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Correlation of the interleukin-29 levels in crevicular fluid and plasma with the genetic polymorphism in chronic and aggressive periodontitis patients

B.M. Shivaprasad [a,b,*], A.R. Pradeep [c]

[a] Ph.D Research Scholar, Department of Periodontology, Government Dental College and Research Institute, Bangalore, India
[b] Reader, Department of Periodontology, Rajarajeswari Dental College and Hospital, Bangalore, India
[c] Professor and Head, Department of Periodontology, Government Dental College and Research Institute, Bangalore, India

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ABSTRACT

Objective: To assess the effect of single nucleotide polymorphism (SNP) on the Interleukin (IL-29) quantity in gingival crevicular fluid (GCF) and plasma of chronic and aggressive periodontitis patients.

Design: Patients with periodontal health ($n = 30$), chronic generalized periodontitis ($n = 30$) and generalized aggressive periodontitis ($n = 30$) were subjected to IL-29 quantity estimation in GCF and plasma using enzyme linked immunosorbent assay and was correlated with IL-29 SNP (rs30461) using polymerase chain reaction.

Results: IL-29 concentration in GCF and plasma was highest in aggressive periodontitis patients (114.17 ± 95.07 pg/ml and 149.69 ± 109.90 pg/ml respectively). The least concentration was found in subjects with healthy periodontium (47.50 ± 37.75 pg/ml and 54.52 ± 37.53 pg/ml) and in chronic periodontitis it was found to be 65.01 ± 41.26 pg/ml and 81.17 ± 46.23 pg/ml. The difference in the quantity of IL-29 in GCF and plasma among different groups was statistically significant ($p < 0.001$ and $p < 0.001$ respectively). rs30461 polymorphism of IL-29 analysis revealed that difference in the prevalence of A/A, A/G and G/G genotype among three groups was not statistically significant ($p = 0.097$).

Conclusion: Increased quantity of IL-29 in GCF and plasma of subjects with periodontitis suggests a role in pathogenesis of periodontitis and the SNP (rs30461) is not related to susceptibility to periodontitis in this population of Indian individuals.

1. Introduction

The pathogenic process of periodontitis includes dynamic interactions among various infectious agents and interconnected cellular and humoral host responses.[1] Proinflammatory cytokines including interleukin 1β (IL-1β), IL-6, IL-8, tumour necrosis factor α and anti-inflammatory cytokines such as IL-4 and IL-10 modulate the constitutional factors on which the inflammation is dependent.[2–4]

Cytokines play a critical role in modulating the innate and adaptive immune systems against microorganisms.[5] A family

* *Corresponding author at*: Department of Periodontology, Rajarajeswari Dental College and Hospital, Bangalore 560074, India.
E-mail addresses: drshivaprasad2008@yahoo.co.in (B.M. Shivaprasad), periodonticsgdcri@gmail.com (A.R. Pradeep).
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of three cytokines, designated interleukin 28A (IL-28A), IL-28B and IL-29 have been recognized from the human genomic sequence that are known as interferon-λ_1 (IFN-λ_1), IFN-λ_2 and IFN-λ_3 respectively. It was also found that like type I IFNs, IL-28 and IL-29 were induced by viral infection and showed antiviral activity.[5,6]

IL-29 is mainly produced by dendritic cells in response to viral proteins or toll-like receptor (TLR) agonists, although it can be produced by any nucleated cell type following viral infection or activation with bacterial components[7–9] and can inhibit the replication of various viruses.[9–11] IL-29 possesses potential antiviral activity against Herpes Simplex virus-1 (HSV-1) infection in human macrophages and dendritic cells.[11] Further, IL-29 mediate its effects via the IL-28R1/IL-10R2 receptor complex and the target cell populations of IL-28/IL-29 are restricted and mainly include epithelial cells and hepatocytes.[11] It was asserted that proper functional niche of the type-III IFNs appears to lie where epithelial surfaces interact with the immune system (i.e., at the interface between the body and the outside world), in keeping with their apparent early evolutionary origins.[8,12]

IL-29 has immunomodulatory properties similar to IFN-α but with few differences.[8] Macrophages and plasmacytoid and monocyte-derived dendritic cells (pDC and MDDC) produce IL-29 in response to infiuenza virus infection and to bacterial and viral molecular mimics like lipopolysaccharide, toll-like receptor agonists and poly I:C.[13–15]

IL-29 activates monocytes and macrophages to produce a restricted panel of cytokines.[16] Whole PBMC (peripheral blood mononuclear cell) exposed to IL-29 increase the production of IL-6, -8, and -10 and human macrophages also respond to IL-29 by producing cytokines IL-6, -8, and -10.[16] Induction of IL-6 by IL-29 might suggest a role in linking the innate immune response to the adaptive immune response.[17] Th2 cytokines consist of IL-4, IL-5, and IL-13 and IL-29 has important immunoregulatory properties with regard to the Th2 responses.[17] IL-29 has been shown to inhibit the production of IL-13 by T cells in an IFN-γ independent manner, which is mediated in part via MD-DC.[18]

Recently IFN-lambdas were measured by enzyme linked immunosorbent assay (ELISA) detecting IL-29, IL-28A and IL-28B. IL-29 levels were substantially lower in patients with chronic hepatitis C than in healthy controls and patients with spontaneously resolved hepatitis.[19] Carriers of the rs12979860C allele associated with resolution of HCV infection exhibited increased IFN-lambda levels ($p < 0.02$).[19]

IL-29, having immunomodulatory and antiviral properties might play a role in the pathogenesis of periodontal diseases. Further, single nucleotide polymorphism of IL-29 gene might lead to the imbalance in the production of IL-29 in periodontitis patients. Quantitative changes in the composition of IL-29 in GCF and plasma could have a diagnostic and therapeutic significance. The analysis of cytokine production levels is used as tool for studying the local host response to microbial challenge.[20,21] Therefore the present study was aimed at quantitative analysis of IL-29 in gingival crevicular fluid and plasma of chronic periodontitis and aggressive periodontitis patients and also to assess the effect of single nucleotide polymorphism on the IL-29 quantity in gingival crevicular fluid and plasma. According to the authors' knowledge the present research is the first study conducted in this regard.

2. Materials and methods

Study subjects were selected from outpatient Department of Periodontics, Government Dental college and Research Institute, Bangalore, India from January to April 2010. A total of 90 subjects ($n = 90$; 46 males and 44 females; age ranging from 19 to 47 years, mean age of 31.50 ± 6.84) were divided into three groups, viz., healthy subjects, chronic generalized periodontitis patients and generalized aggressive periodontitis patients.

Chronic periodontitis patients and generalized aggressive periodontitis patients were diagnosed based on the criteria of American Academy of Periodontology classification of periodontal disease (1999). The subjects for sampling were selected at random from individuals scheduled for a routine oral examination. Periodontal evaluation included gingival index (GI), probing pocket depth (PPD), and clinical attachment level (CAL). All clinical measurements were performed by a single examiner (SBM). PPD and CAL were measured using a graduated William's periodontal probe.

Subjects were categorized into three groups based on the GI, PPD, CAL, and radiographic evidence of bone loss for screening purposes. Healthy subjects ($n = 30$, age range of 21–42 years, mean age 30.07 ± 5.75 years) had clinically healthy periodontium, GI < 1 and PPD < 3 mm, and CAL $= 0$, showed no radiographic evidence of bone loss. Chronic generalized periodontitis patients ($n = 30$, age range of 26–47 years, mean 37.63 ± 5.08 years) had signs of clinical inflammation, GI > 1, PPD ≥ 4 mm, and CAL ≥ 2 mm, with radiographic evidence of bone loss. Generalized aggressive periodontitis patients ($n = 30$, age range of 19–35 years, mean 26.8 ± 4.55 years) had signs of clinical inflammation, GI > 1, PPD ≥ 4 mm, and CAL ≥ 2 mm, with radiographic evidence of bone loss.

All patients were systemically healthy and had not received periodontal treatment or antibiotics for at least 6 months prior to the clinical examination and sampling. Patients were excluded from the study if they had diabetes, smoking habit, any other systemic disease or conditions such as pregnancy that could alter the course of periodontal disease. Subjects who satisfied the inclusion criteria of the study were selected and ethical approval was obtained from the Institutional review board. Furthermore, each patient received a detailed explanation regarding the study procedure, and written informed consent was obtained from those who agreed to participate voluntarily in the study.

2.1. Site selection and GCF sample collection

In the periodontitis group, only one site per subject was selected as a sampling site; whereas in the healthy group, multiple sites (three to five sites per subject) with an absence of inflammation were sampled to ensure the collection of an adequate amount of GCF.[22] In chronic and aggressive periodontitis patients, sites showing the greatest CAL and signs of inflammation, along with radiographic bone loss were selected for sampling using graudated Williams periodontal probe. On the subsequent day, the sample collection site was well-isolated and without touching the marginal gingiva, the supragingival plaque was removed. GCF was collected by placing the microcapillary pipette (Sigma–Aldrich, India) at