Kronik Hepatit B'li Hastalarda Serum İleri Oksidasyon Protein Ürünleri (AOPP) Düzeyi İle Laboratuvar Bulguları Arasındaki İlişkinin Araştırılması

The investigation of the relationship between serum advanced oxidation protein product (AOPP) levels and laboratory findings in patients with chronic hepatitis B

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ABSTRACT

Aim: The aim of the present study was to evaluate the relationship between advanced oxidation protein products (AOPP), AST, ALT, HBV DNA levels, Knodell histologic activity index, fibrosis score, and platelet counts in patients with chronic hepatitis B (CHB) infection.

Material and Methods: The study included 25 patients and 20 healthy controls. Serum AOPP levels were analyzed with the spectrophotometric method using an ELISA kit. AST, ALT, and HBV DNA levels, platelet counts, Knodell histological activity index, and fibrosis scores of the patients were determined.

Results: There was no statistically significant difference between the patients with CHB infection and the control group in terms of gender, age, ALT, AST, HBV DNA, Knodell activity index, fibrosis score, platelet count and serum AOPP levels (81.42 ± 32.0 micro M/mL and 73.2 ± 21.8 micro M/mL) (P = 0.38),

Conclusion: Although no significant association was found between serum AOPP levels and liver transaminases, viral load, liver histologic activity index, fibrosis score, and platelet count, the authors consider that further comprehensive studies are required to determine the importance of serum AOPP levels in patients with CHB infection.

Keywords: Chronic hepatitis B, oxidative stres, advanced oxidation protein products (AOPP) , clinical findings.

Özet

Amaç: Bu çalışmanın amacı, kronik hepatit B (KHB) infeksiyonu olan hastalarda serum ileri oksidasyon protein ürünleri (AOPP) düzeyleri ile karaciğer transaminazları olarak bilinen alanin aminotransferaz (ALT), aspartat aminotransferaz (AST)) düzeyleri, viral yük (HBV-DNA düzeyi), karaciğer histolojik aktivite indeksi (Knodell aktivite indeksi), fibrozis skoru ve trombosit sayıları arasındaki ilişkinin araştırılmasıdır.

Yöntem ve Gereçler: Çalışmaya tedavi öncesi olan 25 KHB hastası ve 20 sağlıklı kontrol grubu dahil edildi. Hasta ve kontrol grubunun serum AOPP düzeyleri spektrofotometrik yöntemle ticari ELISA kiti kullanılarak belirlendi. Hastaların AST, ALT, HBV-DNA düzeyleri, trombosit sayıları ile karaciğer biyopsisini kabul eden ve biyopsi yapılan hastalarda Knodell histolojik aktivite indeksi, fibrozis skoru belirlendi.

Bulgular: Kronik hepatit B hastaları ile sağlıklı kontrol grubu arasında cinsiyet ve yaş ortalaması açısından anlamlı farklılık saptanmadı.

KHB hastalarında serum AOPP düzeyleri ile karaciğer transaminazları (ALT, AST), düzeyleri, viral yük (HBV-DNA düzeyi), karaciğer histolojik aktivite indeksi (Knodell aktivite indeksi), fibrozis skoru ve trombosit sayıları arasında istatistiksel olarak anlamlı ilişki saptanmadı.

Kronik hepatit B hastaları ile kontrol grubunun serum AOPP düzeyleri (sırasıyla; 81.4 ± 32 mikro M/mL ve 73.2 ± 21 mikro M/mL) arasında istatistiksel olarak anlamlı farklılık yoktu.

Sonuç: Çalışmamızda serum AOPP düzeyleri ile karaciğer transaminazları, viral yük, karaciğer histolojik aktivite indeksi, fibrozis skoru ve trombosit sayısı arasında istatistiksel olarak anlamlı ilişki saptanmadı.

Yeni oksidatif stres göstergesi olan serum AOPP düzeylerinin KHB'li hastalarda öneminin belirlenmesi için daha fazla örneklem sayılı kontrollü çalışmalara gereksinim olduğu görüşündeyiz.

Anahtar Kelimeler: Kronik hepatit B, ileri oksidasyon protein ürünleri (AOPP), klinik bulgular

Introduction

Hepatitis B virus (HBV) infection is an important public health problem resulting in significant morbidity and mortality in Turkey, and throughout the world, as well. According to the data of World Health Organization, there are more than 400 million carriers of the virus around the world and 75% of this population is from Asian and East Pacific zones (1-3).

The carriage rate of hepatitis B (HBs Ag positivity) was found to be 4% in a study of 5471 subjects conducted in Turkey in 2010 (4,5). Severe complications such as liver cirrhosis and hepatocellular carcinoma occur in adults who acquire HBV early in life, and develop chronic hepatitis B (CHB) infection (1,3).

The oxidative stress is an imbalance between antioxidant defense of the body and the production of free radicals (6). Oxidative stress is also explained as the damage caused by reactive oxygen species (ROS) in the cells, tissues, and organs (7). Intracellular and extracellular conditions that result in chemical or metabolic production of ROS are called oxidative stress (6). ROS are mostly derived from endogenous sources and are produced during energy production in the mitochondria. The free radicals and non reactive radical derivatives are measured at low levels in the cells and tissues. The production and clearance of free radicals is balanced by various antioxidant compounds and enzymes. Activated oxidation protein products (AOPP) are recently identified as oxidative stress markers (7). The substance was first described by Witko-Sarsat et al. in a patient with chronic kidney insufficiency in 1996 (8).

The advanced oxidation protein products (AOPP) are toxins produced as a result of the reaction between plasma proteins and chlorinated oxidants such as chloramine and hypochlorous acid during oxidative stress. AOPP exhibits similar structural features with advanced glycosylation end products (AGE). AOPP levels were reported to be increased in patients with renal complications, atherosclerotic heart disease, diabetes mellitus, systemic sclerosis, and HIV positive patients (9-10).

The antioxidant system and oxidative stress might play a role in the pathogenesis of chronic liver diseases (11). Based on this assumption, it is reported that antioxidant therapies could contribute to the available therapies for CHB infection (12).

In the present study, serum AOPP levels in patients with CHB infection were compared with healthy controls. Furthermore, the association between AOPP levels and liver transaminases (aspartate aminotransferase [AST], alanine aminotransferase [ALT]), viral load (HBV DNA levels), liver histologic activity index, fibrosis score, and platelet count in patients with CHB infection was analyzed.

Material and Methods

Participants: The study included 25 patients (14 females and 11 males, mean age: 41.3 ± 15.6 years) and 20 healthy controls (11 females and 9 males, mean age: 34.9 ± 10.3). The patients with CHB infection were receiving therapy and none of the patients had findings of acute or chronic liver insufficiency. Serological HBV markers were studied with the ELISA method, and viral load (HBV DNA) was studied with the real time PCR method. The serum AOPP levels were analyzed with the direct spectrophotometric method using Oxi Select AOPP Assay Kit in accordance with the recommendations of the manufacturer (Cell Biolabs, Inc, San Diego, USA).

Method

The AOPP containing specimen, control material, and standard chloramine solutions prepared in particular concentrations were treated with reagent that would initiate a color reaction. After a short incubation period of five minutes, the reaction was stopped with the addition of stop solution. The optic density of the specimen, standards, and control materials were read at 340 nm in the spectrophotometer (Shimadzu UV-120-01, Columbia). The AOPP levels in the specimens were determined from the calibration curve constructed with OD values corresponding to standard concentrations, and serum levels were expressed as μM (10).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) was used to analyze the data. The categorical variable (gender) was demonstrated as % and continuous variables as mean ± standard deviation. Chi-square, students T-test, and MWU test, were used to compare the differences among the groups. The relationship between AST, ALT, AOPP, and several variables were analyzed using the Spearman correlation test. A P value < 0.05 was considered statistically significant.

Ethical consideration: The study protocol was approved by the Ethics Committee of the Kırıkkale University.

Results

There was no significant difference between patients with CHB infection and healthy controls in terms of gender and age distribution (P = 0.94, P = 0.32). There was no statistically significant difference between the patients with CHB infection and the control group in terms of mean serum AOPP levels (81.4 ± 32.0 microM/ml and 73.2 ± 21.8 microM/ml, P = 0.38) (Figure 1, Table 1).

Table 1. Demographic and laboratory features of patients with CHB infection and healthy subjects.

	Patients with CHB* infection n: 25	Healthy subjecsts n: 20	P
Gender (female/male)	14 / 11	11 / 9	0.94
Age	41.3 ± 15.6	34.9 ± 10.3	0.32
AST	37.4 ± 29.2	21.7 ± 10.8	0.29
ALT	54.3 ± 47.7	21.5 ± 12.6	0.10
AOPP	81.4 ± 32.0	73.2 ± 21.8	0.38
PLT	230.0 ± 67.6	195.0 ± 120.6	0.99
HBV DNA	1.722 ± 3.9		0.64
HAI (Histologic Activity Index)	Score - number (%) 0 - 9 (36%) 3 - 1 (4%) 5 - 3 (12%) 6 - 3 (12%) 7 - 2 (8%) 8 - 1 (4%) 9 - 1 (4%) 10 - 4 (16%) 11 - 1 (4%)		0.62
Fibrosis Score	Score - number (%) 0 - 10 (40%) 1 - 7 (28%) 2 - 5 (20%) 3 - 3 (12%)		0.83
Diabetes	4 (16%)	4 (20%)	0.51
Comorbid disease	5 (20%)	8 (40%)	0.06

(*) CHB: Chronic hepatitis B

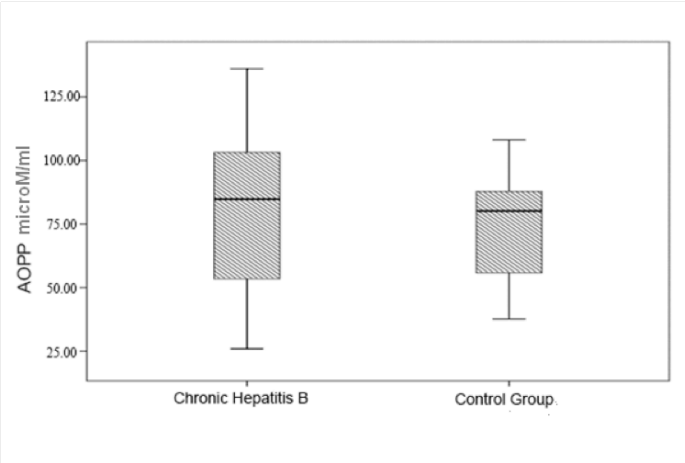

Figure 1. Serum AOPP levels in patients with CHB infection and the control groups.

There was no significant association between serum AOPP levels and liver enzymes (AST, ALT), viral load (HBV DNA level in IU / mL), and platelet count in patients with CHB infection (P = 0.29, P = 0.10, P = 0.64, P = 0.99).

The examination of biopsy specimens revealed no significant difference between the patients with CHB infection and the control group in terms of histologic activity index (Table 1) (P = 0.29).

In patients with CHB infection, the fibrosis score was 0 in 40% (10 / 25), 1 in 28% (7 / 25), 2 in 20% (5 / 20), and 3 in 12% (3 / 25) (Table 1).

The comparison of the patients and the control group in terms of the presence of diabetes revealed that 16% of the patients with CHB infection and 20% of the controls had type 2 diabetes, and there was no statistically significant difference between the groups (P = 0.51).

The comparison of the patients and the controls in terms of the presence of comorbid conditions revealed that 20% of the patients with CHB infection and 40% of the controls had comorbid condition, and there was no statistically significant difference between the groups (P = 0.06).

Discussion

In CHB infection, liver injury can occur as a result of various factors such as the virus itself, immunological mechanisms, or oxidative stress (6-11).

Toxic free oxygen radicals play a role in the pathogenesis of many disorders. The advanced oxidation protein products (AOPP) are a recently described oxidative stress parameter. An increased level of oxidative stress and impaired defense mechanisms have been reported in patients with chronic hepatitis C (CHC) infection (7).

Özenirler et al. (7) studied 29 patients with CHC with persistent elevation in transaminase levels and 46 healthy controls, and measured serum AOPP and malonyldialdehyde (MDA) levels. In that study, serum AOPP levels were significantly higher in patients with CHC infection compared to the control group (235.0 ± 142.8 microM and 116.7 ± 79.51 microM, respectively). Serum MDA levels were also higher in patients with CHC infection compared to the control group. However, no statistically significant difference was found in total radical trapping antioxidant parameters. Total radical trapping antioxidant parameter / AOPP index was lower in patients with CHC infection than in healthy controls. There was no significant association between AOPP level, MDA levels and hepatosteatosis. Furthermore, there was no relationship between serum AOPP and MDA and necroinflammatory activity, fibrosis, and liver transaminase levels. In conclusion, the authors reported increased oxidative stress and insufficient antioxidant capacity in patients with CHC infection, and therefore, AOPP and MDA levels could be used in the follow up of patients with CHC infection.

In the present study, no significant association was found between serum transaminase levels, liver histopathology (Knodell index score), fibrosis score, HBV DNA levels, and platelet count in patients with CHB infection. In this respect, the findings of the present study was similar to the findings of Özenirler et al. (7); however, the present study differs due to a lack of a significant difference between the patient and control group in terms of AOPP levels. This can be caused by the lack of significant hepatosteatosis in CHB infection as in CHC infection. The susceptibility to hepatosteatosis and oxidative stress caused by steatosis might be more prominent in hepatitis C infection.

AOPP is a recently described marker for oxidative stress indicating protein damage mediated by oxidation, and plays a role in inflammatory processes. There is an increased level of AOPP in patients with chronic kidney insufficiency and diabetes mellitus (8,13-15). The studies conducted in patients with chronic kidney insufficiency reported AOPP levels as an important mediator of atherosclerosis related to renal fibrosis and uremia (15).

Liu et al. (9) investigated serum AOPP levels before and after plasma exchange therapy in 50 patients with acute and chronic liver insufficiency, 30 patients with compensated cirrhosis, 30 patients with CHB infection, and 50 healthy controls. In that study, baseline AOPP levels were found to be higher in patients with acute and chronic liver insufficiency compared to the patients with liver cirrhosis, CHB infection, and the control group. A positive correlation was found between AOPP level and total bilirubin, Child-Pugh end-stage liver insufficiency score, and serum cytokeratin-18. AOPP levels were reported to be an independent risk factor for prognosis. Furthermore, AOPP levels decreased after plasma exchange therapy. Consequently, the authors reported that serum AOPP levels were an appropriate and prognostic marker of oxidative

stress in acute and chronic liver insufficiency, and the levels were decreased after plasma exchange therapy. The finding of significantly higher AOPP levels in patients with liver insufficiency can be explained by increased oxidative as the impairment in liver functions further progressed and therefore increased AOPP levels as one of the markers of oxidative stress .

Atik et al. (11) evaluated the correlation between inducible nitric oxide synthase (iNOS) levels and histopathological findings in 56 patients with chronic hepatitis comprising 38 patients with CHB infection and 18 patients with CHC infection. The study found increased iNOS levels in all liver biopsy specimens with a diffuse distribution pattern and the authors reported a correlation between iNOS and disease severity.

In the present study, AOPP levels did not differ between patients with CHB infection and healthy controls. This situation may be due to relatively higher prevalence of comorbid disease in the control groups. Furthermore, there was no significant relationship between serum AOPP levels and liver enzymes, HBV DNA levels, liver histopathology, fibrosis score, and platelet count in patients with CHB infection. This might be due to lack of severe deterioration of liver function in the study patients. The oxidative stress increases with the further deterioration of the liver functions and therefore serum AOPP levels increase with increasing oxidative stress. The patient group in the present study was comprised of those who were receiving therapy for CHB infection but who did not have severely affected liver functions (acute or chronic liver insufficiency). It is likely that oxidative stress may have occurred earlier (before the initiation of liver failure) due to hepatosteatosis being more prominent in patients with CHC infection. This may allow for the prediction of prognosis by measuring AOPP levels in patients with CHC infection but not in patients with CHB infection before the development of severe liver injury. Due to the authors' assumption that serum AOPP levels are increased in later stages of the disease in CHB infection, the finding of elevated AOPP levels may suggest advanced stages of liver insufficiency.

In conclusion, although no statistically significant difference was found between patients with CHB infection and the control group in terms of serum AOPP levels, patients with CHB infection had higher serum AOPP levels compared to the control group (81.4 ± 32.0 microM /ml and 73.2 ± 21.8 microM/ml, respectively, P = 0.38). Multicenter, controlled studies with larger sample sizes are required to determine the importance of AOPP levels in patients with CHB infection.

Conflict of interest

The authors report no conflict of interest

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