

RELATIONSHIP BETWEEN TOTAL THIOL STATUS AND THROMBOCYTOPENIA IN PATIENTS WITH CRIMEAN-CONGO HEMORRHAGIC FEVER

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Abstract. The objective of this study was to investigate the relationship between serum total thiol level and total oxidant status (TOS) and thrombocytopenia among patients with Crimean-Congo hemorrhagic fever (CCHF). Eighty-three subjects and 56 controls were enrolled in the study. Thiol levels were measured with the DTNB method and TOS was measured with the Erel's method among subjects and controls. Thiol levels were lower in subjects than controls and TOS levels were higher in subjects than controls. There was a significant correlation between total thiol levels and platelet counts ($r=0.84$, $p<0.0001$) among subjects. Further investigations are needed into the link between total thiol level and TOS and the pathogenesis of hemorrhage in CCHF.

Keywords: Crimean-Congo hemorrhagic fever, aPTT, TOS, thiol, thrombocytopenia

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne viral disease. The mortality rate with CCHF has been reported to be between 5% and 30% (Gale *et al*, 2010; Leblebicioglu, 2010; Maltezu

et al, 2010). The pathogenesis of CCHF remains unclear due to the lack of a suitable animal model to study the disease. The high virulence of CCHF requires its study to be performed under Biosafety Level 4 conditions (Bente *et al*, 2010). Most of what is known today about CCHF is derived from human case studies and some *in vitro* experiments. Much is extrapolated from what is known about other viral hemorrhagic fevers (Bodur *et al*, 2010).

The virus is thought to damage the host's immune response by affecting the

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cells which initiate the antiviral response; it also causes endothelial dysfunction (Bodur *et al*, 2010; Ozturk *et al*, 2010; Saksida *et al*, 2010).

Thrombocytopenia is a consistent finding with CCHF (Onguru *et al*, 2008; Yilmaz *et al*, 2008; Tasdelen Fisgin *et al*, 2009; Hatipoglu *et al*, 2010; Onguru *et al*, 2010). The cause of thrombocytopenia in CCHF is unclear but thought to be due to reactive hemophagocytosis, deficient marrow production of thrombocytes, and sequestration of thrombocytes in infected organs (Schnittler *et al*, 2003; Karti *et al*, 2004; Yilmaz *et al*, 2008).

Thiols are a class of organic sulfur derivatives characterized by the presence of a sulfhydryl group (-SH) at their active site (Sen and Packer, 2000; Moriarty-Craige and Jones, 2004). There is a strong relationship between platelet function and thiol status (Bayele *et al*, 2002). Protein disulfide isomerase enzyme found in platelets cannot function properly in thiol deficiency and is associated with a decrease in platelet number and function (Jordan *et al*, 2005; Jordan and Gibbins, 2006). Since thiols play an important role in the intrinsic coagulation pathway, low levels of thiol are associated with prolonged aPTT values (Bayele *et al*, 2002).

Thiol deficiency is seen in some viral and bacterial diseases. In some infectious diseases it has been reported that oxidant levels increases; as a response, thiol is oxidated and TOS levels increase while thiol level is decreasing (Markovic *et al*, 2004; Dulger *et al*, 2011; Duygu *et al*, 2012; Usta *et al*, 2012). Thiol and total oxidant status (TOS) levels in CCHF are unknown. We studied thiol and TOS levels and platelet counts among patients with CCHF to determine if an association exists between them.

MATERIALS AND METHODS

Eighty-three subjects and 56 controls were enrolled in the study. All the subjects included in the study were treated for CCHF at the Ankara Ataturk Education and Research Hospital, Department of Infectious Diseases and Clinical Microbiology. CCHF was diagnosed by either detection of CCHF viral RNA with reverse transcriptase-polymerase chain reaction (RT-PCR) or by detecting IgM antibodies to CCHF. The tests were conducted at the reference Virology Laboratory of Refik Saydam Hygiene Central Institute, Ankara, Turkey. Tests for other possible infectious agents were carried out on all 83 subjects for *Leptospira* spp, *Rickettsia* spp, *Brucella* spp, *Toxoplasma* spp, *Coxiella burnettii*, agents of Lyme disease, malaria, rubella, herpes viruses, cytomegalovirus and hepatitis A, B and C viruses. No other agents were found. The control group consisted of healthy volunteers. The study was carried out after approval by the ethics committee of Ankara Ataturk Education and Research Hospital.

Biochemical and hematological methods

Blood samples for hematological, biochemical and microbiological analysis were taken. The blood was centrifuged at 1,800g for 10 minutes and stored at -80°C until analysis. Platelet counts were determined with a Beckman Coulter LH850 automatic hemocounter. Activated partial thromboplastin time (aPTT) values were determined with a Dade Behring BCS. Total serum thiol levels were measured with a commercially available kit (Rel Assay Diagnostic, Turkey) based on DTNB reduction (Costa *et al*, 2006). Serum TOS levels were determined using a commercial available kit (Rel Assay Diagnostic, Turkey) based on a ferrous ion xylenol orange assay (Erel, 2005).

Table 1
Comparison of measured parameters between subjects with Crimean–Congo hemorrhagic fever and controls.

	CCHF <i>n</i> = 83	Controls <i>n</i> = 56	<i>p</i> -value
Total thiol level in mol/l	220 ± 78	543 ± 52	<0.0001
Platelet count/ l ^a	63,063 ± 36,696	239,845 ± 54,735	<0.0001
Total oxidant atatus in mol H ₂ O ₂ wquiv/l ^a	16.63 ± 11.32	7.90 ± 3.45	<0.0001
aPTT in seconds	35.6 (17.5-118)	27.6 (20.4-38.2)	0.001

^aThe comparisons were performed after log transformations. Data are given as mean ± standard deviation or median (minimum–maximum).

Descriptive statistics were conducted on all studied variables. The data were analyzed using the Kolmogorov–Smirnov test. Normally distributed data were analyzed with the Student's *t*-test, and those without normal distribution were analyzed with the Mann-Whitney *U* test. Relationships among parameters were determined by a correlation analysis test.

A receiver operating characteristic curve was determined, and the area under the curve was estimated. SPSS version 11.5 (SPSS, Chicago, IL) and MedCalc version 10.0.2.0. (MedCalc, Mariakerke, Belgium) were used for the calculations.

RESULTS

The mean age of the 83 subjects was 47 years old; 36 patients (43.3%) were male. The most common symptoms reported were fatigue (90.4%), fever (85.1%), myalgia (80.9%) and headache (72.3%). The median duration between onset of symptoms and admission to the hospital was 3 (1-7 days) days. Hemorrhagic findings were present in 56 of 83 subjects (67.5%); the CCHF mortality rate was 6.4% (6 of 83 cases).

The mean total thiol level among

subjects was considerably lower than controls (Table 1). The maximum thiol value among subjects was lower than the minimum level in the control group (Fig 1). The sensitivity and specificity levels for CCHF using a total thiol level of 435 mol/l were 100% for both and the area under the curve (AUC) was 1.0 (Fig 2). A strong positive correlation was seen between thiol level and the platelet count among subjects (Fig 3). A significant negative correlation was seen between the total mean thiol level and the mean TOS level. The thiol level fell as the aPTT increased (Table 2).

DISCUSSION

In this study the total serum thiol level among CCHF subjects was considerably lower than among controls. The total serum thiol level had a strong positive correlation with the platelet count.

The cause of the decrease in total serum thiol level among patients with CCHF is unclear. In most infectious diseases, as a part of the host's immunological defense mechanism, oxidants generally increase and antioxidants generally decrease (Araujo *et al*, 2008; Cepins-

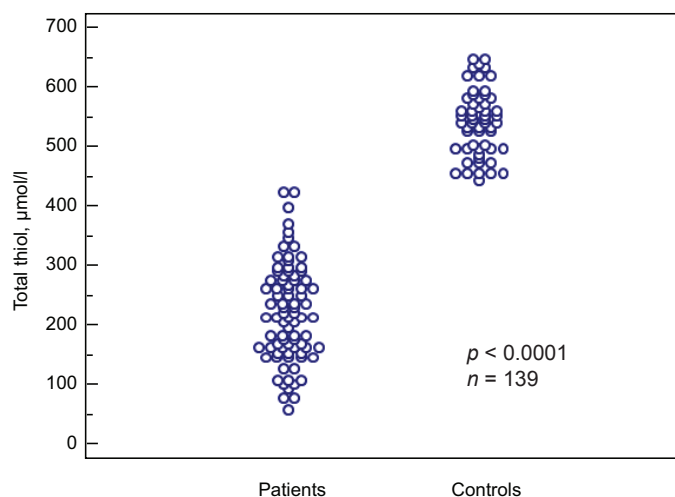


Fig –Total thiol levels among patients and controls.

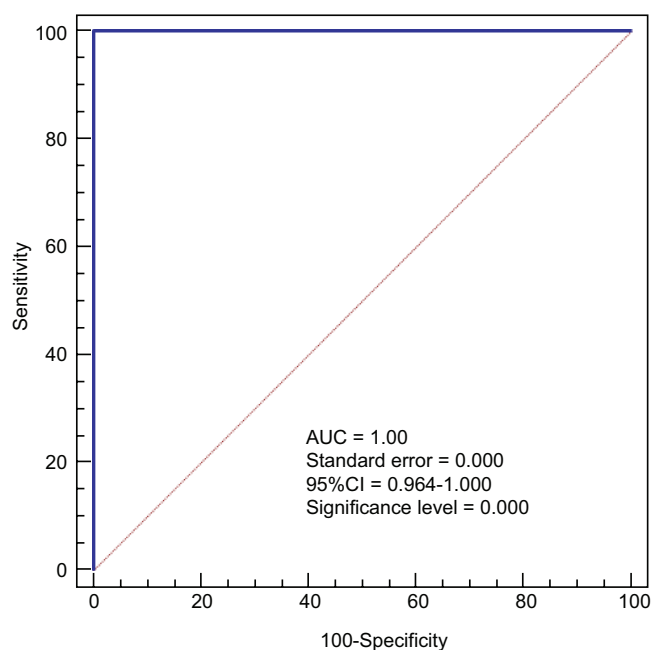


Fig2–Receiver operating characteristic of total thiol levels.

kas and Wilson, 2008; Farinati *et al*, 2008; Victor *et al*, 2009; Coaccioli *et al*, 2010). In this study, TOS levels were considerably higher among CCHF patients (Table 1).

Celik *et al* (2010) found a ten-fold increase in the activity of xanthine oxidase, a source of oxidant molecules for superoxide and hydrogen peroxide and Tütüncü *et al* (2010) found high nitric oxide levels among CCHF patients.

Thiols constitute the main part of the non-enzymatic antioxidant system in the blood and are the first molecules to oxidize in response to a rise in oxidant molecules (Erel, 2004). In this study a significant negative correlation was found between TOS and total thiol levels (Table 2).

For fusion between the virus envelope and host cell to occur the thiol level should be appropriate (Jain *et al*, 2009). When chemical agents that oxidize the reduced thiols in the medium, such as DTNB and p-mercuribenzoic acid, are introduced, viruses are not able to merge with the host cell (Ryser *et al*, 1994). Protein disulfide isomerase (PDI) enzyme, located on the surface of the cell, regulates the oxidized/reduced thiol status of the integral proteins. Fusion of the virus is inhibited when the PDI enzyme is inhibited with protein disulfide isomerase antibody (Jain *et al*, 2007).

PDI activity in patients with CCHF has not been reported before. In our opinion, in this viral disease, in addition to the increase in oxidants and decrease in thiols, the PDI enzyme may play an important role

Table 2
Relationships among measured parameters.

	Platelet count	Total oxidant status	aPTT
Total thiol level in mol/l	$r = 0.84$ $p < 0.0001$ $n = 139$	$r = -0.368$ $p < 0.0001$ $n = 139$	$r = -0.394$ $p = < 0.0001$ $n = 139$
Platelet count/ l		$r = -0.426$ $p < 0.0001$ $n = 139$	$r = -0.377$ $p = < 0.0001$ $n = 139$
Total oxidant status in mol H ₂ O ₂ equiv/l			$r = 0.260$ $p = 0.003$ $n = 139$

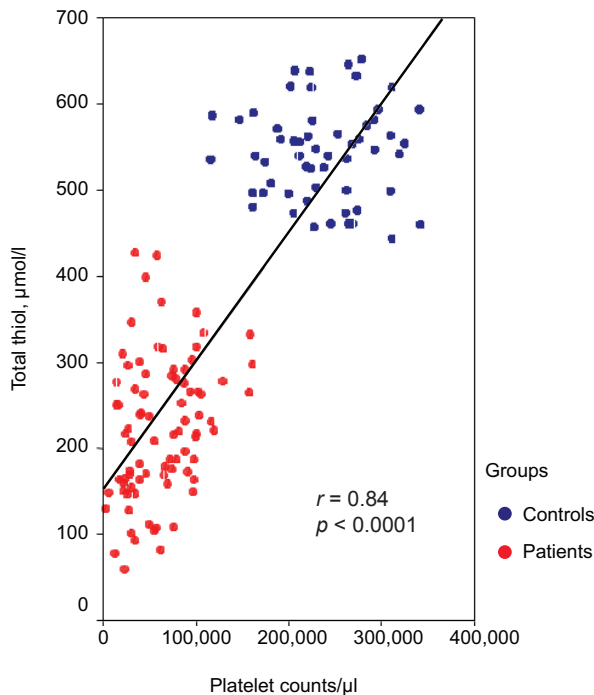


Fig 3–Relationship between total thiol levels and platelet counts.

in breaking the thiol/disulfide balance and providing thiol oxidation. In these patients we observed that while the decrease in thiol levels was in millimoles, the increase in oxidants was in micromoles.

The increase in oxidants does not explain the amount of thiol oxidation; therefore, some other enzymatic factor may be responsible. PDI, in our opinion, is a strong candidate. Further studies are required to explain our pathogenetic hypothesis.

Platelets are involved in primary hemostasis, aggregating at sites of vascular damage to form a hemostatic plug and promoting the cessation of bleeding. In platelets, reagents that react with cell surface sulfhydryl groups are capable of blocking a number of functional responses, including integrin-mediated aggregation, adhesion and granule secretion (Jordan *et al*, 2005; Manickam *et al*, 2011; Margaritis *et al*, 2011). In the majority of these studies, specific functions have been inferred following the inhibition of PDI activity.

We found a strong correlation between total serum thiol level and platelet count among CCHF patients ($r=0.84$, $p<0.001$) (Fig 3 and Table 2). Besides the low number of platelets, their functional degradation may also be responsible for the low levels of serum thiol.

There also are interactions between coagulation mechanisms and thiol status.

While factors II (prothrombin), VII, IX, XII and XIII are insensitive to oxidation, factors I (fibrinogen), V, VIII and X are inactivated by oxidation (Stief, 2000). The negative correlation between prolonged aPTT and total thiol status may be related to these interactions (Table 2).

In conclusion, the total thiol status significantly decreased in patients with CCHF. Besides its occurrence as a response to the increased oxidants in this disease, it may also be related to thiol/disulfide homeostasis moving to the oxidative side as part of the host's immunochemical response to the viral disease. Further investigation is needed into the link between these levels and the pathogenesis of hemorrhage in CCHF.

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