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Original Article

The Effect of Fetuin-A and Other Laboratory Paremeters on Prognosis and Mortality in Crimean-Congo Hemorrhagic Fever

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Abstract

Background: Crimean-Congo Hemorrhagic Fever (CCHF) is a disease with high morbidity and mortality, which is an important health problem in the world. Therefore, we aimed to evaluate the effects of plasma fetuin-A (FA) level, which is a new parameter in terms of prognosis and mortality of CCHF.

Methods: A total of 87 patients were included who presented to the Emergency Department, Bağcılar Training and Research Hospital, İstanbul, Turkey with the diagnosis of CCHF from Feb 1, 2019 to Feb 1, 2020. The patients were divided into three groups as tick bite, contact history, and endemic area travel according to the transmission type, and two groups according to the presence of mortality or not. The laboratory data of the patients were compared within these groups. Relationship of hemogram, C-reactive protein (CRP), D-Dimer, sedimentation, lactate, and FA levels between groups were evaluated.

Results: The average age of the patients was 62.52±14.94 years and 27(31%) of them were women. Mortality rates were in 6(6.9%) patients from endemic areas (P=0.015). While the FA level of the mortality group was 171.6±30.0 mg/L, it was 230.3±25.0 mg/L in the survivors (P=0.001). There was a moderate and strong negative correlation of FA level with mortality, tick history, and hospitalization. In ROC curve analysis of mortality and FA levels, parameters were determined as sensitivity 97.4% and specificity 96.2%.

Conclusion: In addition to FA levels, as anticipated by our hypothesis, lactate, CRP, and sedimentation values can be used to predict prognosis and mortality in cases of CCHF.

Keywords: Crimean Congo hemorrhagic fever; Emergency department; Mortality; Fetuin-A; Lactate

Introduction

Crimean Congo Hemorrhagic Fever (CCHF) is a disease of viral origin and its causative agent is included in Orthonairovirus species of the Bunyaviridae family. It is a negative-polar, enveloped, single-stranded ribonucleic acid (RNA) virus. The disease is usually transmitted through the attachment of the tick that carries the virus. The most common tick type is *Hyalomma marginatum*. Also, it can be transmitted from degraded skin or mucous membranes, by contact with blood, body fluids, and tissues of people and animals with viremia, and by appropriate non-sterilized medical equipment (1-3). It can be mortal with hemorrhagic and toxic syndromes and its mortality can vary between 10-40% (4).

Symptomatic CCHF disease has four stages: the incubation stage, the prehemorrhagic stage, the hemorrhagic stage, and the recovery stage (3). Symptoms start suddenly before hemorrhagic findings and can be confused with other bacterial and viral infections. There may be signs and symptoms such as fever, chills, muscle pain, headache, mood disorders, photophobia, sore throat, neck stiffness, lymphadenopathy and nausea, vomiting, abdominal pain, diarrhea (3,5). The pre-bleeding disease can be misdiagnosed or overlooked. In advanced stages, bleeding can be seen in the gastrointestinal or genitourinary system as petechiae, ecchymosis, and mucosal hemorrhage. While 30% of cases result in death, usually in the second week of the disease, recovery usually begins after 9-10 days among survivors (5). Specific viral isolation, detection of specific antibodies, and detection of the viral genome or antigen are used for the diagnosis. Apart from these, especially neutropenia, leukopenia, thrombocytopenia, and increased alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase levels have also been reported (6).
The main part of the treatment is the supporting part. Ribavirin has been given to many patients with CCHF, although its efficacy is uncertain. In addition, methods such as high-dose steroid administration, plasma transfusion from patients in a recovery period, intravenous immunoglobulin administration, and plasma exchange are included, but there is no clear data on their efficacy (7,8).
Vaccine and new parameter studies are carried out for CCHF patients. Plasma fetuin-A (FA) protein has been used for this reason recently. Fetuin was first obtained from fetal bovine serum by purification in 1944 and is the main plasma protein of the fetus (9). It is secreted from many organs such as the liver, brain, and gastrointestinal system during fetal development. In adults, the liver is the main secretion site. Being a positive or negative acute-phase protein, FA regulates against inflammatory responses that occur with injury and infections (10-12). Laboratory data, which are as important as clinically in patients, are very effective in early evaluation, diagnosis, and prediction of prognosis.
In our study, we aimed to evaluate the effects of FA level on morbidity and mortality in CCHF cases.

Materials and Methods

Study Design and Population

A total of 87 patients were included who presented to the Emergency Department, Bağcılar Training and Research Hospital, İstanbul, Turkey with the diagnosis of CCHF from Feb 1, 2019 to Feb 1, 2020.
Demographic data, tick contact histories, laboratory values, diagnostic information, hospitalization and survival status of the cases were obtained from the hospital data recording system. The hospital is a tertiary education and research hospital and its data recording system is secure.
Cases with complete data in terms of demographic, laboratory, clinical, hospitalization or mortality in the data processing system were included in the study. Patients with a tick bite, contact history, and CCHF clinical symptoms and indicators from an endemic area, over the age of 18 yr were included in the study, as were patients with positive CCHF IgM and RT-PCR tests. Cases, under the age of 18 yr and, that did not have enough information in any category were taken out of the study. Also, the study excluded patients with known heart valve diseases, arrhythmia, and bypass surgery at admission, patients taking anticoagulants for cerebrovascular disease, patients with chronic liver disease, patients receiving dialysis for chronic renal failure, and who had recently received erythrocyte suspension in last six months. The study also eliminated patients whose real-time polymerase chain reaction (RT-PCR) and CCHF IgM results reported as negative.
For the purpose of determining how the cases were exposed to the disease agent, patients diagnosed with CCHF were divided into three groups for according to their anamnesis: tick bite, contact history, and endemic area. "Tick bite" cases

with direct contact with ticks, "contact history" cases that are related to meat, milk, and livestock, have contact with the CCHF patient, and direct tick contact is not reported, and "endemic area" cases with a history of travel and lodging in endemic areas according to the CCHF and who did not report direct contact were grouped as cases. Besides, all patients diagnosed with CCHF were hospitalized, and patients were divided into two groups according to survival or mortality.

The study was conducted in accordance with the Helsinki Declaration on Human Research after obtaining Institutional Local Ethics Approval (Decision No:2020-02/18). After all, patients were informed, their consent was obtained for inclusion in the study.

Laboratory Design

Blood samples were taken for hemogram, biochemistry, sedimentation, lactate, D-Dimer, C-reactive protein levels in the emergency department application of the patients who come with suspicion about the related diagnosis. They were studied at the time of admission to the emergency department, resulting in 45-90 minutes. Real-time polymerase chain reaction (RT-PCR) and CCHF IgM values were the tests planned to confirm the diagnosis. FA levels were determined from the serum of the patients with positive CCHF IgM and RT-PCR results, which were centrifuged and stored at -80 ^{0}C.

A Beckman Coulter Automated CBC Analyzer was used to evaluate the hemogram. Biochemistry values were checked with the Cobas 6000 device. D-Dimer values were analyzed with HemosIL D-Dimer HS (CuagulationAnalizer, IL ACL TOP 700 CTS, USA), and the normal range is 0-500 ng/dL. For testing FA, 5 ml of venous blood from each patient was spun in a centrifuge at 4000 rpm (revolutions per minute) for 5 minutes to separate the serums. Serums were kept in Eppendorf tubes at a temperature of -80 ^{0}C. The Human FETUA (FA) Sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) Kit was used to measure FA levels (96-Fine Test, EH0218, Wuhan Fine Biological Technology, China). For measuring FA, polyclonal goat anti-human FA antibodies were used with an immunoturbidimetric method. This method was tested along with an enzyme-linked immunosorbent assay, which had an intra-assay coefficient of variation (CV) of 3.5% and an inter-assay CV of 5.4%. This kit had a measuring range of 9.38–350 mg/L. The "in-house" RT-PCR method was used with a one-step enzyme mixture (Invitrogen Superscript Platinum III, USA) on a real-time PCR device to look for CCHF in clinical samples. Anti-CCHF IgM ELISA test was studied by ELISA method using antigen and reagents provided by CDC (Centers for Disease Control and Prevention, Atlanta, USA). After the ELISA microplates were read with a spectrophotometer at a wavelength of 405–410 nm, the cut-off values were calculated and the results were analyzed.

Statistical Analysis

The data were analyzed with SPSS 20 (IBM Corp., Armonk, NY, USA). Kolmogorov-Smirnov test was used while investigating the normal distributions of the variables. Descriptive statistics were presented as mean±standard deviation or median (minimum-maximum) for continuous variables and as the number of cases and percentage (%) for nominal variables. When examining the differences between groups, Mann-Whitney U and Kruskal-Wallis H tests were used because the variables did not come from the normal distribution. Chi-square analysis was used when examining the relationships between groups of nominal variables. Spearman's Rho analysis was used for the correlation of FA with variables. Receiver operating characteristic (ROC) curve analysis was performed to predict mortality development. When interpreting the results, values below the significance level of 0.05 were considered statistically significant.

Results

The mean age of 87 patients admitted for Crimean-Congo Hemorrhagic Fever was 62.52±14.94 years. Twenty seven (31%) of these patients were female and 60(69%) were male. While the rela-

tionship between the mode of transmission of the disease and age was insignificant, the relationship between white blood cell, neutrophil, and C-reactive protein levels was significant (*P*=0.001). The mean FA level was 224.25±31.07 mg/L. Plasma FA level was the highest with 239.64±24.94 mg/L in the group with tick bites, 203.93±15.42 mg/L in contact patients, and the lowest with 186.09±20.69 mg/L in patients with a history of endemic regions (*P*=0.001, Table 1).

Table 1: The relationship of age, hospitalization and laboratory values with disease transmission type

Variable	*All Patients (n: 87)*	*Tick Bite (n:56)*	*Contact History (n:18)*	*Endemic Area (n:13)*	*P-value*
Gender (F/M)	27/60	14/42	7/11	6/7	0.015
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
Age (yr)	62.52±14.94	63.55±14.45	58.56±18.05	63.54±12.32	0.587
Hospitalization(day)	7.77±2.29	7.43±1.99	8.06±2.62	8.85±2.79	0.192
Laboratory Findings					
WBC(10^3/μL)	4.99±3.42	3.80±1.09	3.53±0.97	12.13±3.49	0.001
PLT(10^3/μL)	58.84±26.15	61.71±26.09	68.11±23.00	33.61±13.23	0.001
NEU(10^3/μL)	4.90±2.28	4.41±2.03	4.57±2.25	7.48±1.64	0.001
LYM(10^3/μL)	1.95±0.95	1.87±0.91	2.15±1.05	2.00±0.98	0.611
GLC(mg/dL)	141.95±56.65	142.84±60.44	142.56±50.91	137.31±50.62	0.926
ALT(U/L)	117.25±47.08	115.37±46.92	123.39±45.97	116.85±52.24	0.780
AST(U/L)	124.68±33.80	124.73±34.29	123.61±28.80	125.92±40.28	0.994
Fibrinogen(mg/dL)	252.28±55.31	259.21±56.54	233.61±48.20	248.23±56.72	0.216
D-dimer(ng/ml)	577.01±122.65	560.43±116.49	573.33±123.84	653.54±127.41	0.077
Sedimentation(mm/h	27.76±12.86	27.45±12.56	27.83±15.91	29.00±10.09	0.626
CRP(mg/dL)	5.03±1.68	4.79±1.55	4.47±1.35	6.84±1.57	0.001
Lactate(mmol/L)	2.68±1.49	2.60±1.44	2.98±1.94	2.65±0.99	0.360
Fetuin A(mg/L)	224.25±31.07	239.64±24.94	203.93±15.42	186.09±20.69	0.001

SD:standard deviation, WBC:White Blood Cell, PLT:Platelet, NEU: Neutrophil, LYM:Lymphocyte, GLC:Glucose, ALT:Alanine Aminotransferase, AST:Aspartate Aminotransferase, LDH:Lactate dehydrogenase, CK:Creatine kinase, CRP:C-Reactive Protein, p:Statistical significance (<0.05) (The chi-square test was used for gender, and the kruskal wallis-h test was used for other variables)

While mortality was observed in 9(10.3%) patients, 6(6.9%) of them were female and 3(3.4%) were male (*P*=0.015). Although the mean age was high in the mortality group, no significant relationship was found (*P*=0.581). While the lactate level was 2.40±0.94 mmol/L in the surviving group, it was 5.14±2.79 mmol/L in the mortality group (*P*=0.001). While the mean FA level was 171.65±29.98 mg/L in the mortality group, it was 230.32±24.98 mg/L in the survivors (*P*=0.001). Also, sedimentation and C-reactive protein were also higher in the mortality group (p=0.002, Table 2).

In the correlation analysis of plasma FA level with variables, a moderate negative correlation was observed between hospitalization time, C reactive protein, and lactate levels. Also, a strong negative correlation was found between mortality and type of transmission (*P*=0.001, Table 3).

Table 2: The relationship of age, gender, hospitalization and laboratory values with mortality

Variable		*Mortality (-)*	*Mortality (+)*	*P value*
		n(%)	n(%)	
Gender	Female	21(24.1)	6(6.9)	0.015
	Male	57(65.5)	3(3.4)	
	Total	78(89.7)	9(10.3)	
		Mean ±SD	Mean ±SD	
Age (yr)		62.19±15.20	65.33±12.83	0.581
Hospitalization(day)		7.64±2.14	8.89±3.30	0.263
Laboratory Findings				
WBC(10^3/μL)		4.63±3.05	8.09±4.94	0.070
PLT(10^3/μL)		60.01±25.48	48.67±31.22	0.138
NEU(10^3/μL)		4.76±2.26	6.11±2.16	0.069
LYM(10^3/μL)		1.95±0.95	1.87±0.99	0.845
GLC(mg/dL)		139.36±57.74	164.44±42.10	0.061
ALT(U/L)		118.13±46.15	109.67±57.08	0.499
AST(U/L)		125.44±33.48	118.11±37.96	0.572
Fibrinogen(mg/dL)		252.32±54.71	251.89±63.81	0.775
D-dimer(ng/ml)		577.69±126.75	571.11±83.78	0.656
Sedimentation(mm/h)		26.18±11.57	41.44±15.99	0.002
CRP(mg/dL)		4.88±1.68	6.36±1.09	0.002
Lactate(mmol/L)		2.40±0.94	5.14±2.79	0.001
Fetuin A(mg/L)		230.32±24.98	171.65±29.98	0.001

SD:standard deviation, WBC:White Blood Cell, PLT:Platelet, NEU:Neutrophil, LYM:Lymphocyte, GLC:Glucose, ALT:Alanine Aminotransferase, AST:Aspartate Aminotransferase, LDH:Lactate dehydrogenase, CK:Creatine kinase, CRP:C-Reactive Protein, p:Statistical significance (<0.05). (Chi-square test was used for gender and Mann Whitney U test was used for other variables)

Table 3: Correlation of parameters with Fetuin A level

Variable	*r*	*P value*
Age (yr)	0.025	0.822
Gender	0.192	0.074
WBC(10^3/μL)	-0.175	0.105
PLT(10^3/μL)	0.022	0.843
NEU(10^3/μL)	-0.223	0.038
Fibrinogen(mg/dL)	0.111	0.305
D-dimer(ng/ml)	0.005	0.966
Sedimentation(mm/h)	-0.97	0.369
CRP(mg/dL)	-0.211	0.050
Lactate(mmol/L)	-0.292	0.006
Hospitalization	-0.263	0.014
Mortality	-0.446	0.001
History	-0.715	0.001

SD:standard deviation,WBC:White Blood Cell, PLT:Platelet, NEU:Neutrophil, LYM:Lymphocyte, GLC:Glucose, ALT:Alanine Aminotransferase, AST:Aspartate Aminotransferase, LDH:Lactate dehydrogenase, CK:Creatine kinase, CRP:C-Reactive Protein, p:Statistical significance (<0.05) (Spearman's Rho test was used for correlation*)*

In Fig. 1, the ROC curve analysis of mortality is shown. According to this analysis, FA and lactate optimal cut-off values to predict mortality development; 97.4% sensitivity and 96.2% specificity for FA (AUC, 0.078; 95% CI, -0.067-0.222, *P*<0.001), 88.9% sensitivity and 80.6% specificity for lactate (AUC, 0.855; 95% CI, 0.715-0.995, *P*=0.001), 98.7% sensitivity and 97.3% specificity for CRP (AUC, 0.811; 95% CI, 0.715-0.904, *P*=0.002), 94.9% sensitivity and 82.9% specificity for sedimentation (AUC, 0.809; 95% CI, 0.689-0.929, p=0.002) have been detected over 45%.

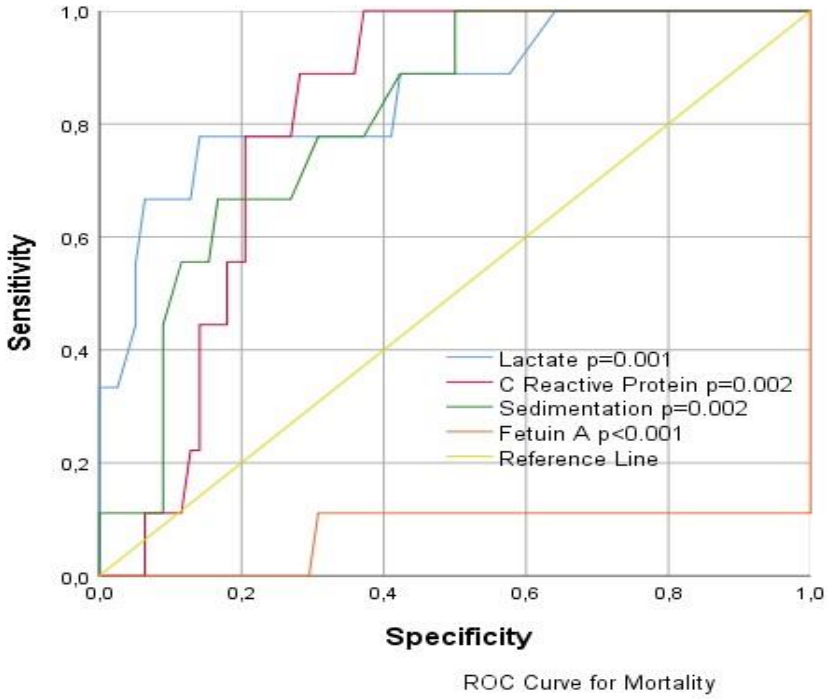

Fig. 1: ROC curve analysis based on the mortality relationship between lactate, CRP, and sedimentation and plasma Fetuin-A levels

Discussion

CCHF disease, which occurs in patients from endemic areas without contact or tick bites, is one of the important medical conditions frequently encountered in emergency rooms. CCHF, which can lead to mild symptoms to severe bleeding, severe psychological trauma, and fatal consequences, contains difficulties due to the high number of transmission routes and causes, the presence of many ticks, and a wide range of age. Factors such as climate, culture, development level, season, gender, and age are effective in the development of CCHF in humans. We aimed to reveal the relationship of plasma FA levels with inflammatory parameters and disease activity and in CCHF patients, to determine early diagnosis and prognosis. Our study is the first to report that decreased FA levels are an independent predictor of disease activity in CCHF patients and that plasma FA levels are negatively correlated with CRP and lactate levels. It shows that FA may have a possible inflammatory function in CCHF and potentially be used as a biological marker.

The virus that causes CCHF reaches the endothelial cells and damages the cells (13,14). This damage occurs either directly by the effect of the virus or indirectly by the activation of immunological and inflammatory pathways (15). Cytokines, chemokines, and other pro-inflammatory mediators are released, vascular permeability increases as a result of endothelial cell activation and endothelial damage, the intrinsic coagulation system is activated, and disseminated intravascu-

lar coagulation (DIC) develops. As a result of the development of DIC, a clinical condition leading to bleeding may develop (16,17). The most important events in the pathogenesis include the degree of endothelial damage, cytokine storm, thrombocytopenia, DIC, hemophagocytosis, and liver cell necrosis. It has been shown that the main target of the virus is mononuclear phagocytes, endothelial cells, and hepatocytes. As a result of the widespread infestation of hepatocytes by the CCHF virus, liver enzymes increase, edema and necrosis in the cells occur. Consequently, bleeding occurs as a result of hemophagocytosis and liver dysfunction (16,17).

The clinical course of the disease can be mild or have fatal consequences. Not every tick bite, contact, or endemic area travel results in illness. Mortality can vary between 10-40% (4). 21.6% of 97 patients with PCR (+) admitted to emergency services resulted in mortality (18). In a study of 220 patients, Baştuğ et al. reported the mortality rate as 16.4% (19). In our study, 9 out of 87 patients (10.3%) resulted in mortality. The average mortality rate in Turkey is at the level of 4-5%, but our study found a higher mortality rate (20). We think that this high rate is due to the advanced level of the hospital and intensive care service. In addition, geographical location and mode of transmission have been shown to have effects on mortality (14). Similarly, in our study, the mortality of patients coming from endemic regions without a history of tick bites was found to be higher than those with a history of tick bite and contact.

Baştuğ et al. reported a significant relationship between high white blood cell (WBC) values and mortality in their study (19). Onguru et al. found a significant relationship between WBC and mortality in their study in which 9(17.6%) of 51 patients resulted in death (21). However, in our study, high WBC and neutrophil counts were found in patients coming from endemic regions, but their relationship with mortality could not be determined. Also, sedimentation and C-reactive protein among acute phase reactants were found to be higher in the mortality group.

The parameter used in predicting mortality in patients with sepsis due to infections is blood lactate level. The mortality rate increases at high lactate levels. Jones et al. showed that lactate level above 2 mmol/L in patients with sepsis and septic shock is an important finding in the increase in mortality (22). Arnold et al. evaluated 166 sepsis patients over the age of 17, and they found that mortality increased with the lactate level above 4.7 mmol/L (23). In our study, the lactate level was found to be 5.14±2.79 mmol/L in the mortality group.

The FA is one of the molecules on which many studies have been conducted. It directly affects animal and human adipose tissue cells, causing subclinical inflammation and cytokine release (24). It can be synthesized extrahepatically in the kidneys, choroid plexus, and all major organs during fetal development. There are no strictly proven standardized values for the reference range. A serum concentration is in the range of 140-297 mg/L (25). The serum level is independent of age and gender after the neonatal period (26). FA was first noted as a negative acute-phase protein in acute inflammation situations. Among the factors affecting FA secretion in humans, conditions such as acute viral hepatitis, cirrhosis, and severe liver damage have been reported (27). Proinflammatory cytokines released in the early period in endotoxemia or sepsis have been shown to decrease FA secretion from the liver. It is stated that a 50-100 ng/mL increase in TNF-α decreased FA levels by 50-60% (28). Altınışık et al. found a significant decrease in FA levels at the 24th hour following the diagnosis of a sepsis patient (29).

In our study, the FA value in patients with mortality was found to be significantly lower than the survival group. A strong negative correlation was found between mortality and FA levels. In addition, the sensitivity of FA for mortality was 97.4% and specificity was 96.2%. In CCHF, an inflammatory process, these data are consistent with similar studies showing a decrease in plasma FA levels. Released FA in CCHF patients resembles cytokines, chemokines, and other proinflammatory mediators. It tries to have a protec-

tive effect like these parameters, but we think that there is a decrease in plasma FA level as a result of increased inflammation. It was determined that this decrease was more pronounced in patients with poor general conditions such as DIC pathology and with mortal outcomes. This may be due to the fact that FA originates from the liver and its release from the liver decreases in the progressive disease process. Plasma FA level decreases in patients coming from an endemic area with high mortality due to the process described above. Plasma FA level of CCHF patients to be evaluated during their admission to the emergency department can be used as a predictive marker in evaluating morbidity and mortality. The limitations of the study include the single-center nature of the study, the inability to include every patient due to the selection of patients with no missing records, the need to re-study some laboratory results, and the inclusion of patients whose stored samples were corrupted.

Conclusion

In CCHF patients, a significant relationship was found between other inflammatory parameters, especially plasma FA level, which is a negative acute-phase reactant. Especially in endemic area cases, hospitalization time is longer and FA levels are lower. In addition, FA level was found to be significantly lower in mortality group patients. On the contrary, high lactate, CRP and sedimentation rates, which are known to increase in infection processes, are risk factors for mortality in CCHF. Indeed, in contrast, FA provides a dose-dependent and long-lasting protection against mortal systemic inflammatory diseases. Therefore, the measurement of serum FA levels of CCHF patients can be used as a test for predicting morbidity and mortality.

Journalism Ethics Considerations

Ethical issues (Including plagiarism, informed-consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors have no conflict of interest.

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