CA125	0.002	0.001	0.046

4. Discussion

This study aimed at determining the serum level of CA-125 and AFP to predict chronic liver disease severity amongst chronic liver disease patients in Jos University teaching hospital. From the results, the study found out the age distribution of 40-49 and 50-59 with the highest frequency of 20 each with a record of 25%. This suggests the age range where the disease is mostly active. This result is consistent with the finding made by Ugo et al. [13] which recorded the age range of 40-59 as the age with highest activity of chronic liver disease, and Echejoh et al. [14] with age range of 41-50 as the most active ages for chronic liver disease.

The gender distribution saw a higher number of male participants (48) than females (32). This can be traced down to the lifestyles of the male gender as they are mostly predisposed to the common risk factors of chronic liver disease such as heavy alcohol consumption, smoking and drugs of different trends. This finding is consistent with Muhammad et al. [15]; Riccardo et al. [16] and Han et al. [17]. However, Abdul et al. [18] reported a higher rate for female gender than male counterpart in Jos, Nigeria. The marital status distribution in this study recorded married participants with an outrageous representation of 72 out of a possible 80 subjects. This conforms to the finding made by Liang et al. [19] and Xing et al. [20] respectively who reported a higher number for married participants.

Educational attainment in the study recorded over half, 53.8% (43 participants), had attained tertiary education. This is also consistent with the findings by Mbang et al. [21] who reported a higher number of participants with Higher education when compared to others. However, this finding negates the report recorded by Tommaso et al. [22] and Yuanyue et al. [23], who recorded a higher prevalence for participants with non-formal education respectively. In terms of occupation, civil servants constituted the largest group at 31.3% (25 participants). Farmers made up 28.8% (23 participants), followed by business people at 22.5% (18 participants). Artisans accounted for 7.5% (6 participants), while retirees and students each represented 5.0% (4 participants each) of the total sample. However, Gabriel et al. [24] reported a higher prevalence for Artisans in eastern Nigeria.

In this study, alcohol consumption is the highest risk factor with 15% (12 participants). This study conforms to Asrani et al. [25] who reported a high prevalence for alcohol users. This result contradicts that of Mbang et al. [21] who reported HBsAg and HCV as the major risk factors for CLD cases. The clinical staging of the disease base on this study showed that fibrosis has the highest number of participants 27 (33.7%) of the study population and cirrhosis with 21 (26.3%). However, the study by Ashish and Shivaraj [26] contradicts this finding as it records cirrhosis as the most active stage of the disease.

Similarly, the scoring of the diseases base on the CTP, records class A (mild) as the most common scoring class, this class has the highest survival rates for CLD patients across the globe, this finding is in concordance with that of Sabrina et al. [27].

The demographic characteristics of the study participants, for gender, the AFP for females was significantly higher than the male counterparts while CA-125 was higher in males than females, this result is not consistent with Zhou et al. [28], who documented otherwise. For Marital status, the married class had a higher significance than the other groups this can be traced down to the higher number of participants in this category. Educational attainment recorded a higher number of participants for those with non-formal education in the two indicators when compared to other class. Occupation had a high significance for retiree in AFP while that of CA-125 had a high significance among farmers this has proven that the demographic information has great influence on the progression of the disease, this finding is in agreement with Shashank et al. [29], who recorded a similar report.

The mean levels of CA-125 (74.6±99.7) and tAFP (67.2±153.3) suggests that both indicators have a clear predictive significance for evaluating chronic liver disease severity. This finding is in agreement with Hogendorf et al. [30] and Kim et al. [31] respectively. The relationship between the two indicators across the different stages of chronic liver disease showed that at fibrosis stage that the indicators were statistically significant with a Spearman's rho of = 0.446 and a p-value of = 0.020. However, there are no documented studies that confirm this significance at the said stage of the disease hence this finding will be the first to suggest such valuable information concerning the excruciating disease.

The relationship between CA-125 and AFP using the child-turcottepugh score. C-125 was significantly a better indicator of chronic liver disease severity with $P < 0.001$ while AFP has $P = 0.311$. This result is in concordance with Qureshi et al. [32], who documented a high level of CA-125 for liver cirrhosis patients with ascites. To determine the predictive abilities of these indicators while AFP is a very useful marker in chronic liver disease management, CA-125 is a better marker for predicting chronic liver disease severity. CA-125 had a P-value of <0.05 which shows that it is statistically significant. This result is consistent with Zhou et al. [28], who reported that CA-125 levels have high predictive value for determining severity and progression of liver disease.

5. Conclusion

This study found out a significant positive relationship between AFP and CA-125 levels, indicating a good association between the two indicators when combine together in predicting chronic liver disease severity. Similarly, this can be confirmed statistically by the Spearman's rho for correlation of the two indicators which is 0.445, confirming the strength and significance of this relationship.

Abbreviations

ALP	Alpha-Fetoprotein (AFP)
CA	Cancer Antigen
CLD	Chronic Liver Disease
HCC	Hepatocellular Carcinoma

Author Contributions

Fador Nimfa Geofery: Conceptualization, Data curation, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing– review & editing

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Data Availability Statement

Data are available with the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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