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The Role of β_2 Microglobulin Levels in Monitoring Chronic Hepatitis B

SELMA YEGANE, MELEK REVANLI[1] and FATMA TANELI[2]

Clinical Microbiology Laboratory, [1]Clinical Biochemistry Laboratory, International Moris Sinasi Children's Hospital, Manisa, Turkey, and
[2] Department of Biochemistry and Clinical Biochemistry, Celal Bayar University Faculty of Medicine, Manisa, Turkey

YEGANE, S., REVANLI, M. and TANELI, F. *The Role of β_2 Microglobulin Levels in Monitoring Chronic Hepatitis B*. Tohoku J. Exp. Med., 2004, **203** (1), 53-57 —— β_2 microglobulin is one of the domains of the histocompatibility class I human leukocyte antigen (HLA) antigen. In hepatitis infection the presentation of the viral antigen on the hepatocyte in the presence of class I HLA antigens plays a significant role in the elimination of the virus. The aim of the study was to estimate the serum β_2 microglobulin levels in cases of chronic hepatitis B infection. Serum β_2 microglobulin levels were assessed in 65 cases with chronic hepatitis B infection including 29 pediatric and 36 adult patients as the study group and in 30 cases as seronegative control group. β_2 microglobulin level was found significantly higher in chronic active Hepatitis B virus (HBV) patients compared to the asymptomatic HBV carriers and also in the chronic active HBV patients compared to control group. We are of the opinion that β_2 microglobulin concentration is an indicator for monitoring chronic active HBV infections at the asymptomatic hepatitis B virus carrier patients, thus would lead to early initiation of Interferon (IFN) treatment and to monitor the effectiveness of the therapy. ——— β_2 microglobulin; chronic active hepatitis B infection: asymptomatic HBV carriers
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Beta$_2$ microglobulin (β_2-MG) is a protein of low molecular weight found in all nucleated cells and is one of the domains of the histocompatibility class I human leukocyte antigen (HLA) antigen. This antigen is present on the surface of the T and B lymphocytes and in the tissue of many organs (Lapinski et al. 2002). As a free polypeptide, β_2-MG can be detected in serum and being a small molecule it passes through the glomerular membrane and less than 1% of it is being excreted in urine and the rest is being reabsorbed at the proximal tubuli. The serum level of β_2-microgrobulin (MG) is used in studying the excretion and reabsorption functions of the kidneys. Significantly high serum levels are said to be encountered in a variety of inflammatory and neoplastic

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Address for reprints: Fatma Taneli, Department of Biochemistry and Clinical Biochemistry, Celal Bayar University Faculty of Medicine, Manisa, 45020, Turkey.
e-mail:fatma.taneli@bayar.edu.tr

diseases, in acute and chronic hepatitis, and in the presence of hepatic cirrhosis with no renal disturbances (Revilard 1980; Nakamura et al. 1981; Cylwik and Szmitkowski 1997). β_2 microglobulin, as part of HLA-I complex, is responsible for transportation of the viral antigens on the hepatocyte surface. In HBV infection the presentation of the viral antigen plays a significant role in the elimination of the virus. There are still no clear observations on β_2 microglobulin levels in hepatic diseases in pediatric and adult age groups. Thus, we aimed to estimate the serum concentration of β_2-MG as a marker of viral expression and its possible difference between pediatric and adult HBV infected patients. In the present non interventional cross sectional study, serum β_2-MG levels were estimated in cases with chronic active hepatitis B infection and in asymptomatic HBsAg carriers in the pediatric and adult age groups.

Materials and Methods

Subjects

A total of 65 cases, 29 children and 36 adults, diagnosed as asymptomatic HBsAg carriers and chronic active hepatitis B infection, aged 5-44 (21.8±10.8 years), referred to our hospital between January 2000 and September 2001 were recruited. Nineteen patients with chronic active hepatitis B infection and 46 asymptomatic HBsAg carriers were taken into the study. Asymptomatic HBsAg carriers were diagnosed by normal serum aspartate amino transferase (AST), alanine amino transferase (ALT), and gammaglutamiltranspeptidase (GGT) enzyme levels and negative HBeAg and HBV DNA and positive antiHBsAg markers. Chronic active hepatitis patients were diagnosed with positive HBeAg or antiHBe, positive HBV DNA and high hepatic enzyme levels. Histological activity index were evaluated by liver biopsies. Exclusion criteria were acute HBV cases, and patients with kidney insufficiency to prevent the possible β_2-MG interference. HBsAg positive patients were further assessed for total antiHBc, antiHBc IgM, HBeAg, antiHBe, HBV DNA and liver enzymes including ALT, AST, GGT levels were assessed.

The control group, aged 4-47 (22.5±13.6 years) consisted of 15 children and 15 adults with negative HBV and HCV markers. Blood was drawn after an overnight fasting between 08:30-10:00 AM. The characteristics of the patient and control subjects were summarized in Table 1. All sera were kept at −20°C until assayed.

Assents from the children in addition to informed consent from the parents were obtained. The study protocol was approved by the Ethics Committee of the Faculty of Medicine. The study was conducted according to the Helsinki Declaration.

Biochemical assessments

Serum β_2-MG levels were assessed by immunoturbidimetric method within 15 days. Assessments were made using commercial reagents (Roche Diagnostics Corporation, Indianapolis, USA) by enzymatic methods on autoanalyzer (Hitachi 902, Kobe). Serum HBsAg, total antiHBc, antiHBc IgM, HBe Ag levels were studied by enzyme-linked immunosorbent assay: micro enzyme-linked immuno-sorbent assay (ELISA) with commercial reagents (Biokit-Spain, Barcelona, Spain).

Statistical analysis

Statistical analyses were done by Statistical Package for the Social Sciences Program (SPSS) software. Data distributions were evaluated by Kolmogorov and Smirnov test. Student-*t* test was used for the comparison of patient and control group and a value $p<0.05$ was considered to be significant.

Results

Serum β_2-MG levels in adult and pediatric age groups of the chronic active hepatitis B patients, asymptomatic HBV carriers and the control groups are depicted in Table 1. The mean age±S.D. of total 65 patients included in the study was 22.8 ±10.8 (range: 5-44) years. The mean age±S.D. of

TABLE 1. *Serum β_2 microglobulin levels in chronic active HBV infected patients, asymptomatic hepatitis B carriers and the control groups*

β-2 microglobulin (mg/liter)	Chronic Active HBV Infection	Asymptomatic HBsAg carriers	Control Group
Pediatric Age Group (n=29)	2.40±0.70[a] (n=10)	1.94±0.52 (n=19)	1.76±0.31[a] (n=15)
Adult Age Group (n=36)	2.25±0.42[b,d] (n=9)	1.82±0.60[c,d] (n=27)	1.60±0.39[b,c] (n=15)
All Patients (n=65)	2.34±0.59[e,f] (n=19)	1.84±0.58[e] (n=46)	1.75±0.30[f] (n=30)

Values are indicated by mean±S.D.
[a] p=0.003, [b] p=0.007, [c] p=0.036, [d] p=0.016 [e] p=0.005, [f] p=0.0001.

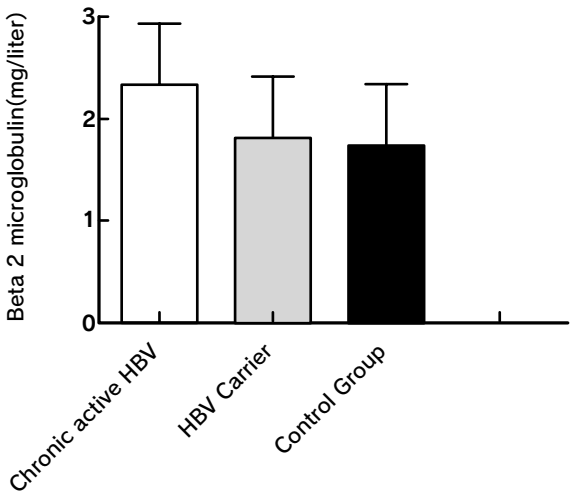

Fig. 1. Serum β_2 microglobulin levels in the total (n=65) patients and total (n=30) controls. The statistical differences between the groups are p=0.0001 compared chronic active hepatitis B patients to control group, and p=0.005 chronic active hepatitis B patients to HBV carrier patients.

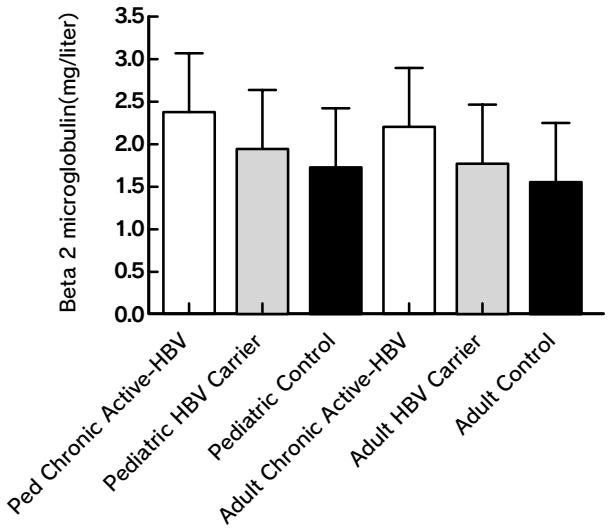

Fig. 2. Serum β_2 microglobulin levels in the pediatric and adult age patients. The statistical differences between the pediatric chronic active hepatitis B patients and pediatric controls are p=0.003, between adult chronic active hepatitis B patients and adult controls are p=0.007 and between adult chronic active hepatitis B patients compared to the HBV carrier group is p=0.016 by Mann-Whitney-U test.

pediatric patients included in the study was 11.8± 4.1 (range: 5-17) years. The mean age±S.D. of adult patients included in the study was 29.8±6.7 (range: 19-44) years. The mean age±S.D. of controls included in the study was 22.5±13.6 (range: 4-47) years.

Overall 65 patients and 30 control subjects were analyzed and the serum β_2-MG levels were found to be significantly higher in 19 chronic active hepatitis B patients compared to 46 asymptomatic HBV carriers (2.34±0.59 vs. 1.84±0.58 mg/liter, p=0.005). Similarly serum β_2-MG levels were significantly higher in chronic active hepatitis B patients compared to the control subjects (2.34±0.59 vs. 1.75±0.30 mg/liter, p=0.0001) as shown in Fig. 1.

When β_2-MG levels were analyzed further only in the pediatric age group (<18 years); serum β_2-MG levels were significantly higher in chronic

active hepatitis B patients compared to the control subjects (2.40±0.70 vs. 1.76±0.31 mg/liter, p=0.003).

In analysis of the adult patients (≧18 years); serum β_2-MG levels were significantly higher in chronic active hepatitis B patients compared to the control subjects (2.25±0.42 vs. 1.60±0.39 mg/liter, p=0.007) and also serum β_2-MG levels were significantly higher in chronic active hepatitis B patients compared to the adult asymptomatic HBV carrier subjects (2.25±0.42 vs. 1.82±0.60 mg/liter, p=0.036). Results of the pediatric and adult groups are shown in Fig. 2.

Discussion

The major finding of this study is the increase of serum β_2-MG levels in chronic active hepatitis B infected patients and in asymptomatic hepatitis B carrier patients compared to healthy controls. To date, the increasing number of scientific and medical data supports the evidence that increased serum β_2-MG levels are associated with the development of hepatitis (Hallgren 1979; Revilard 1980; Amodio et al. 1984; Cylwik and Szmitkowski 1997; Akdogan et al. 2003). In line with this fact, as a part of HLA Class 1 complex, β_2-MG is responsible in the transport of viral antigens to the hepatocyte surface. In a report of 160 cases with hepatic disease 63 normal controls and in 75 asymptomatic HBsAg carriers, serum β_2-MG levels are found significantly elevated in cases with acute viral hepatitis, chronic persistent hepatitis, and hepatic cirrhosis and in asymptomatic hepatitis B carriers (Beorchia et al. 1981).

Zhang and Zhang (1990) and Nagafuchi and Scheuer (1986), evaluated the β_2-MG expression in hepatocyte membrane of biopsy materials; although no expression of β_2-MG was seen in normal hepatic membrane, significantly increased β_2-MG expression was seen in acute and chronic hepatic infections. In cases with acute viral infection when a prostaglandin analogue, misoprostol is instituted, the initially high β_2-MG levels are seen to decrease with shortened hospitalization period which could be related to the cessation of the cell surface expression of β_2-MG (Flisiak and Prokopowicz 1999). In addition, Tomasievicz et al. (1996), report significantly high levels of serum β_2-MG levels in 14 cases with acute hepatitis, in 16 cases with acute hepatitis B and in 10 cases with hepatitis C. In hepatitis A and B, β_2-MG levels were normal during convalescence, whereas it remained high in hepatitis C. These data confirm our results of higher serum β_2-MG levels in chronic active hepatitis B infection patients in comparison to HBV carriers and the healthy control group. It appears that an increase in the β_2-MG concentration in serum is an index of an-going inflammatory changes in the liver. We believe that convalescence period is responsible in reduction of the β_2-MG levels to their original states.

A number of studies of chronic hepatic diseases due to hepatitis B and hepatitis C, revealed increases in serum β_2-MG levels in children and in adults (Rashid et al. 1981; Miyaoka 1991; Malaguarnera et al. 1997). Serum β_2-MG level is compared along with some other tumor markers used in the follow-up studies of neoplastic diseases and is shown to be also applicable to cases with hepatitis, progressing to hepatocellular carcinoma. Thus, in 50 cases with chronic hepatitis C plus hepatocellular carcinoma, in 50 cases of chronic hepatitis C and in 20 cases of control group serum β_2-MG levels are assessed and the results are compared with alpha-fetoprotein (AFP) levels, and tomographic, ultrasonographic findings and significant elevations are seen in the serum β_2-MG levels when related to the AFP levels. In addition, a positive relation is seen between serum β_2-MG levels; AFP level and the tumor mass (Malaguarnera et al. 2000).

Recently Lapinski et al. (2002) report that among patients with chronic HCV infection treated with interferon (IFN-α), the β_2-MG concentration is an indicator of the medication's activity and the effectiveness of treatment. They conclude that in unsuccessfully treated chronic HCV infected patients β_2-MG levels shows a steady increase where as after successful IFN-α therapy β_2-MG

levels decreases. They propose that an increase in the β_2-MG concentration in serum is an index of on-going inflammatory changes in the liver. Our own results confirm the observations of Lapinski et al. (2002).

Akdogan et al. (2003) determined the fluctuations in transaminase, β_2-MG levels, HBV DNA levels and histological activity indexes before during and after interferon α therapy. They conclude that serum β_2-MG levels before and during treatment may be useful in predicting the outcome of chronic hepatitis B patients similar to our observations.

In conclusion, we are of the opinion that, the serum β_2-MG concentration is one of the indicators for monitoring chronic active hepatitis B infections at the asymptomatic HBV carrier patients, thus would lead to early initiation of IFN treatment.

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