Crimean-Congo hemorrhagic fever-induced liver injury: a systematic review and meta-analysis

Abstract

Background:

Crimean-Congo hemorrhagic fever (CCHF) is a fatal acute tick-borne viral infection and a substantial emerging global public health threat. This illness has a high case fatality rate of up to 40%. The liver is one of the important target organs of the CCHF virus.

Objective:

The aim of this meta-analysis to evaluate the correlation between CCHF and liver injury and draw more generalized inferences about the abnormal serum markers of liver injury such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) in CCHF patients.

Methods:

A literature search was accomplished for published eligible articles with MEDLINE/PubMed and Embase databases. All eligible observational studies and case series were included from around the world. The inclusion criteria were articles describing liver injury biomarkers AST and ALT amongst patients diagnosed with CCHF.

Results:

Data from 18 studies, consisting of 1238 patients with CCHF were included in this meta-analysis. The overall pooled prevalence of at least one raised liver injury biomarker was 77.95% (95% CI, $I^2 = 88.50\%$, $p < 0.0001$). Similarly, pooled prevalence of elevated AST and ALT was 85.92% (95% CI, $I^2 = 85.27\%$, $p < 0.0001$) and 64.30% (95% CI, $I^2 = 88.32\%$, $p < 0.0001$) respectively. Both Egger and Begg-Mazumdar's tests detected no apparent publication bias in all three meta-analyses($p > 0.05$).

Conclusion:

These elevated liver injury biomarkers have been identified as significant prognostic factors. Hence, Physicians must recognize and continuously monitor these biomarkers, since these aid

early stratification of prognosis and the prevention of severe outcomes in infection with such a high case fatality rate.

Abbreviations: CCHF, Crimean-Congo hemorrhagic fever; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PCR, Reverse transcription-polymerase chain reaction

Review criteria

- A literature search was accomplished for published eligible articles with MEDLINE/PubMed and Embase databases.
- The scanning was focused on unique keywords and accomplished for articles published from 1970 through February 20, 2021.
- The study selection and data extraction embodied in the methodology section.

Message for the clinic

- The liver is one of the important target organs of the Crimean-Congo hemorrhagic fever virus
- High proportion of CCHF patients shows elevated liver injury biomarkers such as AST and ALT.
- These elevated liver injury biomarkers have been identified as significant prognostic factors and hence their continuous monitoring is required for early stratification of prognosis and the prevention of severe outcomes.

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is a fatal acute tick-borne viral infection and a substantial emerging global public health threat. The virus is geographically widespread, and CCHF is prevailing in regions of Africa, Asia, Southeastern Europe, and the Middle East.[1,2] However, its true incidence is substantially under-reported, and diagnosis is frequently prolonged. Till now it has affected more than 30 countries around the globe, with most cases being reported from Turkey.[3] Case fatality rate in CCHF often high and ranges from 10% to 40%.[4]

The causative agent behind this illness is the Crimean-Congo hemorrhagic fever virus (CCHFV), a constituent of the Nairovirus genus of the Bunyaviridae family. Virus structure consists of three segments of negative-sense RNA and an RNA-dependent RNA polymerase, It is wrapped inside a lipid envelope which also contains two viral glycoproteins [Gn and Gc].[5] CCHFV, a major tick-borne virus, may spread to human hosts by multiple tracks involving the tick bite or the crushing of bloated ticks, or by percutaneous or per mucosal encounters with viremic body fluids and tissues of humans and animals.[6] A reservoir of this virus has been found in domestic and wild animals including cattle, sheep, goats, hedgehogs, and hares.[7] CCHF virus has been isolated from several tick species, but only a few of them are capable of transmitting disease. Hyalomma species is the most significant tick vector since the virus was isolated from it and it shows the same geographic distribution as the disease.[8] During the acute stage of infection, another transmission route of the virus in humans is by contact with the body fluids of an infected person through the percutaneous or per mucosal route.[9] This is of particular concern to healthcare workers who might get infected when dealing with patients affected by CCHF and this has been associated with several nosocomial outbreaks.[10,11]

The spectrum of clinical presentations in CCHF virus infection ranges from moderate febrile illness with nonspecific symptoms to a serious life-threatening condition characterized by vascular dysfunction, hemorrhage, and multi-organ failure ultimately leading to death. The clinical spectrum of CCHF passes through four stages, incubation, pre-hemorrhagic, hemorrhagic, and convalescence. When infection is acquired by tick bite, incubation time ranges from 1 to 9 days; however, if the infection is contracted from infected tissues or blood, incubation time is 5 to 13 days.[9,12] In the pre-hemorrhagic stage, the temperature of the body may

rise substantially (39–41°C), along with a feeling of chilliness, photophobia, muscle aches, and severe headache.[12,13] The hemorrhagic stage is short lasting about two to three days, has a variety of features, such as petechiae and ecchymoses, and, in some cases, gastrointestinal, urinary, cerebral, and respiratory tract hemorrhages. Approximately after 20 days, the convalescence stage occurs and is distinguished by fatigue, tachycardia, respiratory difficulty, and loss or change of hearing, visual, memory, and other functions.[12,14] Early diagnosis is critical in CCHF cases, and RT-PCR and real-time PCR are first-line methods and currently capable of efficiently diagnosing this illness. Roughly 7 days after infection onset, serological methods such as ELISA and immunofluorescence assays may provide a sensitive and specific diagnosis. And are often employed for diagnosis.[2]

One of the important target organs of the CCHF Virus is the liver and Hepatocyte infection leads to decreased albumin synthesis and abnormal liver function tests and raised level of liver injury biomarker enzymes such as Aspartate Aminotransferase(AST) and Alanine Aminotransferase (ALT).[15] Liver infection is characterized by massive hepatocellular necrosis. These elevated biomarkers of liver injury have been recognized as prominent prognostic factors for poorer outcomes in Crimean-Congo hemorrhagic fever and their raised level is often associated with the severity of the disease.

To date, however, little literature exists regarding the relationship between CCHF and liver dysfunction. To our knowledge, at this time, there is no current systematic review and meta-analysis that compiles the published studies that document liver chemistry in patients with CCHF virus infection. The aim of this meta-analysis to evaluate the correlation between CCHF and liver injury and draw more generalized inferences about the abnormal serum markers of liver injury such as ALT and AST in CCHF patients.

METHODS

The current systematic review and meta-analysis are conveyed out and inscribed in conjunction with Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[16]

Search strategy:

We accompanied a literature quest using MEDLINE/PubMed, and Embase databases for studies published from 1970 through February 20, 2021. We united Medical Subject Headings (MeSH) terms and keyword and subsequent search terms were used [[Crimean–Congo], [Crimean hemorrhagic fever], [Crimean–Congo hemorrhagic fever], [Crimean–Congo hemorrhagic fever AND Liver], [Crimean–Congo hemorrhagic fever AND AST],[Crimean–Congo hemorrhagic fever AND ALT], [Crimean–Congo hemorrhagic fever AND Liver biomarker]]. Studies were included from all around the globe, with no language constraints. For more qualifying studies, we checked the reference lists of the incorporated studies and the relevant literature manually. Duplicate citations were eliminated and all residual articles were examined by their titles and abstracts to appraise eligibility. Figure 1 illustrates the PRISMA flow diagram.

Eligibility Criteria

All eligible observational studies and case series were included for this meta-analysis. To be qualified for this meta-analysis, the article must satisfy the subsequent inclusion criteria: (a) Observational study or case series ; (b) article describes liver injury biomarkers including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) amongst cases diagnosed with Crimean-Congo hemorrhagic fever (CCHF). These studies were incorporated irrespective of age, gender, ethnicity of the included patients.

Exclusion criteria were: (a) less than or equal to three patients, (b) if no data regarding relevant liver functions is given (c) any case reports, letters to the editor, reviews article, commentaries. Following the implementation of these provisions, a thorough interpretation of the residual studies and data extraction were performed in an Excel Table.

Study selection and data extraction:

Three of us (S.S.R.), (M.S.), and (Q.W.) separately reviewed the titles and abstracts of the earlier found articles. Based on the preset eligibility criterion, both authors distinguished studies separately. The conflict was resolved by negotiation and a previous understanding that a third author(A.H.M) would assess the unresolved dispute.

The data extraction for each study was autonomously progressed by five authors (S.S.R, R.P, J.H, I.H., S.T.) and Cross verified to depreciate errors. From each study, several details were retrieved including the First author name, year, the origin country of study, study design, total sample size, Median age, gender (female sex proportion), the proportion of patients with at least one raised liver injury biomarker, the proportion of patients with an elevated level of AST and ALT.

Quality assessment of included studies and Publication bias:

The risk of bias assessment and quality appraisal of included studies was done with help of the Newcastle-Ottawa Scale (NOS).[17] Three of us (A.M.U, N.K.A, D.M.P) independently employed the Newcastle-Ottawa Scale (NOS) for evaluating the individual quality of every study. The following sections were rated per study: low bias risk (8-9 points), moderate bias risk (5-7 points), and high bias risk (0-4 points).NOS is compiled in supplementary table 1. Assessment of publication bias was accompanied with the help of Egger's regression test and Begg-Mazumdar's rank correlation test with $p< 0.05$, as a significant result indicating publication bias.[18,19]

Statistical analysis:

MedCalc® Statistical Software version 19.6.4 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021) was used to conduct statistical analysis. The overall prevalence of abnormal liver injury biomarkers (AST And ALT) according to the laboratory cut-offs specified by each study and relevant 95% confidence interval (CI) was employed to estimate the pooled effects of the studies. Due to expected heterogeneity, review statistics were computed using a random-effects model. Statistical heterogeneity was assessed using Q value and I^2 statistics. For the I^2 statistic, heterogeneity was designated as low (25%-50%), moderate (50%-75%), or high (>75%).

RESULT

Characteristics of the studies:

Preliminary scans in multiple databases generated 1279 studies. Of these, after removing duplicates, 735 studies were assessed. 631 reports were further excluded after taking into consideration the title and abstract, leading to 104 studies for full article review for conceivable consideration in this report. Finally, 18 articles consisting of 1238 Crimean-Congo hemorrhagic

fever-positive patients were included in this meta-analysis based on thorough evaluation and inclusion criteria. The baseline characteristics of the included studies are outlined in Table 1. From these included studies, 12 articles were retrospective study design, two were prospective and two studies were case series. It was additionally noticed that a large portion of these articles (n=12) was from Turkey, nevertheless, two articles were included respectively from India and Iran, and one article each from Pakistan and South Africa. The median age of included patients was 38 years with 40% of them being female.

The Pooled prevalence of various marker of liver injury in CCHF:

At least one elevated liver injury marker:

Overall random effects estimate of at least one elevated liver marker across studies was 77.95% (95% CI, 69.70 to 85.21). Test statistics results revealed high heterogeneity (I^2 = 88.50%, $p < 0.0001$). (Figure 2) This result was pooled from 18 studies which included 1237 patients. This raised liver marker could be serum alanine aminotransferase (ALT) or serum aspartate aminotransferase (AST).

Serum Aspartate Aminotransferase (AST):

Ten studies including 531 patients described outcome data on AST. The pooled prevalence of Aspartate Aminotransferase reported in various studies was 85.92% (95% CI, 74.30 to 94.46). (Figure 3) Test statistics results revealed high heterogeneity (I^2 = 85.27%, $p < 0.0001$).

Serum Alanine Aminotransferase (ALT):

Ten studies including 760 patients described outcome data on ALT. The pooled prevalence of Alanine Aminotransferase reported in various studies was 64.30% (95% CI, 51.44 to 76.19). (Figure 4) Test statistics results revealed high heterogeneity (I^2 = 88.32%, $p < 0.0001$).

Publication bias:

The inverted funnel plot was relatively symmetrical for all three analyses. (Figure 5) Due to associated heterogeneity, evaluation of publication bias was accompanied with the help of Egger's regression test and Begg-Mazumdar's rank correlation test. There was no apparent publication bias detected in the case of all three meta-analyses tested by both Egger's and Begg-Mazumdar's tests ($p > 0.05$). The result of publication bias calculation is illustrated in table 2.

DISCUSSION:

The World Health Organisation has classified Crimean-Congo hemorrhagic fever (CCHF) as a key global disease threat.[4] This virus causes severe viral hemorrhagic fever outbreaks, with a very high case fatality rate of 10–40% and may lead to hospital and medical facility outbreaks, and is troublesome to curb and treat. Despite recent advances in research efforts, CCHF pathophysiology remains recognized to a limited degree, especially in vulnerable groups.[37] Liver seems to be a major target organ affected in CCHF.[12] In spite of this true prevalence of liver injury in CCHF remains unknown. In this meta-analysis, we aimed at determining the true proportion of liver injury present in CCHF patients. For this, we included 18 studies, reporting elevated liver injury markers such as Aspartate Aminotransferase(AST) and Alanine Aminotransferase (ALT).

We found that AST and ALT elevations were a considerable burden in patients with Crimean-Congo hemorrhagic fever. The overall pooled prevalence of elevated at least one liver injury marker, AST, and ALT, was 77.95%, 85.92%, and 64.30%, respectively. It was also perceived that the incidence of raised AST levels was higher than the levels of ALT.

The pathological mechanism behind the liver injury in CCHF patients is poorly understood, although several mechanisms have been speculated. It is believed that the association between the CCHF virus and the host cells is liable for the pathogenicity of the disease. The principal target cells in Crimean-Congo hemorrhagic fever are considered to be the hepatocytes and endothelial cells. Receptor-mediated endocytosis between viral glycoprotein and Virus receptor on the human cell called Nucleolin helps in the entry of CCHF virus into a human cell.[15] Owing to fenestrated liver sinusoids and the lack of a basement membrane, it is smooth for entry of the virus into the basolateral membrane of hepatocytes.[38] Hepatocyte infection leads to decreased albumin synthesis leading to edema, one of the clinical features of the disease, and hepatocyte infection also leads to abnormal liver function tests and raised level of liver injuries marker enzymes such as AST and ALT.[15] The existence of CCHF virus antigen within both hepatocytes and non-parenchymal liver cells has been exhibited by liver immune-histochemistry in CCHF patients.[39] In specific, Kupffer cell hyperplasia and hepatocellular necrosis are peculiarities of liver injury.[41] This has been supported by an in-vitro study on Huh 7 hepatocyte-like cell line in

which ER-stress and apoptosis were seen in this cellular line infected by the CCHF virus.[41] A report from Spain in 2016, performed a necropsy on CCHF patients and noticed massive hepatic necrosis, in the inadequacy of inflammatory infiltrates.[42] A recent mice model study by Lindquist et al. utilized antibodies to disrupt IFN-I signaling in mice to inspected the virus-induced hepatic injury. This study reported extensive apoptosis of hepatocytes and the activation of tumor necrosis factor (TNF) superfamily components on day 4 after exposure.[43] CCHF virus provokes apoptosis of hepatocytes by both intrinsic and extrinsic pathways.[37] Liver damage along with vascular endothelial injury, thrombocytopenia, diminished levels of coagulation factors and disseminated intravascular coagulation (DIC) leads to hemorrhage and subsequently leading to multiple organ failures and ultimately death.

Raised aspartate aminotransferase(AST) and alanine aminotransferase (ALT) and other markers of liver injury have been recognized as prominent prognostic factors for poorer outcomes in Crimean-Congo hemorrhagic fever. Swanepoel et al. (1989) suggested AST level of ≥200 U/L, and ALT level of ≥150 U/L through the first 5 days of the illness was about 90% predictive of a fatal outcome.[14] Yilmaz et al., 2010 reported cut-off values for AST of 117 U/L as independent biochemical markers of the severity of the illness. AST level of ≥117 U/L was associated with 2.95 times greater risk of critical illness.[44] In a multivariate analysis of the laboratory attribute on day 1, an ALT level of > 119.5 U/L distinguished as independent predictors of mortality with an odds ratio of 7.26.[45] Similarly, Ergonul et al. reported that increased level ALT was a predictor of fatality (OR 1.003; 95% CI 1.001-1.005).[46] These laboratory parameters are Indications of liver damage. Abnormal liver injury markers thus assist as a prognostic factor to evaluate the severity of Crimean-Congo hemorrhagic fever disease. From a medical standpoint, based on existing evidence, it is recommended that hospitalized patients with CCHF be routinely evaluated for liver biomarkers, which could be a convenient method for monitoring patients and for early stratification of the prognosis.

There are several strengths of this article. This is the first systematic review and meta-analysis in our knowledge that summarizes the available literature distinguishing the prevalence of abnormal liver biomarkers in Crimean-Congo hemorrhagic fever globally. This systematic study of 18 indexed studies was performed in order to more reliably and accurately quantify the worldwide prevalence of abnormal levels of liver biomarkers in CCHF patients. Nevertheless,

there are some limitations of this meta-analysis that should be considered. Firstly, the largest proportion of the article incorporated in the meta-analysis were retrospective studies in nature, and thus bias in data aggregation is an inherent concern. The results must be viewed with foresight, as the current study didn't consider any potential con founders, such as age, gender, and comorbidities. However, our study may be utilized as a guide for health professionals to use abnormal liver injury biomarkers as prognostic tools for the outcome of the disease and for epidemiologists to proactively make efforts in doing further large-scale studies addressing CCHF associated liver injury.

CONCLUSION

This meta-analysis target to evaluate the correlation between CCHF and liver injury and draw more generalized inferences about the abnormal serum markers of liver injury such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) in CCHF patients. The analysis revealed that elevated level AST and ALT, are common in CCHF virus infection and correlates with hepatocyte damage caused by the infection. These elevated liver injury biomarkers have been identified as significant prognostic factors. Hence, recognization and continuous monitoring of these biomarkers are important for physicians for early stratification of the prognosis and to avoid severe disease and poorer outcomes in disease with such a high case fatality rate.

Author Contributions

All authors contributed equally to the work.

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Table 1: Baseline study demographics and characteristics

Study	**Year**	**Country**	**Study design**	**Sample size(n)**	**Mean age (Years)**	**Female(%)**	**Diagnostic technique**	**Proportion of raised at least one raised biomarker**	**Proportion of raised ALT**	**Proportion of raised AST**
Bakir et al.[20]	2012	Turkey	Retrospective	237	52	40	PCR or Serology	0.47	0.29	N/M
Belet et al.[21]	2014	Turkey	Prospective	54	12.8	37	PCR	0.61	N/M	0.61
Doğan et al.[22]	2017	Turkey	Prospective	40	57	46	PCR	1.00	N/M	1.00
Duran et al.[23]	2013	Turkey	Retrospective	46	47	54.3	PCR	0.76	N/M	N/M
Ergonul et al.[24]	2004	Turkey	Prospective	35	43	51	PCR or Serology	0.86	0.74	0.86
Ertugul et al.[25]	2009	Turkey	Retrospective	26	30.7	42	PCR or Serology	0.77	N/M	N/M
Gozalan et al.[26]	2007	Turkey	Retrospective	29			PCR or Serology	0.79	0.48	0.79

Gozdas et al.[27]	2019	Turkey	Retrospective	31	52.8	61.3	PCR or Serology	0.68	N/M	N/M
Hekimoglu et al.[28]	2016	Turkey	Retrospective	19	54	52.6	PCR or Serology	0.84	N/M	N/M
Jamil et al.[29]	2005	Pakistan	Retrospective	8	26.4	0	PCR	0.66	0.5	0.66
Kara et al.[30]	2016	Turkey	Prospective	9	9.7	11	PCR	1.00	N/M	1.00
Karakecili et al.[3]	2018	Turkey	Retrospective	206	53	49.5	PCR	0.82	N/M	N/M
Kilinic et al.[32]	2016	Turkey	Retrospective	102	46.5	46	PCR or Serology	0.65	N/M	N/M
Mostafavi et al.[33]	2014	Iran	Retrospective	334	21	30	PCR or Serology	0.71	0.63	N/M
Mourya et al.[34]	2017	India	Retrospective	21	38	23	PCR or Serology	0.82	0.83	0.82
Sharif-Mood B et al.[35]	2007	Iran	Case series	6	27	100	PCR	0.66	N/M	N/M
Swanepoel et al.[9]	1986	South Africa	Retrospective	31		29	Serology	1.00	1	1.00
Yadav et al.[36]	2016	India	Case series	4	26	0	PCR or Serology	1.00	1	1.00

Abbreviations- AST: aspartate aminotransferase; ALT: alanine aminotransferase; PCR: Reverse transcription polymerase chain reaction

Table 2: Test results for publication bias

Analysis	**Egger's test**	**Begg's test**
At least one elevated liver injury marker	P = 0.1152	P = 0.3065
Serum Aspartate Aminotransferase	P = 0.1279	P = 0.9287
Serum Alanine Aminotransferase	P = 0.4504	P = 0.4042

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram

Figure 2: Forest plot for at least one raised liver biomarker meta-analysis

Figure 3: Forest plot for raised AST meta-analysis

Figure 4: Forest plot for raised ALT meta-analysis

Figure 5: Funnel plot for publication bias (a) At least one raised liver biomarker (c) Raised Aspartate Aminotransferase (c) Alanine Aminotransferase