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Comparison between glypican-3 and alpha-fetoprotein in discrimination of hepatocellular carcinoma from cirrhotic patients

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ABSTRACT:

Background: Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Liver cirrhosis progression could be a consequence for developing HCC. Although alpha-fetoprotein (AFP) is widely used as, a marker for detection of HCC, but it has poor sensitivity. **Objective:** Evaluate the diagnostic power of serum AFP and Glypican-3 (GPC3) as biomarkers of development of HCC. **Subjects and Methods**: A total of 182 patients, 110 patients with HCC and 72 patients with liver cirrhosis were included. AFP and GPC3 were determined using ELISA. The diagnostic power **was evaluated using Area under Roc curve (AUC). Results**: levels of AFP and GPC3 in sera of HCC patients were higher than in those with liver cirrhosis ($p < 0.0001$). AFP had Area under curve (AUC) = 0.772 with sensitivity 39.1%, specificity 97.2%, positive predictive value (PPV) 97.7%, negative predictive value (NPV) 34.3% and efficiency 53.4% while GPC3 had AUC=0.841 yielded sensitivity 76.4%, specificity 86.1%, PPV 94.4%, NPV 64.3% and efficiency 78.8%. There was significant weak correlation ($r = 0.241$; $P < 0.001$) between AFP and GPC3. **Conclusions:** GPC3 is good marker for HCC diagnosis. Therefore, GPC3 may be more useful than AFP in differentiating HCC from cirrhotic patients.

KEYWORDS: AFP, Glypican-3, Liver cirrhosis, HCC, Blood markers, Diagnosis

1. INTRODUCTION

Worldwide, HCC represents one of malignant tumors that consider the third most cause of death (Zhu et al., 2016). HCC may be cured when it is diagnosed early (Wu et al., 2016). HCC remains one of the main malignancies with a poor-prognosis (Zhu et al., 2016). The HCC diagnosis include non-invasive, objective, and reproducible assessments, which consist of biomarkers are potential tools for surveillance and HCC early diagnosis (Berretta et al., 2017). Widely, AFP is the most marker used for HCC during the past several decades with sensitivity of 41-65% and specificity of 80-90% (Lou et al., 2017). AFP levels elevation is not noted in an approximately 80% of the small HCC tumors (Saffroy et al., 2007). There are many tumor markers could be substituted or complemented of AFP in the HCC detection. GPC3 is a cell surface proteoglycan, it was normally detected in the liver of fetal while not detected in the liver of healthy adult. However, in patients with HCC, the GPC3 is overexpressed at both the protein and gene levels (Zhou et al., 2018). In this study, we evaluated serum AFP and GPC3 as predictors for development of HCC.

2. MATERIALS AND METHODS

Subjects

According to clinical examination, the 182 patients classified into 72 patients with liver cirrhosis, 44 male and 28 female had mean age ± SD (standard division); 52.5±7.1 years and 110 patients with HCC, 82 males and 28 females with age 54.6±10.5 years**.** The patient's samples collected from Tropical medicine department at Mansoura University after written consent from all patients. Diagnosis of Liver cirrhosis was performed based on laboratory test, historical features, physical examination and computed tomography (CT) or ultrasonography (US) scan. Diagnosis of HCC patients were initially diagnosed by image studies were included US, CT or magnetic resonance (MRI). We excluded

the patients whom underwent intent-to-cure treatment and patients with double primary cancer from this study.

3. METHODS

Biochemical and hematological tests

Blood samples collected from patients with HCC before any curative treatment from patients with liver cirrhosis at the clinic visit time. Serum aliquots were stored at -20^{o} C until time of candidate marks measurements after freshly tested for Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin (total), serum albumin using an automated biochemistry analyzer (Hitachi 917; Roche Diagnostics, Mannheim, Germany). Complete blood counts (CBC) including platelets counts were determined using automated hematology analyzer (KX-21 Sysmes, System Corporation, Japan).

Determination Alpha-fetoprotein and Serum glypcan-3 levels

Serum AFP was measured using Immu-lite AFP-1000 ELISA kit (Diagnostic Products Corporation, Los Angeles, CA, USA). GPC3 was determined by human GPC3 ELISA kit (Wuhan EIAab Science Co., Ltd., Hubei, China).

Statistical analysis:

The numerical data presented as means±SD (standard division) and mean±SE (standard error) while categorical data presented as percentage. Chi-square and Mann-Whitney U tests were used when appropriate. Statistically the significance difference considered if P-value was ≤ 0.05. On the other hand, the correlation was evaluating using Pearson's correlation coefficient. In addition, area under curve (AUC) used to describe the diagnostic performance of each marker. In addition, the diagnostic accuracy was evaluate by comparing the sensitivity, the specificity, the PPV and the NPV; respectively.

4. RESULTS

Laboratory data

As shown in **Table 1**; serum AST, ALT, ALP, total bilirubin and INR (International Normalized Ratio) in patients with HCC were higher ($p < 0.0001$) than liver cirrhotic patients while albumin and platelets counts in patients with HCC were lower than in liver cirrhotic patients.

Determination of serum AFP and GPC3

In HCC patients, levels (mean ± SE) of AFP and GPC3 were 2340.4±632.9 and 11.71±0.92 higher than cirrhotic patients 60.2±9.8 and 3.68±0.39 were; respectively with an extremely significant difference ($P < 0.0001$ for both) **Fig. 1A and 1B.** There was significant weak correlation ($r = 0.241$; $P < 0.001$) between AFP and GPC3 in HCC and liver cirrhosis patients; **Fig. 2.**

Diagnostic performance of serum GPC3 and AFP

The AFP had AUC=0.77 with sensitivity 39.1% and specificity 97.2% and PPV 97.7%, NPV 34.3% and efficiency 53.4% at cut-off 200 ng/mL to differentiate HCC from liver cirrhosis; **Fig. 3A and Fig. 4.** GPC3 differentiate HCC from liver cirrhosis with AUC=0.84 and yield sensitivity 76.4% and specificity 86.1% and

PPV 94.4%, NPV 64.3% and efficiency 78.8% at cut-off value 5.0 ng/mL; **Fig. 3B and Fig. 4.**

5. DISCUSSION

For successful clinical management HCC diagnosis in the early stages is important (Kiyokawa and Yasuda, 2017). Several factors affect the degree of reliability of ultra-sonographic findings and it is operator dependent (Mikami et al., 2015). The diagnostic accuracy of tumor markers is not fully sensitive and specific for effective surveillance (Sterling et al., 2012). Hence, we need noninvasive method for early HCC detection. AFP is the most widely investigated serum tumor marker for diagnosis of HCC. AFP elevation has been shown to be a risk factor for developing HCC and is used to help define the at-risk populations (Huang et al., 2013); therefore, suggesting that production AFP increasing in chronic liver diseases patients might reflect abnormal or altered regeneration of liver cell. Our findings, the levels of AFP and GPC3 in HCC patients were higher ($P < 0.0001$ for both) than liver cirrhotic patients agreed with finding of **Chu et al., (2001)** serum AFP levels were high with the sever portal necr-inflammation as well as severity of fibrosis and liver cirrhosis. The production of AFP is enhanced in presence of the injury, possibly resulting from hepatocyte turnover increasing (Chu et al., 2001). In addition, **Hu et al. (2004)** found that, there was the same correlation between the liver disease activity and elevation of AFP levels (Hu et al., 2004). The increased of serum AFP and biochemical values indicated that necrosis, inflammation, and hepatocellular injury occured (Mousa et al., 2012). (Chan et al., 2014) found that, the data of a total of 805 subjects of HCC were evaluated in their study, at a cut-off = 200-ng/ml, the sensitivity 47.7%, the specificity 97.1%, and PPV 97.5% and NPV 44.4% similar with our results.

GPC3 is highly expressed in HCC tissues and regulate the signaling activity of several growth factors, including Wnts signaling (Xu et al., 2013). (Liu et al., 2010) found that, GPC3 is overexpressed in most HCC tumors, and used as an indicator for HCC diagnosis and prognosis. Three studies ((Tangkijvanich et al., 2010; Youssef et al., 2010; Zhang et al., 2010) found that, the GPC3 was more accurate than AFP HCC diagnosis. (Beale et al., 2008; Özkan et al., 2011) found an opposite result, while the other studies did not tell the difference. Meta-analysis indicated that, the pooled sensitivity of AFP and GPC3 is 52.0 % and 60.2 %, respectively, while the pooled specificity of serum AFP and GPC3 is 93.9 % and 84.8 %; respectively. Moreover, we found, significant weak correlation ($r=0.241$; $P < 0.001$) between AFP and GPC3 in HCC and liver cirrhosis patients. This weak correlation means that each of them gives independently varied data about abnormalities in HCC. Serum GPC3 may be a marker for diagnosis HCC and show that serum GPC3 may be used to distinguish the HCC patients with AFP-negative from liver cirrhotic patients.

In conclusion: GPC3 may be helpful in improving diagnosis of HCC and in differentiating diagnosis between AFP negative HCC and cirrhotic nodules.

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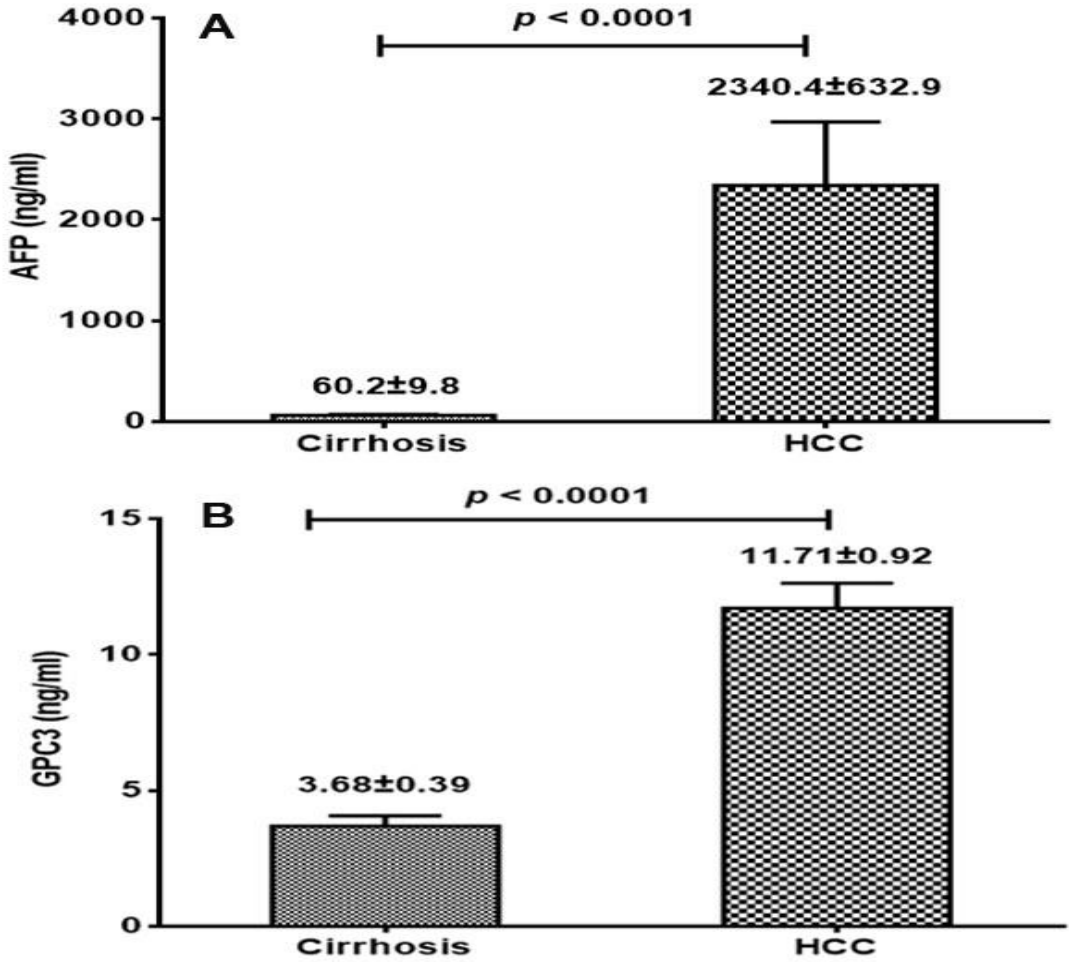

Figure 1: (A) Level of serum AFP in HCC compared with Cirrhosis. There was an extremely high significant difference ($p < 0.0001$). **(B)** Level of serum GPC3 in HCC compared with Cirrhosis. There was an extremely high significant difference ($p < 0.0001$).

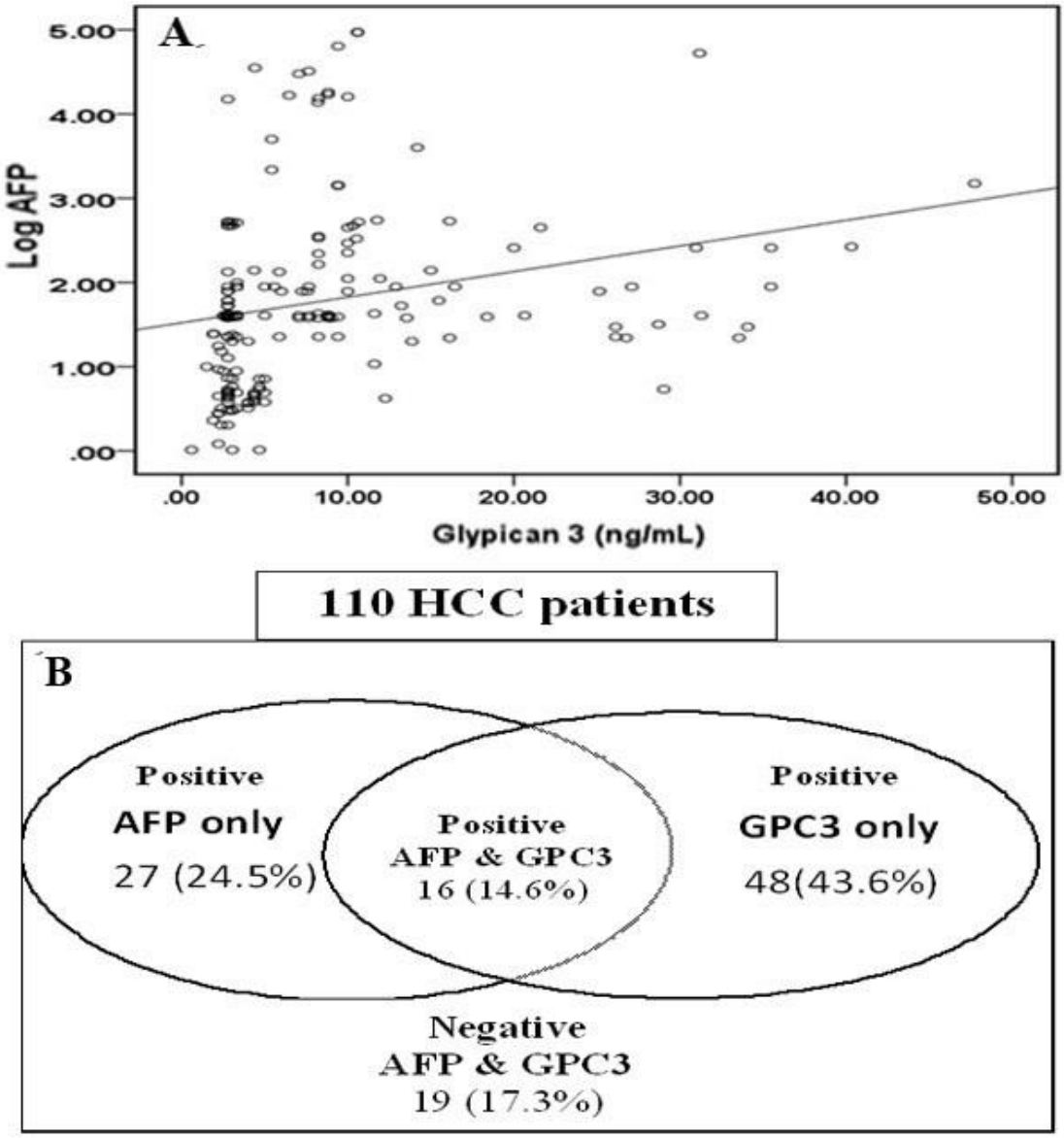

Figure 2: (A) Correlation between serum GPC3 (ng/ml) and AFP (ng/mL) in HCC and liver cirrhosis patients. Weak significant correlation (r = 0.241 and P < 0.001). **(B) Relation between detection rates of GPC3 and AFP in HCC patients.**

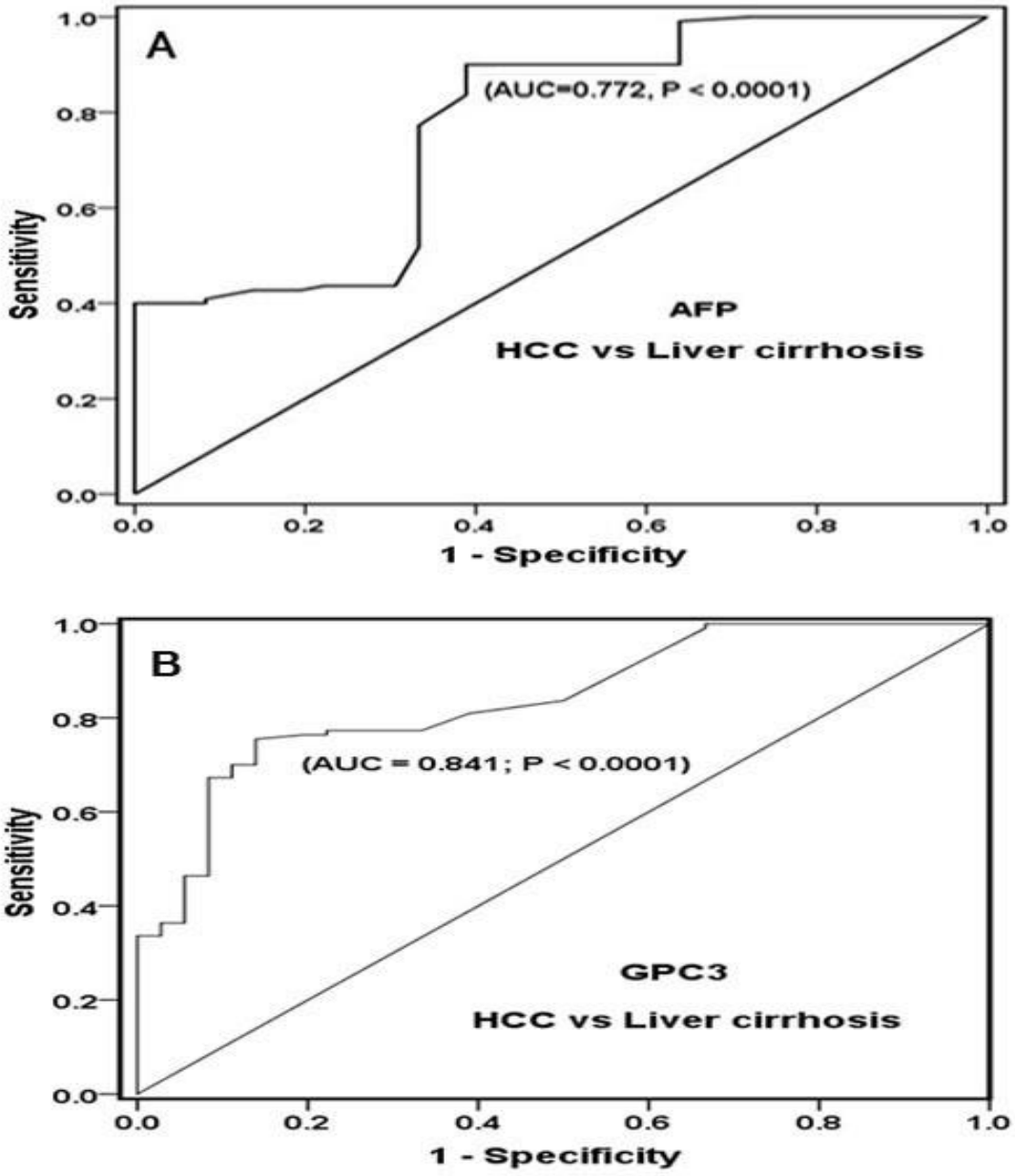

Figure 3: (A) AFP ROC curve for HCC detection. The AUC of serum AFP in liver HCC *vs* Cirrhosis was 0.772; $P < 0.0001$. **(B)** ROC curve for circulating GPC3 in HCC detection. The AUC in HCC *vs* liver cirrhosis was 0.841; $P < 0.0001$.

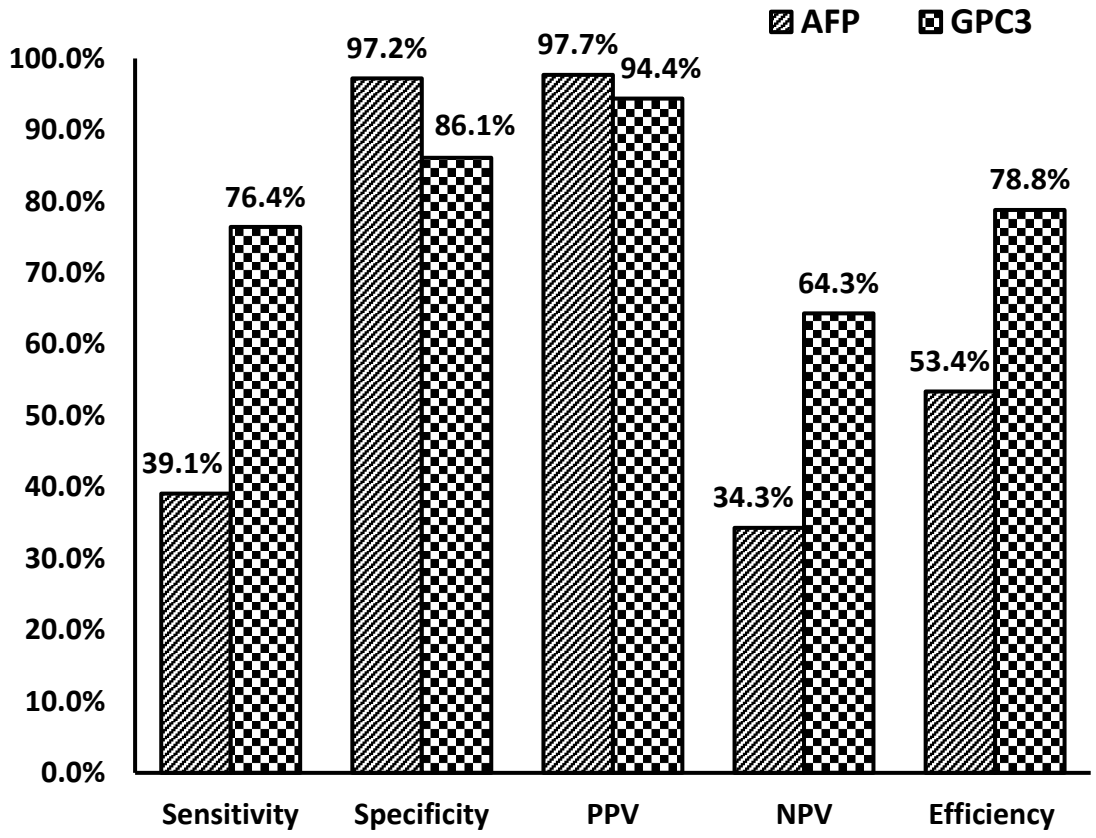

Figure 4: Diagnostic performance of serum AFP and GPC3 differentiate HCC from compensated cirrhosis.

Table 1: Laboratory data of patients and healthy individuals

	Cirrhosis	**HCC**	**P value**
Number	72	110	-
Gender (M/F)	44/28	82/28	-
Age (years)	52.5±7.1	54.6±10.5	P > 0.05
ALT (U/L)	52.8±20.5	47.7±19.2	P > 0.05
AST (U/L)	57.8±20.3	75.2±34.9	P < 0.001
ALP (U/L)	109.4±29.7	183.4±90.3	P < 0.001
T. Bilirubin	1.67±0.98	2.37±1.55	P < 0.01
S. Albumin	3.58±0.66	3.20±0.62	P < 0.001
INR	1.36±0.19	1.38±0.20	P > 0.05
Platelets	152.0±96.0	138.0±81.8	P > 0.05

Data are presented as means ± SD. **SD:** Standard deviation, **M:** Male, **F:** Female, **HCC:** patients with hepatocellular carcinoma and. P: probability (one-way ANOVA). **Reference ranges:** ALT up to 45 U/L; AST up to 40 U/L; ALP 22-92 U/L, total bilirubin: up to 1 mg/dl; serum albumin 3.8-5.4 g/dL and INR (International Normalized Ratio) 0.8-1.3, Platelets: 150–400×10^9/L.