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Serum Level of Cathelicidin in Covid-19 Patients in Relation with Oral Heath

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ABSTRACT

Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the microorganism that responsible for the pandemic coronavirus disease 2019 (COVID-19). Cathelicidin is an important host defenses antimicrobial peptide, plays essential roles in both types of immunity (specific and non-specific) and possess antiviral properties.

Aim of study: The present study was performed to evaluate the serum level of cathelicidin in COVID-19 patients group in comparison to healthy group and to assess the relationship of cathelicidin level with oral hygiene and clinical features of disease.

Materials and Methods: Fifty patients with confirmed COVID-19 by reverse transcriptase polymerase chain reaction (29 males and 21 females) were enrolled in this study, their age range (18-77) years. And healthy volunteers their ages and sexes were identical to patients consisted of 35 individuals who were considered as control (16 males and 19 females), their ages ranged between (18-73) years. The serum obtained from COVID-19 patients and healthy controls was analyzed by using commercially available ELISA kit to evaluate the level of cathelicidin.

Results: The findings found that there was significant decrease (P<0.01) in level of cathelicidin in patients group as compared to that in healthy group. While there were no significant differences (P>0.05) in levels of cathelicidin nor according to oral hygiene, and not according to disease severity.

Conclusions: Low Level of cathelicidin in COVID-19 patients may give additional evidence that immune response is impaired in SARS-CoV-2 infection.

Key words: COVID-19, Antimicrobial Peptide, Cathelicidin LL-37.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 is an RNA virus with exemplary crown-like due to exist of glycoprotein spikes on its envelope. SARS-CoV-2 was determined as the cause of the disease, and Chinese Scientists named the disease COVID-19 [1, 2]. SARS-CoV-2 belongs to the group of beta-CoVs containing 82% nucleotide with human SARS-CoV and similar to SARS-CoV that utilizes angiotensin-converting enzyme-2 (ACE2) as the receptor to enter into the host cell [3, 4]. The presence of COVID-19 is demonstrated by many symptoms, varying from asymptomatic/mild symptoms, severe disease and death. Fever, cough and shortness of breath are the common symptoms. Other symptoms are weakness, respiratory distress, malaise, sore throat muscle pain, and loss of taste and/or smell [5].

Antimicrobial peptides (AMPs) a small cationic peptide is a broad class of host-defense molecules, that acting to encounter the microbial invasion and challenge. These peptides that play an essential role in the progressing of innate immunity [6]. The major classes of host defense oral peptides include defensins and the cathelicidin LL-37, these peptides are expressed in neutrophil granules by many tissue types and they have direct and indirect antimicrobial activity, the ability of these peptides to act as chemokines and stimulate chemokine productions [7, 8]. Human cathelicidin antimicrobial peptide (CAMP) and its effective form, LL-37, plays a critical role in infectious diseases such as; viral, bacterial and fungal infections as well as autoimmune diseases [9, 10]. The LL-37-induced activation of membrane receptors and signaling pathways leads to the changing of cellular functions [11]. LL-37 can be produced not only by macrophages but also by epithelial cells and has antiviral activity especially against enveloped viruses such as SARS-CoV-2 [12]. Cathelicidins are produced by mammals in response to different pathogen; human cathelicidin antimicrobial peptide (CAMP/hCAP-18) is the lone member of the cathelicidin family of protein in humans [13]. The hCAP-18 are expressed in most epithelia in spite of its expression in keratinocytes is produced by infection, vitamin D, short-chain fatty acids, butyrate and endoplasmic reticulum stress signalling. HCAP-18 can be down regulated by

pathogens including Shigella and Neisseria, and some bacterial exotoxin [14]. Vitamin D triggers the receptors of vitamin D which is a transcription factor that impacts transcription of hundreds of genes as well enhancing transcription of the hCAP18 gene that encodes cathelicidin. Certain regulating genes of vitamin D are a key to balanced responses of the immune system against several viral and bacterial infections. The new publications bind Vitamin D deficiency to severity of COVID-19 [15, 16]. These studies supposed that with sufficient of Vitamin D that LL37 aid to clear the SARS-CoV-2 virus and help to regulate the immune system responses. The aim of this study used a case control approach to evaluate the serum level of cathelicidin in COVID-19 patients as compared to controls and to assess the relationship of cathelicidin with oral hygiene and clinical features of disease.

PARTICIPANTS AND METHODS

Ethical clearance

From Ethical Committee, College of Dentistry\ University of Baghdad.

Inclusion and exclusion criteria

The patients participate in this study and considered eligible must have met the following criteria; signs and symptoms of COVID-19 infection (fever, generalized malaise, cough and shortness of breath) and positive RT-PCR for COVID-19. While pediatric and pregnant patients, patients with chronic viral infection and systemic diseases, allergic rhinitis and chronic sinusitis, and patients who could not give informed consent were excluded from this study.

Oral hygiene index

Oral examination was performed by the specialist dentist according to [17].

Blood sample collection

Four ml of venous blood will be drawn from each subject under aseptic technique. Blood was transferred to sterile plain tube, and serum was separated by centrifugation at 3000 rpm for 10 min, then divided into small aliquots and kept at -20ºC until used for analysis.

Measuring of Cathelicidin

The level of cathelicidin LL-37 was determined by the ELISA kit (Shanghai/China).

Statistical analysis

The data was non-parametric and calculated by Mann-Whitney test, p-value of $p<0.05$ was considered significant.

RESULTS

The demographic pictures of patients and controls groups were presented in (Table 1), the study showed non-significant differences between two groups according to age and gender, and the most age group frequency was (40+ years) which comprised (62%) of the patients. The current results revealed that 24 (48%) of patients have mild disease, 16 (32%) have moderate disease, while the remaining 10 (20%) of the patients have severe disease as shown in (Table 2).

Table 1: Distribution of the patients and controls according to the age groups.

Age group (years)	Study groups				P-value
	Patients group (N=50)		Control group (N=35)		
	N	%	N	%	0.106NS
<30	9	18	5	14	
30-40	10	20	16	46	
40+	31	62	14	40	
Range	(16 - 77)		(18-73)		
Mean ± SD	44.26 ± 16.57		40.08 ± 12.64		

Table 2: Frequency distribution of patients according to clinical features.

Clinical features (N=50)	N %
Mild	24 (48%)
Moderate	16 (32%)
Severe	10 (20%)

Moreover, the results demonstrated that 30 (60%) of the patients had good oral hygiene, while 20 (40%) were with poor oral hygiene (Table 3). In this study we found that all patients with severe case had bad oral hygiene 10 (50%), in mild 6 were with poor oral health (30%), and 4 (20%) for moderate case, (Table 4).

Table 3: Case-control differences in oral hygiene and number of teeth.

Oral hygiene	Study groups				P-value
	Patients group (N=50)		Control group (N=35)		
	N	%	N	%	0.024*
	Oral hygiene				
Good	30	60	29	83	
Poor	20	40	6	17	

Table 4: Distribution of oral hygiene according to severity of COVID19.

		Severity of COVID-19			
Oral Hygiene		Severe	Moderate	Mild	P-value
Good	N	0	12	18	0.000**
	%	-	40%	60%	
Poor	N	10	4	6	
	%	50%	20%	30%	

As shown in Table 5 there is significant decrease (P<0.01) in median serum level of cathelicidin in patients group (11.72 ng/ml) as compared to that in healthy control group (12.67 ng/ml). While, there is no significant difference (P>0.05) in serum level of cathelicidin among three groups of patients according to severity of disease, However, the median level for severe, moderate and mild cases were (15.7 ng/ml, 12.12 ng/ml and 16.56 ng/ml) respectively, as observed in (Table 6). Likewise this work the current study failed to show statistically significant differences in median serum cathelicidin levels between patients group with good oral hygiene (25.52 pg/ml and 25.31 ng/ml) and patients group with bad oral hygiene (25.84 pg/ml and 28.4 ng/ml), as observed in (Tables 7).

Table 5: Case control difference in serum levels of cathelicidin (ng/ml).

Antimicrobial Peptides	Study groups		P-value
	Covid-19 patients (N=50)	Healthy control (N=35)	
	Serum Cathelicidin		0.013*
Min	3.28	9.79	
Max	26.38	25.72	
Median	11.72	12.67	
Mean Rank	37.45	50.93	

Table 6: Comparison the levels of Serum Cathelicidin (ng/ml) in patients group according to Severity disease.

Serum Cathelicidin	Patients group			
	Severe (N=10)	Moderate (N=16)	Mild (N=24)	P-value
Min	9.37	3.28	5.77	0.433NS
Max	23.32	23.49	26.38	
Median	12.195	10.875	11.44	
Mean Rank	15.7	12.12	16.56	

Table 7: Comparison the levels of serum Cathelicidin (ng/ml) in patients group according to oral hygiene.

Serum Cathelicidin	Good (N=30)	Poor (N=20)
Min	3.28	8.03
Max	26.38	23.32
Median	11.235	12.13
Mean Rank	25.31	28.4
P-value	0.477NS	

DISCUSSION

Cathelicidin LL-37 is a small cationic peptide that plays major role in antimicrobial defenses, which kills a wide-range of infective agents by disrupting their membranes, involving viruses [18]. The present results showed that the serum cathelicidins level was significantly decreased in COVID-19 subjects as compared to controls and the level was non-significantly decrease as the severity of disease increase. Evidence has reported that cathelicidin can be deemed as a potent sign for response of immune system in bacterial or viral infections [19]. Low level of serum cathelicidin in this study may be due to a disruption of the immune system in COVID-19 patients, or could be attributed to vitamin D deficiency that leads to reduced cathelicidin production. This is in agreement with Crane-Godreau et al., (2020) who reported, it is possible that vitamin D deficiency may lead to reduced LL-37 levels and a weakened antimicrobial response to SARS-CoV-2 [20]. Dixon et al. revealed that the active 1, 25-dihydroxy metabolite stimulates transcription of the cathelicidin gene, and there is a positive correlation between circulating bioactive vitamin D and hCAP18/ LL-37 levels in healthy subjects [21]. Similarly, White et al., (2010) illustrated that 1,25D is a direct promoter of antimicrobial peptides genes expression, in particular cathelicidin [22]. Vitamin D and LL-37 play important roles in lung immunity and response to respiratory diseases [23]. Vitamin D stimulates the production of antiviral molecules such as cathelicidins and defensins, which reduces the rate of viral replication, decrease the pro-inflammatory cytokine concentration, and increase of anti-inflammatory molecules production [24]. In similar way, previous study conducted on asthmatic patients reported that a low level of cathelicidin may lead to contagious complications in healthy children and children with asthmatic [25].

This study also showed that the level of cathelicidin decrease as covid-19 disease progress with no significant differences. There were no available studies on this correlation in covid-19 to compare with it. Anyhow, [26] observed relationship among LL-37, vitamin D and severity of COVID-19. Analysis of Surface Plasmon resonance showed that LL-37 links to SARS-CoV-2 (S) protein and prevents linking to ACE2 receptor, and probably the virus enters into cell. This study supports the protective utilize of vitamin D to encourage LL-37 which keep from SARS-CoV-2 infection, and administration of vitamin D as a therapy for the COVID-19 patients treatment. Furthermore, their results give proof that the direct utilize of LL-37 by inhalation and systemic application may decrease the severity of disease.

Additionally, the current study failed to show significant differences in the levels of cathelidicin in COVID-19 patients with good oral health compared to those patients with poor oral health. A possible explanation for no significant difference could be due to that AMP levels were not correlated with oral health status and the presence of oral bacteria. However; Shin and Choi reported that oral bacteria in dental plaque as Treponema denticola inhibits expression pathway and secretion of AMP by inhibition secretion of TNF-alpha and TLR-2 [27]. One limitation of the current study is to study only one type of AMP, as other types also has antiviral role. In conclusion low Level of cathelicidin in patients with COVID-19 may give additional evidence that immune response is impaired in SARS-CoV-2 infection.

CONFLICTS OF INTEREST

The authors have disclosed no potential conflicts of interest.

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