Progress in Nutrition 2021; Vol. 23, N. 2: e2021055 DOI: 10.23751/pn.v23i2.10190

Original article

Salivary IL17 and IL35 suppresses cell progression in periodontal disease manipulation induced by TNBs via NF-κB signaling pathway

IL17 and IL35 in periodontal disease and systemic osteoporosis

Yunxiao Wang, Ling Wang
Department of Stomatology, Xingtai People 's Hospital, Xingtai, Hebei, China, 054001.

Abstract. *Background:*In periodontal disease prognosis relies primarily on clinical and radiographic parameters. In present study association of interleukins IL17 & IL35 levels in GCF and serum among healthy, gingivitis, and chronic periodontitis (CP) individuals is examined and possibility of using them as possible prognosis marker for periodontal disease activity is explored. Expressions of TNF-α, AKT and NF-κB. Inflammatory cells were evaluated in both cases. *Materials and Methods:* This prospective study was accomplished on patients with osteoporosis and a comprehensive periodontal examination was performed including standardized digital dental periapical radiographs and bone mineral density (BMD) evaluation. ELISA kit was used for quantifying levels of IL-17 and IL-35 in the patients' plasma. ELISA was used to check the expressions level of TNF-α, and NF-κB. Inflammatory cells in both cases. STATA 15.0 was used for statistical analysis and a p value ≤0.05 was considered statistically significant. *Results:* The mean of GI of healthy and osteoporotic group were much lower than periodontitis group. PI, PPD and CAL were almost comparable between healthy control group and osteoporotic group. Plasma IP-17 level and IL35 level where raised in periodontitis group compare to health control and also were significant different from osteoporosis groups. Although IL17 value of osteoporosis group was comparable to health group, IL 35 had significant high value than healthy. Expressions of TNF-α were increased in periodontitis group. Expressions of phosphorylated PI3K and AKT were reduced in periodontitis group. Meanwhile, expressions of NF-κB were decreased periodontitis group compared to systemic osteoporosis. *Conclusion:* Various studies characterized the host response to infection in periodontal/periapical diseases, and they are found to be very different from those of systemic osetoporesis, however this is first to study IL17 and Il35 in such cases. Periodontitis inhibit inflammatory cell process and regulate related factors during pathogenesis, suggesting that through PI3K/AKT/NF-κB signaling pathway disease progression in periodontitis are modulated.

Key words: Periodontal disease, IL17, IL35, chronic periodontitis (CP), inflammatory markers, NF-κB

Introduction

Periodontal disease is the most common chronic inflammatory disorder of bone pathology characterized by destruction of tooth-supporting structures (1). The tissue destruction is mainly caused due to activation of host response to the etiologic microorganism through a variety of inflammatory cytokines (2). Bone loss is characterized both in periodontal disease and osteoporosis which is a systemic process which are modulated by numerous factors such as hormonal changes and cytokines levels. Interleukins influence the progression of any disease so affecting host susceptibility (3). There is considerable reason to examine

the possible relationship between interleukins concentration during development of periodontal disease. Interleukins may modify the host response to disease symptoms, and modulated various other immune cells and their action thus make periodontitis susceptible to the progress and progression of pathogenesis (4).

Identification and validation of host biomarkers is requisite for early diagnosis, evaluation of prognosis and disease severity analysis. Cytokines play crucial roles in organizing varied immune cell types through stimuli to signal transduction networks and mediate critical processes such as differentiation, cell growth, and development of target cells. Among various interleukins, IL-35 which belongs to IL-12 cytokine family is an anti-inflammatory cytokine which suppresses the immune response and there is suggestive of a possible role of IL-35 in chronic inflammatory disorder such as periodontitis (5). Like all other cytokines, IL17 and Il-35 too are not constitutively expressed in all body parts tissues. Gene encoding IL-35 is transcribed mainly by vascular endothelial cells, monocytes and smooth muscle cells after instigation with pro-inflammatory stimuli (6). Apart from IL35, IL17 also has been shown to stimulate fibroblastic cells and induces the osteoblasts, influence osteoblastic bone reabsorption through initiation of nuclear factor-kappa β ligand (7). This present study examined the association of interleukins IL17 & IL35 during systemic osteoporosis and periodontal disease. The purpose of the current study was to associate the cytokines levels in GCF and serum of healthy, and chronic periodontitis (CP) individuals and to explore the possibility of using IL17 and IL35 as a possible prognosis marker for periodontal disease activity. This study also evaluates immune function mechanism during periodontitis and factors in inflammations.

Materials and Methods

This prospective study was performed at Xingtai People 's Hospital with approval of Research Ethics Committee. The study was appraised and permitted by the institutional review board (IRB) and written informed consent of all the participants were obtained with the approval of the IRB). The drive of the study was explained to all every participants and immediate consent in written was obtained.

Subjects: The study population was classified into three groups as described below.

Exclusion Criteria for the Study

Individual with no precise medical history and, with a history of surgically induced menopause, smokers, bone destructive lesions, diabetes mellitus (DM), alcohol abuse, thyroid related growth diseases, renal disease and connective tissue disorders were excluded from selection. Patients on treatment for hormone replacement therapy, chemotherapy, corticosteroids, and/or radiotherapy were not considered in this study. Patients earlier diagnosed for osteoporosis or periodontitis who are on treatment were also excluded from this study. Individuals were considered normal with T score at least −1 SD or more and excluded from osteoporosis Group. Patients confirmed for periodontitis by radiograph were excluded from this group. Patients diagnosed for osteoporosis or with any other systemic disease were not taken in this group.

Inclusion Criteria

Osteoporosis Group: The diagnosis of osteoporosis was followed according to criteria set up by World Health Organization (WHO) and patients with a bone mineral density (T score) less than −2.5 SD. Individuals with GI <1, PD < 3 mm, and CAL<3 were considered in osteoporotic group.

Periodontitis group: Based on Gingival index (GI > 1), Probing pocket depth (PD > 3 mm) and Clinical attachment level (CAL > 3 mm), were diagnosed for periodontitis which were further confirmed by radiographs analysis.

Healthy Individual: Individuals no systemic disease or illness with T score at least −1 SD or more on the basis of GI score, PPD, and CAL measurements, in-

dividuals with GI <1, PD < 3 mm, and CAL<3 were considered as healthy.

Clinical assessment

A comprehensive periodontal inspection was performed for each patients includeds gingival index (described by Löe and Silness 1963), clinical attachment level (CAL) and Probing pocket depth (PPD), (described by Ramfjord, 1967). A standardized digital dental periapical radiograph were taken for every patient to confirm periodontitis diagnosis. Bone mineral density (BMD) evaluation was done on every patient at two body sites preferably tibia and femur using X-ray absorptiometry. The T-score of –2.5 or less outlines osteoporosis.

Laboratory Testing

In this laboratory testing, whole blood of each participant was collected during their first visit clinic. Genomic DNA of fresh whole blood samples from each participant was extracted using Blood Mini Kit (QIAamp DNA) following the manufacturer's instructions. Expression profile of IL35 and IL-17 was evaluated using gene specific primers and Minor Groove Binder (MGB) Eclipse® probes which was performed in RT PCR machine of AB 7500 real time PCR system (Applied Biosystems, USA). The validation of gene expression was done using internal control GAPDH as a reference gene.

ELISA for IL-17 and IL-35

Human CXCL10/IP-17 and IP-35 DuoSet ELISA kit was used for quantifying levels of IL-17 and IL-35 in the patients' plasma. ELISA was performed on all the samples in triplicate and the normal value was taken as a quantity of interleukines level in plasma. The assay was performed as per the manufacturer's instructions. DuoSet ELISA kit was used to estimate the TNF-α, and NF-κB.

Statistical Analysis

STATA 15.0 (StataCorp, College Station, TX) was used for statistical analysis in this study. The data were presented as average, percentage and quartiles. Non-parametric test were used to test the statistical significance of the association of expression profile and circulating interleukines levels with the patient's characteristics wherever appropriate. A p value ≤0.05 was considered statistically significant.

Results

The mean age group of participants varied from 38 to 48 while minimum mean age was in periodontitis group the max mean age was seen in osteoporosis group. Male were dominating the healthy and periodontics group while osteoporosis group did not have much variation in sex ratio (Table 1). Among the 200 study participants, 121 were males and rest was females.

Clinical results

The clinical results of the present study presented that the mean ± SD of GI of healthy control group and osteoporotic group were 0.78±19 and 0.87±13respectively, which are much lower than periodontitis group (2.01±33). While there was no statistically significant difference between healthy and osteoporotic group, both were significantly lower compare to periodontitis group.The mean ± SD of PI, PPD and CAL were almost comparable between healthy control group and osteoporotic group there was no statistically significant difference between these two group with periodontitis (Table 1).

Gene Expression Profiling of IL17 & IL35 in Different Group Studied

Although both the genes were detected in each group, they were preferentially expressed in Periodontitis. There was significant association between plasma IP-17 level and IL35 level where in periodontitis

Table 1. Clinical examination of group subjects and analysis

Factor's	Healthy	Periodontitis Group	Osteoporosis Group	*P* Value
	81 (40.5%)	62 (31%)	57 (28.5%)	
Age	42 (23-52)	38 (25-48)	48 (23-58)	
Gender (n)				
Female	31 (38.18%)	22 (35.48%)	26 (45.61%)	
Male	50 (6.72%)	40 (64.51%)	31 (54.38%)	
BMI	17.1 ± 2.19	17.9 ± 3.11	18.1 ± 3.24	
Clinical parameters				
GI	0.78±19	2.01±33	0.87±13	
PI	0.59±32	2.42±41	0.79±21	
PPD	2.15±43	4.61±51	1.99±63	
CAL	1.89±49	3.79±49	2.54±33	
BMD				
T score	-0.79	-0.85	-3.11	
IP 17 Level (pg/mL)	1.65± 0.19	1.81± 0.22	1.71± 0.14	
IP 35 Level (pg/mL)	**61.31±10.9**	**101.31±18.3**	**81.31±9.1**	
IP 17 Fold changes (pg/mL)	NA	0.5	0.1	
IP 35 Fold changes (pg/mL)	NA	2.5	1.5	

a. Values given in (%) and Median (1st Quartile to 3rd Quartile).

b. Fisher Exact and K-Wallis test were used to test the statistical significance

group the interleukins were raised to higher compare to health control and also were significant different from osteoporosis groups. Although IL17 value of osteoporosis group was comparable to health group, IL 35 had significant high value than healthy (Table 2).

In Periodontitis, Expressions of Cytokines and Signaling Markers were Modulated

Enhanced expressions of TNF-α were detected in periodontitis, which indicated that levels of TNF-α were significantly increased compared to systemic osteoporosis (Figure 1). In order to figure out the mechanism of immune inflammation, phosphorylated AKT and PI3K expressions were analyzed. Expressions of p-PI3K and P-AKT were measured in periodontitis and osteoporosis group, which showed periodontitis promote expressions of p-AKT and p-PI3K (Figure 2). Levels of NF-κB expressions were also detected using ELISA and results showed that NF-κB protein level in periodontitis group was higher than other group (Figure 3).

Discussion

Periodontal disease such as periodontitis is considered confined tissue damage whereas while osteoporosis is a systemic disorder. Both the disease condition are characterized by bone reabsorption influenced by various risk factors such as hormonal imbalance and cytokines expression (8). Although both condition are chronic diseases of dental, it is not that these two have similar diseases. Immunologic responses are in process during any inflammation such as periodontitis and in-

Table 2 Interleukins in different group

IL17	**Healthy**	**Periodontitis Group**	**Osteoporosis Group**
Age			
≤ 25	1.65± 0.19	1.85± 0.21	1.73± 0.19
25 - 50	1.58± 0.18	1.78± 0.13	1.70± 0.17
> 50	1.52± 0.13	NA	1.61± 0.19
Gender			
Female	1.64± 0.21	1.82± 0.19	1.71± 0.21
Male	1.60± 0.18	1.80± 0.15	1.68± 0.17
IL35	**Healthy**	**Periodontitis Group**	**Osteoporosis Group**
Age			
≤ 25	**60.89±13.9**	**95.31±15.2**	**83.31±10.1**
25 - 50	**65.53±11.0**	**107.31±17.9**	**87.31±13.9**
> 50	**59.79±12.11**	**101.31±18.5**	**77.31±13.3**
Gender			
Female	**62.59±12.8**	**17.31±17.8**	**85.31±10.3**
Male	**64.84±15.09**	**105.31±19.6**	**81.31±12.6**

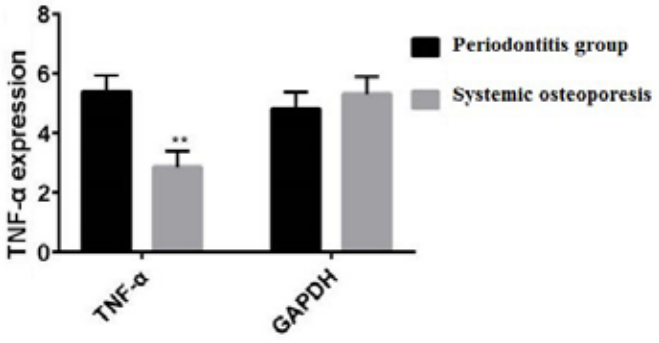

Figure 1. Enhanced expressions of TNF-α in inflammatory cells of periodontitis.
Enhanced expressions of TNF-α were detected in periodontitis, which indicated that levels of TNF-α were significantly increased compared to systemic osteoporosis.

flammatory propagation might leads to necrosis and danger to health (2, 9).

In this study, work was initiated to determine cytokine gene expression in both disease condition and to characterize the role of immune in inflammation process. Th17 cells the synthesizer of IL17 contribute to the expansion of periodontal/periapical lesions via modulating MMPs, and indirectly by amplifying the secretion of the classic proinflammatory cytokines by other cell types. IL17 is known to have strong correlation with severity and extent of inflammation and in particular in the oral cavity (5), and therefore, if that

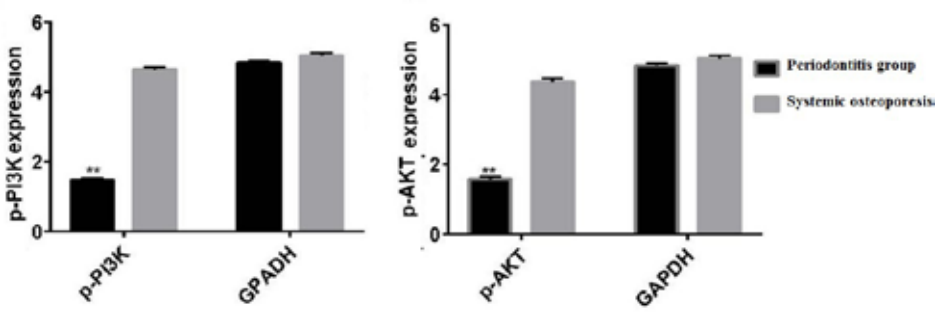

Figure 2. Periodontitis inhibited expressions of p-PI3K and p-AKT during inflammation (significant at $P<0.05$).
Expressions of p-PI3K and P-AKT were measured in periodontitis and osteoporosis group, which showed periodontitis promote expressions of p-AKT and p-PI3K.

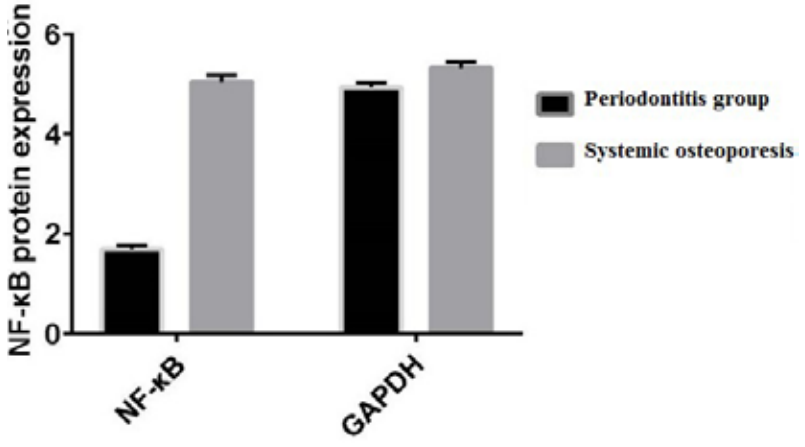

Figure 3. Periodontitis group had inhibited level of NF-κB during inflammation.
Levels of NF-κB expressions were also detected using ELISA and results showed that NF-κB protein level in periodontitis group was higher than another group.

is minimized it may help in improving the inflammatory condition in the oral cavity. This was the though behind assessing the levels of salivary IL17 to evaluate the difference in levels of periodontitis. IL-17A and IL-17F cytokines plays crucial in protecting the host from inflammation, also deregulated IL-17 production can modulate expression of various pro-inflammatory cytokine expression and chronic inflammation, leading to tissue damage (5, 10).

IL-17A is considered to be one of the mark pro-inflammatory product of Th17 population, which have dichotomous roles bone turnover affects the bone diseases. At one side IL-17A has crucial role in the bone loss of inflammatory conditions such as psoriasis, rheumatoid arthritis, periodontal disease, and IBD (11, 12) it also promotes protection against bone destruction in a periodontal disease model caused due to pathogen (13). Th17 cells are the utmost osteoclastogenic subsets of T cells, distinct their capacity by production of IL-17. Th17 cells are constitutively present at mucosal surfaces, especially in the intestinal lamina propria (14, 15, 16). IL17 role in osteoporosis and inflammation is studied in details but IL35 role in both conditions yet to be explored.

TNF Alpha is a multi-directional factor, produced by mononuclear-macrophages (17). It can help

expressions of adhesion molecules through activating NK-KB to promote correlation between neutrophil granulocytes and epithelial cells in patients with inflammatory bowel diseases (18). NK-KB is an important factor produced and released by mononuclear-macrophages and various other nuclear cells, which could promote occurrence of inflammatory reactions and immunoregulations (19). The mechanism of TNF-α involved in cytokines inflammation injury is that TNF-α can promote the release of platelet activating factors and produce leukotriene and oxygen free radicals to cause damages of enterocytes and thrombus in patients' bodies (18). Moreover, TNF-α could enhance injuries of intestinal mucosa after the interaction with inflammatory cells. Studies proved that abnormally activated PI3K/AKT signaling pathway played an important role in occurrence of inflammation of periodontitis. AKT is the direct downstream factor of PI3K, which could regulate signaling pathway about tumors and inflammations (20). Thus, Phosphorylation of AKT was an indicator for judging activation of PI3K. NF-κB was activated by active PI3K/AKT and regulated expressions of genes related to inflammation (21). In process of inflammation, NF-κB could promote transcription of inflammatory factors like IL-8, TNF-α, IL-1b and improve the expressions to accelerate occurrences of colitis and severity (22). In this study, expressions of PI3K, AKT and NF-κB in protein level were tested. In periodontitis, expressions of ATK were increased while expressions of NF-κB were reduced.

Conclusion

Periodontitis could inhibit TNF alpha and upregulated p-AKT expression with inhibiting expression of NF-κB. Besides that, related inflammatory factors IL-6 and TNF-α were also suppressed. Thus, these findings suggest that periodontitis suppress the activation of PI3K/AKT in inflammation and inhibit NF-κB expressions to inhibit process of healing. As the periodontitis and osteoporosis have similar kind of expression of interleukins it is also necessary to have an interleukin to mark the differences between these two conditions.

Compliance with Ethical Standards

Disclosure of Potential Conflicts of Interest

Authors have nothing to disclose.

Informed Consent

Written informed consent was obtained from the participants with the option to withdraw them from the study at any time.

Acknowledgment

None

References

1. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *The lancet*. 2005; 366(9499):1809–20.
2. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontology* 2000. 2014; 64(1):57–80.
3. Lockhart PB, Bolger AF, Papapanou PN, Osinbowale O, Trevisan M, Levison ME, Taubert KA, Newburger JW, Gornik HL, Gewitz MH, Wilson WR. Periodontal disease and atherosclerotic vascular disease: does the evidence support an independent association? A scientific statement from the American Heart Association. *Circulation*. 2012; 125(20):2520–44.
4. Stabholz A, Soskolne WA, Shapira L. Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. *Periodontology*. 2010; 53(1):138–53.
5. Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu. Rev. Immunol*. 2007; 25:821–52.
6. Sakkas LI, Mavropoulos A, Perricone C, Bogdanos DP. IL-35: a new immunomodulator in autoimmune rheumatic diseases. *Immunologic research*. 2018:1–8.
7. Saito S, Nakashima A, Ito M, Shima T. Clinical implication of recent advances in our understanding of IL-17 and reproductive immunology. *Expert review of clinical immunology*. 20117(5):649–57.
8. Garcia RI, Henshaw MM, Krall EA. Relationship between periodontal disease and systemic health. *Periodontology*. 2001 Feb; 25(1):21–36.
9. Hahn CL, Liewehr FR. Innate immune responses of the dental pulp to caries. *Journal of endodontics*. 2007; 33(6): 643–51.
10. DeSelm CJ, Takahata Y, Warren J, Chappel JC, Khan T, Li X, Liu C, Choi Y, Kim YF, Zou W, Teitelbaum SL. IL-

17 mediates estrogen-deficient osteoporosis in an Act1-dependent manner. *Journal of cellular biochemistry*. 2012; 113(9):2895–902.
11. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. *New England Journal of Medicine*. 2009; 361(9):888–98.
12. Martinez GJ, Nurieva RI, Yang XO, Dong C. Regulation and function of proinflammatory TH17 cells. *Annals of the New York Academy of Sciences*. 2008; 1143(1):188–211.
13. Goswami J, Hernández-Santos N, Zuniga LA, Gaffen SL. A bone-protective role for IL-17 receptor signaling in ovariectomy-induced bone loss. *European journal of immunology*. 2009; 39(10):2831–9.
14. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*. 2006; 126(6): 1121–33.
15. Sato K, Suematsu A, Okamoto K, Yamaguchi A, Morishita Y, Kadono Y, Tanaka S, Kodama T, Akira S, Iwakura Y, Cua DJ. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *Journal of Experimental Medicine*. 2006; 203(12):2673–82.
16. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFβ in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity*. 2006; 24(2):179–89.
17. Tanaka, T., M. Narazaki, and T. Kishimoto, *IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol*, 2014. **6**(10): p. a016295.
18. Xiao, Y.T., et al., *Neutralization of IL-6 and TNF-alpha ameliorates intestinal permeability in DSS-induced colitis. Cytokine*, 2016. **83**: p. 189–192.
19. Lopetuso, L.R., et al., *Harmful Effects and Potential Benefits of Anti-Tumor Necrosis Factor (TNF)-alpha on the Liver. Int J Mol Sci*, 2018. **19**(8).
20. Wymann, M.P. and L. Pirola, *Structure and function of phosphoinositide 3-kinases. Biochim Biophys Acta*, 1998. **1436** (1-2): p. 127–5
21. Sui, L., J. Wang, and B.M. Li, Role of the phosphoinositide 3-kinase-Akt-mammalian target of the rapamycin signaling pathway in long-term potentiation and trace fear conditioning memory in rat medial prefrontal cortex. *Learn Mem*, 2008. **15**(10): 762–76.
22. Hozumi, H., et al., Endoscopic finding of spontaneous hemorrhage correlates with tumor necrosis factor alpha expression in colonic mucosa of patients with ulcerative colitis. *Int J Colorectal Dis*, 2013. 28(8): 1049–55.

*Corresponding author:
Yunxiao Wang,
Department of Stomatology,
Xingtai People 's Hospital,
No. 16 Hongxing Street, Xingtai, Hebei, China, 054001.
Email: yunxiao_2@126.com; Tel/Fax: 0086-0319-3286592