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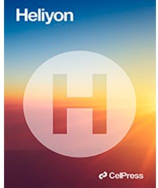

Research article

Association between dysbiotic perio-pathogens and inflammatory initiators and mediators in COVID-19 patients with diabetes

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A R T I C L E I N F O

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Inflammation
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A B S T R A C T

It has been suggested that a corona virus infection is linked to chronic periodontitis (COVID-19). Our objectives were to look at the expression of angiotensin-converting enzyme-2 (ACE2) in periodontal compartments containing periodontal infections to determine if ACE2 is directly or indirectly responsible for the inflammation in periodontal tissues getting worse. In this study, six non-COVID-19 periodontitis patients without diabetes served as controls, and 23 hospitalized periodontitis patients were admitted with PCR-confirmed COVID-19 with diabetes mellitus (Group 1/G1, n = 10), and without diabetes (Group 2/G2, n = 13). We evaluated the mRNA expression of ACE2, IL-6, IL-8, complement C3, and LL-37, as well as the relative proportion of *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Veillonella parvula* to represent the dysbiosis condition in periodontal microenvironment using subgingival plaque and gingival crevicular fluids (GCF) samples and quantitative real time PCR (qPCR). Every analysis was done to ascertain how they related to one another. The area under the curve (AUC) and receiver operating characteristic (ROC) curve were used to determine the sensitivity and specificity of inflammatory indicators. All the grouped patients had ACE2 detected, according to our findings, but only the G1 patients had a positive correlation ($p < 0.05$) between ACE2 expression and the inflammatory

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markers. The combination of IL-6 and C3 mRNAs was found to be 0.78 and 0.55 for the G1 group and the G2 group, respectively, based on the ROC and AUC values. According to our research, the relationship between complement C3 and IL-6 may be able to predict the degree of periodontal inflammation in COVID-19 patients who also have diabetes.

1. Introduction

Diabetes mellitus is one of the most prevalent comorbidities seen in COVID-19 patients, as evidenced by published research [1,2]. Diabetes and other disorders like hypertension are connected to the activation of the renin-angiotensin system in several tissues, despite the fact that the exact relationship between diabetes and COVID-19 is unknown [3].

Additionally, recent research has demonstrated a correlation between the severity and presence of COVID-19 infection and periodontitis [4,5]. Given that the oral cavity is home to the causative viruses of severe acute respiratory syndrome (SARS-CoV-2) and its receptor, angiotensin-converting enzyme-2 (ACE2) [6], we hypothesized that the conditions in the periodontal milieu would also be conducive to COVID-19. Thus, our goal was to investigate whether or not the subgingival marginal niche harbors SARS-CoV-2 infection determinants, as well as any potential links to periodontitis in the presence or absence of diabetic sequelae. In order to achieve this, we contrasted the prevalence of three distinct periodontal pathogens, *P. gingivalis*, *Fusobacterium nucleatum*, and *Veillonella parvula*, in the subgingival microflora. The percentage of major pathogens (*P. gingivalis*) [7], transcript levels of ACE2, and specific inflammatory determinants, such as complement C3, interleukins IL-6 and IL-8, and chronic periodontitis patients with or without diabetes, were analyzed in COVID-19-infected periodontitis patients. The association with the cathelicidin antimicrobial peptide LL-37 gene was also examined.

2. Methods

2.1. Selection of patients

The investigation complied with the requirements provided in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [8]. The study complied with the guidelines of the Medical Research on Human Subjects Act (reference: 0042/SKPE/KKO/2021/00). It was approved by the Ethical Review Committee of the Medical Ethics Committee of the University of Indonesia Hospital. Every sample process was completed in compliance with the committee's rules and guidelines.

Following the methods' explanation, the study only included participants who provided written consent. Data on the patient's age, gender, chronic history of concomitant conditions, clinical periodontitis symptoms, and COVID-19 was gathered. The selection criteria included being older than eighteen, having moderate to severe periodontitis [9], not having received periodontal therapy in the preceding year, and being pregnant or having received an antibiotic prescription during the previous six months. Reverse transcription-polymerase chain reaction (RT-PCR) was used to confirm the diagnosis of COVID-19 in periodontitis patients who were readmitted between June 15 and July 25, 2021, had no respiratory symptoms for at least two weeks, and had no sinus symptoms. Based on hospital medical data, patients were classified as either diabetic (G1) or non-diabetic (G2). Patients with periodontitis who attended the University Hospital of Indonesia's dentistry clinic were also chosen to serve as the control group.

Table 1
Primers used in this study.

Name	Primer sequence (5′-3′)	References
Bacteria	5′-ATAGTAGCGTGTCCGGCTTC-3′	[27]
P. gingivalis	5′-ATCGTAGGCGGATTGGAGA-3′	[27]
F. nucleatum	5′-TCCCAGCAAATGTTGGAAG-3′	[28]
V. parvula	5′-TTCATCATCAAATTCGTCATAGTCT-3′	[29]
Total bacteria	5′-GTAACAAAGGTGTCGTTTCTCG-3′	
Genes	5′-CGTAACATCTTCCGAAACTTTC-3′	
	5′-TTAAACTCAAAGGAATTGACGG-3′	
	5′-CTCACGACACGAGCTGACGAC-3′	
ACE2	5′-ACAGTCCACACTTGCCCAAAT-3′	[30]
IL-6	5′-TGAGAGCACTGAAGACCCATT-3′	[31]
IL-8	5′-ACAGCCACTCACCTCTTCAG-3′	[32]
C3	5′-CCATCTTTTTCAGCCATCTTT-3′	[33]
LL-37	5′-ATGACTTCCAAGCTGGCCGTGGCT-3′	[34]
GAPDH	5′-TCTCAGCCCTCTTCAAAAACTTCTC	[35]
	5′-GCTGCTCCTGCTACTAACCCA-3′	
	5′-AAAGGCAGTTCCCTCCACTTT-3′	
	5′-GTCACCAGAGGATTGTGACTTCAA-3′	
	5′-TTGAGGGTCACTGTCCCCATA-3′	
	5′-AATGGAAATCCCATCACCATCT-3′	
	5′-CAGCATCGCCCCACTTG-3′	

2.2. Assessment and gathering of samples

Ascertaining the precise course of tissue degradation or indicators of tissue damage in impacted areas is challenging; therefore, probing pocket depth (PPD) was established as a reference metric for attachment level [10]. Gingival crevicular fluids (GCF) samples were taken from diseased locations with PPD >3 mm, bleeding during probing, and bacterial sampling. Paper points were used to collect GCF, which was done by inserting them into the periodontal pocket for 2 min each time. Paper points were collected and immediately pooled into microcentrifuge tubes with 100 μL of phosphate buffer and RNAlater (Invitrogen, Carlsbad, CA, USA). These tubes were then maintained at −70 °C until additional analysis was conducted. A registered dentist evaluated periodontitis and collected samples from every patient in the hospital and dental clinic.

2.3. Poriodontal pathogen proportion in the subgingival microflora

Primers and quantitative real-time PCR (qPCR) were utilized to ascertain the relative amounts of *P. gingivalis*, *F. nucleatum*, and *V. parvula* in the periodontal microenvironment, as indicated in Table 1. Following the manufacturer's instructions, GCF samples were used to extract pathogens' deoxyribonucleic acid (DNA). The reagent used was GENEzolTM (General, Ltd., New Taipei City, Taiwan). With the use of the Qubit assay reagent (Invitrogen, Carlsbad, CA, USA), DNA concentration and quality were assessed. Before being processed further, DNA was dissolved in Tris-EDTA buffer and chilled to −20 °C. Using the ABI StepOnePlus Real-Time PCR Master Mix (Applied Biosystems) and the manufacturer's instructions, qPCR analysis was carried out in triplicate. Pre-denaturation thermal cycling settings included 95 °C for 10 min, followed by 40 repetitions of 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 15 s. Using the relative computation of the $2^{-\Delta\Delta Ct}$ method [11], the relative abundance of each bacteria was determined, with the control set at $2^{-\Delta\Delta Ct} = 1$.

2.4. Using qPCR to quantify the transcription of ACE2, IL-6, IL-8, C3, and LL-37

The goal of this work was to identify and measure the mRNA levels that corresponded to the genes encoding complement C3, LL-37, IL-6, IL-8, and ACE2. Following the manufacturer's instructions, RNA was extracted from GCF using the GENEzolTM reagent (General, Ltd., New Taipei City, Taiwan) and a reverse transcription kit (Applied Biosystems). Using particular primers, the resultant cDNA was subsequently amplified by qPCR (Table 1), and non-transcribed RNA samples were utilized as controls to check for contamination with genomic DNA. Every test was run in trplicate. Pre-denaturation PCR settings were 95 °C for 5 min, then 40 cycles of 95 °C for 10 s, 60 °C for 30 s, 72 °C for 30 s, and lastly, extension at 72 °C for 5 min. Lastly, the melting curve profile was 60 s at 60 °C, 15 s at 95 °C, and 15 s at 95 °C. The level of d-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used in this investigation to normalize gene expression of each target gene. Each target gene's mRNA expression in the GCFs of non-COVID-19 patients was employed as a control. Every result collected from the patient groups undergoing testing was normalized and contrasted with the control values.

2.5. Analysis of statistics

For statistical analyses, we utilized GraphPad PRISM 9.0 (GraphPad Software, San Diego, CA, USA), with $P < 0.05$ regarded as the significant value. One-way analysis of variance (ANOVA) test and unpaired Student's t-test were used to compare means between and within groups accordingly. Spearman's correlation coefficient (r) was determined for correlation analysis, and the line of best fit with 95 % confidence intervals was produced by linear regression. In addition, we employed receiver operating characteristic (ROC) curve analysis to ascertain the ideal threshold for highly specific and sensitive detection of inflammatory markers.

3. Results

A total of 29 periodontitis patients were involved in the study, with an age range between 45.1 ± 15.37-years-old and, 10 (43.5 %) were males, and 13 (56.5 %) were females (Table 2). Ten patients reported having diabetes mellitus type 2.

Table 2
The severity of periodontitis * The previous published Clinical Attachment Loss (CALL) scale was implemented to define the severity of periodontitis [9].

	Age (year)		Periodontitis (CAL)*	
Group	27-49	50-73	Moderate	Severe
Male	2	8	5	5
Female	8	5	11	2
Total	10	13	16	7

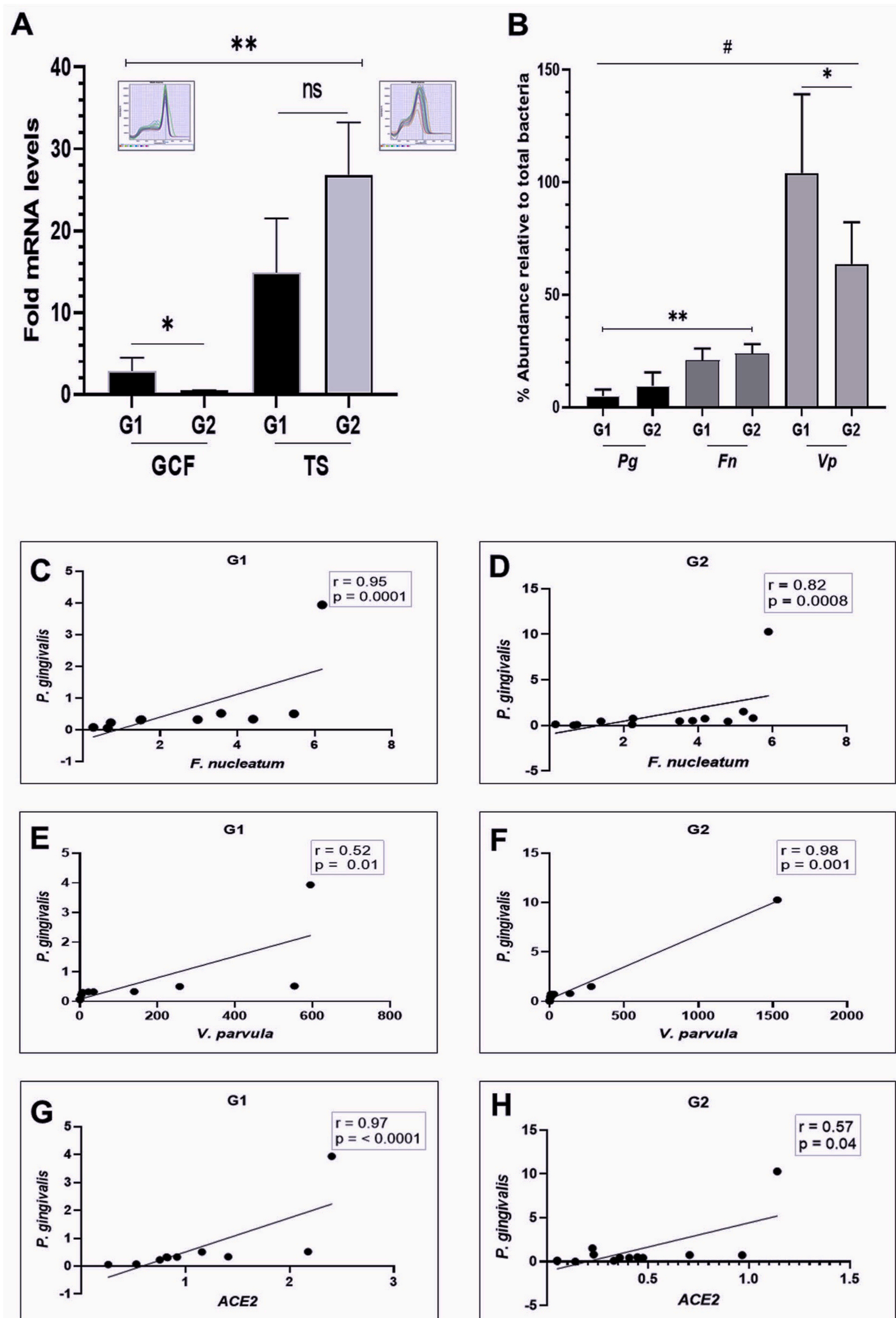

Fig. 1. *ACE2* expression, the proportion of *P. gingivalis* (Pg), *Fusobacterium nucleatum* (*Fn*), *Veillonella parvula* (Vp), and their relationship in different patient groups of COVID-19. The transcription level of ACE2 mRNA was higher in COVID-19 patients with (G1) diabetes than those without (G2) diabetes, but the reverse was found in tongue surface/TS (**A**). The proportion of Pg was found to be the lowest compared to the other two species (Fn and Vp). However, the relative abundance of Pg was not significant in either G1 or G2 patient group (**B**). In both groups, the correlations were

significant moderate (**E** and **H**) and strongly positive, **C**, **D**, **F**, and **G**). All data are expressed as mean ± SE. * significant difference in the gene expression within the group (G1 and G2). ** significant difference of the gene expression between GCF and TS or between *Pg* and *Fn*. ns = not a significant difference. # significant difference in the bacterial abundance *Vp* and *Pg* and *Fn*. The inserts denote the melting curve of qPCR in GCF (left) and TS (right) samples.

3.1. ACE2 mRNA levels and bacteria relative abundance

Our data showed that the ACE2 mRNA was able to be detected in GCF samples collected from all tested subjects (G1, G2, and control). On average, the transcription level of ACE2 was lower in the GCF than that in the tongue surface (TS) ($P < 0.05$). When the gene expression was compared between the G1 and G2, we found that the expression of ACE2 in GCF was significantly higher in the G1 by 2-fold ($P < 0.05$), but the difference was not significant in TS (Fig. 1A). In addition, all surveyed bacteria were detected in the subgingival microbiota in all patient groups. The qPCR results showed that in both G1 and G2, the proportions of *P. gingivalis* and *F. nucleatum* were comparable, whereas the proportion of *V. parvula* was significantly higher. Furthermore, the number of *P. gingivalis* detected in both groups was the lowest compared to the abundance of the other two bacterial species. The proportion of *F. nucleatum* and *V. parvula* was >30 % and >50 % higher than that of *P. gingivalis,* respectively (Fig. 1B).

The proportion of *P. gingivalis* and *F. nucleatum* in the G1 and G2 groups showed a strong positive correlation ($r = 0.95$, $P = 0.0001$, and $r = 0.82$, $P = 0.0008$) (Fig. 1C and D). In contrast, there were moderate ($r = 0.52$, $P = 0.01$) and strong ($r = 0.98$, $P = 0.001$) correlations between *P. gingivalis* and *V. parvula* in the groups (Fig. 1E and F). Finally, in G1 ($r = 0.97$, $P = 0.0001$) and G2 ($r = 0.57$, $P = 0.04$), respectively, there was a high and moderate linear positive connection between the transcription of ACE2 mRNA and the

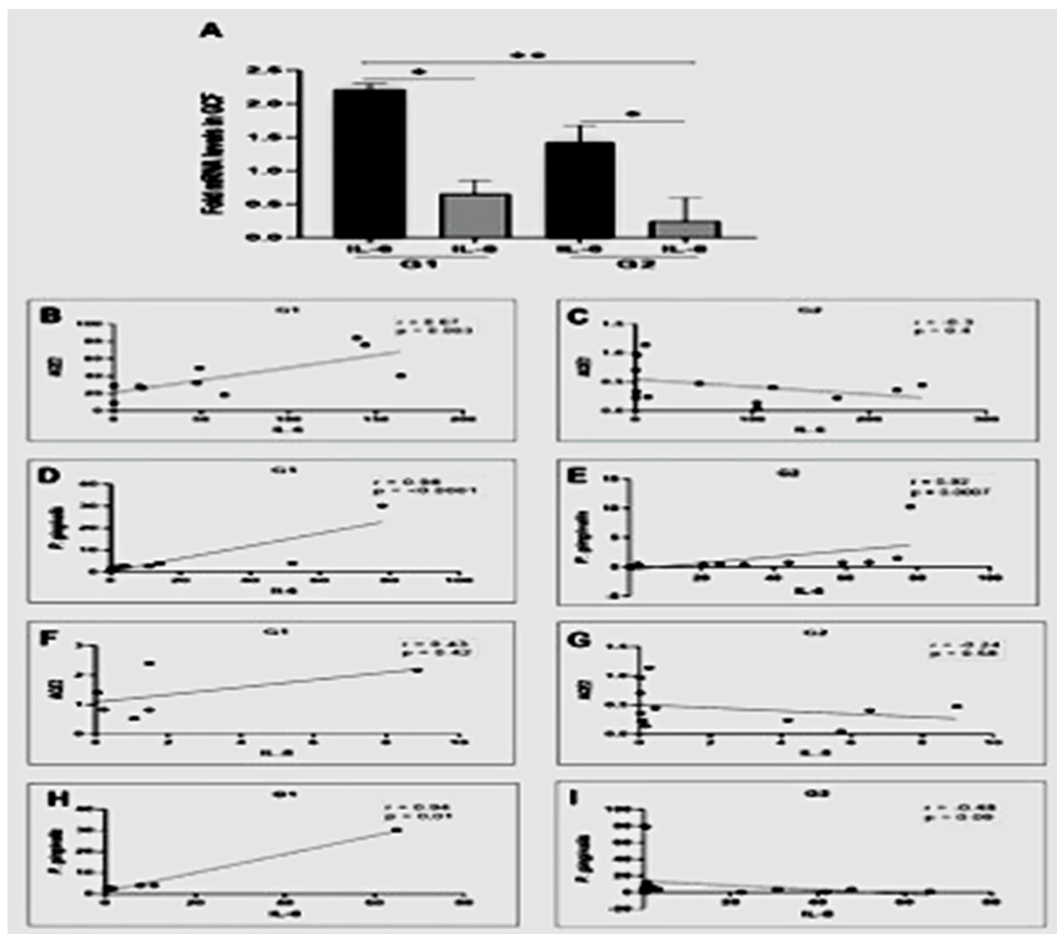

Fig. 2. Transcription levels of Il-6 and IL-8 genes and the correlation between the mRNA expression of *ACE2* or the proportion of *P. gingivalis* and IL-6/IL8 in gingival crevicular fluids (CGF) of COVID-19 patients with (G1) and without (G2) diabetes. In general, mRNA expression of Il-6 and Il-8 was higher in G1 than in G2 group. However, in either group, the transcription level of IL-6 was higher than IL-8 (**A**). This study shows, that in the G1 group, *ACE2* transcription level and *P. gingivalis* abundance show a strong positive correlation with the transcription levels of IL-6 (**B** and **D**). In G2 group, a negative correlation was observed between ACE2 and Il-6 transcripts (**C**), whereas a strong positive relationship was observed between *P. gingivalis* abundance and the transcription level of IL-6 (**E**). *$P < 0.05$ within groups, **$P < 0.05$ between group.

relative abundance of *P. gingivalis* (Fig. 1G and H).

3.2. The correlation between the relative concentrations of ACE2 and P. gingivalis and the IL-6 and IL-8 transcript levels

While only 6/10 (or 60 %) of the G1 group and 10/13 (or 77 %) of the G2 group had detectable amounts of IL-8, all GCF samples exhibited significant levels of IL-6. Statistical analysis revealed that there was a 2-fold increase in IL-6 gene expression in the G1 group compared to the G2 group, with a 1.5-fold increase ($P < 0.001$). The low levels of IL-8 mRNA expression in both groups were found to be comparable ($P > 0.05$) (Fig. 2A).

We also discovered correlations between ACE2 mRNA expression, IL-6 transcription levels, and *P. gingivalis* percentage. Specifically, we found that in G1, there was a strong positive significant correlation between the mRNA levels of the inflammatory cytokine IL-6 and ACE2 ($r = 0.67$, $P = 0.003$) and *P. gingivalis* ($r = 0.098$, $P = 0.0001$) (Fig. 2B and D). Conversely, in G2, there was a negative correlation between IL-6 and ACE2 mRNA levels ($r = -0.3$, $P = 0.4$). Conversely, a strong positive correlation was discovered between IL-6 mRNA and relative amounts of *P. gingivalis* ($r = 0.92$, $P = 0.0007$) (Fig. 2C and E).

For IL-8, a moderate positive correlation was found between ACE2 mRNA and IL-8 gene expression in G1, but the association was not significant ($r = 0.43$, $P = 0.42$) (Fig. 2F). The proportion of *P. gingivalis* and IL-8 mRNA transcription in G1 showed a substantial and significant positive association ($r = 0.94$, $P = 0.01$) (Fig. 2H), but ACE2 and IL-8 mRNA levels in G2 showed a negative correlation ($r = -0.24$, $P = 0.58$). A comparable correlation ($r = -048$, $P = 0.09$) was discovered between the levels of IL-8 mRNA and *P. gingivalis* infection. In G2, neither correlation was significant (Fig. 2G and I).

3.3. Correlation between P. gingivalis and ACE2 mRNA and transcription levels of C3 and LL-37

The levels of C3 mRNA expression were significantly different between the two groups ($P = 0.001$ and $P = 0.01$, respectively), with

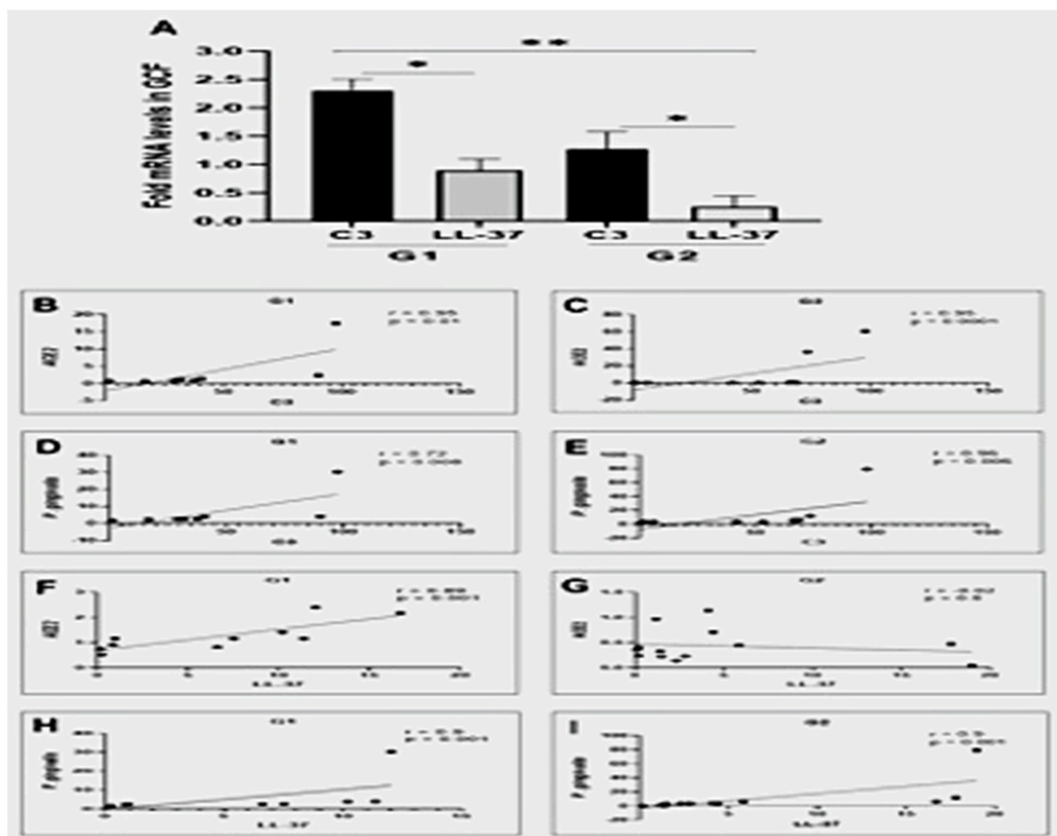

Fig. 3. Transcription levels of C3 and LL-37 genes and the correlation between the mRNA expression of *ACE2* or the proportion of *P. gingivalis* and C3/LL-37 in gingival crevicular fluids (CGF) of COVID-19 patients with (G1) and without (G2) diabetes. In general, mRNA expression of C3 and LL-37 was higher in G1 than in G2 group. However, in either group, the transcription level of C3 was higher than LL-37 (**A**). This study indicates, that in G1 and G2 groups, the transcription *ACE2* mRNA and *P. gingivalis* abundance show a strong positive correlation with the transcription levels of C3 mRNA (**B**, **C**, **D**, and **E**). For LL-37, its associations with *ACE2* mRNA or *P. gingivalis* abundance were strongly positive in G1 (**F** and **H**). In G2, the correlation was found to be negative, not significant with *ACE2*, but strong positive with *P. gingivalis* abundance (**G** and **I**). *$P < 0.05$ within groups, **$P < 0.05$ between groups.

the detection rate of C3 mRNA expression being 8/10 (80 %) in G1 and 10/13 (77 %) in G2. (Fig, 3A). Correlation analysis was done for G1 and G2, respectively, to further explore the relationship between ACE2 mRNA transcription levels, *P. gingivalis* bacterial counts, and C3 and LL-37 mRNA transcription.

Between ACE2 mRNA and C3 transcript levels in G1 ($r = 0.95$, $P = 0.01$) and G2 ($r = 0.95$, $P = 0.0001$), a significant positive connection was found. For *P. gingivalis*, a linear positive connection was found between their percentage and C3 transcript levels in the correlation analyses for G1 ($r = 0.72$, $P = 0.008$) and G2 ($r = 0.96$, $P = 0.006$), respectively. Finally, there was a considerable positive correlation between the relative expression of ACE2 mRNA and LL-37 transcription in G1 ($r = 0.89$, $P < 0.001$) but not a significant negative correlation in G2 ($r = -0.02$, $P = 0.6$). However, *P. gingivalis* abundance and LL-37 mRNA showed a substantial positive correlation in both the G1 and G2 groups ($r = 0.9$, $P = 0.001$) (Fig. 3B–I).

Based on these findings, ROC curves were used to assess the accuracy of the combination of IL-6 mRNA and C3 mRNA, with AUC values of 0.78 and 0.55 in the G1 and G2 groups, respectively (Fig. 4A and B).

4. Discussion

With this work, we first demonstrated that all of the subjects in the study had subgingival niche expression of the functional receptor for SARS-CoV-2-associated molecule (ACE2). The ACE2 transcription in tongue epithelial cells verified this result [6]. Our research thus shows that SARS-COV-2 may infiltrate periodontal tissue, resulting in the distinct features of the periodontal microenvironment. This finding corroborates earlier studies that the virus can multiply and infect oral structures directly, including periodontal tissue [12,13].

The periodontal tissue of our subjects was probably exposed to SARS-CoV-2 infection because there is a correlation between ACE2 expression and susceptibility to the virus [14]. Furthermore, while assessing the percentage of specific periodontal infections, we discovered that *P. gingivalis*, *F. nucleatum*, and *V. parvula* were identified in the periodontal niche of every participant in this investigation (G1, G2, and control). The findings point to keystone species (*P. gingivalis*) that is significant for periodontitis or other species that were previously present in preceding clinical conditions, such as *Veillonella* spp. [15,16].

Our findings demonstrated a favourable correlation between the lowest *P. gingivalis* abundance with comparatively larger promotes of *F. nucleatum* and *V. parvula* in both studied groups. This correlation suggests that *P. gingivalis* needs another microbiota to cause periodontitis because it is a keystone pathogen [7]. Given that SARS-CoV-2 has been found in GCF, it is possible that the aggressive pathogenic subgingival flora in our COVID-19 patients also includes the virus, given the significant correlations shown between the *P. gingivalis* fraction and the expression of ACE2 mRNA [17].

Studies conducted in vitro have indicated that *P. gingivalis* plays a role in increasing the expression level of ACE2 [18]. Furthermore, our findings supported the findings of an in vitro investigation that revealed ACE2 was upregulated in alveolar epithelial cells following exposure to *F. nucleatum* [19]. It was unable to conclude from the current investigation whether percentage of the species found in the subgingival microbial population was responsible for the pathogenesis or replication of SARS-CoV-2. Nonetheless, we

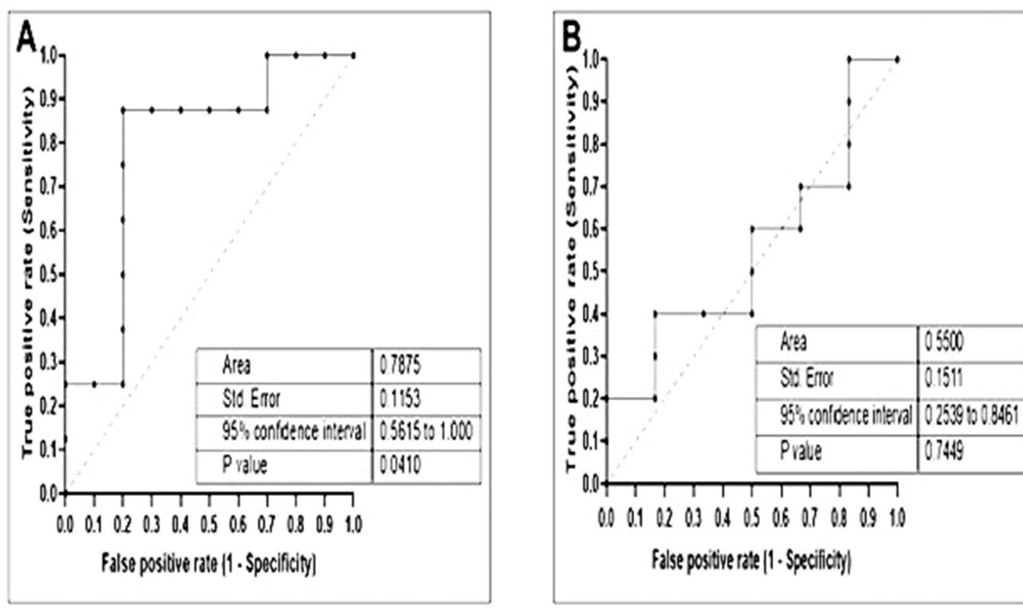

Fig. 4. Receiver operating characteristic curve (ROC) showing the plot and the best cut-off point of the relationship between IL-6 and C3. The combination of IL-6 and C3 mRNA expression could discriminate the aggressive of periodontitis in COVID-19 patients with diabetes (**A**) (area under curve/AUC, 0.78; $P = 0.04$) and without diabetes (**B**) (area under curve/AUC, 0.5; $P = 0.7$).

hypothesized that the periodontal niche's inflammatory process would affect how the virus (SARS-CoV-2) enters the body. Furthermore, the presence of SARS-CoV-2 in the periodontal pocket may be explained by the percentage of *P. gingivalis* and the low expression of ACE2 in GCF (compared to TS).

Periodontitis is caused and progressed by *P. gingivalis* and other dysbiotic-related bacteria. We found that its low proportion is consistent with the presence of infection and the inflammatory reactions that go along with it. Interestingly, we discovered that in the GCF of COVID-19 patients with and without diabetes, respectively, IL-6 and C3 were present at high (>2-fold) and intermediate (>1-fold) levels. The transcription levels of IL-6 vs. IL-8 and C3 vs. LL-37 also showed the similar pattern in both patient groups, with mRNA expression of IL-6 and C3 being usually greater in COVID-19 patients with diabetes. Since *V. parvula* has been demonstrated to promote the production of this proinflammatory cytokine, the elevation of IL-6 is consistent with the larger proportion of this species [20]. Furthermore, there was a positive correlation seen between low levels of IL-8 or LL-37 mRNA expression and both ACE2 transcription levels and *P. gingivalis* abundance. The degree of bacterial infection in periodontal environments may be indicated by this association [21]. Particularly in COVID-19 patients with diabetes, SARS-CoV-2 and *P. gingivalis* may paralyze the local production of chemokine (IL-8) due to the presence of mixed bacteria, specifically *F. nucleatum* and *V. parvula* [22]. In fact, the reduced expression of antimicrobial peptide (LL-37 mRNA) in our results suggested a more severe bacterial infection of periodontitis.

It is challenging to evaluate the periodontal status during COVID-19 infection, which is typically supported by radiographic data to define the bone level. In these cases, pathological characteristics are crucial variables. Thus, in individuals suffering from periodontal disease, the cytokine IL-6 functions as a biomarker [23]. On the other hand, complement C3 is an important inflammatory factor that makes infections persist in the microbial population because it causes a damaging inflammatory response in the periodontal tissue [24]. Additional information is provided by our data, which show a substantial link between the dysbiotic-related keystone bacterium *P. gingivalis* and the increase of C3 mRNA expression and IL-6, two markers of inflammatory amplification in the periodontal niche. We observed that the over expression of ACE2 mRNA and the comparatively reduced abundance of *P. gingivalis* in the periodontal milieu of COVID-19 patients with diabetes were positively correlated with the transcription levels of both IL-6 and C3 in the periodontal niche. Assume that the transcription levels of ACE2 are positively correlated with the associated proteins' expression. Our results therefore suggest that the activation of ACE2 in a milieu of dysbiosis (periodontal niches) in response to the presence of SARS-CoV-2, which in turn allows the virus to infect cells and accelerate the release of inflammatory mediators such as C3 and IL-6. Similar biological phenomena have been reported for dysbiosis of the gut microbiota [25]. Therefore, in order to increase the prediction accuracy of the combination of IL-6 and C3 markers in the G1 and G2 groups, it is required to determine the sensitivity and specificity to acquire the most informative features. Therefore, we discovered that in COVID-19 patients with diabetes, both IL-6 and C3 mRNA expression had a better predictive value for periodontitis activity based on ROC curve analysis. Given that severe COVID-19 infection has been shown to be significantly predicted by IL-6 levels [26]. Our hypothesis is that the underlying mechanism of periodontal disease activity in COVID-19 patients with diabetes is a synergistic interaction between IL-6 and complement C3. These findings suggest that, in COVID-19 patients with diabetes, complement C3 may be a somewhat accurate indicator of periodontal inflammation and disease severity. Nonetheless, it is believed that SARS-CoV-2, which is present in pathogenic bacterial periodontal compartments, contributes indirectly to the degeneration of periodontal tissue. While the current investigation did not allow us to assess the role of IL-6 and C3 as protective or pathogenic associated mediators in our COVID-19 participants, both inflammatory markers are probably similar in their ability to predict the activity of periodontal disease in patients infected with SARS-CoV-2.

5. Conclusion

The transcription levels of inflammatory markers (IL-6 and C3) in the periodontal microenvironment may be able to stabilize the inflammatory process in COVID-19 patients with diabetes and periodontitis, both locally and systemically. Therefore, while keeping an eye on the health of COVID-19 patients, these inflammation markers may be used to differentiate between active and non-active periodontitis states.

There are inherent limitations to our investigation. Firstly, it is challenging to rule out all possible confounders in an observational study. As a result, the degree to which some variables influence the outcome prediction may be overstated. Second, because there were so few participants in the study, we were unable to determine if our COVID-19 patients had already experienced the changed microbial composition in the periodontal niche at the time GCF samples were taken. Rather than using actual levels, we used the relative abundance of each targeted microbe as a fraction in our investigation. Lastly, while GCF is a direct indicator of the serum immune response [17], the findings of this investigation do not support a direct correlation between the degree of inflammatory determinants in GCF and the systemic inflammatory mechanism of COVID-19. Nonetheless, given the limitations of our investigation, our findings may contribute to a better understanding of the fundamental relationship between COVID-19 and periodontal disease.

Author Contribution

Hari - Sunarto: Resources, Validation, Visualization. Yuniarti - Soeroso: Investigation, Supervision. Boy Muchlis Bachtiar: Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. Ardiana - Kusumaningrum: Data curation, Resources. Irandi Putra Pratomo: Investigation, Supervision. Benso - Sulijaya: Project administration, Software, Validation. Citra Fragrantia Theodorea: Data curation, Project administration. Efa - Apriyanti: Data curation, Investigation. Shahida Mohd Said: Conceptualization, Writing – review & editing. Endang Winiati Bachtiar: Conceptualization, Methodology, Writing – review & editing. Yudhistira -.: Investigation, Validation. Defi - Efendi: Conceptualization, Writing – review & editing

Data availability

The data that support the findings of this study are available from the Oral Science Research Centre, Faculty of Dentistry Universitas Indonesia, Jakarta Indonesia. Data are available from the corresponding authors upon reasonable request and permission of Dental Centre Universitas Indonesia Hospital.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] O.H. Al-Hussain, Complications and comorbidities in COVID-19 patients: a comparative study, Cureus 14 (8) (2022) e28614.
[2] A. Sanyaolu, et al., Global pandemicity of COVID-19: situation report as of June 9, 2020, Inf. Disp. 14 (2021) 1178633721991260.
[3] R.C.W. Ma, R.I.G. Holt, COVID-19 and diabetes, Diabet. Med. 37 (5) (2020) 723–725.
[4] Z.T. Bloomgarden, Diabetes and COVID-19, J. Diabetes 12 (4) (2020) 347–348.
[5] N. Marouf, et al., Association between periodontitis and severity of COVID-19 infection: a case-control study, J. Clin. Periodontol. 48 (4) (2021) 483–491.
[6] H. Xu, et al., High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa, Int. J. Oral Sci. 12 (1) (2020) 8.
[7] G. Hajishengallis, et al., Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement, Cell Host Microbe 10 (5) (2011) 497–506.
[8] E. von Elm, et al., The strengthening the reporting of observational studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies, Int. J. Surg. 12 (12) (2014) 1495–1499.
[9] P.N. Papapanou, et al., Periodontitis: consensus report of workgroup 2 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions, J. Periodontol. 89 (Suppl 1) (2018) S173–S182.
[10] L.F. Zhuang, et al., Subgingival microbiota of Sri Lankan tea labourers naive to oral hygiene measures, J. Clin. Periodontol. 41 (5) (2014) 433–441.
[11] B. Navidshad, J.B. Liang, M.F. Jahromi, Correlation coefficients between different methods of expressing bacterial quantification using real time PCR, Int. J. Mol. Sci. 13 (2) (2012) 2119–2132.
[12] J. Amorim Dos Santos, et al., Oral manifestations in patients with COVID-19: a 6-month update, J. Dent. Res. 100 (12) (2021) 1321–1329.
[13] D.A. Brandini, et al., Covid-19 and oral diseases: crosstalk, synergy or association? Rev. Med. Virol. 31 (6) (2021) e2226.
[14] C.H. Mazucanti, J.M. Egan, SARS-CoV-2 disease severity and diabetes: why the connection and what is to be done? Immun. Ageing 17 (2020) 21.
[15] S.J. Leishman, H.L. Do, P.J. Ford, Cardiovascular disease and the role of oral bacteria, J. Oral Microbiol. 2 (2010).
[16] M.J. Mammen, F.A. Scannapieco, S. Sethi, Oral-lung microbiome interactions in lung diseases, Periodontol 83 (1) (2000) 234–241.
[17] S. Gupta, et al., SARS-CoV-2 detection in gingival crevicular fluid, J. Dent. Res. 100 (2) (2021) 187–193.
[18] K. Sena, et al., Altered expression of SARS-CoV-2 entry and processing genes by Porphyromonas gingivalis-derived lipopolysaccharide, inflammatory cytokines and prostaglandin E(2) in human gingival fibroblasts, Arch. Oral Biol. 129 (2021) 105201.
[19] Y. Takahashi, et al., Expression of the SARS-CoV-2 receptor ACE2 and proinflammatory cytokines induced by the periodontopathic bacterium Fusobacterium nucleatum in human respiratory epithelial cells, Int. J. Mol. Sci. 22 (3) (2021).
[20] B. van den Bogert, et al., Immunomodulatory properties of Streptococcus and Veillonella isolates from the human small intestine microbiota, PLoS One 9 (12) (2014) e114277.
[21] O. Turkoglu, et al., Gingival crevicular fluid levels of cathelicidin LL-37 and interleukin-18 in patients with chronic periodontitis, J. Periodontol. 80 (6) (2009) 969–976.
[22] J. Brunner, et al., The capsule of Porphyromonas gingivalis reduces the immune response of human gingival fibroblasts, BMC Microbiol. 10 (2010) 5.
[23] R.P. Darveau, Bacteria modulate host-cell responses by capitalizing on the lipid raft structure, Future Microbiol. 4 (2) (2009) 155–157.
[24] T. Maekawa, et al., Genetic and intervention studies implicating complement C3 as a major target for the treatment of periodontitis, J. Immunol. 192 (12) (2014) 6020–6027.
[25] M.C. Howell, et al., SARS-CoV-2-Induced gut microbiome dysbiosis: implications for colorectal cancer, Cancers 13 (11) (2021).
[26] M. Aziz, R. Fatima, R. Assaly, Elevated interleukin-6 and severe COVID-19: a meta-analysis, J. Med. Virol. 92 (11) (2020) 2283–2285.
[27] I. Tomas, et al., Quantification by qPCR of pathobionts in chronic periodontitis: development of predictive models of disease severity at site-specific level, Front. Microbiol. 8 (2017) 1443.
[28] I. Mashima, et al., Identification of Veillonella species in the tongue biofilm by using a novel one-step polymerase chain reaction method, PLoS One 11 (6) (2016) e0157516.
[29] C.M. Sedgley, et al., Quantitative real-time PCR detection of oral Enterococcus faecalis in humans, Arch. Oral Biol. 50 (6) (2005) 575–583.
[30] W. Sakaguchi, et al., Existence of SARS-CoV-2 entry molecules in the oral cavity, Int. J. Mol. Sci. 21 (17) (2020).
[31] S. Salemi, et al., Detection of interleukin 1beta (IL-1beta), IL-6, and tumor necrosis factor-alpha in skin of patients with fibromyalgia, J. Rheumatol. 30 (1) (2003) 146–150.
[32] Y.K. Huang, et al., IL-8 as a potential therapeutic target for periodontitis and its inhibition by caffeic acid phenethyl ester in vitro, Int. J. Mol. Sci. 22 (7) (2021).
[33] K. Li, et al., Expression of complement components, receptors and regulators by human dendritic cells, Mol. Immunol. 48 (9–10) (2011) 1121–1127.
[34] L. McMahon, et al., Vitamin D-mediated induction of innate immunity in gingival epithelial cells, Infect. Immun. 79 (6) (2011) 2250–2256.
[35] M.S. Zhang, F.W. Niu, K. Li, Proflavin suppresses the growth of human osteosarcoma MG63 cells through apoptosis and autophagy, Oncol. Lett. 10 (1) (2015) 463–468.