Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

Evaluation of salivary beta-2 microglobulin as HBV proliferation marker in HBS Ag^+, HBV DNA PCR^+ and HBV DNA PCR^- subjects

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ABSTRACT

Aim: The aim of this study was to determine the concentration of salivary B_2M as a marker of viral proliferation in HBS Ag^+, HBV DNA PCR^+ and Hbs Ag^+ and HBV DNA PCR^- subjects.

Background: Beta-2 microglobulin (B_2M) is responsible for transmission of viral antigens such as Hepatitis B (HBV) on the surface of liver cells as part of an HLA complex.

Patients and methods: In this case control study, 25 PCR^+ and 2 PCR^- patients were included. 5 mL of the saliva sample was obtained from all patients and salivary B_2M level was measured using nephelometer. The data was evaluated by the descriptive, chi square and t tests.

Results: 72% of the PCR^+ patients received medications and in contrast, 85.7% of the patients with PCR^- did not take any medication ($P<0.001$). The average salivary concentration ofBeta-2 microglobulin in the PCR^+ group (5.28 ± 5.45 mg/deciliter) was more than PCR^- group (1.51 ± 0.77) and this difference was statistically significant ($P=0.003$).

Conclusion: the **s**alivary B_2Mlevel can be used as a marker of viral proliferation in patients with hepatitis B.

Keywords: Beta-2 microglobulin, Saliva, Hepatitis B, Virus proliferation marker.

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Introduction

Hepatitis means inflammation of the liver and includes wide range of clinical and pathological conditions (1, 2). According to the latest statistics of World Health Organization, hepatitis has chronically affected more than 350 million people in the world and is one of the major problems of the health care system (3-5). The infected people are the main source and the main factor of disease transmission (6). According to data from fourth international congress of Hepatitis, Iran is an endemic region of hepatitis B with average

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prevalence of 2.3%. In some regions of country such as Golestan and Sistan-va-Baloochestan provinces, prevalence of the disease reaches 6%. In the meantime, 35% of the Iran population is at the risk of infection with the disease and 3.4% of the population chronically carries hepatitis B virus (7). The main mechanism of damaging the hepatic cells in hepatitis is reaction of the immune system (8). The studies on cellular immune system shows that the presence of HLA antigen and cohesion molecules in hepatocytes level for provision of HBV is the key factor and results in activity of T lymphocytes against HBS Ag and HBe Ag(9,10). There is also direct relationship between HLA-1 of the hepatocytes and inflammation of the hepatic cells in hepatitis B(10). When the person become infected with HBV, the first virologic marker which can be identified in the serum, saliva and sperm is HBs-Ag within 8-12 weeks after contamination (1,3). In addition, there is another important and reliable diagnostic test such as HBV DNA using PCR method. Patients with HBV DNAvalues of more than 20000 units in milliliter or 100000 copies in milliliters are at the risk of high infectivity and possible hepatic cirrhosis, hepatic dysfunction and hepatocellular carcinoma (1, 2). B_2M is a single polypeptide chain including 18 amino acids lacking carbohydrate with molecular weight of 11.8 kilodalton which forms Beta HLA-1 chai. It is located on surface of most nucleated cells and also in most body fluids such as blood, saliva, urine, sperm, cerebrospinal fluid, synovial liquid and milk(11,12). B_2M is responsible for transmission of viral antigens on the surface of liver cells as a part of the HLA complex (13). Normal concentration of B_2M is 1.5-3 ng/l in serum and 0-0.38 ng/l in the saliva(14). According to the studies on acute hepatitis, stable chronic hepatitis, hepatic cirrhosis and transmitter without symptoms of hepatitis B , a considerable increase of the B2M concentration in serum has been reported (8,10).

Unfortunately there is limited information about salivary B_2M serum levels in patients with hepatitis B despite of the fact that spitting has some advantages compare to blood drawing. Spit sample is a non-aggressive method, easier to access and the ability to be performed by the patients; therefore, it seems that salivary B_2M monitoring can be considered as a reliable index to study the disease trend, response to the therapy and follow-up. This issue was one of the goals of this research.

Patients and Methods

In this case control study, the patients who had positive HBS Ag at least for 6 months, and had a PCR test for investigating HBV DNA were included and were referred to Farshchian hospital affiliated with Hamadan University of Medical Sciences. Patinets' refusal, the dialysis patients, presence of other systemic diseases and medication intake not related to hepatitis within the past 3 months were the exclusion criteria. After completing other required tests including HbeAg, HbeAb, LFT and HBV DNA, the subjects were treated and they were followed periodically. The samples were collected after taking the informed consent from the participants and their charts were investigated. The cases were selected after investigating 400 patients' charts related to all kinds of hepatitis. 150 cases of hepatitis B subjects were selected of whom 90 of the cases were excluded from the study for different reasons such as lack of PCR result, lack of the accurate information and patients' refusal. From all the cases, 60 patients were selected and the saliva samples were taken from 46 patients after coordination and they were referred to the infectious diseases ward. 21 out of 46 subjects were in negative PCR group and 25 subjects were in positive PCR group. HBV DNA of more than 20000 unit/milliliters or 100000 copies/milliliters were considered as positive PCR sample and the

lower values were considered as negative PCR (1,2). Saliva was collected using Spitting method (15). All samples were collected in a definite time interval i.e. between 8 and 10 AM and all were taken in winter to have the minimum salivary changes. About 5cc of the saliva was taken using the above method and was immediately placed in the ice pack and was transferred to the laboratory. ALL the samples were kept at -20°C until all were collected. Later, all samples were de-frozen and centrifuged for 10 min at 4°C and with speed of 800 g to separate the squamous cells and cellular debris. 12ml of the centrifuged fluid was used for measuring values of β_2M in the lidded and sterile plastic tubes (falcon) while they were put in the ice. β_2M level was measured with HUMAN MININEPH kit made by Binding site Company , England and with nephelometer method (11,16).

All data were analyzed by SPSS software, version 16 and using descriptive, chi square and t tests and value of $P<0.05$ was considered statistically significant.

Results

In this study, 21 patients with positive HBS Ag and 25 patients with positive HBS Ag and with HBV DNA PCR^+ were investigated including totally 25 males (54.3%) and (45.7%) females. The ratio of males to females was obtained to be 1/2 and which was not significant in term of gender (P=0.86). The average age of the participants in this study was 86.11±72.35 years and age range was 14-61 years and this difference was not statistically significant (P=0.48).

72% of the PCR^+ patients took medication for the treatment in contrast to 85.7% of the PCR^- who did not take any medication ($P<0.001$). There was statistically significant difference between PCR^+ and PCR^-groups in concentration of salivary beta-2 microglobulin (P=0.003) which has showed in table 1, however concentration of this index was not significantly different in males and females (P=0.572). Pearson Correlation Coefficient between concentration of salivary Beta-2 microglobulin and age was -0.229 that was not statistically significant considering P=0.126. Considering table 2, results of this study showed that concentration of the salivary Beta-2 microglobulin was significantly high in the patients who received pharmacotherapy compared with the patients who didn't take medication (P=0.002).

Table 1. Average concentration of salivary β2M in patients with HBS Ag+ in two PCR+ (n=25) and PCR- (n=21) groups

	mean±SD	Min	Max	P-value
Concentration β_2M				0.003
PCR^+	5.28±5.45	0.76	20.76	
PCR^-	1.51±0.77	0.76	2.43	

Table 2. Mean (± standard deviation) concentration of salivary β2M in patients with HBS Ag+ based on the record of medicine taking

	No (%)	Concentration	P-value
medicine taking			0.002
Yes	21 (45.7)	5.6±5.88	
No	25 (54.3)	1.8±1.01	

Discussion

HBV-DNA is known as a viral marker in simulation of HBV and has the highest diagnostic value in terms of sensitivity and quantity (1,2). Negative HBeAg can indicate reduction of the viral simulation but virus simulation and cell proliferation continue in some patients despite $HBe\text{-}Ab^+$. This status can be due to mutation changes in viral Pre- core area. Therefore, this study tries to use HBV-DNA as a virus proliferation factor (13). There is direct relationship between HLA-1 of hepatocytes and inflammation of hepatic cells in hepatitis B (17,18). β_2M shows Beta HLA-1 chain and is available lactated on in cellular surface of most nucleated cells and liquids of the body (19). The

present study showed increase of β_2M in PCR + group.

β_2M production increases in response to several factors, such as inflammation, acidosis, therapy with calcitriol and dialysis (12,16,20,21), acute and chronic cirrhosis(13,23,22) and in many viral diseases (23,24), renal disorders (20,16,25,26), autoimmune diseases and lymphoproliferative diseases. This increase is found in plasma cells dyscrasia and all kinds of Solid tumors (14,17,22,27,28). β_2M is responsible for transmission of viral antigens such as Hepatitis B(HBV) on the surface of hepatic cells as a part of the HLA complex. β_2M can be an index in incidence of the HBV virus. According to the performed studies, there is considerable increase of β_2M in viral acute hepatitis, stable chronic hepatitis (11,17,18,20). Hepatic cirrhosis and transmitters without symptoms of hepatitis B (11,13,18,29). Therefore, it seems that increase of serum β_2M can be an important index in liver inflammatory changes. Some studies about the chronic hepatitis B and C indicated serum values of β_2M (23,28,29). In the patients suffering from hepatitis C who were cured with interferon α, it was specified that β_2M could indicate activity of the disease and effectiveness of the hepatitis treatment (13,18).

It was also specified that values of β_2M before and during hepatitis treatment could help predict the therapeutic results (22-24).

Results of this study showed that concentration of B_2M in the subjects with positive HBSAg and HBV-DNA PCR was higher than that of PCR group and indicated that the subjects with hepatitis B had more B_2M than PCR group in case of higher viral proliferation. In this study, 72% of the PCR patients took medicine (lamivudine and adefovir) and 85.7% of the PCR patients didn't take any medicine and this statistically significant difference ($P<0.001$) between two groups indicated therapeutic follow-up of the patients and medical team for the PCR subjects. Study of Lapinski (22) on 24 patients with cured chronic hepatitis C introducing β_2M and INF-γ as the activity index and therapy effectiveness, is consistent with our study. Elefsiniotis also concluded that there was a specified relationship between β_2M serum reduction and virologic level of disease in patients with chronic hepatitis and monotherapy with lamivudine (29). HBV-DNA serum is a predictor of virological response of the disease and HBV-DNA quantitative measurement presents good prognostic information butit requires high quality laboratory test. Salivary B2M it is inexpensive and easy to access and increase of the salivary level of B_2M in hepatitis B can be a good method for assessment of virus proliferation instead of PCR in serum (17,24,29). Akdogum studied the changes in hepatic transaminases, β_2M and HBV-DNA and stated that values of β_2M can be effective and applicable for prediction of the therapy results (22) so that salivary concentration of β_2M in our study was higher in PCR group compared with PCR group despite therapy with medicines other than INF-γ. Yegane used β_2M for disease monitoring (17). In this study, average serum values of β_2M in the (symptomatic) patients was higher than the average values in patients without symptoms and also in patients with progressing of the disease and this difference was comparable with the present study considering. On the other hand, in a study done by Shayegan, serum β_2M values in the patients with chronic hepatitis B PCR and PCR were compared among 35 healthy subjects. In PCR group, average concentration of B_2M was higher than PCR and this difference was statistically significant (4). In our study, 72% of the PCR patients received medications and average concentration of salivary Beta-2 microglobulin in PCR was higher than that in PCR group regardless of their therapeutic duration.

In 2002, Lapinski announced that concentration of β_2M was predictor of the disease activity and response to the therapy among the patients with

chronic hepatitis C who were cured with interferon-α. He stated that the patients who received unsuccessful therapy showed increase of β_2M level. This result was different compare to the previous studies (13,18) which was due to the hepatic inflammation changes. Results of our study showed increase of β_2M in the patients after therapy (22).

Cooper has studied 158 serum specimens collected from the patients with CMV, infectious mononucleosis, HSV and VZV in virology section of blood bank. Their study showed that increase of β_2M value has been affected by different forms of viral infection (24). In the patients with AIDS, increase of serum β_2M occurs in the cases that are infected with *Pneumocystis carinii* or sarcoma kaposis. The role of CMV in etiology of sarcoma kaposis can indicate the relationship between CMV and increase of serum β_2M (24). Elevation of serum β_2M indicates either the deficiency in glomerular filtration or increase of its production. Increase of β_2M production is due to effect of the disease on patients' immune system. Previous studies have demonstrated the highest rate of β_2M at the beginning of acute hepatitis phase. A considerable increase of β_2M also occurs during rejection of the liver allograft relating to the hepatocytes damage (8,25). Serum β_2M values are dependent on age. In this study, patients with active hepatitis C are older than the patients with stable β_2M. Increase of serum β_2M with aging may be due to dysfunction of the liver, other factors can be explained by factor of age (23,24). In the current study, the rate of enzyme (what enzyme? liver) has decreased with age but this difference was not statistically significant. Michelis studied saliva β_2M in patients with diabetes mellitus (DM), chronic kidney disease (CKD) and hemodialysis (HD) and determined an increase of serum β_2M value in all of these three groups but salivary β_2M in HD patients was similar to the values before hemodialysis. Uncontrolled leakage of β_2M into the saliva in hemodialytic patients is probably due to the absence of relationship between concentration of saliva and serum β_2M. The excessive and uncontrolled leakage of β_2M in saliva causes the inappropriate concentration in the saliva which has no relationship with its main values in the serum. In contrast, the relationship between serum β_2M in saliva in CKD patients is strong; therefore, saliva analysis is a good adjunct method of evaluation of the CKD versus serum study based on the current results (21).

As mentioned before, serum HBV DNA PCR levels presents good diagnostic information but it is an expensive test and required high quality laboratory equipment. In contrast, it is cheaper and easier to measure serum β_2M using microparticle enzyme immunoassay technology. Therefore, evaluation of β 2M saliva level in hepatitis B can be replaced the assessment of the virus proliferation using PCR method. It is a suitable and easy to performed diagnostic method which is cost- effective compared with other methods.

Our study only investigates salivary β_2M level and its relationship with hepatitis B. the salivary β_2M in CKD was also assessed which was found to be similar to increase in the saliva. Considering very limited studies on saliva in the future studies, it is better to compare saliva and serum B_2M rate in the patients to specify advantage of using the saliva and study relationship between virus and enzyme quantity. In addition, it is better to compare the group with hepatitis with the healthy control group in the future studies.

Concentration of β_2M in positive HBV-DNA is higher than that in negative HBV-DNA, therefore, it seems that it can be considered as an index for virus proliferation.

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