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ORIGINAL ARTICLE / ÖZGÜN MAKALE

PCR Diagnosis, Epidemiological and Clinical Data of Crimean-Congo Hemorrhagic Fever Virüs in Tokat/Türkiye

Kırım Kongo Kanamalı Ateşi Virüsünün Tokat/Türkiye'de PCR Tanısı, Epidemiyolojik ve Klinik Verileri

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Abstract

Objectives: To contribute to case management algorithms and guidelines by evaluating the clinical symptoms, laboratory data, risk factors and mortality rates of patients admitted to health institutions with tick bite in Tokat.

Methods: The virus determination was made from the blood by conventional PCR in 141 patient. Epidemiological data such as socio-demographic variables and risk factors were compared with clinical symptoms, biochemical and hematological parameters.

Results: Of the patients, 83 (58.9%) were male, 84(59.6%) were positive. Five patients (5.95%) died in the PCR-positive group. Fever and tick contact history rates was found to be significantly higher in the PCR positive patients than the PCR negative group. Thrombocytopenia, leukopenia, aspartate transaminase (AST) elevation were found to be significantly higher in PCR positive patients. Despite the mean alanine transferase (ALT) was not significantly higher, the increase in the number of patients exceeding the reference range was found to be significantly higher in PCR-positive group ($p<0.05$).

Conclusion: PCR diagnosis has an active role in the incubation and prehemorrhagic period diagnosis of cases with a history and risk factors, clinical symptoms and compatible hematological and biochemical parameters in the endemic region. The place of ALT and AST elevation in the case management algorithm should be better clarified. Mortality rates of the disease can be reduced by providing the health services quality and a well-functioning surveillance system. Efforts to increase the knowledges of people living in rural areas about the disease may be effective in controlling the disease.

Keywords: PCR Diagnosis, Crimean-Congo Hemorrhagic Fever, Epidemiological and Clinical Data

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Öz

Amaç: Tokat ilinde kene ısırığı ile sağlık kuruluşlarına başvuran hastaların klinik semptomları, laboratuvar verileri, risk faktörleri ve mortalite hızlarını değerlendirerek vaka yönetimi algoritmalarına ve kılavuzlarına katkıda bulunmaktır.
Yöntem: 141 hastanın kanında konvansiyonel PCR ile virus tayini yapılmıştır. Sosyodemografik değişkenler ve risk faktörleri gibi epidemiyolojik veriler, klinik semptomlar, biyokimyasal ve hematolojik parametreler ile karşılaştırıldı.
Bulgular: Hastaların 83'ü (%58,9) erkek ve 84'ü (%59,6) pozitifti. PCR pozitif grupta beş hasta (%5.95) öldü. Ateş ve kene temas öyküsü oranları PCR pozitif hastalarda PCR negatif gruba göre anlamlı olarak yüksek bulundu. Trombositopeni, lökopeni, aspartat transaminaz (AST) yüksekliği PCR pozitif hastalarda anlamlı olarak yüksek bulundu. Ortalama alanin transferaz (ALT) anlamlı olarak yüksek olmamasına rağmen, referans aralığı aşan hasta sayısındaki artış, PCR pozitif grupta anlamlı olarak yüksek bulundu ($p<0.05$).
Sonuç: PCR tanısı, endemik bölgede öyküsü ve risk faktörleri, klinik semptomları ve uyumlu hematolojik ve biyokimyasal parametreleri olan olguların inkübasyon ve prehemorajik dönem tanısında etkin bir role sahiptir. ALT ve AST yükselmesinin vaka yönetimi algoritmasındaki yeri daha iyi aydınlatılmalıdır. Sağlık hizmetlerinin kalitesi ve iyi işleyen bir sürveyans sistemi sağlanarak hastalığa bağlı ölüm oranları azaltılabilir. Kırsal kesimde yaşayan insanların hastalık hakkındaki bilgilerinin artırılmasına yönelik çalışmalar hastalığın kontrol altına alınmasında etkili olabilir.

Anahtar Kelimeler: PCR Teşhisi, Kırım-Kongo Kanamalı Ateşi, Epidemiyolojik ve Klinik Veriler

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF), endemic to Türkiye, is a tick-borne viral zoonosis with Hyalomma marginatum as the main vector, which is widely seen in parts of Africa, Eastern Europe, the Mediterranean, Northwest China, Central Asia, Southern Europe, the Middle East and India (1). CCHF has a mortality rate of approximately 30% and has a potential for hospital-acquired spread (2). Early diagnosis of CCHF is important for case management and prevention of nosocomial infections (3). Crimean-Congo hemorrhagic fever virus (CCHFV) is a lipid-enveloped segmented, linear, single-stranded, negative-sense RNA virus belonging to the order Bunyavirales, family Nairoviridae, genus Orthonairovirus (4). Although their sensitivity is discussed, PCR-based tests have become the gold standard in the diagnosis of CCHF (5). The Ministry of Health of The Republic of Türkiye has developed an approach and case management algorithms for people who come with tick bites (6).

In this study, epidemiological data such as mortality rates, socio-demographic variables (gender, age groups, occupation) and risk factors, as well as clinical symptoms and biochemical/hematological parameters that determine the characteristic findings of the disease, were aimed to investigate to contribute to these algorithms and guidelines.

MATERIALS AND METHODS

Case Definition

CCHF case definition criteria were established by the CCHF working group of the Ministry of Health of the Republic of Türkiye (6). Accordingly, patients with the following two criteria were evaluated as suspected CCHF cases and included in the study:

Patients with a history of epidemiological risk factors for CCHF:

Two of the symptoms of sudden onset fever (axillary>38°C), headache, widespread body pain, arthralgia, weakness, diarrhea and bleeding

Living in an endemic area or within the last 2 weeks; endemic area visit, history of contact with ticks, contact with animal bodily fluids, history of contact with a patient diagnosed with CCHF.

Acute disease picture suggestive of Viral Hemorrhagic Fever

Thrombocytopenia (blood platelet count <150x109 cells/Liter) and/or

Leukocytes below 4,000

Tests

Serum samples of 141 suspected-possible CCHF disease cases who applied to Tokat Gaziosmanpaşa University Health Research and Practice Center and health institutions in Tokat were included in the study. 10 ml venous blood samples were taken from each patient in gel tubes for PCR and biochemical tests and 2.5 ml in EDTA tubes for hematological parameters.

In order to detect CCHF virus from blood samples, RNA isolation was performed according to the manufacturer's instructions using the Ribo-Sorb RNA/DNA extraction kit of Sacace Biotechnologies (Italy). Following RNA isolation, RNA was converted to cDNA by the Reverse Transcriptase step and PCR (iCycler IQ - BioRad Lab., USA) was performed.

Hemogram (Cell-Dyn 3700 (Abbott Diagnostics, Abbott Park, Ill., USA) and ALT and AST tests were studied on the "Dimension® Clinical Chemistry System" DADE BEHRING RxLMax (Dade Behring Inc. Newark, DE 19714, USA) device.

Statistical Analysis

Data analyse was made using the Statistical Package for the Social Sciences (SPSS) V.15 package program. Continuous data are shown with their medians and interquartile ranges because they do not show normal distribution. The Man Whitney-U test was used to determine whether there was a statistically significant difference between the variables in the PCR positive and PCR negative groups and those that did not show normal distribution. Chi-square (Fisher's Exact Test) analysis was used to examine the distribution differences of categorical data in both groups.

In this study, epidemiological data such as mortality rates, socio-demographic variables (gender, age groups, occupation) and risk factors were compared with clinical symptoms, biochemical and hematological parameters that determine the characteristic findings of the disease.

RESULTS

Of the patients, 83 (58.9%) were male, 84 (59.6%) were PCR-positive. Five (5.95%) patients in the PCR positive group, and two in the PCR negative group died, and there was no statistically significant difference between the groups (p= 0.701). The ages of those who died in the PCR positive group were 24, 34, 45, 64 and 75, and it was not seen in the pediatric age groups.

The mean age in PCR positive and PCR negative groups were, respectively, 41.3 and 36.0. No significant difference were found between the groups in terms of gender, age, and occupation (p>0.05 for all) (Table 1).

Fever (p=0.036) and tick contact history (p<0.001) rates was found to be significantly higher in the PCR positive patients than the PCR negative group (Table 2).

Thrombocytopenia (p=0.001), leukopenia (p=0.001) aspartate transaminase (AST) elevation (p=0.01) were found to be significantly higher in PCR positive patients. Despite the mean alanine transferase (ALT) was not significantly higher, the increase in the number of patients exceeding the reference range was found to be significantly higher in PCR-positive group (p=0.001) (Table 3).

Table 1. Socio-demographic distribution

	PCRnegative (n=57)		PCRpositive (n=84)		Total (n=141)	p
	n	(%)	n	(%)	n	
Gender						
Male	31	(54.4)	52	(61.9)	83	0.388
Female	26	(45.6)	32	(38.1)	58	
Age groups						
1-15	10	(17.5)	8	(9.5)	18	0.552
16-25	10	(17.5)	16	(19)	26	
26-45	17	(29.8)	22	(26.2)	39	
46-65	16	(28.2)	28	(33.3)	44	
65+	4	(7)	10	(12)	14	
Occupation						
Farmer	24	(42.1)	27	(32.1)	51	0.071
Housewife	16	(28.1)	27	(32.1)	43	
Student/re-school	13	(22.8)	12	(14.4)	25	
Other	4	(7)	18	(21.4)	22	

Table 2. Clinical symptoms

	PCR negative (n=57) n (%)	PCR positive (n=84) n (%)	Total (n=141) n	p
Semptom				
Fever	50 (90.9)	83 (98.8)	133	**0.036**
Weakness	48 (87.3)	79 (95.2)	127	0.088
Vomiting	9 (16.4)	18 (21.4)	27	0.305
Headache	42 (76.4)	74 (88.1)	116	0.057
Nausea	31 (56.4)	46 (54.8)	77	0.496
Stomachache	31 (56.4)	54 (64.3)	85	0.224
Diarrhea	4 (7.3)	9 (10.8)	13	0.349
Common Body Pain	45 (81.8)	73 (86.9)	118	0.280
Hemorrhage	6 (10.9)	7 (8.3)	13	0.410
History				
Tick contact / attachment	34 (59.6)	79 (94.0)	113	**<0.001**
Animal theme	29 (50.8)	56 (66.7)	85	0.071
Rural story	37 (64.9)	61 (72.6)	98	0.312
Animal blood contact	1 (1.7)	1 (1.2)	2	0.637
patient blood contact	1 (1.7)	0 (0)	1	0.396
Similar complaint around	15 (26.3)	14 (17.7)	29	0.099

Table 3. Hematological and biochemical tests

		PCR negative (n=57)	PCR positive (n=84)	p
Platelets	**Median (IQR*)**	149.000/mm^3 (81.500-247.500)	103.500/mm^3 (59.500-182.250)	**0.014**
	n (%) (cytopenia)	33 (57.8)	80 (95.2)	**0.001**
Leukocyte	**Median (IQR)**	4.200/mm^3 (2.900-6.450)	3.350/mm^3 (2.225-4.925)	**0.008**
	n (%) (cytopenia)	31 (54.3)	80 (95.2)	**0.001**
AST	**Median (IQR*)**	41 U/L (28.5-78.5)	73.5U/L (42.5-154.5)	**0.010**
	n (%) (over RI)**	33 (57.8)	60 (71.4)	**0.003**
ALT	**Median (IQR)**	36 U/L (23-57)	50.5 U/L (29.25-83.50)	0.071
	n (%) (over RI)	22 (38.5)	54 (64.2)	**0.001**

*Inter Quartile Range (IQR)
** Reference Interval(RI)

DISCUSSION

In the meta-analysis conducted by Nasirian in 2020, it was determined that there was a linear increase in CCHF cases between 1944 and 2017 (7). CCHF disease was detected in 59.6% of the samples obtained from patients who met the criteria in our region. Türkiye has already been defined as an endemic region by the Centers for Disease Control and Prevention (CDC) (8).

In this study, conventional RT-PCR was performed and 84 (59.6%) of the 141 investigated patients were found to be positive. However, this rate does not reflect the general population, as the study was conducted in a selected patient group, following the criteria specified by the

Ministry of Health. However, it was reported in the comparative studies of Duh et al. that these rates may be higher with the possibility of Real-time RT-PCR (9). It is important that the result is obtained between 4-6 hours with the method used. The described criteria can be revised.

A total of 11,041 CCHF cases were reported between 2002 and 2018 in Türkiye, and the case-mortality rate is 4.8% (10). In this study, the rate was 5.95%, which was consistent with Türkiye in general. According to the data of the Ministry of Health in the years the study was conducted, the mortality rate was 6-8% in Türkiye [11]. In the Nasirian meta-analysis, mortality rates were regionally higher than in our study; it was found 33.5% in Asia, 33.8% in Europe, and 22% in Africa (12). While the low number of cases in some regions may indicate high rates, high mortality rates are also common in regions with low access to healthcare. Phylogenetically, the Kosovo strain is close to the Türkiye strain (13). Considering the similarity of European strains and strains in Türkiye, the 25% high mortality rate in Kosovo can be explained by the quality of health care services. Similarly, as a result of the development of serological and molecular diagnostic methods in the neighboring country Iran, it was reported that the death rate decreased from 20% in 2000 to 2% in 2007 (14,15).

In the study, no deaths were observed in the pediatric age group. In a study conducted between 2000 and 2016 in the pediatric age group in Iran, the mortality rate was 11.8%. This may be associated with the higher incidence of mild to moderate cases, the quality of health care services, and a good surveillance system (16).

It has been shown in many studies that there is no statistically significant difference in the distribution according to gender distribution and age groups, and it is similar to this study (17).

In the research, when the occupational group that describes themselves as housewives is examined, it has been determined that they are related to agriculture. Farmer (32.1%) and housewife (32.1%) group constitute the largest group as the same group. In the study conducted in Gümüşhane (18) and in the study conducted in Bolu (19), the occupational group dealing with agriculture and animal husbandry was the group in which the disease was most common. Again in Iran, the farmer group is the group in which the disease is most common (15).

Fever (98.8%), weakness (95.2%), headache (88.1%) and diffuse body pain (86.9%) were the most common clinical symptoms, and they were very consistent with the case definition of the Ministry of Health (7). The only finding that differed statistically from the negative group was fever. CCHF should be considered in individuals living in an endemic region or staying in this region for more than 2 weeks and presenting with these four most common clinical complaints. In a study conducted in Iran, fever was reported as 96.5%, muscle pain as 90.2% and hemorrhage at 89% (20). In this study, hemorrhage was 8.3%. The patients we studied may have applied in the early stages of tick exposure, in the incubation period or in the prehemorrhagic period. It is thought that informing the public about the disease through the press plays an important role.

The most common risk factors in the study were tick exposure/attachment (94%), rural history (72.6%), and animal contact (66.7%). The risk factor that differed statistically from the negative group was tick contact/attachment history. In a study conducted in Elazığ, tick contact history was found as 72.1% and rural history as 98.4% (21). Rural life and contact with livestock were noted as the most common risk factors in a study examining Afghanistan and Pakistan. The higher prevalence of vectorial diseases in rural people has been associated with social turmoil, economic situation, and ignorance of the potential risk of disease transmission (22).

In the study; thrombocytopenia and leukopenia were clearly observed in the PCR positive group. These two findings have been shown as prominent findings in many studies for CCHF disease (23). Hematological findings in the studies of Bakır (24) and Yılmaz (25) from our country were also quite compatible with the findings of this study. According to the results of the study,

the coexistence of thrombocytopenia and leukopenia shows the clinician that CCHF should be considered in the differential diagnosis as well as other hematological diseases.

In this study, in the PCR positive group, the AST level remained high and the number of patients exceeding the reference range increased. While the ALT level did not increase statistically, the number of patients above the reference range increased. Copper (24) and Swanepoel (26) showed in their studies that AST was similarly higher in patients with confirmed CCHF disease. In patients with leukopenia and/or thrombocytopenia with elevated AST and ALT, it is absolutely necessary to investigate CCHF disease, taking into account the history and risk factors. Compared to ALT, AST elevation is more significant for CCHF disease.

Thrombocytopenia, elevation of ALT and AST, which are criteria defined by Swanepoel et al. (26) to predict severity and mortality of the disease, were consistent with this study, whereas leukocytosis was inconsistent. Unlike the study of Swanepoel et al. (26), leukopenia was observed in this study. In parallel with the study of Swanepoel et al. (26) our mortality rate is 5.95% in patients with thrombocytopenia with ALT and AST levels exceeding the reference range.

CCHF is a fatal disease that is endemic in Türkiye. PCR plays an active role in the incubation and prehemorrhagic period diagnosis of cases with a history and risk factors, clinical symptoms, and compatible hematological and biochemical parameters in the endemic region. The place of ALT and AST elevation in the case management algorithm of the Ministry of Health of the Republic of Türkiye should be made more specific. Mortality rates of the disease can be reduced by providing quality health services and a well-functioning surveillance system. Although the disease is more common in rural areas, efforts to increase the knowledge level of people in these regions about the disease may be effective in controlling the disease. Epidemiological data, history of the disease, risk factors and clinical complaints are important data for case diagnosis and management. In this study, it was determined that the disease did not discriminate between sexes, it was common in rural areas and agricultural workers, and tick contact, rural history and animal contact were the most common risk factors. The most common clinical symptoms are fever, weakness, generalized body pain, and headache. CCHF disease should be investigated in case one or more of thrombocytopenia, leukopenia and AST/ALT elevations are observed together with these findings.

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Ethical Declaration:

Before starting the study, the purpose of the study and the procedures to be performed were explained to the patient or their relatives, and their written consent was obtained, after obtaining the approval of the ethics committee of Gaziosmanpaşa University Faculty of Medicine with the decision no. 38 dated 27/03/2007.

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Conflict of Interest:

All authors declare no conflict of interest.

Authorship Contributions

Concept: MÖ, YB, Design: MÖ, YB, Supervising: MÖ, YB, Financing and equipment: MÖ, YD, YB, Data collection and entry: MÖ, YD, Analysis and interpretation: MÖ, YD, YB Literature search: MÖ, Writing: MÖ, Critical review: MÖ, YB

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