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BIOCHEMICAL AND CLINICAL PROFILES OF HEPATOCELLULAR CARCINOMA (HCC) PATIENTS STRATIFIED BY SERUM ALPHA-FETOPROTEIN (AFP) LEVELS

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Abstract: Alpha-fetoprotein, or AFP, is one of the most important biomarkers for hepatocellular carcinoma, widely used to assess prognosis and monitor disease recurrence. The present study aimed to classify patients according to their serum AFP levels, with etiological backgrounds such as HBV and HCV infection, alcoholism, and cirrhosis, and correlate these levels with biochemical and relevant clinical features. This was a prospective study done from December 2023 to December 2024. Comprehensive clinical and laboratory data were gathered in detail, including hepatitis profiling, cirrhosis assessment, CLD status, and hepatitis serology of HBV and HCV, on all the subjects and organized for analysis. A total of 110 patients (82 males and 28 females), aged 25–78 years, were enrolled according to predefined inclusion criteria. Patients were stratified into three groups based on their AFP concentrations: Group I: AFP < 20.0 IU/mL, Group II: AFP 20–400 IU/mL, Group III: AFP ≥ 400 IU/mL. Serum AFP was measured by ECL technology on the fully automated Cobas 6000 e601 analyzer (Roche Diagnostics, Basel). Biochemical parameters including alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (T. Bil), alkaline phosphatase (ALP), gamma-glutamyl transferase (γ-GT), albumin, and prothrombin time (PT) were determined using internationally standardized protocols on Cobas c512 and c503 chemistry analyzers (Roche Diagnostics) and Sysmex systems. Hepatitis B and C profiles were analyzed using AXSym (Abbott, USA) analyzer, following both normal and pathological reference standards. Among the 110 patients, n = 80 were diagnosed with HBV, n = 10 with HCV and n = 20 had cirrhosis. The overall mean AFP concentration across all participants was 360.10 ± 101.20 IU/mL. The mean AFP levels in Groups I, II, and III were 10.15 ± 2.50 IU/mL, 251.40 ± 55.45 IU/mL, and 575.45 ± 120.35 IU/mL, respectively, with a statistically significant difference ($P < 0.001$). High AFP levels (Group III, ≥ 400 IU/mL) were predominantly observed in ~~52.32% (n = 40) of~~ patients with HBV (HBsAg-positive) infections, followed by ~~34% (n = 30) of~~ patients in Group II (20–400 IU/mL). Total bilirubin, ALP, AST, ALT, γ-GT, albumin, and PT values were significantly elevated ($P < 0.05$ to $P < 0.01$), particularly in Groups II and III. The study demonstrates that markedly elevated AFP levels (≥ 400 IU/mL) serve as a valuable diagnostic and prognostic biomarker for hepatocellular carcinoma. Furthermore, patients with HBV infection exhibit a significantly higher propensity for elevated AFP levels due to HBV complex and hasten inflammatory and genetic intriguing capacity as compared with those suffering from HCV infection or other hepatic disorders.

Key words: Hepatocellular carcinoma (HCC), Hepatitis B Viral infection (HBV), Hepatitis C Viral infections (HCV)

INTRODUCTION

Hepatocellular carcinoma (HCC) represents the third most fatal malignant neoplasm and ranks as the fifth most prevalent carcinoma worldwide (Mazza *et al*., 2024; Kalyuzhnyy *et al*., 2025; Bai, *et al*., 2017; Saber, *et al*., (2017; Yang and Roberts, 2010). In the United States, its mortality rate is approximately 4.52 deaths per 100,000 individuals, marking HCC as a major global public health concern (Mazza *et al*., 2024; Zhang, *et al*., 2016; Ikai, *et al*., 2004). Chronic and persistent infections with hepatitis B virus (HBV) or hepatitis C virus (HCV) are recognized as the predominant etiological factors underlying HCC (Bai, *et al*., 2017; Caccamo *et al*., 2014). Effective management of HCC depends largely on timely diagnosis, accurate staging, and proper evaluation of therapeutic efficacy (Mazza *et al*.,

2024; Zhang, *et al*., 2016; Peng, *et al*., 2004).Alpha-fetoprotein (AFP) remains one of the most widely utilized biomarkers for HCC, serving not only as a diagnostic tool but also for prognostic evaluation and monitoring of disease recurrence (Zhang, *et al*., 2016; Song, *et al*., 2016; Yao *et al.*, 2016). AFP, a member of the albuminoid multigene family located on chromosome 4q (Tilghman, and Belayew, (1982; Nomura, *et al*., 1989; Alam, *et al*., 2004; Alam, *et al.*, 2009) is physiologically expressed in the yolk sac and fetal liver but is typically absent in normal adult tissues (Peng, *et al*., 2004; Tilghman, and Belayew, 1982). Allelic deletions within chromosome region 4q are frequently observed in HCC cases with elevated AFP expression, which are associated with poor tumor differentiation, increased tumor burden, and an unfavourable prognosis (Peng, *et al*., 2004). In the serum AFP levels were analyzed in patients with varying present study, etiologies, including HBV, HCV, alcohol-induced liver disease, and cirrhosis. Participants were categorized into three groups based on AFP concentrations, and their biochemical as well as clinical characteristics were subsequently assessed and statistically correlated with AFP groupings.

MATERIALS AND METHODS

Study protocols and selection criteria

The idea of conducting the study made its way into discussions regarding the evolution of research; consequently, the study itself was conducted prospectively, from Dec 2023 to Dec 2024, with patients selected by screening data either from laboratory information system (LIS) analysis or by reviewing outpatients and clinics, when available. All laboratory patients' clinical data, including hepatitis profiles, were collected and grouped in order to conduct comparisons and analysis. Each individual's data will be compiled relate to the standardization of design utilized in previously published studies (Alam, *et al*., 2004a; 2009b; Baig et al., 2009) and by Tyson and colleagues (Tyson, *et al.*, 2011).Collaborative patients were grouped concerning assigned designate of male or female, but individual data were considered collectively assessed, in order to assist evaluation of population status. The age of all participating patients in the study ranged from 25-78 years old. To compare levels of our objective parameters, AFP being the sole outcome measure, we classified patients in to three groups based on their AFP levels, Group I (< 20.0 IU/ml), Group II (20-400 IU/ml), and Group III (≥ 400 IU/ml). Demographic data including pertinent medical history, as well as clinical data including cirrhosis, chronic liver disease (CLD) and hepatitis status (HCV or HBV) were required of all patients in this study. We started with approximately 200 patients (males = 129 and females = 71); however, as the study progressed, we selected a total of 110 patients (82 males and 28 females) as per our specifications. Patients with prior surgical procedures, immuno-compromised, alcoholic, on steroidal therapies, < 20 years old or > 80 years were excluded from the study.

Clinical and Diagnostic Assessments

All clinical information and laboratory investigations and or any other assessment possible, where possible in detail, were obtained and documented for group purposes. Other data and information were obtained as detailed above (Alam, *et al*., 2004)..

Sample collection and analysis

Using defined procedures, blood was collected in red top (clot activator) tubes for AFP, gel top for alanine transaminase (ALT), asparatate transaminase (AST), Total Bilirubin (T.Bil), Alkaline Phosphatase (ALP), insulated gamma-glutamyl transpeptidase (gGT), and albumin and other specialized tubes for Prothrombin Time (PT), and for hepatitis profiling. Serum was separated, if appropriate. Aliquots were made of serum from separate to conduct separate analytical parameters and were kept at -20^{o}C until analysis was undertaken. AFP was analyzed by two point calibration on ECL technology using fully automated Cobas411 and Cobas6000 e601 [Roche-Diagnostics, Basil), whilst ALT, AST, T. Bil, ALP, GGT, Albumin, and PT was analyzed methodologically according to international recommendations on automated chemistry analyzer Cobas c512 (Roche-Diagnostics, Basil) and on Sysmex analyzers. An AFP value of >20 ng/ml for smokers and >10 ng/ml for non-smokers was required for significance. Tests for hepatitis profiling included both B and C using a testing methodology on AXSym (Abbott-USA) as recommended and via both normal and pathological standards. Data was then analyzed by using One way ANOVA with post HOC test [Tukey's HSD (Honestly Significant Difference)] [SPSS ver 24]. [SPSS ver 24].

RESULTS

Results are summarized in Tables I and II. A total of 110 patients, Males 82 (74.54%) and females 28 (25.45%) were included in the study. Patients were divided into three groups I, II and III according to AFP levels defined in material and methods. Number of patients in Group I were 15, 28 in Groups II and 67 in Groups III. As per gender wise distribution, males were distributed as 10, 22, 50 and females as 05, 06, and 17 respectively. Distribution of total number of patients according to clinical etiology was 80 with HBV, 10 with HCV and 20 patients with Cirrhosis. Sub-divisions of patients are detailed in Table I. Over all mean level of AFP in all three groups was 360.10 ± 101.20 IU/ml, whereas as per Groups I, II III were 10.15 ± 2.50 IU/ml, 251.40 ± 55.45 IU/ml and 575.45 ± 120.35 IU/ml, respectively with significance of P <0.001. Biochemical characteristics were also determined by assessing hepatic enzymes and metabolic components such as Total Bilirubin (mg/dl), alkaline phosphatase IU/L, AST, ALT, gGT, Albumin and Prothrombin Time. All parameters were noted to be elevated significantly (P<0.05 to P< 0.01), specifically in patients in Groups II and III (Table II).

Table 1: Demographic and Clinical Characteristics of HCC patients according to AFP levels*****

Demographic and Clinical Characteristics	Total of all three groups	Group I AFP < 20 IU/ml	Group II AFP 20-400 IU/ml	Group III AFP ≥ 400 IU/ml	P < 0.05
Number of patients	110	15	28	67	<0.05
Gender Males	82 (74.54%)	10	22	50	--
Females	28 (25.45%)	05	06	17	--
Etiology/Diagnosis					
• HBsAg (+)	80	11	29	40	<0.01
• Anti-HCV (+)	10	02	03	05	<0.01
• Cirrhosis	20	03	10	07	<0.01
Mean AFP levels (IU/ml)	360.10 ± 101.20	10.15 ± 2.50	251.40 ± 55.45	575.45 ± 120.35	< 0.001

Table 2: Biochemical Characteristics of HCC patients according to AFP levels*

Biochemical Characteristics	Group I AFP < 20 IU/ml	Group II AFP 20-400 IU/ml	Group III AFP ≥ 400 IU/ml	P < 0.05
Total Bilirubin (mg/dl)	0.45 ± 0.15	2.45 ± 0.20	3.60 ± 0.35	<0.01
Alkaline phosphatase IU/L	389.45 ± 86.55	448.10 ± 101.55	589.60 ± 111.15	<0.01
AST (IU/L)	101.25 ± 25.55	145.35 ± 30.45	189.40 ± 31.65	<0.01
ALT (IU/L	89.40 ± 15.15	112.45 ± 25.50	155.65 ± 20.70	<0.01
gGT (IU/L)	54.25 ± 4.55	85.10 ± 10.15	125.65 ± 15.55	<0.05
Albumin (g/dl)	3.5 ± 0.55	3.8 ± 1.15	3.7 ± 1.10	NS
Prothrombin Time (sec)	12.15 ± 2.40	13.25 ± 1.85	14.55 ± 2.10	<0.05

DISCUSSION

It is well established that elevated serum α-fetoprotein (AFP) levels or AFP gene overexpression in patients with hepatocellular carcinoma (HCC) are positively associated with etiological backgrounds of hepatitis B virus (HBV) or hepatitis C virus (HCV) infections (Haque & Yasmin, 2024; Mazza, *et al*., 2024; Peng, *et al*., 2004; Matsumoto, *et al*., 2016; Miyagawa, *et. al* ,2003; Imamura *et al.,* 2003). Furthermore, AFP has been strongly advocated as a valuable biomarker for HCC, as AFP mRNA overexpression has been frequently observed and consistently correlated with HCC-positive cases (Mazza, *et al*., 2024; Zhang, *et al*., 2016; Peng *et al*., 2004). Large cohort studies have reported that approximately 47% of HCC patients exhibit elevated AFP levels, among whom 93.5% demonstrated AFP mRNA overexpression in conjunction with HBV or HCV antigen titers, younger age (≤45 years), and p53 mutations as major contributing risk factors (Peng *et al*., 2004).

In our study, markedly elevated AFP levels (≥400 IU/mL) were observed in 52.32% (n = 45) of patients with HBV (HBsAg-positive), followed by 34% (n = 30) of patients with moderate AFP levels (20–400 IU/mL). Substantially increased levels of liver enzyme markers such as AST, ALT, ALP, and γGT were also observed in individuals with higher AFP level concentrations, suggesting a strong correlation between higher AFP levels and worsening HCC. The results are consistent with previously published findings which show increased chronicity and aggressiveness of HBV infections leading to increased AFP levels, as well as the higher frequency of HBV etiology over HCV (Kurniawan *et al*., 2024; Saber *et al*., 2017; Zhang *et al*., 2016; Peng *et al*., 2004; Tangkijvanich *et al.,* 2000; Wang *et al*., 2013). In a recent study, it has been documented that around 18 million people are infected with HBV in Indonesia, out of which estimated 3.5 to 5.2 million shall be expected to be with liver cirrhosis or cancer (Kurniawan *et al*., 2024).

Moreover, the literature has pointed to an inverse relationship between elevated AFP and patient age, indicating that younger HCC patients carry more aggressive tumors (Matsumoto et al., 2016; Tangkijvanich *et al.,* 2000; Sauzay *et al*., 2016; Shijo et al., 1991; Trevisani *et al*., 1996). Younger individuals often have a higher stage of HCC which commonly is associated with chronic HBV infection (Zhang *et al*., 2016; Zhu *et al*., 2015; Yaprak *et al*., 2012). Additionally, HBV-HCV co-infection has been shown to accelerate disease development and clinical aggressiveness in HCC, and this may also contribute to concurrent AFP overexpression (Zhang *et al*., 2016; Tangkijvanich *et al*., 2000; Sauzay *et al*., 2016; DiBisceglie *et al*., 1988).

A more recent review elaborated correlation between HVB and development of HCC (Adugna et al., 2025). It has been reported that development of HCC might have the underlying mechanism of continuous inflammation by HBV infection causing persistent hepatocyte damages emerging from that similar protracted immunological reactions to HBV. This continual cycle of inflammatory reactions, redevelopment, and cellular injuries results in genetic modifications and transmutations in cellular system, causing HBV-related HCC, in most of reported cases (Agunda et al., 2025). Extracellular matrix remodeling and cell types proliferation that always takes place during HBV infections and hepatocyte transformation which is a convoluted process involving multiple stages and genetic connections, results in more accelerated transition from inflammatory status to carcinogenesis (Agunda *et al*., 2025; Kurniawan et al., 2024). It has been indicated that the altered protein components from HBV infections hasten the growth of hepatocyte remodeling and ultimate outcome as cancer, more definitely than other hepatic viral infections. We have seen similar finding in our study where

AFP is important for diagnosis and prognosis in HCC at the clinical level. A number of studies have shown that AFP is an independent prognostic factor (Mazza *et al*., 2024; Zhang *et al*., 2016). Data has shown that the incorporation of AFP into HCC staging systems improves prognostic prediction beyond AJCC (Mazza *et al*., 2024; Zhang *et al*., 2016; Li *et al*., 2013). It has also been shown that AFP promotes HCC metastasis through the up regulation of metastasis-associated proteins (Zhu *et al*., 2015; Lu *et al*., 2016). As a

recent multivariate regression analysis showed, AFP is an independent predictive factor in HCC. Furthermore, AFP has been demonstrated to be a predictive biomarker of treatment responsiveness and a marker of poor prognosis in liver transplant patients (Zhang *et al*., 2016).

CONCLUSIONS

Our research concludes that significantly high AFP levels (≥400 IU/mL) are a useful diagnostic and prognostic indicator for HCC patients. AFP concentrations are considerably higher in people with HBV infection than in people with HCV infection. However, managing HCC clinically is still a difficult task. Clinical decision-making may be improved and more successful, evidence-based treatment approaches may be made possible by including AFP testing into HCC prognostic evaluation and staging systems.

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