RESEARCH ARTICLE

Serum Beta-2 Microglobulin: a Possible Marker for Disease Progression in Egyptian Patients with Chronic HCV Related Liver Diseases

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Abstract

__Background:__ Egypt has the highest prevalence of HCV infection in the world (~14.7%). Around 10-15% of HCV-infected persons will advance to cirrhosis within the first 20 years. The incidence of HCC is expected to grow in the next two decades, largely due to HCV related cirrhosis, and detection of HCC at an early stage is critical for a favorable clinical outcome. No simple reliable non-invasive marker has been available till now. B2M, a non-glycosylated polypeptide composed of 99 amino acids, is one of the components of HLA class I molecules on the surfaces of all nucleated cells. It has been reported that the level of serum B2M is elevated in patients with chronic hepatitis C and HCV-related HCC when compared to HCV-negative patients or healthy donors. Determining the clinical utility of serum B2M as a marker for disease progression in Egyptian patients with HCV related chronic hepatitis, cirrhosis and hepatocellular carcinoma was the aim of the present study. __Materials and Methods:__ In this analytical cross sectional study 92 participants were included in 4 equal groups: Group (1) non cirrhotic chronic HCV; Group (2) HCV related liver cirrhosis; Group (3) HCC on top of HCV,; and Group (4) healthy controls. History taking, clinical examination, routine labs and abdominal ultrasound were conducted for all patients, PCR and Metavir scores for group (1) patients, and triphasic CT abdomen and AFP for Group (3) patients. B2M levels were measured in serum with a fully-automated IMX system. __Results:__ The mean serum B2M level of Group (1) was 4.25±1.48 μg/ml., Group (2) was 7.48±3.04, Group (3) was 6.62±2.49 and Group (4) was 1.62±0.63. Serum B2M levels were significantly higher in diseased than control group (p<0.01) being significantly higher in cirrhosis (7.48±3.04) and HCC groups (6.62±2.49) than the HCV group (4.25±1.48) (p<0.01). There was a significant correlation between B2M Level and ALK, total and direct bilirubin and INR (p<0.05), and a significant inverse correlation between B2M level and albumin, total proteins, HB andWBCS values (p<0.05). There was no significant correlation between B2M level and viral load or Metavir score, largest tumour size or AFP (p>0.05). The best B2M cut-off for HCV diagnosis was 2.6 with a sensitivity of 100%, a specificity of 92%, a positive predictive value (PPV) of 97% and a negative predictive value (NPV) of 100%. The best B2M cut-off for HCC diagnosis was 4.55 which yielded sensitivity, specificity, positive predictive value, negative predictive values of 74%, 62%, 39.5, 87.8% respectively (p-value <0.01) while best cut-off for cirrhosis was 4.9, with sensitivity 74 % and specificity 74%.The sensitivity for HCC diagnosis increased upon B2M and AFP combined estimation to 91%, specificity to 79%, NPV to 95% and accuracy to 83%. __Conclusions:__ Serum B2M level is elevated in HCV related chronic liver diseases and may be used as a marker for HCV disease progression towards cirrhosis and carcinoma.

__Keywords:__ Serum- B2M-HCV- HCC- progression- marker

Introduction

About130-170 million people are chronically infected with HCV and more than 350,000 people die from HCV-related liver diseases each year (Chung and Baumert 2014; Cuadros et al., 2014; Feld et al., 2014). The rate of progression to cirrhosis is highly variable, and is influenced by several factors, including age of initial HCV infection, degree of inflammation and fibrosis on liver biopsy, and co-morbid conditions Those with cirrhosis are at increased risk of developing HCC (Kanwal and Bakon , 2012; Hajarizadeh et al., 2013; Flemming et al., 2014).

The problem of the development of biomarkers capable of non-invasive staging of fibrosis in the liver is difficult due to the fact that the process of fibrogenesis is a component of the normal healing response to injury, or invasion by other pathogens (Baranova et al., 2011). Serum AFP levels of more than 200 ng /ml are considered diagnostic for HCC; however, such high values are observed only in a small percentage of patients. Ultrasound surveillance even performed at three months interval could not improve detection of small HCC because of limitations in recall procedures (Behne and Sitki, 2012; Mourad et al., 2013).

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ß2 microglobulin (B2M) is an amino acid peptide, a component of MHC class I molecules, which are present on all nucleated cells that present self and non self antigens to cell surfaces. A high serum level of B2M was detected in many infectious diseases including HCV (Riolobos et al., 2013; Changhoon et al., 2015). Serum B2M was elevated in HCV patients and HCV-related HCC when compared to HCV-negative patients or healthy donors (Saito et al., 2010; Saleh, 2012; Huckans et al., 2014). Yet, other studies claim no true relation between serum B2M and disease progression (Tabayoyong and Zavazava , 2007).

Materials and Methods

Clinical samples

A total of 92 blood samples (Group (1): 23 chronic HCV; Group (2): 23 cirrhotics on top of HCV, Group (3): 23 HCC on top of HCV, andGroup (4): 23 healthy controls) were withdrawn after informed consent from patients and volunteer visitors at the Department of Endemic Medicine, KasrAlainy Hospital (Cairo, Egypt) from April till October 2013. Laboratory tests were done in the Clinical pathology and Medical Microbiology and Immunology departments, Kasr Alainy, Cairo University.

All patients and healthy controls were HBsAg -ve, with normal urea and creatinine level, no history of auto-immune diseases or malignancies other than HCC. Patients with liver disease other than HCV such as acute hepatitis, metabolic hepatitis, and history of alcohol consumption or hepatotoxic drug use were excluded.

All subjects were subjected to full medical history taking, clinical examination, routine Laboratory investigations (CBC, Liver function tests, Kidney function tests, coagulation profile) and abdominal Ultrasound examination. HCV Quantitative PCR and Metavir score of fibrosis were obtained from Group (1) patients, Triphasic CT abdomen and AFP were required to confirm HCC diagnosis in Group (3).

Specimen collection, Preparation, and Storage

A volume of 10 ml of blood was withdrawn from each participant under complete aseptic conditions, divided equally into 2 volumes for the sake of routine laboratory investigations and serum separation by centrifugation. Each separated serum sample was divided into 4 aliquots to prevent repeated freezing and thawing of the same aliquot which may alter the results. Only clear, non haemolyzed serum samples were used. Sera were stored at -20°C till processing. Sera were brought to room temperature prior to testing. Frozen samples were completely thawed and mixed well prior to testing.

ELISA Testings

All serum samples were subjected to HBsAg detection using a commercial third generation enzyme immunoassay (Murex HBsAg Version 3, S. Africa), and HCV Ab detection using a commercial third generation enzyme immunoassay (Murex anti-HCV, version 4.0, S. Africa), based on enzyme immune assay (sandwich technique). The tests were done according to manufacturers' instructions.

B2M Detection in serum

B2M was estimated by the IMX ß2-MG assay using fully-automated IMX system (Abbott Laboratory, USA), based on the microparticle immunoassay (MEIA).

Statistical methods

Quantitative variables presented by number and percent. They were compared by chi-square or Fisher's exact test. These were presented by mean and standard deviation (SD) if normally distributed and by median and inter quartile range (IQR) if not normally distributed.

Table 1. Demographic Data and Investigations for Studied Groups

Mean ± SD	HCV	Cirrhosis	HCC	Controls	P value
Age	42.2±8a	54.8±7 b	58.6±8b	32.8±8c	<0.01
Sex(females)	9 (39.1%)	9 (39.1%)	7 (30.4%)	10 (43.5%)	>0.05
Hb(g/L)	13.7±1.7a	10.7±2.2b	11.2±2.3b	13.4±1.8a	<0.01
RBCs (103/l)	4.7±0.9a	3.8±1.3b	3.7±0.95b	4.8±0.64a	<0.01
WBCs (103/l)	6.4±1.9c	5.8±3.8c	5.5±2.1b	7.2±2c	>0.05
Platelets (103/l)	211±80a	113±94b	131±62b	267±82c	<0.01
AST(15-37)	49±38a	58±35a	79±40b	24±8c	<0.01
ALT(30-65)	56±44a	40±21a	51±28a	17±6b	>0.01
ALK	143±81a	125±57a	247±134 b	75±20c	<0.01
T.Bil(mg/dl)	0.9±.3a	4.9±4.8b	2.8±3c	0.4±.2a	<0.01
D.Bil (mg/dl)	0.23±.11a	2.5±2.6b	1.4±1.9c	0.08±.05a	<0.01
Albumin(g/l)	4.3±.47a	2.7±.53b	3±.8b	4.2±.7a	<0.01
T.Proteins	7.5±0.8a	6.5±0.9b	6.7±1.1b	7.8±0.6a	<0.01
INR	1.2±.3c	1.5±.4b	1.3±.3c	1.1±.08d	<0.01
Metavir A1/A2	15,8	-	-	-	NA
Met. F1-2/ F3	14,9	-	-	-	NA
MELD <20/≥20	-	14,9	-	-	NA
AFP (ng/L)	8.±3.21	5.6±1.6	1409.4±4174.8.	-	>0.05
HFL (n) S/M	-	-	9,14	-	NA
B2M (µg/ml)	4.25±1.48a	7.48±3.04b	6.62±2.49b	1.62±.63c	<0.01

AST: Aspartate Transaminase; ALT: Alanine Transaminase; ALK: Alkaline Phosphatase; T. &D. Bil: Total& Direct Bilirubin; INR: International Normalization Ratio; S/M:Single/Multiple; All values are expressed in Mean ±SD; Different letters mean significant difference at the level of 0.05 or 0.01

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Multiple groups were compared by ANOVA or Kruskill Wallis test respectively. Pearson or Spearman Correlations were used to correlate two continuous variables. Receiver operator characteristic (ROC) curve was constructed to assess B2 micro globulin in discriminating 2 groups. In all tests, p value was considered significant if less than 0.05.

Results

Demographic data and investigations for studied groups are shown in Table (1)

Serum B2M levels were significantly higher in diseased groups than in control group (p<0.01), and significantly higher in Cirrhosis (7.48±3.04) and HCC groups (6.62±2.49) than HCV group (4.25±1.48) (p<0.01) (Figure 1).

There was a significant correlation between B2M level and age (p<0.01). There was a significant inverse correlation between B2M level and Hb level, Platelet Count, serum albumin and total protein among the studied
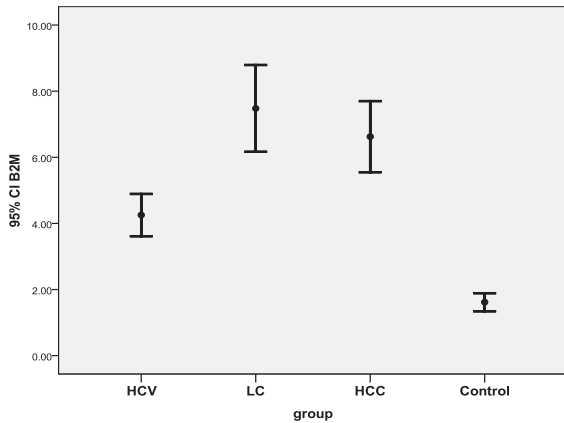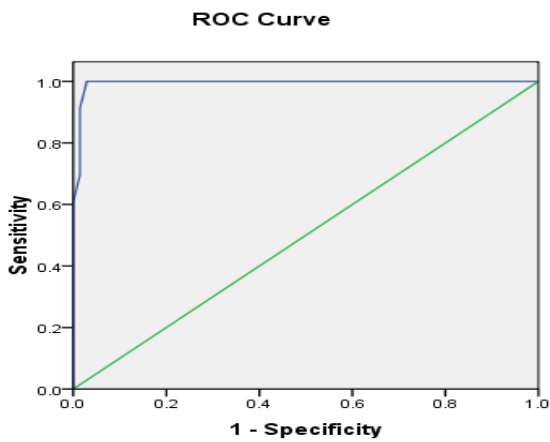
Figure 3. Diagnostic Performance of B2M for Liver Cirrhosis. AUC 0.81 (95% CI 0.71-0.92), P<0.01, best cut-off 4.9, at which sensitivity o.74 and specificity 0.74

Figure 1. Serum B2M Levels of Studied Groups. Serum B2M levels were significantly higher in diseased than control group (p<0.01) being significantly higher in Cirrhosis (7.48±3.04) and HCC groups (6.62±2.49) than HCV group (4.25±1.48) (p<0.01)

Figure 4. Diagnostic performance of B2M for HCC. The best B2M cut-off for HCC diagnosis was 4.55 which yielded sensitivity, specificity, positive predictive value, negative predictive values of 74%, 62%, 39.5, 87.8% respectively (p value<0.01)

Figure 2. Diagnostic Performance of B2M for Chronic HCV Patients. The best B2M cut-off for HCV diagnosis was 2.6 with sensitivity of 100%, specificity 92%, positive predictive value (PPV) 97% and negative predictive value(NPV) 100%
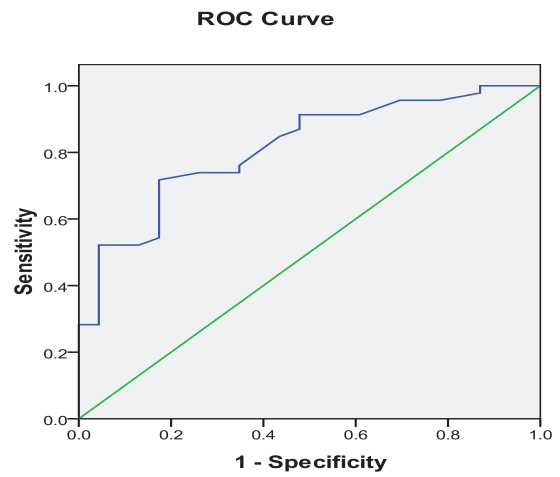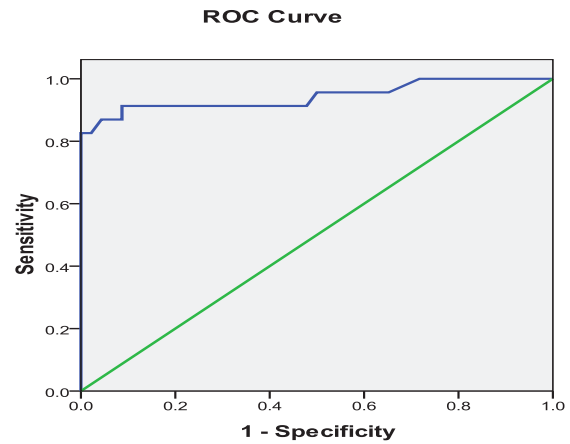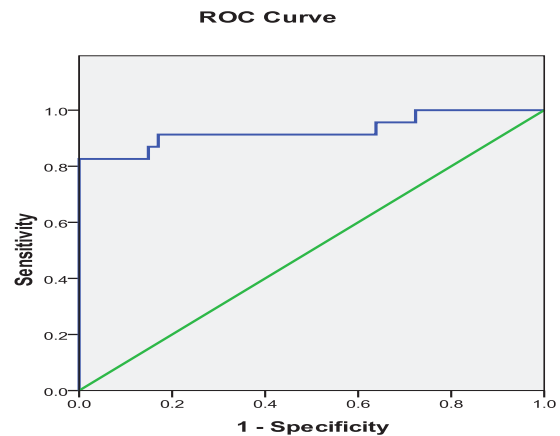
Figure 5. Diagnostic Performance of Combined B2M and AFP for HCC. The sensitivity for HCC diagnosis increased upon B2M & AFP combined estimation to 91%, specificity to 79% , NPV to 95% and accuracy to 83%

groups (p<0.01).There was a significant correlation between B2M level and INR level (p<0.01), ALK (p<0.05), total and direct bilirubin levels (p<0.01 and p<0.05 respectively). There was no significant correlation between B2M level and viral load or Metavir score in HCV group (p>0.05). No significant correlation between B2M level and tumor size or AFP in HCC Group (p>0.05) was noted.

Performance characteristics of B2M in studied patients

By categorizing patients into normal and diseased Performance Characteristics of B2M in diagnosis of the HCV related diseases was plotted (Figure 2). At cut-off value of 2.6, the AUC was 0.99 with sensitivity of 100%, specificity 92%, positive predictive value (PPV) 97% and negative predictive value (NPV) 100%.

By categorizing patients into cirrhotics and non cirrhotics, Performance Characteristics of B2M in diagnosis of the HCV related cirrhosis was plotted (Figure 3). At cut-off value of 4.9; the AUC was 0.81 with sensitivity of 74%, specificity 74%.

By categorizing the patients HCC *vs* non HCC Figure (4), ROC analysis of serum B2M was performed to determine its diagnostic accuracy for HCC.AUC was 0.74,the best cutoff was 4.55 which yielded sensitivity, specificity, positive predictive value, negative predictive values of 74%, 62%, 39.5, 87.8% respectively (p value<0.01).

Value of combined B2M and AFP in HCC diagnosis

The AUC increased significantly to 0.93 upon combined B2M / AFP estimation (P<0.01) for HCC diagnosis (Figure 5). The sensitivity, specificity, negative predictive value and accuracy of this combination were 91%, 79%, 95% and 83% respectively.

Discussion

Serum markers have been proposed as a simple and convenient means to estimate chronic liver disease progression.Although some markers may be effective, the clinical utility of these markers is still limited (Behne and Sitki,2012) .Detecting simple, non invasive, accurate and reliable marker that is linked to clinically important milestones in liver disease progression, such as cirrhosis and HCC, is currently an area of active investigation (Chatterjeea and Mitra 2015).

In the current study, the aim was to assess the possible role of serum B2M level as a marker of disease progression in Egyptian patients with chronic HCV related liver disease.

Serum B2M levels were significantly higher in patients than control group (p<0.001). This finding goes with Huckans et al. (2014) who conducted a study on altered expression of peripheral immune factors associated with neuro-psychiatric symptom severity in adults with and without chronic hepatitis C virus infection, and found that B2M levels were higher in in adults with hepatitis C virus (n=39) than those without (n=40) (p<0.001). This might be attributed to the increased endogenous production of interferons and/or other cytokines associated with viral infection. High serum B2M level is associated with an activated immune response and release by activated lymphocyte (T4/T8 cells), so increase in its level might indicate increasing HCV replication- related cell death. In HCV chronicity, many differentially expressed genes involved in the pathways of immune system, fibrosis, proliferation, cell growth, and apoptosis have been found to be up- regulated, including major histo-compatibility, and B2M genes (Kim and Wang, 2003; Elgendy et al., 2005; Khaled et al., 2012; Saleh, 2012).

Serum B2M levels were significantly higher in HCC group (6.62±2.49) than chronic HCV group (4.25±1.48) (p<0.01). Other studies reported high serum B2M in HCC patients on top of chronic HCV and suggested that the B2M in plasma could be used as an early marker to detect imaging-invisible HCC (Maliguarnera et al., 2000; Migliaresia et al., 2000; Saito et al., 2010). In histologically normal liver, HLA class1 antigens were mainly expressed on liver sinusoidal lining cells rather than on hepatocytes (Asanza et al., 1997). The reason why the phenomenon that histologically normal hepatocytes have no or only weak HLA class1 antigens expression though liver cancer cells strongly express HLA class antigens, may be related to the unique lymphocytes distribution in liver and NK-cell escape. About 31% of the liver resident lymphocytes are NK cells. Therefore, re-expression or enhanced expression of HLA class1 antigens on HCC cells would be helpful for inhibiting the non-specific cytotoxicity of NK cells through their inhibitory KIR receptors (Huang, 2002).

In the current study there was a significant correlation between B2M level and age (p<0.01). This goes with Hamidreza et al. (2013) who reported that serum B2M values are dependent on age in patients with chronic active hepatitis which may be due to dysfunction of the liver.

A significant correlation was found between B2M level and some biochemical parameters (ALK, bilirubin and INR) in the study groups (p<0.05). A significant inverse correlation was found between B2M level and serum Albumin ,Total proteins, Hb level, WBCs count, and Platelet count in the study groups (p<0.05). However, No significant correlation was found between B2M and viral load among group 1 patients (p>0.05). This goes with Asanza et al. (1997) who performed a study on 32 cases to assess the immune-histochemical evidence of immune-pathogenetic mechanisms in chronic hepatitis C recurrence after of liver transplantation, and reported that in severe hepatic inflammation, high numbers of activated cytotoxic T cells were found along with marked hepatocellular expression of B2M. Yet, the level of viremia did not correlate with the degree of liver damage.

In this study, no significant correlation was found between B2M and the Metavir score in group1 patients (p>0.05). This doesn't go with Quiroga et al. (1994) who found significant correlations between beta 2-microglobulin concentration and Knodell's index (r=0.638, P=0.00045), the score of piecemeal necrosis (r=0.572, P=0.0023), and the degree of fibrosis (r=0.527, P=0.0056) in their HCV patients than healthy group. This difference in the results may be due to genotypic variation among study subjects that has an influence on HCV core Ag level as reported by Agha et al. (2004) where they

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found the mean HCVcAg level was significantly higher with genotypes 1, 2, and 3 and lower with genotype 4, with altered immune reactivity that plays a role in the pathogenesis of chronic hepatitis C.

No significant correlation was found between B2M and tumor size or serum AFP level (p>0.05) in group 3 patients. This doesn't go with Malaguarnera et al. (2000), who found significant correlation between B2M and tumor size (r=+0.3; P=0.02), and alpha-fetoprotein (r=+0.4; P=0.005) in their HCC patients. Upon which they concluded that serum levels of B2M reflect tumor size. This discrepancy might be due to small sample size in our study. Larger scale studies may be needed to verify the correlation.

Combined AFPand B2M estimation improved AUC for HCC diagnosis from 0.74 to 0.93.This goes with Ward et al. (2006) where B2M was the most significantly HCC associated proteomic finding in their study. They mentioned that B2M might add power to a multi-marker HCC diagnostic panel and concluded that combined estimation of AFP and B2M might add a significant yield rather than B2M values alone for diagnosis of HCC.

Thus, this hypothesis needs to be explored further by the close monitoring of patients cohorts with HCV chronic active hepatitis and cirrhosis who are at high risk of HCC and repeated B2M serum profiling in order to reach more confirmatory results.

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