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Relationships between High-mobility Group Protein B1 and Triggering Receptor Expressed on Myeloid Cells Concentrations in Gingival Crevicular Fluid and Chronic Periodontitis

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ABSTRACT

One of the inflammatory mediators which is secreted by inflammatory cells is high-mobility group protein B1 (HMGB1). Interaction of HMGB1 and toll-like receptors (TLRs) leads to increased production of inflammatory cytokines. On the other hand, it was shown that triggering receptor expressed on myeloid cells (TREM-1) also can be activated by TLRs, and its soluble form (sTREM-1) can be formed by cleaving of membrane-bound form of TREM-1 proteinases. Since there is not enough knowledge about the precise role of HMGB1 and sTREM-1 in periodontal diseases, the aim of this study was to evaluate the concentration of HMGB1 and sTREM-1 in gingival crevicular fluid (GCF) samples of patients with chronic periodontitis.

Gingival crevicular fluid (GCF) samples were obtained from a total of 24 individuals with clinically healthy gingiva and 24 patients with moderate to severe chronic periodontitis. For collecting GCF samples, periopapers were placed at the entrance of the crevice and left in position for 30 seconds. Then, they were stored at -80°C. Enzyme-linked immunosorbent assay (ELISA) was used for measuring the concentration of HMGB1 and sTREM-1 in GCF samples.

The concentration of HMGB1 ($p<0.001$) and sTREM-1 ($p<0.017$), was significantly higher in chronic periodontitis group. In addition, there was a significant positive correlation between HMGB1 and sTREM-1 concentration in chronic periodontitis group ($p<0.05$). We also found significant positive correlation between PD (Pocket depth) and the concentration of HMGB1 ($p<0.001$) and sTREM-1 ($p<0.015$).

It is concluded that both HMGB1 and sTREM-1 are released during the inflammatory response of periodontal tissues and they can promote inflammatory process, which leads to tissue destruction.

Keywords: Alarmins; Chronic periodontitis; DAMP; High-mobility group protein; HMGB1; Inflammation; sTREM1

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INTRODUCTION

Periodontitis is one of the poly-microbial inflammatory diseases that affects supportive tissues around the teeth, or periodontium.[1] Periodontitis is regarded as the second most common dental disease and has a prevalence of 30-50% in United States.[2] Periodontitis has been linked to some of the systemic diseases such as diabetes, atherosclerosis and rheumatoid arthritis. Although it has been well known that the initiation of periodontal diseases depends on periodontopathic bacteria and their products. It is has been clear that severity of tissue destruction is not necessarily according to bacterial load. In fact, the main determinant of the periodontium destruction is inflammatory responses of host in an attempt toprotection itself.[3]

One of the inflammatory mediators secreted by inflammatory cells is high-mobility group protein B1 (HMGB1). Interaction of HMGB1 and toll-like receptor2 (TLR2), TLR4 and TLR9 leads to increased production of inflammatory cytokines. Therefore, HMGB1 may have a possible role in destructive changes of periodontal tissues.[4-13]

It was found that in patients with periodontal diseases, there is higher concentration of HMGB1.[14,15]

In addition several studies have suggested that HMGB1 can play a role in the osteoclastogenesis, which in turn can lead to alveolar bone loss.[15]

Triggering receptor expressed on myeloid cells (TREM-1) was first described in 2000.[17] It was shown that TREM-1 can be activated by binding to TLR2 and TLR4, which results in potentiating inflammation.[18,19] sTREM-1 or soluble form of TREM-1, can be formed by cleaving membrane form of TREM-1 using matrix metalo-proteinases.[20,21] sTREM-1 can be measured in biological fluids such as GCF.[22] The highest levels of sTREM-1 were found in sepsis and other inflammatory diseases.[22,23]

Since periodontal diseases are inflammatory disorders and there is not enough knowledge about the precise role of HMGB1 and sTREM-1, the aim of this study was to evaluate the relationship between moderate to severe chronic periodontitis and the concentration of HMGB1 and sTREM-1 in GCF samples.

MATERIALS AND METHODS

Patients

GCF samples were obtained from a total of 24 individuals with clinically healthy gingiva and 24 patients with moderate to severe chronic periodontitis. The diagnosis was made by a periodontist. All subjects received clinical examination including the following periodontal parameters: plaque index (PI), bleeding on probing (BOP), PD and clinical attachment loss (CAL). PD was measured from the free-gingival margin to the base of the periodontal pocket or sulcus and CAL was measured from the cementoenamel junction (CEJ) of the tooth to the base of the periodontal pocket or sulcus. The inclusion criteria included: 1) CAL≥3 millimeter at least in 30% of periodontal tissues 2) alveolar bone loss in radiograph. Exclusion criteria were: 1) systemic disease, 2) chronic medication which affects the periodontal status, 3) antibiotic therapy and periodontal therapy within 6 months prior to sampling, 4) pregnant or lactating women, 5) smoking, 6) oral diseases other than periodontal diseases.

The study design was approved by Ethics Committee of Tehran University of Medical Sciences (No. 23919-70-01-93). Informed consents was obtained from all participants.

Methods

For collecting GCF samples, following the careful removal of supragingival biofilm, areas were washed with water spray; the selected sites were isolated with cotton rolls and gently dried by a fine-bore high-power suction tip. GCF was collected by placing 2 perio-papers (Oraflow Inc., Amityville, NY, USA) into the mesial and distal of the buccal surface of each selected site, until a slight resistance was felt and then left in place for 30 seconds. Periopapers contaminated by blood or saliva were excluded. Immediately, the volume of the sample was measured with a calibrated periotron 800 (Oraflow Inc., Amityville, NY, USA). After volume measurements, the strips were placed into sterile eppendorf tubes (Eppendorf, Hamburg, Germany) and were stored at -80°C.

The GCF blotted periopapers were allowed to thaw at room temperature for 30 minutes. For elution of GCF samples, 200 microliter of phosphate-buffered saline (PBS) was added to each micro-centrifuge tube containing one periopaper and they were centrifuged at 1000 rpm for 30 minutes. Following elution, each

GCF sample was analyzed separately. Then enzyme-linked immunosorbent assay (ELISA) was used in order to measure the concentration of HMGB1 (BlueGene, human HMGB1/E01H0009, Shanghai, China). Sandwich ELISA measured the amount of HMGB1 by colorimetric method.

Measurement of sTREM-1 level in GCF samples was done by and sTREM-1 ELISA kit (R&D Systems, human sTREM-1, DY12788, Abingdon, UK). At first, 50 µL (microliter) of anti-TREM-1 antibody was coated at 10 µg/mL (microgram per milliliter) in coating buffer ($Na_2HPO_4 \times 2H_2O$ 0.1 M pH = 9.3) at 4 °C over night and 37 °C for one hour, respectively. Then plates were blocked with 200 µL blocking buffer (PBS 1%, BSA 1%) for 1.5 hours at room temperature. Afterwards the standard (recombinant human TREM-1 in 7.5% BSA-PBS) and the samples were added and the plates were incubated for 1.5 hours at room temperature. After 1.5 hours incubation, plates were washed and the biotinylated detection polyclonal Ab anti-TREM-1 at 5 μ g/mL was added for 1 hour. After incubation for 1.5 hours plates were washed and the biotinylated detection polyclonal Ab anti-TREM-1 at 5 μ g/mL was added for 1 hour. Plates were then washed and streptavidine-HRP (Horse radish peroxidase) was added for 20 minutes at room temperature. Plates were washed again, they were developed using the Tetramethylbenzidine Peroxidase Substrate System (KPL, Gaithersburg, Md, USA) and reaction was stopped by adding H_2SO_4. All dilutions were carried out in blocking buffer. The absorbance was measured at 450 nanometer (nm).

Statistical Analysis

Data analysis was performed using SPSS16.00. At first, the mean of concentration of HMGB1 and sTREM-1 was estimated for each group. Regarding normal distribution of data, parametric statistical tests were done including t-test and Pearson's correlation coefficient tests. A *p* value less than 0.05 was considered statistically significant.

RESULTS

Patients Characteristics

The mean age in case and control groups were 40±2.5 and 46±3 years, respectively. The case group consisted of 15 men and 9 women and the control group consisted of 13 men and 11 women.

Para-clinical Evaluation

In table1 descriptive statistics for concentration of HMGB1 and sTREM-1 have been summarized, respectively.

Statistical analysis by t-test, showed that the concentration of HMGB1 ($p<0.001$) and sTREM-1 ($p<0.017$), was significantly higher in chronic periodontitis group. In additionas shown in Table 2, there is a significant positive correlation between HMGB1 and sTREM-1 concentration in chronic periodontitis ($p<0.05$).

Table 1. Descriptive statistics on concentration of HMGB1 and sTREM1 (ng/ml)

	Groups	Number	Minimum	Maximum	Mean	Standard Deviation (SD)
HMGB1	Chronic periodontitis	24	1.34	1.73	1.544	0.085
	Clinically healthy gingiva	24	0.43	0.971	0.671	0.135
sTREM1	Chronic periodontitis	24	1.11	2.27	1.784	0.312
	Clinically healthy gingiva	24	0.57	2.04	1.540	0.363

Table 2. Correlation between HMGB1and sTREM1 concentrations

		sTREM-1	HMGB1
sTREM-1	Pearson Correlation	1	.351*
	Sig. (2-tailed)		.014
	N	48	48
HMGB1	Pearson Correlation	.351*	1
	Sig. (2-tailed)	.014	
	N	48	48

* Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

The main objective of this study was to evaluate the concentration of HMGB1, as one of the important danger-associated molecular pattern (DAMP) and sTREM-1, as a soluble form of an important receptor for DAMPs in chronic periodontitis. We found significant positive correlations between HMGB1 and sTREM-1 concentration; HMGB1 concentration and PD; and sTREM-1 concentration and PD in chronic periodontitis, which suggests that we can use HMGB1 and sTREM-1 concentration as useful diagnostic tool for degree of tissue destruction in chronic periodontitis.

In several previous studies, higher expression of HMGB1 gene or concentration of HMGB1 in GCF of patients with chronic periodontitis has been reported. It should be considered that evaluating the concentration of a given mediator is more specific than its gene expression for determining the role of related mediators in pathogenesis of the disease.

Lou et al. have shown that there is an increased concentration of HMGB1 in aggressive (7217 pg/ml) and chronic (18924 pg/ml) periodontitis compared to healthy control group (196 pg/ml).[24] We also found higher concentration of HMGB1 in chronic periodontitis.

Ito et al. found that the concentration of HMGB1 and Receptor for advanced glycation and products (RAGE) in gingival epithelial cells treated with Interleukin-1beta (IL-1β) in vitro, are higher compared to healthy or un-treated cells.[25] Our results also showed that higher levels of HMGB1 in GCF samples of chronic periodontitis and the formation of GCF only occurs in the presence of inflammatory stimulation in the related gingiva. In addition, we showed correlation between PD and the level of HMGB, which indicates that this molecule could be used for clinical evaluation.

As like as our study, Zhao et al. found that HMGB1 levels in GCF samples of patients with chronic periodontitis were higher than those in healthy group.[26]

In the case of sTREM-1 the results were as follows; we found higher level of sTREM-1 in GCF samples of patients with chronic periodontitis than healthy gingival. Since, there was a positive significant correlation between sTREM-1 level and PD; it also can be used as a disease marker. Our findidngs are also compatible with the results of the following studies.

Bisson et al. showed that sTREM-1 levels are higher in GCF samples of severe periodontitis compared to healthy gingiva. They also claimed that sTREM-1 can be considered as a disease marker.[27]

Belibaskis et al. found that the levels of sTREM-1 in GCF samples of chronic periodontitis and aggressive periodontitis patients are significantly higher than subjects with healthy gingiva.[28]

It is concluded that both HMGB1 and sTREM-1 are released during the inflammatory response of periodontal tissues and can promote inflammatory process, which leads to tissue destruction. In addition, they can be considered as markers for determining the clinical status of periodontal diseases. It is noteworthy that HMGB1 which is considered as a DAMP is recognized by TLRs especially TLR4, which results in progression of inflammatory responses in periodontal tissue. Of course, more studies are needed in order to prove the above hypothesis.

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