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**Original Article**

# Evaluation of Redox Status in Patients with Hepatitis B Virus in Zahedan, Iran

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## ABSTRACT

**Background and objectives:** Redox status can be defined as an increase in oxidant and/or a decrease in antioxidant capacities. There is limited information about the redox status of patients with hepatitis B virus (HBV) infection. Therefore, we aimed to evaluate the redox status of patients with HBV infection in Zahedan, Southeast of Iran.

**Methods:** This study was carried out in 2019 on 25 HBV patients and 25 healthy individuals. The level of malondialdehyde (MDA) was measured as a marker of lipid peroxidation. Superoxide dismutase (SOD) activity was also measured. Data were analyzed using SPSS software (version 17) at significance of 0.05.

**Results:** The MDA level in patients (5.3 ± 2.511 n/ml) was significantly higher than in controls (3.48 ± 0.516 n/ml) (P <0.01). Moreover, SOD activity was significantly lower in HBV patients (125.05±55.545 n/µl) compared to healthy controls (271 ± 74.236 n/µl) (P<0.01).

**Conclusion:** Our results show that patients with HBV infection have higher serum MDA level and lower SOD activity compared to healthy individuals. This can cause liver damage and aggravate the complications in hepatitis B patients.

**Keywords:** Redox status; hepatitis B; malondialdehyde; superoxide dismutase

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. Generally, any chronic inflammatory liver disease has the potential to cause HCC (1-3). The most common pathophysiological process associated with this cancer is liver cirrhosis, which could be caused by hepatitis B virus (HBV) infection. Chronic HBV infection is considered as the most important etiological factor of HCC (4,5). Reactive oxygen species (ROS) are oxygen containing molecules that are produced during normal metabolism and has the potential to react with polyunsaturated fatty acids. This in turn leads to the release of toxic and reactive aldehyde metabolites, such as malondialdehyde (MDA), a biomarker of lipid peroxidation (6). Moreover, MDA has also been reported to be involved in tumor promotion as it can interact with the functional groups of a variety of cellular compounds. Different antioxidant systems, including superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase as well as non-enzymatic antioxidants such as glutathione and vitamin A, C and E are required to lessen the damage of ROS. It has been suggested that ROS and lipid peroxidation products may contribute to both onset and progression of hepatic fibrosis (7). In addition, oxidative stress can cause DNA damage (8), which might be associated with the development of HCC in patient with chronic viral hepatitis. In several studies, the hepatocellular damage caused by chronic hepatitis B infection (CHB) has been attributed to increased oxidative stress. Therefore, we evaluated changes in redox status, MDA level and SOD activity of HBV patients in Zahedan, Southeast of Iran.

## MATERIALS AND METHODS

Blood sample collection and preparation

The study population consisted of individuals who were referred to Ali ibn Abi Taleb hospitaol in Zahedan, Sistan and Baluchestan Province, Southeast of Iran. Fasting blood samples were collected from healthy controls (n=25) and HBV patients (n=25) after diagnosis and right before any substantial treatment, especially chemo- or radiotherapy. The control subjects had no history of diseases such as diabetes mellitus, rheumatoid arthritis or malignancies, which could affect the oxidant or antioxidant status. The blood samples were collected in tubes containing ethylenediamine tetraacetic acid (EDTA). The SOD activity was measured using whole blood samples. To analyze MDA level, plasma was separated by centrifugation at 3,000 rpm for 10 min at 4 $^{o}$C. All samples were stored at -20 $^{o}$C until analysis.

### Measurement of MDA

All chemicals and reagents used in the study were of analytical grade. Reaction with thiobarbituric acid (TBA) is a commonly used method of assessing lipid peroxidation (8). As a stable end product of fatty acid peroxidation, MDA reacts with TBA at acidic conditions to form a complex with maximum absorbance at 532 nm. For this purpose, 300 µl of each sample were mixed with 1.5 ml of 0.05 mmol/l HCl and 0.5 ml of 0.67% TBA. The mixture was then boiled at 95 $^{o}$C for 30 min. After cooling, the products were extracted in 2 ml of 15% butanol and centrifuged at 2,500 rpm, 4 $^{o}$C for 30 min. Finally, the absorbance of the supernatant was read at 532 nm.

### Measurement of SOD activity

The SOD activity was measured using Randox commercial kits and spectrophotometry. This method of SOD assay is based on $O_2$ generation by xanthine and xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye that can be measured by a spectrophotometer (Beckman Coulter, CA, USA). The degree of reaction inhibition represented SOD activity.

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### Statistical analysis

Data are expressed as mean + standard deviation. All statistical analyses were carried out using SPSS (version 17). Differences were assessed using t-test at significance of 0.05.

## RESULTS

The mean age of the case group (45.30± 13 years) and the control group (42 .68 ± 16.04 years) did not differ significantly. The MDA level in the HBV patients was significantly higher than in the controls (P<0.01, Table 1).

**Table 1. MDA level in HBV patients and healthy controls**

| Parameter | Patients | Controls | t | P-value |
|---|---|---|---|---|
| MDA (n/ml) | 5.3 ± 2.511 | 3.48 ± 0.516 | 20.047 | <0.01 |

The SOD activity in the HBV patients was significantly lower than in the controls (P< 0.01, Table 2).

**Table 2. SOD activity in HBV patients and healthy controls**

| Parameter | Patients | Controls | t | P-value |
|---|---|---|---|---|
| SOD (n/µl) | 125.05±55.545 | 271 ± 74.236 | —11.356 | <0.01 |

## DISCUSSION

It is well-established that excessive ROS generation with exhausted antioxidant defense systems can cause oxidative damage (9-11), which may subsequently lead to carcinogenesis. Previous studies reported ROS overproduction and high rate of lipid peroxidation in some neoplastic lesions, including liver cancer (12, 13). In a study by Custer et al., MDA level was significantly higher in patients with chronic HBV compared to healthy subjects (18). However, Kökoğlu et al. found no significant difference in the serum MDA level between patients with nonalcoholic fatty liver disease and healthy individuals (19). In a study by Kaya et al., MDA level was higher in hepatitis C patients than in control subjects (20). As a product of lipid peroxidation, MDA is an important indicator of oxidative damage. In our study, MDA level was significantly higher in the blood of patients compared to that of controls. Previous studies have also reported increased MDA level in tissue or plasma samples of breast cancer (14) and lung cancer (15) patients.

It is known that SOD plays an important role in the defense against oxidative stress. In a previous study, SOD was lower in HCC cells compared to normal hepatocytes (16). In our study, the SOD activity was significantly lower in the patients compared to the healthy controls. However, we believe that HBV patients experienced more oxidative stress. It should be noted that the differences in the dietary habits of the patients might have influenced the oxidative status.

## CONCLUSION

Our results show that patients with HBV infection have higher serum MDA level and lower SOD activity compared to healthy individuals. This can cause liver damage and aggravate the complications in hepatitis B patients.

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## DECLARATIONS

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### Ethics approvals and consent to participate

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The study protocol was approved by the Ethics Committee of Islamic Azad University of Zahedan, Iran (AE-MLS-WI-064-02). Written consent was obtained from all participants.

## Conflict of interest

The author declares that there is no conflict of interest regarding publication of this article.

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