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Research Article

Comparison Between Salivary and GCF Myeloperoxidase in Periodontal Disease Screening Effect

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Abstract

Objective: This study was undertaken to compare the association of the activity and level of Myeloperoxidase (MPO) from saliva and from gingival crevicular fluid (GCF) in screening of periodontal disease.

Methodology: Saliva and GCF were collected from 88 subjects at baseline before periodontal treatment: 26 with chronic periodontitis (CP), 32 with gingivitis and 30 with healthy periodontium. Full mouth plaque index (PI), bleeding on probing (BOP), periodontal pocket depth (PPD) and clinical attachment level (CAL) were also measured and recorded. MPO activity and level were measured by colorimetric method and indirect ELISA.

Results: Increase in MPO activity, level and ratio to total protein was seen in patients with chronic periodontitis. Significant difference in MPO was seen in both saliva and GCF of periodontally healthy and gingivitis patients compared with patients with periodontitis. The calculated cut-off value from GCF gave higher sensitivity, specificity and predictive values than saliva samples.

Conclusions: MPO activity, MPO level and ratio between MPO to total protein from GCF or saliva might be considered as a diagnostic and prognostic biomarker of periodontal bone destruction.

Keywords: Myeloperoxidase, Periodontal diseases, Saliva, GCF and biomarker

Introduction

Periodontal diseases are chronic infection and inflammation diseases. While gingivitis causes mild to severe inflammation limited only to gingival tissues, periodontitis leads to destroy different amounts of alveolar bone support which can lead to tooth loss [1]. A natural characteristic of the periodontal diseases is an irregular intermittent inflammation of soft and hard tissue. Therefore, all the patients need a correct differential diagnosis and careful periodic follow-up through-out one's life time [2,3]. In most circumstances, early detection of the disease follow by timely treatment is required for reducing disease severity and complications [4]. In any community, average incidence of periodontal diseases is nearly 80%. Currently, for Thai population over 90% have different levels of periodontal diseases where 48% of the adults already have alveolar bone loss due to the periodontitis [5]. Myeloperoxidase (MPO) is a critical enzyme from neutrophils, one of the most effective microbicidal and cytotoxic mechanism, which related to be a promising biomarker to periodontal disease [6]. Increased MPO activity in gingival crevicular fluid (GCF) and whole mouth saliva from patients with periodontal diseases during the chronic inflammatory stage has been reported [7-10]. The objective of this study was to determine and compare if MPO activity and MPO concentration from GCF and saliva has the ability to screen periodontal status.

Materials and Methods

Study Population and Selection Criteria

Eighty-eight systemically healthy Thai subjects (21 males and 67 females), aged 20-60 years who sought treatment at the Department of Periodontology, Faculty of Dentistry, Khon Kaen University, Thailand from June 2013-Jan 2015 were enrolled in this study. This cross-sectional study was approved by the human ethics committee, Khon Kaen Univeristy (HE551372). Informed consent was obtained from all subjects after the study objectives, design and study plan has been explained.

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Periodontal Examination using clinical parameters namely, Gingival Index (GI), bleeding on Probing (BOP), Plaque Index (PI), Probing Pocket Depth (PD) and Clinical Attachment Loss (CAL) were recorded by one examiner using a periodontal probe (UNC-15, Hu-Friedy, Chicago, IL). All subjects who had at least 15 remaining teeth were divided into 3 groups: Group1 (n=25) were generalized chronic periodontitis patients (BOP > 20% of sites, PD > 4 mm, CAL > 1 mm), Group 2 (n=32) were gingivitis patients (BOP > 20% of sites, PD ≤ 4 mm, CAL ≤ 1 mm) and Group 3 (n=30) were periodontally healthy subjects taken as control. Those with history of any systemic disease, smoking, current pregnancy or lactation, periodontal therapy or use of antibiotics or mouth rinse in the previous 3 months were excluded.

Saliva Collection

Collection of unstimulated whole saliva samples were obtained for measuring MPO activity and MPO concentration were performed as described previously [10]. Briefly, unstimulated whole saliva samples were collected in the morning. No food and soft drink was allowed for at least 90 minutes before collection of saliva. After rinse the mouth thoroughly with normal saline, followed by expectorating whole saliva into a centrifuge tube for 15-20 minutes. Measurement of salivary pH and MPO activity was performed immediately.

GCF Collection

GCF samples from healthy and gingivitis patients were collected from mesial sites of tooth numbers 16, 21, 24, 36, 41 and 44 using 6 PerioStrips. For chronic periodontitis patients, GCF samples were obtained from the most severe sites of each quadrant using 4 strips. All strips were gently inserted to the bottom of the gingival sulcus and left for 30 sec. Strips were then transferred into tubes containing 640µl of TrisHCL. Optimum protein elution was performed by using the most optimum condition for total protein elution (data not shown).

Colorimetric Assay of MPO Activity

The MPO activity was measured immediately after sample collection by using chemical reagents and methods as described before [10]. Briefly, each sample was pipetted into 0.5 mM 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution and hydrogenperoxide (H_2O_2) was added to initiate the reaction. After incubation 20 µl of 0.1 mM sodium azide was added to stop the reaction. The color of the MPO enzyme product ranged from almost colorless in healthy subjects, moderately brown in gingivitis patients and dark brown in periodontitis patients. By using a spectrophotometer at wavelength 586 nm (Genesys 20 Thermo Scientific, USA), the differences of color intensity were detected.

Enzyme-linked Immunosorbent Assay (ELISA) of MPO Concentration

ELISA indirect technique was performed by using 4ug/ml mouse monoclonal antibodies to Myeloperoxidase (Abcam, England) as a capture antibody. One ug/ml rabbit polyclonal antibodies to Myeloperoxidase and donkey anti-rabbit IgG linked with HRP was added as detecting and secondary antibody. Phosphate-buffered saline with 0.05%Tween was used as washing solution as describe before [10]. Absorbances were measured at 450 nm.

Statistical Analysis

Statistical analyses were performed using SPSS program (version 11.5). Kruskal-Wallis analysis followed by Mann-Whitney test with Bonferroni's test were carried out for a comparison between the groups. $P < 0.05$ was considered statistically significant.

Results

Characteristics of Study Subjects

Demographic characteristics and periodontal clinical parameters of the study subjects are provided in table 1. Mean age of the population is 34.48 years old with majority of the subjects being females (76%). While all periodontal clinical parameters (BOP, PD, and CAL) in periodontitis patients were significantly higher than other groups, only BOP from gingivitis group were significantly higher than in periodontally healthy group.

Concentration of Total Protein in Salivary and GCF Sample

The mean and standard deviation of total protein and MPO concentration from all groups are presented in table 2. The results

Characteristics	Healthy (n=30)	Gingivitis (n=32)	Periodontitis (n=26)
Demographic characteristics			
Age in years *	32.13 ± 9.92	31.84 ± 9.06	45.54 ± 13.11 [a,b]
Female (%)	83.33	87.5	57.69
Periodontal parameters			
PD *	1.887± 0.196	1.948 ± 0.155	2.634 ± 0.376
(median)	1.865	1.935	2.495 [a,b]
CAL *	0.521 ± 0.168	0.534 ± 0.123	2.345 ± 1.064
(median)	0.495	0.56	2.155 [a,b]
BOP (%)	15.42 ± 3.58	38.36 ± 9.83	51.10 ± 15.19
(median)	16.071	36.558 [a]	50.253 [a,b]

* = mean ± SD, PD = probing depth, CAL = clinical attachment loss, BOP = bleeding on probing

[a]Significant difference from healthy group (Kruskal Wallis test, p < 0.05, Mann-Whitney U test, p <0.006).

[b]Significant difference from gingivitis group (Kruskal Wallis test, p <0.05, Mann-Whitney U test, p <0.006).

Table 1: Demographic characteristics and periodontal clinical parameters of the subjects

	Healthy (n=30)	Gingivitis (n=32)	Periodontitis (n=26)
Total Protein			
Saliva *	490.64 ± 252.76	550.68 ± 128.03	1,016.46 ± 1,307.38
(median)	465.33	527.00 [a]	672 .00 [a,b]
GCF *	446.62 ± 138.29	436.56 ± 116.31	432.46 ± 68.38
(median)	425.83	405.00	412.00
MPO			
Saliva *	0.485 ± 0.267	0.676 ± 0.891	2.458 ± 1.622
(median)	0.422	0.543	2.624 [a,b]
GCF *	0.371 ± 0.126	0.537 ± 0.358	1.659 ± 0.688
(median)	0.383	0.450	1.71 [a,b]
MPO : total protein			
Saliva *	1.17 ± 1.02 ($x10^{-3}$)	1.19 ± 1.15 ($x10^{-3}$)	3.93 ± 3.77($x10^{-3}$)
(median)	0.92 ($x10^{-3}$)	0.93 ($x10^{-3}$)	2.9 ($x10^{-3}$) [a,b]
GCF *	0.87 ±0.34($x10^{-3}$)	1.33± 1.03($x10^{-3}$)	3.96 ±2.01($x10^{-3}$)
(median)	0.90($x10^{-3}$)	1.03($x10^{-3}$)	4.19 ($x10^{-3}$) [a,b]

* = mean ± SD, MPO = Myeloperoxidase enzyme, GCF= gingival crevicular fluid

[a]Significant difference from healthy group (Kruskal Wallis test, p < 0.05, Mann-Whitney U test, p <0.006).

[b]Significant difference from gingivitis group (Kruskal Wallis test, p <0.05, Mann-Whitney U test, p <0.006).

Table 2: Total protein and MPO levels from samples (Unit: mg/ml)

show that total protein levels in saliva were lowest in healthy group and highest in periodontitis group. On the contrary, there was little difference observed in the total protein levels from GCF samples. A statistically significant difference in the median total protein level was observed from the saliva of periodontitis group but not from the GCF.

Concentration of MPO in Salivary and GCF Sample

The MPO levels both in salivary and GCF of periodontitis patients were dramatically higher when compared with the 2 other groups **(**Kruskal Wallis test, $p < 0.05$, Mann-Whitney U test, $p < 0.006$**).** The ratio of MPO level to total protein concentration from periodontitis group showed statistically significant difference from the 2 other groups with $p < 0.05$. Clinically, a cut-off value of 0.857 ug/ml in saliva samples yields 84.6 % sensitivity and 86.7% specificity. In addition, a cut-off value of 0.542 ug/ml in GCF samples produces higher sensitivity and specificity at 92%. Data shown in table 3.

MPO Activity in Salivary and GCF Sample

The differences of color intensity were detected and shown in table 4. It was found that the median MPO activity in saliva and GCF from periodontitis patients is statistically significantly higher compared to 2 other groups **(**Kruskal Wallis test, $p < 0.05$, Mann-Whitney U test, $p < 0.006$**).** The cut-off value of 8.2 x 10^{-3} in saliva samples yields 69 % sensitivity and 70% specificity. In comparison, a higher sensitivity (73%) and specificity (77%) was obtained when utilizing the cut-off value of 11.3 x 10^{-4} in GCF samples (Table 5).

	Sensitivity (%)	Specificity (%)	Predictive value (%)	
			Positive	Negative
Saliva	84.6	86.7	86.4	84.9
GCF	92.3	93.3	93.2	92.3

The cut-off value 0.857 and 0.542 ug/ml for saliva and GCF samples were calculated using the receiver operating characteristic curve (ROC) method.

Table 3: Sensitivity, specificity, positive and negative predictive values of the MPO level

	Healthy (n=30)	Gingivitis (n=32)	Periodontitis (n=26)
Saliva * (median)	11.8± 17.1 5.9	14.3 ± 15.6 11.4 [a]	37.8 ± 33.2 38.2 [a,b]
GCF * (median)	2.9 ± 2.8 1.4	5.4 ± 1.5 2.5	7.3 ± 8.1 6.4 [a,b]

Data are shown in 10 -3, * = mean ± SD, MPO = Myeloperoxidase enzyme, GCF= gingival crevicular fluid

[a]Significant difference from healthy group (Kruskal Wallis test, $p < 0.05$, Mann-Whitney U test, $p < 0.006$).

[b]Significant difference from gingivitis group (Kruskal Wallis test, $p < 0.05$, Mann-Whitney U test, $p < 0.006$).

Table 4: MPO activities from samples (Wavelength: 586nm)

	Sensitivity (%)	Specificity (%)	Predictive value (%)	
			Positive	Negative
Saliva	69	70	69.6	69.3
GCF	73	77	76.0	74.0

The cut-off value 0.008 and 0.00113 for saliva and GCF samples were calculated using the receiver operating characteristic curve (ROC) method.

Table 5: Sensitivity, specificity, positive and negative predictive values of the MPO activities

Discussion

MPO is often demonstrated to be a significant inflammatory marker for early detection of the pathogenesis of inflammatory periodontal diseases [11-14]. Our previous article was successful in demonstrating direct association of increased GCF and salivary MPO activity with severity of periodontitis [10]. Even the major function of MPO in saliva was not reported, the highest MPO activity was observed in saliva from periodontitis patients, followed by gingivitis patients and finally periodontally healthy individuals. Findings of this study are consistent with previous reports that MPO activity in saliva and GCF samples detected by colorimetric method are related to the grading of clinical destruction in periodontal disease.

In the present study, GCF total protein from all subject groups remained almost the same, but significant increase was observed in saliva samples. On the other hand, MPO concentration from both saliva and GCF were highest in periodontitis patients, about 6 times greater than control group, followed by gingivitis patients and least on periodontally healthy subject. Even this is an observational cross-sectional study, but from all the results may led us to consider that the higher ratio of MPO to total protein concentration has correlation with chronic periodontitis.

While salivary sample collection has its own advantage in being more practical with less skill required than GCF collection, GCF collection is a more effective screening test [15-17]. In this present study, when focusing on percentage of sensitivity, specificity and predictive value, GCF samples brought more pleasant statistical data set. This will be more beneficial in terms of clinical diagnosis. The power of differentiation between healthy subjects and periodontal disease patients was estimated at the cut-off value of 0.857 and 0.542 ug/ml for saliva and GCF samples, by the receiver operating characteristic curve (ROC) method. The area under ROC curve (AUC) was 0.843 (95% CI = 0.646-0.923). Additionally, 92.3% sensitivity, 93.3% specificity, 93.2% positive predictive value and 92.3% negative predictive value was observed.

Conclusions

The present study demonstrated the significant increase in level of MPO activity and MPO concentration from saliva and GCF in periodontitis patients. After calculating the cut-off value, GCF samples gave higher sensitivity and specificity values than saliva samples. The positive predictive value from MPO level in GCF and salivary samples were 93% and 86% and the negative predictive value were 92% in GCF and 84% in saliva samples. Our finding suggested that MPO activity, MPO level or ratio between MPO to total protein may be used as adjunctive indicators for screening of chronic periodontitis in large community.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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