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Evaluation of AFP for diagnosis of HCC in Egyptian patients

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Abstract:

Background: It was initially suggested in the 1960s to use AFP as a tumor marker for hepatocellular carcinoma (HCC), AFP still plays a big part in HCC monitoring. **Patients and methods:** this study was conducted on 90 patients with liver diseases and 25 healthy control G1, patients were divided into 4 groups, (G2) 25 patients with HCV infection, (G3) 25 HCC+HCV infection, (G4) 25 patients with HBV infection, (G5) 15 patients with HCC + HBV. **Results:** there was a statistically significant difference between the five groups p-value <0.001*, compared to the control group the three groups G2, G3, and G5 have the same statistically significant difference with the same p-value <0.001*, while group 4 HBV patients have not statistically significant difference with p-value 0.254 compared to the control group, Serum AFP highly significant elevated in HCC patients associated with HCV or HBV infection patients.

Keywords: Alpha-fetoprotein, AFP, HBV, HCV, HCC, Liver diseases

1. Introduction:

Hepatocellular carcinoma (HCC) is the most often primary malignancy of the liver, accounting for about 90% of all primary liver malignancies, and is very prevalent in the world's most populous regions (1).

In terms of individual countries, Egypt was the country with the second-highest risk of liver cancer in 2018 after Mongolia (Mongolia about four times that of men in China and the Republic of Korea) (2). Almost 50% of all cases of HCC are associated with HBV infection and 25% are associated with HCV, the lifetime risk of

HCC development among HBV carriers being from 10% to 25% (3).

In Egypt, there are two methodologies for HCC primary and secondary prevention: the HBV vaccination program (4), and, more recently, HCV eradication through a national campaign (5).

Alpha-fetoprotein (AFP) is created by the yolk sac, fetal liver, and gastrointestinal system during fetal and newborn life. Due to adults' quick reduction in AFP, just a trace quantity can be tested in adults (6). It was initially suggested in the 1960s to use AFP as a tumor marker for hepatocellular carcinoma (HCC). Due to its

poor sensitivity and specificity, AFP has been criticized for its efficacy in surveillance and diagnostic testing. However, in addition to ultrasonography (US) and other imaging modalities, AFP still plays a big part in HCC monitoring (7).

Clinically speaking, AFP-positive HCC patients had a worse prognosis than AFP-negative HCC patients due to their high malignancy, quick development, and poor prognosis (8). According to prior studies, individuals with AFP-positive HCC had a 5-year survival percentage of 26.7% while those with AFP-negative HCC had a 5-year survival rate of 56.5% (9).

For the early diagnosis of HCC, alpha-fetoprotein (AFP) screening and ultrasonography are frequently employed. However, there are certain restrictions with AFP and ultrasonography in HCC (10). Most patients with primary HCC are detected at late stages, which is linked to a poor prognosis and a low survival rate of the illness.

This research aims to study AFP levels in Egyptian patients with liver diseases such as hepatitis C infection HCV, hepatitis B infection HBV, and hepatocellular carcinoma HCC. to detect the role of AFP in the diagnosis of liver disease and its ability to serve as a biomarker for diagnosis of HCC.

2. SUBJECTS AND METHODS

2.1. Subjects

This study was conducted on 90 Egyptian hepatic patients and twenty-five healthy subjects. They matched in gender, social standard, and residency, all participants were selected from outpatient clinics, the National Liver Institute, Menofya University at the time from October 2019 to December 2021 (During the COVID-19 pandemic), The study was conducted according to the ethical approval of the Research Ethics Committee, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt

All subjects were classified into five groups as follows:

Group (1): This group consisted of 25 healthy subjects which served as a control group.

Group (2): This group consisted of 25 patients with HCV infection.

Group (3): This group consisted of 25 patients with hepatocellular carcinoma (HCC) associated with HCV infection.

Group (4): This group consisted of 25 patients with HBV infection.

Group (5): This group consisted of 15 patients with hepatocellular carcinoma (HCC) associated with HBV infection.

2.2. Methods

2.2.1. Blood sample collection and preparation

3 milliliters of venous blood were taken from each patient in all five groups using a vacutainer system and septic venipuncture. Allow samples to coagulate for 30 minutes in a serum separator tube (SST) before centrifuging for 15 minutes at 1000 g. Removed serum was stored at -20°C or -80°C to the analytical time.

2.2.2. Quantification determination of AFP by Enzyme-Linked Immunosorbent Assay (ELIZA):

AFP was analyzed using (Chemux Bioscience, Inc. USA) Lot No: 319091802, according to the manufacturer's guidelines by using the Strip reader Stat Fax USA.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using numbers and percentages. The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR). The values with $P < 0.05$ were considered significant.

3. Results:

AFP ranged from 1.10 – 4.0 ng/dl in the control group with a mean of 2.80 ± 0.96 ng/dl, while in group 2 HCV patients it ranged from 4.0 – 32.0 ng/dl with a

mean of 15.07 ± 8.08 ng/dl, in group 3 HCV+HCC patients it ranged from 4.10 – 8756.0 ng/dl with mean of 2496.7 ± 2417.3 ng/dl, in group 4 HBV patients it ranged from 1.10 – 9.0 ng/dl with mean of 4.07 ± 2.23 ng/dl, while in group 5 HBV+HCC patients it ranged from 2210.0–78571.0 ng/dl with mean of 11620 ± 19321 ng/dl, there was a statistically significant difference between the five groups p value $<0.001^*$, compared to the control group the three groups G2 G3 G5 have the same a statistically significant difference with the same p-value $<0.001^*$, while group 4 HBV patients have not statistically significant difference with p-value 0.254 compared to

the control group.

Comparing between HCV and HCV+HCC groups AFP was highly increased in HCV+HCC from 15.07 ± 8.08 ng/ml to 2496.7 ± 2417.3 ng/ml with a significant statistical difference between p-value 0.006^* , AFP highly increasing from HBV group 4.07 ± 2.23 ng/ml to 11620 ± 19321 ng/ml in HBV+HCC group with a significant statistically difference between p-value $<0.001^*$. But group HCV+HCC and group HBV+HCC, the two groups have a markedly high level of AFP and no statistically significant difference between the two groups with a p-value of 0.207. as shown in Table (1) and figure (1).

Table (1): Comparison between the different studied groups according to AFP

	Control (n = 25)	HCV (n = 25)	HCV+HCC (n = 25)	HBV (n = 25)	HBV+HCC (n = 15)	Test of Sig.	P
AFP							
Min. – Max.	1.10 – 4.0	4.0 – 32.0	4.10 – 8756.0	1.10 – 9.0	2210.0–78571.0		
Mean \pm SD.	2.80 ± 0.96	15.07 ± 8.08	2496.7 ± 2417.3	4.07 ± 2.23	11620 ± 19321	H= 94.339*	$<0.001^*$
Median (IQR)	2.40 (2 – 3.5)	17.0 (8 – 21)	1200.0 (590 – 4458)	3.50 (2.4 – 5)	3251.0 (2731 – 10730)		
p₁		$<0.001^*$	$<0.001^*$	0.254	$<0.001^*$		
Sig. bet. Grps.		$p_2=0.006^*, p_3=0.001^*, p_4<0.001^*, p_5<0.001^*, p_6=0.207, p_7<0.001^*$					

IQR: Inter quartile range

SD: Standard deviation.

F: F for One-way ANOVA test, pairwise comparison bet. each 2 groups were done using a Post Hoc Test (Tukey)

H: H for Kruskal Wallis test, pairwise comparison bet. each 2 groups were done using a Post Hoc Test (Dunn's for multiple comparisons test)

p: p-value for comparing the different studied groups.

p₁: p-value for comparing between Control and each other groups.

p₂: p-value for comparing between HCV and HCV+HCC.

p₃: p-value for comparing between HCV and HBV

p₄: p-value for comparing between HCV and HBV+HCC.

p₅: p-value for comparing between HCV+HCC and HBV

p₆: p-value for comparing between HCV+HCC and HBV+HCC.

p₇: p-value for comparing between HBV and HBV+HCC * : Statistically significant at $p \leq 0.05$.

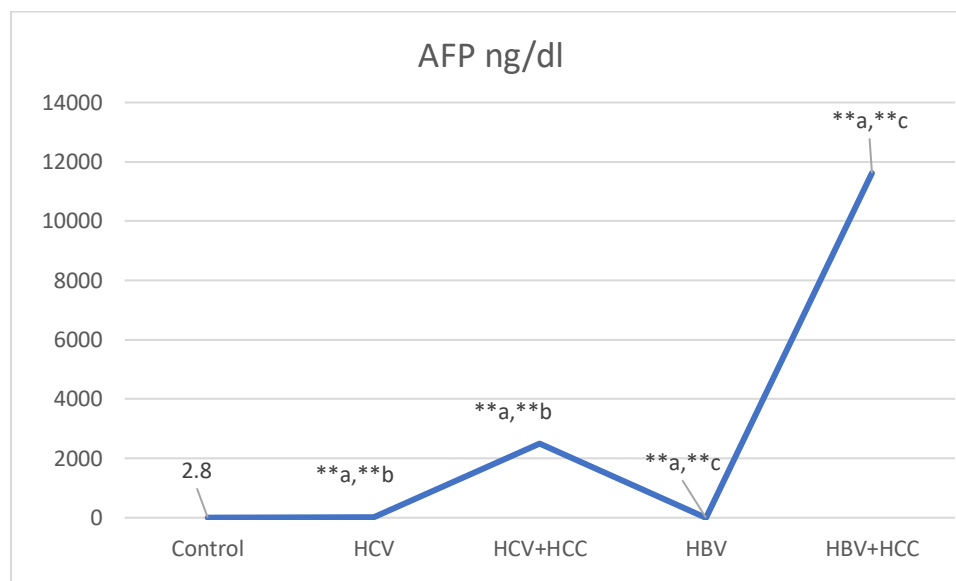


Figure (1): Levels of serum AFP in all studied five groups.

a: Significant from a control group
 b: Significant from between HCV & HCC+HCV
 ** (P<0.01)

4. Discussion:

In this study, the AFP levels had a statistically significant difference between the five groups (Control group, HCV, HCV+HCC, HBV, HBV+HCC Groups) with p -value<0.001*, compared to the control group the three groups G2 G3 G5 have the same a statistically significant difference with the same p -value <0.001*, while group 4 HBV patients have not statistically significant difference with p -value 0.254 compared to the control group.

Comparing between **HCV** and **HCV+HCC** groups AFP was highly increased in **HCV+HCC** from 15.07 ± 8.08 ng/ml to 2496.7 ± 2417.3 ng/ml with a significant statistical difference p -value 0.006*, AFP highly increasing from HBV group 4.07 ± 2.23 ng/ml to 11620 ± 19321 ng/ml in **HBV+HCC** group with a significant statistically difference between p -value <0.001*. But group **HCV+HCC** and group **HBV+HCC**, the two groups have a markedly high

level of AFP and no statistically significant difference between the two groups with a p -value of 0.207.

In this study AFP was highly elevated in HCC which is associated with HBV or HCV infection from healthy patients and infected patients without HCC, then AFP can be used as a biomarker for the diagnosis of HCC, Previous research has demonstrated that AFP-positive patients had poorer biological behavior and lower survival rates than AFP-negative patients (11), While AFP has been utilized as a marker for HCC diagnosis and prognosis, only 60% of HCC patients show positive AFP, according to (12).

Alpha-fetoprotein (AFP) is the most widely used biomarker for the detection of HCC, however, its diagnostic relevance is progressively being questioned because of its limited sensitivity, especially for early HCC, according to (13). 20% of HCC patients can often have readings over 400 ng/mL to confirm the

diagnosis so much research on miRNA-122 and other biomarkers for early diagnosis of HCC (14).

In this study, AFP elevation is more greatly associated with HBV-associated HCC (11620±19321) than HCV-associated HCC (2496.7± 2417.3), which is not correlated with Shen Q et al., who reported that AFP elevation is more greatly associated with HCV-associated HCC than HBV-associated HCC (15).

This study showed a significant elevation of AFP in HCV infected group to the healthy group and this elevation was less than in the HCC groups,

The incidence of elevated AFP in chronic hepatitis C (CHC) patients ranges from 10%-43% (16), AFP and HCV RNA levels were not correlated, according to Yang et al.'s analysis of 279 CHC patients (17).

In the current study, AFP levels are normal in the HBV infection without HCC progresses group with no significant increase compared with the healthy control group p-value was 0.254, This result is not correlated with the study by Toyoda H et al., who reported AFP levels that are elevated are typically seen as CHB without HCC progresses. Exacerbation of the underlying liver disease, with or without changes to the state of hepatitis B virus (HBV) replication, was the most frequent cause of AFP rise (18).

According to Kim et al., AFP normalization (< 20 ng/mL) was attained in 89.5% of treated patients, although it remained abnormal in 40.6% of patients who had never received antiviral therapy (16).

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Conflicts of Interest:

The authors affirm that there are no conflicts of interest.

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