Role of AFP-L3 in Diagnosis of Atypical Hepatocellular Carcinoma in Upper Egypt Patients

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Abstract: Background: Hepatocellular carcinoma (HCC) is a common worldwide health problem. The diagnosis is based on typical radiological imaging pattern and elevated tumor markers. Sometimes, atypical HCC on imaging represents a diagnostic challenge. **Aims of the work:** To evaluate the role of AFP-L3 in diagnosis of HCC in atypical imaging patients in upper Egypt. **Patients and methods:** The study included fifty three cirrhotic patients with hepatic focal lesions and atypical radiological picture by triphasic CT, recruited from Hepatology Clinic of Sohag University Hospital. Forty three patients were cirrhotic with positive HCV and ten were cirrhotic with positive HBV. All of them were diagnosed as HCC by percutaneous core biopsy at the Internal Medicine and Tropical Department of Sohag University Hospital. All patients were subjected to history taking, complete clinical examination, abdominal ultrasound, CT and laboratory tests including liver function tests, prothrombin time, Anti-HCV, HBs-Ag, AFP and AFP-L3. **Results**: The mean value of AFP in all patients was 86.61±11.31ng/ml. Patients were classified into two groups based on AFP concentration: group (1), included 20 patients with AFP concentration up to 20 ng/ml (39.62%) with a mean value of 15.07±0.73. Group (2): included 33 patients with AFP concentration > 20 ng/ml (60.38%) with a mean value of 129.97±13.36.The mean value of AFP-L3 concentration in all patients was 11.62±1.13ng/ml with mean percentage of 12.29±2.19%. The mean value of AFP-L3 concentration in group (1) was 1.3±0.27 ng/ml and mean percentage 7.37±1.34%. The mean value of AFP-L3 concentration in group (2) was 18.95±2.97 ng/ml and mean percentage 14.2±1.46%. The number of patients with AFP-L3% >10% were twenty four patients (45.28%) out of all patients. In group (1) Seven patients out of twenty (35%) had AFP-L3 level >10%. In group (2) seventeen patient out of thirty three (51.5%) had AFP-L3 level >10%. **Conclusion:** AFP-L3 has an important role in diagnosis of HCC with atypical radiological picture. In addition, AFP-L3 ≥ 10 % could be a diagnostic tool for HCC and can replace the invasive liver biopsy specially in patients with atypical radiological picture by CT.

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1. Introduction:

Hepatocellular carcinoma (HCC) is one of the most prevalent cancer in humans. It is the fifth most common neoplasm in the world and the third most common cause of cancer- related death **[1]**. The estimated incidence of a new cases is about 500.000 - 1000.000 per year, causing 600.000 deaths globally per year **[2]**. Chronic infection with HBV and HCV are major risk factors for HCC. HCV is the most important risk factor for HCC in Western European and North American countries, liver cancer has a higher prevalence in patients with HCV associated cirrhosis than in non-viral etiologies of chronic liver disease **[3]**.

Egypt has the highest prevalence of HCV infection in the world**[4]**. However, we don't have a national screening program for early diagnosis of HCC in cirrhotics. Early detection of HCC is an essential issue since HCC patients can receive effective and curative treatment such as hepatic resection, percutaneous ablation and liver transplantation **[5].** Advances in imaging, such as a Multidetector Computed Tomography (MDCT) and Magnetic Resonance Imaging (MRI), have improved the early radiologic diagnosis of HCC**[6]**. However, atypical radiological findings in some patients can be challenging. AFP is the only generally available serologic marker for HCC surveillance, diagnosis and monitoring, serum AFP < 10 ng/ml is expected in healthy men and non- pregnant women**[7]**. AFP has limited sensitivity and specificity for HCC, as transient increase and fluctuations in serum AFP may occur in a considerable number of patients with chronic liver disease and cirrhosis, especially during exacerbation of hepatitis**[8]**. Two-third of patients with small a symptomatic tumors will have an AFP < 200 ng/ml **[9]**.

The lens culinary is agglutinin-reactive fraction of AFP (AFP-L3) have become widely used for HCC diagnosis and follow up as serological tumor maker in

Japan. AFP-L3 is a variant of AFP, based on the sugar chain structure and has an additional alpha 1-6 fructose residue appended to N-acetyl glucosamine at the reducing end, and AFP-L3 production is observed mainly in malignant cells **[10,11]**. AFP-L3 is highly specific for HCC, one study reported a specificity of 100% and sensitivity of 73% for the disease**[12]**. Malignant liver cells produce AFP-L3 even when HCC is at its early stages. AFP-L3 can be detected in the serum of approximately 35% of the patients with small HCC (< 2cm) and it has shown to be associated with the aggressiveness of HCC**[13]**. HCC patients with an increased AFP-L3% have a poorer prognosis than patients with AFP-L3 negative, and should be more aggressively treated and closely followed up **[14]**. Recently, many publications discussing the role of AFP-L3 in diagnosis of HCC **[14-18]**, but no reports on the significance of AFP-L3 in HCC among Egyptians patients especially in atypical HCC on dynamic imaging.

Aim of the work:

To study the role of AFP-L3 level in HCC patients with atypical radiological findings.

2. Patients and methods:

Patients:

This study included fifty three cirrhotic patients with hepatic focal lesions and atypical radiological picture by triphasic CT, recruited from hepatology clinic of Sohag University Hospital. Thirty nine males and fourteen females, their ages ranged from 40-62 year with a mean value of 52.96±0.74. Forty three patients were cirrhotic and positive HCV and ten were cirrhotic with positive HBV. All of them were diagnosed as HCC by percutaneous core biopsy at the Internal Medicine and Tropical Department of Sohag University Hospital. The study was approved by the Ethical committee and a written informed consent was obtained from all cases in accordance with Sohag University Hospital ethical committee guide lines.

Inclusion Criteria:

1-All patients with HCC who have atypical radiological findings on dynamic imaging.

2- Histologically proven HCC by needle biopsy.

Exclusion criteria:

Patients with typical radiological findings diagnostic for HCC according to guidelines**[5]**.

Methods:

Blood was drawn into standard citrate, lithium heparin and plain vacutainers for the assay of prothrombin, liver function, AFP and AFP-L3 respectively. Separated serum was divided in aliquots of 400 u, one aliquot was used for AFP estimation, the rest of aliquots were stored at -70 to be used for AFP-L3 estimation.

All cases were subjected to the following:

History taking and complete clinical examination, abdominal ultrasonography, CT and routine lab tests including: liver function tests (ALT, AST, total bilirubin, albumin) were done by the use of Cobas-C311 (Roche Diagnostics, Mannhium, Germany), Prothrombin time (PT) was done by the use of Siemens thrombore lS reagent on Siemens Sysmex CA-1500 autoanalyzer (Siemens Healthcare Diagnostics Gmbh, Eschbom, Germany),HCV antibodies and HBs-Ag were done by the use of Architect anti HCV and HBs-Agkits using Architect 2000 system (ABBOTT Diagnostics, Dallas, USA). Specific investigations including: AFP and AFP-L3. AFP was done by the use of AFP kits using Architect 2000 system (ABBOTT Diagnostics, Dallas, USA).

AFP-L3 level determination was done using ELISA kit (Wuhan EIA-ab science co; Ltd. Wuhan, China). The micro titer plate provided in this kit has been pre-coated with an antibody specific to AFP-L3. Standards or samples are then added to the appropriate micro titer plate wells with a biotin-conjugated polyclonal antibody preparation specific for AFP-L3. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each micro plate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain AFP-L3, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is determined by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of AFP-L3 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Serum AFP-L3%, which is the ratio of AFP-L3 to total AFP was calculated. The cut-off value for a positive test was set at 10% according to previous studies.

Statistical analysis:

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS-version 19). Quantitative data were expressed as mean ± SE. Qualitative data were described in the form of number and percentage. Correlation Coefficient (r) was used for showing positive and negative correlation between variables.

3. Results:

This study included fifty three cirrhotic patients with histologically proven HCC, thirty nine male (73.58%) and fourteen female (26.42%), forty three were cirrhotic with positive HCV-antibodies (81.13%) and ten were cirrhotic with positive HBV-surface antigens (18.87%).

Liver function tests and PT of the patients are presented in Table (1).

Table (1): liver function tests and PT of all patients

	Mean±SESE+SD
ALT(IU/ml)	45.5±0.98
AST(IU/ml)	66.94±1.6
S.Albumin(g/dl)	2.6±0.09
Totalbilirubin(mg/dl)	4.2±0.36
PT(seconds)	17.4±0.49

The mean concentration of AFP in all patients was: 86.61±11.31ng/ml. Patients divided into two groups: group 1, included 20 patients with AFP concentration up to 20 ng/ml (39.62%)and a mean value of 15.07±0.73ng/ml and group 2, included 33 patients with AFP concentration > 20 ng/ml (60.38%) and a mean value of 129.97±13.36ng/ml. Group 2 subdivided according to AFP concentration as follow: group-A included seventeen patient with AFP level from > 20-100 ng/ml (30.19%), Group-B included ten patients with AFP level from >100-200 ng/ml (18.87%) and group-C included six patients with AFP level from> 200-300 ng/ml(11.32%). Table (2).

Table (2): Serum concentration of AFP in all patients

	Number of patients	Frequency (%)	AFP (ng/ml)Mean±SE
Total patients:	53	100	86.61±11.31
Group-1	20	39.62	15.07±0.73
Group-2	33	60.38	129.97±13.36
A:Up to 100 ng/ml	17	30.19	±5.8567.59
B:> 100-200 ng/ml	10	18.87	±7.41161
C:> 200-300ng/ml	6	11.32	±12.11255

Mean AFP-L3 concentration in all patients was 11.62±1.13ng/ml (12.29±2.19%). The number of patients with AFP-L3> 10% were twenty four patients (45.28%). In group-1 seven patients out of twenty (35%) had AFP-L3 level >10% with a mean concentration of 1.3±0.27ng/ml and a mean percentage of (7.37±1.34%). In group-2 seventeen patient from thirty three (51.52%) had AFP-L3 level >10% with a mean concentration of 18.95±2.97ng/ml and a mean percentage of 14.2±1.46%.

In group 2 (A) nine patients out of seventeen had AFP-L3% > 10% representing 52.94% from the patients in this group. In group 2 (B) five patients out of ten had AFP-L3% > 10% representing 50% from the patients in this group. In group 2 (C) three patients out of six had AFP-L3% > 10% representing 50% from the patients in this group, table (3).

Table (3): The AFP-L3in all patients

Patients groups	Number of AFP-L3> 10% (%)	AFP-L3(ng/ml) Mean±SE	AFP-L3 % (Mean±SE)
Total patients(53)	24 (45.28)	11.62±8.21	12.29±2.19
Group-1 (20)	7 (35)	1.3±0.27	7.37±1.34
Group-2(33)	17 (51.52)	18.95±2.9718.95±17.08	14.2±1.46
A:(17)	9 (52.94)	11.38±0.95	±1.5818
B: (10)	5 (50)	38.8±**5.33**	±2.6424.2
C: (6)	3 (50)	48.3±6.67	±3.1219.2

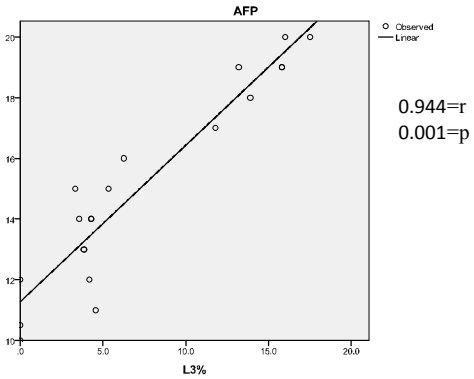

Fig. 1: Correlation between AFP and AFP-L3% in group one patients.

Correlation between AFP concentration and AFP-L3% in our patients were performed. There was highly significant positive correlation between AFP and AFP-L3% in patients with AFP up to 20 ng/ml (group 1), whereas there were insignificant correlation between them in patients with AFP level > 20 ng ml (group 2), (r: 0,944, 0.117 p=0.001, 0.517)respectively, fig. (1).

4. Discussion:

HCC is the commonest primary cancer of the liver and the fifth commonest malignancy worldwide and the third leading cause of cancer-related death worldwide exceeding only by cancers of the lung and stomach**[4]**.

Detection of HCC at early stages is critical for good clinical outcome as the prognosis of HCC patients is very poor when diagnosed at late and symptomatic stages**[13]**. Although serum AFP is the most established tumor markers in HCC it was found to be normal in about 30% of the patients, especially those who are at early stages and its sensitivity depend on the cut-off value**[13]**. All these factors limit its utility as an early indicator of HCC**[19]**. Although AFP-L3% are widely recognized as useful tumor markers for HCC in Japan and USA no comparative data on AFP-L3% in Egyptian HCC population have been published therefore, an assessment of this marker in Egyptian HCC population is necessary to determine the clinical usefulness of AFP-L3% in diagnosis of HCC in atypical HCC on dynamic imaging.

In this study, we found that the percentage of males and females with HCC was 73.58% and 26.42% respectively and this result agreed with Parkin *et al.* **[20]**, who reported that about of 71% of the new cases of HCC are men. Also our result reported that 81.13% of the cases were HCV positive and 18.87% were HBV positive and this result is in accordance with another study, Gomaa *et al.* **[4]**, who reported that liver cancer has a higher prevalence in patients with HCV associated cirrhosis than in non viral etiologies of chronic liver disease and reported that HBV and HCV account for over than 80 % of liver cancers world wide. The current result also matched with El-Serag and Mason **[3]**, who reported that HCV infection was detected in 76% of patients with HCC.

In our study we found that 39.62% of the cases have AFP level up to 20 ng/ml, and this agree with the study of Yao *et al.* **[21]**, who reported that AFP level may remain in the normal range in 15 to 30% of patients with HCC. Also we found that 60.38 % of patients have AFP level from > 20-300 ng/ml. Dusheiko**[3]**, reported that two- third of patients with small asymptomatic tumors will have an AFP < 200 ng/ml. In our study we reported that twenty four patients out of fifty three histologically proven HCC patients had AFP-L3% >10%, this means that the sensitivity of AFP-L3% in the diagnosis of HCC inpatients with atypical radiological picture was 45.28%, this sensitivity decreased to 35% in patients with AFP level up to 20 ng/ml and increased to 51.52% in patients with AFP>20 ng/ml. Different studies as the study of Stefaniuk *et al.* **[13]**, reported that AFP-L3 can be detected in the serum of approximately 35% of the patients with small HCC (< 2cm), Kobayashi *et al.* **[22]** and Oda *et al.* **[23]**,reported that the sensitivity for AFP-L3% in diagnosis of HCC was 40.3% and 44.6% respectively at a cut off 5% with AFP concentration less than 20 ng/ml, using highly sensitive AFP-L3 (hs-AFP-L3), by the use of fully automated immunoassay system using microchip capillary electrophoresis, micro-total analysis system (uTAS). Kobayashi reported that conventional method using automated analyzer called LiBASYs (Liqued-phase Binding Assay System)was enable to determine AFP-L3%in patients with AFP less than 20 ng/ml, whereas Oda reported that the sensitivity of AFP-L3% by conventional method in diagnosis of HCC in patients with AFP less than 20 ng/ml was 12.5%. Some found low sensitivity of AFP-L3%, as a result of Nouso *et al.* **[24]**, who reported that the positivity of AFP-L3 in HCC patients was 13.3% at a cut-off 10%, however they used hs-AFP-L3. Others detected high sensitivity of AFP-L3, as a result of Haydon *et al.* **[12]**, who reported that the sensitivity of AFP-L3% in the diagnosis of HCC is 73% and the result of Carr *et al.* **[25]**, who reported that the sensitivity of AFP-L3 is 61.6%. The discrepancy between previously mentioned studies and our study can be explained by the using of different cut off values, as some used 5%**[22,23]** versus 10% in our study. Also the discrepancy may be due to different measuring techniques, some used hs-AFP-L3**[22,23,24],** other used conventional method by LiBASYs**[12,13,25]** versus ELISA used by us. The discrepancy may be also explained by the patients criteria as our patients had atypical radiological finding.

Conclusion:

AFP-L3 has an important role in diagnosis of HCC with atypical radiological picture.

AFP-L3 ≥ 10 % could be diagnostic for HCC and can replace the invasive liver biopsy in patients with atypical radiological picture by CT.

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