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ORIGINAL ARTICLE

Diagnostic Value of Serum Beta 2 Microglobulin in Cirrhotic Patients with Hepatocellular Carcinoma

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ABSTRACT

Background: Early and accurate diagnosis of hepatocellular carcinoma (HCC) in cirrhotic patients remains challenging, particularly in high-risk populations. Serum beta-2 microglobulin (β2M) has emerged as a potential biomarker for disease severity and malignant transformation in chronic liver disease. The present study aimed to evaluate the diagnostic role of serum β2M in differentiation between cirrhosis and HCC and to assess its performance in combination with alpha-fetoprotein (AFP).

Methods: This case-control study was performed on 84 patients categorized into two groups: cirrhosis without HCC (n=42) and cirrhosis with HCC (n=42). Diagnosis was confirmed by radiological and laboratory findings. Serum β2M was assessed utilizing enzyme-linked immunosorbent assay (ELISA) and AFP by chemiluminescent immunoassay. Clinical, laboratory, and imaging data were collected and analyzed.

Results: β2-microglobulin was significantly correlated with markers of advanced liver and kidney dysfunction in both groups. In cirrhosis patients without HCC, β2M correlated negatively with platelets and albumin and positively with aspartate aminotransferase (AST), total bilirubin, international normalized ratio (INR), blood urea nitrogen (BUN), and creatinine. In HCC with cirrhosis, β2M showed negative correlations with hemoglobin, platelets, AST, alanine aminotransferase (ALT), and albumin, and positive correlations with white blood cells (WBCs) count, bilirubin, and INR. β2M levels were highest in Child-Pugh C in both groups ($p < 0.001$). At a 7.15 mg/L cutoff, β2M predicted HCC among cirrhotic patients with 97.6% sensitivity, 88.1% specificity, and 95.1% accuracy.

Conclusion: Serum β2M was significantly higher among cirrhotic patients, with the highest levels observed among patients with HCC. Its increase is correlated with disease severity and progression. Combined with AFP, β2M may enhance diagnostic accuracy for HCC; however, its utility is primarily as an adjunct marker reflecting disease severity and overall progression rather than as a standalone diagnostic test. Its use should be interpreted within the broader clinical and laboratory context to support earlier detection and surveillance in high-risk patients.

Keywords: Cirrhotic Patients; Hepatocellular Carcinoma; Serum Beta 2 Microglobulin.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a major cause of cancer-related morbidity and mortality worldwide, most frequently arising in the setting of chronic liver disease and cirrhosis, particularly related to hepatitis B and C virus infections [1]. Early detection of HCC remains a critical challenge, as curative

therapies are only feasible at initial disease stages. Accordingly, routine surveillance for HCC in cirrhotic patients is recommended by major clinical guidelines [2].

Among serum biomarkers, alpha-fetoprotein (AFP) remains the most widely used in clinical practice for HCC screening, diagnosis, and monitoring. However, its sensitivity, especially for early-stage HCC, is suboptimal, with only 40-60% of HCC cases showing elevated AFP, and even lower rates in early-stage disease [3]. AFP-L3, a glycoform of AFP, and des-gamma-carboxy prothrombin (DCP, also known as PIVKA-II) have been introduced to improve diagnostic accuracy. AFP-L3 is considered more specific for HCC and, when combined with total AFP, may provide incremental diagnostic value [4]. DCP has also demonstrated utility, particularly for larger tumors and advanced stages, but is less sensitive in early HCC and can be elevated in non-malignant liver conditions [5]. Despite these advances, current biomarkers are limited by variable sensitivity, poor specificity for small lesions, and confounding by underlying hepatic inflammation or regeneration.

Recent research has explored additional serum markers and composite diagnostic algorithms, including glypican-3, osteopontin, and circulating microRNAs. Still, these are not yet widely adopted in routine clinical care due to limited validation and standardization [6, 7]. In this context, there is a persistent need for reliable, noninvasive biomarkers that can improve the early diagnosis and risk stratification of HCC in high-risk populations.

Beta-2 microglobulin (β2M) is a low molecular weight protein that forms part of the human leukocyte antigen (HLA) class I molecule on all nucleated cells. Elevated serum β2M levels have been reported in chronic liver disease, HCV-related cirrhosis, and HCC, reflecting increased immune activation, cellular turnover, and disease severity [8]. However, its diagnostic utility relative to established markers remains underexplored. The present study was designed to evaluate the performance of serum β2M in distinguishing between cirrhosis and HCC and to compare its role with established markers such as AFP in a cohort of cirrhotic patients.

METHODS

This case-control study included adult patients (aged 18–65 years) of both sexes, recruited from the inpatient and outpatient clinics of the Internal Medicine and Clinical Pathology Departments at Zagazig University Hospitals and Al-Ahrar Teaching Hospital, over twelve months (June 2024 to June 2025).

Patients were consecutively screened and enrolled if they met the inclusion and exclusion criteria. All participants were required to provide written informed consent prior to study procedures and demonstrate willingness to participate in the study.

Institutional Review Board (ZU-IRB# 284/7-April-2024) clearance was obtained, and informed consent was collected from all patients who participated in the study. The research was conducted following the World Medical Association's Code of Ethics (Helsinki Declaration) for studies involving human subjects.

Sample size calculation

Based on a previous study, where the mean ± SD of serum β2M was 6.6±1.49 mg/L in HCC patients and 7.4±1.04 mg/L in cirrhotic patients [6], the minimum required sample size was calculated to be 82 subjects (41 per group), with a 95% confidence level and 80% power, using OpenEpi software.

Inclusion criteria

Eligible participants for this study were adults aged between 18 and 65 years, of either sex, who were diagnosed with liver cirrhosis of any etiology based on at least three radiological criteria on abdominal ultrasonography, including altered hepatic echotexture, irregular liver margins, splenomegaly (splenic diameter >12 cm), dilated portal vein (>12 mm), and the presence of ascites. These imaging features were supplemented by clinical and laboratory data (such as hypoalbuminemia, thrombocytopenia, or prolonged international normalized ratio (INR)) as appropriate. All

diagnoses were reviewed and confirmed by experienced hepatologists [6].

Patients with HCC were required to have a diagnosis established by triphasic computed tomography (CT) in addition to cirrhosis.

The participants were categorized into two groups: the cirrhosis group (n=42), including patients with liver cirrhosis without HCC, and the HCC/cirrhosis group (n=42), including patients who had liver cirrhosis and HCC.

Exclusion criteria

Patients were excluded if they had evidence of secondary hepatic metastases, any extrahepatic malignancy (including but not limited to lymphoproliferative disorders), prior treatment for HCC, chronic kidney disease (defined as an estimated glomerular filtration rate below 60 mL/min/1.73m² or elevated serum creatinine), or recent antibiotic use within the preceding three months. Additional exclusion criteria included the presence of acute or chronic inflammatory diseases, autoimmune disorders, active systemic infection at the time of recruitment, or neurological disorders. Potential participants with laboratory or clinical evidence suggestive of immune or lymphoproliferative disorders—identified through comprehensive medical history, physical examination, complete blood count, and additional investigations as warranted (e.g., C-reactive protein, erythrocyte sedimentation rate, or autoimmune serology)—were also excluded to minimize confounding factors that might influence serum β2M levels.

History taking & clinical examination

Upon inclusion, all participants underwent thorough history taking, including demographic data (age, sex, residence, smoking status), comorbidities (diabetes mellitus, hypertension), present illness, previous medical and surgical history, and detailed medication review. A complete clinical examination was performed, with particular attention paid to abdominal findings.

Blood sample collection

Venous blood samples were obtained from each participant using strict aseptic techniques and distributed into appropriate collection tubes for subsequent analyses. Whole blood was collected in EDTA tubes for complete blood count (CBC) determination. In contrast, sodium citrate tubes were used to assess the coagulation profile, involving prothrombin time (PT) and INR. Additionally, blood was dispensed into plain vacutainer tubes, with one aliquot allocated for biochemical and serological investigations and another for measurement of AFP and β2M levels. The sera were separated by centrifugation at 1200 x g for 10 minutes, then aliquoted into sterile tubes and stored at −20°C until further assay.

Routine laboratory investigations

Routine laboratory investigations included liver function tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, albumin), CBC, renal function tests (urea, creatinine), and coagulation profile (PT, INR). All analyses were performed using automated analyzers (e.g., Sysmex XN-1000 from Sysmex in Japan for CBC, Sysmex CS2100i from Siemens in Germany for coagulation profile, and Roche Cobas 8000-c702 analyzer from Roche Diagnostics in Germany for biochemistry), following manufacturer protocols.

Measurement of serum alpha-fetoprotein level

Serum AFP levels were evaluated using an electro-chemiluminescent immunoassay on an automated platform (Cobas 8000-e602 analyzer, Roche Diagnostics, Germany). The assay has a linearity extending up to 1200 ng/mL. Results were interpreted as positive for HCC based on the laboratory's reference cutoff (>20 ng/mL), as recommended in clinical guidelines [7]. Assay controls and calibrators were included with each run to ensure accuracy and reproducibility.

Measurement of serum Beta-2 microglobulin level

A quantitative sandwich measured serum β2M concentrations using enzyme-linked immunosorbent assay (ELISA), employing commercially available kits Human beta 2-Microglobulin Parameter Assay Kit [Catalog number: KGE019] (R&D Systems, Minnesota, USA), according to the manufacturer's protocol.

This assay demonstrates high sensitivity (as low as 0.13 mg/L) with a dynamic range suitable for clinical samples (typically 0.2–10 mg/L). All samples and standards were run in duplicate, and absorbance was measured at 450 nm using a Sunrise™ absorbance reader (Tecan Trading AG, Männedorf, Switzerland). Normal adult reference values for β2M range between 0.7–1.8 mg/L; values above this suggest increased cell turnover, inflammation, or malignancy [8]. The results were interpreted in the context of clinical and radiological findings, with higher levels supporting the diagnosis of HCC in cirrhotic patients.

Imaging techniques & assessment of liver disease severity

An abdominal ultrasound was used to assess the initial liver. HCC diagnosis relied on multiphasic CT imaging. The severity of liver disease was evaluated for all patients using the Child-Turcotte-Pugh (CTP) score, including both the numerical score (range 5–15) and the Child-Pugh class (A, B, or C), based on total bilirubin, serum albumin, PT/INR, presence of ascites, and degree of hepatic encephalopathy [9].

Statistical analysis

Data were collected and analyzed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Quantitative variables were presented as mean ± SD and median (range), while qualitative data were shown as counts and percentages. The independent samples t-test or Mann-Whitney U test compared two groups, and one-way ANOVA was used for more than two groups. Chi-square or Fisher's exact test assessed categorical variables. Pearson correlation coefficient examined associations, with values near 1 indicating strong correlation and values near 0 indicating weak correlation. All tests were two-sided, with $p < 0.05$ considered statistically significant.

RESULTS

Analysis of demographic and clinical variables revealed non-statistically significant differences between the two studied groups regarding demographic data, comorbidities, or clinical data. All patients in the HCC with cirrhosis group exhibited smaller liver sizes compared to 40.5% in the cirrhosis group ($p < 0.001$), with no cases of normal or enlarged liver size among HCC patients ($p < 0.001$ and $p = 0.011$, respectively). In terms of splenic size, none of the HCC with cirrhosis group had normal spleen, whereas 23.8% of the cirrhosis group did ($p = 0.001$). Additionally, the incidence of splenectomy was significantly higher among HCC patients (28.6% vs. 9.5%, $p = 0.026$) (Table 1).

The HCC with cirrhosis group had significantly lower hemoglobin ($p = 0.003$) and platelet counts ($p < 0.001$), as well as higher ALT ($p < 0.001$), BUN ($p < 0.001$), and creatinine levels ($p = 0.001$). Non-statistically significant differences were found in WBCs, INR, AST, albumin, total or direct bilirubin between the two groups (all $p > 0.05$) (Table 2).

The HCC with cirrhosis group had significantly higher AFP and β2M levels than the cirrhosis group ($p < 0.001$ for each) (Table 3).

In the cirrhosis group, β2M showed significant negative correlations with platelet count ($r = –0.678$, $p < 0.001$) and albumin ($r = –0.750$, $p < 0.001$), and significant positive correlations with AST ($r = 0.332$, $p = 0.032$), total bilirubin ($r = 0.610$, $p < 0.001$), INR ($r = 0.725$, $p < 0.001$), BUN ($r = 0.515$, $p < 0.001$), and creatinine ($r = 0.529$, $p < 0.001$) (Table 4).

In the HCC with cirrhosis group, β2M had significant negative correlations with hemoglobin ($r = –0.348$, $p = 0.024$), platelet count ($r = –0.558$, $p < 0.001$), AST ($r = –0.456$, $p = 0.002$), ALT ($r = –0.613$, $p < 0.001$), and albumin ($r = –0.820$, $p < 0.001$). Significant positive correlations were observed with WBCs ($r = 0.397$, $p = 0.009$), total bilirubin ($r = 0.586$, $p < 0.001$), direct bilirubin ($r = 0.358$, $p = 0.020$), and INR ($r = 0.704$, $p < 0.001$) (Table 5).

There was a statistically significant difference in β2M levels across Child-Pugh classes in both the cirrhosis and HCC with cirrhosis groups ($p < 0.001$ for each group). β2M concentrations increased progressively with worsening liver function, showing the highest levels in patients classified as Child C (Table 6).

Beta 2 microglobulin, at the level of 7.15 mg/L, had a sensitivity of 97.6% and specificity of 88.1% for predicting HCC on top of cirrhosis, with an overall accuracy of 95.1% (Table 7 & Supplementary Figure 1).

Table (1): Basic characteristics and clinical data of the studied groups

Variables		Cirrhosis Group (n=42)	HCC/Cirrhosis Group (n=42)	t	P-value
Age (in years) Mean ± SD		56.14 ± 6.46	58.19 ± 4.68	-1.661	0.100
		N (%)	**N (%)**	**X^2**	**P-value**
Sex					
Male		25 (59.5%)	28 (66.7 %)	0.460	0.498
Female		17 (40.5%)	14 (33.3 %)		
Residence					
Rural		27 (64.3%)	25 (59.5%)	0.202	0.653
Urban		15 (35.7%)	17 (40.5%)		
Smoking		8 (19%)	12 (28.6%)	1.050	0.306
HTN		16 (38.1%)	17 (40.5%)	0.050	0.823
DM		17 (40.5%)	20 (47.6%)	0.435	0.510
Jaundice		9 (21.4%)	11 (26.2%)	0.263	0.608
Pallor		11 (26.2%)	13 (31%)	0.233	0.629
Ascites		20 (47.6%)	24 (57.1%)	0.764	0.382
LL edema		18 (42.9%)	23 (54.8%)	1.191	0.275
Encephalopathy		16 (38.1%)	17 (40.5%)	0.050	0.823
Liver Size	**Normal**	19 (45.2%)	0 (0%)	24.554	<0.001*
	Enlarged	6 (14.3%)	0 (0%)	6.462	0.011*
	Shrunk	17 (40.5%)	42 (100%)	35.593	<0.001*
Spleen Size	**Normal**	10 (23.8%)	0 (0%)	11.351	0.001*
	Enlarged	28 (66.7%)	30 (71.4%)	0.223	0.637
	Removed	4 (9.5%)	12 (28.6%)	4.941	0.026*

t: Independent samples t-test
X^2: Chi-square test
****Statistically significant (p-value <0.05)***
DM, Diabetes Mellitus; HCC, Hepatocellular Carcinoma; HTN, Hypertension; LL, Lower Limb; SD, Standard Deviation.

Table (2): Comparison of CBC, INR, liver function tests, and kidney function tests results of the studied groups

Variables	Cirrhosis Group (n=42)	HCC/cirrhosis Group (n=42)	t	P-value
	Mean ± SD	Mean ± SD		
Hb (g/dL)	10.52 ± 1.01	9.85 ± 1.01	3.052	**0.003***
WBCs (10^3/µL)	5.79 ± 1.48	5.70 ± 1.28	0.299	0.765
PLTs (10^3/µL)	133.4 ± 32.68	105.88 ± 18.92	4.723	**<0.001***
INR	1.64 ± 0.4	1.64 ± 0.3	-0.028	0.978
AST (U/L)	38.57 ± 5.81	40.90 ± 5.34	-1.917	0.059
ALT (U/L)	32.09 ± 5.42	38.26 ± 5.30	-5.270	**<0.001***
Albumin (g/dL)	3.20 ± 0.64	3.03 ± 0.59	1.209	0.230
BUN (mg/dL)	24.49 ± 5.13	29.46 ± 5.05	-4.477	**<0.001***
Creatinine (mg/dL)	1.05 ± 0.22	1.20 ± 0.18	-3.358	**0.001***
	Median (IQR)	**Median (IQR)**	**U**	**P-value**
Total bilirubin (mg/dL)	1.80 (1.1-2.9)	1.80 (1.2 -3)	-0.233	0.816
Direct bilirubin (mg/dL)	0.45 (0.3-1.2)	0.65 (0.5- 1.2)	-1.305	0.192

t: Independent samples t-test
U: Mann–Whitney U test
****Statistically significant (p-value <0.05)***
ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BUN, Blood Urea Nitrogen; CBC, Complete Blood Count; Hb, Hemoglobin; HCC, Hepatocellular Carcinoma; INR, International Normalized Ratio; IQR, Interquartile Range; PLTs, Platelets; SD, Standard Deviation; WBCs, White Blood Cells.

Table (3): Comparison of alpha fetoprotein and Beta 2 microglobulin results between the studied groups

Variables	Cirrhosis Group (n=42)	HCC/cirrhosis Group (n=42)	Test	P-value
AFP (ng/mL) Median (IQR)	7.35 (4.60-19)	795(30.2-1264)	(MW) -6.017	**<0.001***
β2M (mg/L) Mean± SD	6.22 ± 0.87	8.31 ± 0.83	(t) -11.173	**<0.001***

MW: Mann-Whitney U test
t: Independent sample t-test
**** Statistically significant (p-value <0.05)***
AFP, alpha fetoprotein; β2M, Beta 2 microglobulin.

Table (4): Correlation between Beta 2 microglobulin and different parameters in cirrhosis group

Variables	Beta 2 microglobulin	
	r	p-value
Age	0.258	0.100
Hb	-0.264	0.091
WBCs	0.176	0.266
PLTs	-0.678	<0.001*
AST	0.332	0.032*
ALT	0.170	0.280
Albumin	-0.750	<0.001*
Total bilirubin	0.610	<0.001*
Direct bilirubin	0.282	0.071
INR	0.725	<0.001*
BUN	0.515	<0.001*
Creatinine	0.529	<0.001*
Alpha fetoprotein	-0.017	0.915

r: Pearson Correlation Coefficient
** Statistically significant (p-value <0.05)*
ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BUN, Blood Urea Nitrogen; Hb, Hemoglobin; INR, International Normalized Ratio; PLTs, Platelets; WBCs, White Blood Cells.

Table (5): Correlation between Beta 2 microglobulin and different parameters in HCC with cirrhosis group

Variables	Beta 2 microglobulin	
	r	p-value
Age	0.073	0.645
Hb	-0.348	0.024*
WBCs	0.397	0.009*
PLTs	-0.558	<0.001*
AST	-0.456	0.002*
ALT	-0.613	<0.001*
Albumin	-0.820	<0.001*
Total bilirubin	0.586	<0.001*
Direct bilirubin	0.358	0.020*
INR	0.704	<0.001*
BUN	0.220	0.162
Creatinine	0.293	0.060
Alpha fetoprotein	0.279	0.073

r: Pearson Correlation Coefficient
** Statistically significant (p-value <0.05)*
ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BUN, Blood Urea Nitrogen; Hb, Hemoglobin; INR, International Normalized Ratio; PLTs, Platelets; WBCs, White Blood Cells.

Table (6): Relation between Beta-2 Microglobulin Levels and Child Score in the studied groups

Items	Child A (n=28)	Child B (n=28)	Child C (n=28)	F-test	P-value
	Mean ± SD	Mean ± SD	Mean ± SD		
Cirrhosis Group	5.19 ± 0.39	6.32 ± 0.35	7.16 ± 0.24	124.3	<0.001*
HCC/Cirrhosis Group	7.58 ± 0.24	7.96 ± 0.23	9.39 ± 0.33	173.24	<0.001*

f: One-way ANOVA test
* Statistically significant (p value <0.05)
HCC, Hepatocellular Carcinoma.

Table (7): Validity of Beta 2 microglobulin for prediction of HCC on top of cirrhosis.

	AUC	95%CI	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
B2M	0.988	0.973-1.0	<7.15 mg/L	97.6%	88.1%	89.1%	97.3%	95.1%

AUC, Area under curve; CI, Confidence Interval; PVN, Predictive value for Negative; PVP**,** Predictive value for positive.

DISCUSSION

Recent advances in HCC biomarker research have highlighted several novel candidates beyond traditional AFP, including AFP-L3 (a glycoform of AFP with increased specificity for HCC), des-gamma-carboxy prothrombin (DCP or PIVKA-II), glypican-3, osteopontin, and various circulating microRNAs [4, 5]. While some of these biomarkers demonstrate higher specificity or improved performance in early-stage disease when used in combination, none have fully replaced AFP in standard surveillance protocols. AFP-L3 and DCP are incorporated into certain guidelines in East Asia, but are limited by cost, accessibility, and variable sensitivity, especially outside large tumors or high-prevalence settings [6].

Compared to these emerging biomarkers, β2M is attractive because of its established use in other disease contexts, routine laboratory availability, and its reflection of both tumor burden and immune activation. However, as our study and others suggest, β2M lacks disease specificity. It can be elevated in a variety of inflammatory and neoplastic disorders, limiting its use as a stand-alone diagnostic tool for HCC. The integration of β2M into existing HCC screening algorithms may be most valuable when used as part of a multimarker panel (e.g., in conjunction with AFP and/or imaging modalities) and in risk stratification among high-risk cirrhotic patients. Prospective validation and standardization, as well as cost-effectiveness studies, are needed before β2M can be recommended for routine inclusion in HCC surveillance programs [4, 5].

In the present study, the mean age of cirrhosis patients was 56.1 years, while patients with HCC had a mean age of 58.2 years. The difference between the groups regarding age, sex, and residence was not statistically significant. This finding disagrees with the results reported by Moucari et al. [10], who found that most HCC patients were older than 57 years and that age was a significant differentiator between HCC and cirrhosis. This discrepancy may reflect different genetic backgrounds, population selection, or environmental exposures affecting the rate of hepatocyte transformation with aging.

Clinically, a higher percentage of patients in the HCC with cirrhosis group exhibited pallor, jaundice, ascites, and encephalopathy compared to those with cirrhosis alone, but these differences were not significant. These observations were in line with Biomy et al. [11], who showed no significant difference in symptoms such as jaundice or encephalopathy

between HCC and cirrhosis groups. By contrast, Hagrasy et al. [12] disagreed with the current findings by reporting that HCC patients were less likely to present with ascites and encephalopathy, a difference possibly explained by their selection of patients across Child-Pugh classes. In contrast, the current study included patients with a broader range of disease severity.

Ultrasound imaging in our study revealed universal liver shrinkage in the HCC and cirrhosis group, while only 40% of the cirrhosis group had this finding. Splenomegaly or previous splenectomy was common in both groups. These results agreed with Shehata et al. [13], who documented significant differences in liver size and echotexture between HCV, cirrhosis, and HCC, and Patel et al. [14], who highlighted that reduced liver volume and enlarged spleen accompany more advanced cirrhosis. Such findings coincide with the progressive architectural changes of chronic liver disease.

Hematological parameters in the current study showed lower hemoglobin and platelet counts in the HCC with cirrhosis group compared to cirrhosis alone, with no significant differences in WBCs or INR. These results were in line with Ibrahim et al. [15], who observed significantly lower platelets in HCC. This is usually explained by increased splenic sequestration, reduced thrombopoietin, and enhanced platelet destruction. Chan et al. [16] also coincided with these observations, adding that autoantibody production and hemodilution may further contribute. These findings agreed with Shehata et al. [13] and Elgamal et al. [17], who also found lower hemoglobin and platelet counts in HCC, reflecting the multifactorial impact of advanced liver disease and portal hypertension.

The current study findings revealed higher ALT in the HCC and cirrhosis group, while AST, albumin, and bilirubin levels did not show significant differences between the groups. These results were in line with Abdel-Fatah et al. [18], who demonstrated higher ALT and AST levels in both HCC and cirrhosis compared to controls, and with Shehata et al. [13], who found greater transaminase elevation in advanced cirrhosis and HCC. This is consistent with Maulidia et al. [19], who reported that ALT is higher in chronic hepatitis B patients with HCC complications, indicating progressive hepatocellular injury.

The renal function test in our study, measured by BUN and creatinine, was more impaired in the HCC and cirrhosis group. This finding coincides with the study by Hassan et al. [20], who found higher BUN and creatinine in HCC and cirrhosis, and with Cox et al. [21], who showed that HCC patients have altered metabolic profiles, including changes in creatinine, possibly reflecting the hepatorenal syndrome or multisystem involvement in advanced disease.

Alpha-fetoprotein was significantly higher in the HCC with cirrhosis group compared to cirrhosis alone, which is in line with Ibrahim et al. [15], Shehata et al. [13], and Elnakeeb et al. [22], all of whom found higher AFP in HCC and demonstrated correlations with tumor size. This finding was also supported by Liu et al. [23], who showed pronounced differences in AFP between HCC and cirrhotic patients, confirming the role of AFP in diagnosis but also underscoring its limitations as a universal marker.

A key finding of this study was that β2M levels were significantly higher in the HCC and cirrhosis group than in cirrhosis alone. This agreed with the results of Saito et al. [24], who demonstrated that β2M is a useful marker for early, imaging-invisible HCC. The current findings were also in line with Shehata et al. [13], who observed significantly higher β2M in both HCC and cirrhosis versus controls. Huckans et al. [25] supported this by showing higher β2M in HCV patients, likely reflecting increased interferon and cytokine production and immune activation. The current findings coincide with the theory that β2M elevation is linked to immune system activation, lymphocyte turnover, and hepatocyte apoptosis processes that are upregulated in chronic liver

disease and especially in the context of neoplastic transformation.

Ouda et al. [6] also reported that serum β2M was significantly higher in HCC than in chronic HCV, aligning with the current study results. Furthermore, Saito et al. [24] noted that plasma β2M can help detect early, imaging-negative HCC. Malaguarnera et al. [26] observed significantly higher β2M in HCC than in chronic hepatitis C or healthy controls, supporting the present study. In a normal liver, HLA class I antigens (of which β2M is a component) are minimally expressed on hepatocytes. Still, their upregulation in HCC is thought to reflect both malignant transformation and mechanisms of immune escape. These findings were in line with current results, reinforcing β2M's value as a marker of cellular and immunological changes in liver cancer.

The present study identified significant positive correlations between β2M and creatinine, BUN, INR, AST, and total bilirubin in cirrhosis. Significant negative correlations were found between β2M and platelets and albumin in cirrhosis. These results agreed with Shehata et al. [13], who described negative correlations between β2M and platelets or albumin in cirrhosis, and positive correlations with inflammatory markers. Ouda et al. [6] found that β2M correlated with alkaline phosphatase, bilirubin, and INR, and was inversely correlated with albumin, total protein, hemoglobin, WBCs, and platelets, which is in line with the current study's findings. Such associations confirm that higher β2M levels are linked with worse hepatic function and advanced disease, making β2M a potential marker for severity in cirrhosis and HCC.

In the HCC and cirrhosis group, β2M was positively correlated with INR, direct and total bilirubin, and WBCs, and negatively with hemoglobin, albumin, ALT, AST, and platelets. These findings coincide with Shehata et al. [13], who described positive correlations between β2M and markers such as hemoglobin, WBCs, AST, ALT, and AFP in HCC. This is in line with Malaguarnera et al. [26], who found a positive correlation between β2M and AFP in HCC, highlighting the complementary diagnostic value of both markers. The relationship between β2M and inflammation, liver injury, and tumor burden emphasizes the role of β2M as a multifaceted marker reflecting both immune response and oncogenic processes. However, Ouda et al. [6] disagreed with this, as they did not find a significant correlation between β2M and AFP, considering that they selected only HCV-related cirrhosis and HCC. In contrast, other causes of cirrhosis were excluded.

Within cirrhotic patients, the present study found that β2M levels were highest in Child-Pugh C compared to Child-Pugh A or B. This was also observed in the HCC and cirrhosis group. This finding coincides with Shehata et al. [13], who demonstrated significantly higher β2M in Child C groups, supporting the use of β2M as a marker of advanced liver dysfunction.

Regarding diagnostic accuracy, a β2M cutoff of 7.15 mg/L in this study provided a sensitivity of 97.6% and specificity of 88.1% for HCC diagnosis in cirrhotic patients, with an overall accuracy of 95.1%. This was in line with Ouda et al. [6], who found a cutoff of 4.9 mg/L for distinguishing cirrhosis and a cutoff of 4.55 mg/L for HCC, with good diagnostic performance. The current study agreed with Shehata et al. [13], who showed that combining β2M and AFP increased the area under the curve (AUC) for HCC diagnosis. Malaguarnera et al. [26] confirmed that β2M is higher in HCC than in chronic hepatitis or controls and suggested that altered HLA antigen expression (in which β2M is a core component) is used by tumor cells for immune evasion. This study's findings were also in line with those of Ward et al. [27], who found β2M to be a significant HCC-associated serum protein and recommended its inclusion in multi-marker panels.

Despite these strengths, the current study has some limitations. The small sample size and the single-center design could limit the generalizability of the results. Despite our efforts to exclude patients with known renal

dysfunction, extrahepatic malignancy, and recent infections, we recognize that subclinical or undiagnosed inflammatory, immune, or lymphoproliferative conditions may have influenced β2M levels in some participants. We attempted to minimize this risk through careful clinical evaluation and laboratory screening, but acknowledge this remains a limitation. Additionally, although our results demonstrate a strong association of β2M with HCC in cirrhotic patients, the absence of a combined diagnostic model (e.g., integrating β2M and AFP using logistic regression or combined ROC analysis) limits the assessment of the true added value of β2M in clinical practice.

Furthermore, the cross-sectional nature of our study restricts our ability to assess β2M as a marker for disease progression or to evaluate its prognostic value. Due to the limited sample size and single-center, cross-sectional design, our study's findings should be regarded as preliminary. Additional large-scale, multicenter studies with longitudinal follow-up are warranted to confirm the diagnostic performance and clinical utility of serum β2M, define its optimal cutoffs, and clarify its role alongside other established and emerging biomarkers in the surveillance and early diagnosis of HCC.

It is important to recognize that serum β2M is not a disease-specific marker. Although our findings demonstrate higher levels among patients with HCC superimposed on cirrhosis, β2M may be elevated in various chronic inflammatory, neoplastic, and renal conditions. These confounders limit its role as a diagnostic marker, and the observed associations with HCC likely reflect its broader relationship to severity of illness and increased cell turnover. Therefore, while β2M may contribute to the diagnostic workup when combined with other established markers such as AFP, it should not be considered a specific or definitive test for HCC. Rather, β2M may be most appropriately utilized as an adjunct marker to help assess disease severity and monitor progression, in alignment with previous reports and current clinical practice.

CONCLUSION

Serum beta-2 microglobulin was significantly higher in cirrhotic patients, with the highest levels seen in those with hepatocellular carcinoma. Its elevation was associated with greater severity of liver dysfunction at a single time point. However, due to the cross-sectional design of this study, we cannot conclude disease progression. Longitudinal studies are needed to determine whether β2M may serve as a prognostic or monitoring marker over time. Combined with AFP, β2M may enhance diagnostic accuracy for HCC and could serve as a valuable adjunct in distinguishing HCC from cirrhosis, supporting earlier detection and surveillance in high-risk patients.

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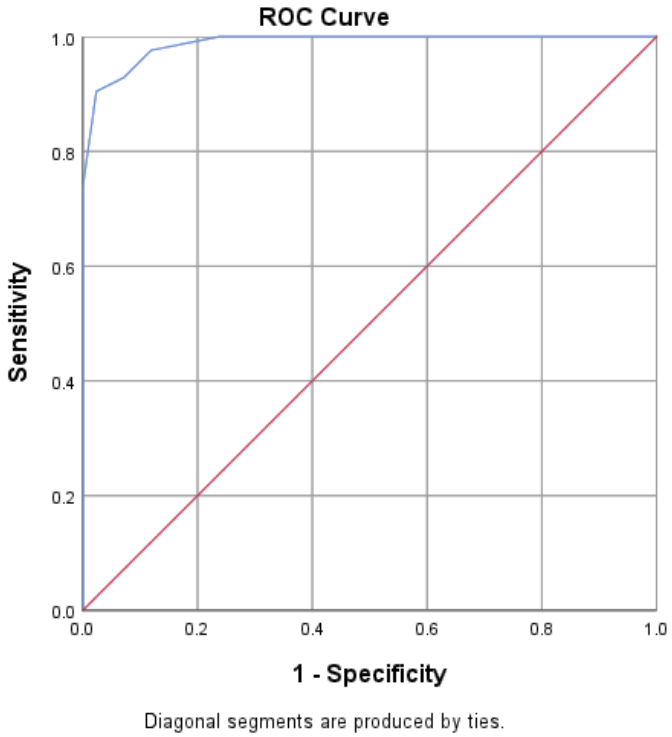

Supplementary Figure (1): Roc curve illustrating validity of Beta 2 microglobulin for prediction of HCC on top of cirrhosis.

Citation

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