Original Article

Interleukin-21 and its association with chronic periodontitis

Rani Vilas Lokhande, Jeevan Gangadhar Ambekar, Kishore Gajanan Bhat,[1] Nilima Nandkumar Dongre

Department of Biochemistry, Shri B. M. Patil Medical College, Vijayapura, [1]Department of Microbiology, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgavi, Karnataka, India

Abstract:

Context: Interleukin-21 (IL-21) is a pleiotropic cytokine, well documented to contribute to the development of Th17 cells which have been shown to play an important role in the pathogenesis of periodontitis. Periodontal disease is a chronic infection of tooth-supporting tissue. **Aim:** This study evaluates the saliva and serum levels of IL-21 in patients with chronic periodontitis and periodontally healthy individuals. **Settings and Design:** The present study was carried out in the Department of Microbiology in association with Department of Oral Medicine and Radiology, Maratha Mandal's N.G.H Institute of Dental Sciences and Research Centre, Belgavi, Karnataka. **Materials and Methods:** Fifty samples of each group were included in the present study. The levels of IL-21 were assessed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit and the results were expressed as pg/mL. **Statistical Analysis Used:** Statistical analysis was performed using SPSS 17.0 software. Data were expressed as mean ± standard deviation and interquartile ranges and comparison of controls and cases by Mann–Whitney test. **Results:** Serum and salivary levels of IL-21 were significantly higher in chronic periodontitis group than in controls ($P < 0.001$). Clinical periodontal parameters correlated positively with serum IL-21 levels. **Conclusions:** IL-21 is highly expressed in patients with chronic periodontitis and correlated well with clinical parameters of periodontal destruction. Therefore, IL-21 appears to play a role in tissue destruction and can be used as diagnostic biomarker in chronic periodontitis. Saliva can be considered to be a useful alternative to serum as a diagnostic sample.

Key words:

Chronic periodontitis, diagnostic biomarker, enzyme-linked immunosorbent assay, interleukin-21

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INTRODUCTION

Periodontitis is an inflammatory disorder of periodontium, the supporting tissue of teeth which is characterized by the formation of pockets and loss of attachment between the tooth and gums.[1,2] Chronic periodontitis is the common form of periodontitis that mainly occurs in young adults and is a complex disease having multifactorial etiology.[3]

Even though different microbes are known to initiate the disease, it has been proved that exaggerated and disproportionate immune response of host is responsible for damage of periodontium in affected patients. The adaptive immune response is brought about mainly due to the involvement of various pro-inflammatory cytokines produced by activated T-cells in diseased gingival and periodontal tissue.[4]

The role of several cytokines as interleukin-1 (IL), IL-6, IL-8, IL-10, and IL-12 has been evaluated in chronic periodontitis and their involvement in the destruction of periodontal tissues.[5] In recent years, interest is focused on the study of a newly identified cytokine called as IL-21.[6] Investigations have shown that activated T-cells produce IL-21 which has the ability to act on multiple cells of the immune system causing impacts on immunity to infection.[5,7] There are not many studies conducted to find out the role of IL-21 in chronic periodontitis.

However, most of them have used gingival crevicular fluid and periodontal tissue as the clinical sample. The current study was intended to estimate IL-21 in the salivary and serum of chronic periodontitis patients and compare with those of healthy individuals and determine its diagnostic utility aid in periodontal disease.

MATERIALS AND METHODS

The present study conducted in the Department of Microbiology in association with the Department of Oral Medicine and Radiology in our Institute. Ethical clearance was taken from RGHUS, the

Address for correspondence:
Ms. Rani Vilas Lokhande, Department of Biochemistry, Maratha Mandal's NGH Institute of Dental, Sciences and Research Centre, Belgavi, Karnataka, India.
E-mail: ranivl@rediffmail.com

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Institutional Review Board of Institution before initializing study.

The study involved a total of 100 participants that included 50 cases of chronic periodontitis and 50 healthy individuals between the age ranges of 26 and 60 years belonging to both sexes. Participants with the known systemic disease, malignancies, and blood disorders, those with the habit of smoking, alcoholism, and drug abuse were excluded from the study.

In addition, those with <20 teeth and/or had undergone any dental treatment or received antibiotic therapy during the last 3 months were not considered for the study. Inclusion basis for case selection was at least 4 sites with >5 mm pocket probing depth (PD) and attachment loss. The corresponding criteria for the healthy control group were no sites with >3 mm pocket PD and attachment loss.

Written consents form was obtained from each participant before enrolling for the study. The standard operating procedure was followed for sample collection.

Blood collection

Blood samples collected in serum separator vacutainers. The site of venepuncture was cleaned with an alcohol swab. Gently, a 5 cc syringe with a 23G needle inserted in the vein and approximately 3 ml of blood collected in tubes, inverted tubes for 2–3 times to mix with clot activator in tubes. Once blood had drawn, the tourniquet was removed and kept sterile cotton swab over needle insertion and the needle was removed. Blood samples allowed to clot for 30 min at room temperature. Tubes transported to the laboratory and centrifuged at 15,000 rpm for 10 min. Serum samples were collected in multiple aliquots and stored at - 80°C until laboratory analysis.

Saliva collection

Navazesh spitting method was followed for collection of unstimulated saliva.[8]

The saliva collection procedure was explained to the participants. Participants asked to abstain from food or any drinks for minimum 1 h. Participants asked to rinse the mouth with water. Sterile wide-mouth containers were labeled and given for saliva collection. Participants were asked to accumulate the saliva in the floor of the mouth and asked to spit in a container for every 60 s. In this way, 2–3 mL saliva collected and transported to the laboratory on an ice pack. The saliva samples were vortexed for 2 min. The saliva samples transferred in 10 mL Falcon tubes and centrifuged for 10 min at 10,000 rpm. The supernatants were collected and stored as multiple alliquotes at - 80°C.

Laboratory measures

IL-21 levels were assessed by means of a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Krishgen Biosystems 15375 Ashley Ct. Whittier, CA Lot No 1211015). Manufacturer's instructions were strictly adhered while performing the assay.

Briefly, 100 µL of standards and samples were added to labeled microtiter wells and incubated for 2 h at room temperature. Unbound antigen removed by washing step. Following this, the wells were kept for 2 h with 100 µL detection antibody at room temperature. Moreover, washing step repeated as mentioned earlier. Then, to each well, 100 µL Avidin-HRP was added and waited for 30 min.

Wells have washed again and then incubated with tetramethylbenzidine substrate for 20 min. Then, 100 µL of stop solution was added to stop the reaction and optical density was read at 450 nm in microplate LISA reader. The IL-21 concentration was determined as pg/ml. Results of the test samples were calculated using the standard curve produced in an assay.

RESULTS

This study includes 100 participants which included 50 periodontally healthy controls and 50 cases with chronic periodontitis [Table 1].

The IL-21 levels quantified using ELISA technique from samples of each participant from control and study groups. The values of serum IL-21 in control groups ranged from 15.8 to 193.3 pg/mL (mean 65.34 pg/mL). Salivary levels of IL-21 in controls varied from 15.0 to 86.0 pg/mL (mean 21.98 pg/mL). Serum levels of IL-21 in chronic periodontitis patient ranged from 67.0 to 694.5 pg/mL (mean 497.78 pg/mL). Salivary IL-21 levels were 32.0–812.0 pg/mL (mean 214.02 pg/mL) in chronic periodontitis [Table 2].

The mean IL-21 levels were compared in chronic periodontitis patients and periodontally healthy participants by Independent sample *t*-test. Statistical analysis was significant with mean levels across test groups with $P < 0.005$. Clinical parameters for chronic periodontitis PD and clinical attachment loss (CAL) were assessed for correlation with IL-21 levels in the serum and saliva. It was found that there was significant correlation of PD with serum IL-21 levels (rs= -0.341, P =0.016) but no significance could attached to IL-21 levels in both test fluids with CAL [Table 3].

Table 1: Age and sex distribution pattern of chronic periodontitis patients and healthy individuals

Characteristics	Periodontitis (*n*=50)	Healthy (*n*=50)
Age (years mean±SD)	46.20±14.86	35.07±5.62
Males (%)	40	50
Females (%)	60	50

SD – Standard deviation

Table 2: Comparison between interleukin-21 levels of healthy and chronic periodontitis groups by Independent samples *t*-test

Interleukin levels	Group	*n*	Mean±SD	*P*
IL-21 levels serum (pg/ml)	Periodontal disease	50	497.78±297.06	<0.001** (significant)
	Healthy	50	65.34±42.66	
IL-21 levels saliva (pg/ml)	Periodontal disease	50	214.02±188.54	<0.001** (significant)
	Healthy	50	21.98±15.67	

**P<0.001 (highly significant). IL-21 – Interlukine-21; SD – Standard deviation; P – P-value; n – 50

In Table 4, the present study was compared with other previous studies.

DISCUSSION

Among the inflammatory diseases, chronic periodontitis is commonly found in human beings. The disproportionate adaptive response toward microbial pathogens present in the dental plaque is known to be the reason for the destruction of periodontal tissues in diseased patients.[9] Both innate and adaptive immune responses are involved in the progression of periodontal disease.

Literature survey shows the participation of various T-cells subsets in periodontal diseases. There are three subtypes of T helper cells – Th1, Th2, and Th17 cells. Investigations have shown that T-cells produce inflammatory cytokines which are causative agents of the symptoms and complications of periodontitis.[10] Recently, the role of Th17 cells and IL-21 has been the topic of investigation in periodontitis and several other systemic infectious disorders.[11,12] IL-21 was discovered in 2000 as a CD4 + T-cell-derived cytokine. It belongs to common gamma chain of cytokines family.

Table 3: Pearson's correlation to compare pocket probing depth, loss of attachment with the Interleukin-21 levels in serum and in saliva

Group	IL-21 levels serum	IL-21 levels saliva
Periodontal disease (*n*=50)		
PPD (*n*=50)		
Pearson correlation	−0.341*	0.201
Significant (two-tailed)	0.016 (significant)	0.161 (not significant)
LOA		
Pearson correlation	0.022	0.026
Significant (two-tailed)	0.881 (not significant)	0.858 (not significant)
IL-21 levels serum		
Pearson correlation	-	−0.634**
Significant (two-tailed)	-	<0.001 (significant)

*Correlation is significant at the 0.05 level. **Correlation is significant at 0.01 level. Interpretation: Serum and salivary levels of IL-21 were significantly correlated with each other. Pocket depth was significantly and negatively correlated with IL 21 levels. PPD – Pocket probing depth; LOA – Loss of attachment; IL-21 – Interleukin-21; *P* – *P*-value; *n* – 50

It is a pleiotropic cytokine and has the tendency to impact on immunity. IL-21 involved in the development of Th17 cells, which play a major role in the disease process of periodontitis.[13-15] We investigated the utility of serum and salivary IL-21 levels in patients with chronic periodontitis and periodontally healthy individuals which has not been tried so far.

Most of the previous studies used the gingival tissue, gingival crevicular fluid for detection of IL-21 levels using different methods including immunohistochemistry, Western blot, polymerase chain reaction, and ELISA.[16] The most of these studies showed significantly raised levels of IL-21 in chronic periodontitis patients when compared to healthy individuals.

Several investigators have shown IL-21 levels correlated significantly with periodontal parameters.[5,13] The literature survey has shown only one study, wherein levels of salivary IL-21 was decreased after induced physical stress.[17] In Table 4, the present study was compared with other previous studies.

The present study analyzed IL-21 levels in serum and salivary samples by ELISA technique which is the most sensitive, speedy, easy to carry out, and reproducible method.[18] The study found a significant difference between periodontally healthy and chronic periodontitis patients for both serum and salivary IL-21 levels ($P < 0.001$). The levels of serum IL-21 in chronic periodontitis cases were correlated negatively with probing pocket depth by Pearson's correlation.

it was interesting to find that serum and salivary IL-21 levels showed no correlation with CAL. Saliva is a diagnostic medium having many advantages in comparing with other body fluids. The proteins and RNA contents of both saliva and serum are similar.

Collection of saliva sample is convenient and safe for both technicians and patients. Saliva is readily available, noninvasive, and easier to handle.[19-21] There are upcoming newly methods are arising for diagnosis of periodontitis. At present, detection of the periodontal pathology depends predominantly on the clinical and radiological examinations, which are useful in

Table 4: The comparison of results from various studies

Name of author	Number of samples	Sample	Method	Results	Reference
Dutzan *et al*., 2011	Case-15 Control-19	Gingival tissue	IHC, ELISA Western blot	Gingival IL-21 significantly higher in CP group	[7]
Zhao *et al*., 2011	Case-30	GCF	ELISA	Downregulation of IL-21	[15]
Napimoga *et al*., 2011	Case-15 Control-15	Gingival tissue	PCR	mRNA levels of IL-21 higher in CP group	[11]
Isaza Guzman *et al*., 2015	Case-105 Control-44	Saliva	PCR	No significant domination in periodontitis	[22]
Nizam *et al*., 2014	OSP mild-17 OSP severe-22 Control-13	Saliva	ELISA	Significant correlation with CAL and IL-21	[12]
Present study	Case-50 Controls-50	Serum and saliva	ELISA	Significant difference between periodontally healthy and chronic periodontitis patients positive correlation serum IL-21 with PD	

CP – Chronic periodontitis; CAL – Clinical attachment loss; OSP – Obstructive sleep apnea; ELISA – Enzyme-linked immunosorbent assay; GCF – Gingival crevicular fluid; PCR – Polymerase chain reaction; IHC – Immunohistochemistry; PD – Probing depth; *P*<0.05; *n* = number of samples

tracing data of the past disease, present periodontal health, but suggest incomplete information about diseases and sites at threat for the future periodontal disease risk.

Several diagnostic parameters in the saliva have proposed and use as an analytical aid for periodontal pathology. Early diagnosis and management reduce the severity and possible complication of the disease process.

CONCLUSIONS

These data concluded that IL-21 levels were significantly increased in chronic periodontitis patients and linked with clinical periodontal parameters of tissue destruction.

Therefore, IL-21 levels might responsible for tissue damage processes that characterize chronic periodontitis. IL-21 levels used as one of the diagnostic and prognostic aids in chronic periodontitis.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Kim J, Amar S. Periodontal disease and systemic conditions: A bidirectional relationship. Odontology 2006;94:10-21.
2. Pussinen PJ, Könönen E, Paju S, Hyvärinen K, Gursoy UK, Huumonen S, *et al.* Periodontal pathogen carriage, rather than periodontitis, determines the serum antibody levels. J Clin Periodontol 2011;38:405-11.
3. Consolaro A. In adults: 47.2% have periodontitis! How about in orthodontic patients? Dental Press J Orthod 2013;18:3-5.
4. Seymour GJ. Importance of the host response in the periodontium. J Clin Periodontol 1991;18:421-6.
5. Dutzan N, Vernal R, Vaque JP, García-Sesnich J, Hernandez M, Abusleme L, *et al.* Interleukin-21 expression and its association with proinflammatory cytokines in untreated chronic periodontitis patients. J Periodontol 2012;83:948-54.
6. Parrish-Novak J, Foster DC, Holly RD, Clegg CH. Interleukin-21 and the IL-21 receptor: Novel effectors of NK and T cell responses. J Leukoc Biol 2002;72:856-63.
7. Dutzan N, Rivas C, García-Sesnich J, Henríquez L, Rivera O, Dezerega A, *et al.* Levels of interleukin-21 in patients with untreated chronic periodontitis. J Periodontol 2011;82:1483-9.
8. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci 1993;694:72-7.
9. Hajishengallis G. Immunomicrobial pathogenesis of periodontitis: Keystones, pathobionts, and host response. Trends Immunol 2014;35:3-11.
10. Glimcher LH, Murphy KM. Lineage commitment in the immune system: The T helper lymphocyte grows up. Genes Dev 2000;14:1693-711.
11. Napimoga MH, Nunes LH, Maciel AA, Demasi AP, Benatti BB, Santos VR, *et al.* Possible involvement of IL-21 and IL-10 on salivary IgA levels in chronic periodontitis subjects. Scand J Immunol 2011;74:596-602.
12. Zhao L, Zhou Y, Xu Y, Sun Y, Li L, Chen W. Effect of non-surgical periodontal therapy on the levels of th17/Th1/Th2 cytokines and their transcription factors in Chinese chronic periodontitis patients. J Clin Periodontol 2011;38:509-16.
13. Nizam N, Basoglu OK, Tasbakan MS, Nalbantsoy A, Buduneli N. Salivary cytokines and the association between obstructive sleep apnea syndrome and periodontal disease. J Periodontol 2014;85:e251-8.
14. Yi JS, Cox MA, Zajac AJ. Interleukin-21: A multifunctional regulator of immunity to infections. Microbes Infect 2010;12:1111-9.
15. Kratholm SK, Iversen MB, Reinert L, Jensen SK, Hokland M, Andersen T. Interleukin-21 receptor signalling is important for innate immune protection against HSV-2 infections. PLoS One 2013;8:e81790.
16. Mootha A, Malaiappan S, Jayakumar ND, Varghese SS, Toby Thomas J. The effect of periodontitis on expression of interleukin-21: A systematic review. Int J Inflam 2016;2016:3507503.
17. Moreira A, Bacurau RF, Napimoga MH, Arruda AF, Freitas CG, Drago G, *et al.* Salivary IL-21 and IgA responses to a competitive match in elite basketball players. Biol Sport 2013;30:243-7.
18. Yolken RH. Enzyme-linked immunosorbent assay (ELISA): A practical tool for rapid diagnosis of viruses and other infectious agents. Yale J Biol Med 1980;53:85-92.
19. Patil PB, Patil BR. Saliva: A diagnostic biomarker of periodontal diseases. J Indian Soc Periodontol 2011;15:310-7.
20. Spielmann N, Wong DT. Saliva: Diagnostics and therapeutic perspectives. Oral Dis 2011;17:345-54.
21. de Almeida Pdel V, Grégio AM, Machado MA, de Lima AA, Azevedo LR. Saliva composition and functions: A comprehensive review. J Contemp Dent Pract 2008;9:72-80.
22. Isaza-Guzmán DM, Cardona-Vélez N, Gaviria-Correa DE, Martínez-Pabón MC, Castaño-Granada MC, Tobón-Arroyave SI, *et al.* Association study between salivary levels of interferon (IFN)-gamma, interleukin (IL)-17, IL-21, and IL-22 with chronic periodontitis. Arch Oral Biol 2015;60:91-9.