

Prognostic value of Golgi P 73 and Beta 2-microglobulin in patients with Viral Hepatitis B

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Previous studies detected higher Golgi protein 73 levels in the serum of patients with chronic liver disease. The Beta-2 microglobulin levels were also observed to be higher in patients with chronic hepatitis B infection compared to the inactive carriers and the protein plays an important role in the response to viral infections. The aim of the present study was to assess the liver fibrosis through non-invasive methods in chronic hepatitis B patients. Three groups were included in the study. The first group comprised of the patients who were admitted to the Infectious Diseases and Clinical Microbiology clinic to undergo a liver biopsy, while the second group included the patients who were admitted inactive hepatitis B carriers. The third group comprised the healthy controls. The Golgi p-73 and Beta-2 microglobulin levels in the plasma were determined using the ELISA method. Beta-2 microglobulin level was highest in the patients group and the difference was statistically significant. No significant difference was observed between the carriers group and the group of healthy controls. The Golgi P-73 values were significantly higher in the patients group in comparison to both other groups. However, the mean Golgi p-73 value was also significantly higher in the carrier group compared to the control group. In patients who are followed up with the diagnosis of chronic hepatitis B and who have undergone biopsies as candidates for treatment, the Beta-2 microglobulin and Golgi p-73 values may be important markers since they indicate the extent of the liver damage.

Key words: Golgi protein-73, Beta-2 microglobulin, prognosis, Hepatitis B

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Abbreviations: HBV, Hepatitis B virus, GP-73, Golgi protein 73, CHB, chronic hepatitis B, β -2 MG, Beta-2 microglobulin, ALT, alanine aminotransferase, AST, aspartate aminotransferase

INTRODUCTION

The hepatitis B virus (HBV) is a highly contagious virus affecting approximately two billion people worldwide (EASL, 2012). About 240 million people have chronic hepatitis B infections and 600 thousand deaths occur due to acute and chronic hepatitis B every year (WHO, 2013). HBV is also an important risk factor in acute and chronic liver disease (Zhou *et al.*, 2009). Chronic HBV infection leads to progressive liver damage and liver fi-

bro sis. Therefore, the description of an accurate method to evaluate liver damage and liver fibrosis is vital for the patients with chronic HBV infection. Currently, the lack of an accurate, reproducible and practical method to show the degree of the liver damage and liver fibrosis in patients with HBV is a serious limitation in the clinical management of this disease. The existing clinical methods for this purpose are ultrasonography, liver function tests, coagulation tests, and the serum markers of liver fibrosis which may reflect the disease progression in patients with chronic HBV (Rotman *et al.*, 2009). Liver biopsy indicates the progress of the liver disease in the patients with chronic hepatitis B (CHB) (EASL, 2012). However, liver biopsy is a method with limited application in the clinic due to its invasive nature. Therefore, simple and non-invasive serum markers should be determined in chronic hepatitis B patients in order to evaluate the disease progress. The Golgi protein 73 (GP-73) is a newly-described transmembrane protein released from the human epithelial cells (Kladney *et al.*, 2000). In normal liver tissue, GP-73 is primarily released from the biliary epithelial cells and rarely from the healthy hepatocytes (Riener *et al.*, 2009; Kladney *et al.*, 2002). Studies showed that the hepatocellular GP-73 mRNA levels and protein release increase independently from the etiology and the concurrent disease in patients with acute and chronic hepatitis (Kladney *et al.*, 2002; Sun *et al.*, 2011). Higher GP-73 protein concentrations were also demonstrated to be associated with chronic liver disease in various studies (Sun *et al.*, 2011; Iftikhar *et al.*, 2004). The Beta-2 microglobulin (β -2 MG), which is an integral part of the human leukocyte antigen class 1 (HLA-1), is a protein with low molecular weight present in every cell that contains a nucleus. HLA-1 is found on the surface of the T- and B-lymphocytes and in various organs (Elefsiniotis *et al.*, 2004). Studies reported higher serum β -2 MG levels in patients with acute hepatitis B, chronic hepatitis B, hepatic cirrhosis, and in inactive hepatitis B carriers (Kieslichova *et al.*, 2009; Kim *et al.*, 2011). The aim of the present study was to assess the usefulness of the non-invasive GP-73 and β -2 MG tests as markers of liver damage in patients with hepatitis B.

MATERIAL AND METHOD

The study was conducted in a prospective manner on patients who had undergone biopsies due to chronic hepatitis B (Group 1), on inactive HBsAg carriers (Group 2), and healthy controls (Group 3) at the Dicle University Medical Faculty, Infectious Diseases and Mi-

crobiology Department between January 2012 and January 2014.

Inclusion criteria (13 Anna *et al.*, 2011).

Group 1: Patients who had undergone biopsies due to chronic Hep B

- (1) HBsAg-positive for more than 6 months,
- (2) ALT value greater than 1.5 times the upper normal value limit (normally, the ALT value is below 40 IU/mL),
- (3) HBV DNA $\geq 100\,000$ copies/mL (20 000 IU/mL) in those positive for the hepatitis Be antigen (HBeAg-positive),
- (4) HBV DNA $\geq 10\,000$ copies/mL (2 000 IU/mL) in those HBeAg-negative, and
- (5) Fibrosis ≥ 2 in the histopathological evaluation of the liver

Group 2: Inactive carriers

- (1) HBsAg-positive,
- (2) Normal ALT values,
- (3) HBeAg-negative, and
- (4) HBV DNA $\leq 10\,000$ copies/mL (2 000 IU/mL)

Group 3: Control group comprising HBsAg-negative and antiHBe-total negative patients.

Exclusion criteria. Diabetes mellitus, liver cirrhosis, hypertension, coronary artery disease, chronic obstructive pulmonary disease, corticosteroid treatment, malignancies, morbid obesity, pregnancy, liver and kidney failure, and smoking were the criteria for the exclusion from the study.

All the patients with chronic hepatitis B had undergone biopsies before the treatment. The patients' age, gender, and HBsAg, HBeAg, antiHBe, ALT, AST and HBV DNA values were recorded. In addition, the fibrosis scores of the chronic hepatitis B patients based on the biopsy results were also recorded. The ALT and AST tests were conducted at the central laboratory of the Dicle University Medical Faculty through the spectrophotometric method (Architect CT16000 Abbott, USA). The DNA levels were tested using the COBAS®AmpliPrep Total Nucleic Acid Isolation Kit (Roche Molecular Systems Inc., Branchburg, NJ, USA). After the DNA was isolated, the HBV DNA levels were evaluated with the COBAS®AmpliPrep/COBAS®Taqman® HBV Test v2.0 (Roche Molecular Systems Inc., Branchburg, NJ, USA). The HBV DNA levels of the patients were expressed in IU/ml. The sera collected during the study were stored at -70°C . Subsequently, the GP-73 in the serum was studied with the Human Golgi Protein 73 (GP-73) ELISA (enzyme-linked immunosorbent assay) kit (Hangzhou Eastbiopharm Co., LTD, Hangzhou, China). The β -2 MG test was carried out using Human β 2 microglobulin (β -2 MG) ELISA (enzyme-linked immunosorbent assay) kit (Hangzhou Eastbiopharm Co., LTD, Hangzhou, China).

The approval of the Dicle University Medical Faculty, Non-Invasive Clinical Studies Ethics Committee was obtained for the study. The GP-73 and β -2 MG ELISA kits required for the study were financed with the

grant of the Dicle University Medical Faculty Scientific Research Project Coordinatorship.

Statistical Analyses. The obtained data were entered into the SPSS 15.0 statistical software. Categorical data were analysed using the Chi-square test. The normality of the distribution of the numeric data was tested with the Kolmogorov-Smirnov test. The normal data were analysed with Student's *t*-test, while those not displaying normal distribution were analysed using the Mann-Whitney U test. Statistical significance was based on a value of $p < 0.05$.

RESULTS

For the purpose of the study, a total of 165 patients were enrolled, of which 60 were in Group 1, 55 were in Group 2, and 50 were in Group 3. There was no statistically significant difference between the groups in terms of the mean age and gender ($p=0.318$ and $p=0.133$, respectively) (Table 1). The mean GP-73 value was highest in Group 1, while the lowest value was observed in Group 3. A statistically significant difference was observed between the three groups in terms of the mean GP-73 values ($p < 0.001$) (Table 1). There was no relationship between the GP-73 value and the fibrosis score, and the GP-73 value increase was not correlated with the fibrosis score increase. On the other hand, there was a statistically significant correlation of the HBV DNA level and the fibrosis score ($r=0.295$, $p=0.022$), and the fibrosis score was observed to increase together with the HBV DNA level. The β -2 MG values were statistically significantly different in the three groups. The highest mean value was found in Group 1, while the lowest mean value was observed in Group 3 (Table 1). There was no significant correlation between the fibrosis scores and the β -2 MG levels (Table 2). In terms of the association of the HBV DNA levels with the β -2 MG levels, the β -2 MG values were not observed to be positively correlated with the HBV DNA levels. This result was also statistically significant (Table 2).

DISCUSSION

GP-73 is primarily released from the biliary epithelial cells and rarely from the healthy hepatocytes (Riener *et al.*, 2009; Kladney *et al.*, 2002). Various studies demonstrated that higher GP-73 protein levels are correlated with chronic hepatic disease (Sun *et al.*, 2011; Iftikhar *et al.*, 2004). In patients with chronic hepatitis, the correlation between the serum GP-73 protein levels and the pathological grading of the liver or the fibrosis staging is yet to be cleared. The controversy on the relationship between the serum GP-73 protein levels and HBV replication still continues (Sterling *et al.*, 2007; Chen *et al.*, 2010). In the study by Zhengju *et al.* (Zhengju *et al.*, 2015), the serum GP-73 levels and the pathological grading of the liver were compared in patients with chronic hepatitis B.

Table 1. The clinical and demographic data of the study groups

	Group 1 n=33 (%)	Group 2 n=33 (%)	Group 3 n=33 (%)	<i>P</i>
Mean age	33.21 \pm 10.20	36.73 \pm 11.54	33.00 \pm 11.71	0.318
Gender (M/F)	21/12	21/12	14/19	0.133
GP-73	31.38 \pm 3.01	8.30 \pm 1.09	6.66 \pm 7.62	<0.001
β -2 MG	22165.20 \pm 22764.4	5715.78 \pm 10169.09	4056.80 \pm 5620.63	<0.001

β -2 MG, Beta-2 microglobulin; GP-73, Golgi protein 73. Statistical significance was based on a value of $p < 0.05$

Table 2. Relationship of the HBV DNA and fibrosis scores with the GP-73 and β -2 MG levels

	r (HBVDNA)	p (HBVDNA)	r (fibrosis score)	p (fibrosis score)
GP-73	0.040	0.762	-0.160	0.223
β -2 MG	0.061	0.642	-0.134	0.307
Fibrosis score	0.295	0.022	None	None

β -2 MG, Beta-2 microglobulin; GP-73, Golgi protein 73; r: correlation coefficient. Statistical significance was based on a value of $p < 0.05$

According to the results of this study, the serum GP-73 levels were correlated with the pathological grading of the liver and the GP-73 levels increased together with the pathological grade (Zhengiu *et al.*, 2015). In a study conducted on the diagnostic and prognostic efficiency of the GP-73 protein in chronic liver disease due to chronic hepatitis B, GP-73 was demonstrated to be a beneficial marker both in the serum and histopathologically in the liver (Zhengiu *et al.*, 2015). In our study, no correlation was observed between the serum GP-73 levels and the fibrosis score. This result was attributed to the limited number of patients in our study. We did not study the GP-73 levels histopathologically in our patients. In a study by Liu and coworkers (2011) where they studied the serum GP-73 levels as a progression marker in patients with CHB, no relationship was observed between the HBV DNA and the serum GP-73 levels. However, a correlation was found between the fibrosis score and the serum GP-73 levels (Liu *et al.*, 2011). In our study, we observed a correlation between the HBV DNA levels and the fibrosis score and the fibrosis score increased together with the HBV DNA level. As part of the HLA complex, β -2 MG is responsible for the transmission of the viral antigens of HBV onto the surface of the liver cells. Also, β -2 MG may be an indicator of HBV virus prevalence. (Abdolsamadi *et al.*, 2013). In previous studies, significant increase was observed in the β -2 MG levels both in acute viral hepatitis and in patients with chronic hepatitis (Man *et al.*, 1989; Yegane *et al.*, 2004; Westral *et al.*, 1984; Malaguarnera *et al.*, 2000). In the study by Gunbay and coworkers (2014) where they evaluated β -2 MG as a prognostic factor in the management of chronic hepatitis B, patients were divided into two groups with Group 1 comprising those who had biopsies due to the diagnosis of CHB and Group 2 including inactive HBsAg(+) carriers. When the β -2 MG levels in each group were compared, the β -2 MG levels in Group 1 were found to be higher. Among the patients in Group 1 who received pegylated interferon therapy, the β -2 MG levels were observed to diminish significantly in the second month of the therapy in comparison to pre-treatment values (Gunbay *et al.*, 2014). Also in our study, the highest β -2 MG levels were found in the patients who had undergone biopsies with the diagnosis of CHB, which was in line with the previous studies. In a study conducted on patients with chronic hepatitis C, a significant correlation was observed between the histology activity index and the β -2 MG levels (Malaguarnera *et al.*, 1994). No relationship was observed between the β -2 MG levels and the fibrosis score in our study. In a study investigating the relationship between HBV proliferation and β -2 MG from the saliva of the patients with CHB, the β -2 MG levels were higher in the patients who were classified as HBV DNA positive with PCR test (Abdolsamadi *et al.*, 2013). However, no relationship was observed between the HBV DNA level and the β -2 MG values in our study.

The greatest limitation of our study is the omission of a histopathological method to test the above-mentioned

markers in the hepatic cells. However, we believe that our study may lead the way for further research in the future.

In conclusion, the β -2 MG and GP-73 levels may be the most important non-invasive diagnostic markers used to monitor the disease progression in patients to undergo a biopsy based on the diagnosis of CHB. Still, we are of the opinion that they cannot replace the biopsy, which is the gold standard of the diagnosis.

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