Research Article

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Importance of biochemical parameters in order to predict clinical severity in patients diagnosed with Crimean-Congo haemorrhagic fever

Kırım kongo kanamalı ateşi tanılı hastalarda klinik şiddeti tahmin etmede biyokimyasal parametrelerin önemi

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Abstract

Objective: We aimed to investigate new biochemical indicators to predict the clinical course of patients following the diagnosis of Crimean-Congo haemorrhagic fever (CCHF).
Material and methods: We retrospectively evaluated patients diagnosed with CCHF. They were divided into three groups based on a scoring system known as severity grading score in order to predict severity. Red cell distribution width (RDW), mean platelet volume (MPV), creatinine phosphokinase (CPK), alkaline phosphatase (ALP), glutamyl transferase (GGT) and C-reactive protein (CRP) levels were evaluated on the first day of admission. These biochemical parameters may predict the clinical course of our three patient groups.
Results: In our study, there were 38 (70.4%) male and 16 (29.6%) female patients, and the mean age was 44.33 ± 16.94 years. Based on our scoring system, 17 (31.4%), 30 (55.5%) and 7 (12.9%) patients were in group 1, 2 and 3, respectively. Statistically significant difference was observed between groups 1–3 and groups 2–3 for ALP values; however, a statistically significant difference was observed among all three groups for GGT values. Significant differences were not observed among the groups for RDW, MPW, CPK and CRP levels ($p > 0.05$).
Conclusion: ALP and GGT values can be used as auxiliary indicators to predict the clinical course for patients with CCHF. However, CPK, CRP, MPV and RDW values were not observed to be important for prognosis.

Keywords: Fever; Crimean-Congo haemorrhagic fever; RDW; MPV; ALP; GGT.

Öz

Amaç: Kırım Kongo kanamalı ateşi (KKKA), insanlarda görülen en sık kene ile ilişkili hastalıktır. Çalışmamızda KKKA tanısı ile takip edilen hastaların klinik seyrini ön görmek için yeni belirteçlerin araştırılması amaçlanmıştır.
Materyal ve metod: Bu çalışmada 2011–2017 tarihleri arasında KKKA tanısı ile takip ettiğimiz hastalar retrospektif olarak değerlendirildi. Klinik şiddeti tahmin etmede kullanılan skorlama sistemine göre hastalar üç ayrı gruba ayrıldı. Gruplar arasında klinik seyri tahmin etmede yararlı olabileceği düşünülen hastaneye başvuru gününde saptanan eritrosit dağılım genişliği (RDW), ortalama trombosit hacmi (MPV), kreatinin fosfokinaz (CPK), alkalen fosfataz (ALP), gama glutamil transferaz (GGT) ve C-reaktif protein (CRP) düzeyleri değerlendirildi.
Bulgular: Çalışmamıza dahil edilen 54 olgunun 38 (%70.4)'i erkek, 16 (%29.6)'sı kadındı. Yaş ortalaması 44.33 ± 16,94 bulundu. Hastaların 17 (%31,4)'si grup 1'de, 30 (%55,5)'u grup 2'de, 7 (%12,9)'si ise grup 3'te yer aldı. Gruplar arası karşılaştırmada ALP değerleri açısından grup 1–3 ve grup 2–3 arasında, GGT düzeyleri açısından ise tüm gruplar arasında istatistiksel olarak anlamlı fark saptandı. Gruplar arasında RDW, MPV, CPK, CRP düzeyleri açısından anlamlı farklılık gözlenmedi ($p > 0.05$).

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Sonuç: Klinik seyri tahmin etmede kullanılan skorlama sisteminde ALP ve GGT değerleri de yardımcı marker olarak kullanılabilir. Ancak CPK, CRP, MPV, RDW değerleri prognoz açısından önemli bulunmamıştır.

Anahtar kelimeler: Ateş; Kırım Kongo kanamalı ateşi; RDW; MPV; ALP; GGT

Introduction

Crimean-Congo haemorrhagic fever (CCHF) is a life-threatening, tick-borne zoonotic disease common in Africa, Asia, East Europe and the Middle East. The Crimean-Congo haemorrhagic fever virus (CCHFV) is an enveloped RNA virus (*Nairovirus* belonging to the *Bunyaviridae* family) [1, 2]. CCHFV infects humans via the bite of a *Hyalomma* tick harbouring the virus or coming into contact with the blood and bodily fluids of previously infected animals or humans [3]. In Turkey, many studies have revealed various seropositivity rates (2.3%–19.6%).

Thrombocytopenia and leucopoenia are the primary clinical presentations of CCHF, and there is also increased levels of aspartate transferase (AST), alanine transferase (ALT), lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), alkaline phosphatase (ALP), gamma (γ) glutamyl transferase (GGT) and mean platelet volume (MPV) [4]. Definitive diagnosis of CCHF is established with real time reverse transcriptase polymerase chain reaction (RT-PCR) of viral nucleic acid in blood and body fluid samples or detection of IgM positivity or IgG seroconversion via enzyme-linked immunosorbent assays [5].

CCHF can vary in disease severity and may result in mortality; however, subclinical infections are typically present in patients living in endemic areas [6]. A scoring system was recently developed to predict severity of CCHF (Table 1). Depending on this scoring system, disease severity is classified as mild (≤4), intermediate (5–8) or severe (≥9) [7].

Determining complete blood counts is practical and cost-effective, and it includes parameters important for several diseases. For example, red cell distribution width (RDW) shows the heterogeneity of circulating erythrocytes. Large cohort studies have demonstrated a positive correlation of RDW levels with inflammation [8] and infectious diseases such as acute pancreatitis, sepsis and septic shock [9].

In our study, 54 patients diagnosed with CCHF were grouped according to the aforementioned scoring system based upon the first day of admission. We aimed to determine the relationship between RDW, MPV, CPK, ALP, GGT and C-reactive protein (CRP) levels with scoring system and to detect their effect on prognosis.

Table 1: CCHF clinical severity scoring system [7].

Items	Classification	Points
Age	<60 years	0
	≥60 years	1
Haemorrhage	No	0
	Yes	1
Hepatomegaly	No	0
	Yes	1
Organ failure	No	0
	Yes	1
AST	<5 × ULNV	0
	≥5 × ULNV	1
ALT	<ULNV	0
	≥ULNV	1
LDH	<3 × ULNV	0
	≥3 × ULNV	1
WBC	<10,000 cells/μL	0
	≥10,000 cells/μL	1
PT	<3 s	0
	≥3 s, <6 s	1
aPTT	<70	0
	≥70	1
INR	<1.6	0
	≥1.6	1
PLT	≥100,000	0
	≥50,000, <100,000	1
	<50,000	2

ULNV, Upper limit of normal value; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; WBC, white blood cell; PT, prothrombin time; aPTT, activated partial tromboplastine time; INR, international normalised ratio; PLT, platelet.

Materials and methods

We included 54 patients hospitalised at XXX University Faculty of Medicine Infection Diseases and Clinical Microbiology Department from 2011 to 2017 diagnosed with CCHF via RT-PCR or anticors with IgM. The XXX University Non-invasive Clinical Researches Ethical Board approved this study.

Patient information, clinical and physical examination results, laboratory findings, diagnostic methods and results were obtained from patient records and epicrises. Laboratory and clinical findings and physical examinations obtained upon hospital admission were evaluated. Patients were grouped according to the scoring system presented in Table 1. The resulting score is known as the severity grading score, and based on the score, disease was classified as mild (≤4), intermediate (5–8), or severe (≥9) [7]. In addition, RDW, MPV, CPK, ALP, GGT and CRP values were compared amongst the patient groups.

Statistical analysis

Data were analysed using the IBM Statistical Package for Social Sciences v22 (SPSS, Inc., Chicago, IL, USA). Descriptive statistics, such as frequencies or percentages for categorical variables and mean (±standard deviation) and median (min–max) for continuous variables, were used to describe baseline demographic data and clinical characteristics. The variables were investigated using visual (histograms, probability plots) and analytic (Shapiro-Wilk's test) methods to determine whether they were normally distributed. In addition, the differences in variables were analysed using the Kruskal-Wallis (Dunn) tests. The χ^2-test was used to compare the proportions in different groups. p-Values of <0.05 were considered statistically significant for all analysis.

Results

Our patient cohort consisted of 38 (70.4%) male and 16 (29.6%) female patients. Mean age was 44.33 ± 16.94 years. Additionally, 53 (98.1%) patients resided in rural regions of Turkey, and 42 (77.8%) patients had a history of tick contact. Table 2 presents our laboratory results. Patients were divided into three groups based on scoring system mentioned by Bakir et al. (Table 1). From the total, 17 (31.4%), 30 (55.5%) and 7 (12.9%) patients were in group 1, 2 and 3, respectively. Significant differences were detected among the groups with respect to AST, ALT, LDH, aPTT and platelet levels. Significant differences were not detected for age, white blood cell counts or PT and INR levels (Table 3).

GGT and ALP values, not included in the scoring system yet expected to increase during CCHFV infection, were significantly different among the three patient groups (GGT: $p < 0.001$, ALP: $p = 0.020$). There was a statistically significant difference only between groups 1–3 and groups 2–3 for ALP values ($p = 0.014$, $p = 0.007$, respectively). Moreover, there were statistically significant differences among all groups for GGT levels. However, significant differences were not detected in CPK, RDW, MPV and CRP values among the patient groups (Table 4). The mean duration of hospitalisation was 9.76 ± 2.27 days in group 1, 9.73 ± 2.47 days in group 2 and 11.14 ± 3.5 days in group 3. One patient in group 3 died, whereas all other patients in our study were discharged with full recovery.

Table 2: Laboratory results of patients during consultation within upper border of normal values.

Parameter	Median (min–max)	Reference ranges
Hb (g/dL)	14.1 (10.3–20.2)	11.1–17.1
WBC (mm^3)	2.27 (0.63–21.17)	3800–8600
Plt (mm^3)	73 (10–300)	140–360
ALT (U/L)	62 (20–841)	5–40
AST (U/L)	105 (15–1500)	5–40
LDH (U/L)	403 (163–3112)	120–246
CPK (U/L)	384 (41–11,988)	24–195
ALP (U/L)	66.5 (11–267)	30–120
GGT (U/L)	49 (5–881)	0–55
Total bilirubin (mg/dL)	0.5 (0.2–1.7)	0–1.10
Direct bilirubin (mg/dL)	0.2 (0.07–1)	0–0.35
PT (s)	12.3 (10.2–16.2)	10–14
INR	1.05 (0.85–1.59)	0.8–1.20
aPTT (s)	32.37 (20.7–133.3)	21–36
CRP (mg/L)	3.45 (0.2–44)	0–5
MPV (fL)	8.9 (7.40–11.40)	7–9
RDW (%)	13.8 (11.9–17.9)	12–15

Hb, Haemoglobin; WBC, white blood cell; Plt, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CPK, kreatinin fosfokinaz; ALP, alkalen fosfataz; GGT, gama glutamil transferaz; PT, prothrombin time; INR, international normalised ratio; aPTT, aktive parsiyal tromboplastin; CRP, C-reaktif protein; MPV, mean platelet volume; RDW, red cell distribution width.

Discussion

CCHF was the first haemorrhagic fever virus detected in Turkey, with the first known symptomatic case in the country coming from the Tokat province, Kelkit Valley, in 2002 [10]. The disease has immensely spread in recent years, with cases reported all over the country [11]. Most cases are reported in rural areas, either from patients living in such areas or patients who have visited these areas. In our study, all patients except one had an history of living in a rural region. Additionally, 60% of CCHF patients had a previous history of tick bites [12]. In our study, 77.8% of patients had previous exposures to tick bites. However, the absence of contact with ticks in a number of patients as well as the non-specificity of indicators collectively reveal the need for relevant biochemical parameters in early phase diagnosis.

In our study, 38 (70.4%) patients were male and 16 (29.6%) were female. Bakir et al. noted 58.7% male and 41.3% female patients in their research [7]. Higher incidence of disease in males may be because CCHF in endemic regions mainly afflicts shepherds, butchers and slaughter house employees. These occupations are dominated by males.

As aforementioned, leucopoenia and thrombocytopenia are observed in CCHFV infection. In addition, there are

Table 3: Groups based on scoring system.

	Group 1 n = 17 (31.4%)	Group 2 n = 30 (55.5%)	Group 3 n = 7 (12.9%)	p-Values
Age (mean)	39 ± 3.3	45 ± 3.2	52 ± 7.1	p = 0.288
Haemorrhage	2 (11.7)	15 (50)	7 (100)	p < 0.001
Hepatomegaly n (%)	–	1 (3.33)	1 (14.2)	
Organ failure n (%)	–	–	1 (14.2)	
AST (median/min–max)	67 (15–212)	114 (31–1252)	712 (182–1500)	p < 0.001
ALT (median)	35 (20–156)	75 (26–841)	331 (105–627)	p < 0.001
LDH (median/min–max)	285 (163–888)	410 (294–1578)	1271 (650–3112)	p < 0.001
WBC (median/min–max)	2330 (1580–12,320)	1950 (670–21,170)	4110 (630–9030)	p = 0.094
PT (median/min–max)	13.31 (10.8–16.39)	12.37 (10.2–16.2)	14.8 (11.2–17.9)	p = 0.064
aPTT (median/min–max)	31.19 (21.5–71)	31.9 (20.7–133.3)	56.5 (37.5–70.7)	p = 0.002
INR (median/min–max)	1.09 (0.85–1.35)	0.99 (0.85–1.35)	1.19 (0.9–1.5)	p = 0.024
Plt (median/min–max)	119,000 (54,000–300,000)	63,500 (21,000–128,000)	21,000 (10,000–23,000)	p < 0.001

AST, Aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; WBC, white blood cell; PT, protrombin time; aPTT, aktive parsiyal tromboplastin; INR, international normalised ratio; Plt, platelet.

Table 4: Relationship between GGT, ALP, CPK, RDW, MPV and CRP values of the groups.

Parameter (median/min–max)	Group 1 (n = 17)	Group 2 (n = 30)	Group 3 (n = 7)	p-Values
GGT	21 (9–72)	56 (5–233)	129 (55–881)	p < 0.001
ALP	66 (21–172)	59 (11–137)	87 (53–267)	p = 0.020
CPK	216 (41–11,988)	423 (78–4151)	424 (293–1421)	p = 0.171
RDW	14.1 (12.1–15.7)	13.7 (11.9–17.9)	13 (12.4–16.6)	p = 0.550
MPV	8.4 (7.4–9.9)	9.1 (7.8–11.1)	9.4 (7.5–11.4)	p = 0.060
CRP	3.4 (0.2–30.7)	8.6 (2–44.1)	16.1 (10.3–29.4)	p = 0.149

GGT, Gama glutamil transferaz; ALP, alkalen fosfataz; CPK, kreatinin fosfokinaz; RDW, red cell distribution width; MPV, mean platelet volume; CRP, C-reaktif protein.

increased AST, ALT, LDH and CPK levels. Infection also resulted in elongated PTZ, aPTT and other coagulation test times [13]. Numerous studies have stated that when CCHF results in mortality, AST, ALT, LDH, CPK and INR levels are high, and platelet levels are low [4, 14]. Hence, it is critical to assess these parameters, especially in known infectious tick-bite cases. Impaired consciousness, agitation, hepatorenal deficiency, respiratory failure, disseminated intravascular coagulopathy, shock and coma may develop in patients with CCHF, resulting in mortality [15, 16]. Predicting the clinical course before the onset of disease lifesaving [13]; therefore, the development of the CCHF scoring system to achieve such measures is important. Patients are divided into three groups based on disease severity scores as mild, intermediate and severe, with this last group presenting with the worst clinical outcomes [7]. In our research, we also separated patients into these three groups (Table 3). Haemorrhagic symptoms, key prognostic factors affecting the clinical course of disease and AST, ALT, LDH and aPTT values were significantly high and platelet values were significantly low in group 3 ($p < 0.05$). There was no statistically significant difference in leucocyte values in our study. Low leucocyte values are considered as early indicators of poor prognosis in elderly patients with CCHF according to Taşdelen et al. [17]. In our study, the mean age was 44 (min: 15; max: 81), and there was no significant difference detected regarding age ($p > 0.05$).

There is a relationship among platelet activation, MPV value and the inflammatory response [18]. In previous studies, MPV values were shown to be significantly higher in CCHF groups than in control groups, and thrombocytopenia was commonly observed [19, 20]. In our work, we did not detect significant differences in intragroup MPV ratios. ALP and CRP values are known risk factors for CCHF-induced fatality. However, while GGT is detected as higher than reference values in the context of CCHFV infection, it is not considered a risk factor for fatality [4]. In our present work, GGT and ALP levels were significantly high in group 3, the severe disease group.

Although mean CPK levels in groups 2 and 3 were higher than reference range values, there were no significant differences noted (Table 4). The effect of our assessed parameters on mortality was not evaluated given the low rate of mortality in our study. Only one patient in group 3 died due to haemorrhaging. This patient was 81 years old and was referred to our hospital 13 days after the onset of symptoms; therefore, the patient was diagnosed late. On the day of admission, ALP and GGT values were 130 and 145 U/L, respectively.

Numerous studies have strived to shed light on CCHF pathogenesis. Direct or indirect interaction of CCHFV with endothelial and immune cells results in disease onset. Endothelial cell damage, cytokine storm, thrombocytopenia, disseminated intravascular coagulation, haemophagocytosis and hepatocellular necrosis play vital roles in pathogenesis. Hepatocellular necrosis is responsible for increased AST, ALT, ALP and GGT levels.

RDW is correlated with various diseases, highlighting its role in inflammatory intestinal diseases, coronary artery diseases, acute pancreatitis, bacteraemia and sepsis [9]. However, no studies have assessed if RDW affects CCHF pathology and disease. In our study, we did not detect a significant relationship among our patient groups regarding RDW values. Thus, we could not conclude RDW as a critical parameter for CCHF prognosis.

As noted, our country is endemic for CCHF [10]. Predicting the clinical course of the disease is very important for taking necessary precautions. In our research, there were no significant differences for age, white blood cell, PTZ and INR among groups. It was seen that CPK, CRP, MPV, and RDW were not effective parameters to predict clinic course. ALP and GGT values are significantly different among groups. This situation shows that they are useful markers for predicting the clinical course of CCHF. However, low mortality was a limitation of our study, and studies with larger number of patient are warranted to clearly understand the impact of these parameters on CCHF mortality.

Conflict of interest: The authors confirm that this article content has no conflicts of interest.

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