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Cutaneous Findings of Crimean-Congo Haemorrhagic Fever: A Study of 269 Cases

Running Title: Crimean-Congo Haemorrhagic Fever

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Key words: Crimean-Congo haemorrhagic fever, Crimean-Congo haemorrhagic fever virus, cutaneous findings.
Summary

Crimean-Congo haemorrhagic fever (CCHF) is a viral zoonotic disease. We aimed here in this study to investigate the cutaneous manifestations of CCHF and reveal their associations with fatality. Two hundred and sixty-nine patients diagnosed with CCHF were recorded. Skin findings were seen in 170 (63.1%) patients. A facial rash was most common cutaneous finding (82 (30.4%)). In the severe cases, haemorrhagic cutaneous manifestations (petechiae and ecchymoses) were recognised. A statistically significant correlation was obtained between the cutaneous manifestations and fatality, and it was determined that there was a strong positive correlation between the fatality and ecchymosis (r=567, p<0.001). In addition, a logistic regression analysis was performed, and death occurred 4.69 times more in those with skin signs than in those without. We hypothesize that CCHF patients with ecchymosis are at the highest risk, and that the cutaneous findings can contribute the prognosis of CCHF.
1. Introduction

Crimean-Congo haemorrhagic fever (CCHF) is an acute zoonotic infection with a 5–10% fatality. The CCHF virus (CCHFV) is a single-stranded RNA virus in the Nairovirus genus of the Bunyaviridae family (1). The first case in Turkey was identified in the Tokat province in the Kelkit river valley in 2002, and the Turkish fatality rate ranges from 4–5% (2,3). This disease can be mild or mortal (4), and the early signs typically include fever, tachypnoea, hypotension, relative bradycardia, pharyngitis and sometimes conjunctivitis. The early stage of this disease is called the prehaemorrhagic phase, which is characterized by elevated liver enzymes, an increased bleeding duration and thrombocytopenia. Hyperaemia can be seen in the area where the tick was attached. A petechial rash is the first symptom, followed by petechiae and ecchymoses on the skin and petechiae on the internal mucosal surfaces (4-7). After several days, it is followed by the haemorrhagic phase, in which epistaxis, hematemesis, haematuria, melena, haemoptysis and bleeding from venepuncture sites are common. Moreover, bleeding can occur in the other organs, including the brain (8). Given that the symptoms of CCHF are non-specific and may mimic a broad spectrum of infectious diseases.

It is crucial to correctly identify this disease, particularly in regions where it occurs frequently, in order to take the necessary precautions and initiate the treatment without delay. The aim of this study was to investigate the cutaneous manifestations of CCHF and reveal their associations with fatality. This is one of the studies which evaluate a great number of CCHF cases according to skin findings.

2. Materials and Methods

Two hundred and sixty-nine patients who were admitted to the Infectious Diseases and Dermatology Clinics of Tokat State Hospital between April 1 and September 1 of 2011 were hospitalized with clinical and laboratory findings compatible with CCHF and confirmed as a
CCHF according to the diagnostic criteria, were enrolled in this prospective cohort study. The patients’ demographic data, dermatological and epidemiological findings, and disease courses were analysed. Dermatological findings were examined with dermatology specialist and recorded. Dermatological findings were defined as follows;

A macule is a flat blemish or discoloration that measures less than 1 cm. A papule is an elevated lesion measuring less than 1 cm. Combining the two terms, a maculopapular rash is a smooth skin rash or redness covered by elevated bumps. Petechiae/purpura are pink colored lesions caused by extravasation of blood. Those smaller than 5 mm are called petechiae and those larger than 5 mm are called purpura. Ecchymosis is commonly characterized by skin discoloration of reddish or bluish color that measures more than 1 cm.

In patients prediagnosed with CCHF diagnostic criteria were as follows;

1- Clinical findings:

At least two symptoms (fever, headache, myalgia, nausea/vomiting, arthralgia, weakness, bleeding) and leukopaenia (<4000/μL)/thrombocytopenia (<150 000/μL), elevation of serum aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), lactate dehydrogenase (LDH) and creatinine phosphokinase (CK).

2- Supportive findings:

Haemorrhagic-purpuric rash and other haemorrhagical symptoms

3- Epidemiological history one or more of the following exposures within the 3 weeks before onset of symptoms:
Living in-or travel to endemic area, history of tick exposure, contact with blood or other body fluids of an animal, contact with blood or other body fluids of confirmed CCHF patient, work in a laboratory that handles CCHF specimens

Suspected case definition: Case meets the clinical and epidemiologic linkage criteria

Probable case: Case meets the clinical and epidemiologic linkage criteria and meets two supportive findings or Case meets the clinical and epidemiologic linkage criteria in endemic areas for CCHF.

Confirmed case: Case meets the clinical+ demonstration of viral RNA in blood and tissue samples, specific IgM positivity, four-fold increase in specific Ig G titre, epidemiological association with confirmed CCHF patient.

For all participants, the case definition forms and serum samples were submitted to the National Reference Laboratory by the Public Health Institution of Turkey.

The specimens were tested for anti-CCHF immunoglobulin (Ig)M and IgG antibodies using an enzyme-linked immunosorbent assay, while a reverse transcription polymerase chain reaction (RT-PCR) and direct sequence analysis were carried out for the detection of CCHFV RNA. RT-PCR and/or anti-CCHF IgM positivity were suggestive of CCHF in those patients. A total of two hundred and sixty-nine patients who were CCHF PCR and/or IgM antibody positive were diagnosed and hospitalized with the CCHF, and were included in the study. We did not use ribavirin in our treatments. All patients were treated with supportive care.

The exclusion criteria were other dermatological problems and systemic collagen tissue diseases. The patients were allowed to take their own photographs. All enrolled patients provided written informed consent prior to study inclusion. The study population has been described before. The study was performed in accordance with the 1975 Declaration of
Helsinki for prospective biomarker studies. Ethics committee approval was obtained for this study.

Statistical Analysis

The statistical analyses were conducted using the Statistical Package for the Social Sciences version 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). If the continuous variables were normal, they were described as the mean±standard deviation (SD) [p>0.05 in the Kolmogorov-Smirnov or Shapiro-Wilk’s test (n<30)]. An independent samples t test (t test in the independent groups) was performed on the quantitative data showing a normal distribution. However, since the variables did not have a normal distribution, a Mann-Whitney U test was applied. The qualitative data comparison was done by using a chi-squared test, and a P value of less than 0.05 was considered to be statistically significant. The correlation between two continuous variables was analyzed using Spearman’s bivariate correlation, and the correlation was significant with the level at 0.01 (2-tailed). The factors affecting fatality were evaluated by a logistic regression analysis, and Pearson’s chi-squared test was used to compare the categorical variables between the groups.

3. Results

A total of two hundred and sixty-nine CCHF cases and 17 deaths (6.4%) were identified during the study period. Of the included cases, 159 (59.1%) were males and 110 (40.9%) were females. The mean age of the patients was 36.28 years old (SD 10.21, min-max 15-73). Skin findings were seen in 170 patients. The epidemiological findings were similar, with or without cutaneous findings, and a statistically significant difference was not found. The evaluation of other viral infections and drug histories was negative in our patients.

A facial rash was most common cutaneous finding, and it was recognised in 82 (30.4%) of the CCHF patients. Twenty-one (7.8%) patients had petechiae, 23 (8.5%) patients had
maculopapular rashes, and 8 (2.9%) patients had purpura. The cutaneous findings of the patients are summarised in Table 1. In the severe cases, haemorrhagic cutaneous manifestations were recognised, which included petechiae (Picture 1) and ecchymoses (Pictures 2 and 3).

A statistically significant correlation was obtained between the cutaneous manifestations and fatality, and it was determined that there was a strong positive correlation between the fatality and ecchymosis ($r=0.567$, $p<0.001$). In addition, a logistic regression analysis was performed, and death occurred 4.69 times more in those with skin signs than in those without.

Based on the correlation analysis, there was a significant positive correlation between the fatality and the skin findings ($p<0.001$, $r=0.381$). The correlations between the fatality and the laboratory findings were as follows: alanine aminotransferase $p<0.001$ and $r=0.704$, aspartate aminotransferase $p<0.001$ and $r=0.787$, creatine kinase $p<0.001$ and $r=0.458$, lactate dehydrogenase $p<0.001$ and $r=0.719$, white blood cell count $p=0.004$ and $r=0.176$, platelet count $p=0.009$ and $r=-0.158$, prothrombin time $p=0.044$ and $r=0.123$, partial thromboplastin time $p<0.001$ and $r=0.277$, and international normalized ratio $p<0.001$ and $r=0.366$.

4. Discussion

A distinctive rash, fever and influenza-like symptoms may indicate a tick-borne disease (9-11). The diseases caused by tick-borne pathogens in the Turkey include babesiosis, anaplasmosis, Lyme borreliosis, tularemia, CCHF and Mediterranean spotted fever. Mediterranean spotted fever is characterized by a maculopapular rash (involving the palms or soles) and/or a black mark at the site of the tick bite. In CCHF patients, maculopapular rashes are not involving the palms and soles and the absence of an eschar (tache noir) can help to differentiate. With these features, the spotted mediterranean is separated from the fire. Nonspecific skin rashes have been reported in babesiosis and anaplasmosis cases (9-11). In
addition, in the literature, there have been reported cases of erythema figuratum due to septic babesiosis (12-15). The three characteristic cutaneous manifestations of Lyme disease are erythema migrans, borrelial lymphocytoma and acrodermatitis chronica atrophicans (16). Erythema multiforme, ulcers, urticaria, erythema nodosum and cellulitis can be seen in tularemia cases (17).

The dermatological signs of CCHF are morbilliform eruptions, petechial lesions, purpura, ecchymosis and oral erythema-petechiae (7,11). However, there have been few studies describing the cutaneous manifestations and their relationships with fatality in CCHF patients. In recent years, Akyol et al. (18) investigated the cutaneous manifestations (31.4% morbilliform eruptions) of CCHF, and they found a correlation between the morbilliform eruptions and a reduced platelet number. In another study conducted in another hospital at different period in 2011, 176 CCHF patients were examined, and a significant relationship was found between the skin findings and fatality (p<0.01, r=0.435) (19).

In a previous study, 281 patients with CCHF prediagnoses were evaluated, and 10 patients (3 CCHF +) had bruises on the body, 20 patients (8 CCHF +) had skin eruptions, 8 patients (3 CCHF +) had petechiae, and 4 patients (2 CCHF +) had ecchymosis. However, there was no significant difference between the CCHF positive and negative patients with regard to the skin findings (20). In another study, the clinical findings of 99 patients with CCHF were reviewed, and a total of 36 patients had maculopapular rashes (7 patients exitus). However, no significant difference was found in terms of the cutaneous findings when the exitus patients were compared with the others (4).

Humans can also develop inflammatory responses to tick bites in the dermis, including infiltrates of neutrophils, eosinophils, histiocytes and lymphocytes, as well as vascular thrombi, erythrocyte extravasation and neutrophil damage to the blood vessels consistent with vasculitis. Despite these data, it remains unclear how tick-induced changes at the tick-dermal interface
may enhance pathogen transmission, and how the host responses to repeated tick bites might inhibit such transmission, especially as a result of CCHF. Repeated tick exposure is associated with the interruption of tick feeding, early tick detachment and the prevention of tick-borne pathogen transmission (12-15).

The main contributors to the pathogenesis of CCHFV are endothelial cells and immune cells. Following the entry of the CCHFV into the host, it encounters innate immune system cells, including monocytes and dendritic cells (21). The infected cells produce various types of cytokines, chemokines and inflammatory factors. An exaggerated proinflammatory cytokine response or a "cytokine storm" can cause endothelial cell activation and increased vascular permeability, resulting in hypotension, shock, multiple organ failure and death (22-25). The proinflammatory cytokines may be the reason for the CCHF dermatological signs (morbilliform eruptions, petechial lesions, purpura, ecchymosis and oral erythema-petechiae), and they are secreted after endothelial injury. One case of CCHF with erythema nodosum has been reported in the literature (26,27). In the study conducted by Kilinc et al. (20), CCHF positive and negative patients were compared, and 40% of the CCHF positive patients had skin eruptions, 36.3% had petechiae and 36.1% had ecchymoses. However, no significant difference was found. In one previous study conducted in 220 CCHF patients in 2016, petechiae were found in 21.4%, ecchymoses in 15.9% and maculopapular rashes in 11.4% of the patients. The petechiae and ecchymoses were reported to be significantly higher in the exitus patients (p=0.018 and p<0.001, respectively) (28). The limitations in this research were that we did not investigate the site of tick bite in our records of examinations and, we did not investigate the possible dermatological effects of drugs such as paracetamol, anti-emetics, blood and blood products which were used in supportive care.

CCHF is a public health problem that is especially important in certain regions of Turkey. The distribution of the diseases may not be limited to the reported areas and may be
underdiagnosed in many regions. CCHF viruses cause a variable skin lesions and these lesions play important role for clinical diagnosis of CCHF. Similar skin lesions have also been presented in other endemic zoonotic diseases in Turkey so we confirmed the diagnosis by PCR. The proper diagnosis is important to facilitate early treatment to decrease morbidity and fatality, even though the treatment should often be initiated before a definitive diagnosis is made. The dermatological findings should be examined carefully, because they can provide ideas about the patient's fatality. We hypothesize that CCHF patients with ecchymosis are at the highest risk, and that the cutaneous findings can contribute the prognosis of CCHF; however, future studies are needed.

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**Conflict of interests:** None to declare

**References:**


Table 1. The cutaneous and laboratory findings of the patients.

Picture 1. Petechiae of CCHF patient on the dorsalis pedis and anterior tibia.

Picture 2. Ecchymosis of CCHF patient on the upper extremity on flexor surface.

Picture 3. Ecchymosis of CCHF patient on the upper extremity on flexor surface.
Table 1. The cutaneous and laboratory findings of the patients.

<table>
<thead>
<tr>
<th></th>
<th>Total case N=269 (%)</th>
<th>Fatality (N=17) (%)</th>
<th>Recovery (N=252) (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean±SD</td>
<td>36.28±10.21</td>
<td>56.70±10.45</td>
<td>49.18±17.82</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Male</td>
<td>159 (59.1)</td>
<td>9 (52.9)</td>
<td>150 (59.5)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Living in rural area</td>
<td>259 (96.3)</td>
<td>16 (94.1)</td>
<td>243 (96.4)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Skin findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facial rash</td>
<td>82 (30.4)</td>
<td>2 (11.8)</td>
<td>80 (31.8)</td>
<td></td>
</tr>
<tr>
<td>Maculopapular rash</td>
<td>23 (8.5)</td>
<td>0</td>
<td>23 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Petechia</td>
<td>21 (7.8)</td>
<td>0</td>
<td>21 (8.3)</td>
<td></td>
</tr>
<tr>
<td>Purpura</td>
<td>8 (2.9)</td>
<td>0</td>
<td>8 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Ecchymoses</td>
<td>22 (8.1)</td>
<td>13 (76.5)</td>
<td>9 (3.6)</td>
<td></td>
</tr>
<tr>
<td>Facial rash and petechia</td>
<td>22 (8.1)</td>
<td>11 (64.7)</td>
<td>11 (4.4)</td>
<td></td>
</tr>
<tr>
<td>Facial rash and purpura</td>
<td>3 (1.1)</td>
<td>0</td>
<td>3 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Ecchymoses and petechia</td>
<td>2 (0.7)</td>
<td>2 (11.8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Facial rash and ecchymoses</td>
<td>11 (4.1)</td>
<td>11 (64.7)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Multiple skin findings</td>
<td>60 (22.3)</td>
<td>13 (76.5)</td>
<td>47 (18.7)</td>
<td></td>
</tr>
<tr>
<td>ALT* (U/L) median (IQR)</td>
<td>159 (84-297)</td>
<td>1335 (872-3151)</td>
<td>146.5 (81.2-256)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST* (U/L) median (IQR)</td>
<td>295 (144-640.5)</td>
<td>7087 (2329-10672)</td>
<td>267.5 (138.2-544.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CK* (mg/dL) median (IQR)</td>
<td>353 (168.5-794)</td>
<td>2560 (1389.2-4414.2)</td>
<td>306 (142.2-657.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDH* (U/L) median (IQR)</td>
<td>605 (428-1087)</td>
<td>4818 (3429-7430)</td>
<td>574 (416-912)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WBC (10^9/L) median (IQR)</td>
<td>1650 (1270-2200)</td>
<td>1300 (1170-2820)</td>
<td>1600 (1292.5-2177.5)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Plt (10^9/L) median (IQR)</td>
<td>32.7 (16.9-60.2)</td>
<td>12.9 (10.2-16.5)</td>
<td>34 (18-63.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PT median (IQR)</td>
<td>11.9 (10-14.3)</td>
<td>15.4 (14.3-18.8)</td>
<td>11.7 (10-13.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>aPTT median (IQR)</td>
<td>41.1 (35-52)</td>
<td>81.1 (51.4-95.7)</td>
<td>40.95 (34.5-49.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PT INR median (IQR)</td>
<td>1 (0.9-1.2)</td>
<td>1.39 (1.2-1.7)</td>
<td>1 (0.9-1.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
WBC: White blood cell count, Plt: Platelet value, PT: Prothrombine time, aPTT: Active tromboplastine time, INR: The international normalized ratio, IQR: Interquartile range, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CK: Creatine kinase, LDH: Lactate dehydrogenase

* Normal values: CK 21-232 mg/dL, AST 15-37 mg/dL, ALT 30-65 mg/dL, LDH 110-240IU/L
Picture 1. Petechiae of CCHF patient on the dorsalis pedis and anterior tibia.
Picture 2. Ecchymosis of CCHF patient on the upper extremity on flexor surface.
Picture 3. Ecchymosis of CCHF patient on the upper extremity on flexor surface.