

Changes in Plasma Amino Acid Levels in Crimean-Congo Hemorrhagic Fever Patients

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ABSTRACT

Objective: Crimean Congo Hemorrhagic Fever (CCHF) has an important place in viral hemorrhagic fever. Plasma amino acid (AA) levels of patients who were diagnosed with CCHF in the acute and convalescent period of the disease were investigated in this study.

Methods: 35 patients were included in the study specific polymerase chain reaction (PCR) and/or IgM antibody positivity for CCHF virus. AA levels were measured in the plasma derived from the blood samples of the patient and control groups, using liquid chromatography-mass spectrometry (LC-MS/MS) technique.

Results: In our study, we observed that plasma aspartate, glutamate, histidine, leucine, phenylalanine, tyrosine levels increased statistically significantly ($p<0.05$), while some AA levels decreased ($p<0.05$) in acute stage patients compared to the control groups. In addition, while there was an increase in plasma glutamate levels of convalescent patients compared to the control groups ($p<0.05$), there was a significant decrease in other AA levels ($p<0.05$).

Conclusion: Further studies to investigate the relationship between increased or decreased AAs in the plasma levels of these patients and the immune system are likely to contribute to a better understanding of the pathogenesis of the disease and to guide the approach to whether AA supplementation is necessary for treatment.

Keywords: biochemical analysis, Crimean-Congo hemorrhagic fever virus, metabolic analysis, research and analysis methods

INTRODUCTION

Crimean-Congo Hemorrhagic Fever (CCHF) has an important place among the viral hemorrhagic fevers in the world. This disease occurs when infected with the CCHF Virus, which is classified in the Orthonairovirus genus of the Nairovirus

family of the Bunyavirales group [1,2]. CCHF disease has a wide geographical distribution among tick-borne, medically important, viral diseases [2]. The disease is transmitted to humans through the attachment of ticks, unprotected contact with the blood, body fluids or tissues of infected people or

animals [3].

In healthy people, the AA concentration, which is in balance, is disrupted in various pathological conditions and many metabolic pathways are affected [4,5]. The need for AAs changes due to metabolic changes seen in infections or infectious diseases [4].

Currently, there is an increase in viral infections worldwide. CCHF disease is a viral infection that increases exponentially every year. It has been known for a long time that plasma AA levels are affected by the increase in catabolic and anabolic reactions in many diseases, especially infections [6,7]. In a study conducted with CCHF patients, Aydin and colleagues. showed that plasma glutamine levels decreased in patients [8]. Due to the relationship between AA level and distribution, infectious diseases, and immune response, we investigated the levels of different AAs in the plasma of CCHF patients in the acute and convalescent period in this study.

MATERIALS AND METHODS

A total of 70 people, 35 of whom were patients and 35 of whom were healthy individuals, were included in this study. The patient group consisted of adult patients who were followed up in the Infectious Diseases and Clinical Microbiology Service of Cumhuriyet University Medical Faculty Research Hospital with the diagnosis of CCHF and who wanted to participate in the study. The diagnosis of CCHF was made by detecting CCHF-specific polymerase chain reaction (PCR) and/or CCHF IgM antibody positivity in the blood of the patients. Approximately 2 ml of venous blood was taken from the patients (in the acute

and convalescent periods) and the control group into hemogram tubes with EDTA. Blood samples were taken on the first day of hospitalization for the acute stage patient group and on the day of discharge for the convalescent stage patient group. A control group was formed from 35 volunteers who did not have any disease with similar characteristics to the patient group in terms of age and gender. For this study, permission was obtained from the *Ethics Committee of Sivas Cumhuriyet University Faculty of Medicine* (dated 02/04/2019 and numbered 2019-04/02). Informed consent forms were read to the patients and healthy volunteers and their signatures were obtained.

Exclusion Criteria

Alcohol and substance use, autoimmune diseases and those with unusual eating habits (for example, those who eliminate certain food groups from the diet) were not included in our study. In addition, those with any acute or chronic disease (diabetes mellitus, hypertension, chronic renal failure, malignancy, hematological disorders, etc.) in the patient group, except CCHF disease, were not included in our study.

Amino Acid Analysis

The blood samples were taken with a centrifuge device at 4°C and 4000 rpm for 10 min. it was centrifuged. The obtained plasma was stored at -80°C until the eppendorf tubes were portioned and the analyzes were performed. When the number of patients required for the study was reached, AA levels were measured at once after all samples were brought to room temperature. Quantitative AA analysis kit was used with the LC-MS/MS device.

Statistical Analysis

Statistical analysis was performed by loading the data obtained in the study into the SPSS (version 23.0) program. Descriptive statistics for continuous variables in our study; mean, standard deviation, minimum and maximum; Categorical variables were expressed as numbers and percentages. Shapiro-Wilk ($n < 50$) normality test was applied to numerical variables. Parametric tests were applied because the measurements were normally distributed as a result of the analysis. Independent T-test and F (ANOVA) were used to compare measurements according to patient groups. In our study, Wilcoxon Sign Test was used to compare two-category variables in groups with a correlation. Chi-square test was used to determine the relationship between categorical variables. The statistical significance level (α) was taken as 5% in the calculations.

Main Points;

- In the article, the importance of plasma amino acid values in CCHF disease was discussed.
- In this study, we found that the plasma levels of some AAs decreased and some increased in CCHF patients during the acute and recovery periods.
- It is likely that it will contribute to future studies between increasing or decreasing plasma AA levels and the immune system.
- The article will contribute to a better understanding of the pathogenesis of the disease and will provide information for further research on whether AA supplementation is necessary for treatment.

RESULTS

In the study, the mean age of the patients was 44.00 ± 20.171 (17-80 years), while the healthy volunteers were 44.60 ± 12.793 (19-68 years). While 22 (63%) of the patient group were male and 13 (37%) were female, 23 (63%) of the control group were male and 12 (37%) were female. There was no significant difference between the patient and healthy individuals in terms of age and gender ($p > 0.05$).

The average weight of the study group; was measured as 67.03 ± 9.87 (50-88 kg) in the patient group in the acute period, and 66.11 ± 10.09 (49-86 kg) in the patient group in the convalescent period. The amount of protein consumed daily was calculated based on the National Food Composition Database [9], the Turkish Ministry of Health Nutrition Guide 2015 (TUBER) [10], and the Bebis 7.1 program used in the Nutrition and Dietetics Department Research and Practice Hospital. Daily protein consumption was calculated as 55.97 ± 8.63 gr in the control group, 39.12 ± 12.29 gr in the acute period patient group, and 43.26 ± 9.59 gr in the convalescent patient group (Table 1). The dietary habits of the patients before hospitalization were also questioned, and the average daily protein intake was calculated. The mean hospital stay of the patients was calculated as 7.69 ± 1.92 days. The average amount of protein consumed daily in the patient and control groups is given in Table 1.

In this study, plasma AA aspartate, glutamate, histidine, leucine, phenylalanine, and tyrosine levels were significantly increased ($p < 0.05$) in the acute period patient group compared to the control group, and the decrease in arginine, glutamine, proline and tryptophan levels was significant. ($p < 0.05$). While an increase was observed in plasma glutamate levels in the convalescent group compared to the control group ($p < 0.05$), the decrease in alanine, glutamine, asparagine, histidine, arginine, serine, tryptophan, tyrosine, methionine, and valine levels was statistically significant ($p < 0.05$).

DISCUSSION

AAs are supplied from two sources, exogenous and endogenous. In a healthy person who is fed regularly, plasma AA concentration is in the balance depending on anabolic and catabolic reactions. However, this imbalance due to various pathological conditions affects metabolic pathways. Changes in metabolic pathways affect morbidity and mortality, especially immune response [4,5]. In infectious diseases, the effect of plasma AA levels of patients may be a clinical indicator of the magnitude of the immune response to the pathogen [6].

Exogenous sources are one of the most important factors determining the plasma level of branched-chain AAs (BCAA), which include leucine, isoleucine and valine AAs [11]. In our study, we found that the daily protein consumption of the acute period patient group was lower compared to the pre-disease period and the control group (table 1). Plasma levels of BCAAs were highest in the acute period ($684,68$), followed by the control group ($667,66$), followed by the convalescent patient group ($589,44$). The difference between the groups was found to be statistically significant ($p = 8,57 \times 10^{-30}$) (table 2). Although the daily protein consumption of the acute group was lower than that of the control and convalescent groups, the high plasma BCAA level in the acute group may be of endogenous origin due to the increase in catabolic reactions at the onset of the disease. The decrease in the convalescent period may have developed due to the decrease in the catabolic/anabolic reaction rate and its use as an energy source. Because studies have reported that anabolic reactions increase during the recovery period of viral and bacterial infections [7], and BCAAs are used as an energy source by the muscles during fasting [11,12]. The amino group released as a result of BCAA catabolism in skeletal muscle is converted to alanine if it gives pyruvate, to glutamate if it gives α -keto glutarate, and to glutamine if it gives glutamate and is given to the peripheral circulation [11,13]. In our study, we found the difference between plasma alanine levels ($p = 0.0037$)

Table 1. Daily Average Protein consumption (gr)

	Mean(gr) \pm SD	Min.-Maks.	p
Control Group (n=35)	55,97 \pm 8,64	36,0-72,0	1,07x10 ⁻⁹
Acute Period Patient (n=35)	39,13 \pm 12,29	16,1-56,4	
Convalescent Period Patient (n=35)	43,26 \pm 9,59	20,6-58,3	
Before Illness (n=35)	47,17 \pm 08,32	28,0-60,0	

and glutamate levels ($p=2,60 \times 10^{-18}$) statistically significant. When the groups are compared in pairs; There was an increase in plasma alanine ($p>0.05$) and glutamate ($p<0.05$) levels in the acute patient group compared to the control group, and the plasma glutamate level of the convalescent group increased compared to the control group ($p<0.05$), but the alanine level was lower ($p<0.05$). Plasma glutamine levels were highest in healthy subjects; When compared to the control group, a statistically significant decrease was observed in both the acute and convalescent patient groups (Table 2,3). Aydin et al. suggested that the plasma level of glutamine decreased due to the increase in the use of glutamine in CCHF patients [8]. Increases in alanine and glutamate levels may be due to BCAA catabolism as mentioned above. However, no increase in glutamine level; It may be due to excessive catabolism of BCAAs and increased use of glutamine. In a study of patients with sepsis, it was suggested that plasma alanine levels increased, which was related to BCAA metabolism [14]. This study supports our opinion.

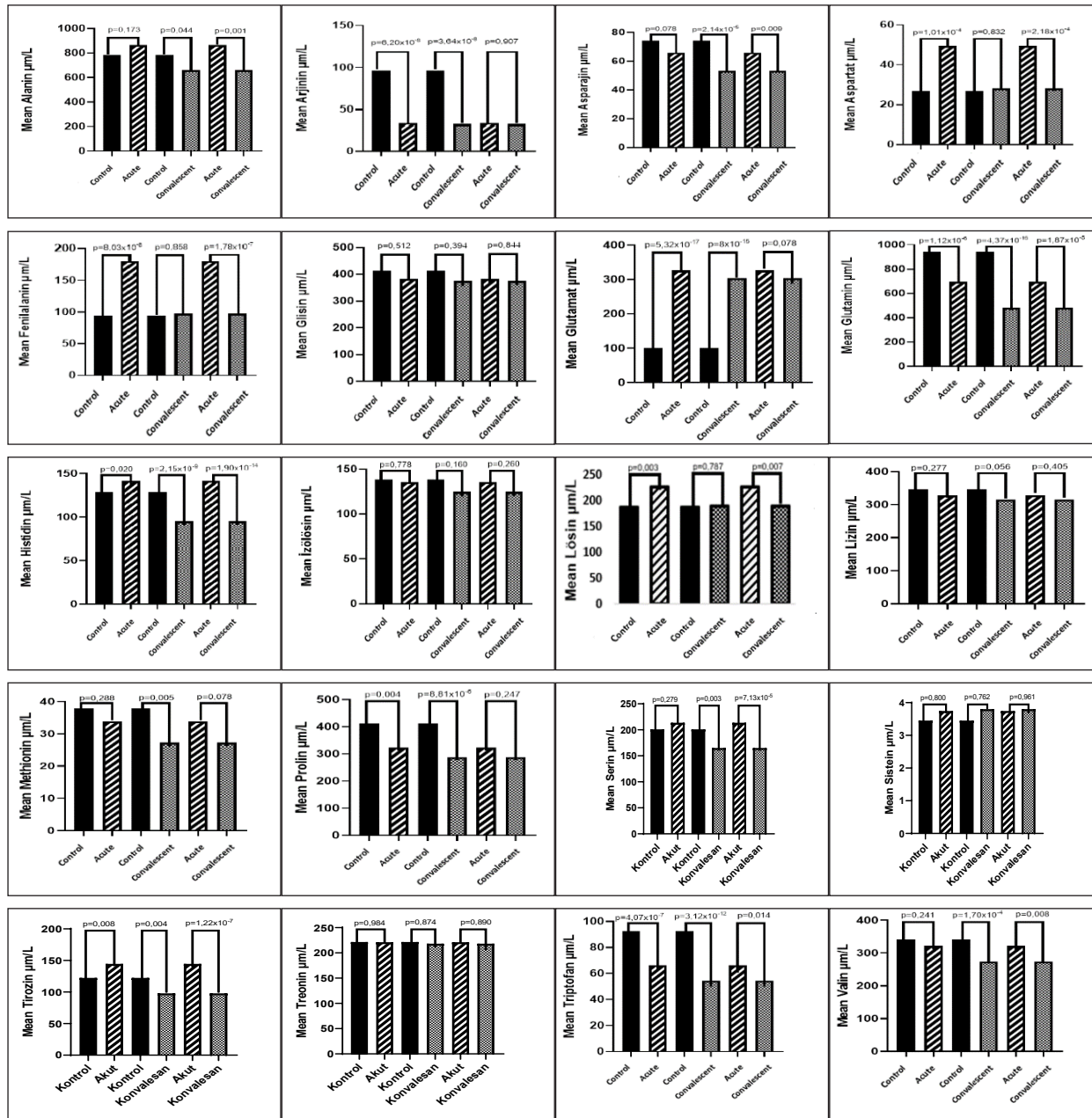
Aspartate and glutamate are non-essential AAs that play an important role in the metabolism and function of leukocytes. The AA aspartate contributes to the synthesis of purine and pyrimidine nucleotides by the proliferation of lymphocytes [15] and to the immune response with glutamate [16]. In addition to their role in leukocyte metabolism, these AAs are excitatory neurotransmitters in the central and peripheral nervous systems [15]. Glutamate is a substrate for GABA synthesis in both lymphocytes [17] and macrophages [18]. As a precursor of glutathione synthesis, it plays a role in the removal of oxidants and thus in the regulation of the immune response [19]. It has been reported that while plasma aspartate level increases in some viral diseases, it decreases in others [20,21,22]. We found that plasma aspartate levels of CCHF patients in the acute phase were significantly increased compared to healthy volunteers. We did not detect any difference between plasma aspartate levels in patients with convalescent periods and individuals in the control

Table 2. Plasma AA Levels of Control Group and CCHF Patients (Acute and Convalescent).

Amino acid	Control (µmol/L)			Acute (µmol/L)			Convalescent (µmol/L)			p
	Mean	SD±	Min.-Maks.	Mean	SD±	Min.-Maks.	Mean	SD±	Min.-Maks.	
Ala*	782,84	33,94	325-1144	864,38	51,44	331-2126	661,61	38,62	242-1212	0,0037
Arg*	96,3	10,18	17-302	33,71	6,63	7-190	32,45	4,95	3-135	$4,89 \times 10^{-9}$
Asn*	73,83	2,89	44-108	65,94	3,86	16-144	53,41	3,11	10-95	$1,43 \times 10^{-4}$
Asp*	26,99	2,42	10-84	49,46	5,76	8-185	26,16	2,69	9-64	$7,95 \times 10^{-5}$
Phe*	94,65	2,25	69-124	179,89	17,30	83-614	97,30	4,60	59-190	$1,47 \times 10^{-8}$
Glu*	99,35	4,27	61-148	326,69	19,46	198-589	304,50	19,11	140-607	$2,60 \times 10^{-18}$
Gln*	939,63	23,45	655-1205	695,03	37,90	281-1162	482,99	36,85	65-902	$3,72 \times 10^{-15}$
Gly	412,41	21,32	197-733	383,37	32,42	20-1151	374,65	37,61	17-1252	0,670
His*	129,11	3,37	97-176	141,19	4,05	92-182	95,44	3,40	60-126	$3,16 \times 10^{-14}$
İle	138,60	7,77	83-319	135,82	6,97	59-245	124,71	5,93	63-224	0,330
Leu*	186,99	8,03	119-308	227,32	10,46	113-368	190,61	9,6	109-329	$4,49 \times 10^{-3}$
Lys	346,43	11,83	214-519	328,67	9,92	183-462	315,06	12,57	209-481	0,159
Met*	37,83	1,57	21-60	33,88	3,64	0,5-87	27,29	2,19	6-51	0,019
Pro*	412,83	25,67	168-781	323,62	18,25	117-562	288,10	20,16	134-584	$0,28 \times 10^{-4}$
Ser*	200,92	8,97	114-287	213,69	9,57	119-385	165,10	5,87	121-246	$2,08 \times 10^{-4}$
Cys	3,45	0,97	0-25	3,74	0,67	0-21	3,80	0,78	0-18	0,94
Tyr*	122,35	4,35	75-173	144,50	7,52	81-243	98	4,97	60-211	$7,76 \times 10^{-7}$
Thr	251,58	19,28	9-410	221,16	11,63	90-396	218,23	12,69	14-406	0,985
Trp*	92,40	3,21	46-128	66,44	4,14	17-123	54,46	2,64	24-82	$1,04 \times 10^{-11}$
Val*	342,07	12,98	223-567	321,54	13,04	184-525	274,12	10,77	191-436	0,001
BCAA*	667,66	27,83	466-1186	684,68	28,94	357-1075	589,44	9,60	109-329	$8,57 \times 10^{-30}$

* $p<0,05$

Table 3. Pairwise Comparison of Plasma AA Levels of Groups.



group (Table 3).

Although glutamin (Gln) and alanine constitute only 6-8% of the structural muscle protein, Gln and alanine constitute 70% of the AAs released by skeletal muscle during stress and sepsis [23]. Studies have reported that the plasma Gln level of HIV, Dengue Fever, and CCHF patients is decreased [8,21,22]. In another study, it was shown that oxidative stress increased in CCHF patients [24]. During stress and sepsis, serum and intracellular Gln concentration decrease, and under these conditions it becomes an essential AA [23]. In addition, Gln is an important energy source for immune system cells [25] and is very important for the synthesis of glutathione [26] and

nucleotides (purine and pyrimidine) [27]. In our study, we found that plasma Gln level was statistically significantly lower in acute (p=1,12x10⁻⁶) and convalescent period (p=4,37x10⁻¹⁶) patients when compared to the control group consisting of healthy individuals. When we look at these results, it is seen that our results are compatible with the studies mentioned above. We thought that the endogenous synthesis of glutamine increased in our patient group, but the low plasma level could be due to oxidative stress, purine, pyrimine synthesis, and the glutaminolysis pathway.

Asparagine (Asn) is a non-essential AA synthesized from

aspartate and Gln by the ATP-dependent asparagine synthase enzyme. Petra et al. reported a decrease in plasma asparagine level in Dengue Fever [22]. In our study, plasma asparagine level in the acute period patient group was compared with the control group and the convalescent period; accordingly, while the decrease in acute period patients was not significant compared to the control group ($p=0.078$), it was significant compared to the convalescent period patients ($p<0.01$). When the plasma asparagine level in the convalescent period patients was compared with the control group; The decrease in patients with the convalescent period was found to be statistically significant ($p=2,14 \times 10^{-5}$). While many non-essential AAs require intermediates of Gln metabolism for de-novo synthesis, Asn is dependent only on Gln [28]. It is possible that the low level of Asn is due to the decrease in the level of plasma Gln. It is also possible that the plasma Asn level is due to decreased activity of the enzyme. Because the enzyme Asparagine synthetase, which is ATP dependent, is the only enzyme that catalyzes the biosynthesis of Asn [28]. The need for ATP increases in viral diseases [29]. Therefore, the decrease in the Asn level may also have resulted from the energy deficit.

In the Wannemacher study, it was reported that plasma tryptophan and phenylalanine (Phe) levels increase in bacterial or viral infections [6]. Tryptophan, the precursor of serotonin and melatonin, is an essential AA [30]. It has been suggested that tryptophan metabolites affect both innate and acquired immunity [31]. Petra et al. reported that plasma tryptophan levels decreased in dengue fever [22]. Hortin and colleagues showed that there was a significant decrease in plasma tryptophan concentration in patients with HIV [32]. In our study, plasma tryptophan levels in CCHF patients in the acute phase of the disease were decreased compared to the control group ($p<0.05$). The results of Petra and colleagues [22] and Hortin and colleagues [32] are similar to our study results. Hortin et al. attributed the decrease in tryptophan to the response to HIV infection [32]. A similar situation is possible for CCHF patients. In other words, the low plasma tryptophan level we detected in CCHF patients may be related to the immune response of the host against infection.

The change in plasma AAs seen in infections is markedly different from the change seen in fasting. In many studies, it has been shown that plasma phenylalanine (Phe) level is affected in the presence of infection. Wannemacher et al. [6] reported increased levels of Phe in bacterial or viral infections, Yang et al. [20], in acute and chronic hepatitis, and Petra et al.

[22] in dengue fever. Freund et al. reported that it increased significantly in patients with sepsis, and this rate decreased in patients who recovered [14]. Ziegler et al., in their study, found the plasma phenylalanine amount in HIV-infected individuals to be significantly lower than in healthy individuals [21]. In our study, plasma Phe levels in the control group and patients in the convalescent period were similar. However, plasma Phe levels in acute CCHF patients were increased by compared to the control group ($p=8,03 \times 10^{-8}$). In addition, in our patient group, we found that the plasma Phe level in the convalescent period decreased compared to the acute period. In the studies mentioned above, it has been suggested that the increase in plasma phenylalanine level may be due to liver failure, muscle mass proteolysis, and changes in the activity of phenylalanine hydroxylase/dihydropteridine reductase enzymes. Powanda et al. suggested that phenylalanine hydroxylase activity decreased in mice infected with tularemia [33], while Wannemacher suggested that liver phenylalanine hydroxylase activity did not change in mice with pneumonia [6]. While our study results are consistent with the studies mentioned above [6,20,22], they show full parallelism with the study of Freund et al. [14] We did not look at muscle mass proteolysis and changes in the activity of phenylalanine hydroxylase/dihydropteridine reductase enzymes in CCHF patients, as this was not within the scope of our study. Further studies investigating how the expression or activity of phenylalanine hydroxylase/dihydropteridine reductase enzymes are affected in CCHF patients are likely to contribute to a better understanding of the pathogenesis of the disease.

Tyrosine ceases to be essential in the presence of sufficient phenylalanine in the organism. In the fasted state, only a small part of phenylalanine participates in tyrosine synthesis, while the majority participates in protein synthesis [6]. Tyrosine is the precursor of many biomolecules (catecholamines and thyroid hormones) [30]. Hortin and colleagues [32] and Ziegler and colleagues reported a decrease in plasma tyrosine levels in HIV [21], and Perte and colleagues in Dengue Fever [22]. Yang and colleagues found that plasma tyrosine levels increase in acute hepatitis, and increase in chronic hepatitis in proportion to the severity of the disease (moderate, severe) [20]. Freund and colleagues reported that plasma tyrosine levels increased in patients with sepsis, and this rate decreased in survivors after treatment [14]. In our study, we found that plasma Tyr levels in CCHF patients increased in the acute period compared to the healthy group ($p=0.008$), and decreased significantly ($p=0.004$) in patients who were discharged after treatment. While our

results were consistent with the study results of Yang and Freund's colleagues, they were not compatible with the results of other researchers.

Glycine is required for many metabolic pathways such as glutathione synthesis and purine nucleotides [34, 35]. Although it is generally known as a nonessential AA, it is also called a conditionally essential AA because it can be synthesized endogenously in a certain amount [32]. It is also a powerful antioxidant that scavenges free radicals [36]. Hortin and colleagues reported an increase in plasma glycine value in HIV [32]. Yang's team reported that plasma glycine levels decreased in acute and chronic hepatitis [20], and Petra in Dengue [22]. Ziegler and colleagues reported that there was no significant change in HIV [21]. In our study, acute and convalescent plasma glycine levels were decreased in our patient group compared to the control group, but this decrease was not statistically significant ($p>0.05$). The plasma glycine level in our study was in line with the results of Petra and Yang and their team, while not in agreement with the results of Hortin. It has been known for a long time that replication and oxidative stress increase in viral infection. Various studies have shown that oxidative stress is increased in CCHF patients and the antioxidant system is activated to prevent oxidative damage [24]. It is possible that the decrease in plasma glycine levels of CCHF patients is due to the activation of these metabolic pathways.

Proline and hydroxyproline make up one-third of the AAs in collagen, and they are important for the immune system [37,38]. In studies, it has been reported that plasma proline levels decrease in acute/chronic hepatitis [20] and HIV [21]. In our study, a significant decrease ($p<0.01$) was found in plasma proline levels in the patient group (in the acute and convalescent period) compared to the control group. However, no significant difference was found between the acute and convalescent patient groups ($p=0.247$). Our study results are similar to those mentioned above. Considering the increase in plasma glutamate level in the acute and convalescent period; It can be thought that proline is actively used to support immunity in the acute and convalescent period and the proline-P5C cycle is maintained in the direction of catabolisation. However, since the effect of this cycle on CCHF patients is not fully known, more detailed studies by looking at the concentrations of intermediates in this pathway will contribute to a better understanding of the pathogenesis of the disease. Collagen, in which proline is located, is an important structure that protects vascular structures. Damage

to vascular structures may occur due to decreased collagen synthesis as a result of proline deficiency [38]. It is known that the main pathology in CCHF patients is endothelial damage. In our study, it is possible that the plasma proline level, which we found lower in CCHF patients compared to the control group, may contribute to the endothelial damage in the patients. Of course, further studies on this subject will be useful for our better understanding of the pathogenesis of the disease.

One of the important metabolic pathways in which arginine participates is nitric oxide (NO) synthesis. NO plays an important role in the immune response [39]. Freund et al. While in sepsis [14], Hortin reported a decrease in arginine level in HIV [32], Ziegler and colleagues reported no significant change in HIV [21]. In our study, we found a statistically significant decrease ($p<0.01$) in plasma arginine levels of CCHF patients in the acute and convalescent periods compared to the control group. From endogenous arginine sources; Despite the significant increase in glutamate ($p<0.01$), we saw a decrease in the amount of arginine; It may be due to the increased response to infectious pathogens in the acute and convalescent period in CCHF patients.

Plasma contains glycoproteins rich in histidine [40]. Hortin reported a statistically insignificant increase in plasma histidine levels in HIV [32]. Ziegler did not detect any changes [21]. Yang reported a significant decrease in plasma histidine levels in acute and chronic hepatitis [20], while Petra reported a significant decrease in dengue fever [22]. In our study, a statistically significant increase ($p=0.02$) in plasma histidine levels was observed in acute CCHF patients, while a decrease was found in the convalescent period ($p=2,15 \times 10^{-9}$). In addition, histidine can be converted to histamine under the influence of histidine decarboxylase [38]. In our study, in CCHF patients, the amount of protein consumed during the convalescent phase of the disease was higher than in the acute phase. Despite the increased protein consumption in the convalescent period, the decrease in plasma histidine amount may be due to the increase in histamine production to support the immune system. Further studies are needed for the detailed regulation of the histidine-histamine pathway and a clearer understanding of its role in CCHF.

Serine is a non-essential AA. There are many ways in which the series is used; these; are purine and pyrimidine, ethanolamine, ceramide, and phosphatidylserine synthesis [4,34]. Hortin found that plasma serine level was increased in HIV [32], and Yang

in acute and chronic hepatitis [20]. However, Petra reported that plasma serine levels decreased in Dengue Fever [22]. In our study, we found that plasma serine levels increased in the acute phase ($p=0.279$) and decreased in the convalescent period ($p=0.003$) in CCHF patients. While our results were consistent with the results of HIV and hepatitis patients in the studies mentioned above, they were not compatible with those of Dengue Fever patients.

While more AAs increased in the acute period in CCHF patient plasmas, it was observed that they decreased more in the convalescent period. We thought that this may be due to the increase in catabolic reactions in the acute period and the increase in anabolic reactions in the convalescent period. It is thought that AAs that decrease in the acute period are used in cellular and humoral immune responses (such as cytokine, interleukin, acute phase reactant synthesis, purine, and pyrimidine synthesis). Since AAs participate in many metabolic pathways, it is difficult to discuss them individually. In this study, we determined the plasma AA profile of CCHF patients in the acute and convalescent periods.

As seen above, there is no consensus on the effects of AA supplementation on disease prognosis, pathophysiology, and mortality in many diseases. Since there were no patients who died in our study, we could not discuss the effect of plasma AA concentrations on mortality. In this study, we determined the increased/decreased plasma AA levels of CCHF patients and discussed their possible effects on metabolic pathways and the immune system. One of the weaknesses of our study is that we did not look at the levels of AAs and metabolites in the urine of the patients. However, it is an important study in terms of investigating the plasma levels of many AAs in the acute and convalescent period in CCHF patients. Considering our study results, there is a need for more comprehensive studies planned for this purpose to interpret the effect of AA supplementation on metabolic pathways, immune response, and mortality in CCHF patients.

It is known that AAs play an important role in immune response and the protection of energy metabolism. Protein loss occurs in the body due to catabolism caused by infection, and this loss can be balanced by increasing protein intake during recovery. Malnutrition can weaken the body's response to infection. While many studies have recently been found suggesting that protein/AA supplementation may help in the treatment of infectious

diseases [7,12,14,21,35], studies showing which metabolic problems will result from excessive intake of protein/AAs are scarcely any.

CONCLUSION

In this study we did, we found that the plasma levels of some AAs decreased and some increased during the acute and convalescent period in CCHF patients. The differences we detected in plasma levels of AAs are likely to be due to different metabolic pathways during the acute and convalescent phases of the disease. Further studies, which will be planned to investigate the relationship between AAs with increasing or decreasing plasma levels and the immune system, will contribute to a better understanding of the pathogenesis of the disease and provide information on whether AA supplementation is necessary in terms of treatment.

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Author Contributions: ZE: Constructing the hypothesis and idea of research, interpretation of data, drafting of the manuscript, research of the literature, Collecting of patients and controls data. HA: Drafting of the manuscript, research of the literature, reviewing the article before submission scientifically. AE: Reviewing the article before submission scientifically, analysis and interpretation of the data, research of the literature. Authors have agreed on the final version of the manuscript for publication. Availability of data: All data and materials regarding to this manuscript are available from ZE.

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