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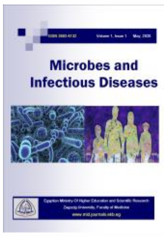

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Original article

The association between CMV infection and COVID-19 clinical outcomes in critically ill patients

Nesreen Hanafi [1] , Rewan Atta [1,2*], Ehab El Reweny[3], Sara lofty Asser [1]

1- Department of Microbiology, Faculty of Medicine, Alexandria University, Egypt

2- Norfolk and Norwich University Hospitals, Norwich NR4 7UU, UK

3 - Department of Critical Care, Faculty of Medicine, Alexandria University, Egypt

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ABSTRACT

Background: COVID-19 pandemic impacted all areas of daily life, including medical care. In particular, delivering care for critically ill patients in the ICUs during the crisis was challenging given the competing risks of death serious complications from SARS-CoV-2, and the likely higher lethality of COVID-19 in immunocompromised patients. The mortality associated with COVID-19 is greatly influenced by known risk factors such as elderly age, cardiovascular disease, hypertension, diabetes, and immunosuppression. As cytomegalovirus reactivation in critically ill patients has been linked with higher morbidity and mortality in intensive care settings, it has been suggested that cytomegalovirus reactivation might lead to worse clinical outcomes of patients with COVID-19. This study aims to assess the frequency of cytomegalovirus infection in critically ill COVID-19 patients. **Methods** : Our study population consists of 90 critically ill COVID-19 patients admitted to the ICUs of Alexandria Main University Hospitals. All the patients underwent serological testing for CMV IgG to assess seroprevalence followed by real time PCR testing. **Results** : CMV IgG seroprevalence rate was 100%. Among the CMV reactivated patients (n = 68), days of hospital stay ranged from 7-43 days compared to 4-26 days in non CMV reactivated patients (n =22) and there was a statistically significant difference between the two results. 61.8 % of the CMV reactivated patients died compared to 36 % of the patients who died in the non CMV reactivated group and this difference was statistically significant. **Conclusion**: CMV infection was found to be associated with worse clinical outcomes.

Introduction

COVID-19 evolved into a global pandemic. The virus, initially labelled as a novel coronavirus by experts, was officially named COVID-19 by the WHO [1] . Currently, there are seven known human coronaviruses (hCoVs): SARS-CoV, MERS-CoV, hCoV-HKU1, hCoV-OC43, hCoV-NL63, hCoV-229E and SARS-CoV-2, . While some cause mild illnesses like common colds, the severe impact was recognized after the SARS-CoV-1 outbreak [2]. Genetic studies show SARS-CoV-2's relation to the 2003 SARS outbreak and its classification within Betacoronavirus. Notably, three highly dangerous hCoVs are SARS-CoV, MERS-CoV, and SARS-CoV-2.

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* *Corresponding author:*Rewan Mohamed Atta
E-mail address: *ruanatta@gmail.com*

Egypt has faced challenges in managing the COVID-19 pandemic. Various factors, including population density, healthcare infrastructure, public health measures, and socioeconomic conditions, have influenced the virus's epidemiology in Egypt [3]. At the onset of the pandemic, Egypt implemented measures such as travel restrictions, social distancing, and lockdowns to mitigate the spread of the virus. However, like many countries, it struggled to balance public health concerns with economic impacts [4]. The number of cases reported in Egypt until July 2023 is 516023 cases, among which 24830 confirmed deaths with a mortality rate of 4.8 % [5].

Multiple factors have played a role in the spread of COVID-19 in Egypt, including high population density, urbanization, poverty, and limited access to healthcare in certain areas. Cultural practices, such as large gatherings and multigenerational living arrangements, may have also facilitated transmission [6]. The effects of COVID-19 on various age groups in Egypt varied as older adults and individuals with co-morbidities were at greater risk of severe illness and death. Additionally, healthcare workers have faced increased risk due to their exposure to infected patients [7].

The prevalence of nosocomial infection in patients admitted to hospitals with COVID-19 had been at least 10% of cases [8]. Cytomegalovirus (CMV) reactivation has been reported in patients with complications and long-term admissions to the intensive care units (ICUs) [9]. The human cytomegalovirus (hCMV) is a widespread DNA virus that is highly prevalent worldwide with a universal seroprevalence of 60-90% among all populations (about 90% in Africa and Asia and 66% in the European region [10]. hCMV is a persistent latent virus following subclinical primary childhood infection, with the monocytes and macrophages are being the main reservoirs [11]. This state of latency allows hCMV to reactivate when host defenses become compromised, such as in critical illness or immunosuppression [12].

We conducted a study in the ICUs at Alexandria University Hospitals between November 2020 to January 2022 to assess the frequency of CMV reactivation in critically ill COVID-19 patients. We investigated the correlation between the impact of the impact of CMV reactivation on the clinical outcomes in a cohort of critically ill COVID-19 patients on who were on mechanical ventilation**.**

Methods and Materials

Patient cohort

We conducted a prospective observational study. Ninety confirmed critically ill COVID-19 patients admitted to the ICUs of Alexandria Main University Hospitals were included. The following data were collected for all patients included in the study: i) Demographic data: name, sex, age, residence, ii) Medical history: underlying diseases including any comorbidities, any immunosuppressive conditions, any immunosuppressive medications.

Inclusion criteria

The laboratory confirmed 90 critically ill COVID-19 cases by real-time reverse-transcription PCR performed on nasopharyngeal swabs, requiring hospitalization and mechanical ventilation, which were included in the study.

Exclusion criteria

We excluded patients who were below 20 years of age or pregnant.

Sample Size

It was calculated using OpenEpi [13], which is a free and open-source software for epidemiological statistics. we have estimated the minimum number of cases in each group considering the CMV infection as exposure and the alpha value of 5% and Power of 80%. The calculated sample number would be able to detect the difference of outcome in each group with a confidence interval of 95%.

Samples

Plasma samples

Sixty peripheral blood samples (2 ml blood on EDTA, plasma) were collected from the COVID-19 patients to identify the presence of the CMV viral genome.

MiniBAL samples

Thirty miniBAL samples were collected from COVID-19 patients in the ICU.

Methods

Sample collection:

Each blood sample was collected in a vacutainer with a 3% EDTA solution. Plasma was separated by centrifugation, aliquoted into three labeled sterile Eppendorf tubes, and stored at -70°C. Serum samples were obtained in heparin tubes.

Serology testing:

The CMV seroprevalence was detected using the Elecsys® CMV IgG , (Roche Diagnostics, USA) according to the manufacturer 's instructions.

Molecular testing:

DNA was extracted from 200 µL of plasma using Thermo Fisher Scientific GeneJET Viral DNA and RNA Purification Kit® (Vilnius, Lithuania) according to manufacturer 's instructions.

Real-time PCR amplification was performed with a thermal cycler (Rotor- Gene Q MDx) with specific primers to detect CMV.

The primer used was obtained from previous study by Habbal et al. The sequence of the forward CMV primer was 5' ACC CGA GAG ATG ATT TTG CG 3' and the reverse CMV primer was 5' GCA GAA GAC AGC AGC GAG AT 3' [14]. We used the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a "housekeeping" gene to ensure the quality of the samples and used as an internal control was added to each sample to ensure the quality of the nucleic acid extraction process and its suitability for PCR. The Sequence of forward GAPDH primer was 5' AGC TCA TTT CCT GGT ATG TGG 3' and the reverse GAPDH primer was 5 'TTG TCA GGG CCC TTT TTC TG 3'. The primers used were synthesized by (Thermo Fisher Scientific, Invitrogen, UK). Their specificity was checked by BLAST® [15].

Statistical analysis

The gathered data were reviewed, organized, and analysed using the Statistical Package for Social Science (SPSS V20 for Windows). The statistical analysis was carried out based on the type of data for each parameter. For numerical data, the mean, standard deviation (± SD), and range (minimum and maximum values) were calculated. For nonnumerical data, frequency and percentage were determined. *p*-value: Statistical significance was defined as p <0.05.

Results

In this study, we included 90 COVID-19 patients admitted to the ICUs of Alexandria University Hospitals with varying clinical severities. All patients enrolled in the study signed an informed written consent after explaining the study's nature, steps and aim to them before enrollment. The study was conducted after approval of the Medical Ethics Committee of Alexandria Faculty of Medicine.

The demographic characteristics of the studied COVID-19 patients are shown in **table (1)**. Males constituted 52 % of the patients and females constituted 47%. Most of the patients around 68 % were aged above 60 years, 19 % of the patients fall between 41-60 years, and 13% fell between 20-40 years.

All patients tested positive for CMV IgG making the seroprevalence rate 100%. **Table 2** shows the distribution of the COVID-19 patients according to the presence or absence of comorbidities. Out of 90 patients, 78 had positive different comorbidities and 12 patients were not associated with any comorbidity. 51% of the patients had hypertension as a comorbidity followed by diabetes in 32% of the patients.

The mean laboratory parameter values in the 90 COVID-19 patients are shown in **table (3)**. This table shows descriptive analysis of the inflammatory markers where CRP showed values ranging from (12.48 – 86.35) with median of [42.23] while procalcitonin showed values ranging from (0.06 – 0.32 ng/ml) with median [0.15]. Whereas D-dimer showed values ranging from (753 – 4380 ug/ml) with median [1412] and LDH showed values ranging from (4.3 – 1019 U/L) with median [332]. Moreover, Ferritin showed values ranging from (361 – 10,000 ng/ml) with median [1893.5] and IL6 showed values ranging from (5.59 – 67.67 pg/ml) with median [16.40]

Table 5 demonstrates the relation between the CMV reactivation and the clinical outcomes of the patients. Among the CMV reactivated patients (n = 68), days of hospital stay range from 7-43 days compared to 4-26 days in non CMV reactivated patients (n =22) and there was a statistically significant difference between the two results. 61.8 % of the CMV reactivated patients died compared to 36 % of the patients who died in the non CMV reactivated group and this difference was statistically significant. The maximum length of ICU stay was 26 days in the CMV reactivated group and 22 days in the non-CMV reactivated group, there was no statistically significant difference between the length of the ICU stay between both the CMV reactivated group and the non CMV reactivated group.

Table 1. Demographic characteristics of COVID-19 patients according to age and sex (n = 90).

	Number	**Percentage (%)**
Sex		
Male	47	52.2
Female	43	47.8
Age (Years)		
20 – 40	12	13.3
41 – 60	17	18.9
>60	61	67.8
Min. – Max.	20.0 – 96.0	
Mean ± SD.	61.74 ± 16.19	
Median (IQR)	65.0 (54.0 – 73.0)	

IQR: Inter quartile range, **SD**: Standard deviation

Table 2. Distribution of the studied cases according to the presence or absence of comorbidities (n = 90)**.**

	Number	**Percentage (%)**
Absence	12	13.3
Presence	78	86.7
Comorbidities		
Hypertension	46	51.1
Diabetes	29	32.2
Cardiac	8	8.9
Respiratory	3	3.3
CKD	2	2.2
Cancer	5	5.6
Collagen	2	2.2
Others	32	35.6

CKD : Chronic Kidney disease

Table 3. Descriptive analysis of the studied cases according to laboratory investigations in COVID-19 patient group (n = 90) .

	Min. – Max.	**Mean ± SD.**	**Median (IQR)**
Complete blood picture			
Platelets (´10^3/ml)	19.14 – 570.71	221.13 ± 107.55	192.0(163.57 – 258.7)
Lymphocyte (%)	1.70 – 81.23	12.57 ± 10.23	8.53 (8.0 – 15.29)
Coagulation profile			
Prothrombin time (sec.)	11.14 – 31.70	14.80 ± 3.98	13.80 (12.67 – 15.14)
Partial thromboplastin time (sec.)	24.29 – 99.74	38.30 ± 10.75	38.69 (31.14 – 41.83)
Renal functions			
Urea (mg/dl)	15.07 – 306.57	68.42 ± 51.69	44.57 (39.57 – 77.71)
Creatinine (mg/dl)	0.46 – 5.34	1.34 ± 1.12	1.03 (0.69 – 1.24)
Liver function testing			
ALT (U/L)	9.86 – 508.86	81.53 ± 86.12	72.0 (35.0 – 81.29)
AST (U/L)	15.14 – 586.86	74.43 ± 70.73	57.29 (35.71 – 105.86)
Other investigations			
D-dimer (ug/l)	181.67 – 23036.67	2762.28 ± 3559.37	1412.0(753.0 –4380.0)
Interleukin-6 (pg/ml)	1.33 – 290.33	43.59 ± 58.41	16.40 (5.59 – 67.67)
Procalcitonin (ng/ml)	0.01 – 3.20	0.35 ± 0.54	0.15 (0.06 – 0.32)
CRP (mg/l)	1.17 – 340.0	62.76 ± 70.88	41.23 (12.48 – 86.35)

IQR: Inter quartile range, **SD**: Standard deviation

Table 4 . Comparison between the three studied days according to D-dimer, Interlukin-6 and CRP in cases group (n = 90).

	Day 1	**Day 4**	**Day 7**	***p*-value**
D-dimer (ug/l)				
Min. – Max.	170.0 – 35200.0	190.0 – 17550.0	185.0 – 16430.0	0.046^*
Mean ± SD.	3444.1 ± 5171.7	2564.8 ± 3227.8	2277.9 ± 2809.2	
Median (IQR)	1035	1650	1280	
	(548.0 – 6800.0)	(720.0 – 3800.0)	(725.0 – 2150.0)	
Inter-days significance	p_1=0.709,p_2=0.021*,p_3=0.053			
Interlukin-6 (pg/ml)				
Min. – Max.	1.50 – 244.0	1.30 – 224.0	1.20 – 700.0	0.438
Mean ± SD.	37.87 ± 49.68	39.67 ± 49.40	53.42 ± 110.2	
Median (IQR)	13.65	18.6	20.05	
	(2.20 – 49.90)	(1.63 – 67.0)	(2.10 – 50.15)	
CRP (mg/l)				
Min. – Max.	0.20 – 340.0	1.50 – 360.0	1.0 – 320.0	$<0.001^*$
Mean ± SD.	75.40 ± 84.47	60.68 ± 75.24	52.15 ± 70.76	
Median (IQR)	31.8	29.1	19.75	
	(11.72 – 111.0)	(10.90 – 68.0)	(6.95 – 76.0)	
Inter-days significance	p_1=0.002*,p_2<0.001*,p_3=0.003*			

QR: Inter quartile range, **SD**: Standard deviation, * Statistically significant
p: p value for comparing between the three studied days
p_1: p value for comparing between **Day 1** and **Day 4**
p_2: p value for comparing between **Day 1** and **Day 7**
p_3: p value for comparing between **Day 4** and **Day 7**
*: Statistically significant at $p \leq 0.05$

Table 5. Relation between PCR and outcomes in total cases group (n = 90).

	PCR				
	Negative (n = 22)		**Positive (n = 68)**		***p* value**
	No.	**%**	**No.**	**%**	
Mortality					
Alive	14	63.6	26	38.2	0.037*
Died	8	36.4	42	61.8	
Hospital stay (days)					
Min. – Max.	4.0 – 26.0		7.0 – 43.0		0.011*
Mean ± SD.	12.14 ± 6.90		15.99 ± 7.20		
Median (IQR)	9.50 (7.0 – 16.0)		15.0 (10.0 – 19.0)		
Length of ICU stay (days)					
Min. – Max.	0.0 – 22.0		0.0 – 26.0		0.123
Mean ± SD.	7.09 ± 6.89		9.31 ± 6.62		
Median (IQR)	5.0 (2.0 – 11.0)		8.0 (4.0 – 12.50)		

IQR: Inter quartile range, **SD**: Standard deviation, * Statistically significant

Table 6. Relation between PCR and mortality in total positive cases group (n = 68)

	No.	PCR		*p*
		Mean ± SD.	**Median (Min. – Max.)**	
Mortality				
Alive	**26**	2587.9 ± 3729.0	571.0 (13.0 – 12135.0)	0.009*
Died	**42**	11370.2 ± 65436.7	190.0 (8.0 – 425083.0)	

IQR: Inter quartile range, **SD**: Standard deviation, * Statistically significant

Discussion

Clinical evidence confirms that COVID-19 can affect every age group, from children to elderly, resulting in a wide variability of disease progression and prognosis [16]. Pneumonia is the most frequent severe complication of COVID-19, characterized primarily by fever, dry cough, dyspnea, and bilateral infiltrates on chest imaging. COVID-19 patients' symptoms range from mild to moderate to severe symptoms requiring ICU admission. It is reported that mortality rates in ICU-admitted patients may reach up to 31% [17]. COVID-19 has been increasingly linked to the reactivation of latent CMV infection [18]. The COVID-19 can result in acute respiratory distress syndrome (ARDS). This has posed immense challenges for the research and medical communities. There is an urgent unmet need to understand why severe disease develops in some people [19].

Cytomegalovirus is a common herpesvirus with a seroprevalence in various populations globally ranges from 45% to 100%, depending on geographical location and socioeconomic status. To establish a dormant infection in the body, the virus adopts tactics to avoid detection by the immune system [20]. In healthy individuals, these viruses are latent as they are suppressed by the host immune system, but reactivation from a latent state can occur in an immunocompromised population [21]. The COVID-19 pandemic has introduced new challenges, particularly concerning the interaction between SARS-CoV-2 and pre-existing infections like CMV. There were many reports on the CMV reactivation before the COVID-19 pandemic critically ill patients admitted to ICU [22].

This study aimed to evaluate the frequency of CMV reactivation in critically ill COVID-19 patients and to examine the correlation between CMV reactivation and clinical outcomes in those requiring mechanical ventilation.

During the study period, plasma samples from 90 COVID-19 patients were subjected to HCMV IgG serological testing. The seroprevalence among the studied group was 100%, and this runs parallel to a study performed in the Middle East and North Africa region by **Al Mana et al.** in 2019, which revealed 100% seropositivity [10]. The seroprevalence of HCMV in different parts of the world is affected by socioeconomic status.

Demographic data retrieved in the current study showed that among the 90 studied COVID-19 patients, the elderly (> 60 years old) was the highest age group in prevalence (67%) with a median age of 65 years. 19% of the patients in the 40-60 age group and 13 % between 20-40 years old. Globally, among various studies performed to track the critically ill COVID-19 patients demographics and the association with CMV reactivation, individuals older than 60 years constituted the highest percentage, including a study done by Talan et al. in 2022 where the median age of the study participants 67.7 years [23]. The two most common comorbidities found in the studied COVID-19 patients were hypertension (51%) and diabetes (32%).

Various studies have discussed the role of D-dimer in COVID-19 diagnosis and its severity. Our study showed a significant increase in D-dimer levels in COVID-19 patients with severe symptoms compared to those with moderate symptoms. This result was comparable to those reported in the literature [17,20,22,24]. These findings identified the D-dimer as a prognostic marker for detecting COVID-19 disease severity.

The study also found that serum levels of inflammation markers, including IL-6 and CRP, were significantly elevated in patients with severe symptoms compared to those with moderate symptoms. Additionally, IL-6 levels were shown to rise from the day of admission to day 4, followed by a gradual decline from day 4 to day 7. In agreement with our results, these studies showed that IL-6 and CRP were elevated in patients with COVID-19 [25].

In our cohort of 90 critically-ill COVID-19 patients admitted to Alexandria University Hospital ICU, CMV reactivation was detected in (68/90) 75% of the COVID-19 patients. Patients with CMV reactivation had longer hospital and ICU stays than non-CMV reactivated patients. This is in concordance with reports that CMV reactivation has led to worse prognosis of COVID-19 cases including extended hospital stay and higher mortality rate [26,27].

Among the CMV-reactivated patients (n = 68), days of hospital stay ranged from 7-43 days compared to 4-26 days in the non-CMV-reactivated patients (n =22), and there was a statistically significant difference between the two results (p=0.011). 61.8 % of the CMV-reactivated patients died compared to 36 % of the patients who died in the non-CMV-reactivated group, and this difference was statistically significant (p= 0.037). The maximum length of ICU stay was 26 days in the CMV-reactivated group compared to 22 days in the non-CMV-reactivated group, and there was no statistically significant difference between the length of the ICU stay in days between both the groups.

Our results were consistent with an Italian study that collected data from three ICUs [28] and reported increased mortality rates when patients had reactivated CMV compared to those who did not have CMV reactivation (67.0% vs. 24.5%). However, the impact of CMV reactivation in critically ill patients is independent of the infectious pathogen, as its impact was reported before the COVID-19 pandemic. **Limaye et al.** discovered that there is a causal relationship between CMV reactivation and lengthy hospital stays or increased mortality [29]. **Heininger et al.** carried out a double-blinded study in patient with severe sepsis, where it was reported that patients who demonstrated CMV reactivation had required extended mechanical ventilation, and spend longer periods in ICU [30]. Another report by **Frantzeskaki et al.** had flagged that 13.75% of critically ill, mechanically ventilated, immunocompetent ICU patients had CMV reactivation which was linked to severe organ dysfunction. The report defined the CMV reactivation as DNAemia of 500 copies/mL or more [31]. Similar prevalence of CMV reactivation in the ICU patients (approximately 18%) was reported in a

Chinese cohort of 71 patients with several negative outcomes, including higher complication rates, extended mechanical ventilation, increased hospitalization costs, longer ICU stays, and a higher mortality rate [8].

Retrospective studies and case reports have highlighted the increased mortality rate in patients co-infected with CMV and SARS-CoV-2. For example, a study on critically ill patients with COVID-19 in the ICU found a significant correlation between CMV co-infection and mortality, emphasizing the need for vigilant monitoring and potential antiviral interventions in these patients [32].

The impact of CMV reactivation on the outcome of COVID19 in our cohort could be contributed to several factors. First is the immune dysregulation, Both CMV and SARS-CoV-2 induce significant alterations in the immune system. CMV is known for causing immune activation and inflammation, which can exacerbate the cytokine storm associated with severe COVID-19. Studies suggest that the immune system's overreaction in response to SARS-CoV-2 is a critical factor in COVID-19 mortality, and CMV reactivation may worsen this response. The CMV infection inhibit the responses of NK, B,T cells by directly inhibiting the antigen presentation leading to immune evasion mechanisms. As the virus escpae the immune system with a high replication rate in ICU patients who is already immunocompromised, the active viral infection may heighten their risk of mortality from SARS-CoV-2 [33].

Secondly, CMV infection is associated with a phenomenon known as "memory inflation," where a major proportion of activated T-cells are directed to CMV, leading to T-cell exhaustion especially CD8+ T which reduces the immune system's ability to respond effectively to new infections, including SARS-CoV-2, potentially increasing the severity and mortality of COVID-19 [34].

Finally, both CMV and COVID-19 can cause endothelial dysfunction. SARS-CoV-2 targets the ACE2 receptor, which is prevalent in endothelial cells, leading to widespread vascular inflammation and coagulopathy. Cytomegalovirus also infects endothelial cells, contributing to vascular inflammation and thrombosis. The combined effect can exacerbate cardiovascular complications, leading to higher mortality in co-infected patients [35] .

The study design may be a limitation of the extrapolation of the results of our study, the limited sample size of 90 patients in a prospective single centre with lack of treatment protocols integration in the data analysis. The low sample size had limited the control of confounding factors due to small sample size in each group if further breakdown analysis is performed for confounding variables such as the severity of COVID-19, comorbidities, and treatments received. We recommend that if a new wave of COVID-19 emerge, a multi-centre study should be carried out prospectively but this would require a national coordination.

The interaction between CMV and SARS-CoV-2 greatly affects the prognosis of COVID-19 patients, leading to higher mortality rates, especially among immunocompromised and critically ill individuals. It is crucial that we understand the mechanisms of this interaction, coupled with vigilant clinical management and preventive strategies, which are crucial in improving outcomes for these high-risk patients. We would recommend monitoring for CMV reactivation in COVID-19 patients, especially those in ICUs or with known risk factors, as this can facilitate early intervention and improve outcomes. This involves frequent testing for CMV DNAemia and preemptive antiviral therapy in patients showing signs of reactivation [36].

The management of CMV in COVID-19 patients presents unique challenges. Antiviral therapies effective against CMV, such as ganciclovir, may be considered in COVID-19 patients showing signs of CMV reactivation. Future research should focus on optimizing therapeutic protocols and exploring novel interventions to mitigate the dual burden of these infections.

Declaration:

Disclosure of potential conflicts of interest

None to disclose.

Financial disclosure

No financial interests, relationships and affiliations relevant to the subject of the manuscript.

Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contribution

Conceptualization: N.H., and S.A., Study Design N.H., Data collection and lab testing R.A., Data Revision and Clinical Diagnosis E.R., Data Revision, Paper Reading and Lab Investigations S.A. All authors have participated in the concept and design, analysis and interpretation of data, and drafting or revising of the manuscript, and they have approved the manuscript as submitted.

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