Serum Matrix Metalloproteinase-2: A Possible Link between COVID-19 and Periodontitis

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Abstract:

Background: Coronavirus disease-19 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is a severe infection primarily targeting the respiratory system. However, many other extrapulmonary body organs are also affected with a varying degree of severity. Some evidence indicated the development of periodontist in patients, although the pathogenesis is not well-defined.

Aims: This study aimed to investigate the association of COVID-19 severity and role of matrix metalloproteinase 2 in development of periodontitis.

Patients and Methods: This is a cross sectional study which included a total of 160 patients with COVID-19. Patients were categorized into severe and mild-moderated according to World Health Organization criteria. Periodontitis was diagnosed in those patients according to clinical criteria. Serum level of matrix metalloproteinase 2 was estimated in all patients using enzyme linked immunosorbent assay (ELISA). Demographic and laboratory data were obtained from the patients' records.

Results: Forty-two patients (26.25%) had severe COVID-19. Demographically, older ages and the presence of comorbidities were significantly associated with COVID-19 severity. Besides the inflammatory markers, the median serum level of MMP-2 was higher in severe than mild-moderate COVID-19 cases (208.12 ng/ml vs. 196.33 ng/ml) with a significant difference. The PO rate in severe and mild-moderate COVID-19 was 23.81% and 10.17%, respectively, with a significant difference. The median serum MMP-2 in patients with PO was 228.5 ng/ml which was significantly higher than those without PO 193.81 ng/ml.

Conclusions: These data indicate the significant association between COVID-19 severity and development of PO. Matrix metalloproteinase-2 could be the possible link between severe COVID-19 and PO.

Key words: Coronavirus disease-19, Periodontist, Matrix metalloproteinase 2, Inflammatory markers

المستويات المصلية للبروتنيز الفلزي للمادة الخلالية-2: أمكانية الربط بين الكوفيد-19 والتهاب دواعم السن

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الخلاصة:

خلفية الدراسة: كوفيد-19 هو مرض فايروسي يسببه فايروس متلازمة الجهاز التنفسي الحادة الوخيمة كورونا- 2 . وهو عدوى شديدة تستهدف الجهاز التنفسي في المقام الأول. ومع ذلك ، تتأثر أيضًا العديد من أعضاء الجسم خارج الرئة بدرجات متفاوتة من الشدة. تشير بعض الأدلة إلى تطور التهاب دواعم السن لدى مرضى الكوفيد-19، على الرغم من أن السبب غير محدد جيدًا.

الأهداف: هدفت هذه الدراسة إلى استقصاء الارتباط بين شدة الكوفيد-19 ودور البروتنيز الفلزي للمادة الخلالية-2 في تطور التهاب دواعم السن.

المرضى وطرائق العمل: شملت هذه الدراسة المقطعية ما مجموعه 160 مريضًا مصابًا بالكوفيد-19 . قسم المرضى إلى حالات شديدة وخفيفة/معتدلة وفقًا لمعايير منظمة الصحة العالمية. تم تشخيص التهاب دواعم السن عند هؤلاء المرضى حسب للمعايير السريرية. اجري تقدير مستوى المصل من البروتنيز الفلزي للمادة الخلالية-2 في جميع المرضى باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم .تم الحصول على البيانات الديموغرافية والمختبرية من سجلات المرضى.

النتائج: كان لدى 42 مريضا (26.25 ٪) كوفيد-19شديد. من الناحية الديموغرافية ، ارتبطت الأعمار الأكبر سنا ووجود الأمراض المصاحبة بشكل معنوي مع شدة كوفيد-19 . اضافة الى الواسمات الالتهابية، فقد كان متوسط مستوى المصل من البروتنيز الفلزي للمادة الخلالية-2 أعلى في الحالات الشديدة من كوفيد-19 مقارنة بالحالات الخفيفة /المعتدلة (208.12 نانوغرام / مل مقابل 196.33 نانوغرام / مل) وبفرق معنوي. بلغ معدل التهاب دواعم السن في حالات الكوفيد-19 الشديدة والخفيفة/المعتدلة 23.81٪ و 10.17٪ على التوالي وبفرق معنوي. وكان متوسط مصل الدم من البروتنيز الفلزي للمادة الخلالية-2 في المرضى الذين يعانون من التهاب دواعم السن 228.5 نانوغرام / مل وهو أعلى معنويا من أولئك الذين ليس لديهم التهاب دواعم السن(193.81 نانوغرام / مل).

الاستنتاجات: تشير هذه البيانات إلى الارتباط المعنوي بين شدة الكوفيد-19وتطور التهاب دواعم السن . يمكن أن يكون البروتنيز الفلزي للمادة الخلالية-2هو الرابط المحتمل بين الكوفيد-19 الشديد و التهاب دواعم السن.

الكلمات المفتاحية: مرض فيروس الكورونا ، التهاب دواعم السن، البروتنيز الفلزي للمادة الخلالية-2 ، الواسمات الالتهابية

Introduction

Even though the majority of COVID-19 patients have moderate symptoms, roughly 14% of verified cases experience severe conditions that necessitate hospitalization and oxygen assistance, 5% require admission to intensive care units, and 2% died from the disease [1]. Several factors have been found to be significantly associated with disease severity such as age and gender [2], while many preventive measures were followed to reduce the spread of infection [3].

Acute respiratory distress syndrome (ARDS), sepsis, and septic shock are common complications in severe cases that can cause organ damage [4]. Patients with severe COVID-19 and ARDS frequently have an aggravated immune response, sometimes known as "cytokine storm syndrome," which is characterized by elevated levels of proinflammatory cytokines and extensive tissue destruction [5].

SARS-CoV-2 was previously identified in saliva of the infected patients which indicated the involvement of oral cavity by the infection. Furthermore, angiotensin converting enzyme 2 (ACE2) receptor has been detected in oral mucosa, particularly on the dorsum of tongue and salivary glands [6].

Oral COVID-19 encompassed ulcer, bulla, erosion, pustule, vesicle, fissured or depapillated tongue, macule, papule, plaque, halitosis, pigmentation, whitish areas, hemorrhagic crust, petechiae, necrosis, erythema, swelling, and spontaneous bleeding. The comment locations of involvement are tongue (38%), labial mucosa (26%), palate (22%), gingiva (8%), buccal mucosa (5%), oropharynx (4%), and tonsil (1%) [7].

There may be a connection between COVID-19 and periodontal disease, according to several theories [6,8]. This notion is strengthened by the discovery of SARS-CoV-2 in gingival crevicular fluid (GCF), which raises the potential of an additional point of entry [9]. There have also been a few studies using patient data that, in general, show periodontal disease to be a factor in less favorable COVID-19-

related outcomes [10]. However, these studies merely use previously gathered patient data or a small number of self-reported oral health markers and correlate them with the participants' current COVID-19 disease process.

Matrix metalloproteinases are a family of proteolytic enzymes containing a zinc ion at the active site of catalysis and can cleave a wide variety of substrates. MMPs are not highly expressed in tissues in steady-state settings. However, their expression is increased in response to damage, inflammatory response, extracellular matrix (ECM) turnover, and repair. For instance, during ARDS, MMP expression is dysregulated, and they may be essential for the development and progression of the illness [11]. MMP-2 and MMP-9 have been shown to mediate the repair of the alveolar epithelial-endothelial gap injury brought on by mechanical ventilation during the acute phases of ARDS [12]. In addition, hypoxia and high-volume mechanical ventilation have been shown to enhance their expression [13].

The present study aimed to investigate the association of COVID-19 severity and role of matrix metalloproteinase 2 (MMP-2) in the development of PO.

Patients and Methods

This was a cross-sectional study including 160 patients with SARS-CoV-2 who were admitted and treated at Al-Shifaa Hospital/ Baghdad during the period from 1st August 2020 to 1st April 2021. The diagnosis of COVID-19 was based on nasopharyngeal swab and detection of SARS-CoV-2 RNA by real-time polymerase chain reaction (RT-PCR). The severity of the disease was determined according to WHO criteria. Severe cases are presented with oxygen saturation< 90% at room air, respiratory rate> 30 breaths/min and signs of severe respiratory distress (accessory muscle use, inability to complete full sentences, and, in children, very severe chest wall indrawing, gruntng, central cyanosis, or presence of any other general danger signs). Non-severe COVID-19 is defined as absence of any criteria for severe COVID-19 [14]. Patients were followed up for one months after admission, during which the oral cavity was examined for PO. The clinical examination included full-mouth probing using a standard periodontal probe, tooth mobility assessment and gingival recession.

Periodontal clinical examination was accomplished by a single calibrated inspector using a 10-mm round-tip manual Williams's periodontal probe. Clinical characteristics such as probing depth (PD> 3 mm), clinical attachment loss (CAL> 3 mm), plaque index (PI>1), gingival index (GI>1), tooth loss, bleeding on probing (BOP> 30%), color and texture of gums (red and swollen) were used as criteria for clinical diagnosis of PO. These clinical evaluations were performed on six sites per tooth and on all of the patient's remaining teeth. This was done by the chief investigators according to preset schedule. Patients were categorized into periodontal healthy, gingivitis and stage I–IV periodontitis, as per the new classification of periodontitis as described by Chapple *et al*. [15].

After describing the study's purpose, each participant signed a consent form prior to sample collection. Each patient was informed to drop from the study at any time. Furthermore, data confidentiality was ensured throughout all the study.

Demographic and clinical data including age, gender, height, weight and comorbidities were collected through direct interview with each participant. The results of routine investigation including total leukocyte count (WBC), absolute neutrophil count, absolute lymphocyte count, serum levels of D-dimer and C-reactive protein (CRP).

Blood Samples

About 3 ml of venous blood were collected from each in plain tubes. Sera were separated for non-coagulated blood by

centrifugation, and kept at -20ºC until be used.

Serum level of MMP-2

A ready commercial kit (Cusabio/China) was used for measuring serum MMP-2 in serum samples base on quantitative Sandwich enzyme immunoassay technique. The manufacturer's protocol was followed precisely where the standards were prepared by serial 2-fold dilution. One hundred µL of standards and serum samples were added to the ascertain wells in the microplate, which then covered by adhesive strip and incubated for 2 hours at 37 ºC. The liquid was then removed and 100 µL of biotin antibody were added to each well with an incubation for 1 hour at 37 ºC. After liquid aspiration, the microplate was washed 3-time with washing buffer. One hundred µL of HPR-avidin were added to each well with an incubation and washing as in the previous step. The TMP-substrate (90 µL) was added to each well, and the plate was incubated at 37 ºC for 30 min. Finally, the stop solution (50 µL) was added to each well with a gentle mixing. The optical density was determined within 5 min using microplate reader at 450 nm.

Statistical Analysis

All statistical analyses were performed by the software Statistical Package for Social Science (SPSS/Chicago/ USA) version 25. A p-value of less than 0.05 was established as the level of significant. Numerical variables were presented as mean± standard deviation (SD) and analyzed with student t-test. Binomial variables were expressed as numbers and percentages and analyzed with Chi-square.

Results

Demographic characteristics of the study population.

One hundred and sixty patients with COVID-19 infection were separated into two groups: those who had severe symptoms, which included 42 patients (26.25%) were aged 57.38±8.41 years. The second group consists of 118 individuals who had mild COVID-19 infection and aged 53.19±9.18 years old, with significant difference between the two groups.

Males made up the bulk of the severe and mild groups (25.77% and 74.23%, respectively), with no significant difference. The BMI was roughly comparable between the two groups with no significant difference. Comorbidities were less common among patients with mild-severe than those with severe disease (26.27% vs. 47.62%) with a significant difference. In particular, hypertension and T2DM were far more common among severe cases (47.62% and 30.95%, respectively) than mild-moderate cases (27.95% and 15.25%, respectively) with significant differences (Table 1).

Table (1): Demographic features of the study population according to the severity of COVID-19 infection.

Variable	Severe infection (n=42)	Mild-moderate (n=118)	p-value
Age, years Mean±SD Range	 57.38±8.41 39-83	 53.19±9.18 35-72	 0.01*
Gender Male Female	 25(25.77%) 17(26.98%)	 72(74.23%) 46(737.02%)	 0.865
BMI, Kg/m^2 Mean±SD Range	 30.69±4.17 21.77-39.21	 30.0±3.23 21.2-39.84	 0.268
Comorbidities* No comorbidity Hypertension Diabetes mellitus IHD	 20(47.62%) 20(47.62%) 13(30.95%) 4(9.52%)	 81(26.27%) 33(27.97%) 18(15.25%) 4(3.39%)	 0.015* 0.027* 0.020* 0.208

*A patient can have more than one comorbidity, BMI: body mass index, IHD: ischemic heart disease

Data were expressed as mean±SD and range. Statistical test used were independent t-test for continuous variables and Chi square for categorical variables.

Peripheral Blood Profile and Inflammatory Markers

Data regarding blood profile and inflammatory markers were found to be non-normally distributed. Accordingly, these data were expressed as median and range, and analyzed with non-parametric Mann Whitney test. With regards to hematological parameters, the median total WBC and neutrophil count were significantly higher in severe cases (11.5× 10^9/L and 8.72× 10^9/L, respectively) than those with mild-moderate cases (8.85× 10^9/L and 7.22× 10^9/L, respectively). Likewise, D-dimer and CRP were significantly increase in severe cases (1233.5 ng/ml and 83.5 mg/L, respectively) than mild-moderate cases (803.5 ng/ml and 50.5 mg/L, respectively). Interestingly, the median serum level of MMP-2 in severe cases was 208.12 (range= 121.6-511 ng/ml) which was higher than that of mild-moderate cases (median= 196.33 ng/ml, range= 104-410.11 ng/ml) with a significant difference (Table 2).

Table (2): Association of peripheral blood profile and inflammatory markers with the severity of COVID-19

Variable	Severe infection (n=42)	Mild-moderate (n=118)	p-value
Total WBC × 10^9/L Median Range	 11.5 3.23-18.5	 8.85 2.72-17.2	0.010*
Neutrophil × 10^9/L Median Range	 8.72 3.69-16.7	 7.22 1.8-16.1	0.027*
Lymphocyte × 10^9/L Mean Range	 1.1 0.22-4.1	 0.93 0.22-4.1	0.310
D-dimer, ng/mL Median Range	 1233.5 100-16754	 803.5 127-12360	0.014*
CRP, mg/L Median Range	 83.5 12-228	 50.5 4-259	0.026*
MMP-2, ng/mL Median Range	 208.12 121.6-511	 196.33 104-410.11	0.041*

WBC: white blood cells, CRP: C-reactive protein, MMP: matrix metalloproteinase
Data were expressed as median and range. Statistical test used was Mann Whitney U test.

Incidence of Periodontitis According to COVID-19 Severity

Generally, the total incidence of PO among patients with COVID-19 was 13.75% (22 patients out of 160). Out of 42 severe cases, 10 (23.81%) had PO. On the other hand, 12 patients out of 118 mild-moderate cases (10.17%) developed PO. Statistically, there was a significant difference in the incidence of PO between the two group (Figure 1).

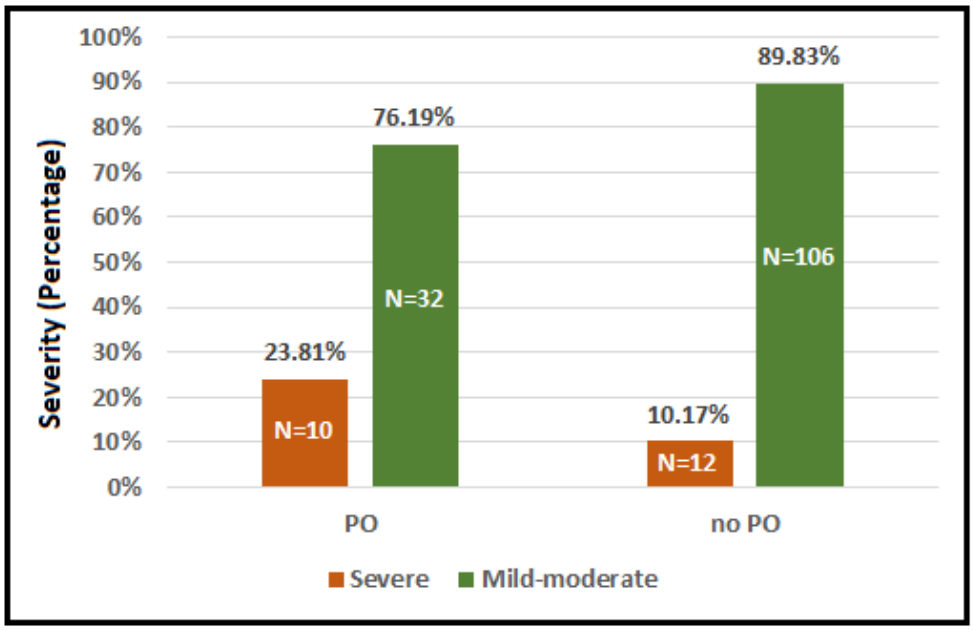

Figure (1): Incidence of PO according to COVID-19 severity

Association of Demographic Factors with the Incidence of PO

Only one demographic factor significantly associated with the development of PO. Patients with PO had older age than those without PO (57.91±8.93years vs. 53.72±9.08 years) with a significant difference. Although T2DM was more common among patients without PO than those with PO (21.74% vs. 4.55%), the difference was not significant (Table 3).

Table (3): Association of demographic features with periodontitis in patients with COVID-19.

Variable	With PO (n=22)	Without PO (n=138)	p-value
Age, years Mean±SD Range	 57.91±8.93 41-72	 53.72±9.08 35-83	 0.046*
Gender Male Female	 14(14.43%) 8(12.70%)	 83(85.67%) 55(87.30%)	 0.756
BMI, Kg/m^2 Mean±SD Range	 30.32±3.44 25.97-39.21	 30.16±3.53 21.2-39.84	 0.838
Comorbidities* No comorbidity Hypertension Diabetes mellitus IHD	 15(86.18%) 7(31.81%) 1(4.55%) 0(0%)	 86(62.32%) 46(33.33%) 30(21.74%) 8(5.8%)	 0.597 0.888 0.079 0.642

IHD: ischemic heart disease

Data were expressed as mean±SD and range. Statistical test used were independent t-test for continuous variables and Chi square for categorical variables.

Association of Peripheral Blood Profile and Inflammatory Markers with the Incidence of PO

None of the blood profile parameters were significantly different between COVID-19 patients with and without periodontitis. Although, serum levels of D-dimer, and CRP were higher in patients with than those without PO, the differences were not significant. On the reverse, the median MMP-2 level was significantly higher in patients with versus those without periodontitis (228.5ng/mL vs 193.81ng/mL) as indicated in table 4.

Table (4): Association of peripheral blood profile and inflammatory markers with the incidence of PO in patients with COVID-19

Variable	With PO (n=22)	Without PO (n=138)	p-value
WBC × 10^9/L Median Range	 11.5 3.23-18.5	 8.85 2.72-17.2	0.140
Neutrophil × 10^9/L Median Range	 8.85 3.69-16.7	 7.25 1.8-16.1	0.162
Lymphocyte × 10^9/L Mean Range	 1.1 0.22-4.1	 0.93 0.22-4.1	0.757
D-dimer, ng/mL Median Range	 1233.5 100-16754	 803.5 127-12360	0.151
CRP, mg/mL Median Range	 83.5 12-228	 50.5 4-259	0.666
MMP-2, ng/mL Median Range	 228.5 117-418	 193.81 104-511	**0.008**

WBC: white blood cells, CRP: C-reactive protein, MMP: matrix metalloproteinase
Data were expressed as median and range. Statistical test used was Mann Whitney U test.

Discussion

The impact of COVID-19 on the oral cavity has been controversial. While some evidence suggests a relevant role of the oral mucosa in the transmission and pathogenicity of SARS-CoV-2 [16], the exposure of oral disease as a risk of increased severity of COVID-19 has not been demonstrated. In the present study, older age and the presence of comorbidities were significantly associated with the severity of COVID-19. This is in accordance with many previous studies worldwide. A study by Ambrosino *et al*. [17] emphasized that age above 60 years is a risk factor for mortality and can be aggravated by the presence of chronic diseases such as hypertension, diabetes, cardiovascular diseases, hypercholesterolemia and obesity conditions. A recent meta-analysis found that about 31% of adult patients had comorbidities, with diabetes mellitus (10.4%) and coronary heart disease (8.5%). These comorbidities were found to be linked with the severity of COVID-19 [18]. The presence of comorbidities may interfere with immune response to the COVID-19 or affect the pulmonary circulation through overproduction of proinflammatory cytokines such as IL-6 and IL-1, and eventually exacerbated the infection.

Both total leukocyte count and absolute neutrophil count were significantly higher in patients with severe disease than those with mild infection in the present study. This association was previously demonstrated where mortality in COVID-19 can be independently predicted by peripheral blood neutrophilia as WBC and neutrophil counts in the deceased patients were substantially greater than those in the surviving patients [19].

Moreover, serum level of D-dimer and CRP was significantly higher in severe than mild cases. These results support the earlier studies in this regard. According to a systematic study by Rostami *et al*. [20],

the average D-dimer level was 0.58 g/ml in patients with mild disease and 3.55 g/ml in patients with severe disease. Another meta-analysis by Gungor *et al.* [21] revealed that patients with elevated D-dimer levels at the time of admission had a higher probability of mortality and a more severe illness. Higher CRP levels were linked to the development of ARDS, cardiac damage, and death in COVID-19 patients [19]. Serum CRP and ferritin levels were found to be considerably higher in died COVID-19 patients, indicating a poor composite outcome, according to Keski *et al.* [22].

An interesting finding in the present study was that MMP-2 was significantly increased in severe COVID-19 cases compared with mild-moderate infection. In a Brazilian study, Avila-Mesquita *et al.* [23] found that COVID-19 non-survivors had higher levels of MMP-2 compared with COVID-19 survivors. They explained such elevation by a higher activation of the renin-angiotensin system (RAS), which is critical in the pathophysiology of COVID-19 [24].

According to the result of the present study PO was more common among severe than those mild-moderate COVID-19 with a significant difference.

The majority of chronic inflammatory illnesses that are known to affect the severity of COVID-19 share risk variables with periodontitis [25]. There are a number of speculative mechanisms that could account for the high correlations between PO and COVID-19 severity. Takahashi *et al.* [26] hypothesized that aspiration of periodontopathic bacteria might aggravate COVID-19 by inducing the expression of ACE2, a receptor for SARS-CoV-2, and inflammatory cytokines in the lower respiratory. Additionally, it was assumed that the oral cavity, particularly periodontal pockets, could serve as a viral reservoir and that these bacteria might increase SARSCoV-2 pathogenicity by cleaving its S glycoproteins [27, 28]. Gupta and Sahni [29] proposed that neutrophil extracellular trap (NET) production is a common feature of both PO and COVID-19, and Sahni Gupta [30] assumed that COVID-19 cytokine storm may become worse due to the robust Th17 response in cases of severe periodontitis. The significant prevalence of periodontal lesions, particularly necrotizing periodontal disease (NPD), during COVID-19 may be predicted by all of these speculative pathways [31].

In the present study, the only factors that was significantly associated with both severe COVID-19 and PO (besides age) was the serum level of MMP-2. In fact, the present study is the first that linked the PO with COVID-19 severity through the increased production of MMP-2. As all included inflammatory markers did not differ significantly between patients with and without PO, it is reasonable to assume that these inflammatory parameters have no direct effect on oral cavities. Rather they induce mediators (like MMP-2) which in some way enhance the development of PO.

Conclusions:

Periodontitis is a frequent complication of severe COVID-19, and serum levels of MMP-2 rise significantly in severe cases of COVID-19 as well as cases complicated with PO compared with mild-moderate cases and cases without PO. Thus, the matrix metalloproteinase -2 could be considered as a link between severe COVID-19 and development of PO. Clinically, the inhibition of MMP-2 could be a promising treatment strategy in severe COVID-19 as well as the PO.

Ethical Clearance

The study was approved by Ministry of Health, Al-Shaab Specialized Dental Health Center Committee.

Conflict of Interest

The authors declare that they have no conflict of interest

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