Original Article

Diagnostic Potential of Salivary Biomarker Profiles in Epidemiological Survey of Periodontitis

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Abstract

Objective: To assess the diagnostic value of salivary biomarker profiles in determining the prevalence of periodontitis in the adult population using the case definition for population-based surveillance of periodontitis (CDC/AAP) criteria.

Methods: Eighty subjects with healthy periodontium, mild, moderate, and severe periodontitis were recruited (20 in each group) using CDC/AAP criteria. Clinical parameters of probing pocket depth (PPD), clinical attachment loss (CAL), plaque index (PI), and bleeding index (BI) were recorded. Salivary samples were analyzed to determine levels of matrix metalloproteinase-8 (MMP8), interleukin 1β (IL-1β), and total protein (TP). ANOVA and Kruskal-Wallis tests were used to compare clinical and salivary parameters. Multinomial regression analysis was used to identify each examined group.

Results: Clinical parameters were significantly higher in periodontitis groups compared to the healthy group ($p< 0.0001$) except in PPD and PI. Both MMP8 and IL-1β were significantly higher in the moderate and severe periodontitis groups compared to the healthy group. No statistically significant differences in TP levels were found between examined groups ($p=0.9$). Multinomial regression analysis showed that the combination of MMP8, IL-1β, and age could accurately identify health, mild, moderate, and severe periodontitis groups with the certainty of 90%, 65%, 60%, 70%, respectively.

Conclusions: This study suggests that age, salivary MMP8, and IL-1β levels offer a way to determine the prevalence of periodontitis using CDC/AAP criteria.

Keywords: *Periodontitis, Diagnosis, Prevalence, Saliva, MMP8, IL-1β, Prediction.*

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Introduction

Periodontitis is a group of inflammatory diseases that affect the supporting tissues of teeth. Bacteria is the main cause; however, most destruction happens because of the immune response[1]. Periodontal diseases are among the six most prevalent diseases affecting humankind, with an estimated 740 million people affected by the severe form of periodontitis[2]. The World Health Organization has reported that severe forms of periodontitis cause tooth loss in about 5-15% of the population worldwide. Consequently, it is considered to be amongst the most common global health problems[3].

Epidemiological studies of periodontal diseases are conducted to determine periodontal health status, etiology, and preventive measures and, ultimately, control these diseases[4]. Furthermore, knowing the prevalence of the periodontal disease in each population is of great value in estimating the burden of periodontal disease and its risk factors on the morbidity rates in the population[5]. According to the latest data from the 2009 UK adult dental health survey, 37% of the adult population suffers from moderate chronic periodontitis levels, and 8% of the population suffers from severe periodontitis[6]. Periodontitis affects more than 47% of the adult population in the United States[7, 8], and severe periodontitis has been found to affect 11% of adults.

The Centers for Disease Control/American Academy of Periodontology (CDC/AAP) recommended the definitions by Eke et al.[9] as standard case definitions for mild, severe, and moderate periodontitis in population-based epidemiological surveys and the full-mouth examination periodontal protocol as the methodology of data collection. The CDC/AAP definition combines different threshold values of PPD and CAL. It requires examining a minimum number of affected sites on separate teeth to diagnose mild, moderate and severe periodontitis[9].

Diagnosis of periodontal diseases mainly relies on the assessment of traditional clinical parameters[10]; however, in epidemiological studies where a large number of subjects will be examined, using these parameters has many shortcomings; for example, it is time-consuming, requires skilled personnel, is prone to error and less accepted by patients[11,12]. To overcome these problems, many studies have looked into biomarker profiles in saliva that are objectively reliable and reflect the physio-pathological status of periodontitis[13-15]. Saliva collection is non-invasive and easy; it contains many useful components that can be used to display the oral health status and the onset of periodontal diseases[11].

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine that is associated with neutrophil recruitment and activation of osteoclasts through its ability to induce chemokines[16], and it has been shown to play a major role in the onset and progression of periodontitis[17]. Matrix metalloproteinase-8 (MMP-8) is regarded as one of the promising candidates for diagnosing and predicting periodontal disease progression in saliva and associated severity of PPD and bone loss[18-20]. Combined elevated salivary levels of IL-1β and MMP-8 have been found to increase the risk of experiencing periodontal diseases by 45-fold[21]. Total protein (TP), a nonspecific measure of the total amount of all the proteins present in saliva occurring as a consequence of the inflammatory process, is elevated in subjects with periodontitis[22].

Periodontitis has a complex nature, and a single biomarker is not sufficient to reflect all aspects of the disease[23]. Identifying potential panels of combined salivary biomarkers is more robust in distinguishing patients with different stages of periodontitis from healthy individuals. Thus, this study aims to assess the value of salivary profiles of IL-1β, MMP-8, and TP for determining the prevalence of periodontitis.

Materials and methods

Patient recruitment

This prospective case-control study was approved by the Scientific Committee, Faculty of Medicine, University of Sulaimani (ethical approval number: 3) on 20/12/2018 following the Helsinki declaration for human researches. Participants were recruited from patients attending the Periodontology Clinic at the Shorsh Dental Center from April to October 2019. Potential study participants were screened and assessed for periodontal disease and inclusion and exclusion criteria before being invited to join the study. A total of 580 subjects were screened, and 80 subjects were accepted after signing a consent form. Inclusion criteria were subjects aged 30 years and older, systemically healthy, with ≥20 sound teeth, and their periodontal status was diagnosed according to CDC/AAP criteria[9]. The patients were allocated to one of four groups: healthy periodontium, mild, moderate, and severe periodontitis, with 20 subjects in each group. Exclusion criteria were subjects with a history of systemic disease

or medication that could affect their periodontal condition, such as diabetes and Nifidepine, smokers, pregnant and lactating women, subjects who had taken antibiotics in the last three months, and subjects without the capacity to give informed consent.

Clinical measurements

Full-mouth clinical parameters of CAL, PPD, PI[24], and BI[24] were recorded at six sites per tooth, excluding wisdom teeth, using a Williams periodontal probe. Clinical attachment loss was calculated as the distance from the CEJ to the base of the pocket. Probing pocket depth was measured as the distance from the gingival margin to the bottom of the pocket[25]. The absence and presence of plaque were detected based on a dichotomous scoring index. The interdental CAL and PPD averages were calculated by dividing all interdental CAL and PPD by the total number of sites with interdental CAL and PPD, respectively. The percentages of sites with CAL, PPD, PI, and BI in each subject were calculated by dividing the number of sites with these clinical signs of the disease into the total number of tooth sites. The intra and inter-examiner reliability were high (Kappa test = ≥98%), and the literature indicates that agreement of 90% for CAL and PPD readings is considered good[26].

Saliva collection and analysis

Three milliliters of unstimulated whole saliva was collected from the participants by spitting method in the morning[27]. All subjects were instructed not to eat a major meal less than 60 minutes before the appointment. Collected samples were placed immediately on ice. The saliva samples were placed into a sterile Eppendorf and centrifuged (1300 rpm for 20 minutes) to obtain clear supernatant. The supernatant was stored at -80C until required for biochemical and immunological analysis. The levels of MMP8 and IL-1β were determined by the ELISA kit of Human MMP8 (Abcam219050, Cambridge, UK) and Human IL-1β (MyBioSource, MBS2510385, USA), respectively, according to manufacturers' instructions. Concentrations of MMP8 and IL-1β were determined using 450 nm filters, and the optical densities were compared to the standard curve to obtain final concentrations as pg/mL. Meanwhile, the Biuret method[28] was used to determine total protein concentration using bovine albumin (6g/dL) and 550 nm filters in which peptide bonds of protein react with Cu^{2+} in alkaline solution to form a blue-violet complex which is proportional to the concentration of the total protein in the specimen. All estimations were conducted using Biuret reagent kits (Biolabo-France).

Statistical analysis

The Shapiro-Wilk test was used to test the normality of data. A one-way ANOVA test was carried out to determine the significant differences in parametric data (interdental CAL, CAL%, PI%, BI%, and MMP8) between the tested groups. Then, LSD tests were done to conduct pairwise comparisons between groups. Kruskal-Wallis test was used to find statistically significant differences in non-parametric data (PPD, PPD%, IL-1β, and total protein) between tested groups. Multinomial regression analysis was used to investigate the clinical data's correlation (patients grouped according to CDC/AAP criteria) as a dependent variable. Age and sex were used as independent variables for biomarker examination. $P \leq 0.05$ was regarded as statistically significant. Data analysis was conducted using the statistical software package IBM-SPSS (Statistical Package for the Social Sciences-version 22.0, Chicago IL, USA).

Results

Subjects

A total of 580 subjects were screened, with the majority either not fulfilling the study criteria or not agreeing to participate in the study. Eighty subjects (24 male, 56 female) were recruited, with mean age 40.9±9.3 years ranging between 30 and 70 years (Healthy group: 42.4 ±9.5 years, mild periodontitis: 37.1 ±6.1 years, moderate periodontitis: 40.1 ±10.8 years, severe periodontitis: 43.9 ±9.4 years).

Clinical data

Full mouth clinical data (CAL, PPD, PI, BI) showed statistically significant differences between healthy group vs. diseased groups except for mean PPD in the healthy group when compared to the mild and moderate periodontitis groups. Furthermore, no statistically significant differences were detected in PI percentages between the healthy and moderate periodontitis groups ($p= 0.3$) (Table 1 and 2). A comparison between the mild and moderate periodontitis groups revealed no statistically significant differences in any clinical parameters ($p>0.05$) apart from PI ($p=0.011$). Whereas, statistically significant differences were found for all clinical parameters in the mild and moderate periodontitis groups when compared to the severe periodontitis group ($p<0.05$) except for average PPD and percentage of sites with PPD ($p = 0.92, 0.11$, respectively) (Table 1 and 2).

Biomarker levels

Statistically significant differences were revealed in both MMP8 and IL-1β for the healthy group compared to the moderate and severe periodontitis groups ($p<0.05$). However, no statistically significant differences were identified when comparing levels of MMP8 and IL-1β in the healthy and mild periodontitis groups ($p= 0.13, 0.17$, respectively), as shown in Table 2. Furthermore, there were statistically significant differences in MMP8 levels between the mild vs. moderate (ANOVA, $p = 0.03$), mild vs. severe (ANOVA, $p= 0.0001$), and moderate vs. severe (ANOVA, $p= 0.014$) periodontitis groups. Whereas, when all the periodontitis groups were compared together, for IL-1β, a statistically significant difference emerged only between the mild vs. severe periodontitis groups (Kruskal-Wallis test, $p=0.0003$). Finally, no statistically significant differences were detected in TP levels ($p>0.05$).

Predictive value

Multinomial regression analysis was used to determine whether a biomarker profile with demographical data (age and sex) is a useful prognostic tool for the prevalence of periodontitis. Groups of patients determined according to CDC/AAP criteria was used as a dependent variable. MMP8 and IL-1β levels, in combination with the age of subjects, was able to predict healthy subjects with a certainty of 90%. Additionally, these three parameters predicted mild, moderate, and severe periodontitis groups with 65%, 60%, and 70% certainty, respectively (Table 3). The effects (β) of IL-1β and MMP8 were directly associated with the severity of periodontitis, and IL-1β had a greater odds ratio than MMP8 in identifying all periodontitis groups (Table 4). Whereas the age of subjects was negatively related to the severity of periodontitis (β was negative), the TP level and sex of subject had no impact on overall prediction levels ($p> 0.05$).

Variables	Health	Mild	Moderate	Severe
CAL (mm)*	0	1.79 (0.1)	2.176 (0.2)	3.546 (0.26)
CAL (%)*	0	12.9 (1.51)	15.5 (2.6)	47.25 (5.14)
PI (%)*	41.2 (4.26)	60.6 (3.59)	46.75 (4.3)	75.2 (2.64)
BI (%)*	17.15 (2.85)	33.6 (4.16)	38.8 (4.03)	57.45 (4.45)
MMP8 (pg/mL)	536.1 (85.2)	978.1 (132.6)	1254.7 (92.7)	1443.7 (109.9)
PPD (mm)**	0	4.05 (0.23)	4.35 (0.51)	4.59 (0.97)
PPD (%)**	0	3 (2)	5 (6)	18 (17)
Interleukin-1β (pg/mL)**	7.06 (5.82)	11.8 (8.48)	27.3 (20.02)	34.98 (17.2)
Total Protein (mg/mL)**	11.77 (2.06)	11.325 (2.85)	12 (1.72)	12 (2.55)

Table 2. Comparison of clinical parameters and biomarkers levels between examined groups.

Variables	Health vs. mild	Health vs. moderate	Health vs. severe	Mild vs. moderate	Mild vs. severe	Moderate vs. severe
CAL (mm)*	0.0001	0.0001	0.0001	0.14	0.0001	0.0001
CAL (%)*	0.004	0.0001	0.0001	0.55	0.0001	0.0001
PI (%)*	0.000	0.3	0.0001	0.011	0.008	0.0001
BI (%)*	0.004	0.0001	0.0001	0.35	0.0001	0.001
MMP8 *	0.13	0.0001	0.001	0.03	0.0001	0.014
PPD (mm)**	0.99	0.19	0.001	0.37	0.006	0.92
PPD (%)**	0.002	0.0001	0.0001	0.80	0.0006	0.11
Interleukin-1β**	0.17	0.0002	0.0001	0.25	0.0003	0.22
Total Protein**	0.99	0.99	0.99	0.21	0.99	0.99

Table 3: Multinomial regression analysis for health, mild, moderate, and severe periodontitis as a dependent variable.

Groups	Predictive %
Health	90
Mild	65
Moderate	60
Severe	70

Table 4. Summary of multinomial regression for each independent variable for subjects with mild, moderate, and severe periodontitis using the healthy group as reference.

Groups	Predictor variable	Effects (β)	Odds Ratio (OR)	95% CI for OR		p-value
				LCL	UCL	
Mild	IL-1β	0.51	1.666	1.114	2.432	**0.008**
	MMP8	0.03	1.003	1.001	1.005	**0.016**
	Total protein	-0.387	0.679	0.4.34	1.061	0.089
	Age	-0.247	0.781	0.666	0.917	**0.003**
	Sex	-.085	0.919	0.066	12.840	0.950
Moderate	IL-1β	0.554	1.740	1.189	2.547	**0.004**
	MMP8	0.04	1.04	1.002	1.007	**0.002**
	Total protein	-0.18	0.982	0.926	1.041	0.540
	Age	-0.205	0.815	0.690	0.963	**0.016**
	Sex	0.940	2.559	0.123	53.205	0.544
Severe	IL-1β	0.578	1.782	1.217	2.610	**0.003**
	MMP8	0.04	1.04	1.002	1.007	**0.001**
	Total protein	-0.317	0.728	0.485	1.095	0.127
	Age	-0.19	0.854	0.720	1.013	**0.05**
	Sex	0.219	1.245	0.055	28.378	0.891

Discussion

This study indicates that combining MMP8, IL-1β, and age-associated with determination the prevalence of periodontitis. Finding biomarkers profile in saliva that can identify the prevalence of periodontitis in different populations would be of significant value in terms of both reducing cost and saving time[29]. Salivary biomarkers have shown the ability to differentiate subjects with different stages of periodontal diseases[30,31]. The underlying rationale of the study was that an individual biomarker is less likely to reflect sufficiently the complex nature of the disease, and no single biomarker is adequate for use in practice[32,33]. Therefore, the present study attempted for the first time to use combined saliva biomarker profiles to determine periodontal status based on CDC/AAP criteria, which are the currently recommended criteria in epidemiological studies. It is important to note that CDC/AAP criteria are based on PPD and interdental CAL to distinguish between mild, moderate, and severe periodontitis. This study indicates that combining MMP8, IL-1β, and age-associated with determination the prevalence of periodontitis.

In this study, saliva has been used as a biomarker source as it is easily accessible, abundant, and large volume samples can be obtained without needing clinical facilities or complex skills. Moreover, the saliva content reflects all aspects of periodontal disease activity and therefore conveys a consensus 'whole mouth' inflammatory status[34]. Furthermore, this study did not attempt to find new biomarkers of periodontal disease, using only biomarkers with clear evidence of association with periodontal disease. Instead, biomarker levels were combined in an attempt to enhance their

diagnostic value and to overcome the problem of a single biomarker being insufficient for clinical use due to the complex nature of the disease.

Salivary MMP-8 levels have previously shown a correlation with clinical signs of periodontitis and radiological bone loss[35] and demonstrated the capability to differentiate the stages of the disease (health, gingivitis, and periodontitis) with 69% sensitivity and 70% specificity[36]. This is in line with the result of the current study, except that MMP8 levels could not differentiate subjects with mild periodontitis from healthy subjects. This could be because this study classified different stages of periodontitis according to CDC/AAP criteria rather than comparing periodontitis subjects as a group to the healthy group. In contrast, mild periodontitis is the first stage of the disease and might be associated with low MMP8 levels. Furthermore, it could also be associated with the CDC/AAP criteria relying upon interdental CAL and PPD for diagnosis; since the former mostly reflects what has happened rather than the current status of the disease, a low MMP8 level might be expected. Indeed, statistically significant differences were found in interdental CAL between all examined groups (except mild vs. moderate). In contrast, no statistically significant differences were found for PPD among the study groups.

Many clinical studies reported the use of IL-1β as a potential biomarker of periodontal diseases. The mean levels of salivary IL-1β were significantly higher in patients with periodontal disease than in controls, and they were correlated with individual clinical parameters of periodontal diseases[37,38]. This agrees with this study's result as IL-1β levels were generally higher in periodontitis groups than the healthy group. However, no statistically significant differences were identified when two consecutive groups (health vs. mild, mild vs. moderate, and moderate vs. severe) were compared. This explains the overlap in IL-1β level when CDC/AAP criteria based on both PPD and interdental CAL are used for determining the diseased groups.

Interestingly, neither were any statistically significant differences in PPD detected when two consecutive groups were compared. One can conclude that IL-1β is more associated with PPD, which represents the active status of the disease rather than interdental CAL that might be a combination of recession and true PPD. Nevertheless, IL-1β demonstrated robust diagnostic value when combined with MMP8.

There is little available data on variations of the TP level in relation to periodontal diseases in the literature. Mohammad (2018)[22] found higher levels of TP in chronic periodontitis subjects than control healthy subjects. This finding is not consistent with this study's result, and the discrepancy can be explained by using different case definitions of periodontitis. Furthermore, TP concentrations can vary significantly in response to saliva flow variations among individual subjects [39].

MMP8 and IL-1β have been investigated in terms of differentiating healthy from periodontitis subjects. However, their diagnostic value for identifying different periodontitis stages using CDC/AAP criteria has not been tested yet. Here we show that combining MMP8 and IL-1β increased their capacity to determine different stages of periodontitis. The additional diagnostic value was obtained by including the subject's age, which is a variable that can be easily applied without looking in the subject's mouth. The TP level and sex of the subject were not statistically significantly associated with periodontal status (Table 4). We think this result can be considered acceptable, given that age and levels of salivary MMP8 and IL-1β can be collected, non-invasively, and with less time consumption, for use as the first line of a screening tool to identify patients that require further clinical examination.

Conclusions

This study has shown that combining salivary MMP8 and IL-1β levels with subjects' age can provide significant value in the diagnosis of subjects with mild, moderate, and severe periodontitis. This is a promising finding supporting conducting studies with a larger sample size with all age groups and including cofounding factors such as diabetes and smoking, which were excluded here. We also recommend that further studies should not rely on TP as a biomarker of periodontal diseases but should consider other biomarkers for enhancing the diagnostic value.

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