ß2-microgloblin as a candidate for diagnosis of Hepatocellular Carcinoma in HCV-4 related liver cirrhosis

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Abstract:

   Background: *Patients with liver cirrhosis are at higher risk for development of hepatocellular carcinoma.* **The present study aimed to** *verify the reliability of ß2-microgloblin as a marker for detection of hepatocellular carcinoma in HCV-4 related liver cirrhosis.*

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Sameh Mohamed Ghaly, Reham Ezzat Al Swaff, Eslam Safwat, Hossam Al Baz and Mohamed Fouad- **ß2-microgloblin as a candidate for diagnosis of Hepatocellular Carcinoma in HCV-4 related liver cirrhosis**

Methods: Serum ß2-microgloblin level was measured in 91 Egyptian subjects who were recruited from the hepatology outpatients' clinic of Ain Shams University, Cairo, Egypt. 39 patients had HCV-4 related liver cirrhosis and hepatocellular carcinoma; 31 patients had HCV-4 related liver cirrhosis (hepatocellular carcinoma was excluded in these patients at time of enrollment in the study) and 21 healthy subjects served as a control group. Serum ß2-microgloblin level was measured by an enzyme linked immunoassay using double antibody sandwich kits.

Results: A highly significant higher mean ß2-microgloblin level was found in patients with hepatocellular carcinoma (P = 0.00). At a cut off value 4.7 mcg/ml; serum ß2-microgloblin had a sensitivity of 60 %, a specificity of 73.3%, a positive predictive value of 75 %, a negative predictive value of 57.9 % and an overall accuracy of 65.7 % for diagnosis of hepatocellular carcinoma. At a cut off value 254 ng/ml; serum AFP had a sensitivity of 37.5 %, a specificity of 100.0%, a positive predictive value of 100.0 %, a negative predictive value of 53.0 % and an overall accuracy of 61.0 % for diagnosis of hepatocellular carcinoma.

Conclusion: Serum ß2-MG is a promising marker for detection of hepatocellular carcinoma in HCV-4 related liver cirrhosis.

Key words: HCC, HCV-4, Cirrhosis, ß2-microgloblin

Introduction:

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide. Patients with liver cirrhosis are at higher risk for the development of HCC. To date diagnostic imaging such as Computer Tomography (CT) or Magnetic Resonance Imaging (MRI) is taken as the gold standard for definitive diagnosis of HCC [1].

Several serum markers developed for the diagnosis of HCC. α –fetoprotein (AFP) and protein induced by vitamin K absence (PIVKA-II) are most widely used as diagnostic serum markers for HCC; however their early diagnostic value is poor

[2]. Up to 40% of HCC patients' have normal AFP. Moreover, AFP can also be elevated in patients with cirrhosis or exacerbation of chronic hepatitis. Prospective studies evaluating the value of AFP in HCC surveillance have reported sensitivities of 39-64%, specificity of 76-91% and positive predictive values of 9 -32% [3].

ß2-microglobulin (ß2-MG) is a non-glycosylated polypeptide composed of 99 amino acids. It is one of the components of major histocompatibility complex HLA class I molecules on the cell surface of all nucleated cells [1]. Increased serum levels of ß2-MG occur in multiple myeloma, lymphoma, Sjogren's syndrome, amyloid fibrils and in patients receiving hemodialysis for long periods [4].High serum levels of ß2-MG were also detected in many infectious diseases including infection with HCV [5].

A significant correlation was found between serum ß2-MG level and interleukin-6 (IL-6), AFP and HCC tumor size. This indicates that the elevation of ß2-MG seems to be a consequence of the stimulation of hepatocytes by humoral components such as IL-6. Weakening of the immune system, due to IL-6, may be responsible for a more severe progression of HCC and over expression of ß2-MG [6].

The aim of the present study was to verify the reliability of ß2-MG as a marker for diagnosis of HCC in patient with HCV-4 related liver cirrhosis.

Patients and methods:

This study was conducted in the Gastroenterology and Hepatology unit, department of Internal Medicine, Ain Shams University hospital, Cairo, Egypt. 91 adult Egyptian patients were recruited from the HCC and Hepatology outpatients' clinic and were divided into 3 groups as follows:

Sameh Mohamed Ghaly, Reham Ezzat Al Swaff, Eslam Safwat, Hossam Al Baz and
Mohamed Fouad- **ß2-microgloblin as a candidate for diagnosis of Hepatocellular
Carcinoma in HCV-4 related liver cirrhosis**

Group I included 39 patients with HCV-4 related liver cirrhosis and well established diagnosis of HCC ; diagnosis of HCC was based on the presence of typical vascular pattern of enhancement of HCC in triphasic spiral computed CT scan of the abdomen.

Group II included 31 patients with HCV-4 related liver cirrhosis; HCC was excluded in these patients at time of enrollment in the study (exclusion of HCC was based on absence of any hepatic focal lesion in abdominal ultrasonography scan).

Group III included 21 "age and sex matched" healthy subjects who served as a control group.

All patients were subjected to the following: history taking, thorough clinical examination, abdominal ultrasonography (Aloka SSD620, Japan), laboratory investigations including: fasting and 2h post prandial blood glucose level, liver function tests, prothrombin time & INR, renal function tests, serum total protein level, complete blood count, CRP, ESR, serum alpha fetoprotein level, ANA, HBsAg, HCV and HIV antibodies using ELISA technique and urine analysis.

Serum ß2-MG level was determined by an enzyme linked immunoassay (ELISA) using double antibody sandwich ELISA Human Beta 2-Microglobulin antibody kits (MBS564037, Version3 L14.0 -- 5, MyBioSource, San Diego, CA 92195-3308, USA).

HCV genotyping was based on epidemiologic assumption [7].

Patients were excluded from the study if they had any of the following conditions: any form of chronic infection, any autoimmune disorder , any other malignancies (solid or humoral), previous history of interferon based treatment for HCV infection , acute or chronic kidney disease, hepato-renal syndrome, I.V. drug users, HIV infection, patients receiving any form of immune-modulating drugs, organ transplant

recipient, patients who received any form of treatment for HCC, female subjects who are pregnant or nursing an infant ,current or past history of heavy alcohol consumption, other liver diseases as alcoholic liver disease, non alcoholic fatty liver disease, drug-induced hepatitis, other viral hepatitis, hereditary haemochromatosis, Wilson's disease, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis and alpha-1 antitrypsin deficiency.

This study was approved by the local ethical committee of Ain Shams University and a written informed consent was obtained from each participant.

Statistical analysis: data were collected, coded, and entered to a personal computer (IBM compatible, 3 GHz). The collected data were analyzed with the program Statistical Package for Social Science version 16 for the Windows operating system. The following tests were used: calculation of mean values and standard deviation (SD), Pearson Correlation coefficient (r) test, one way Analysis of variance (ANOVA) and Chi-square test (χ^2). The probability of error (P) was interpreted as follows:

P>0.05 considered insignificant; P<0.05 considered significant and P<0.01 considered highly significant. Sensitivity, specificity, diagnostic efficiency, positive & negative predictive values and accuracy were calculated as follows:

Sensitivity = True Positive/ True Positive+ False Negative
Specificity = True Negative/True Negative+ False Positive
Positive predictive value = True positive/ True positive+ False positive
Negative predictive value = True negative/True negative +false negative
Accuracy = True Positive +True Negative/ all cases examined

The overall diagnostic performance of a test was assessed by receiver- operating characteristics (ROC) curve analysis.

Results:

This study was conducted in the period from May 2013 to June 2014. After applying the exclusion criteria strictly; only 91 adult subjects were recruited from the HCC and Hepatology outpatients' clinic and were divided into 3 groups as follows: group I included 39 patients with HCV related liver cirrhosis and well established diagnosis of HCC ;the mean age of this group was 55.9 $\pm$5.9 year ranging from44 to 74 years. Group II included 31 patients with HCV related liver cirrhosis; HCC was excluded in these patients at time of enrollment in the study; the mean age of this group was 52.2 $\pm$7.9 year ranging from34 to 69 years. Group III included 21"age and sex matched" healthy subjects who served as a control group; the mean age of this group was 29.8 $\pm$4.8 year ranging from24 to 48 years.

Insignificant differences were found between the three studied groups as regards gender (P=0.5). A detailed description of liver function tests of all participants is shown in table 1.

A highly significant higher mean serum ß2-MG level was found among patients with HCC (group I) as compared to other groups (P = 0.00). Mean ß2-MG level was also significantly higher among liver cirrhosis patients' (group II) as compared to controls (group III) (table 2).

Highly significant higher percentages of positive cases for ß2-MG was found among patients with HCC (92.3%) as compared to group II (54.8%) and group III(0%) (P = 0.00).

Insignificant correlations were found between serum ß2-MG level and different liver function tests among patients with HCC (table 4). However, a borderline significant positive correlation was found between AFP and serum ß2-MG level (P=0.05) (table 3).

In contrary to patients with HCC, patients with decompensated liver cirrhosis (group II) showed significant correlations between serum ß2-MG level in one hand and direct

Sameh Mohamed Ghaly, Reham Ezzat Al Swaff, Eslam Safwat, Hossam Al Baz and
Mohamed Fouad- **ß2-microglobin as a candidate for diagnosis of Hepatocellular
Carcinoma in HCV-4 related liver cirrhosis**

bilirubin, PT and serum albumin levels on the other hand (P = 0.00,0.00 and 0.04 respectively)(table 3).

Receiver-operating characteristic (ROC) curve analysis was applied to determine the best cut-off value of serum ß2-MG and AFP levels for the diagnosis of HCC in patient with HCV-4 related liver cirrhosis.

At a cut off value of 3 mcg/ml; serum ß2-MG level had a sensitivity of 92.5% , a specificity of 100.0% , a positive predictive value of 100% , a negative predictive value 87.0% and an overall accuracy of 95% in detection of HCC (considering healthy subjects as controls) (figure 1:A).

At a cut off value 4.7 mcg/ml; serum ß2-MG level had a sensitivity of 60 %, a specificity of 73.3%, a positive predictive value of 75 %, a negative predictive value 57.9 % and an overall accuracy of 65.7 % in detection of HCC in patients with HCV-4 related liver cirrhosis (considering patients with decompensated liver cirrhosis as controls) (figure 1:B).

Serum AFP level had lower sensitivity and lower negative predictive value in diagnosing HCC than serum ß2-MG level. At a cut off value 254 ng/ml; serum AFP level had a sensitivity of 37.5 %, a specificity of 100.0%, a positive predictive value of 100.0 %, a negative predictive value 53.0 % and an overall accuracy of 61.0 % in detection of HCC in patients with HCV-4 related liver cirrhosis (considering patients with decompensated liver cirrhosis as controls) (figure 2).

Discussion:

Major etiologic factors associated with HCC include infection with HCV and HBV viruses, excess alcohol intake and aflatoxin B1 exposure [8]. HCC and HCV infection represent major health problems in Egypt, where the two pathological conditions are integrated in many cases [9].

The mechanism of HCV-mediated oncogenic insults, required for initiation and/or progression of HCC, is not clear. HCV-RNA does not integrate into the host genome but likely induces HCC through the interaction of its proteins, such as the core, NS3 and NS5A, with the host cell proteins [10]. Over the past decade many reports have demonstrated that HCV proteins regulate growth and apoptosis related genes. These findings suggest that HCV infection is, directly or indirectly, involved in HCV-mediated HCC. Such cross-talk between HCV proteins and host cell regulatory proteins was, extensively monitored, but still unable to clear the role of HCV in HCC initiation and/or progression [6].

HCV infection enhances the elevation of serum ß2-MG levels [5]. This increase is due to stimulation of hepatocytes by humoral components of immunological response, such as IL-6 [11]. The current study aimed to verify the reliability of ß2-MG as a marker for diagnosis of HCC in patients with HCV-4 related liver cirrhosis.

In accordance with previous reports [5,11], the current study revealed a highly significant higher mean serum ß2-MG level among patients with HCC as compared to patients with liver cirrhosis and healthy subjects. Moreover, mean serum ß2-MG level was significantly higher among liver cirrhosis patients' than among healthy subjects. The previous results strongly points to a possible, direct and/or indirect, role of ß2-MG for initiation and/or progression of HCC in patients with HCV-4 related liver cirrhosis and also highlights the importance of serum ß2-MG level as a promising marker for detection of HCC. In agreement with the previous conclusion, Malaguarnera M et al, 2000[5] stated that weakening of the immune system, which may be due to IL6, could be responsible for more severe progression of HCC and hyper- expression of ß2-MG which may allow tumor cells to escape immunological control.

Sameh Mohamed Ghaly, Reham Ezzat Al Swaff, Eslam Safwat, Hossam Al Baz and
Mohamed Fouad- **ß2-microgloblin as a candidate for diagnosis of Hepatocellular
Carcinoma in HCV-4 related liver cirrhosis**

Serum AFP has long been identified as a poor serum marker for diagnosis of HCC. In addition, up to 40% of patients with HCC have normal AFP [3]. A remarkable finding of the current study is the superior role of serum ß2-MG over AFP in the diagnosis of HCC. Serum ß2-MG level was found to be more sensitive and more accurate than AFP in the diagnosis of HCC in patients with HCV-4 related liver cirrhosis. This result markedly adds to the value of serum ß2-MG as a promising marker for detection of HCC.

In contrast to Dennis and Rifkin [12] levels of serum ß2-MG did not correlate with tumor size. This discrepancy may be related to differences in sample sizes, patients' selection criteria and HCV genotypes.

The present clinical trial had few limitations. First, serum ß2-MG level was correlated only with AFP; further studies should be conducted to correlate serum ß2-MG level with other biomarkers for diagnosis of HCC such as PIVKA-II. Second, serum ß2-MG level was not evaluated following treatment of HCC; further studies are needed to evaluate the role of serum ß2-MG as a prognostic factor after treatment of HCC. Third, number of patients included in the present study is relatively small; larger scale studies should be conducted to establish the clinical utility of serum ß2-MG for detection of HCC and to evaluate the best cut off value.

Conclusion:

Serum ß2-MG is a promising marker for detection of HCC in HCV-4 related liver cirrhosis.

Acknowledgment:

Sameh Mohamed Ghaly, Reham Ezzat Al Swaff, Eslam Safwat, Hossam Al Baz and
Mohamed Fouad- **ß2-microgloblin as a candidate for diagnosis of Hepatocellular
Carcinoma in HCV-4 related liver cirrhosis**

Conflict of interest: nothing to display.

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Sameh Mohamed Ghaly, Reham Ezzat Al Swaff, Eslam Safwat, Hossam Al Baz and
Mohamed Fouad- **ß2-microgloblin as a candidate for diagnosis of Hepatocellular
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Tables:

**Table (1) Comparison between the studied groups as regards liver
function tests**

	Mean	SD	F	P	LSD
ALT Group I GroupII Group III	68.1 44.2 20.2	54.5 27.9 6.8	9.9	0.000	I vs. II, III II vs. III
Direct bilirubin Group I Group II Group III	1.9 1.0 0.09	2.7 1.2 0.06	5.7	0.004	I vs. III
Total proteins GroupI GroupII Group III	6.9 6.6 7.5	1.0 1.0 0.4	5.1	0.008	III vs. I, II
Serum albumin Group I Group II	2.8 2.6	0.6 0.9	29.7	0.000	III vs. I, II

Group III	4.1	0.3			
PT					
Group I	16.5	2.8	18.4	0.000	III vs. I, II
Group II	16.0	3.2			
Group III	12.2	0.0			
INR					
Group I	1.4	0.2	14.5	0.000	III vs. I, II
GroupII	1.3	0.3			
Group III	1.0	0.0			

SD: Standard Deviation, LSD: Least significant difference,

ALT: alanine aminotransferase, PT: prothrombin time,

INR: international normalization ratio, vs.: versus

Table (2) Comparison between the studied groups as regards the mean ß2-MG level

	Mean	SD	F	P	LSD
Group I	6.2	3.0			
Group II	1.8	0.3	32.7	0.000	I vs. II, III
Group III	0.7	0.2			II vs. III

SD: Standard Deviation, LSD: Least significant difference, vs.: versus

**Table (3) correlation between ß2-MG level and other studied parameters**

group I	ß2-MG
ALT	r =0.142 P=0.3
Direct bilirubin	r =0.239 P=0.1
Total protein	r =0.206 P=0.2
Serum albumin	r =0.096 P=0.5
PT	r =0.276 P=0.08
INR	r =0.248 P=0.1
AFP	r =0.315 P=0.05
Number of tumor foci	r =0.127 P=0.4
Tumor size	r =0.142 P=0.3

Sameh Mohamed Ghaly, Reham Ezzat Al Swaff, Eslam Safwat, Hossam Al Baz and
Mohamed Fouad- **ß2-microgloblin as a candidate for diagnosis of Hepatocellular
Carcinoma in HCV-4 related liver cirrhosis**

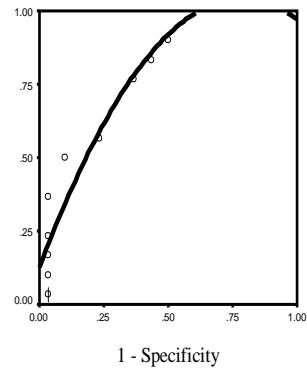| group II	ß2-MG
ALT	r =0.038 P=0.8
Direct bilirubin	r =0.619 P=0.000
Total protein	r =0.134 P=0.4
Serum albumin	r =-0.364 P=0.04
PT	r =0.492 P=0.000
INR	r =0.520 P=0.003

ALT: alanine aminotransferase, PT: prothrombin time,
INR: international normalization ratio, AFP:alpha feto-protein

Figures:

**Figure (1): ROC curve of serum ß2-MG level in detection of HCC (A:
considering healthy subjects as controls, B: considering patients with
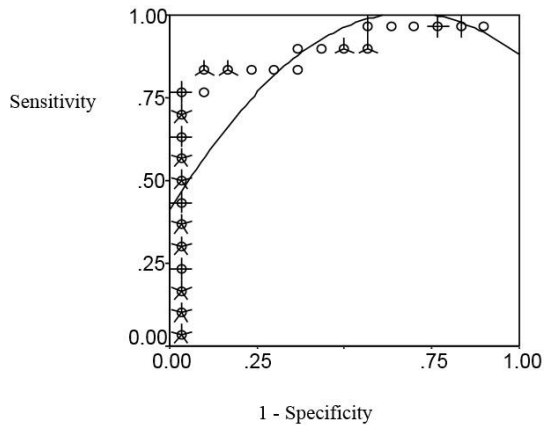decompensated liver cirrhosis as controls)**

1 - Specificity

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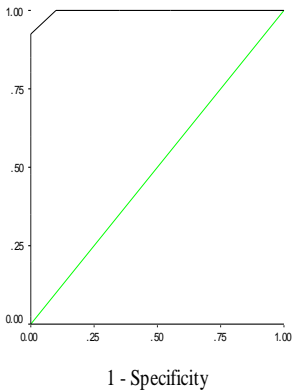
A: Area under the curve= 0.996, 95% CI (0.98-1.0)   **B**: Area under the curve = 0.79, 95% CI (0.69-0.90)

Figure (2) ROC Curve analysis of AFP in detection of HCC (considering patients with decompensated liver cirrhosis as controls)

Area under the curve= 0.90, 95% CI (0.84-0.96)