Some acute phase reactants and cholesterol levels in serum of patient with Crimean-Congo haemorrhagic fever

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Abstract

The purpose of this study is to determine erythrocyte sedimentation rate (ESR), C - reactive protein (CRP), serum amyloid-A (SAA) and cholesterol levels in patients with Crimean-Congo Hemorrhagic Fever (CCHF) and determine the relationship of these parameters with the severity of disease.

By polymerase chain reaction and enzyme-linked immunosorbent assay (ELISA) method 40 patients were diagnosed as CCHF and 39 volunteer without any systemic disease whose blood were taken and their serum separated. SAA, CRP and ESR were measured with ELISA, nephelometry and Mix-Rate x100 vital diagnostic device, respectively, in serum samples. High density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol levels were determined by using autoanalyzer HDL, LDL and total cholesterol kit (Syncron LX20).

Statistically significant difference was determined between patients and controls in terms of the levels of SAA, CRP, HDL, LDL and total cholesterol ($p<0.05$). However, there was no significant difference between the groups in terms of the levels of ESR. In addition, neither SAA, CRP, ESR nor HDL, LDL and total cholesterol levels varied with the severity of disease in the cases assessed ($p>0.05$).

Using of CRP and SAA together might increase the sensitivity of diagnosis of CCHF infection. However, none of the parameters investigated in this study were found to be a proper marker of the prognosis in CCHF. Cholesterol levels were significantly decreased in patients with CCHF, which was suggested to be associated with the increased serum levels of SAA in the patient group.

KEY WORDS: acute phase reactant, cholesterol, Crimean-Congo hemorrhagic fever disease, serum Amyloid A

INTRODUCTION

Crimean-Congo haemorrhagic fever (CCHF) disease occur when individual infected by *Bunyaviridae* family member genus of *Nairovirus*. This virus affects human health and has a high geographic distribution among the disease carried by ticks [1]. The infection of CCHF disease occurrence is possible by biting of tick contact with people who have this infection in their acute phase of CCHF disease and by contacting blood products of infected animals [1, 2]. The clinical symptoms of CCHF are non specific. The typical symptoms are high fever, headache, fatigue, joint aches, muscle ache, abdomen ache and diarrhoea without blood. In addition, patients may have advanced haemorrhagic symptoms [1, 3]. CCHF infection in some individuals causes vascular leakage, fever and the disease which characterized with coagulopathy [1, 4]. To diagnose CCHF, methods based on the isolation of the virus or the virus RNA from blood and tissue samples, and serologic identification of virus antigen and antibodies formed against the virus. Recently, molecular diagnosis methods are also successfully applied [1]. Acute phase response (APR) is a series of inflammatory responses of the host against infection and trauma, and is manifested via pyrogen cytokines that cause fever [5]. The role of APR is to isolate and neutralize pathogens, prevent the entry of further pathogens by minimizing tissue injury, initiate the repair process and thus assure rapid re-institution of normal physiological functioning of the homeostatic mechanisms of the host . Proteins, whose serum or plasma levels increase or decrease in APR are called acute phase proteins or acute phase reactants. Recent studies have suggested that determining the concentrations of acute phase proteins in plasma or serum provides important diagnostic information regarding the diagnosis and prognosis of the disease [6, 7]. The most commonly used acute phase pro-

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Submitted: 26 July 2012 / Accepted: 20 December 2012

teins for this purpose include C - reactive protein (CRP), and erythrocyte sedimentation rate (ESR). Although serum amyloid-A (SAA) is known to be more sensitive compared to both CRP and ESR, its use is not yet widespread [8]. SAA is one of the most important positive acute phase proteins, and an amphipathic apolipoprotein weighing 12 kDa. SAA production is mainly induced in the liver by some cytokins (IL-1β, IL-6 and TNF-α) during APR. As a result, under normal physiological conditions, SAA may increase up to 1000 times the 1–5 μg/ml level [9, 10]. SAA is induced in cases of inflammation, and bacterial and fungal infection, and reaches high levels (up to 1000 mg/L) in the serum. These protein is accepted as a supportive marker for the diagnosis and prognosis of inflammatory and autoimmune diseases [11]. A milder increase (up to 100 mg/L) is observed in SAA expression in various acute and viral infections [12, 13]. Therefore, SAA is a useful inflammatory marker in viral infections where clinical differential diagnosis is difficult and viral isolation is not possible [14]. If the SAA is secreted into circulation, it binds to the plasma high density lipoprotein (HDL) with removed apolipoprotein A-I in a short time [15]. In the absence of HDL, SAA is linked to low density lipoprotein (LDL) or very low-density lipoprotein (VLDL) [16]. It has been expressed that these relations and interactions between HDL and SAA may change the HDL metabolism and cholesterol transport during acute inflammation [17, 18]. Furthermore, SAA can remove excess cholesterol from sites of inflammation [19], and has been suggested to play a role in cholesterol efflux within the adipose tissue [20]. A study by Olsson et al established a transgenic mouse model with adipose tissue-specific expression of human serum amyloid A (hSAA). Olsson et al. [21] demonstrated that increased adipose tissue derived hSAA plasma levels from high fat fed hSAA mice, correlated with amount of adipose tissue and, in plasma, hSAA concentration peaked in HDL containing fractions. Serum levels of the acute phase reactants CRP and ESR have important roles in differentiating between bacterial and viral diseases [8]. However, CRP often does not attain detectable levels in viral diseases [22]. Therefore, serum levels of ESR and CRP are expected to remain normal in CCHF, which is a viral disease. Recent studies have reported SAA to be a more sensitive acute phase reactant in determining viral infections compared to CRP [22, 23]. In this study, our objective was to determine whether SAA was a more beneficial marker in establishing a pre-diagnosis of CCHF compared to ESR and CRP. A recent study has demonstrated that reduced cholesterol levels in plasma membrane prevented infection with Crimean-Congo Hemorrhagic Fever virus (CCHFV) significantly [24]. Therefore, we also aimed to determine cholesterol levels in patients with CCHF, since CCHF virus infection and SAA levels have been suggested to be associated with cholesterol levels. Finally, we aimed to investigate the relationship between these parameters and disease severity.

MATERIAL AND METHODS

Study population

This prospective study was conducted between 2008 and 2010 in Cumhuriyet University Hospital in Sivas, a city located in central Anatolia. The study protocol was approved by the Human Ethics Committee of the Cumhuriyet University Faculty of Medicine. Informed consent was obtained from each patient. This work is supported by the Scientific Research Project Fund of Cumhuriyet University under the project number T-360. Suspected cases with CCHF were defined as those who had clinically observed sign and symptoms (e.g. fever, nausea, vomiting, myalgia, bleeding), epidemiological risk factors (being bitten by tick or being a farmer) and laboratory data consisting of a platelet (PLT) count of <150 000/mm3, white blood cell (WBC) count of <4000/mm3, and elevated liver enzymes. All patients were followed up at the Department of Infectious Diseases and Clinical Microbiology at Cumhuriyet University Hospital. The presence of serum IgM of patients was tested by ELISA and/or of the genomic segments of the CCHF virus by reverse-transcription polymerase chain reaction (RT-PCR). In acute and convalescent phase sera. Acute phase blood sera were collected within 24 hours of hospitalization. A convalescent phase serum sample was obtained from each patient after at least 7 days. Following collection of serum samples were immediately sent to the Virology Laboratory of Refik Saydam National Public Health Agency (RSNPHA) Ankara, Turkey, for serologic and virologic analyses to confirm the diagnosis of CCHF. The presence of CCHFV RNA was tested in blood sera using a TaqMan-based real time RT-PCR assay as described by Yapar et al. [25]. The definitive diagnosis of CCHF infection was based on typical clinical and epidemiological findings and the detection of CCHF virus-specific IgM by enzyme-linked immunosorbent assay (ELISA) or of genomic segments of the CCHF virus by reverse-transcription polymerase chain reaction (RT-PCR). Written informed consent was obtained from patients or their family members. All CCHF patients were classified into two groups in terms of disease severity (severe, non-severe), according to the predictive factors for fatal outcome criteria reported by Swanepoel et al. [26]. Thirty-nine healthy adult volunteers who had no infections or immune system diseases such as rheumatoid arthritis, psoriasis and sarcoidosis, were included in this study as a control group. The control group consisted of volunteers from the Cumhuriyet University staff.

Blood collection

Venous blood samples were collected in tubes after an 8-h fast and immediately stored at 4° C. Next, the serum was separated from the cells and fibrines by centrifugation at 1610 x g for 10 min and stored in several aliquots at -80° C until assayed.

Measurement of SAA levels in sera

Human SAA levels were measured with ELISA kit (Human SAA Cytoscreen ELISA Immunoassay kit Catalog #KHA0011, BioSource International, Camarillo, CA, USA) according to instructions of the manufacturers [27].

Measurement of CRP levels

Serum CRP levels were measured with nephelometric method using 'Beckman Coulter™ Immage Analyzer System at Microbiology Laboratory of Cumhuriyet University Medical School Education and Research Hospital [28].

Determination of ESR

ESR in patient and control groups were determined with Mix-Rate x100 vital diagnostic device at Hematology Laboratory of Cumhuriyet University Medical School Education and Research Hospital.

Statistical analysis

Parametric data are expressed as the mean ± standard deviation and categorical data as percentages. The Statistical Package for the Social Sciences (SPSS) version 14 for Windows (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Parametric data were evaluated by the independent sample t-test and categorical data by the chi-squared test. A p value ≤0.05 was considered as significant.

TABLE 1. Demographic data for Crimean-Congo haemorrhagic fever patients and the control group.

	CCHF (N=40)	Control Group (N=39)	*p* value
Mean age (year)	42.8±20.6	41.3 ± 18.3	0.734
Sex (female/male)	17/23	24/15	0.090

TABLE 2. ESR, SAA, CRP, HDL, LDL, Total cholesterol serum levels in patients with Crimean-Congo haemorrhagic fever and the healthy control group.

	CCHF (N=40)	Control Group (N=39)	*p* value
SAA (µg/mL)	152.9 ± 58.4	4.3 ± 1.5	0.001*
CRP (mg/L)	27.5 ± 42.9	4.0 ± 2.2	0.001*
ESR (mm/saat)	16.6 ± 16.4	11.1 ± 4.2	0.054
HDL (mg/dL)	24.2 ± 8.8	43.2 ± 9.2	0.001*
LDL (mg/dL)	64.3 ± 22.9	87.4 ± 16.1	0.001*
Total Cholesterol (mg/dL)	116.1 ± 32.8	169.9 ± 26.7	0.001*

Data are expressed as the mean ± standard deviation. SAA, Serum amyloid A; CRP, C-reactive protein; ESR, Eritrocyte sedimentation rate; HDL, High density lipoprotein; LDL, low density lipoprotein. * $p < 0.05$.

TABLE 3. ESR, SAA, CRP, HDL, LDL, Total cholesterol levels severe Crimean-Congo haemorrhagic cases with - severe disease and non-severe disease groups.

	CCHF cases with severe disease (N=21)	CCHF cases with non-severe disease (N=29)	*p* value
SAA (µg/mL)	148.1 ± 60.7	157.2 ± 57.3	0 .63
CRP (mg/L)	29.0 ± 43.3	25.9 ± 43.6	0.83
ESR (mm/saat)	18.0 ± 15.8	15.6±17.1	0.66
HDL (mg/dL)	23.4 ± 9.9	24.9 ±7.7	0.59
LDL (mg/dL)	66.5 ± 29.7	62.2 ± 15.0	0.57
Total Cholesterol (mg/dL)	114.6 ± 37.7	117.4 ± 28.5	0.79

Data are expressed as the mean ± standard deviation. SAA, Serum amyloid A; CRP, C-reactive protein; ESR, Eritrocyte sedimentation rate; HDL, High density lipoprotein; LDL, low density lipoprotein. * $p < 0.05$.

RESULTS

Forty CCHF patients and 39 control subjects were recruited for the study. Of the CCHF patients, 17 (42.5 %) were female and 23 (57.5 %) male, and the mean age was 42.75±20.56 years. In the control group, 15 (38.46 %) individuals were male and 24 (61.54 %) were female, with a mean age of 41.26 ± 18.28 years. There were no significant differences in the age or sex ratio between CCHF patients and controls (Table 1; $p>0.05$). ESR, CRP and SAA levels in CCHF patients were significantly higher than were those determined for the control group ($p<0.05$). HDL, LDL and total Cholesterol levels in patients with CCHF were meaningfully less than the control group ($p<0.05$; Table 2). We found no significant difference in serum CRP, SAA, HDL, LDL, total Cholesterol levels and ESR between severe and non-severe patients ($p>0.05$; Table 3). In addition, an inverse correlation coefficient (r=-0.125) was determined between HDL and CRP levels in the patient group. However, this correlation coefficient was statistically insignificant ($p=0.46$; $p>0.05$). A statistically significant correlation coefficient (r=-0.389) was determined between CRP and ESR in the patient group ($p=0.02$; $p<0.05$).

DISCUSSION

CCHF is a viral hemorrhagic fever disease, highly threatening for the health. The mortality rate of the disease may be about 30%. Various mortality rates of 5-50% were also reported [1, 29]. Currently, there is limited information on the pathogenesis of the CCHF disease. Initially, a pre-diagnosis is established in suspected cases of CCHF in most endemic regions of CCHF. Clinical symptoms as well as epidemiological risk factors including history of tick bite and contact with

tick are considered in establishing this pre-diagnosis. However, CCHF might be mistaken for typhus, tularemia and lyme which are other tick-borne diseases with similar clinical pictures [30, 31]. Establishment of an exact diagnosis is necessary, but possible in only a couple of reference laboratories in most countries. Therefore, this process takes a certain period of time which might be as long as 7-10 days. Early diagnosis and initiation of antibiotic treatment in cases of bacterial infections is crucial particularly in patients with poor general condition. Acute phase reactants including CRP and ESR are used in differentiating between bacterial and viral infections since years. Although SAA has been suggested to be more sensitive, it is not used commonly in clinical practice. CRP is the preferred routine follow-up parameter in most centers since it is both practical and less expensive. Although CRP is the most well-known and commonly used marker of infection, CRP-alone sometimes proves inadequate in early diagnosis of infection. Symptoms often arise prior to elevation of CRP levels. Several studies have thus investigated the role of SAA, another acute phase protein, in the diagnosis and follow-up of various diseases. SAA levels have been determined to be significantly elevated in several diseases including common flu and influenza [32], rubella and measles ($p<0.05$) [33]. However, there are no studies in the literature examining the levels of SAA, CRP, ESR and cholesterol levels in patients with CCHF. In this study, our objectives were to determine ESR, SAA, CRP and cholesterol levels in patients with CCHF and to determine the relationship of these parameters with establishment of pre-diagnosis, prognosis and pathogenesis of the disease. SAA and CRP levels were found significantly higher in the patient group compared to the controls ($p<0.05$), whereas no significant differences were determined between the groups in terms of ESR ($p>0.05$; Table 2). CRP is a more sensitive and reliable marker of acute inflammatory events compared to ESR [8]. However, CRP often remains below the detectable threshold in viral infections whereas SAA is detectable. Even slight increases are detectable in SAA since this marker has higher physiological levels than CRP and remains elevated for longer than CRP [22, 23]. Several studies have demonstrated that SAA provides a sooner and more rapid increase, and that it is elevated in the acute phase of 98% of all viral infections [13, 34]. Additionally, SAA expression is more prominently increased in bacterial and fungal infections (up to 1000 mg/L) compared to viral infections (up to 100 mg/L) [9]. This suggests that SAA is a beneficial marker in differentiating viral infections from bacterial ones. In our study, SAA and CRP levels were found significantly higher in the patient group compared to the controls ($p<0.05$), whereas no significant differences were determined between the groups in terms of ESR ($p>0.05$; Table 2). Therefore, we suggest that ESR is not a good marker to determine CCHF which is an acute viral disease and that the use of CRP-alone might be inadequate. That is why, together use of CRP and SAA in establishing a diagnosis of CCHF might increase the rates of sensitivity and accuracy. Our study also compares the HDL, LDL and total cholesterol levels of patient and control groups, finding a meaningful difference ($p<0.05$) for all these parameters between the two groups. In addition, no significant correlations were determined between CRP and HDL (r=-0.181), or ESR and SAA (r=-0.125), despite the presence of significant correlation between SAA and HDL levels (r=-0.389) in our study. As also seen in Table 2 despite a very serious drop in the HDL level in CCHF patients, there is also a remarkable fall in LDL and total cholesterol levels. That is why, lipoprotein and cholesterol metabolism generally change in these patients. And this may be associated with an increase in the level of SAA secreted into circulation as a result of induction of SAA expression in the liver through the secretion of cytokines depending on viral infection in CCHF patients. That is, most probably, the SAA level that increases in circulation as a result of APR which develops in these patients removes more apoA1 from HDL and binds with this lipoprotein. HDL's structure changes as a result of this binding. Therefore, this may be a cause of low HDL level in patients with CCHF. Results obtained from a study where the plasma lipoprotein dynamics are studied in patients with severe sepsis support of our findings. In that study, researchers found that cholesterol and HDL level rapidly and meaningfully fell between days 0 to 3 in patients with severe sepsis. Moreover, a positive correlation (r=0.0684, $p<0.05$) was found between SAA concentration in plasma CRP and HDL. As a result of these findings, it is expressed that SAA was the main apoprotein in HDL during the onset of sepsis. Besides, the reason underlying the drop in total cholesterol is mainly linked to the fall in HDL level [35]. SAA is linked to LDL or VLDL in the absence of HDL in blood circulation [36]. And because the HDL level is very low in CCHF patients, some of SAA is linked to LDL and other lipoproteins. This may be considered as one of the causes of low LDL level in CCHF patients. It is considered that the decrease in the LDL level, especially the HDL, is one of the factors causing fall in total cholesterol level in CCHF patients. We believe that SAA interacts with lipoproteins as a result of inflammation, indirectly contributing to all such falls in the cholesterol levels in CCHF patients. In the in-vitro study of Simon et al. [24] performed with cell cultures, nucleocapsid protein and viral RNA levels of CCHFV have been found significantly decreased in cells with diminished cholesterol via methyl-β-cyclodextrin administration. In other words, reduced cholesterol levels in cellular plasma membrane have prevented CCHFV infec-

tion significantly. On the other hand, this process has been observed to be reversed when exogen cholesterol was added. According to the results of their study, authors have suggested that cholesterol is involved in the early stages of CCHFV replication cycle [24]. Similarly, our results also supported the notion that cholesterol has an important role in the pathogenesis of CCHF. However, further studies should be performed to further clarify this hypothesis. Analysis of all the study parameters in terms of CCHF severity demonstrated no statistically significant differences between severe and non-severe cases of CCHF (p>0.05). Results of our study indicate that ESR, CRP, SAA, HDL and total cholesterol levels are not good markers of prognosis in CCHF disease.

CONCLUSION

CRP is used as a sensitive inflammatory marker in bacterial infections but its value is limited in acute viral infections due to minimal elevation compared to bacterial infections. SAA increases sooner and more markedly than CRP; and contrary to CRP, SAA is elevated markedly in bacterial as well as viral infections. Parallel use of CRP and SAA might increase the rates of sensitivity and accuracy of these acute phase proteins in establishing a diagnosis of CCHF infection. However, ESR, CRP, SAA, HDL and total cholesterol are not deemed as good markers of prognosis in CCHF disease. Additionally, cholesterol levels were significantly decreased in patients with CCHF, which was suggested to be associated with increased circulatory levels of SAA.

DECLARATION OF INTEREST

None of the authors has any conflict of interest relating to this paper. The authors alone are responsible for the content and writing of the paper.

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