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Identifying Collagenase (MMP-1, -8, -13) Expression and Correlation with Periodontitis Progression Using the Rat Model

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ABSTRACT

Collagenase (MMP-1, -8, and -13) is one of the groups of the matrix metalloproteinases (MMPs) that is responsible for the breakdown of collagen, particularly type-I collagen, which is found in profusion in the extracellular matrix (ECM). It is essential to understand the role of a group of biomarkers in the progression of periodontal disease. This study aims to evaluate the expression of MMP-1, -8, and -13 combined in the periodontitis progression induced by wire ligation and *Enterococcus faecalis* inoculation using the rat model. Twelve rats were allocated uniformly between the control group 0-day, experimental group 7- and 14-days. Orthodontic wire (0.2 mm) was placed between the proximal space of the right upper first and second molar tooth area and 0.5 µl of 1.5×10^8 cfu/ml. Rats in the experimental groups received an injection of *E. faecalis* suspension into their gingival sulcus. After the respective induction time, the rats were euthanised. Gingival tissue and maxillary jaw samples were obtained from all rats for quantitative real-time PCR and histological examination. The results showed a significant increase in mRNA expression within the tissue samples from the gingiva of MMP-1 ($p < 0.05$), -8 ($p < 0.01$), and -13 ($p < 0.01$) in 7 days as compared to the control. The MMP-8 expression levels were also significantly reduced ($p < 0.05$). Histological analysis showed a higher inflammatory cell infiltration and the presence of osteoclast in the 7 days, which was reduced in the 14 days. MMP-1, -8, and -13 levels were positively correlated with the presence of inflammatory cells. Therefore, identifying a group of collagenases might be a useful biomarker to detect the progression of periodontitis.

Keywords: Collagenase, matrix metalloproteinase, MMP-1, MMP-8, MMP-13, periodontitis

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INTRODUCTION

The gingiva, periodontal ligament, radicular cementum, and alveolar bone around the teeth are all affected by periodontal disease, a pathological condition (Beck et al., 2020). According to the World Health Organization (WHO), severe periodontitis appears to be the sixth most common disease worldwide, affecting around 10% of the global population (Jacob, 2012). The estimated disability-adjusted life year (DALY) for severe periodontitis was stated to be relatively high, ranking at number 77 (Dom et al., 2016). In Malaysia, moderate and severe periodontitis incidence was 30.3 and 18.2%, respectively, based on the National Oral Health Survey for adults. The disease is more prevalent in the adult population, while adolescents are less likely to be affected. Subgingival microbiota, a specific periodontopathogenic bacteria and host immune-inflammatory response are accountable towards the progression of the disease (Preethanath, 2020). In addition, more than 700 species of oral microorganisms are equally responsible for the disease progression (de Molon et al., 2016). *Enterococcus faecalis* is a facultative, Gram-positive microorganism and an important bacterium in endodontic infection, though it is less important as a periodontal pathogen. However, an association between *E. faecalis*-induced endodontic lesion and periodontal disease has been demonstrated in several studies (Alghamdi & Shakir, 2020; Souto et al., 2006). Bacteria in infected gingival tissue around the teeth cause inflammation by breaking down the barrier between the gingiva and the underlying connective tissue. After bacteria enter the sites, their byproducts cause periods of inflammation exacerbation and remission, which increases the generation of MMPs and proinflammatory cytokines (Ramadan et al., 2020).

Collagenase (MMP-1, -8, and -13) is one of the most significant MMP groups. It breaks down collagen, particularly type-I collagen, in the ECM (Khuda et al., 2021). Collagenase group member MMP-1 is sometimes referred to as fibroblast collagenase, interstitial collagenase, and collagenase 1. Most periodontal tissue matrix is made up of MMP-1, which is typically expressed by fibroblasts, osteoblasts, keratinocytes, macrophages, endothelial cells, platelets, chondrocytes, and tumour cells (Pirhan et al., 2008). Collagenase 2, or neutrophilic collagenase, another name for MMP-8, plays a key contributor in the aetiology of periodontal disease. The key host cell-derived collagenase generated from neutrophils causes gingival and periodontal collagen to break down and tissue destruction (Kraft-Neumärker et al., 2012). Osteoclastic activities, such as bone resorption and destruction, are carried out by MMP-13, which is also known as collagenase 3 (Checchi et al., 2020).

In the periodontal inflammatory process, microorganisms initiate an inflammatory and immune response that leads to tissue damage, particularly in susceptible hosts. Therefore, there is a lot of interest in identifying, validating, and clinically using biomarkers for periodontal disease. Early identification is essential for improving the management of the condition since periodontal disease is a global health problem linked to several systemic disorders (Hajishengallis & Chavakis, 2021). Numerous studies have pointed

to MMP-8 as a prognostic marker for periodontal health and disease (Rathnayake et al., 2017). Furthermore, an MMP-8 point-of-care chairside test kit has also been developed to diagnose periodontal disease. Combining biomarkers can be useful for better diagnostic performance than just a single biomarker. It is crucial to comprehend the part played by this set of biomarkers in developing periodontal disease to improve diagnostic procedures. This study aims to assess the expression of the MMPs-1, -8, and -13 combined in the periodontitis progression by wire ligation and *E. faecalis* inoculation using the rat model.

MATERIALS AND METHODS

Animals

The Universiti Kebangsaan Malaysia Animal Ethical Committee (UKMAEC) and the National Institutes of Health (NIH) both gave their approval for the animal studies to be carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (FD/2018/NURRUL SHAQINAH/28-NOV./967-NOV.-2018-JAN. -2020). The Sprague Dawley rats used in this study were grown under specific pathogen-free settings in the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia, maintained in the standard environment (25 ± 1°C, 55% humidity, 12–12 days–night pattern) with water and food provided ad libitum. Before the experiment, four rats were housed in a cage and given a week to get used to the environment. Twelve male rats, weighing about ~180 g and aged six weeks old, were divided into 0-day (control), 7- and 14-days (experimental) groups. This study followed the ARRIVE 2.0 reporting guidelines (du Sert et al., 2020). The rats were weighed on 0 day and every 3 days throughout the experimental schedule. The degree of freedom used in the analysis of variance (ANOVA) computation to determine sample sizes has an "E" value that must be between 10 and 20 (E = Total number of animals – Total number of groups) (Ilyas et al., 2017). Rats were assigned 4 per group, with no losses of the rats from the groups at the end of the experiment. Two investigators blinded to treatments evaluated the rats for at least one hour each day for behavioural and physical symptoms of animal welfare, and the rats remained within acceptable, humane endpoint criteria, with no indication of pain or discomfort outside of the experimental stimulus.

Experimental Procedure

An intraperitoneal injection of a 10% ketamine (100 mg/kg, Ketamil, Australia) and a 2% xylazine® (Pharmika India Pvt. Ltd., India), (10 mg/kg) body weight combination was used to administer general anaesthesia (Davis, 2001). Anesthesia was installed in 4–5 min after administration. The sterile endodontic file #8, #10 (Dentsply Sirona, USA) was carefully placed between the interdental spaces of the upper right first and second molar teeth. The gentle push and pull movements were repeated a few times to make a space without damaging the gingival tissue (Li et al., 2020). Then, using a needle holder, a 0.2 mm

sterile orthodontic wire with a length of 5 mm was bent into the shape of a "(" and gently placed into the space. The procedure was performed carefully to prevent any damage to the tissue. An oral examination was performed twice a week to check the wire placement.

Enterococcus faecalis strain was obtained from the American Type Culture Collection® (ATCC 29212, USA). The strain was cultured in an anaerobic chamber at 37°C for 24 hr using a brain heart infusion (BHI, Oxoid, United Kingdom) agar medium. The bacterial solution was prepared using sterile PBS in 1.5×10^8 cfu/ml bacteria and standardised using McFarland standards. A Hamilton syringe (Hamilton, USA) was used to carefully inject 0.5 µl of the bacterial inoculation once a week into the gingival sulcus of the upper right first and second molar region over the respective induction period after the insertion of a ligature wire.

Sample Collection

Rats were euthanised at 0-, 7-, and 14-days post-induction by using a mixture of 10% ketamine (200 mg/kg) and 2% xylazine (20 mg/kg) followed by cervical displacement. The control group (0 day) was sacrificed after the 14-day induction period. A gross examination of the oral cavity and the general appearance of the rats was performed. Tissue samples from the gingiva around the molar teeth area were obtained for RNA and DNA extraction. The RNA and DNA extraction samples were rinsed with a cold, sterile saline solution and immediately kept at -80°C until further use. Instantly after being extracted from the maxillary jaw for histopathological analysis, the samples were preserved in 10% neutral buffered formalin (Leica, USA) for at least 48 hr.

RNA and DNA Extraction

The 20 mg of samples from gingival tissue were used to extract and purify total RNA using the Innu PREP® RNA Mini Kit 2.0 (Analytik Jena, Germany). Reverse mRNA transcription to cDNA was performed using a High-quality ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, Japan) according to protocol. Using the Nucleospin® DNA extraction kit (Macherey-Nagel, Germany), total DNA was extracted and purified from 25 mg of gingival tissues in accordance with the manufacturer's guidelines. The quantity and integrity of the extracted total RNA and DNA was measured by a NanoDrop (ND-2000, Thermo Fisher Scientific, USA). The study did not include RNA or DNA samples with 260/280 ratios greater than 1.8.

RT-qPCR Assay

Quantitative real-time PCR (RT-qPCR) was carried out using a ready-to-use 2× concentration of ChamQ Universal SYBR® qPCR master mix (Vazyme, China). The master mix contains dNTPs, Mg^{2+}, Champagne Taq DNA polymerase, and Specific ROX reference

dye. The 10 µl real-time PCR reactions consisted of 5 µl master mix, 0.5 µl of reverse and forward primers, cDNA template of 1 µl, and RNase-free water of 3 µl. The tubes were sealed and briefly centrifuged to remove all air bubbles. The specific gene sequence was amplified using a CFX96 Connect™ RT-PCR Thermal cycler (Bio-Rad, USA). A two-step amplification protocol for collagenase and inflammatory cytokines was set, as shown in Table 1. The primer sequences and their specific qPCR settings are listed in Table 1. All reactions were prepared in triplicate, and any contamination presence was determined by running a no-template control, which contained the reaction mixture without template DNA with every qPCR run. Melting peaks were used to determine PCR specificity. Results were normalised using the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and data were evaluated using the $2^{-\Delta\Delta CT}$ technique (Livak & Schmittgen, 2001).

Table 1
Primer sequence for quantitative real-time polymerase chain reaction assays

Gene name	5'-3' primer sequence	Annealing temperature (°C)	Amplification cycle	Reference
MMP-1	Forward ACAACTGCCAAATGGGCTTGA	60, 30s	40	Hirate et al. (2012)
	Reverse CTGTCCCTGAACAGCCCAGACTTA			
MMP-8	Forward TCCTTGCCCATGCCTTTCAA	60, 30s	40	Matsui et al. (2011)
	Reverse CCAAACTATGCTTACAGAGAACCC			
MMP-13	Forward AGAAGTGTGACCCAGCCCTATC	65, 30s	32	Matsui et al. (2011)
	Reverse GCATACGAGCATCCATCCCGA			
Enterococcus faecalis	Forward GGAATTGTTCTTGCATCCGT	60, 30s	40	Liu (2011)
	Reverse ACAATTAAGTATTCTACGCC			
GAPDH	Forward TGCTGGTGCTGAGTATGTCG	60, 30s	40	Kuo et al. (2019)
	Reverse ATTGAGAGCAATGCCAGCC			

Note. MMP = Matrix metalloproteinases; GADPH = Glyceraldehyde-3-phosphate dehydrogenase

Histological Examination

Tissue samples from the maxillae were collected and processed for histological analysis. Soft tissues that would be examined histopathologically were fixed instantly and immersed in 10% buffered formalin for at least two days. Following a 21-day decalcification period in 10% buffered ethylenediaminetetraacetic acid (EDTA, Sigma-Aldrich, Germany) solution, the maxillary specimens were sectioned into 5 μm and subjected to Hematoxylin and Eosin staining (H&E staining, Leica, USA) in accordance with established processes.

Analysis of Inflammatory Cells

All the tissue sections were examined under a microscope at magnifications of 4×, 10×, and 40×, and the Image-Pro Plus system (Media Cybernetics, USA) digitally captured the images — the distinctive morphology of inflammatory cells (nutrophils, macrophages, lymphocytes) allowed for their identification. Only whole, clearly blue-stained inflammatory cells were counted. The inflammatory cells were counted using the ImageJ software bundled with Java 1.8.0_172 and presented in a bar graph. The histopathology results were qualitatively reported, and the group descriptions were contrasted.

Statistical Analysis

On day 0 and at the conclusion of the experimental period, body weight was analysed through a one-way ANOVA. The data were presented as mean values with either standard deviation (SD) or standard errors (SE), and these analyses were carried out using IBM SPSS data editor version 23.0 (IBM, USA). If a statistically significant difference was observed, a pairwise multiple comparison test (Tukey post-hoc) was executed to assess distinctions among the groups. The RT-qPCR fold change results were logarithmically transformed, and statistical analysis was performed. The ANOVA was employed to assess distinctions in group means, and a Tukey post-hoc test was utilised to identify which means differed significantly from the other groups. In the current study, *P* values less than 0.05 were accepted as statistically significant. The Pearson correlation was performed to evaluate the correlation between collagenase (MMP-1, -8, -13) expression and inflammatory cell count.

RESULTS

Body Weight Measurement

The mean body weight of all the rats at the beginning of the experiment was around 180 g, and there was no significant variation among the groups ($p > 0.05$). The average body weight of the animals in all three experimental groups had increased significantly by the end of the experimental period, with a significant difference between them ($p < 0.05$). The control group (0 day) gained significantly more ($p < 0.05$) weight during the study than the

7- and 14-day groups. The final mean body weight of 0- and 7-day groups after the 7-day experimental period was 231.25 ± 14.34 and 194.50 ± 6.95, respectively, as shown in Figure 1 (a). The final mean body weight of 0- and 14-day groups after the 14-day experimental period was 260.50 ± 15.93 and 221.25 ± 7.68, respectively, as shown in Figure 1 (b).

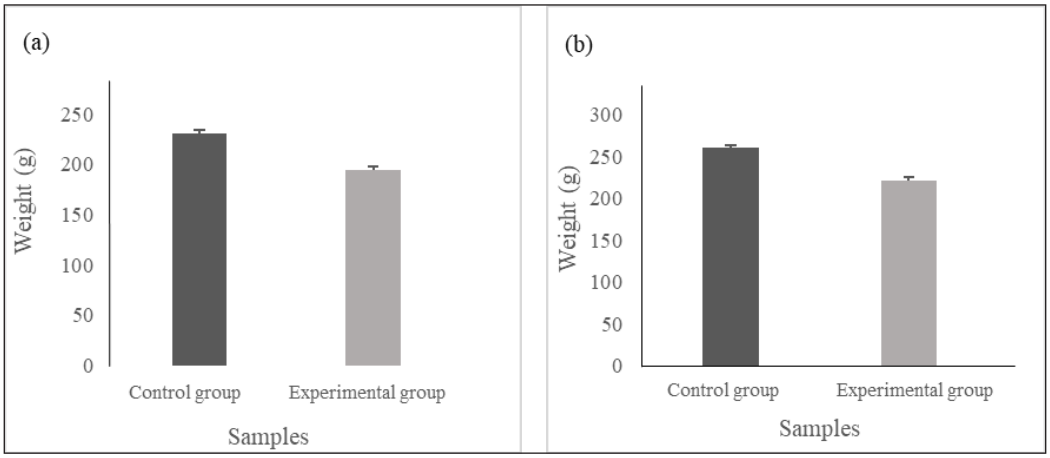

Figure 1. The final mean body weight of (a) 0- and 7-days groups after the 7 days experimental period; (b) 0- and 14-days groups after the 14 days experimental period

Expression of Collagenase (MMP-1, -8, -13) in Gingival Tissue Samples

This study found an upregulation of collagenase significantly, MMP-1 ($p < 0.01$), MMP-8 ($p < 0.01$), and MMP-13 ($p < 0.01$), as shown in Figure 2 in gingival tissue samples at 7 days post-induction. There was a notable variation in the MMP-8 expression between 7 and 14 days ($p < 0.05$), as shown in Figure 2 (b). However, in comparison to the 0-day group, MMP-1 and MMP-8 were substantially elevated at 7 and 14 days ($p < 0.01$); the upregulation of MMP-13 expression at 14 days in the gingival tissue samples was not significant statistically ($p > 0.05$) as shown in Figure 2.

Enterococcus faecalis Bacterial Load Analysis in the Gingival Tissue Samples

The cycle threshold (Ct) value is a semi-quantitative measure of bacterial load, where the greater the amount of bacterial DNA, the lower the Ct value. There was a significant difference in the *E. faecalis* gene Ct values of the gingival tissue samples at the 7 days ($p < 0.01$) and 14 days ($p < 0.05$) post-induction group as compared to the control group (Ct 34.41). Moreover, a considerable difference was seen between the 7- and 14-days groups ($p < 0.01$). At 7 days, the Ct value was lowered (Ct 28.72) but then increased at 14 days (Ct 32.81). All results are shown in Figure 3.

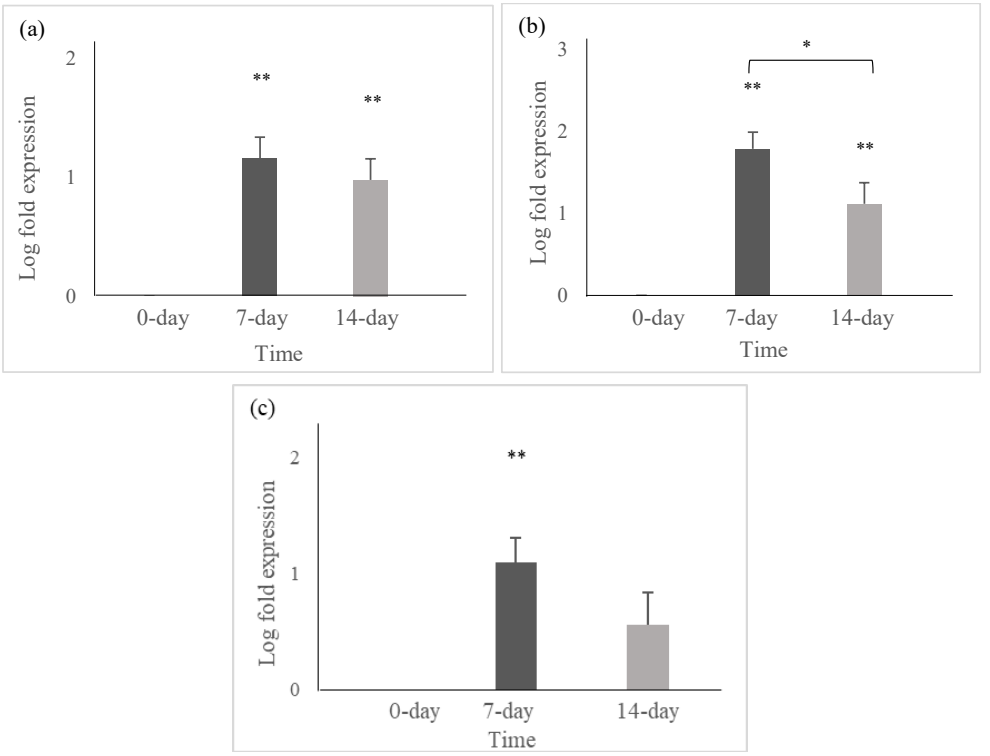

Figure 2. Expression of MMP-1, -8, -13 in gingival tissue samples shows (a) significant upregulation of MMP-1 at 7 and 14 days ($p < 0.01$) as compared to 0-day group; (b) significant upregulation of MMP-8 at 7 and 14 days ($p < 0.01$) as compared to 0-day group, a significant difference between 7 and 14 days group ($p < 0.05$); (c) significant upregulation of MMP-13 at 7 days ($p < 0.01$) as compared to 0-day group
Note. * = $p < 0.05$; ** = $p < 0.001$

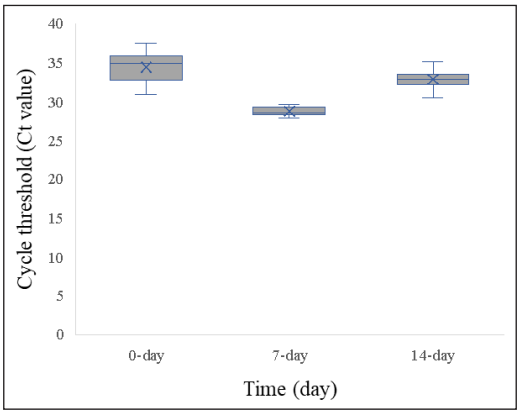

Figure 3. The cycle threshold (Ct) values of the *Enterococcus faecalis* gene in gingival tissue samples. At 7 days, the Ct value was (Ct 28.72), then increased at the 14 days (Ct 32.81). There was a significant difference between the 7- and 14-days groups ($p < 0.01$)
Note. ** = $p < 0.001$

Histopathological Changes in the Periodontium

The histopathological analysis of the periodontium of the 7 days post-induction group showed thin and ulcerated junctional epithelium (JE), migration of the JE apically, loss of attachment at the cementoenamel junction (CEJ) as shown in Figure 4 (b), inflammatory cells infiltration mostly neutrophils, macrophages as shown in Figure 4 (d) as well as the presence of osteoclasts and alveolar bone (AV) resorption as shown in Figure 4 (e). In the 14-day post-induction group, inflammatory cell infiltration was less as compared to the 7-day post-induction group as shown in Figure 4(f), even though the migration of the JE apically and attachment loss from the CEJ were observed as shown in Figure 4 (f). In the control group, the JE was normal; there was no attachment loss from CEJ, inflammatory cell infiltration and resorption of the alveolar bone surface within the periodontium as shown in Figure 4 (a). When compared to the 14-day group, the 7-day group exhibited a notably greater quantity of inflammatory cells ($p < 0.01$), as shown in Figure 4 (g).

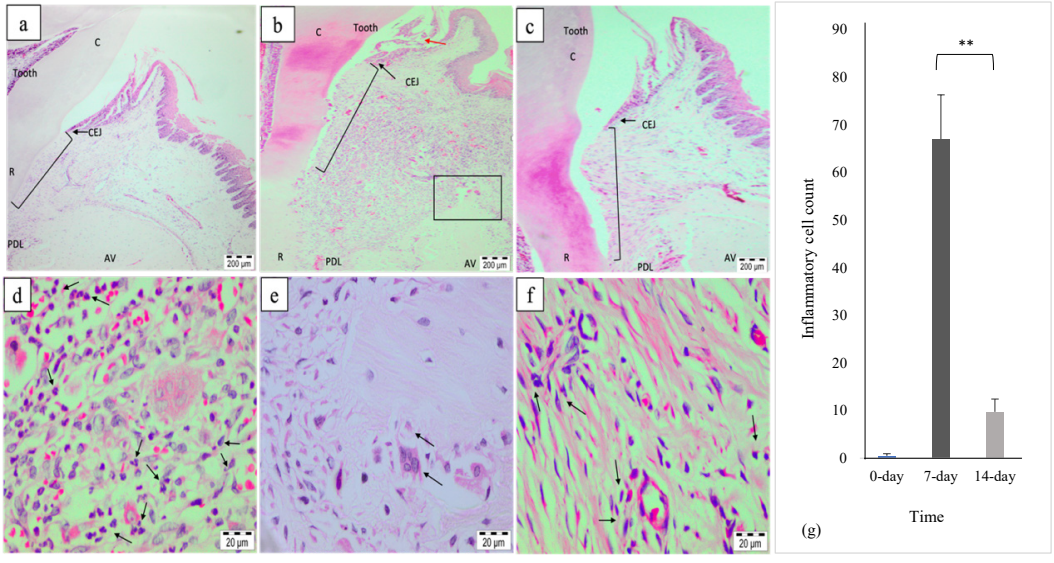

Figure 4. Histopathological images were shown in figures (a, b, c, d, e, f) and the number of inflammatory cells was shown in figure (g). (a) Images of normal histological structure within the periodontium at 0-day; (b) Image of a thin and ulcerated junctional epithelium (JE), apical migration of the JE, attachment loss from the cementoenamel junction (CEJ) at 7 days; (c) Images of attachment loss from CEJ at 14 days; (d) Images of inflammatory cells infiltration mostly neutrophils and macrophages; (e) Images of presence of osteoclasts and alveolar (AV) bone resorption; (f) Images of less inflammatory cells infiltration at 14 days as compared to the 7 days PI group, even though the migration of the JE apically and attachment loss from the CEJ were observed using 4x, 10x, and 40x magnifications; (g) Image of bar graph shows significantly higher inflammatory cell count ($p < 0.01$) in the 7-day group as compared to the 14-day
Note. C = Crown; R = Root; PDL = Periodontal ligament; ** in figure (g) = $p < 0.001$

Correlation Between Collagenase (MMP-1, -8, -13) Expression and Inflammatory Cells Count

The Pearson correlation method was applied to determine if a correlation exists between the gene expression (based on qPCR data) and inflammatory cell count. The analysis showed a highly positive correlation of MMP-1 ($r = 0.926$), MMP-8 ($r = 0.890$), and MMP-13 ($r = 0.823$) in 7-day with the inflammatory cells, as shown in Figure 5 (a, b, c). Furthermore, the correlation between MMP-1 ($r = 0.873$), MMP-8 ($r = 0.906$), and MMP-13 ($r = 0.914$) in 14 days was highly positive with the inflammatory cells, as shown in Figure 5 (d, e, f).

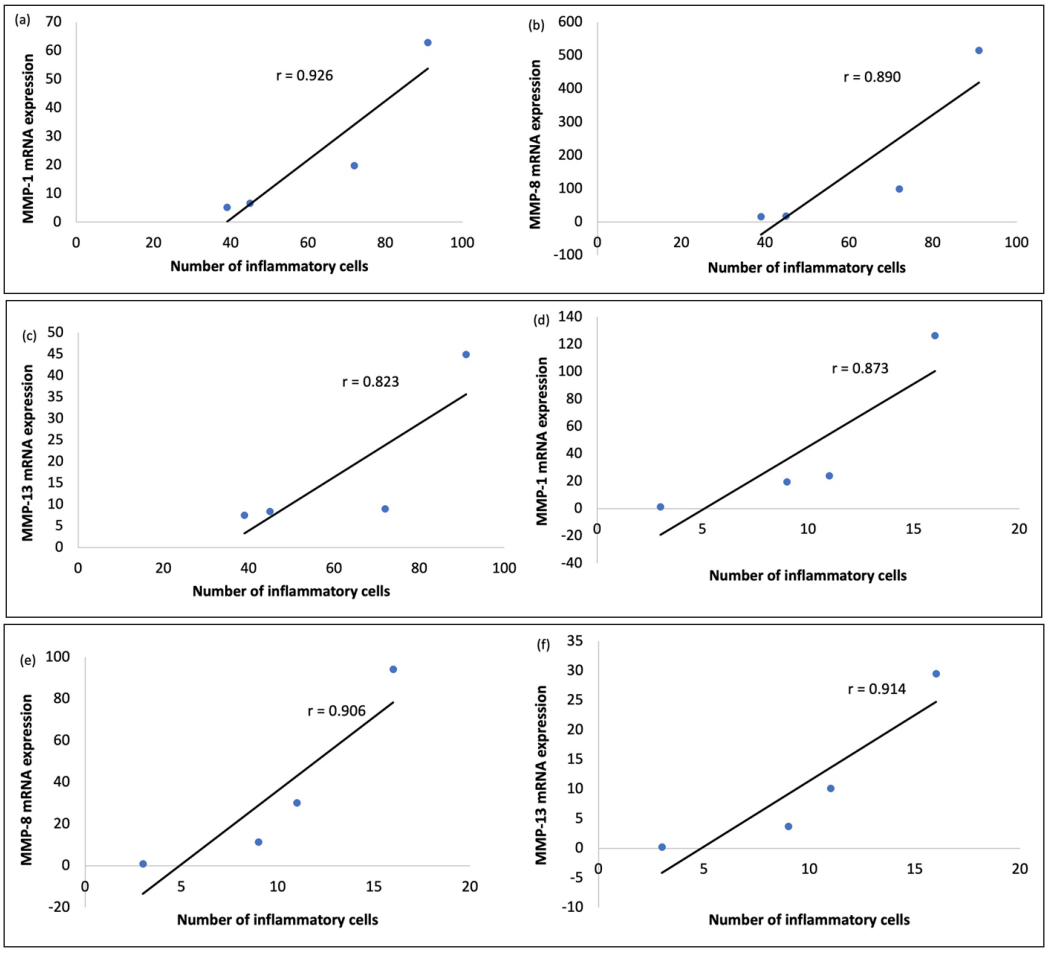

Figure 5. A correlation between collagenase (MMP-1, -8, -13) expression and inflammatory cell count was performed. There was a highly positive correlation of MMP-1, -8, -13 mRNA expression with the number of inflammatory cells at 7 days (a, b, c) and a highly positive correlation of MMP-1, -8, -13 mRNA expression at 14 days with the number of inflammatory cells (d, e, f)

DISCUSSION

MMP-1, -8, -13 expression is generally low; nonetheless, the enzymes are activated in a variety of physiological conditions when ECM remodelling is necessary. The findings of this study highlight the utility of combining disease mediators, such as MMP-1, -8, and -13, to assess a patient's condition more precisely. Based on varied regulatory mechanisms among distinct MMPs, the current study hypothesised that individual MMPs may play a variety of potentially conflicting functions in oral inflammatory conditions. Their varying temporal expression levels are possible. Hence, utilising an experimentally generated periodontitis animal model, the mRNA expression levels of collagenase were examined at various time points following simultaneous injection with *E. faecalis* and ligature wire infection.

Once the gingival surface has been successfully colonised, bacteria multiply within the host and induce immunological responses in local resident cells and circulating immune cells, producing proinflammatory cytokines, prostaglandins, proteinases, and MMPs. The immune system's cells are generally distributed throughout the body, and when an infection develops, the inflammatory response enables the marshalling of immune system components to certain areas. Early stages of the inflammatory response to an infection are typically not clinically detectable. In the current study, a considerable rise in the body weight of the control group shows the healthy states of the animals. However, the experimental group's weight was increased over their respective induction periods, though it was still lower than the control groups, indicating an inflammatory process within the experimental groups (Choubaya et al., 2019).

The current investigation examined gingiva tissue samples taken from 0-, 7-, and 14-days following wire ligature and concurrent infection with *E. faecalis* inoculation to evaluate the bacterial load of the *E. faecalis* gene using qPCR assay. This study has detected a higher bacterial load of *the E. faecalis* gene in the gingival tissue at 7 days post-induction. However, the bacterial load was lower at 14 days compared to the 7 days groups. The lower Ct value of *E. faecalis* in the 7-day post-induction period suggested that periodontal inflammation may favour the establishment of this organism. According to a study conducted by Chidambar et al. (2019), a significantly higher frequency of *E. faecalis* was detected in the subgingival biofilms of the periodontitis group (41.7%), compared to gingivitis (5.9%) and healthy group (0%) (Chidambar et al., 2019). In the current study, wire ligation acts as a biofilm retentive factor, which helps to accumulate complex microbiota within the periodontal pocket. The complex microbiota, along with the inflammatory process, may produce a variety of nutrients and binding sites for developing this microorganism.

Collagenase is a member of the MMP group, capable of degrading nearly every kind of collagen found within the ECM and contributing to tissue destruction. However, the pattern of MMP-8 and MMP-13 expressions in the periodontium is still under research.

The current study has demonstrated upregulated MMP-1, -8, -13 mRNA expression levels in the gingival tissue at 7- and 14-days post-induction. There were significant differences in MMP-1, -8, and -13 mRNA expression levels in gingival tissue at 7 days post-induction compared to the control group. This study also discovered a substantial positive association between the number of inflammatory cells and the levels of the MMP-1, -8, and -13 genes, which suggests that the presence of inflammatory cells that have migrated to the infection site because of experimentally induced periodontitis regulates the gene expression. MMP-8, also known as neutrophilic collagenase, is mostly secreted by neutrophils. Moreover, a significant elevation of the collagenase levels, particularly for MMP-8, within the gingival tissue indicates that the destruction of the periodontal tissue occurred during the event.

Furthermore, a significant decrease in MMP-8 expression at 14 days suggested a reduction in the inflammatory activity within the periodontium (Yang et al., 2013). This finding is supported by the histological analysis of the periodontium, which showed that the infiltration of inflammatory cells, mostly neutrophils and macrophages, was higher within the tissue at 7 days and was notably reduced at 14 days. Yang et al. (2013) observed a significant decrease in MMP-8 staining on days 5 and 7, which continued from days 11 to 21. A significant decrease in MMP-8 expression was observed on days 5 and 7, and this decrease continued from 11 to 21, which is consistent with our findings. Furthermore, we found that the upregulation of MMP-1, also known as fibroblastic collagenase, steadily decreased at 14 days. Compared to the 7 days, histopathological analysis of the 14-day sample showed that the neutrophils and macrophages were significantly reduced, with fibroblast cells present.

The physiologic remodelling of bones and bone resorption are both regulated by MMP-13. So, it is probable that the presence of osteoclasts during the 7 days caused MMP-13 to be upregulated. Interestingly, we did not see any osteoclasts after 14 days, suggesting that the MMP-13 expression was likely lower than at 7 days. According to previous studies, MMP-13 expression peaked on day 7 and subsequently began to decline from days 11 to 21. This study's results are consistent with previous studies (Yang et al., 2013).

The innate immune system is activated while neutrophil and macrophages are deployed to clear the microbial challenge within the periodontal tissue, significantly elevating these collagenases. More importantly, the current study has observed that all the inflammatory processes started to decrease at 14 days, although it was not significant, except for the MMP-8 expression, which was significantly reduced compared to 7 days. When the *E. faecalis* gene expression was compared to the histological analysis, it was noticed that the bacterial loads were lowered at 14 days post-induction, and histologically, fewer inflammatory cells were observed. It is postulated that the inflammatory process started to decrease due to the development of the host's immune response to resist the microbial challenge. The decreased inflammation severity over time could be attributed to a protective

feature of the periodontal tissue in response to microbial challenges. Previous studies by de Molon et al. (2014) and de Souza et al. (2011) have also reported a reduction in the inflammatory response with time, which agrees with the present study (de Molon et al., 2014; de Souza et al., 2011).

CONCLUSION

Our results showed that MMP-1, MMP-8, and MMP-13 were expressed at different levels in each sample of periodontitis, along with the accompanying inflammatory cells. The findings imply that the degree of inflammation was correlated with a reduction in MMP-1, -8, and -13 expressions. The study found that identifying the MMP-1, -8, and -13 groups could be beneficial for detecting numerous aspects of periodontitis progression. Knowing the activities of the groupings of biomarkers in periodontitis might play an essential role in the early identification of periodontitis and more effective disease treatment. This study provides significant insight into the severity of periodontal disease based on its biomarker activities. Future research is still required to develop precise diagnostic tools to improve the predictability of diseases in treatment.

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