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Comparison of the osteopontin gene expression in gingival tissue related to chronic and acute periodontitis mRNA expression of osteopontin in gingival tissue of the chronic and acute periodontitis

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Abstract

Bacterial infection of periodontal tissue refers to periodontal disease which can lead to bone resorption and tooth loss in severe cases. During the disease procedure, both innate and adaptive immune responses involve that trigger inflammation. Osteopontin (OPN) is an extracellular glycosylated phosphoprotein which increases in inflammation status such as periodontitis. Correlation of the OPN expression and severity of periodontal tissue infection does not investigated yet. Therefore this study assessed OPN expression level in chronic and acute periodontitis to introducing a new correlation between them. For this purpose, gingival tissue samples were collected from 20 patients with chronic periodontitis and 10 persons without obvious sign of periodontitis. Total RNA related to each tissue sample was extracted after homogenizing. The expression of OPNgen (amount of corresponding mRAN) was evaluated by real-time PCR method. Expression of the OPN*gene decreased in both types of examined periodontitis and reduced more significantly in acute cases (P<0.05). Statistical analysis revealed presence of significant correlation between negative fold change of gene expression and severity of infectious in periodontal tissue (P<0.05). Results suggest OPN has a possible important role in protection against periodontopathic bacteria and bone lesion, but disease progression accompanied by reduced expression may be due toOPN producer cell death.*

Keywords: Periodontal Immunopathogenesis, Chronic and acute periodontitis, Immunoregulation, Osteopontin, Tissue destruction, Real-time PCR.

Introduction

Periodontal diseases or periodontitis are serious infectious affects gum tissue, range from simple gum inflammation to serious disease that results major damage in the soft tissue and tooth supporting bone. Early signs of periodontal gum disease are swollen and bleeding gums but in the worst cases, teeth are lost. Bacteria, especially gram-negative bacteria play an important role in the development of periodontal diseases. Periodontal bacteria stimulate periodontal ligament cells and induce them to express various types of inflammatory intermediates such as interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), interleukin-6, and interferon-gamma (IFN-γ)[1]. However, the severity of periodontal disease related to the balance between amounts of microorganisms and host immune responses[2]. The presence of inflammatory cytokines and active T cells in developed lesions cause bone lysis through RANK / RANKL interactions[3]. TNF-TNFR protein families are essential factors in bone lysis and deformation such as RANKL, RANK, and osteoprotegrin (OPG)[4,5]. Osteopontin (OPN) is also important in bone regeneration or repair by osteoclasts migration, fixation on the bone surface and activation of them[6-9]. OPN might function as a negative feedback regulator for osteoblastic differentiation[10]. Previous studies confirmed OPN expression increased in some autoimmune diseases such as rheumatoid arthritis[11]. Growing evidence has been suggested hypotheses regarding the relationship between periodontitis and autoimmune diseases also[12,13]. Significant correlation was observed between OPN in GCF (gingival crevicular fluid) and PD (Pocket Depth)[14]. Therefore OPN level could be used as a good indicator to identify the immunopathology in chronic and acute periodontitis or determining the severity of the disease. In this study to clarify the pathologicalchanges in periodontitis, OPN gene expression of the gum tissues related to chronic and acute periodontal disease compared and tried to find a relationship between gene expression and severity of disease.

Methodology

Samples selection method: We have 3 experimental groups in this study as follow: chronic periodontitis (n=20), acute periodontitis (n=20) and control (n=10).

The illnesses werenon-smokers Iranians over the 18 years old that did not use anti-inflammatory drugs before surgery and referred to periodontics department of Shahid Beheshti Dental School and subsidiary clinics during 2012-2013. All individuals completely informed about sampling and assigned agreement

form for participation in the study. The control samples selected between those who needed crown lengthening surgery without any BLE (Bleeding on probing) in sampling area, clinical signs of gingivitis and history of periodontitis. The mouths and teeth of all selected people were cleaned from local stimuli including plaque, gauze dressing pads, decay and possibly interim lining. They were educated to have brushing 3 times a day (modified Stillman method) and use of dental floss once a day at least a week before surgery. Diagnosis of the clinical condition or periodontal disease was done by the periodontist before surgery. Chronic, moderate and acute condition of periodontitis identified according to periodontal disease classification system of American Academy of Periodontology[15].

For patients with chronic and acute periodontitis supraventricular and gingival scraping and root canal smoothing done at least one month before the sampling. All of the persons suffer from systemic disease or use periodontal effective medications (such as calcium channel blockers and antibiotics) or immunosuppressive drugs (cyclosporine A corticosteroids and cytotoxic drugs) during 1 month before sampling, removed from both groups.

Tissue homogenizing and RNA extraction: Gingival tissue samples were collected following surgery and incubated in RNA Later (manufactured by QIAGEN Germany Lot No. 13.175928) at 4°C for 24 hours until the RNA later completely penetrated into the tissue and then transferred to -20°C. The ratio of the tissue to RNA Later was about 100mg tissue per 1 ml of solution. After collection of samples, all amounts of RNA later removed and tissue homogenized. Total RNA extraction was done according to RNA extraction kit (manufactured by ROCHE, lot.1403590). Gel- electrophoresis on 2% agarose was used to check quality of purified RNA.

cDNA synthesis and real-time PCR: cDNA synthesis done according to "cDNA Synthesis Kit Thermo Scientific Revertaid (from Bio-Rad Company)" manual. This kit is a complete system for the efficient synthesis of the primary chain of cDNA from mRNA or total RNA as template. Prepared cDNA stored at -20°C until real-time PCR was performed. Product quantified by Nano drop for equilibration of cDNA in each PCR reaction.

Quantitative RT-PCR was done according previous works by using synthesized cDNA[16]. Briefly, candidate gene sequences were used to primer design by primer 3 software. We tried the primerscover a large intron in each case (>500 bp) as Santa Cruz protocol[17] (Table-1).

Optimal value of products considered 100bp but 50-150bp was acceptable. 57◦C was also set as optimal Tm of primers as usual. 384-wellmicrotiter plates were used to set the reactions in aRotorgene 6000 instrument. RT-PCR Mix synthesis System (Qiagen,Cat. No.981005) was used to convert RNA to cDNA according to the manufacturer's instructions. Total reaction was 10 µl and PCR program was set as follow: 95°C for 10 min, (95°C for 15sec, 60°C for 60sec, 72°C for 20sec)*40. We determined cycle threshold or Ct and relative fold changes rather than endogenous controls (GAPDH is a house kipping gen used as an internal control). Ct was calculated using the standard formula:

$X=2^{-\Delta Ct}$ with ΔCt = Ct(target) – Average [Ct (internal controls)].

Table-1: Sequence of the forward and reverse primers related to goal gen (OPN) and internal control gen (GAPDH).

Primer name	Sequence
OPN-Forward	5'-TTCTTCTAACTTCCTCTCCTGTGA-3'
OPN-Reverse	5'-AGCGGCAACCTTAGCATTC-3'
GAPDH-Forward	CTCTGGTAAAGTGGATATTGT-3'-'5
GAPDH-Reverse	5/-GGT GGAATCATATTGGAACA-3/

Statistical analysis: Average of the Ct values of housekeeping gene, glyceraldehyde dehydrogenase (GAPDH), was used to calculation of Ct values for the goal gens. We done all of the experiments in pairs and relative fold changes were averaged after normalization. Data analysis done according to ΔΔCt by SPSS software (version 16.00). Differences significance were assessed in gene expression between patient samples and controls accordingly and corrected for multiple testing.

Results and discussion

Demographics and periodontal findings of enrolled periodontitis patients (chronic and acute) are shown in Table-1 before sampling. The Mann Whitney U test showed data related to age has significant differences in two groups. But in the case of sex, Chi-Square test revealed that the sex abundance is similar in two groups.

After obtaining data related to Ct and efficiency about the genes (OPN, GAPDH) in the experimental groups, showed in Table-3, some descriptive statistics were determined in two groups of chronic and acute periodontitis. Table-4 compared expression of the OPN gene between three groups.

Results showed OPN expression reduced significantly rather than control. comparison of the fold change in Table-4 revealed reduction of OPN expression in acute periodontitis are significantly more than chronic group (near 5 fold).

It was observed when CAL and PD were greater than 5 mm, OPN gene expression reduction was slight in both groups (decreased 7.205 times for chronic periodontitis ($P < 0.01$) and decreased 19.578 times in acute periodontitis cases ($P < 0.005$).

Table-2: The features of chronic and acute groups. CAL (Clinical attachment loss) and PD (Pocket Depth) amounts also described for each experimental groups.

Variables groups	Sex		Average of age	Average CAL	Maximum CAL	Average PD	Maximum PD
	Female	Male					
Chronic periodontitis	65	35	45.15±13.57	3.92±1.72	2.20±1.61	3.36±1.33	2.35±1.35
Chronic periodontitis	70	30	30.67±11.49	6.80±1.8	7.56±2.68	6.09±1.94	6.89±2.45

Table-3: Descriptive statistical analysis related to gene expression (OPN and GAPDH) in both chronic and acute periodontitis (P<0.05).

Groups	Control		Chronic periodontitis		Acute periodontitis	
Genes	GAPDH	OPN	GAPDH	OPN	GAPDH	OPN
Number	10	10	20	20	20	20
Average	25.11	23.12	22.92	16.57	24.06	12.18
Standard deviation (SD)	1.11	0.34	1.21	0.26	0.99	0.54
Coefficient of variation (CV)	4.44	1.47	5.26	1.54	4.10	4.39

Table-4: OPN gene expression fold change of both groupsin comparison with control group (P<0.05).

Variables	Chronic	Acute
Fold change	-1.824 ± 0.39	-8.385 ± 0.07
Gene absolute adjustment	0.548	0.119
Gene absolute adjustment (2-log)	-0.867	-3.068
Gene absolute adjustment (2-log standard error)	±0.18611	±0.02871

Discussion: Periodontitis is a chronic inflammatory disease characterized by the destruction of periodontal tissue. It is a common disease that resulted from complex interaction between periodontal microorganisms and the host inflammatory response, so primarily initiated by periodontal pathogens. The host response involves proinflammatory cytokines, chemokines, prostaglandins, toll-like receptors and proteolytic enzymes, which have all been demonstrated to play an important role in the pathogenesis of periodontitis. Studies have been performed combining *in vivo* and *in vitro* approaches to identify genes responsible for periodontitis. To date, there are a few published RT-PCR studies investigating the inflammatory gene expression profile in periodontist and correlation of gene expression with severity of disease.

Osteopontin (OPN) is a matricellular protein has critical role in different biological functions. OPN participated in normal physiological processes and is occupied in the pathogenesis of a wide range of disease, including atherosclerosis, glomerulonephritis, cancer, autoimmune disease and several chronic inflammatory diseases. Due to OPN role in inflammation its possible OPN expression has role in periodontitis (inflammatory condition in gingival tissue). This study investigates OPN expression changes in periodontitis. We also compare OPN gene expression in acute and chronic condition of periodontitis. OPN gene expression reduced in both experimental groups suffer from periodontitis. Significant decrease of OPN expression in acute group was observed compared to chronic periodontitis (P <0.05). In the case of subgroups with CAL and PD greater than 5 mm reduced level of OPN observed significantly (P <0.05). In other words, there are a correlation between OPN expression and severity of inflammation in periodontal tissue. Kido et al. measured the amount of OPN by ELISA method and found a weak but significant correlation between OPN in GCF and PD. They conclude that progression of periodontal degradation increased OPN level in GCF[14]. Differences of previous study with our results are possibly due to nature of samples (we used liquid

specimens between the gingival canal not gum tissue) and nature of assessed molecule (they measured OPD protein not related mRNA). Kido et al. were investigated relationship between periodontitis group with PD greater than 4 mm and control group with PD less than 3 mm without considering significant correlation between groups with PD between 3 and 4 mm in GCF, but we considered wide range of data in this study. Its possible if they considered data with PD more than 5, significant correlation does not achieved. Another advantage of our study is considering of the correlation between PD and CAL. We found significant correlation between PD and CAL in this study that is valuable in statistical analysis and confirmed our results.

Previous studies reported minimum levels of OPN in GCF of the control group and the maximum level of it in chronic periodontitis group. They also found a positive correlation between OPN and CAL ($P < 0.05$). Therefore our results are different with these studies used GCF as sample because GCF originate mainly from blood but we used periodontal tissue and assessed gene expression not protein concentration that are different with each other due to some regulatory process in translation. Walker et al. also confirmed essential role of OPN in bone mass formation, repair and bone remodeling[18]. Therefore its possible severe degradation of bone in periodontitis is due to decreased OPN. Attenuated immune system causes apoptosis in OPN producer cells so OPN expression decreased. It is expected destruction of the gum tissue accompanied with reduced OPN producer cells lead to bone destruction also.

Conclusion

Based on the results, it is concluded OPN reduced significantly in acute periodontitis compared with chronic status. Meanwhile, we approved minimal level of OPN expression occurred in severe damages of the periodontal tissues, a reduction was appeared in the expression of all OPN genes. Its possible cell death in periodontal tissue leads to reduced level of OPN that accompanied with disease developing. By considering repairing effects of OPN, developed periodontal damages cause fail of the bone repair and remodeling in sever disease. Our data are inadequate for interpreting the OPN role in bone decay inhibition and it requires further investigations, but our results suggest OPN as a new therapy in periodontal tissue inflammation.

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