BMC Oral Health

RESEARCH

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Evaluation of salivary Ki-67 in health and periodontitis

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Abstract

Background Ki-67, a nuclear protein is found in cells undergoing proliferation during the cell cycle. It has been established as an important tumor proliferation and prognostic marker. An increased expression of Ki-67 is observed in inflammation. Periodontitis tissue biopsy specimens have shown Ki-67 expression. Saliva has become popular as a non-invasive source of biomarkers that may have a clinical utility. Ki-67 has not been examined in the saliva of patients having periodontitis. This cross-sectional study aimed to detect and make a comparative estimation of salivary Ki-67 in health and periodontitis.

Methods Fifty-two participants were divided equally into two groups: Health [systemically and periodontally healthy ($n=26$)] and Periodontitis [systemically healthy periodontitis patients ($n=26$)]. Study volunteers were recruited based on the selection criteria. Plaque index, modified gingival index, bleeding on probing, probing pocket depth and clinical attachment loss were recorded. Saliva was obtained and Ki-67 was estimated with a commercially available ELISA kit.

Results The periodontitis group had significantly higher levels of Ki-67 than the healthy group. Overall, except with plaque index, there were significant positive weak correlations between Ki-67 and modified gingival index, bleeding on probing, probing pocket depth, and significant positive strong correlation with clinical attachment loss. Receiver operating characteristic analysis of the diagnostic accuracy of Ki-67 in periodontitis showed that the area under the curve was statistically significant, and the cutoff value was > 787.67 pg/ml.

Conclusion Ki-67 can be detected in saliva and has a role in periodontitis. This implies that saliva can be a non-invasive source for Ki-67 as a potential biomarker in periodontitis.

Keywords Ki-67, Periodontitis, Saliva, Biomarkers

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Background

Periodontitis is a multi-factorial inflammatory disease caused due to the accumulation of dysbiotic dental biofilm and featuring destruction of the teeth-supporting tissues including the loss of periodontal ligament and alveolar bone, demonstrated by the presence of gingival bleeding, periodontal pockets, clinical attachment loss (CAL) and radiographically evidenced loss of alveolar bone [1, 2]. The destruction of periodontal tissues mainly happens due to the host's inflammatory and dysregulated immune response [3]. The chronic inflammatory response in periodontal disease results from periodontal pathogens (mainly the red complex gram-negative bacteria found in subgingival dental biofilm in the susceptible host), that stimulate inflammatory cells, to produce pro-inflammatory mediators such as prostaglandin E2 (PGE2), interleukin (IL)-1β and tumor necrosis factor (TNF)-α. These pro-inflammatory mediators and virulence factors lead to the production of proteinases that destroy collagen fibers in the periodontal ligament and alveolar bone destruction by activation of osteoclasts [4]. The sustained physiological responses initiated by periodontitis can influence a systemic inflammatory response; different biomarkers have been well-studied in the literature and have been linked to systemic inflammation [5].

In this background, an inflammatory biomarker of interest is Ki-67. It is a nuclear protein that is found in the active phases of the cell cycle and has increased expression in different conditions such as diabetes mellitus, rheumatoid arthritis, pancreatitis, oral dysplastic conditions such as leukoplakia, erythroplakia, leukoerythroplakia, and squamous cell carcinoma [6, 7]. Ki-67 has a short half-life (about sixty to ninety minutes) that indicates its balanced regulation during the cell cycle [8]. Ki-67 is a cell proliferation-specific protein first identified in the 1980s and is now considered a reliable predictive and prognostic indicator for the assessment of biopsies from cancer patients [9]. Investigations about the role of Ki-67 as an inflammatory biomarker in periodontitis are few (Ki-67 was evaluated as a tissue proliferative marker in drug induced gingival overgrowth, in the formerly known "aggressive periodontitis" "chronic periodontitis", "early onset periodontitis", "adult periodontitis", smokers with periodontitis, and periodontitis patients with type 2 diabetes mellitus, respectively), and no studies have reported its assessment in the saliva of patients having periodontitis. Saliva collection has the advantage of being non-invasive, and relatively simple and it is a multipurpose biological fluid whose analysis can reflect both the local oral, and systemic environment related to health and disease offering the foundation for a convenient patient-specific biomarker to evaluate periodontitis and other systemic diseases [10].

Therefore, the objective of this study was to evaluate salivary Ki-67 to find out if saliva is a source of this protein and whether there is a variation in its levels in health and periodontitis.

Methods

This cross-sectional case-control study was conducted at the College of Dental Medicine at the University of Sharjah, UAE from October 2021 to October 2022. The study protocol was approved by the Research Ethics Committee at the University of Sharjah (Reference No.: REC-21-09-28-02-S). All the clinical procedures were performed in full accordance with the revised Declaration of Helsinki and the Good Clinical Practice Guidelines. The study aim was described to all the subjects and written informed consent was obtained; they were also provided a participation information sheet before enrolment.

Sample size calculation

An a priori analysis was done to calculate the sample size using two-tailed t-tests with an effect size of 0.8 and α error probability of 0.05, power (1-β error probability) of 0.8, allocation ratio N2/N1 equaling 1 gave a non-centrality parameter $\delta = 2.884$ and critical $t = 2.008$ with a differential of 50. This required a sample size of 26 per group, i.e., a total of 52 with an actual power = 0.807. Therefore, 52 study volunteers were equally divided into two groups: group 1 (Health)-systemically and periodontally healthy ($n = 26$) and group 2 (Periodontitis)-periodontitis patients who were systemically healthy ($n = 26$).

Study design

Individuals visiting the postgraduate clinic of Periodontology, the College of Dental Medicine at the University of Sharjah and the Clinics of the University Dental Hospital Sharjah were included in the study. An initial exam, including medical, and dental history, and clinical and radiographic examination was done to evaluate participant eligibility for recruitment in the study. Inclusion criteria were volunteers in the age group of 20 to 55 years who were systemically healthy. Health group: participants with absence or minimal bleeding on probing (BoP) < 10%, no CAL, no redness, no probing depth of more than 3 mm and no clinical swelling/edema or pus, and no bone loss evidenced in radiographs. Periodontitis group: Participants having generalized periodontitis (based on the 2017 classification) [2], stages II, III, IV, grade B, probing pocket depth (PPD) > 4 mm, CAL ≥ 3 mm, with BoP, and bone loss evidence on the radiographs. Exclusion criteria were individuals who were immunocompromised or with systemic diseases, requiring prophylactic antibiotics before dental treatments, tobacco smokers/smoke-less tobacco users, pregnant/breastfeeding/menopausal females, patients who

underwent previous periodontal therapy and/or administered antibiotics/ anti-inflammatory agents in the last 3 months.

All the clinical parameters were recorded by one periodontist after intra-examiner calibration was found to be acceptable (kappa statistic value of 0.89, 95% agreement). The following parameters were recorded on the whole dentition. Full mouth plaque (oral biofilm) score (Pl)was assessed wherein six sites per tooth were evaluated for the presence or absence of supragingival plaque using Plaqsearch (Oraldent Ltd., Huntingdon, UK) two-tone disclosing solution. The presence (+) / absence (–) of plaque was recorded and (+) was calculated as a percentage [11]. A visual assessment of gingival changes was done to measure the severity of inflammation using the modified gingival index (MGI) [12]. Using a scale of 0–4, the color, texture, edema, and spontaneous bleeding of marginal and papillary gingiva were scored. Then the average score was taken. PPD, CAL measurements (recorded at six aspects per tooth: mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) were rounded off to the nearest mm using a periodontal probe (UNC 15, Hu-Friedy® Manufacturing Inc., Chicago, IL, USA). BoP was calculated as a percentage of sites examined.

Saliva collection

The saliva collection procedure followed recommendations in the literature [13]. Before the collection of saliva, the participants in the study were told to refrain from foods that may be highly acidic or sugary, avoid caffeine, not use any medications/mouthwash, nor consume alcohol (for a minimum of twelve hours before the collection of saliva), no major meal (before one hour of saliva collection), no toothbrushing (for at least forty-five minutes prior to collection of saliva), no dental procedure done on them in the past twenty-four hours, as this may affect the assay. The saliva samples were collected at a pre-defined time of the day (8:00 a.m. to 10:00 a.m.) from every participant after rinsing with saline for thirty seconds. Thereafter they were instructed to expectorate in a basin to eliminate any food/debris and wait for ten minutes after rinsing, before saliva collection to prevent sample dilution. After this, the study volunteers were instructed to gently spit unstimulated saliva (approximately 10 to 15 ml) into sterile and plain collection tubes. The samples were then centrifuged for 20 min at 1000×g at 2–8℃. The supernatant was collected and stored at -80℃ to be used for further analysis by the assay.

Table 1 Demographics and characteristics of variables

Parameter			Periodontitis	Health	*p*-value
Sex	Male	n	18	11	0.094
		%	69.2%	42.3%	
	Female	n	8	15	
		%	30.8%	57.7%	
Age (years)	Mean ± SD		37.00 ± 8.21	36.10 ± 10.21	0.728
Pl	Mean ± SD		62.97 ± 32.45	31.14 ± 29.19	< 0.001*
MGI	Mean ± SD		1.38 ± 0.85	0.04 ± 0.05	< 0.001*
BOP (%)	Mean ± SD		62.49 ± 34.69	2.99 ± 3.37	< 0.001*
PPD (mm)	Mean ± SD		3.30 ± 1.20	1.90 ± 0.38	< 0.001*
CAL (mm)	Mean ± SD		3.00 ± 1.99	0.06 ± 0.10	< 0.001*
Ki-67(pg/ml)	Mean ± SD		847.73 ± 171.70	698.23 ± 79.94	= 0.001*

SD: Standard deviation; Pl: Plaque Index; MGI: Modified Gingival Index; BOP: Bleeding on probing; PPD: Probing Pocket Depth; CAL: Clinical Attachment Loss; pg/ml: picogram/milliliter

* Statistically significant (Mann-Whitney U test)

Analysis of Ki-67 by ELISA

Quantitative determination of Human Ki-67 concentrations from saliva was done using a commercially available ELISA kit (Elabscience Biotechnology Inc., Houston, TX, USA; catalog number: E-EL-H5432) per the manufacturer's instructions. The detection range per the manufacturer is 0.3 ~ 20ng/ml with a sensitivity of 0.19 ng/ml. The concentrations of human Ki-67 in the samples were measured in pg/ml by comparing the optical densities of the samples to the standard curve.

Statistical analysis

Categorical data (i.e., sex) were presented as frequencies and percentages and were analyzed using the chi-square test. Numerical data (i.e., marker and periodontal parameters) were presented as mean and standard deviation values. They were explored for normality by viewing the distribution and using Shapiro-Wilk's test. Data were non-parametric and were analyzed using the Mann-Whitney U test. Correlations were analyzed using Spearman's rank-order correlation coefficient with exact p-values reported for significance testing. Diagnostic accuracy in calculating the salivary Ki-67 concentrations for the predictive value was determined using receiver operating characteristic (ROC) analysis, and a z-test was used to assess the statistical significance of the AUC. The significance level was set at $p < 0.05$ for all tests.

Results

The demographic data/characteristics of parameters are in Table 1. The study was performed on 52 participants (i.e., 26 in each group). Eighteen (69.2%) males and 8(30.8%) females were in the periodontitis group, and in the health group, there were 11(42.3%) males and 15(57.7%) females. The difference between both groups was not statistically significant regarding sex ($p = 0.094$) and age ($p = 0.728$). Based on the effect size calculated from the comparisons of marker levels between both groups (d = 1.12) (i.e., large effect size), the achieved power was (0.977), and p-value = 0.001, indicating that the current sample size was adequate.

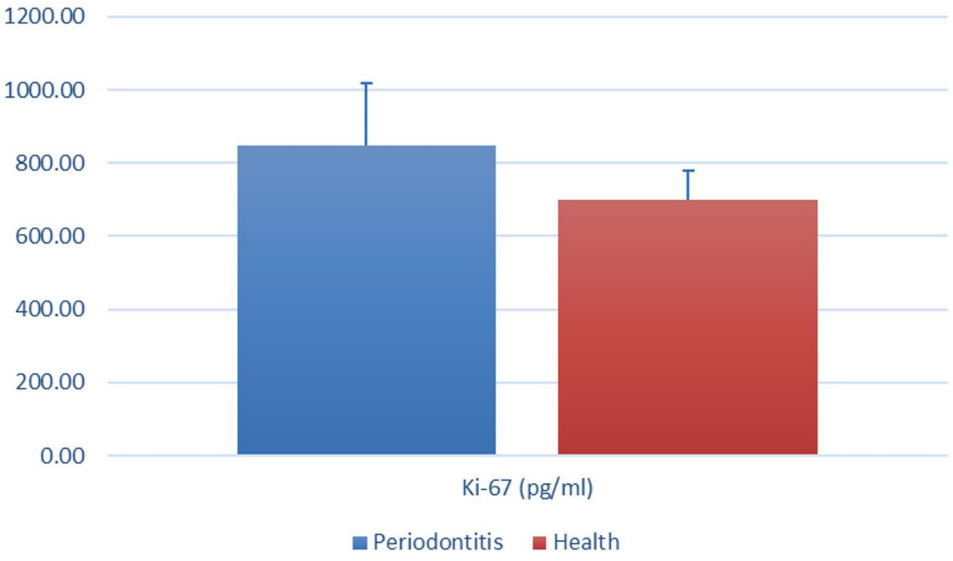


Fig. 1 Comparison of Ki-67 levels in health and periodontitis. pg/ml:picogram/milliliter

Table 2 Subgroup Ki-67 levels in the stages/grades of periodontitis

		Numbers (Percentage)	Ki-67(pg/ml)	*p*-value
Periodontitis stage	I	0 (0.00%)	NA	0.113
	II	5 (19.23%)	810.27 ± 249.80*	
	III	19 (73.08%)	838.68 ± 151.68*	
	IV	2 (7.69%)	1027.40 ± 14.53*	
Periodontitis grade	A	0 (0.00%)	NA	0.598
	B	18 (69.23%)	841.89 ± 187.08	
	C	8 (30.77%)	860.87 ± 141.47	

NA: Not Applicable; * significantly different (Mann-Whitney U test)

Table 3 Correlations between different periodontal parameters and Ki-67 (pg/ml) level

Group	Parameter	Correlation coefficient(*r*)	*p*-value
Periodontitis	PI	-0.013	0.949
	MGI	0.270	0.185
	BOP (%)	0.120	0.567
	PPD (mm)	0.160	0.421
	CAL (mm)	0.240	0.240
Health	PI	0.098	0.636
	MGI	-0.160	0.425
	BOP (%)	-0.079	0.700
	PPD (mm)	-0.025	0.904
	CAL (mm)	0.140	0.498
Overall	PI	0.250	0.075
	MGI	0.440	0.001*
	BOP (%)	0.410	0.003*
	PPD (mm)	0.420	0.002*
	CAL (mm)	0.490	< 0.001*

* Significant ($p < 0.05$) (Spearman's rank-order correlation test)

The periodontitis group had significantly higher values of periodontal parameters and salivary Ki-67. Intergroup comparison of Ki-67 (pg/ml) levels is in Table 1; Fig. 1. Subgroup analysis of Ki-67 levels in periodontitis are shown in Table 2. Correlations between different periodontal parameters and Ki-67 levels are presented in Table 3; Figs. 2, 3 and 4.

For the periodontitis and healthy groups, all correlations were not statistically significant. Overall, there were significant positive weak correlations between Ki-67 and MGI, BoP, PPD, and significant positive strong correlation with CAL. Results of the ROC analysis of the diagnostic accuracy of Ki-67 in periodontitis prediction showed that the area under the curve (AUC) was (0.763 [95%CI (0.624:0.870]) which was statistically significant ($z = 3.90$, $p < 0.001$), sensitivity was 61.54, specificity was 88.46 and the cutoff value based on Youden was > 787.67. The ROC curve is presented in Fig. 5.

Discussion

Ki-67 has made its presence as a tumor marker in the past few decades. Ki-67 was discovered in the nuclei of cells in Hodgkin lymphoma. The "Ki" stands for Kiel, Germany, where the identification was done, and "67" for the number of the original clone [14] Ki-67 has been detected in the nucleus of proliferating cells in active phases of mitosis, however, it is not present in non-proliferating cells and is associated with apoptosis [15]. Ki-67 has been established as an important marker of tumor proliferation and as a prognostic marker [16, 17]. It is a 319–358 kDa protein that is consistently detected based on the number of proliferating tumor cells that help predict the behavior of the tumor [18, 19]. Because Ki-67 is detected and specific to proliferative cells,

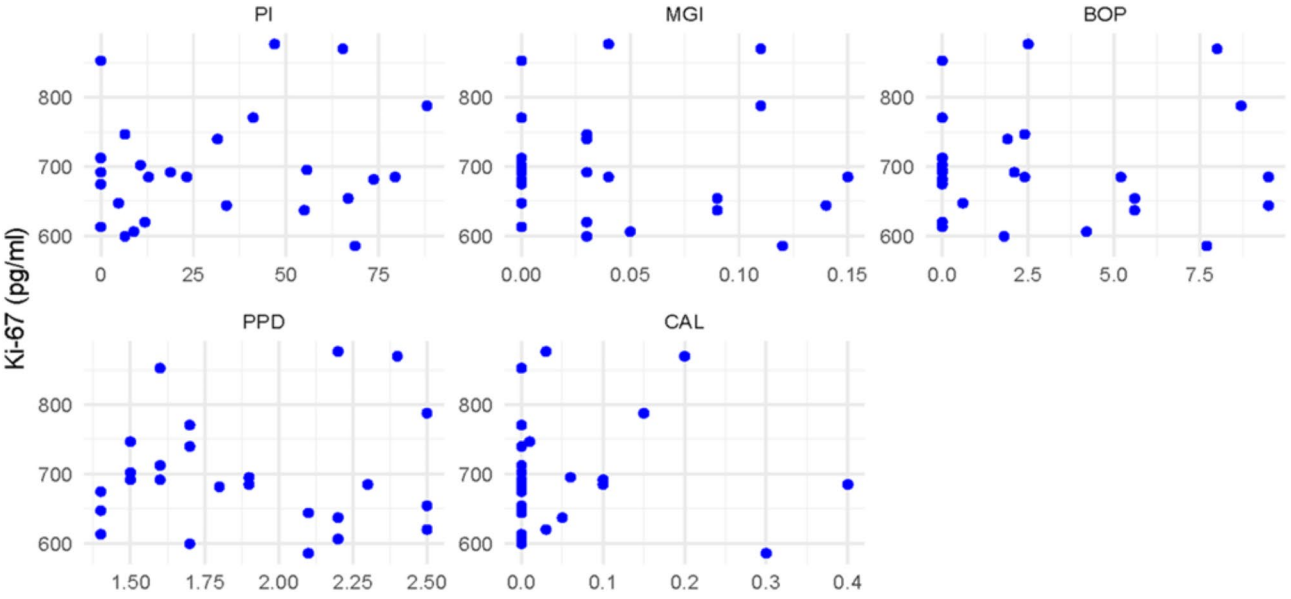


Fig. 2 Scatter plot showing the correlations between different periodontal parameters and Ki-67 (pg/ml) level in the healthy group. PI: Plaque Index; MGI: Modified Gingival Index; BOP: Bleeding on probing; PPD: Probing Pocket Depth; CAL: Clinical Attachment Loss; pg/ml:picogram/milliliter

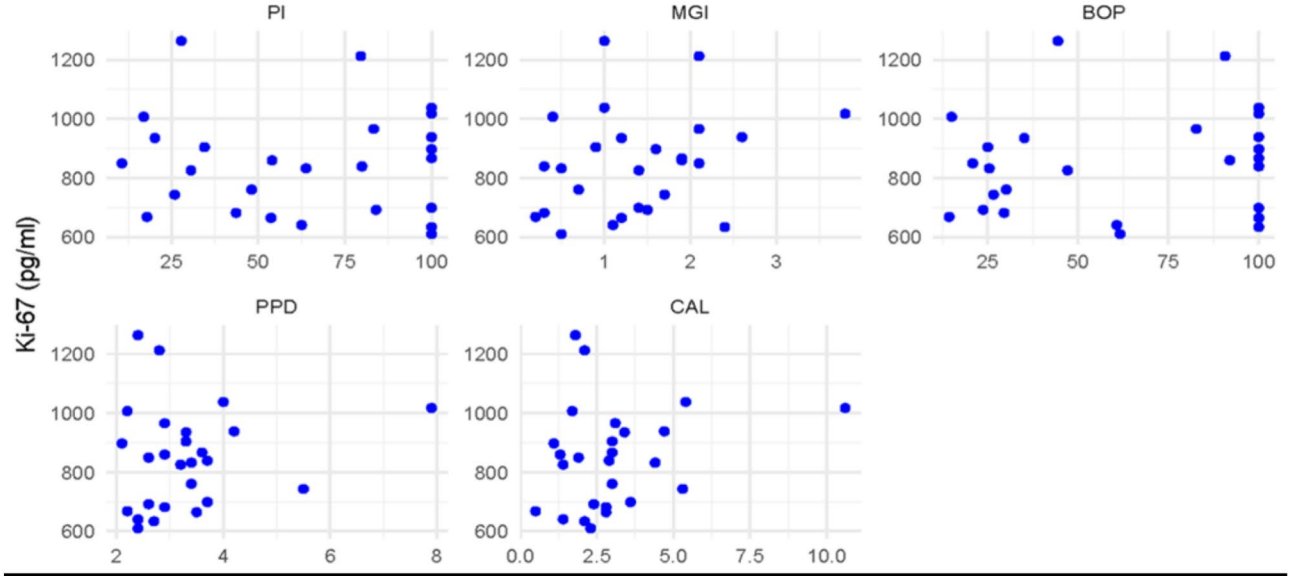


Fig. 3 Scatter plot showing the correlations between different periodontal parameters and Ki-67 (pg/ml) level in the periodontitis group. PI: Plaque Index; MGI: Modified Gingival Index; BOP: Bleeding on probing; PPD: Probing Pocket Depth; CAL: Clinical Attachment Loss; pg/ml:picogram/milliliter

various carcinomas have been investigated using this marker [20–22]. About the oral environment, a systematic review and meta-analysis concluded that Ki-67 was higher in odontogenic keratocyst and malignant odontogenic tumors [23]. Ki-67 was significantly observed in the invasive front of oral squamous cell carcinoma [24]. Inflammation is considered a risk factor for various cancers [25]. Also, inflammation induces the proliferation of cells [26], which may be relatable to a local inflammatory condition like periodontitis that is initiated by a polymicrobial etiology. Inflammation is regarded as a common platform in the association between periodontitis and cancers due to cell proliferation [27, 28]. It has been reported that Ki-67 is expressed lower in health as compared with inflammation and cancers [29]. Ki-67 has a role in inflammation, wherein its specific antibody has been employed in analyses to identify cells undergoing division and proliferation which is a typical feature of the inflammatory process [30].

Hence, the rationale for evaluating Ki-67 in health and periodontitis is based on the premise that it is a cell proliferation marker and may be higher in inflammatory

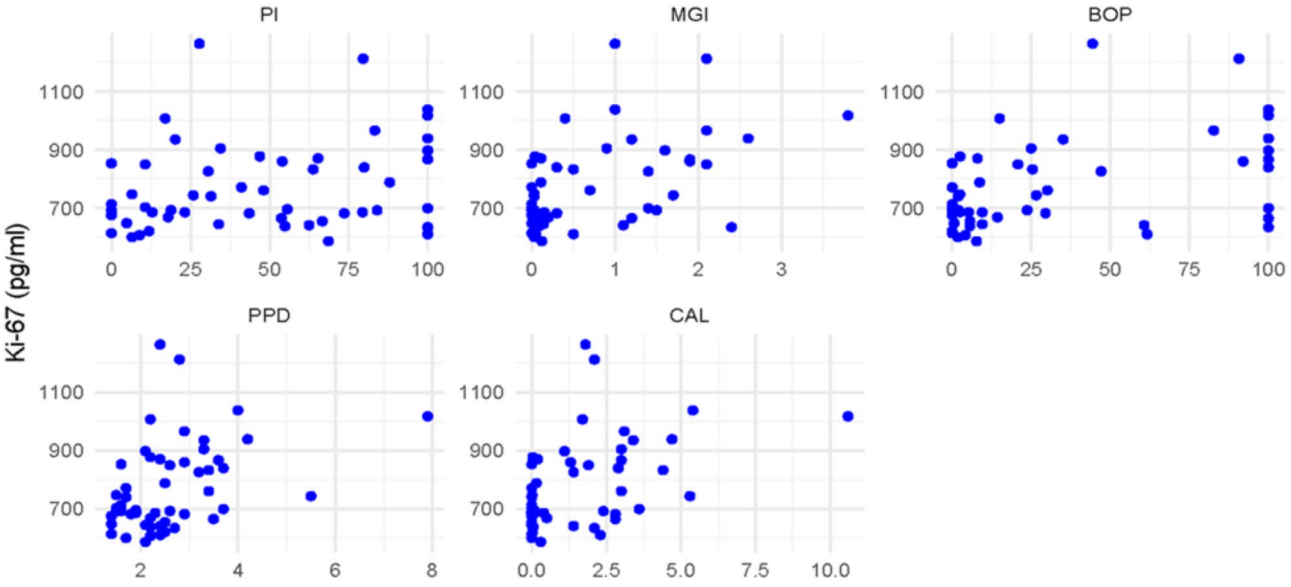
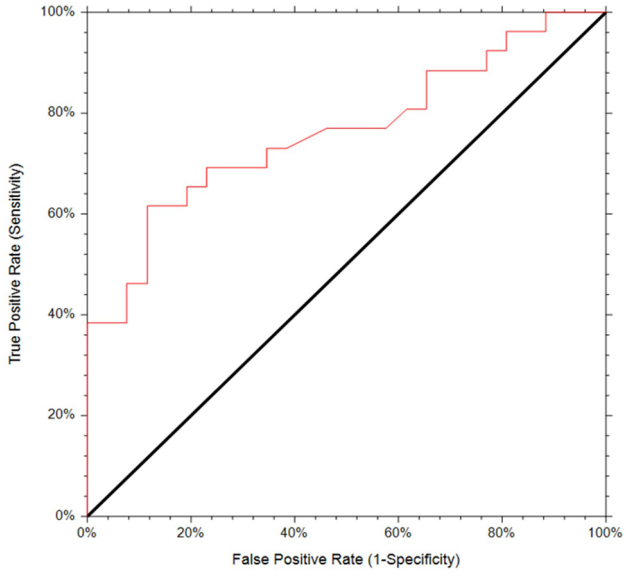


Fig. 4 Scatter plot showing the correlations between different periodontal parameters and Ki-67 (pg/ml) level in both groups. PI: Plaque Index; MGI: Modified Gingival Index; BOP: Bleeding on probing; PPD: Probing Pocket Depth; CAL: Clinical Attachment Loss; pg/ml:picogram/milliliter



	Value (95% CI)
Sensitivity	61.54% (40.6%,79.8%)
Specificity	88.46% (69.8 %,97.6%)
Cut off point	≥787.67
Negative predictive value (PPV)	69.7% (58.1%,79.2%)
Positive predictive value (NPV)	84.2% (63.80%,94.20%)
Area under the curve (AUC)	0.763 (0.624,0.870)

Fig. 5 ROC Curve for Ki-67. ROC analysis, and a z-test was used to assess the statistical significance of the AUC

conditions, although it has been specifically and extensively studied in cancer research.

From a few studies available, the literature primarily shows the evaluation of Ki-67 from periodontal tissue specimens/biopsies which are invasive procedures. Immunohistochemistry is widely accepted as the gold standard method to evaluate Ki-67 [31], but in this research project, saliva was chosen as the source due to the advantage of having a relatively simple, non-invasive collection procedure. One research group concluded that saliva may be more useful than serum for diagnostic and therapeutic reasons [32, 33].

In the current study, a significant difference was seen between the healthy and periodontitis groups for the periodontal parameters assessed, i.e., PI, MGI, BOP, PPD, CAL, and salivary Ki-67 levels. It is challenging to compare our findings as there seems to be no data about salivary Ki-67 in the literature to the best of our knowledge.

The literature provides reports concerning the presence of Ki-67 in periodontal tissues. Buduneli et al., [34], investigated cyclosporine induced-, phenytoin induced-, and nifedipine induced- gingival overgrowth tissue specimens and compared them with oral biofilm (plaque) induced- gingivitis and systemically, periodontally healthy specimens. They noted Ki-67 was expressed significantly higher in phenytoin induced-, nifedipine induced-, followed by cyclosporine induced-gingival overgrowth, gingivitis, and healthy specimen. They found no differences in the proliferating cells and apoptosis between the gingivitis and healthy specimen. According to them, cell proliferation and apoptosis may exhibit an imbalance in drug-induced gingival overgrowth. A direct/indirect effect of the drug cyclosporin on the nuclear

factor-kB (NF-kB) has been proposed. They concluded that the effect of the drug causing gingival overgrowth and a concomitant high expression of Ki-67 makes it a reliable marker of tissue growth in non-tumor lesions of the periodontal structures. In another study, Artese et al., [35] examined gingival biopsies of the formerly known "aggressive periodontitis", "chronic periodontitis", and healthy tissue for vascular endothelial growth factor (VEGF), micro-vessel density (MVD), nitric oxide synthetase (NOS)-1 and, – 3, and Ki-67. The results were a significantly elevated expression of all the aforementioned markers, being highest in "aggressive periodontitis", higher in "chronic periodontitis" and lowest in the healthy samples. The specific reason for including Ki-67 amongst the selected markers by these investigators was to evaluate the proliferation of endothelial cells and they observed maximum endothelial cell proliferation in the sulcular epithelium of "aggressive periodontitis" based on Ki-67 values. Also, Koulouri et al., [36] reported higher numbers of cells (epithelial and fibroblast-like) with Ki-67 expression in granulation tissue of "early onset periodontitis" and "adult periodontitis" when compared with gingival tissue of "adult periodontitis". Nagarkanti et al., [37] detected higher Ki-67 expression in epithelial cells of gingival biopsies of periodontitis patients as compared with healthy specimens. Fanali et al., [38] assessed Ki-67, in addition to VEGF, MVD, NOS-1, -3, CD3, CD 20, and CD68 in gingival biopsies immediately below implants welded with titanium bars and compared them with controls and observed higher expression of Ki-67 in tissues of the test group indicating higher proliferative tissue in association with the inflammatory infiltrate. An immunolocalization study by Preethi et al., [6], noted positive Ki-67 epithelial cells to be highest in a group of smokers with periodontitis, followed by periodontitis, smokers without periodontitis, and, lowest in health. These authors stated that the elevated expression of Ki-67 in smokers is due to the effect of tobacco products on cell proliferation and also, regenerative compensation for loss of cells and tissue because of tobacco products. Kranti et al., [39], reported higher Ki-67 expression in groups of patients with periodontitis and, periodontitis with controlled type 2 diabetes mellitus as compared with health.

The above-mentioned studies indicate a role for Ki-67 in periodontal diseases. It also seems that Ki-67 expression may not necessarily be restricted to conditions such as cancer which has extremely high proliferative cells, but also in drug-influenced gingival overgrowth that has increased rates of proliferative cells. Tissue expression of Ki-67 in different forms of periodontitis, including periodontitis with type 2 diabetes mellitus, implies a proliferative status of the inflamed periodontal tissues.

Concerning our investigation, saliva has proven to be a reliable source of Ki-67 where it was significantly increased in periodontal disease samples as compared to health. The general objective of evaluating and comparing salivary Ki-67 levels in periodontitis and health was achieved. With regard to the specific objectives of the levels of salivary Ki-67 and severity of periodontitis, an overall significant strong positive correlation was noted with CAL. No significant correlations were arrived at for the periodontitis group per se. The diagnostic accuracy of the Ki-67 concentrations based on ROC analysis stands at a cut-off value of > 787.67 pg/ml.

The discussion now leads to the reasons why Ki-67 is elevated in salivary samples of periodontitis patients. It may be explained based on the factors/mediators that may upregulate Ki-67 in individuals with periodontitis. As the current investigation did not evaluate any other potential upregulators of Ki-67, some reports to validate the possibility are cited herewith.

Platelet-derived growth factor (PDGF) is associated with the elevation of Ki-67 [40], and Pinheiro et al., [41], have observed high concentrations of PDGF in inflamed gingival tissues as compared with healthy controls. Nitric oxide is another mediator that upregulates Ki-67 [42], and it was reported earlier that elevated nitric oxide is evident in gingivitis and periodontitis [43]. Gurkan et al., [44] have reported elevated transforming growth factor (TGF)-β in the gingival crevicular fluid (GCF) and gingival tissues of periodontitis patients. TGF-β in turn is associated with upregulating Ki-67 [45]. Hepatocyte growth factor (HGF) is also involved in the upregulation of Ki-67 and is elevated in the GCF of periodontitis cases [46, 47]. The aforementioned literature provides insights regarding the mediators that may be responsible for the upregulation of Ki-67. It is to be noted that these mediators have been studied in periodontal tissue and GCF samples.

From the perspective of our study that examined Ki-67 in saliva, the reasons for its increased levels in periodontitis may also be due to higher salivary concentrations of those mediators that are closely related to the upregulation of Ki-67. Some of the studies regarding such mediators were detected in the saliva of periodontitis. A systematic review and meta-analysis by Chen et al., [48] concluded that nitric oxide in the saliva is elevated in periodontitis patients. Similarly, PDGF is elevated in the saliva of periodontitis patients as per Wu et al., [49]. Another interesting result put forth by Khalaf et al., [50], shows elevated levels of TGF-β in not only the saliva of periodontitis patients, but also in serum. HGF has also been shown to be higher in the saliva of periodontitis patients [51, 52]. Also, Kim et al., [53], have shown increased concentrations of salivary IL-1β and TNF-α (amongst others). It is known that these cytokines activate NF-kB that mediates damage to periodontal tissue [54]. NF-κB (p50/p65) and IκB in gingival tissues [55],

and receptor activator of NF-kB (RANK)/RANK ligand (RANKL) in saliva have been reported to be elevated in periodontitis [56]. Elevated NF-kB may upregulate the Ki-67 levels and speed up cell proliferation. As there is an interaction between Ki-67 and NF-kB [57], it may be hypothesized that the higher salivary Ki-67 levels in periodontitis may also be mediated by NF-kB. Although our study did not evaluate these mediators, it is clear from the literature that potential Ki-67 upregulators are increased in the saliva of individuals who have periodontitis. Therefore, as per our results that show an elevated salivary concentration of Ki-67 in periodontitis, it is implied that it is because of the association of Ki-67 with these elevated mediators.

This study had the primary objective of detecting and comparing Ki-67 in saliva of health and periodontitis which was achieved. However, the limitations are lack of a larger sample size, and that it was not longitudinal. Also, it did not include evaluation of markers related to Ki-67 that could have clarified the mechanism of action of Ki-67 vis-à-vis RANK/RANKL pathway. The inclusion of comorbidities in this study may have broadened the scope of understanding the influence of such conditions on salivary Ki-67 levels. Hence, this study has been able to provide cross-sectional information regarding salivary Ki-67 restricted to clinical inflammatory periodontal factors.

Investigations evaluating inflammatory biomarkers from saliva and putative periodontal pathogens from the oral biofilm have reported noteworthy effectiveness in the diagnosis of periodontitis [58, 59]. A recent systematic review [60] has concluded that dual biomarker combinations show excellent diagnostic ability in diagnosing periodontitis. Our study may have provided better evidence by including related inflammatory mediators and microbial samples.

Generally, our investigation shows that salivary Ki-67 is elevated in periodontitis compared to health. Confirmation of this observation needs future research involving assessment of salivary Ki-67 before and after periodontal therapy with a larger sample size. Furthermore, concomitant evaluation of markers closely associated with Ki-67 may provide clarity about its behavior.

Conclusion

Salivary Ki-67 is a good indicator of periodontitis and may provide a pathogenic perspective and serve as a useful biomarker. This study also suggests the use of saliva as a reliable source of Ki-67 in future investigations.

Abbreviations

AUC	Area under curve
BOP	Bleeding on probing
CAL	Clinical attachment loss
ELISA	Enzyme linked immunosorbent assay
GCF	Gingival crevicular fluid
GI	Gingival Index
HGF	Hepatocyte growth factor
IL	Interleukin
MGI	Modified gingival index
MVD	Micro-vessel density
NF-kB	Nuclear factor-kappa B
NOS	nitric oxide synthetase
PDGF	Platelet-derived growth factor
PGE2	Prostaglandin E2
PI	Plaque Index
PPD	Probing pocket depth
RANK	Receptor activator of nuclear factor kappa beta
RANKL	Receptor activator of nuclear factor kappa beta ligand
ROC	Receiver operating characteristics
SD	Standard deviation
TGF	Transforming growth factor
TNF	Tumor necrosis factor
UNC	University of North Carolina
VEGF	Vascular endothelial growth factor

Acknowledgements

The authors thank the University of Sharjah, Sharjah, UAE, for its support.

Author contributions

MAAA, ABA contributed to conception, design, collection of data, analysis, interpretation of the results, and drafting of the manuscript. SS, BR contributed to analysis, interpretation of the results, and drafting of the manuscript. ARG and AAI directed the biochemical analysis of samples and interpretation of results. MAAA and ABA contributed to formatting and critically revising the manuscript. All authors gave final approval and agreed to be accountable for the work done, ensuring its integrity and accuracy.

Funding

The University of Sharjah supported this work.

Data availability

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

Declarations

Ethics approval and consent to participate

An ethical clearance was obtained from the research ethics committee of the University of Sharjah (REC-21-09-28-02-S) and a written informed consent was obtained from all the participants prior to the investigation. All experimental protocols were approved by the research ethics committee of the University of Sharjah. The study adhered to the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 15 September 2024 / Accepted: 24 February 2025
Published online: 10 March 2025

References

1. Slots J. Periodontitis: facts, fallacies and the future. Periodontol 2000. 2017;75:7–23.
2. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, 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. 2018;89(Suppl 1):S173–82.
3. Trindade F, Oppenheim FG, Helmerhorst EJ, Amado F, Gomes PS, Vitorino R. Uncovering the molecular networks in periodontitis. Proteom Clin Appl. 2014;8:748–61.

4. Kwon T, Lamster IB, Levin L. Current concepts in the management of periodontitis. Int Dent J. 2021;71:462–76.
5. Liu CH, Abrams ND, Carrick DM, Chander P, Dwyer J, Hamlet MRJ, et al. Biomarkers of chronic inflammation in disease development and prevention: challenges and opportunities. Nat Immunol. 2017;18:1175–180.
6. Preethi PL, Rao SR, Madapusi BT, Narasimhan M. Immunolocalization of Ki-67 in different periodontal conditions. J Indian Soc Periodontol. 2014;18:161–5.
7. Takkem A, Barakat C, Zakaraia S, Zaid K, Najmeh J, Ayoub M, Seirawan MY. Ki-67 prognostic value in different histological grades of oral epithelial dysplasia and oral squamous cell carcinoma. Asian Pac J Cancer Prev. 2018;19:3279–86.
8. Davey MG, Hynes SO, Kerin MJ, Miller N, Lowery AJ. Ki-67 as a prognostic biomarker in invasive breast Cancer. Cancers (Basel). 2021;13:4455.
9. Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep. 2015;11(3):1566–72.
10. Taba M Jr, Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. Dent Clin North Am. 2005;49:551–71.
11. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. J Periodontol. 1972;43:38.
12. Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L. A modified gingival index for use in clinical trials. Clin Prev Dent. 1986;8:3–6.
13. Navazesh M, Kumar SK, University of Southern California School of Dentistry. Measuring salivary flow: challenges and opportunities. J Am Dent Assoc. 2008;139 Suppl:35S-40S. https://doi.org/10.14219/jada.archive.2008.0353. PMID: 18460678.
14. Rigillo A, Fuchs-Baumgartinger A, Sabattini S, Škor O, Agnoli C, Schwendenwein I, Bettini G, Rütgen BC. Ki-67 assessment-agreeability between immunohistochemistry and flow cytometry in canine lymphoma. Vet Comp Oncol. 2021;19:551–66.
15. Kill IR. Localisation of the Ki-67 antigen within the nucleolus. Evidence for a fibrillarin-deficient region of the dense fibrillar component. J Cell Sci. 1996;109:1253–63.
16. Richards-Taylor S, Ewings SM, Jaynes E, Tilley C, Ellis SG, Armstrong T, et al. The assessment of Ki-67 as a prognostic marker in neuroendocrine tumours: a systematic review and meta-analysis. J Clin Pathol. 2016;69:612–8.
17. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. International Ki-67 in breast Cancer working group. Assessment of Ki67 in breast cancer: recommendations from the international Ki67 in breast Cancer working group. J Natl Cancer Inst. 2011;103:1656–64.
18. de Paula AM, Carvalhais JN, Domingues MG, Barreto DC, Mesquita RA. Cell proliferation markers in the odontogenic keratocyst: effect of inflammation. J Oral Pathol Med. 2000;29:477–82.
19. Alsaegh MA, Altaie AM, Zhu S. p63 expression and its relation to epithelial cells proliferation in dentigerous cyst, odontogenic keratocyst, and ameloblastoma. Pathol Oncol Res. 2020;26:1175–82.
20. Choong PF, Akerman M, Willén H, Andersson C, Gustafson P, Baldetorp B, Fernö M, Alvegård T, Rydholm A. Prognostic value of Ki-67 expression in 182 soft tissue sarcomas. Proliferation–a Marker Metastasis?? APMIS. 1994;102:915–24.
21. Tretiakova MS, Wei W, Boyer HD, Newcomb LF, Hawley S, Auman H, et al. Prognostic value of Ki67 in localized prostate carcinoma: a multi-institutional study of > 1000 prostatectomies. Prostate Cancer Prostatic Dis. 2016;19:264–70.
22. Soliman NA, Yussif SM. Ki-67 as a prognostic marker according to breast cancer molecular subtype. Cancer Biol Med. 2016;13:496–504.
23. Jabbarzadeh M, Hamblin MR, Pournaghi-Azar F, Vakili Saatloo M, Kouhsoltani M, Vahed N. Ki-67 expression as a diagnostic biomarker in odontogenic cysts and tumors: A systematic review and meta-analysis. J Dent Res Dent Clin Dent Prospects. 2021;15:66–75.
24. Tumuluri V, Thomas GA, Fraser IS. The relationship of proliferating cell density at the invasive tumour front with prognostic and risk factors in human oral squamous cell carcinoma. J Oral Pathol Med. 2004;33:204–8.
25. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883–99.
26. Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Finkelman FD, van Rooijen N, et al. Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. Science. 2011;332:1284–8.
27. Fitzpatrick SG, Katz J. The association between periodontal disease and cancer: a review of the literature. J Dent. 2010;38:83–95.
28. Corbella S, Veronesi P, Galimberti V, Weinstein R, Del Fabbro M, Francetti L. Is periodontitis a risk indicator for cancer? A meta-analysis. PLoS ONE. 2018;13:e0195683.
29. Teresa DB, Neves KA, Neto CB, Fregonezi PA, de Oliveira MR, Zuanon JA, et al. Computer-assisted analysis of cell proliferation markers in oral lesions. Acta Histochem. 2007;109:377–87.
30. Sawhney N, Hall PA. Ki67–structure, function, and new antibodies. J Pathol. 1992;168:161–2.
31. Rigillo A, Fuchs-Baumgartinger A, Sabattini S, Škor O, Agnoli C, Schwendenwein I, et al. Ki-67 assessment-agreeability between immunohistochemistry and flow cytometry in canine lymphoma. Vet Comp Oncol. 2021;19:551–66.
32. Mozaffari HR, Sharifi R, Sadeghi M. Interleukin-6 levels in the serum and saliva of patients with oral lichen planus compared with healthy controls: a meta-analysis study. Cent Eur J Immunol. 2018;43:103–8.
33. Mozaffari HR, Zavattaro E, Saeedi M, Lopez-Jornet P, Sadeghi M, Safaei M, et al. Serum and salivary interleukin-4 levels in patients with oral lichen planus: A systematic review and meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2019;128:123–31.
34. Buduneli N, Buduneli E, Cinar S, Lappin D, Kinane DF. Immunohistochemical evaluation of Ki-67 expression and apoptosis in cyclosporin A-induced gingival overgrowth. J Periodontol. 2007;78:282–9.
35. Artese L, Piattelli A, de Gouveia Cardoso LA, Ferrari DS. Onuma tet al. Immunoexpression of angiogenesis, nitric oxide synthase, and proliferation markers in gingival samples of patients with aggressive and chronic periodontitis. J Periodontol. 2010;81:718–26.
36. Koulouri O, Lappin DF, Radvar M, Kinane DF. Cell division, synthetic capacity and apoptosis in periodontal lesions analysed by in situ hybridisation and immunohistochemistry. J Clin Periodontol. 1999;26:552–9.
37. Nagarakanti S, Ramya S, Babu P, Arun KV, Sudarsan S. Differential expression of E-cadherin and cytokeratin 19 and net proliferative rate of gingival keratinocytes in oral epithelium in periodontal health and disease. J Periodontol. 2007;78:2197–202.
38. Fanali S, Perrotti V, Riccardi L, Piattelli A, Piccirilli M, Ricci L, et al. Inflammatory infiltrate, microvessel density, vascular endothelial growth factor, nitric oxide synthase, and proliferative activity in soft tissues below intraorally welded titanium bars. J Periodontol. 2010;81:748–57.
39. Kranti K, Mani R, Elizabeth A. Immunoexpression of vascular endothelial growth factor and Ki-67 in human gingival samples: an observational study. Indian J Dent. 2015;6:69–74.
40. Rollman O, Jensen UB, Ostman A, Bolund L, Gústafsdóttir SM, Jensen TG. Platelet derived growth factor (PDGF) responsive epidermis formed from human keratinocytes transduced with the PDGF beta receptor gene. J Invest Dermatol. 2003;120:742–9.
41. Pinheiro ML, Feres-Filho EJ, Graves DT, Takiya CM, Elsas MI, Elsas PP, Luz RA. Quantification and localization of platelet-derived growth factor in gingiva of periodontitis patients. J Periodontol. 2003;74:323–8.
42. Hussain SP, He P, Subleski J, Hofseth LJ, Trivers GE, Mechanic L, et al. Nitric oxide is a key component in inflammation-accelerated tumorigenesis. Cancer Res. 2008;68:7130–6.
43. Batista AC, Silva TA, Chun JH, Lara VS. Nitric oxide synthesis and severity of human periodontal disease. Oral Dis. 2002;8:254–60.
44. Gürkan A, Emingil G, Cinarcik S, Berdeli A. Gingival crevicular fluid transforming growth factor-beta1 in several forms of periodontal disease. Arch Oral Biol. 2006;51:906–12.
45. Piekarska A, Piekarski J, Omulecka A, Szymczak W, Kubiak R. Expression of Ki-67, transforming growth factor beta1, and B-cell lymphoma-leukemia-2 in liver tissue of patients with chronic liver diseases. J Gastroenterol Hepatol. 2006;21:700–10.
46. Nagaraja C, Pradeep AR. Hepatocyte growth factor levels in gingival crevicular fluid in health, disease, and after treatment. J Periodontol. 2007;78:742–7.
47. Kanayama M, Takahara T, Yata Y, Xue F, Shinno E, Nonome K, et al. Hepatocyte growth factor promotes colonic epithelial regeneration via Akt signaling. Am J Physiol Gastrointest Liver Physiol. 2007;293:G230–9.
48. Chen M, Cai W, Zhao S, Shi L, Chen Y, Li X, et al. Oxidative stress-related biomarkers in saliva and gingival crevicular fluid associated with chronic periodontitis: A systematic review and meta-analysis. J Clin Periodontol. 2019;46:608–22.
49. Wu YC, Ning L, Tu YK, Huang CP, Huang NT, Chen YF, et al. Salivary biomarker combination prediction model for the diagnosis of periodontitis in a Taiwanese population. J Formos Med Assoc. 2018;117:841–48.
50. Khalaf H, Lönn J, Bengtsson T. Cytokines and chemokines are differentially expressed in patients with periodontitis: possible role for TGF-β1 as a marker for disease progression. Cytokine. 2014;67:29–35.
51. Rudrakshi C, Srinivas N, Mehta DS. A comparative evaluation of hepatocyte growth factor levels in gingival crevicular fluid and saliva and its correlation

with clinical parameters in patients with and without chronic periodontitis: A clinico-biochemical study. J Indian Soc Periodontol. 2011;15:147–51.
52. Anil S, Vellappally S, Preethanath RS, Mokeem SA, AlMoharib HS, Patil S et al. Hepatocyte growth factor levels in the saliva and gingival crevicular fluid in smokers with periodontitis. Dis Markers. 2014; 2014:146974.
53. Kim JY, Kim KR, Kim HN. The potential impact of salivary IL-1 on the diagnosis of periodontal disease: A pilot study. Healthc (Basel). 2021;9:729.
54. Chapple IL. Reactive oxygen species and antioxidants in inflammatory diseases. J Clin Periodontol. 1997;24:287–96.
55. Ambili R, Santhi WS, Janam P, Nandakumar K, Pillai MR. Expression of activated transcription factor nuclear factor-kappaB in periodontally diseased tissues. J Periodontol. 2005;76:1148–53.
56. Tabari ZA, Azadmehr A, Tabrizi MA, Hamissi J, Ghaedi FB. Salivary soluble receptor activator of nuclear factor kappa B ligand/osteoprotegerin ratio in periodontal disease and health. J Periodontal Implant Sci. 2013;43:227–32.
57. Doger FK, Dikicioglu E, Ergin F, Unal E, Sendur N, Uslu M. Nature of cell kinetics in psoriatic epidermis. J Cutan Pathol. 2007;34:257–63.
58. Zhang Y, Kang N, Xue F, Qiao J, Duan J, Chen F, Cai Y. Evaluation of salivary biomarkers for the diagnosis of periodontitis. BMC Oral Health. 2021;21:266.
59. Chew RJJ, Tan KS, Chen T, Al-Hebshi NN, Goh CE. Quantifying periodontitis-associated oral dysbiosis in tongue and saliva microbiomes-An integrated data analysis. J Periodontol. 2025;96:55–66.
60. Blanco-Pintos T, Regueira-Iglesias A, Seijo-Porto I, Balsa-Castro C, Castelo-Baz P, Nibali L, et al. Accuracy of periodontitis diagnosis obtained using multiple molecular biomarkers in oral fluids: A systematic review and meta-analysis. J Clin Periodontol. 2023;50:1420–43.

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