Diagnostic Value of Serum Heat Shock Protein 70 in Hepatocellular Carcinoma Patients

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Background and study aim: Hepatocellular carcinoma (HCC) is the commonest essential hepatic threat among adult. Nowadays, the HCC determination without obsessive relationship is done by imaging methods. To elucidate the role of heat shock protein 70(HSP70) in the diagnosis of HCC.

Subjects and Methods: This case control study was achieved in Internal Medicine and Clinical Pathology Departments, Zagazig University, Egypt. It involved 99 participants divided into three groups; control group, cirrhotic patients and cirrhotic patients with HCC. Participants underwent complete history taking, comprehensive clinical examination, laboratory investigations including viral markers and alpha-fetoprotein. HSP 70 level was calculated via the enzyme-linked immunosorbent assay (ELISA) technique. Radiological investigations including abdominal ultrasonography and triphasic CT scan were done.

Results: There was a non-significant difference between the studied groups concerning demographic characteristics. There was a significant difference between them regarding hemoglobin, platelet count, liver and kidney function tests and coagulation profile($p < 0.05$). Also, there was a significant difference between them as regards HSP 70, and AFP with the maximum values in HCC group. HSP 70 at cutoff ≥ 120 ng/ml can diagnose HCC at sensitivity 85%, specificity 50%, and accuracy 84% ($p < 0.05$). AFP at cutoff ≥ 20 ng/ml can recognize HCC with sensitivity 87.5%, specificity 75.8% and accuracy 89%. Combined HSP 70 and AFP increase the sensitivity of diagnosis at 91.5% and accuracy to 93%.

Conclusion: HSP 70 as a serum biomarker can be used with AFP to increase the accuracy of HCC diagnosis.

INTRODUCTION

Hepatocellular carcinoma is the fifth most common tumor among males and the seventh most malignant growth among females. Hepatitis B and hepatitis C diseases are the most basic hazard factors for HCC [1].

In 2001, non-invasive imaging technique was accepted to diagnose HCC in presence of a cirrhotic liver [2].

As early small lesions are asymptomatic and additionally, there is shortage of satisfactory diagnostic and screening strategies, most patients (>80%) present with an advanced phase. At the present, serum alpha-fetoprotein (AFP) level and ultrasonography are the most utilized screening procedures in cirrhotic patients [3].

There are about 30% of those patients with normal serum AFP levels are hardly diagnosed before any clinical manifestations appear, so, AFP alone is restricted and poorly reliable for early diagnosis of HCC [4].

This highlights the necessity for emerging prognostic and accurate diagnostic biomarkers for HCC. Tumor markers detection in human serum is the most reliable method because it is suitable, noninvasive, inexpensive and clear-cut**.** The seventy kilodalton warm stun proteins (Hsp70s) are a bunch of ubiquitously communicated warm stun proteins exist in for all intents and

purposes all living beings. HSP 70 family of proteins are thought to be effective buffering framework for cellular strain, either from outward (physiological, viral and natural) or inborn (replicative or oncogenic) boosts. As such, this family serves a genuine survival work in the cell. Not incredibly, cancer cells depend on this buffering framework for survival [5-8].

Serum HSP70 levels serially increased in patients with chronic hepatitis, liver cirrhosis, and liver carcinomas, revealing a possible prognostic value [6], and are also typically positive in Intrahepatic Cholangiocarcinoma (IH-ChCa) and metastatic tumors [7].

HSP70 expression was higher in the four sorts of HCC cell lines compared to the normal cells, in agreement within HCC tissues specimens and other types of malignant tumors, including lung cancer, breast cancer and colorectal carcinoma [8]. So our study aimed to illuminate the role of HSP 70 in the diagnosis of HCC.

SUBJECTS AND METHODS

Study design and site:
A case control study was done in Internal Medicine and Clinical pathology Departments, Faculty of Medicine, Zagazig University, during the period from January 2017 to August 2018.

Study population:
A total number of 99 contributors were involved and categorized into three main groups: Group 1 apparently healthy individuals, Group 2 liver cirrhosis and Group 3 HCC.

Inclusion criteria for patients:
1- Patients with Post-hepatitis C Cirrhosis [9].

2- Patients with newly diagnosed HCC (Stage A and B) following Barcelona clinic liver cancer staging [10].

Exclusion criteria for patients:
History of other malignancies, autoimmune liver diseases, chronic HBV, NAFLD and primary biliary cirrhosis. History of acute and chronic inflammatory diseases and sepsis. Also, patients with COPD, bronchial asthma, glomerulonephritis, diabetes mellitus, stroke and seizure-related events were excluded.

All subjects of the study were subjected to the following:
Full history taking, detailed clinical examination and routine laboratory investigations (complete blood picture, liver function tests, renal function tests,HbA1C,random blood glucose, Coagulation profile (PT, PTT, and INR), viral marker: (HBs Antigen, HBc antibody and HCV Antibody). Also the other investigations required for accomplishing the exclusion criteria. Pelvi abdominal ultrasonography was done for all participants but abdominal Triphasic CT was done for all patients.

Measurement of AFP:
Blood sample (3cm) was taken from every subject and then centrifuged, and serum was used for quantifying AFP by cobas 8000 (e602).

Measurement of Heat Shock Protein 70 by ELISA:
Four ml of peripheral venous blood samples were taken by venipuncture from all patients and healthy controls. One ml blood in container enclosing EDTA for CBC then the samples were left to clot serum was separated. It was stored at -80°C until measuring HSP 70 level.

Statistical Analysis
All information were collected, arranged and factually analyzed utilizing SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Quantitative information were expressed as the cruel ± SD& middle (extend), and subjective information were communicated as outright frequencies (number) & relative frequencies (%). Nonstop information were checked for ordinariness by utilizing the Shapiro Walk test. Mann-Whitney U was utilized to compare two bunches of non-normally conveyed information. To compare implies of more than two bunches, one way ANOVA was utilized (when information is ordinarily disseminated). Kruskal Wallis test was utilized to compare medians of more than two bunches (when data isn't regularly dispersed). ROC bend was used to identify the most excellent cutoff value of HSP 70 and AFP within the conclusion of HCC. A p-value <0.05 is considered statistically significant, while p≤0.001 is considered statistically highly significant.

RESULTS

There's a measurably non-significant distinction between the considered patients as respect age, sex and BMI (Table 1).

There was a statistically significant difference between the studied groups as concern hemoglobin level, PT, PTT, INR; platelets count total and direct bilirubin, ALT, AST, serum albumin and creatinine . However, there was a statistically non-significant difference between them regarding TLC (Table 2).

There was non- significant difference in Child-Pugh score classification between HCC and the liver cirrhotic patient groups (Table 3).

Our study displayed that there is a highly statistically significant difference between the studied groups as regard Heat shock protein 70 and AFP. The group of patients with HCC had the maximum levels of both markers (Table 4).

Performance of HSP 70 and AFP in the diagnosis of HCC among the studied patients at a cutoff of HSP 70 more than or equal 120ng/ml, it can diagnose HCC with sensitivity 85%, specificity 50%, PPV 80%, NPV 75% and accuracy 84%.At a cutoff of ≥ 20 ng/ml, AFP can diagnose HCC at a sensitivity of 87.5%, specificity 75.8%, PPV 66.7%, NPV 89% and accuracy 89%. Using combined cutoff of both markers, the sensitivity increases up to 91.5% with specificity 65.8%, PPV 76.7%, NPV 91 and accuracy 93% (Fig 1 and Table 5).

Table (1): Comparison between the studied groups regarding demographic and anthropometric data

Variable	Group1 Control(33)	Group2 Cirrhotics (33)	Group3 HCC (33)	F test	p
Age(years) mean ± SD Range	56.7±5.1 (45-64)	57.6±5.2 (49-67)	59.9±4.7 (52-68)	1.2	0.08
BMI(kg/m$^{2)}$) mean ± SD Range	23.9±2.3 (21.5-28)	23.9±2.7 (19.5-29.5)	21.4±2.2 (17.5-26.3)	1.5	0.07

Variable	Group1 N (%)		Group2 N (%)		Group3 N (%)		χ^2	p
Sex: Male (58) Female (41)	15 18	45.5 54.5	22 11	66.7 33.3	21 12	63.6 36.4	3.5	0.16

Table (2): Comparing laboratory investigations between the studied groups

Variable	Group 1 Control (33) mean ± SD Range	Group 2(33) Cirrhotic patients mean ± SD Range	Group 3 HCC (33) mean ± SD Range	F	p
Hb (g/dl)	12.6±1.3 (10.9-14.7)	11.2±1.1 (9.2-14)	10.4±1.3 (8.1-14.4)	F=33	**0.001**
TLC ($x10^3/mm^3$)	7.1±0.9 (5.6-9.1)	7.2±1.8 (4.2-10.5)	7.4±8.7 (1.9-54)	KW 3.7	0.8
Platelets ($x10^3/mm^3$)	287.70±63.14 (179-423)	105.09±41.24 (63-254)	110.21±48.48 (58-225)	F=133.2	**<0.001**
PT (Sec)	10.38±0.45 (10.0-11.3)	14.29±1.60 (11.7-18.0)	14.72±1.66 (11.7-18.0)	102.8	**<0.001**
PTT (Sec)	29.63±0.91 (28.8-32.3)	38.55±4.72 (31.0-45.0)	39.97±3.98 (31.0-45.0)	79.7	**<0.001**
INR	1.04±0.13 (0.9-1.2)	1.51±0.40 (1.0-2.7)	1.68±0.51 (1.0-2.8)	24.7	**<0.001**
Total bilirubin (Mg/dl)	0.92±0.15 (0.6-1.2)	2.20±0.76 (0.8-3.6)	2.38±0.76 (1.0-4.0)	53.1	**<0.001**
Direct bilirubin (Mg/dl)	0.30±0.11 (0.1-0.5)	0.87±0.38 (0.2-1.7)	0.99±0.43 (0.3-1.8)	40.3	**<0.001**
ALT (IU/L)	24.3±4.5 (15-31)	25.3±7.8 (15-50)	43.9±12.3 (35-80)	42	**0.001**
AST (IU/L)	23.8±4.9 (13-31)	28.3±6.2 (13-42)	40.9±18.8 (30-92)	67.2	**0.001**
Albumin (g/dl)	4.3±0.3 (3.8-5)	3.1±0.48 (2.1-4)	2.8±1.4 (1.8-3.4)	26	**0.001**
Creatinine(Mg/dl)	0.81±0.2 (0.4-1.2)	1.08±0.13 (0.9-1.5)	1.2±0.1 (0.9-1.6)	35	**0.001**

**Table (3):** Comparing Child Pugh classification between HCC and the liver cirrhosis patients groups

Child Pugh classification	Group2 Cirrhotic patients NO(33) %		Group3 HCC NOv(33) %		χ^2	p-value
A	14	42.4	12	36.4	0.254	**0.614**
B	19	57.6	21	63.6		

Table (4): Comparing Heat shock protein 70 and AFP between the studied groups

Variable	Group 1 Control (33)	Group 2 (33) Cirrhotic patients	Group 3 HCC (33)	F-test	p
Heat shock protein 70 (ng/ml) mean ± SD Range	91.5±13.9 (62-121)	116.2±18.3 (87-156)	143.9±33.1 (97-217)	41.8	**0.001**
AFP (ng/ml) mean ± SD Range	5.9±0.9 (3-9)	11.7±6.6 (4-25)	465.1±280 (49-1120)	8.7	**0.001**

Table (5); Performance of HSP 70, AFP and their combination in the detection of HCC

Variable	Cutoff	AUC	Sensitivity	Specificity	PVP	PVN	Accuracy	P
HSP 70	120	0.84	85	50	80	75	84	<0.001
AFP	20.	0.89	87.5	75.8	66.7	89	89.0	<0.001
Combined cutoffs for HSP70 & AFP			91.5	65.8	76.7	91	93.0	<0.001
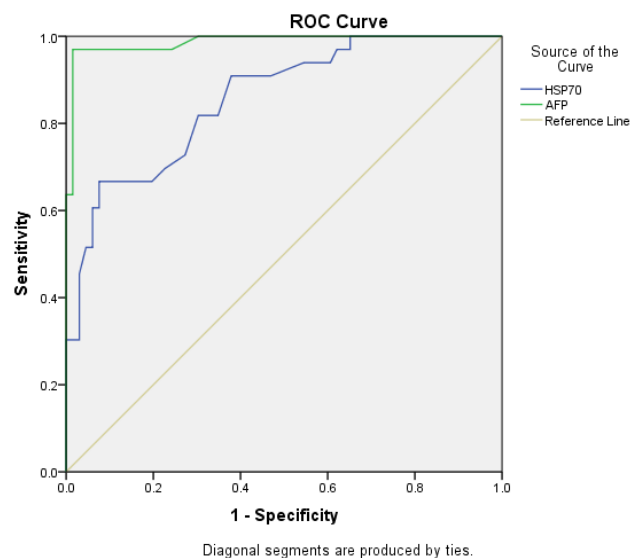
Fig. (1) : ROC curve showing the performance of HSP 70
and alpha fetoprotein detection of HCC.

DISCUSSION

HCC is one of the commonest malignancies [11]. It is estimated to be in charge for almost 746,000 deaths in 2012 (9.1% of the total patients with cancer) [12].

HCC diagnosis at early stage has a far better prognosis owing to availability of potentially curative therapies. Hence, screening and diagnosis of HCC in patients with liver cirrhosis is extremely important.AFP still lacks adequate sensitivity and specificity for active HCC screening [13].

Frequent other biomarkers such as des-gamma carboxy-prothrombin, glypican-3, human hepatocyte growth factor, and insulin-like growth factor-1 are promising, but none of these markers has been licensed for clinical use. Another potential biomarker for HCC is Heat Shock Protein 70 (HSP70). HSPs have been conveyed to be over-expressed in a wide range of human tumors. HSPs expression was related to tumor cell growth, differentiation, resistance to apoptosis, and poor prognosis [14].

So, the aim of our study was to illuminate the role of heat shock protein 70 in the diagnosis of Hepatocellular carcinoma.

In our study, the mean age of patients with HCC was 59.9±4.7 years. Larger percentage of them was male. Due to appropriate randomization, there was a statistically non-significant difference between the three groups concerning age or gender.

There is a move to younger age in the developing countries which may be accredited to the rise of both hepatitis B and or C infections at a younger generation. Old age is an independent risk factor for HCC, especially in areas where HCV infection is endemic [15]. El Zayadi and his colleagues[16] stated that the most predominant age group was (40-59 years).

Zakhary et al. [15] found that males constituted 70.8% of all patients in the HCC group, with 83.3% of patients over 50 years. HCC was common in men in different studies [17-18].

Concerning laboratory data; hemoglobin level, platelet count and serum albumin were comparable between patient groups, while serum AST, ALT, total, direct bilirubin, creatinine were significantly higher among patients with HCC.

As regards AFP, there was non-significant difference between cirrhotic patients as opposed to the control, but there was a highly significant difference between HCC versus the control and cirrhotic patients. It was significantly greater among patients with HCC.

The low specificity of AFP as a biomarker for HCC surveillance could be explained by the temporary rise in AFP levels in patients with cirrhosis reflecting an exacerbation of the hepatitis or in patients with chronic liver disease; and the flares of underlying liver disease such as HBV, HCV or HCC advance [19].

In our work, there was measurably critical distinction between the three groups, mean + standard deviation level of HSP 70 in group I (Control) was (91.5±13.9)μg/ml whereas for group II (Cirrhosis) (116.2±18.3) μg/ml and group III (HCC) (143.9±33.1) μg/ml. 20. Gehrmann et al. [20] found that higher serum HSP70 level was found in HCC and cirrhotic patients whereas the least HSP70 levels were found in patients with unremitting hepatitis.

We found that for HSP 70 at the cutoff esteem 120μg/ml has affectability 85%, specificity 50% and exactness 84%, whereas AFP had affectability 87.5%, specificity 75.8% and precision 89.0% but in combination with each other affectability is 91.5%, specificity is 65.8% and exactness is 93.0%.

HSP70 serum level in all HCC patients were through higher compared to a controls [21]. In expansion, a subgroup of patients with liver cirrhosis who in this way made HCC had higher HSP70 serum levels than patients with liver cirrhosis [22].

Gehrmann and his colleagues found that there's no distinction between HSP 70 level in patients without liver infection and sound human volunteers. Alternately, the HSP70 in patients with liver illnesses such as CH (incessant hepatitis), LC (liver cirrhosis), and HCC differentiated through and through from that of solid volunteers and patients without liver disease. The most elevated serum HSP70 levels in patients were found in HCC (N= 47, 6.5 ± 3.1 ng/ml)and LC patients (N= 46, 6.6 ± 5.2 ng/ml). The least HSP70 levels were found in patients with CH (N=50, 3.9 ± 2.4 ng/ml). These values were essentially lower than those of HCC and LC patients [20].

HSP70 can moreover be utilized in separating hepatocellular adenoma and hepatocellular carcinoma. This study detailed that HSP70 immunohistochemistry is positive within the most of well-differentiated hepatocellular carcinomas cases and a subset of cases with atypical HCC. This may offer assistance within the separating normal hepatocellular adenomas from atypical tumors and hepatocellular carcinoma [23].

Karlsson et al. [24] have recognized that HSP70 as an important regulator for multiple steps of metastasis in human cancer.HSP70 drop significantly inhibits HCC cell invasion and metastasis in the two cell lines; however, the possible underlying mechanism necessitates additional exploration [25].

We concluded that HSP 70 is an accepted valid serum biomarker that can be used in combination with AFP help in the diagnosis of HCC in cirrhotic patients.

There were also some limitations, including a moderately small sample and being applied in a single center. For more evidence, we recommend that large scale prospective multicenter studies should be done to confirm the role of HSP 70 in the diagnosis of HCC.

Administrative and ethical design:

The study protocol was accepted by the ethical committee of the faculty of medicine Zagazig University and institutional review board under the number of ZU-IRB #4217/18-2-2018 prior to the study. All required official permissions to complete the study were obtained from the directors of the Internal Medicine and Clinical Pathology Departments. Informed consent was taken from all members in this study.

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