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Lens Culinaris Agglutinin Reactive 3 Alpha Fetoprotein as a Diagnostic Serum Marker for Hepatocellular Carcinoma with Normal Alpha Fetoprotein Level

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Abstract: Hepatocellular carcinoma (HCC) is the second common cause of cancer incidence and death among Egyptian men. á-fetoprotein (AFP) may be elevated in many liver diseases and is not elevated in all patients with HCC, So more sensitive serum markers are needed for HCC diagnosis. AFP glycoform measuring is more important than measuring of AFP. There are three types of glycoforms, the most important one in diagnosis of HCC is lens Culinaris agglutinin reactive 3 alpha fetoprotein (AFPL3), as it is generated from malignant liver cells, its measurement helps to differentiate HCC from benign hepatic diseases. The aim of the present study was to improve outcome of patients with HCC by early diagnosis, through detection of AFP L3 in patients with normal level of AFP. This study included 82 patients divided into two groups: Group (A): 41 HCC patients with normal Alpha fetoprotein level, Group (B): 41 HCC patients with high level of Alpha fetoprotein. Tri-phasic liver Computed Tomography (CT) is a standardized procedure for the detection and characterization of HCC. Measurement of Human AFP-L3 by ELISA in serum by the kit (AFPL3 EIAab®, Catalog No: E1117h). Results showed that out of 82 patients 18 patients were females and 64 were males with mean age 63.5years old. AFP L3 at cutoff 2.89 had sensitivity of 100% and specificity of 87.8%, AUC=0.515 and accuracy of 93.2% according to this cutoff 95% of patients with AFP <30 ng/mL had AFP L3 =2.89 versus 5% of patients with AFP <30 ng/mL had AFP L3 >2.89, while 100% of patients with AFP >30 ng/mL had AFP L3 >2.89 versus 0% of patients with AFP >30 ng/mL had AFP L3 =2.89, this indicates that AFP L3 is a good diagnostic marker for HCC when AFP level is more than 30 ng/ml. Conclusion: There is a significant direct correlation between AFP level and AFPL3 level in patients with HCC. AFP L3 is a good diagnostic marker for HCC only when AFP level is more than 30 ng/ml.

Key words: HCC · Alpha fetoprotein · Alpha fetoprotein L3

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common types of cancers [1]. HCC is the second common cause of cancer incidence and death among Egyptian men [2].

Serum oncofetal glycoproteins like α-fetoprotein (AFP), is the primary monitor for liver disease progression into liver cancer. However, α-fetoprotein (AFP), can be produced in many liver diseases and is not elevated in all patients with HCC, so more sensitive serum markers are needed for HCC diagnosis [3].

AFP glycoform measuring is more important than measuring of AFP. There are three types of glycoforms, the most important one in diagnosis of HCC is lens Culinaris aglutinin reactive 3 alpha fetoprotein (AFPL3) [4]. As α-fetoprotein L3 (AFP-L3) is generated from malignant liver cells, its measurement helps to differentiate

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HCC from benign hepatic diseases [5].The aim of the present study was to improve outcome of patients with HCC by early diagnosis, through detection of AFP L3 in patients with normal level of AFP.

MATERIALS AND METHODS

This study was conducted on 82 patients admitted to Hepatology department, Theodor Bilharz Research Institute in the period from January 2016 till December 2016.

All patients were diagnosed as chronic liver disease (based on clinical, laboratory and imaging criteria) and HCC (based on triphasic CT Abdomen), NO biopsies were taken from the focal lesions for histopathology and the diagnosis of HCC was based only on the triphasic CT and patients were further divided into two groups:

- Group (A): 41 patients with HCC with normal Alpha fetoprotein level.
- Group (B): 41 patients with HCC with high level of Alpha fetoprotein.

Patients who had a history or recently discovered any other tumors were excluded from the study.

Clinical and laboratory workup was done to all patients including CBC, liver and kidney function tests, coagulation profile, hepatitis markers and measurement of serum AFP*(α-FP Ray Biotech®, Catalog No: ELH-AFP:)* which was measured by ELISA Kit for Alfa –fetoprotein (α-FP) provided by Ray Biotech, Inc. The upper limit of normal AFP is 30 ng/ml.

Measurement of Human α-fetoprotein Lens Culinaris Agglutinin 3 (AFPL3 EIAab®, Catalog No: E1117h): Intended use of this immunoassay kit allows for the *in vitro* quantitative determination of human Alpha-fetoprotein Lens Culinaris agglutinin 3, concentrations in serum, Plasma, Urine, tissue homogenates and Cell culture supernates and other biological fluids. Test principle; the microtiter plate provided in this kit has been pre-coated with an antibody specific to AFPL3. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for an Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of ?? in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Detection Range: 0.624 to 40 ng/mL.

All patients were subjected to abdominal ultra-Sonography (U/S).

Tri-phasic liver Computed Tomography (CT) is a standardized procedure for the detection and characterization of HCC. Tri-phasic spiral CT technique was developed to image the entire liver in arterial, portal and delayed phases. HCC has hyper enhancement arterial phase with rapid wash out in both portal and delayed phases.

Statistical Analysis: All data were analyzed using SPSS 19.0 for windows *(SPSS Inc., Chicago, IL, USA)* & MedCalc 13 for windows *(MedCalc Software bvba, Ostend, Belgium)*. Continuous variables were expressed as the mean ± SD & median (range) and the categorical variables were expressed as a number (percentage).

Continuous variables were checked for normality by using Shapiro-Wilk test.

Mann Whitney U (MW) test was used to compare non-normally distributed variables between two groups.

Percent of categorical variables were compared using the Chi-square (χ^2) test.

Spearman's rank correlation analysis was done between Alpha Fetoprotein L3 (AFP L3) and selected study parameters.

Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of Alpha Fetoprotein L3 (AFP L3) with maximum sensitivity and specificity in diagnosis of HCC in patients with normal AFP level.

All tests were two sided. $P< 0.05$ was considered statistically significant (S), $p < 0.005$ was considered highly statistically significant (HS) and $p \geq 0.05$ was considered not statistically significant (NS).

RESULTS

In the current study, as regard age and sex, there was no statistical difference in the mean of age and sex in both groups (Table 1).

Table 1: Demographic data of patients of both groups

	Group A (HCC with normal AFP) (N=41)		Group B (HCC with high AFP) (N=41)			
Demographic data	No	%	No	%	Test	p-value
Age (years)						
Mean ± SD	63.93 ± 11.07		63.59 ± 7.26		-0.845•	0.398 (NS)
Sex						
• Male	29	70.7%	35	85.4%	2.563‡	0.109 (NS)
• Female	12	29.3%	6	14.6%		

Table 2: Clinical picture of patients of both groups

	Group (A) (N=41)		Group (B) (N=41)			
Clinical picture	No	%	No	%	Test	p-value
Asymptomatic	7	17.1%	10	24.4%	0.668‡	0.414 (NS)
Right hypochondrial pain	28	68.3%	30	73.2%	0.236‡	0.627 (NS)
Anorexia & weight loss	23	56.1%	30	73.2%	2.614‡	0.106 (NS)
Splenomegaly	32	78%	35	85.4%	0.734‡	0.391 (NS)
Low grade fever	8	19.5%	13	31.7%	1.600‡	0.206 (NS)
L.L edema	28	68.3%	31	75.6%	0.544‡	0.461 (NS)
Hepatic Encephalopathy	16	39%	26	63.4%	4.881‡	0.027 (S)
Jaundice	27	65.9%	36	87.8%	5.549‡	0.018 (S)
Ascites	28	68.3%	35	85.4%	3.357‡	0.067 (NS)

Table 3: The associated conditions in both groups of patients

	Group (A)(N=41)		Group (B)(N=41)			
Associated conditions	No	%	No	%	Test	p-value
Smoking	15	36.6%	26	63.4%	5.902‡	0.015 (S)
Diabetes mellitus	14	34.1%	27	65.9%	8.244‡	0.004 (HS)

Table 4: Comparison between the studied groups as regard viral markers

	Group (A)(N=41)		Group (B)(N=41)			
Viral markers	No	%	No	%	Test	p-value
HBV	3	7.3%	0	0%	5.325‡	0.069 (NS)
HCV	36	87.8%	41	100%		
HBV & HCV	2	4.9%	0	0%		

Table 5: Child-Pugh classification in patients of both groups

	Group (A)(N=41)		Group (B)(N=41)			
Child-Pugh classification	No	%	No	%	Test	p-value (Sig.)
A	12	29.3%	3	7.3%	6.739‡	0.034 (S)
B	8	19.5%	9	22%		
C	21	51.2%	29	70.7%		

There is no significant association between level of AFP and clinical picture except for hepatic encephalopathy and jaundice (Table 2)

There was a significant association between level of AFP in relation to smoking and diabetes mellitus (Table 3).

In this study, there was no significant association between level of AFP and viral markers (Table 4).

The etiology of chronic liver disease in both groups was from viral origin either post HBV, HCV or mixed infection.

There was a significant association between level of AFP and Child-Pugh classification where 70.7% of patients with high AFP were Child C in comparison to 51.2% of patients with normal AFP (p=0.034) (Table 5).

In the current study, as regard liver and kidney function tests there were a statistical difference between both groups as regard the mean of AST, ALT and ALP and there were no statistical difference between both groups as regard the mean of albumin, bilirubin, PT and creatinine (Table 6).

Table 6: Liver and kidney function tests among patients of both groups

LFT & KFT	Group (A) (N=41)	Group (B) (N=41)	Test	p-value
Total serum bilirubin (mg/dl)				
Mean ± SD	3.80 ± 5.46	5.52 ± 6.51	-1.717•	0.086 (NS)
Direct serum bilirubin (mg/dl)				
Mean ± SD	2.15 ± 3.20	2.94 ± 3.52	-1.420•	0.156 (NS)
Serum albumin (g/dl)				
Mean ± SD	2.54 ± 0.73	2.36 ± 0.52	-1.115•	0.265 (NS)
AST (IU/L)				
Mean ± SD	108.29 ± 111.59	306.09 ± 541.23	-2.755•	0.006 (HS)
ALT (IU/L)				
Mean ± SD	49.29 ± 22.86	141.65 ± 213.88	-2.710•	0.007 (HS)
Alkaline phosphatase (IU/L)				
Mean ± SD	111.80 ± 175.44	109.68 ± 59.57	-2.752•	0.006 (HS)
PT (seconds)				
Mean ± SD	14.93 ± 3.82	15.77 ± 4.04	-1.620•	0.105 (NS)
Creatinine (mg/dl)				
Mean ± SD	1.34 ± 1.55	1.58 ± 1.20	-1.848•	0.065 (NS)

Table 7: imaging findings in patients of both groups

	Group (A)(N=41)		Group (B)(N=41)			
Imaging findings	No	%	No	%	Test	p-value
Cirrhosis	38	92.7%	40	97.6%	1.051‡	0.305 (NS)
Splenomegaly	32	78%	35	85.4%	0.734‡	0.391 (NS)
Ascites	29	70.7%	36	87.8%	3.636‡	0.057 (NS)
Number of focal lesion						
Mean ± SD	2.17 ± 1.54		1.97 ± 1.31		-0.622•	0.534 (NS)
Unifocal	21	51.2%	24	58.5%	0.443‡	0.506 (NS)
Multifocal	20	48.8%	17	41.5%		
Max. diameter of focal lesion (cm)						
Mean ± SD	4.44 ± 3.03		5.50 ± 4.10		-0.711•	0.477 (NS)
<3 cm	16	39%	16	39%	5.556‡	0.062 (NS)
3 – 5 cm	13	31.7%	5	12.2%		
>5 cm	12	29.3%	20	48.8%		
PVT	10	24.4%	17	41.5%	2.706‡	0.100 (NS)

There was no significant difference between both groups as regard maximum diameter of the focal lesion (Table 7).

There was no significant difference between both groups as regard maximum diameter of the focal lesion (Table 10)

AFP-L3 is expressed as a fraction from the total AFP concentration "(AFP-L3 / total AFP) x 100". Therefore it is an obvious finding that whenever AFP-L3 is elevated the total AFP would be elevated as well. The current study confirm these findings as the mean AFP-L3 in HCC with high AFP group was significantly higher than HCC with normal AFP group (22.98 vs 1.66, p<0.001) (Table 8).

In our study, 95% of patients with AFP <30 ng/mL had AFP L3 =2.89 versus 5% of patients with AFP <30 ng/mL had AFP L3 >2.89, while 100% of patients with AFP >30 ng/mL had AFP L3 >2.89 versus 0% of patients with AFP >30 ng/mL had AFP L3 =2.89, with sensitivity of 100%, specificity 87.8%, positive and negative predictive values (89.1% and 100%, respectively) (Table 9 and Fig. 1).

AFP L3 is a good diagnostic marker for HCC only when AFP level is more than 30 ng/ml.

There was no significant correlation between tumor size and both AFP &AFPL3 level.

In our study, There was a significant direct correlation between AFP and AFPL3 (r=+0.763, p<0.001) (Table 11 and Fig. 2).

And at the end, There was a significant direct correlation between AFP and AST, ALT, TSB, DSB, ESR, WBC (r: +0.310, +0.260, +0.255, +0.246, +0.489, 0.221 respectively) and an indirect significant correlation between AFP and hemoglobin (r: -0.306), but there was no significant correlation between level of AFP and age, serum albumin, PT, creatinine and platelet count (P value: 0.578, 0.139, 0.057, 0.277, 0.155 respectively).

Table 8: AFP and its isoform AFPL3 among patients of both groups

	Group (A)(N=41)		Group (B)(N=41)			
Tumor markers	No	%	No	%	Test	p-value
AFP (ng/dl)						
Mean ± SD	8.77 ± 5.95		8121.22 ± 27449.6		-7.798•	<0.001 (HS)
Median (range)	7 (2.4 – 26.49)		315 (32 – 121000)			
AFPL3 (%)						
Mean ± SD	1.66 ± 0.90		22.98 ± 5.05		-7.796•	<0.001 (HS)
Median (range)	1.83 (0.23 – 3.81)		24.88 (15.61 – 31.80)			
≤2.8	36	87.8%	0	0%	64.174‡	<0.001(HS)
>2.8	5	12.2%	41	100%		

Table 9: Validity of Alpha Fetoprotein L3 (AFP L3) as a diagnostic marker for HCC with normal Alpha Fetoprotein level

Cut-off values	SN % (95% CI)	SP % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy (95% CI)	AUC (95% CI)
AFP L3 >2.89	100% (91.3-100)	87.8% (83.5-99.4)	89.1% (43.5-72.2)	100% (88.5-100)	93.2% (83.8-99.2)	0.515 (0.402-0.627)

*p=0.829 (NS).

SN: Sensitivity; SP: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; AUC: Area under Curve; 95%CI: 95% Confidence Interval; p< 0.05 is significant.

Table 10: Relation between tumor size and both AFP &AFPL3

Size of focal lesion	AFP (ng/dl)	AFPL3 (%)
≤2 cm	253.84 ± 386.73	10.68 ± 11.28
2.1-4.9 cm	884.69 ± 2273.42	8.31 ± 8.75
≥5 cm	8212.30 ± 28562.24	15.22 ± 11.78
Test	1.974•	3.497•
p-value (Sig.)	0.373 (NS)	0.174 (NS)

• Kraskall Wallis H test.

p< 0.05 is significant.

Sig.: Significance.

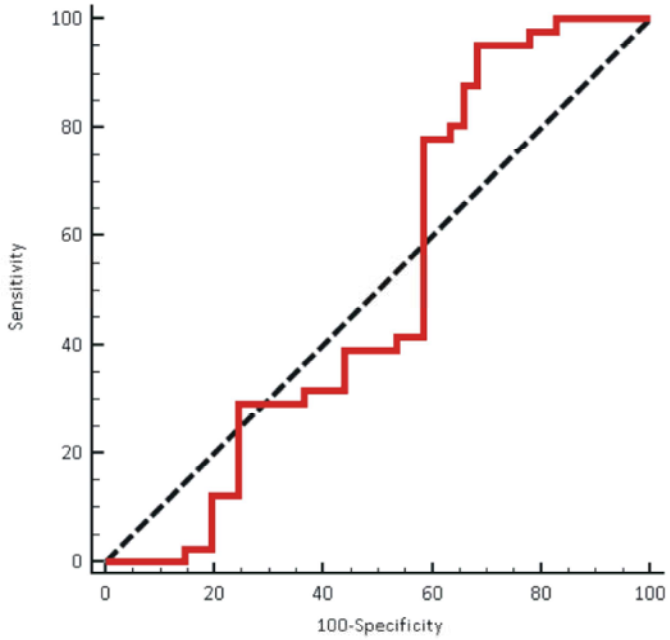

Fig. 1: Receiver operating characteristic (ROC) curve of Alpha Fetoprotein L3 (AFP L3) as a diagnostic marker for HCC with normal Alpha Fetoprotein level

Table 11: Correlation between AFP and AFPL3 in the studied groups

	HCC patients of both groups (N=82)
*r	+0.763
p-value (Sig.)	<0.001 (HS)

*r Spearman's rank correlation coefficient.
p< 0.05 is significant.
p< 0.005 is highly significant.
Sig.: Significance.

Table 12: Correlation between AFP/AFPL3 & selected study parameters.

	AFP (ng/dl)		AFPL3	
	*r	p-value (Sig.)	*r	p-value
Age (years)	+0.062	0.578 (NS)	-0.025	0.826 (NS)
AST (IU/L)	+0.310	0.005 (S)	+0.241	0.029 (S)
ALT (IU/L)	+0.260	0.018 (S)	+0.173	0.121 (NS)
Alkaline phosphatase (IU/L)	+0.203	0.068 (NS)	+0.311	0.004 (S)
*TSB (mg/dl)	+0.255	0.021 (S)	+0.158	0.157 (NS)
*DSB (mg/dl)	+0.246	0.026 (S)	+0.134	0.231 (NS)
Serum albumin (g/dl)	-0.165	0.139 (NS)	-0.096	0.390 (NS)
ESR	+0.489	<0.001 (HS)	+0.438	<0.001 (HS)
PT (seconds)	+0.211	0.057 (NS)	+0.183	0.101 (NS)
Creatinine (mg/dl)	+0.135	0.277 (NS)	+0.188	0.091 (NS)
Hemoglobin (g/dl)	-0.306	0.005 (S)	-0.219	0.048 (S)
Platelet count (x103/mm3)	-0.158	0.155 (NS)	-0.063	0.575 (NS)
WBC (x103/mm3)	+0.221	0.046 (NS)	+0.347	0.001 (S)
AFP (ng/dl)	---	---	+0.763	<0.001 (HS)

*r Spearman's rank correlation coefficient.
*TSB: Total Serum Bilirubin.
*DSB: Direct Serum Bilirubin.

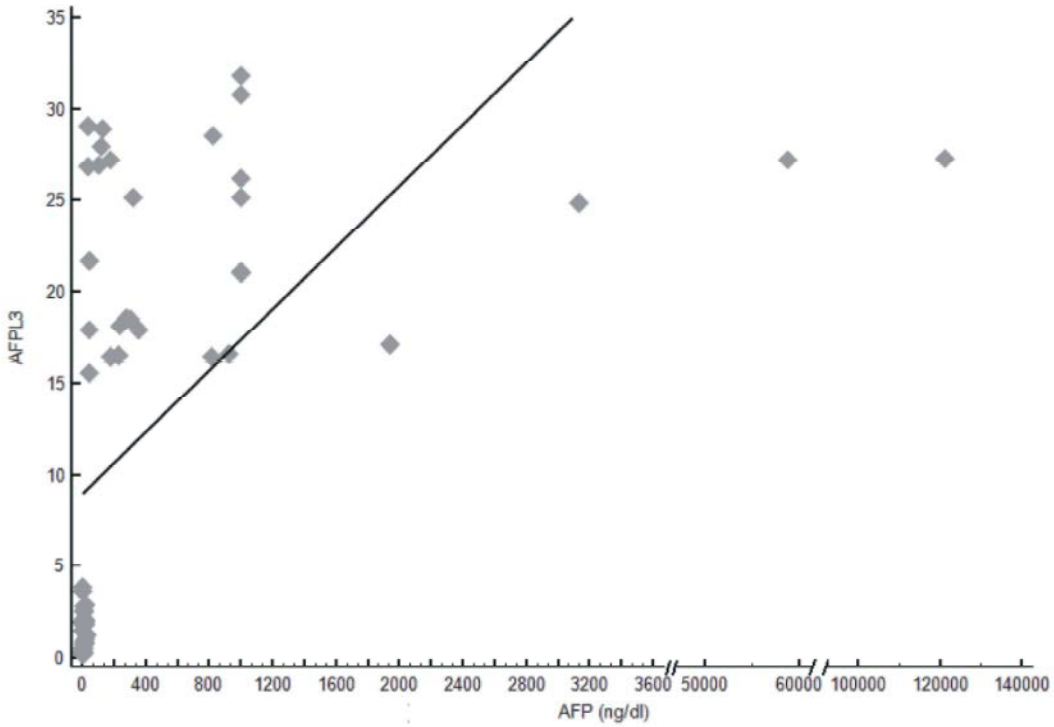

Fig. 2: Scatter plot with regression line showing correlation between AFP and AFPL3.

There was a significant direct correlation between AFPL3 and AST, alkaline phosphatase, ESR, WBC, AFP (r: +0.241, +0.311, +0.438, +0.347, +0.763 respectively) and an indirect significant correlation between AFPL3 and hemoglobin (r: -0.219), but there was no significant correlation between level of AFPL3 and age, ALT, TSB, DSB, serum albumin, PT, creatinine and platelet count (P value: 0.826, 0.121, 0.157, 0.231, 0.390, 0.101, 0.091, 0.575 respectively) (Table 12).

DISCUSSION

Multiple recent biomarkers for HCC have been discovered in recent decades for early detection of HCC, to prevent the danger of this disease, AFPL3 is one of the most important one of these markers which has been studied in details in the present study in comparison to AFP.

In our study, there was no significant association between level of AFP and clinical picture except for hepatic encephalopathy and jaundice (Table 2). These results are in concordance with Zakaria *et al*. [9], study as jaundice was reported in 63.7% of HCC patients. Most of the HCC patients suffered from repeated attacks of jaundice that may be partly of an obstructive type caused by compression of major intrahepatic bile ducts by the primary tumor or the common bile duct from nodal metastasis in the porta hepatis, but Chia-Yang *et al*. [10] study is against these results and it was reported that high serum AFP level was noted in HCC patients with higher grading of ascites ($p<0.001$), this may be due to most of patients selected were child C with decompensated liver.

There was a significant association between level of AFP and smoking (Table 3). These results are in consistent with Siegel *et al*.[11] who reported that 50.2% of patients with HCC had a history of smoking. Heavy smokers have an increasing risk about 50% higher than nonsmokers, this may be due to presence of cytochrome P450 system which is highly inducible by smoking mainly cytochrome 1A1 which is a type of cytochrome p450 that presents in the lung and is important for metabolizing polycyclic aromatic hydrocarbons inhaled by smoking converting these procarcinogen into active carcinogen by hydroxylation reactions [12].

In this study, there was a significant association between level of AFP and diabetes mellitus (Table 3). These results are in harmony with AbdAlla *et al*. [13] who stated that 58.1% of diabetic patients had AFP ≥200 ng/mL. The relationship between DM and the risk of HCC may be explained as early stages of type 2 diabetes, usually associated with production of insulin-like growth factor-1 (IGF-1) which stimulates cellular proliferation and inhibits apoptosis within the liver [14]. Also, insulin resistance leads to increased release of multiple pro-inflammatory cytokines, like tumor necrosis factor alpha (TNF-α), that favors hepatic steatosis, inflammation development and subsequent cancer within the liver [15].

In the current study, there was a significant association between level of AFP and Child-Pugh classification where 70.7% of patients with high AFP were Child C in comparison to 51.2% of patients with normal AFP ($p = 0.034$) (Table 5). This is consistent with Abdel -Aziz *et al*. [16], who found that AFPL3 was significantly($p<0.003$) correlated with child-Pugh classification of HCC, suggesting that it might be associated with the prognosis of the disease, but Abbasi *et al*. [17] study that was done on HCC patients classified into three groups (A, B and C) according to AFP level is against these results, showing a non-significant association between level of AFP and Child-Pugh classification where 50.9% of patients with AFP=400ng/ml (Group A), 72% of patients with AFP 21-399 ng/ml (Group B) and 40.9% of patients with AFP=20ng/ml(Group C) are child C ($p=0.078$).

In the current study, as regard liver and kidney function tests there were a statistical difference between both groups as regard the mean of AST, ALT and ALP and there were no statistical difference between both groups as regard the mean of albumin, bilirubin, PT and creatinine (Table 6). Also, there was a direct significant correlation between level of AFP and liver enzymes (AST and ALT) (Table 12).These results are consistent with Richard *et al*. [18] who reported that, the mean AST and ALT in HCC patients with high AFP group was significantly higher than HCC patients with normal AFP group (114 vs 82.04 IU/L, $p<0.011$) and (81 vs 50 IU/L, $p<0.034$) respectively.

The significant elevation of both AST and ALT with high level of AFP may be due to their release from damaged hepatocytes into blood and their activities have been widely recognized as effective tools to detect liver diseases like HCC [19]. Also, elevation of AST level is more common with HCC as, AST presents in cytoplasm (20%) and mitochondria (80%) and mitochondrial dysfunction has been regarded as a hallmark of HCC [20].

AFP-L3 is expressed as a fraction from the total AFP concentration "(AFP-L3 / total AFP) x 100" [6-8]. Therefore it is an obvious finding that whenever AFP-L3 is elevated the total AFP would be elevated as well. The current

study confirm these findings as the mean AFP-L3 in HCC with high AFP group was significantly higher than HCC with normal AFP group (22.98 vs 1.66, p<0.001) (Table 8), these findings also are in consistency with Zakaria *et al*. [9] who reported that when AFP-L3 is elevated the total AFP would be elevated as well and there is a significant elevation in each of the median AFP 1327 ng/dl (20-12380) and the median of AFP-L3 in HCC patients 44(5.5-77.2)%.

The next logic question to raise, how would AFP-L3 add benefit in the diagnosis of HCC?, Could AFP-L3 offer more diagnostic value in HCC patients?, could it offer more yield in the grey zone patients where AFP is not helpful (10-200 ng/dl)? and finally does it correlate with any of the clinical or laboratory parameters or even the tumor behavior of the studied cases?

In the present study, 95% of patients with AFP <30 ng/mL had AFP L3 ≤2.89 versus 5% of patients with AFP <30 ng/mL had AFP L3 >2.89, while 100% of patients with AFP >30 ng/mL had AFP L3 >2.89 versus 0% of patients with AFP >30 ng/mL had AFP L3 ≤2.89, with sensitivity of 100%, specificity 87.8%, positive and negative predictive values (89.1% and 100%, respectively) (Table 9), this indicates that AFP L3 can't be used as a diagnostic marker for patients with AFP<30 ng/mL as only 5% of these patients had AFP L3 >2.89 but it is a good diagnostic marker for patients with AFP level >30 ng/ml and <200 ng/ml. These findings are in concordance with Toyoda *et al*. [5], Zakaria *et al*. [9], Sterling *et al*. [18], Wang *et al*. [21], Marrero *et al*. [22], Jonghyeon *et al*. [23] and Jiang *et al*. [24].

In Toyoda *et al*. [5] study that compared AFP and AFP-L3% in 685 patients with HCC, they found that when serum AFP was above the cutoff level (20 ng/dL), the serum AFP-L3% was above the cutoff (10%) suggesting the utility of AFP-L3% may be limited to those with elevations in total AFP.

Jonghyeon *et al*. [24] stated thatAFP-L3 in HCC patients with AFP<20 ng/mL was significantly lower than in patients with AFP=20-=500 (Median: 9% vs 21%, p=0.023).

In Zakaria *et al*. [9] study the performance characteristics of total AFP and AFP-L3% for the studied patients showed a sensitivity, specificity, positive predictive value and negative predictive values of AFP at cutoff value of 22.65ng/ml of 83, 83, 79 and 96% respectively. Comparing to AFPL3 at cutoff value of 5% yielded, increasing of sensitivity to 96% and comparable specificity 83%, positive and negative predictive values (78 and 97%, respectively).

Jiang *et al*. [24] demonstrated that AFPL-3 greatly improved the diagnosis of HCC with diagnostic accuracy (AUC=0.953) and diagnostic values of 94.4% sensitivity and 88.9% specificity.

Wang *et al*. [21] reported thatAFP-L3 provides a moderately high sensitivity and high specificity in the detection of HCC for with high AFP levels.

Sterling *et al*. [18] concluded that the high specificity of AFP-L3% persisted among patients with elevated AFP (20-200 ng/mL) and suggests that AFP-L3% has clinical utility in HCV patients with AFP of 20-200 ng/mL.

CONCLUSIONS

- There is a significant direct correlation between AFP level and AFPL3 level. AFP L3 is a good diagnostic marker for HCC when AFP level is more than 30 ng/ml.

Finally we recommend: Extended studies should be done, using AFPL3 in combination with other diagnostic biomarkers of HCC and assess its utility in HCC cases with normal AFP, Another AFPL3 study should be performed to follow up HCC cases after radiofrequency or alcohol injection and compare its results with that of hepatic imaging studies.

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