Research Article

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DOI: 10.4274/vhd.galenos.2022.2021-12-2
Viral Hepatitis Journal 2022;28(2):85-88

Investigation of the Effects of Total Oxidative Stress and Total Antioxidant Capacity on the Prognosis in Patients with Chronic Viral Hepatitis B

Kronik Viral Hepatit B Hastalarında Total Oksidatif Stres ve Toplam Antioksidan Kapasitenin Prognoza Etkilerinin Araştırılması

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ABSTRACT

Objectives: Experimental studies showed the role of oxidative stress in cell destruction and DNA damage in chronic viral hepatitis, In this study, oxidative stress was measured in various clinical forms of chronic hepatitis B (CHB) and the role of oxidative stress was investigated in the development of hepatitis clinic.

Materials and Methods: In total, 33 patients with inactive hepatitis B carrier (IHBC), 33 patients with active CHB infection, and 33 healthy adults were included in the study. Serum transaminases [alanine aminotransferase (ALT), aspartate aminotransferase, total antioxidant capacity, and total oxidative stress (TOS)] were measured and compared in the patient groups.

Results: In 99 patients were included in the study (56 men, 43 women). The mean age of patients in CHB was 33.21±10.20, in IHBC 36.73±11.54, and the control group 33±11.71. The mean ALT value was 40.93±28.28 U/L in the patients with CHB and 36.33±28.99 U/L in the patients with IHBC. The TOS value115.46±139.64 μm H_2O_2 equivalent/L in the CHB and 52.67±40.36-μm H_2O_2 equivalent/L in IHBC.

Conclusion: ALT and TOS levels were significantly higher in the CHB than in the other groups. The increased TOS levels in the CHB may be related to the activity of cell destruction in active cases.

Keywords: Hepatitis B virus, total oxidative stress, total antioxidant capacity, liver fibrosis

ÖZ

Amaç: Deneysel çalışmalar, kronik viral hepatitlerde hücre yıkımında ve DNA hasarında oksidatif stresin rolünü göstermiştir. Bu çalışmada, kronik hepatit B'nin (KHB) çeşitli klinik formlarında oksidatif stres ölçülmüş ve hepatit kliniğinin gelişiminde oksidatif stresin rolü araştırılmıştır.

Gereç ve Yöntemler: Çalışmaya toplam 33 inaktif hepatit B taşıyıcısı (İHBC), 33 aktif KHB enfeksiyonu hastası ve 33 sağlıklı yetişkin dahil edildi. Hasta gruplarında serum transaminazları [alanin aminotransferaz (ALT), aspartat aminotransferaz, toplam antioksidan kapasite ve toplam oksidatif stres (TOS) ölçüldü ve karşılaştırıldı.

Bulgular: Toplam 99 hasta (56 erkek, 43 kadın) çalışmaya dahil edildi. Hastaların yaş ortalaması KHB'de 33,21±10,20, İHBC'de 36,73±11,54 ve kontrol grubunda 33±11,71 idi. Ortalama ALT değeri KHB'li hastalarda 40,93±28,28 U/L, İHBC'li hastalarda 36,33±28,99 U/L idi. TOS değeri CHB'de 115,46±139,64 μm H_2O_2 eşdeğeri/L ve IHBC'de 52,67±40,36 μm H_2O_2 eşdeğeri/L bulundu.

Sonuç: ALT ve TOS seviyeleri KHB'de diğer gruplara göre anlamlı derecede yüksek seyretmektedir. KHB'deki artan TOS seviyeleri, aktif olgularda hücre yıkımının aktivitesi ile ilişkili olabilir.

Anahtar Kelimeler: Hepatit B virüs, toplam oksidatif stres, toplam antioksidan kapasite, karaciğer fibrozu

Cite this article as: Mermutluoğlu Ç, Bekçibaşı M, Deveci Ö, Cerrah S, Kaplan İ, Bozkurt F, Çelen MK. Investigation of the Effects of Total Oxidative Stress and Total Antioxidant Capacity on the Prognosis in Patients with Chronic Viral Hepatitis B. Viral Hepatitis Journal 2022;28(2):85-88

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Introduction

Hepatitis B virus (HBV) is one of the most important pathogens that lead to fibrosis, cirrhosis, and hepatocellular cancer through the damage it causes in the hepatic cells. Despite the efficient vaccine and the advanced diagnostic and treatment methods, 1.5 million people were newly infected with chronic hepatitis B (CHB) infection and 820,000 people are lost every year due to the complications of the HBV (1). However, the exact mechanisms for the pathogenesis of CHB have not been fully clarified.

In recent years, the relationship between oxidants and antioxidants has come into the spotlight and the imbalance among them was observed to play a greater role in cellular damage (2,3,4,5,6). As a part of the metabolic processes, the cells continuously produce free radicals and reactive oxygen species. These free radicals and reactive oxygen species are neutralized through the complex antioxidant system. Increasing oxidant levels or decreasing antioxidant levels disrupt this balance, this situation is called "oxidative stress" (7). The role of oxidative stress in cell destruction and in DNA and RNA damage in chronic viral hepatitis has been established in experimental studies (8). Free radicals show different chemical structures such as hydroxyl superoxide, nitric oxide, and lipid peroxide (9). This study aims to evaluate the total oxidative stress (TOS) and total antioxidant capacity (TAC) in the patients with different clinical forms of hepatitis B and the individuals in the healthy control group.

Materials and Methods

This prospective study was conducted between January 2012 and December 2013 on patients between the ages of 15-61 diagnosed with chronic viral hepatitis B. The patients were divided into three groups:

Group 1: Patients underwent a biopsy due to CHB;

Group 2: Inactive hepatitis B carriers (IHBC);

Group 3: Healthy controls.

Diagnostic criteria (10):

Group 1: CHB;

1. Hepatitis B surface antigen (HBsAg)-positive for more than 6 months,

2. Alanine aminotransferase (ALT) value greater than 1.5 times the normal value (normally, the ALT value is below 40 IU/mL),

3. HBV-DNA value ≥100,000 copies/mL (20,000 IU/mL) in those positive for the hepatitis B e antigen (HBeAg) (HBeAg-positive),

4. In those who are HBeAg-negative ≥10,000 copies/mL (2,000 IU/mL), and

5. Fibrosis ≥2 in the histopathological evaluation of the liver.

Group 2: IHBC;

1. HBsAg-positive,

2. Normal ALT values,

3. HBeAg-negative, and,

4. HBV-DNA ≤10,000 copies/mL (2,000 IU/mL)

The control group consisted of HBsAg-negative and anti-HBc-total negative patients. Patients with diabetes mellitus, liver cirrhosis, hypertension, coronary arterial disease, chronic obstructive pulmonary disease, malignancies, morbid obesity, liver and kidney failure, pregnant patients, those on corticosteroid treatment, and smokers were excluded from the study. Liver biopsy was performed in all patients with CHB and only the patient group that did not receive antiviral therapy was included in the study. The age, gender, HBsAg, HBeAg, anti-HBe, ALT, aspartate aminotransferase (AST), and HBV-DNA values of the patients were recorded. The fibrosis scores of the CHB patients who were applied biopsies were also recorded. The total oxidant status was determined using the automated measurement method developed by Erel (11). The results were expressed in terms of μm hydrogen peroxide equivalent per liter (μm H_2O_2 equivalent/L). The TAC was also determined using the automated method, developed by Erel (5). The results were expressed as micromol (μm) Trolox equivalent/L.

After the approval of the Dicle University Ethics Committee was obtained (approval number: 479, date: 28.03.2012), the TOS and TAC study was supported with the grant of the Dicle University Scientific Research Projects Coordinatorship. Informed consent was obtained.

Statistical Analysis

The obtained data were entered into the SPSS 15.0 statistics software. Categorical data were analyzed using the chi-square test. The normality of the distribution of the numeric data was tested through the Kolmogorov-Smirnov test. The normal data were analyzed with the help of the Student's t-test, while those outside the normal distribution were analyzed using the Mann-Whitney U test. Statistical significance was based on a value of $p<0.05$.

Results

A total of 99 patients divided into three groups of 33 patients were enrolled in the study. Among these patients, 56 (56.5%) were male, while 43 (43.5%) were female. No statistically significant difference in terms of mean age and sex was observed between the three groups (p=0.308, p=0.133, respectively). The characteristics (age, gender), ALT, TOS, and TAC values of the groups are presented in Table 1. While no significant difference in terms of the mean ALT values was observed between CHB and IHBC, the AST values were higher in CHB (p=0.020). When the mean TOS capacity was evaluated among the three groups, the highest level was observed in CHB. This difference was statistically significant (p=0.023). Only when CHB and IHBC were compared in terms of the TOS levels, the mean value in CHB was higher with a statistically significant result (p=0.017). No statistically significant relationship was observed among the mean TAC levels of the three groups (p=0.562). No relationship was observed between the fibrosis score, HBV-DNA, and the TOS and TAC values. No statistically significant relationship was evident between the HBV-DNA value and the TOS and TAC levels (Table 2).

Discussion

The infection caused by HBV manifests itself in various clinical forms from the asymptomatic form to fulminant hepatic failure. It is estimated that one-third of people with chronic HBV infection

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

	CHB (n=33)	IHBC (n=33)	Control group (n=33)	p-value
	Mean ± SD	Mean ± SD	Mean ± SD	
Age	33.21±10.20	36.73±11.54	33.00±11.71	0.318
Gender (M/F)	21/12	21/12	14/19	0.133
TOS (μm H_2O_2 equivalents/L)	115.46±139.64	52.67±40.36	87.91±57.08	0.023[a]
TAC (μm Trolox equivalents/L)	1.47±0.24	1.46±0.20	1.42±0.18	0.562
ALT (U/L)	40.93±28.28	36.33±28.99	None	0.516
AST (U/L)	39.84±29.05	26.51±13.05	None	0.020*

CHB: Chronic hepatitis B, IHBC: Inactive hepatitis B carriers, TOS: Total oxidative stress, TAC: Total antioxidant capacity, ALT: Alanine transaminase, AST: Aspartate transaminase, [a]: There was a statistically significant difference between the CHB group and the other groups but no statistically significant difference between the IHBC group and the control group. *Statistically significant value (p<0.05)

Table 2. The correlation analysis of TAC, TOS with HBV-DNA and fibrosis score

	r (HBV-DNA)	p (HBV-DNA)	r (fibrosis score)	p (fibrosis score)
TOS	0.167	0.437	0.097	0.651
TAC	-0.189	0.377	0.294	0.162

TAC: Total antioxidant capacity, TOS: Total oxidative stress, HBV: Hepatitis B virus

developed liver cirrhosis or hepatocellular carcinoma as a result of long-term disease (12). Various studies have shown that TOS is increased in hepatitis B and hepatitis C infections, and liver disorders (13,14,15). When the generation of free radicals exceeds the antioxidant capacity, various metabolic and functional disorders may occur (16). An increase in oxidative stress leads to necrosis in the hepatocytes, paving the way for the development of fibrosis and cirrhosis in patients with untreated hepatitis C (17,18,19). In a study by Duygu et al. (20), the TOS value was observed to be higher in the groups with HBV infection compared to the HBV-free control group. In the same study, the TOS values in the patient group that have undergone biopsies after they were diagnosed with CHB were found to be higher than the IHBC patients. In another study comparing HBV positive and/or HCV positive, HBV-DNA and HCV-RNA negative individuals with the patients with proven chronic viral hepatitis, the TOS value was found to be significantly higher in the chronic viral hepatitis group (21). According to the results of our study, in line with other studies, the highest TOS value was observed in CHB. However, no statistically significant difference in terms of the TOS values was observed between IHBC and CHB.

TAC measurement is a method used to evaluate the scavenging capacity of free radicals and to estimate the antioxidant capacity *in vivo* (22). In a study comparing the TAC values, the TAC value in the healthy control group was found to be significantly higher compared to the CHB and IHBC groups (20). In the study by Sirmatel et al. (21), the TAC values were lower in the patients with HBV and HCV infections compared to the control group. A recent study revealed that a decrease in TAC and a high oxidative stress index may indicate an imbalance in redox status in HCV-infected patients (23). In our study, no significant result in TAC was observed among the three groups. The lack of difference in TAC levels between the groups can be partially explained by the homogeneity of the patients who underwent biopsy, the exclusion of cirrhotic patients, and the presence of moderate chronic active hepatitis in some patients. In the study by Duygu et al. (20), the relationship between the fibrosis score and the TOS and TAC values was evaluated and the results did not point to any association between the fibrosis score and the TOS and TAC scores. While the TOS score does not increase parallel to the fibrosis score, the TAC score was not observed to diminish either. Parallel to this study, also our study did not demonstrate a significant relationship between the fibrosis score and TOS and TAC values. In recent studies, TAC is a promising biomarker for evaluating the progression of liver fibrosis in patients with HBV, and this finding may indicate the involvement of TAC-composing factors in the pathogenesis of hepatic fibrosis in chronic HBV carriers (24).

Study Limitations

There are some limitations to our study. First, our study was limited to a single-center study, and we had a small number of patients. In addition, the patients were not grouped according to chronic HBV infection stages due to the small number of patients.

Conclusion

The high TOS capacity observed in our study is thought to be associated with HBV activity and disease progression. Further longitudinal and prospective studies are needed to elucidate the mechanisms of the pathophysiological role of TOS and TAC in CHB patients.

Ethics

Ethics Committee Approval: The study was approved by the Dicle University Clinical Research Board of Ethics (approval number: 479, date: 28.03.2012).

Informed Consent: It was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ç.M., Ö.D., S.C., İ.K., M.K.Ç., Concept: Ç.M., M.B., Ö.D., S.C., İ.K., M.K.Ç., Design: Ç.M., M.B., Ö.D., S.C., İ.K., M.K.Ç., Data Collection or Processing: Ö.D., İ.K., F.B.,

M.K.Ç., Analysis or Interpretation: Ç.M., M.B., Ö.D., F.B., M.K.Ç., Literature Search: M.B., Ö.D., Writing: M.B., Ö.D.,

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare no financial support

References

1. World Health Organization, Global progress report on HIV, viral hepatitis and sexually transmitted infections. (2021). [Available from: https://www.who.int/publications/i/item/9789240027077
2. Moriya K, Nakagawa K, Santa T, Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Miyazawa T, Ishibashi K, Horie T, Imai K, Todoroki T, Kimura S, Koike K. Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res. 2001;61:4365-4370.
3. Yamamoto Y, Yamashita S, Fujisawa A, Kokura S, Yoshikawa T. Oxidative stress in patients with hepatitis, cirrhosis, and hepatoma evaluated by plasma antioxidants. Biochem Biophys Res Commun. 1998;247:166-170.
4. Kalender S, Kalender Y, Ogutcu A, Uzunhisarcikli M, Durak D, Açikgoz F. Endosulfan-induced cardiotoxicity and free radical metabolism in rats: the protective effect of vitamin E. Toxicology. 2004;202:227-235.
5. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem. 2004;37:112-119.
6. Janssen YM, Van Houten B, Borm PJ, Mossman BT. Cell and tissue responses to oxidative damage. Lab Invest. 1993;69:261-274.
7. Alavian SM, Showraki A. Hepatitis B and its Relationship with oxidative stress. Hepat Mon. 2016;16:e37973.
8. Dikici I, Mehmetoglu I, Dikici N, Bitirgen M, Kurban S. Investigation of oxidative stress and some antioxidants in patients with acute and chronic viral hepatitis B and the effect of interferon-alpha treatment. Clin Biochem. 2005;38:1141-1144.
9. Cochrane CG. Cellular injury by oxidants. Am J Med. 1991;91:23S-30S.
10. Lok AS, McMahon BJ. Chronic hepatitis B: Update 2009. Hepatology. 2009;50:661-662.
11. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38:1103-1111.
12. Liang TJ. Hepatitis B: The virus and disease. Hepatology. 2009;49(5Suppl):S13-21.
13. Lin CC, Liu WH, Wang ZH, Yin MC. Vitamins B status and antioxidative defense in patients with chronic hepatitis B or hepatitis C virus infection. Eur J Nutr. 2011;50:499-506.
14. Mahmood S, Kawanaka M, Kamei A, Izumi A, Nakata K, Niiyama G, Ikeda H, Hanano S, Suehiro M, Togawa K, Yamada G. Immunohistochemical evaluation of oxidative stress markers in chronic hepatitis C. Antioxid Redox Signal. 2004;6:19-24.
15. Demirdag K, Yilmaz S, Ozdarendeli A, Ozden M, Kalkan A, Kilic SS. Levels of plasma malondialdehyde and erythrocyte antioxidant enzyme activities in patients with chronic hepatitis B. Hepatogastroenterology. 2003;50:766-770.
16. Bast A, Haenen GR, Doelman CJ. Oxidants and antioxidants: state of the art. Am J Med. 1991;91:2S-13S.
17. Chrobot AM, Szaflarska-Szczepanik A, Drewa G. Antioxidant defense in children with chronic viral hepatitis B and C. Med Sci Monit. 2000;6:713-718.
18. Poli G. Pathogenesis of liver fibrosis: role of oxidative stress. Mol Aspects Med. 2000;21:49-98.
19. Bolukbas C, Bolukbas FF, Horoz M, Aslan M, Celik H, Erel O. Increased oxidative stress associated with the severity of the liver disease in various forms of hepatitis B virus infection. BMC Infect Dis. 2005;5:95.
20. Duygu F, Karsen H, Aksoy N, Taskin A. Relationship of oxidative stress in hepatitis B infection activity with HBV DNA and fibrosis. Ann Lab Med. 2012;32:113-118.
21. Sirmatel F, Duygu F, Celik H, Selek Ş, Sirmatel O, Gursoy B, Eris FN. Evaluation of total oxidative level and total antioxidant capacity in cases with chronic viral hepatitis. Klimik J. 2009;22:92-96.
22. Rubio CP, Hernández-Ruiz J, Martinez-Subiela S, Tvarijonaviciute A, Ceron JJ. Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: an update. BMC Vet Res. 2016;12:166.
23. Limongi D, Baldelli S, Santi F, D'Agostini C, Palamara AT, Nencioni L, Ciotti M. Redox alteration in patients infected by different HCV genotypes. Infez Med. 2018;26:249-254.
24. Wang JH, Lee SB, Lee DS, Son CG. Total Antioxidant Capacity in HBV Carriers, a Promising Biomarker for Evaluating Hepatic Fibrosis: A Pilot Study. Antioxidants (Basel). 2021;10:77.