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Alpha-fetoprotein as a predictor of liver disease progression in HBV patients with HIV and HCV co-infections

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Abstract

Introduction: Hepatitis B virus (HBV) infection is a significant health challenge globally, especially in sub-Saharan Africa. Co-infections with HIV and HCV worsen HBV-related liver diseases, complicating clinical management. Alpha-fetoprotein (AFP) is a key biomarker for monitoring liver disease progression and detecting hepatocellular carcinoma (HCC). This study evaluates AFP levels in HBsAg and HBeAg seropositive patients with and without HIV and HCV co-infections over one year in Warri, Delta State, Nigeria. This study aimed to understand the impact of HIV and HCV co-infections on liver disease prognosis in HBV patients by evaluating AFP levels and liver function over one year.

Materials and Methods: This longitudinal cohort study included 200 HBsAg and HBeAg seropositive patients aged 18-65 years, divided into three groups: HBV monoinfection (n=80), HBV/HIV co-infection (n=60), and HBV/HCV co-infection (n=60). Participants were followed for one year with quarterly blood sample collections for AFP measurement using ELISA, liver function tests (ALT, AST, ALP, bilirubin), and viral load assessments. Sociodemographic data were also collected.

Results: AFP levels were significantly higher in the HBV/HCV co-infection group (36.2 ± 12.4 ng/mL) compared to the HBV monoinfection (12.5 ± 4.3 ng/mL) and HBV/HIV co-infection groups (18.7 ± 6.8 ng/mL) ($p<0.001$). Elevated liver function tests, particularly ALT and AST, were more prevalent in the HBV/HCV co-infection group. AFP levels positively correlated with ALT ($r=0.52$, $p<0.01$) and AST ($r=0.47$, $p<0.01$) in the HBV/HCV co-infection group.

Conclusion: The higher AFP levels in HBV/HCV co-infected patients indicate an increased risk of liver disease progression and HCC. The positive correlations between AFP and liver enzymes suggest ongoing liver damage and regeneration in this group. These findings underscore the importance of routine AFP and liver function tests in the early detection and treatment of liver disease among HBV patients, particularly those with HCV co-infection, to enhance clinical outcomes.

Keywords: Alpha-fetoprotein (AFP), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Liver function tests, Hepatocellular carcinoma, Co-infection

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Introduction

Hepatitis B virus (HBV) infection continues to be a significant global health challenge, particularly in sub-Saharan Africa, where the prevalence remains high (1, 2). Complicating the clinical landscape, co-infections with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are common, exacerbating the progression of liver diseases associated with HBV. Co-infections pose additional challenges in clinical management and prognosis, necessitating more in-depth studies to understand their interactions and effects on liver health (1, 2).

Alpha-fetoprotein (AFP) serves as a crucial biomarker in monitoring liver disease progression and detecting hepatocellular carcinoma (HCC). Elevated AFP levels are often associated with liver inflammation, regeneration, and malignancy (3). Despite its widespread use, the dynamics of AFP levels in HBV patients with concurrent HIV and HCV infections remain inadequately explored(4). This study focuses on evaluating AFP levels in HBsAg and HBeAg seropositive patients, both with and without HIV and HCV co-infections, over one year in Warri, Delta State, Nigeria. The findings aim to provide insights into the impact of these viral interactions on liver disease prognosis and AFP variability (3–6).

In-depth knowledge of the implications of anti-HBe in HBV infection is crucial for comprehensive disease management. The presence of hepatitis B e-antigen (HBeAg) in the blood typically indicates active viral replication and high infectivity (7). Conversely, the appearance of antibodies against HBeAg (anti-HBe) usually suggests a transition to a lower replicative state of the virus, which is often associated with a more favorable prognosis (8–11). However, this seroconversion does not necessarily mean that the virus has been cleared from the liver. It signifies that the immune system has responded to the virus in a way that reduces its replication (7–11).

Liver disease in HBV patients, particularly in those with co-infections, poses a complex challenge for clinical management (12–14). Co-infection with HIV and HCV can alter the natural course of HBV infection, leading to more severe liver damage and an increased risk of HCC. HIV co-infection, for instance, can accelerate the progression of liver fibrosis and increase the likelihood of cirrhosis and liver-related mortality. Similarly, HCV co-infection can result in more aggressive liver disease and complicate treatment outcomes. Therefore, scientific knowledge on how these co-infections influence AFP levels and liver disease progression is vital for improving patient outcomes (12–14).

The primary objective of this study is to assess the levels of AFP in HBsAg and HBeAg seropositive patients, with and without HIV and HCV co-infections, over one year. This evaluation will help elucidate the influence of co-infections on liver disease progression and the potential development of HCC. By examining AFP levels longitudinally, this research aims to highlight any significant fluctuations that could be indicative of disease progression or response to therapy (15-17).

Materials and methods

Study Design and Population

This longitudinal cohort study was conducted over one year, involving 200 HBsAg and HBeAg seropositive patients aged 18-65 years, recruited from healthcare facilities in Warri, Delta State, Nigeria.

Sample Size Determination

The sample size was calculated using the formula:

$n = Z^2 * P(1-P) / d^2$

Where:

n = required sample size

Z = 1.96 (for 95% confidence level)

P = 0.109 (10.9% prevalence) (18)

d = 0.05 (5% precision)

$n = (1.96)^2 * 0.109(1-0.109) / (0.05)^2$

n = 3.8416 * 0.109 * 0.891 / 0.0025

n = 149.82

Rounding up to the nearest whole number: 150

To account for potential non-response or dropout, 15% proportion was added to the minimum sample size obtained

150 + (150 * 0.15) = 172.5

Accordingly, a minimum sample size of 173 subjects was appropriate for the study to achieve a 95% confidence level with 5% precision. However, to improve diversity in study participation and to ensure greater precision, 200 subjects were recruited for the study (19).

The study population was categorized into three groups:

1. HBV monoinfection without HBeAb expression (n=80)
2. HBV/HIV co-infection without HBeAb expression (n=60)
3. HBV/HCV co-infection without HBeAb expression (n=60)

Inclusion and Exclusion Criteria

Participants were included if they were seropositive for HBsAg and HBeAg and had no expression of HBeAb. Exclusion criteria included prior liver disease, HCC, or other significant co-morbidities.

Ethical Considerations

This study obtained Ethical Approval from the Delta State Ministry of Health Research and Ethics Review Committee. We adhered to ethical principles, including:

i. Informed Consent: Each participant received a written informed consent form alongside the questionnaire, ensuring their consent to participate.

ii. Data Confidentiality: Findings from the study were kept confidential and shared only among co-investigators.

iii. Beneficence: The results of the findings were provided to the managing clinical team without any charge.

iv. Voluntariness: Both cases and controls had the option to decline participation in the study when approached.

Data Collection

Blood samples were collected quarterly for one year. Alpha-fetoprotein (AFP) levels were measured using enzyme-linked immunosorbent assay (ELISA). Additional tests included liver function tests (ALT, AST, ALP, and bilirubin) and viral load assessments. Sociodemographic data, including age, gender, occupation, and lifestyle factors, were collected using structured questionnaires.

Principles of Assays for Laboratory Analysis

Alpha-fetoprotein (AFP) Measurement

AFP levels were determined using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (Bio-Rad kit). The principle of ELISA involves the following steps:

1. **Antigen-Antibody Binding:** The AFP in the patient's sample binds to the specific antibodies coated on the wells of the ELISA plate.
2. **Washing:** Unbound substances are removed through washing.
3. **Enzyme-Linked Secondary Antibody:** An enzyme-linked secondary antibody specific to AFP is added, which binds to the AFP already captured by the primary antibody.
4. **Substrate Addition:** A substrate is added that the enzyme converts to a detectable signal, typically a color change.
5. **Detection:** The intensity of the color is measured using a spectrophotometer and is proportional to the AFP concentration in the sample.

Liver Function Tests

Liver function tests (LFTs) including ALT, AST, ALP, and bilirubin levels were measured using automated

biochemical analyzers (Biobase BS-230). The principles of these tests are as follows:

1. **Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST):**
    - **Enzyme Activity Measurement:** ALT and AST catalyze the transfer of amino groups from alanine and aspartate to alpha-ketoglutarate, respectively. The reaction produces pyruvate and oxaloacetate, which are then converted to a detectable product.
    - **Spectrophotometry:** The change in absorbance is measured, reflecting enzyme activity.
2. **Alkaline Phosphatase (ALP):**
    - **Enzyme Activity Measurement:** ALP catalyzes the hydrolysis of phosphate esters, releasing inorganic phosphate.
    - **Spectrophotometry:** The release of phosphate is measured, indicating enzyme activity.
3. **Bilirubin:**
    - **Direct and Total Bilirubin Measurement:** Bilirubin reacts with diazo reagent to form azobilirubin, which is measured spectrophotometrically.
    - **Indirect Bilirubin Calculation:** Indirect bilirubin is calculated by subtracting direct bilirubin from total bilirubin.

HBsAg, HBeAg, Anti-HBe, Anti-HCV, and HIVp24 detection

These markers were measured using ELISA kits (Bio-Rad kit), and the principles are similar to the AFP ELISA described above:

1. **Antigen/Antibody Binding:** The specific antigen or antibody in the patient's sample binds to the corresponding antibody or antigen coated on the ELISA plate.
2. **Washing:** Unbound components are washed away.
3. **Enzyme-Linked Secondary Antibody:** An enzyme-linked secondary antibody specific to the target antigen or antibody is added, binding to the antigen-antibody complex.
4. **Substrate Addition:** A substrate is added that is converted by the enzyme into a detectable signal.
5. **Detection:** The resulting color change is measured, which is proportional to the concentration of the target antigen or antibody in the sample.

Quality Control Measures

ELISA Assay: The ApDia ELISA semi-autoanalyzer was used to AFP, HBsAg, HBeAg, Anti-HBe, Anti-HCV, and HIVp24 detection levels in plasma samples. Quality control measures were implemented to ensure the accuracy and reliability of the assay results. External positive and negative controls, provided by the manufacturer, were tested concurrently with each batch of plasma samples. These controls were essential for verifying the proper functioning of the test kits and ensuring that each assay run was valid. Additionally, calibration curves were generated using standard solutions, and the consistency of these curves was monitored across different assay batches.

Liver Function Tests: Liver function tests were conducted by spectrophotometric method using Biobase autoanalyzer (BS-230), which was calibrated regularly to maintain precision. The tests included measurements of ALT, AST, ALP, and bilirubin levels. Quality control was a critical component of the testing process, with both internal and external controls employed. The external controls, provided by the manufacturer, were tested alongside the plasma samples to verify the accuracy of the test kits and the reliability of the results. These controls were run with every batch to confirm the proper performance of the analyzer.

Quality Control Measures

To ensure the robustness of the data, stringent quality control measures were implemented throughout the

study. External positive and negative controls were run concurrently with each assay to verify the correct functioning of the analytical instruments and test kits. Additionally, calibration and internal control procedures were rigorously followed to minimize inter- and intra-assay variability.

Data Cleaning

Before proceeding with data analysis, all collected data underwent a thorough cleaning process. This step involved checking for any inconsistencies, outliers, or missing values that could affect the accuracy of the final results.

Statistical Analysis

The cleaned data were then analyzed using SPSS version 25. AFP levels were compared between groups using one-way ANOVA, followed by post hoc Tukey tests for pairwise comparisons. Pearson's correlation coefficient assessed correlations between AFP levels and clinical parameters. Temporal variations in AFP levels were analyzed using repeated measures ANOVA. A p-value <0.05 was considered statistically significant.

Results

The mean age of the participants was 42 ± 10 years, with a male-to-female ratio of 1.2:1. No significant differences were observed in age, gender, or socioeconomic status between the groups (Table 1).

Table 1. Sociodemographic Characteristics of Study Participants.

Characteristic	HBV Monoinfection (n=80)	HBV/HIV Co-infection (n=60)	HBV/HCV Co-infection (n=60)	p-value
Age (years)	42.3 ± 9.8	41.7 ± 10.2	42.8 ± 9.5	0.87
Gender (M/F)	44/36	32/28	33/27	0.72
Socioeconomic Status (Low/Medium/High)	34/30/16	25/23/12	28/22/10	0.81

AFP Levels and Liver Function Tests

AFP levels were significantly higher in the HBV/HCV co-infection group (36.2 ± 12.4 ng/mL) compared to the HBV monoinfection (12.5 ± 4.3 ng/mL) and HBV/HIV co-infection groups (18.7 ± 6.8 ng/mL) ($p<0.001$) (Table 2). Elevated liver function tests, particularly ALT and AST, were also more prevalent in the HBV/HCV co-infection group.

Table 2. AFP Levels and Liver Function Tests in Study Groups.

Parameter	HBV Monoinfection (n=80)	HBV/HIV Co-infection (n=60)	HBV/HCV Co-infection (n=60)	p-value
AFP (ng/mL)	12.5 ± 4.3	18.7 ± 6.8	36.2 ± 12.4	<0.001
ALT (U/L)	32.4 ± 10.2	45.7 ± 15.3	62.8 ± 20.1	<0.001
AST (U/L)	28.3 ± 9.5	40.2 ± 12.8	59.4 ± 18.6	<0.001
ALP (U/L)	110.7 ± 32.1	122.6 ± 38.4	135.2 ± 41.7	0.02
Bilirubin (mg/dL)	1.1 ± 0.3	1.4 ± 0.4	1.8 ± 0.6	<0.001

Correlation Analysis

AFP levels positively correlated with ALT ($r=0.52$, $p<0.01$) and AST ($r=0.47$, $p<0.01$) in the HBV/HCV co-infection group. No significant correlations were observed in the HBV monoinfection or HBV/HIV co-infection groups (Figure 1).

Figure 2 shows Alpha-Fetoprotein (AFP) levels measured over four quarters, (Q1 to Q4) for two groups of patients: those with both Hepatitis B and C (HBV/HCV co-infection) and those with only Hepatitis B (HBV monoinfection).

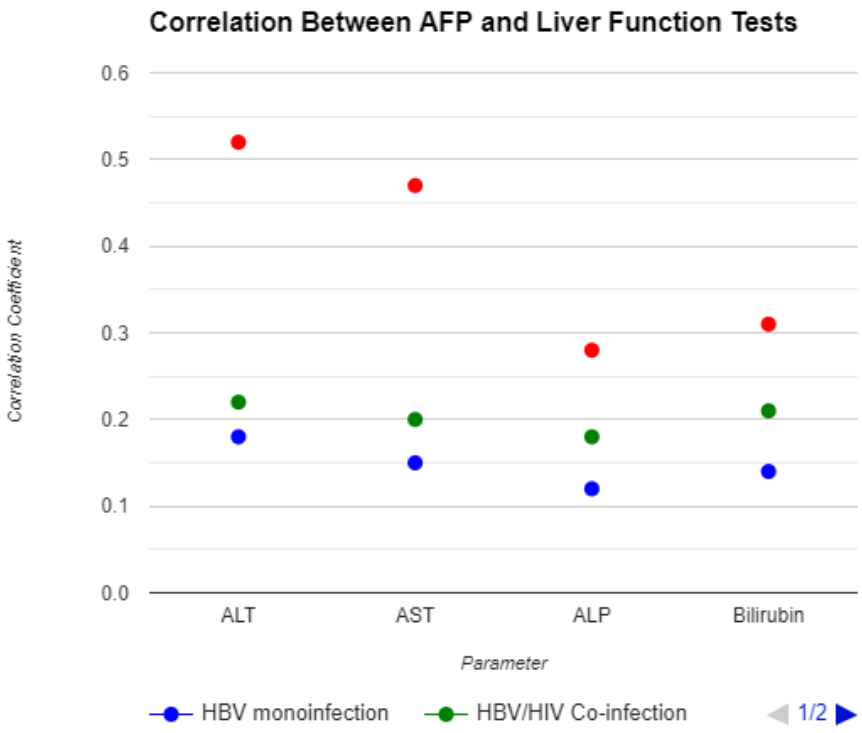

Figure 1. Correlation Analysis Between AFP and Liver Function Tests.

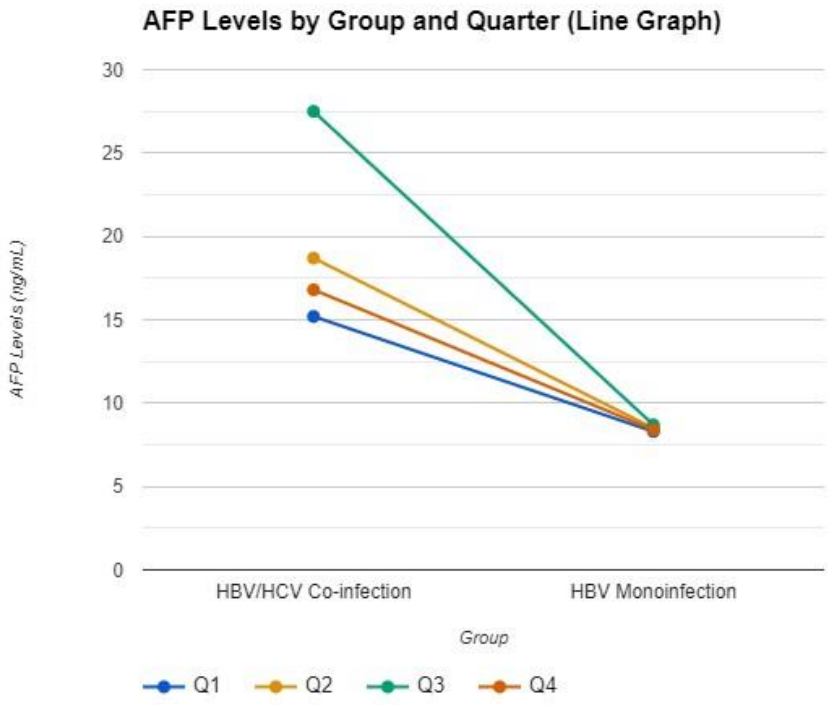

Figure 2. Quarterly Variations of Alpha Fetoprotein Levels in HBV/HCV Co-infection and HBV Monoinfection Groups among Subjects in Warri.

Discussion

This study evaluated Alpha-Fetoprotein (AFP) levels in HBsAg and HBeAg seropositive patients with and without HIV and HCV co-infections in Warri, Delta State, Nigeria, over one year. Our findings indicate that AFP levels are significantly higher in patients with HBV/HCV co-infection compared to those with HBV monoinfection or HBV/HIV co-infection. These elevated AFP levels in the HBV/HCV group suggest a heightened risk of liver disease progression and potential hepatocellular carcinoma (HCC) development. This observation aligns with previous studies that identified HCV co-infection as a factor that exacerbates liver disease in HBV patients (20-23).

The elevated AFP levels observed in HBV/HCV co-infected patients compared to those with HBV monoinfection or HBV/HIV co-infection underscore the additive or synergistic hepatocellular damage inflicted by HCV. HCV is known for its direct cytopathic effects and its ability to exacerbate liver inflammation, leading to more significant liver injury and, consequently, higher AFP levels. This is consistent with previous studies highlighting the exacerbation of liver disease in the presence of HCV, which may explain the pronounced increase in AFP levels observed in our study (24-27).

In contrast, the relatively modest increase in AFP levels among HBV/HIV co-infected patients suggests a different interaction between HIV and HBV in the liver. Although HIV is associated with chronic immune activation and inflammation, its direct impact on hepatocytes may be less pronounced compared to HCV. The immunosuppressive nature of HIV may also modulate the inflammatory response in a manner that does not significantly elevate AFP levels, despite the ongoing liver damage (28-31).

It is also possible that the antiretroviral therapy (ART) used in HIV-infected individuals plays a role in mitigating liver injury and, by extension, AFP production. ART has been shown to reduce HIV viral load and associated immune activation, potentially attenuating the extent of liver damage and AFP elevation in HBV/HIV co-infected individuals (23). However, the hepatotoxic potential of certain ART drugs cannot be ignored, and further research is needed to disentangle these complex interactions (24).

Another factor to consider is the differential immune response elicited by HCV and HIV in co-infected patients. HCV's ability to induce a more robust and sustained inflammatory response in the liver, as opposed to the more systemic immune dysregulation seen in HIV infection, might explain the observed differences in AFP levels. HCV's propensity to cause chronic liver inflammation and fibrosis may lead to increased AFP production as a marker of ongoing liver regeneration and damage (32-38).

Moreover, the role of AFP as a biomarker in these co-infection settings is multifaceted. While elevated AFP is a well-known marker for hepatocellular carcinoma (HCC), its utility in monitoring chronic liver disease progression, especially in co-infected patients, remains an area of active investigation (39-41). The correlation between AFP levels and liver function tests (LFTs) observed in this study further supports its potential role in tracking liver disease severity, particularly in HBV/HCV co-infection (26, 27).

However, the study also highlights the limitations of AFP as a sole biomarker, particularly in distinguishing between benign and malignant liver conditions in co-infected individuals. The modest correlations between AFP and LFTs in the HBV/HIV co-infected group suggest that AFP alone may not be sufficient to fully capture the complexity of liver disease in these patients (28, 29). This finding aligns with existing literature, which advocates for the use of a combination of biomarkers and imaging techniques for a more comprehensive assessment of liver health in co-infected individuals (30).

Our study emphasizes the need for a nuanced understanding of AFP dynamics in HBV co-infection contexts. The differential impact of HIV and HCV on AFP levels reflects the underlying pathophysiological differences in how these viruses interact with HBV and affect liver health. Future research should focus on elucidating the specific mechanisms through which HIV and HCV modulate AFP production and exploring the potential of AFP in combination with other biomarkers for improved clinical management of co-infected patients (42-44).

Limitation

This study has several limitations that should be considered. Firstly, the study population was limited to patients from Warri, Delta State, Nigeria, which may affect the generalizability of the findings to other regions with different demographics or healthcare settings. Additionally, the study's reliance on quarterly blood sample collections may have missed fluctuations in AFP levels occurring between these intervals. The exclusion of individuals with pre-existing liver disease or HCC may also limit the applicability of the results to patients with more advanced liver conditions. Lastly, the study did not account for potential variations in treatment regimens or adherence among participants, which could influence AFP levels and liver function outcomes

Conclusion

The study demonstrates that AFP levels are significantly higher in HBV patients with HCV co-infection compared to those with HBV monoinfection or HBV/HIV co-infection. This elevation in AFP suggests an increased risk of liver disease progression and potential hepatocellular carcinoma (HCC) in the HBV/HCV co-infection group. The observed positive correlations between AFP levels and liver enzymes (ALT and AST) in the HBV/HCV group further indicate ongoing liver damage and regeneration. These findings highlight the need for vigilant monitoring and management of HBV patients with HCV co-infection to address the heightened risk of liver complications.

Recommendations

1. Enhanced Monitoring: Routine AFP and liver function tests should be integrated into the care plans for HBV patients, particularly those with HCV co-infection, to facilitate early detection of liver disease progression and HCC.

2. Differentiated Management Strategies: Tailor treatment strategies based on co-infection status, with a focus on more aggressive monitoring for HBV/HCV co-infected patients. For HBV/HIV co-infected patients, emphasize maintaining immune function and monitoring liver health through regular assessments.

3. Public Health Initiatives: Strengthen public health programs to enhance awareness about the risks of co-infections and promote preventive measures, such as vaccination against HBV and harm reduction strategies to prevent HCV transmission. Implementing comprehensive screening programs can aid in early identification and intervention, improving patient outcomes.

Further Research: Future studies should explore the impact of various treatment regimens and adherence on AFP levels and liver disease progression. Expanding research to diverse populations and healthcare settings will help to validate and generalize the findings.

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Author contribution

MFO conceived the study, and participated in manuscript review, and overall research supervision. **KFA** participated in research design, data analysis, and manuscript writing. **ORO** participated in research design, data collation and manuscript writing. **TBO** participated in research design and sample collection. **TAM** participated in sample collection, data collation and manuscript writing. **AWT** participated in data analysis and manuscript review. **PNK** participated in research design and data analysis. **OJA** participated in research design and research supervision. **OBO** participated in sample analysis, data analysis, data collation and manuscript writing.

Conflict of interest

The authors declare that they have no competing interests.

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