The importance of myeloperoxidase enzyme activity in the pathogenesis of Crimean–Congo haemorrhagic fever

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Crimean–Congo haemorrhagic fever (CCHF) is a disease caused by Nairovirus, from the family Bunyaviridae. It has several symptoms including fever, ecchymosis, thrombocytopenia, hepatic function disorder and high mortality. Myeloperoxidase (MPO) is an enzyme located in neutrophil granulocytes and plays an important role in the destruction of phagocytosed micro-organisms. The aim of this study was to analyse MPO enzyme activity in CCHF cases compared with a control group. A total of 47 randomly selected CCHF patients admitted to the Department of Infectious Diseases of Cumhuriyet University Hospital in Sivas, Turkey, were studied, and as a control group, 41 age- and sex-matched individuals without any systemic disease were included in this study. MPO enzyme activity was measured in plasma and leukocytes for both groups by the ELISA method. MPO plasma and MPO leukocyte values were calculated as 57.62 ± 8.85 and 44.84 ± 9.71 in CCHF patients, and 0.79 ± 0.29 and 0.49 ± 0.11 in the controls, respectively. MPO enzyme activity was statistically significantly higher in patients with CCHF when compared to the control group. In conclusion, MPO enzyme activity is directly related to the activation of phagocytic leukocytes, and increases in both the plasma and leukocytes in CCHF patients. The increase of the MPO enzyme activity in leukocytes due to viral load leads to the destruction of the leukocyte. It is thought that MPO enzyme activity in plasma was higher in CCHF patients due to the destruction of leukocytes. MPO enzyme activity may be important in terms of the prognosis in patients with CCHF; however, more extensive studies are required on this subject.

INTRODUCTION

Crimean–Congo haemorrhagic fever (CCHF) is a disease caused by Nairovirus, from the family Bunyaviridae. It has several symptoms including fever, ecchymosis, haemorrhage, thrombocytopenia and hepatic function disorder (Bakir, 2004; http://www.saglik.gov.tr). The mortality rate is very high. Transmission is usually caused by tick bites.

In our country, CCHF notifications have been made, especially in the Middle Anatolian Region in Tokat, Sivas and Yozgat from the Kızılırmak river basin between 2002 and 2003, and from the Eastern Black Sea Region and from Istanbul in recent years. According to the data obtained from the Ministry of Health of Turkey, the number of cases was determined as 4453 between 2002 and 2009 (http://www.saglik.gov.tr; Mardani & Keshtkar-Jahromi, 2007; Acar, 2006; Karti et al., 2004).

The incubation period of CCHF differs according to the transmission type of the disease and ranges from 2 to 7 days. The disease starts suddenly and develops with fever, trembling myalgia, headache, vomiting and abdominal pain. Haemorrhagic incidents (petechiae, purpura, bleeding in mucosal membranes, epistaxis, haemoptysis, haematemesis, haematuria and melena) occur within the third to fifth days. Death usually results from serious haemorrhage (Mehrabi-Tavana et al., 2002; Kara, 2006). Mortality is high despite modern intensive care techniques, and the death rate varies from 8 to 80% (Acar, 2006; Lupi...
& Tyring, 2003). The mortality rate has been reported as 5–6% in Turkey (http://www.saglik.gov.tr).

Myeloperoxidase (MPO) enzyme is a member of the mammalian peroxidase family, which also includes lactoperoxidase, eosinophil peroxidase and thyroid peroxidase. MPO has three isoenzymes: MPO I, II and III, and plays an important role in the defence mechanism against microorganisms (Wright et al., 1990; Miyasaki et al., 1987; Rocha et al., 2002). This enzyme, purified for the first time by Agner (1941), is one of the important proteins of azurophilic granular leukocytes, is found in their cytoplasm with other antimicrobial proteins and is effective on many micro-organisms, especially viruses (Olsson et al., 2004; Chochola et al., 1994).

The anti-microbial functions of leukocytes occur as part of the phagocytosis process. Respiratory burst occurs via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is localized in the phagosome membrane. In this process, oxygen consumption increases and superoxide radicals (superoxide anions and hydrogen peroxide) occur rapidly (Hampton et al. 1998). The MPO enzyme catalyses 

\[ \text{H}_2\text{O}_2 + \text{halides} (\text{Cl}^-, \text{F}^-, \text{Br}^- \text{and } \Gamma^-) \rightarrow \text{HOCl} + \text{OCl}^- \]

creating hypohalous forms. These materials are very toxic against bacteria, fungus and mammalian cells. The biochemical reactions occurring during the phagocytosis of microorganisms in the activated neutrophils and the location of MPO are shown in Fig. 1 (Inal, 2007).

Generally, MPO activity increases in viral infections (Kulander et al., 2001). The objective of this study was to find out whether MPO enzyme activity is a useful diagnostic and treatment method by the comparison of the MPO enzyme activity level between controls and CCHF patients.

**METHODS**

This study was conducted in the Department of Emergency Medicine and Infectious Diseases, Cumhuriyet University Hospital, Sivas, Turkey, in 2007. Of the samples, 47 cases with a positive diagnosis of CCHF according to the PCR/ELISA result from the Refik Saydam National Public Health Agency Virology Reference and Research Laboratory (Ankara, Turkey) were selected for the study. Forty-one age and gender matched volunteers, who had not any viral or metabolic disease, were enrolled as the control group. Samples were collected into tubes containing EDTA.

**Purification of leukocytes.** Samples were centrifuged at 1000 r.p.m. for 10 min at +4°C and the upper plasma portion was extracted. The precipitate was centrifuged at 3000 r.p.m. for 5 min at +4°C. The white layer of leukocytes that occurred in the upper portion was aspirated with a Pasteur pipette and extracted as a leukocyte suspension. Turk solution (2 ml) prepared with 2 ml glacial acetic acid and 98 ml distilled water was added to the leukocyte suspension to fragment the erythrocytes present. This mixture was centrifuged, and the supernatant portion was removed and the precipitate was retrieved. Isotonic solution (0.9% NaCl) (2 ml) was added to this precipitate, and the mixture was centrifuged at 1000 r.p.m. for 10 min at +4°C. This washing process was repeated four times. Distilled water (1 ml) was added on this mixture, resulting in fragmentation of the leukocytes. Plasma and leukocytes were obtained from these samples. A microscopic view of the fragmented and unfragmented leukocytes is given in Fig. 2(a, b). Plasma and leucocyte MPO enzyme activity was measured using the Rayto 2100 C ELISA reader and MPO ELISA kit (Northwest Life Science Specialties) according to the ELISA method.

**Statistical analysis.** Statistical Package for the Social Sciences, version 14.0, was used for statistical analysis. Inter-group differences were tested with independent-samples t-test, Mann–Whitney U test and Kruskall Wallis test. Data were expressed as arithmetical mean ± SD and P<0.05 values were considered significant.

**RESULTS**

MPO enzyme activity was measured both in the plasma and leukocytes of the patient and control group. The patient group included 47 CCHF patients who had a definite diagnosis by the PCR/ELISA method. The control group consisted of 41 individuals who did not have systemic diseases. The difference between the groups in terms of age and gender was statistically insignificant (P>0.05).

The MPOplasma and MPOleukocyte enzyme activities of CCHF patients were found to be higher than those of the
controls \((P<0.05)\) (Table 1). In terms of MPO enzyme activity, according to the age groups in the control group the difference was statistically significant \((P<0.05)\). When the MPO values were compared according to the age groups, the difference was statistically significant between 14 years and under and 40–49 years, 50–59 years and 60 years and over \((P<0.05)\).

In terms of MPO enzyme activity, according to the age groups in the patient group the difference was not statistically significant \((P>0.05)\). Similarly, the difference between the males and females in the patient group was not statistically significant \((P>0.05)\), and there was an insignificant difference in the control group \((P>0.05)\) (Table 2). When MRIplasma and MRIleukocyte for the males and females in the patient and control groups were compared, the difference between the groups was found to be significant \((P<0.05)\).

### DISCUSSION

The aim of the study was to analyse the MPO enzyme activity in CCHF patients. CCHF disease is seen in nearly 30 countries in the world, including our country where it is especially seen in and around the provinces of Sivas. In our study, the leukocyte and plasma MPO enzyme activity of CCHF patients was found to be higher than in the control group. Currently, there is no information about the MPO enzyme activity measurement of CCHF patients in the literature. Therefore, the data obtained from this study were compared with other clinical research.

Generally, in viral infections, MPO activity increases (Kulander et al., 2001). The MPO enzyme found abundantly in phagocytic leukocytes is one of the most important producers of oxidants. The MPO enzyme catalyses \(\text{H}_2\text{O}_2\) and halides \((\text{Cl}^-, \text{F}^-, \text{Br}^-\text{and I}^-)\), creating hypohalous forms. These materials are very toxic against bacteria, fungus and mammal cells. When the immune system is stimulated by bacteria or viruses, the chemotactic and phagocytic activities of leukocytes increase. After phagocytosis, the leukocytes lead to increased reactive oxygen species through MPO and NADPH oxidase (Altınyazar et al., 2006). The leakage or secretion of the toxic agents from the neutrophils damages the nearby cells. Phagocyte-derived oxidants show toxic, immunosuppressive and mutagenic effects (Valenzuela, 1991). Therefore, the activity level of MPO is directly related to the activation of phagocytic leukocytes. MPO activity is expected to increase with the number of active phagocytic leukocytes. Altınyazar et al. (2006) did not find a significant difference compared with the control group in the neutrophil MPO enzyme activity of patients with recurrent aphthous stomatitis (RAS) disease and they stated that the MPO enzyme activity is at normal levels in the disease cases because the phagocytic active leukocyte number is also at normal levels. Although in our study patients had leukopenia, we found the level of MPO high both in leukocytes and plasma in these patients. The MPO level in the leukocytes suggested increased MPO expression in leukocytes stimulated by viral infection and the plasma level of MPO suggested leukocytes were lysed. Therefore, this was suggestive that leukopenia in CCHF patients might be attributed to lysis.

Iseri et al. (2005) reported that the colon and hepatic MPO enzyme activity increases in rats that have developed sepsis \((P<0.05\) and \(P<0.01\), respectively). In sepsis patients, plasma MPO enzyme activity was shown to be significantly higher (Kothari et al., 2011). Bekheit et al. (2009) reported that a high level of plasma MPO, associated with increased hepatic tissue MPO immunoreactivity, is important and plays a critical role in the development of hepatic cirrhosis.

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### Table 1. Enzyme activity values of MPOplasma, MPOleukocyte CCHF groups and controls

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<thead>
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<th>CCHF group (n=47)</th>
<th>Control group (n=41)</th>
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<tr>
<td>MPOplasma (\text{ng ml}^{-1})</td>
<td>57.62 ± 8.85*</td>
<td>44.84 ± 9.71</td>
</tr>
<tr>
<td>MPOleukocyte (\text{ng ml}^{-1} \text{per leukocyte})</td>
<td>0.79 ± 0.29*</td>
<td>0.49 ± 0.11</td>
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*Higher when compared to the control group \((P<0.05)\).
in hepatitis C virus infection. Zelzer et al. (2009) determined that MPO levels increase in patients with transplants before infection or rejection rather than in patients without any complication. They stated that the high MPO levels can be a determinant of the complications in patients who have an organ transplantation.

In a study conducted *in vitro* it was determined that the oxidant types produced by MPO cause tissue and cell damage, and contribute to many inflammatory diseases including respiration disorder syndrome, glomerulonephritis, arthritis, peptic ulcer and gastric cancer (Winterbourn et al., 2000). Clinical features such as haemorrhage and increased vascular permeability indicate that infection of the endothelium is a major target in CCHF pathogenesis (Swanepoel et al., 1987). CCHF virus targets endothelial cells in two ways. Directly via virus infection and replication in endothelial cells, and/or indirectly via viral factors or infected leukocytes releasing soluble mediators causing endothelial activation and dysfunction (Schnittler & Feldmann, 2003). The involvement of endothelial cells by CCHF virus increases vascular permeability and initiates inflammatory responses (Vestweber, 2007).

The MPO level is an indicator of endothelial dysfunction, inflammation, atherosclerosis and oxidative stress (Michowitz et al., 2008). This enzyme converts the low density lipoprotein into the atherogenic form, many reactive oxidants and free radicals occur and contribute to endothelial dysfunction by decreasing the nitric oxide level. MPO and MPO oxidant products can be a biomarker for the complications occurring during coronary heart disease and chronic heart failure (Tang et al., 2007; Morrow et al., 2008).

Ronald et al. (2009) determined that MPO activity is high in biochemical experiments comparing atherosclerotic plaques to normal arterial walls. They also determined that *in vivo* MPO activity is related to atherosclerotic plaque development and progression. The MPO enzyme is a rising determinant in acute cases, and plaque instability and plasma MPO levels have been found to be high in patients with stroke (Re et al., 1997).

In unstable angina and acute myocardial infarction, MPO is secreted into coronary circulation, thus the plasma levels of MPO increase (Buffon et al., 2002; Deby-Dupont et al., 1999). There is a strong relationship between high leukocyte and blood MPO levels and coronary artery disease (Baldus et al., 2003; Zhang et al., 2001).

Baldus et al. (2003) found that the MPO is significant in patients with a negative troponin test. Kaya et al. (2005) compared plasma levels of MPO enzyme activity and coronary angiography results, and stated that 50 % of the patients with a high MPO have coronary artery diseases, but coronary artery disease was not found in patients with normal plasma MPO levels.

In our study, plasma MPO enzyme activity was higher in the CCHF patient group than the control group. Therefore, increased MPO activity and related oxidant products are thought to contribute to endothelial dysfunction in CCHF patients. However, the presence of vascular disease is unknown in the CCHF patients included in our study. Increased levels of MPO suggested that CCHF disease contributed to the pathogenesis of vascular disorder. Extensive studies on this subject will provide further contribution. In this respect, if CCHF patients have a vascular disease history, during the period from the onset of the infection of the virus to the occurrence of symptoms, MPO enzyme activity levels may contribute to the prognosis.

**Conclusion**

Increased levels of MPO in leukocytes found in the cytoplasm of azurophilic granular leukocytes suggested synthesis related to viral infection, and an increased plasma level of MPO is suggested to be the result of lysis of the leukocytes. Leukopenia in CCHF patients is suggested to be correlated with lysis and not due to the involvement of bone marrow. We concluded that a high level of MPO affects pathogenesis and contributes to increasing oxidative stress. However, further studies are required to determine the significance of MPO in patients with CCHF.

**ACKNOWLEDGEMENTS**

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**REFERENCES**

Myeloperoxidase enzyme activity in CCHF patients


