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ORIGINAL ARTICLE

A COMPARATIVE EVALUATION OF INTERLEUKIN-35 LEVELS IN CHRONIC PERIODONTITIS PATIENTS WITH AND WITHOUT TYPE 2 DIABETES MELLITUS AND ITS RESPONSE TO NONSURGICAL PERIODONTAL THERAPY- A CLINICO- BIOCHEMICAL STUDY

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Abstract

Background:Periodontitis is an inflammatory condition of the tooth supporting structures in which we can discover an imbalance of cytokines. Interleukin-35 (IL-35) is an anti-inflammatory cytokine that has been upregulated in chronic periodontitis. Since Diabetes Mellitus is a systemic condition, where there will be a greater existence of cytokine imbalance, hence the aim of the present study was to investigate the role of Gingival Crevicular Fluid (GCF) IL-35 in chronic periodontitis patients with Type 2 Diabetes Mellitus after Scaling and Root Planing (SRP).

Materials and Methods: A total of 114 patients with an age range of 30-60 years, were divided into 3 groups: Group I (healthy patients), Group II (chronic periodontitis), and Group III (chronic periodontitis with Diabetes Mellitus). Subsequently a thorough Medical & Dental history was recorded alongside the clinical parameters too and followed by the collection of GCF samples to perform ELISA analysis for the detection of IL-35. Latter, the SRP was carried out in Group II & III with a follow up of 6 weeks. Again at the end of 6 weeks the clinical parameters were recorded & GCF samples were collected to perform ELISA analysis for the detection of IL-35.

Results: IL-35 levels were increased in GCF collected from Group II & III compared to Group I. However, after Non-Surgical Periodontal Therapy, there was reduction in the IL-35 levels in GCF collected from Group II & III.

Conclusion: This study clinches that GCF IL-35 levels were higher in Group II & Group III, which later reduced after SRP, suggesting that the role of IL-35 in periodontal disease, hence it can be considered as a Novel Diagnostic Biomarker in the diagnosis of periodontal disease.

KEY WORDS: Biomarker; Chronic periodontitis; Gingival Crevicular Fluid; Interleukin-35; Type 2 Diabetes Mellitus.

1 | INTRODUCTION

Periodontitis is an inflammatory ailment of the tissues contiguous the tooth, affected by a group of explicit microorganisms, resulting in progressive destruction of periodontal tissues.[1] Inflammation in the periodontium starts as an acute inflammatory response subsequently the host-bacterial interaction, latter progresses in to a chronic stage dominated by B lymphocytes and macrophages, ensuing an intense T lymphocytic stage.[2] The transition between the above stages, involving an accumulation and differentiation of immune cells in the inflammatory area ,which are mediated by "cytokines."

Cytokines are soluble mediators contributing for numerous biologic processes such as hematopoiesis, wound healing, systemic and local inflammatory responses.[3] As in other chronic inflammatory diseases; inflammatory cytokines are considered to play an important role in the initiation, progression and resolution of inflammation in periodontal disease as well.

Interleukin-35 (IL-35), a member of the IL-12 family, was discovered by *Stern, et al. in 1990*, and initially named as "cytotoxic lymphocyte maturation factor" (CLMF).[4] IL-35 is composed of the p35 subunit of IL-12 and the Epstein-Barr Virus (EBV) induced gene 3 (EBI3) subunit of IL-27. The anti-inflammatory role of IL-35 was described in 2007 by two separate research groups, *Collison, et al.,* in the USA and *Niedbala, et al*., in the UK .[4]

IL-12 cytokine family consists of four cytokines: IL-35, IL-27, IL-12, and IL-23 (from the inhibitory to the most pro-inflammatory respectively).[5] IL-35 is an effective inhibitory cytokine generated by regulatory T cell (T_{reg}) cell populations and is known for its maximum suppressive effect. It inhibits proliferation of T cells by arresting mitosis in G1 phase without triggering apoptosis.[6,7]

Periodontitis, being a chronic inflammatory lesion is amended by several factors such as smoking, genetics and hormonal factors. The incongruity of one such hormonal factor which has great implications on the integrity of periodontal tissue is Insulin, the deficiency of which leads to Diabetes Mellitus. The risk of periodontitis is increased by approximately three folds in Diabetes Mellitus patient than in Non-Diabetes Mellitus patient.[8]*Loe* suggested that, periodontitis should be considered as the sixth complication of diabetes.[9] Later *Grossi et al*[10] and *Preshaw et al*[11] proposed a two-way relationship for Diabetes Mellitus and periodontitis.

A Successful management of periodontal infection and suppression of periodontal inflammation is always associated with reduced levels of Glycated Hemoglobin. The level of Glycemic control seems to be the key factor in controlling the periodontal infection.[12] The patients with poor Glycemic control had more Clinical Attachment Loss and were more likely to exhibit recurrence of the disease.

A well-known fact is that increase in microbial load leads to periodontal inflammation, but an effective treatment shall help in reducing the microbial load and the degree of inflammation. Few Studies have demonstrated that there is rise in the levels of IL-35 during the course of the periodontitis.[13,14,15,16,17] Also, there are studies on chronic inflammatory conditions like tuberculosis, wherein it was demonstrated that after treatment, the levels of IL-35 were reduced.[18]

On the trail of an extensive literature search and to the best of our knowledge, till date no such studies have been conducted to evaluate the effect of Scaling and Root Planing on GCF IL-35 levels in chronic periodontitis patients with and without Type 2 Diabetes Mellitus. Therefore, the present study was conducted to evaluate and assess the effect of Scaling & Root Planing on Gingival Crevicular Fluid (GCF) IL-35 levels in chronic periodontitis patients with and without type 2 Diabetes Mellitus.

Supplementary information The online version of this article (https://doi.org/10.15520/ijmhs.v10i09.3106) contains supplementary material, which is available to authorized users.

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2 | MATERIALS AND METHODS

Source of data:

A total of 114 patients in the age range of 30-60 years (mean age 42.3 ± 8.59) were selected from the Out-patient Department of Periodontics, Bapuji Dental College & Hospital, Davangere. The study was approved by an Institutional Review Board (IRB) [BDC/Exam/434/2015-16] and all the patients were given the written informed consent in accordance with the *Helsinki* Declaration.[19]

The Inclusion &Exclusion Criteria of the Study were as follows

Inclusion Criteria-

a) Patients of both the gender, b) Patients having at least 20 natural teeth in their mouth, c) Radiographic evidence of alveolar bone loss, d) Chronic periodontitis patients with well controlled type II Diabetes Mellitus (HbA_1C level 6-8%), e) Patients diagnosed with Diabetes Mellitus for more than 3yrs before the beginning of the study.

Exclusion Criteria -

a) Patients with history of any systemic diseases like hypertension, epilepsy, asthma, thyroid, autoimmune diseases and cardiac diseases. (other than DM), b) Patients on antibiotic therapy in past 3 months, c) Patients who have undergone any form of periodontal therapy in past 6 months, d) Patients on immunosuppressive therapy, e) Pregnant and lactating mothers, f) Patients with history of smoking and chewing tobacco.

Clinical Examination:

At baseline, thorough medical and dental history was recorded, periodontal assessment was done using clinical parameters; Plaque index (PI), Gingival index (GI), Probing depth (PD), Clinical attachment level (CAL). Radiographic assessment was done using IOPA and OPG. After 6 weeks, clinical parameters were rerecorded.

The chair-side assessment of HbA1c levels of diabetic patients were assessed by using Chek Diagnostic's A1CNow®+ test kit (FDA cleared). The A1CNow®+ multi-test system provides quantitative measurement of the percentage of glycated haemoglobin (A1C) levels in capillary (fingerstick) or venous whole blood samples. The test system only requires 5μl of blood from the fingertip for analysis and the result was processed in 5 minutes which was displayed on portable handheld device.

GCF Collection

The selected Patients for sampling were made to sit comfortably in the dental chair in an upright position. The selected site was isolated with cotton rolls and air dried. Supragingival plaque was removed gently without touching the marginal gingiva, to avoid contamination and blocking of the micro capillary pipette. GCF was collected by placing white colour-coded 1-5μl calibrated volumetric micro capillary pipettes *(Sigma-Aldrich Chemical Company, USA)*. By using extra-crevicular (unstimulated) method, a standardized volume of 3μl GCF was collected using the calibration on the micropipette from each test site. After collection of GCF, clinical parameters like GI scores, PD and CAL were recorded to confirm the site selection and the sample was assigned to a particular group accordingly. The test sites, which did not express standard volume (3μl) of GCF, or micropipette contaminated with blood/ saliva/ plaque were excluded or discarded. The baseline GCF samples were collected at the initial visit in Group I, Group II, and Group III patients. SRP was performed for Group II and Group III only at the same appointment after GCF collection. After 6 weeks, again along with the recording of clinical parameters the GCF was also collected from same sites in the patients belongs to the Group II and Group III and the collected samples were immediately transferred to Eppendorf tube and stored at –80^{o}C till the time of the assay.

Periodontal treatment

As the group I in the present study, considered as a healthy controls and also the patients belong to the group also exhibited the a very good oral hygiene maintenance, henceforth they were kept under maintenance phase for every 6 months followed by oral hygiene instructions and patient education only.

Later the Group II and III patients were undergone the Periodontal treatment involving the Scaling & Root Planing (SRP) followed by an oral hygiene instruction by a single Periodontist.

STATISTICAL ANALYSIS

The data obtained from clinical examination and bio- analysis was presented as mean ± standard deviation. One-way ANOVA analysis was performed among the 3 study groups for the comparison of PI, GI, PD, CAL and IL-35 levels at baseline. Intergroup comparison of PI, GI, PD, chemical CAL scores & IL-35 levels were assessed by independent‘t’ test. Baseline and post therapy values in individual groups were analyzed by paired t test.

3 | RESULTS

At baseline, all the clinical parameters were significantly higher in Group II and Group III compared to Group I. However, 6 weeks after SRP, clinical parameters were significantly reduced in group II & III (Graph 1& 2). Also, GCF levels of IL-35 were higher in Group II and Group III patients compared to Group I ($p<0.05$). Six weeks after the phase-I therapy, IL-35 levels were significantly reduced in group II and group III ($p<0.05$). However, the decrease in IL-35 was significantly more in Group II as compared to Group III. Also, there was a correlation between IL-35 levels and clinical attachment level (CAL) (Graph 3).

Graph 1:

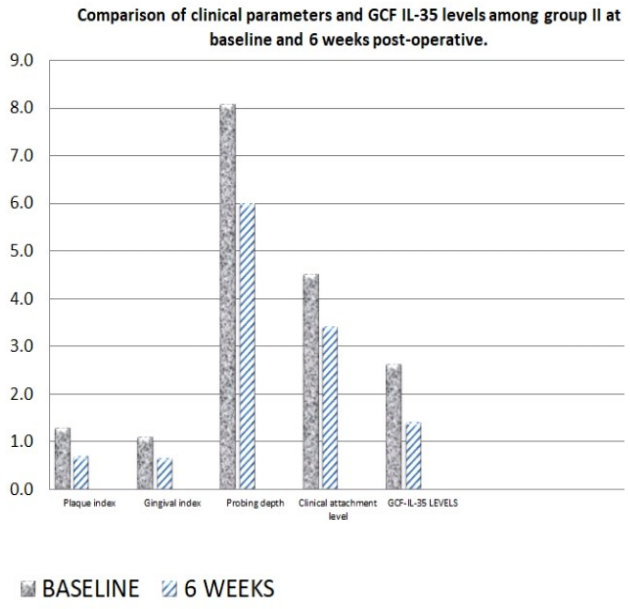


FIGURE 1:

Graph 2:

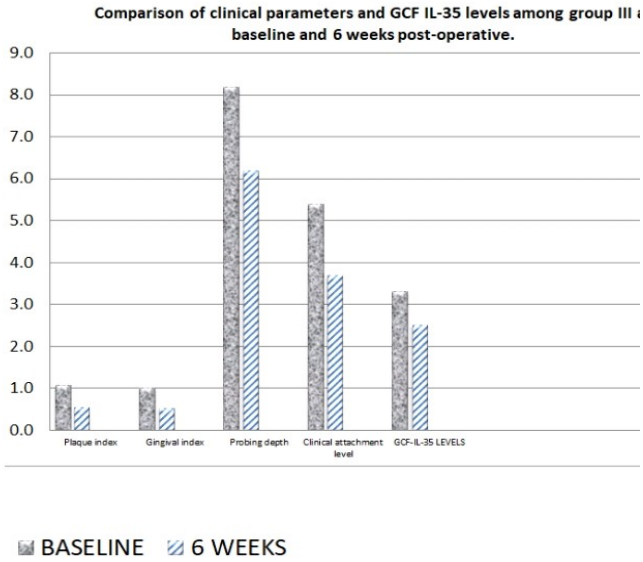
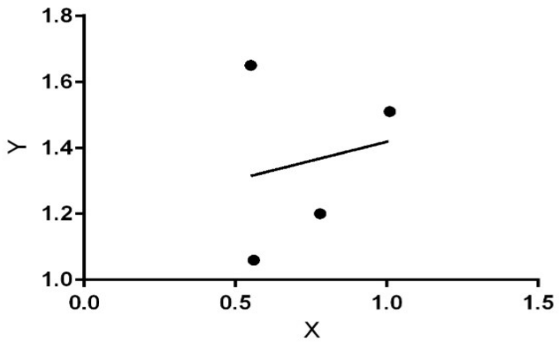


FIGURE 2:

Graph 3
CORELATION COEFFICIENT OF CLINICAL PARAMETER(CAL) AND GCF IL-35 LEVELS AMONG GROUPS II & III AT BASELINE AND 6 WEEKS POST-OPERATIVE.



X-GCF IL-35 LEVELS
Y-CAL VALUES

FIGURE 3: CORELATIONCOEFFICIENT OF CLINICAL PARAMETER(CAL) AND GCF IL-35 LEVELS AMONG GROUPS II& III AT BASELINE AND 6 WEEKS POST-OPERATIVE

4 | DISCUSSION

In the present study, GCF level of IL-35 and clinical parameters were evaluated in chronic periodontitis patients with and without type 2 Diabetes Mellitus before and after Phase-I periodontal therapy. IL-12 is one of the well-studied cytokine family comprising of both pro and anti-inflammatory cytokines, which include IL-12, IL-23, IL-27, and IL-35. Out of these, IL-35 acts as inhibitory cytokine which

can generate and activate T_{reg} cells especially at high inflammation sites.[20] T_{reg} cells are necessary in the maintenance of immune homeostasis and the prevention of autoimmune disease.

There is evidence to suggest that anti-inflammatory T_{reg} cells also play an important role in the development of periodontal disease and are involved in the subsequent inflammation and bone resorption. The infiltration of T_{reg} cells into periodontal tissues reflects their ability to inhibit tissue damage.[21] *Nakijima et al* in their study demonstrated that patients with chronic periodontitis exhibited increased incidence of T lymphocytes and CD4 CD25 T cells in the inflammatory infiltrate of gingival tissues and also demonstrated phenotypic markers of T_{reg}, such as Foxp3.[22] These are the potential markers associated with the severity, as well as the susceptibility of periodontal disease.

Considering the two-way relationship between periodontitis and diabetes, we hypothesized that IL-35 levels may be influenced in cases of periodontitis with diabetes. Production of several inflammatory cytokines, including tumor necrosis factor (TNF), interleukins (ILs), and cytokine-like proteins (adipokines) has been observed in type 2 diabetes.[23] Various studies have concluded that periodontal therapy helps in improving HbA1C level.[9,10]

In the present study, GCF samples were used for estimation of IL-35 as it is non-invasive, easy to collect, and it is a good source of local and systemic biomarkers due to host bacterial interactions.[24] Its composition changes during the development of inflammation.[25,26,27] As a result of inflammation, when GCF leaks from dilated blood vessels within the gingival connective tissue, this fluid flows through the inflamed connective tissue, along with the enzymes and other mediators involved in immune response. This leaked fluid in GCF is an "inflammatory soup" containing subgingival bacteria and host cells. So, it offers a great potential as a source of factors that may be associated with disease activity. Various studies reported that GCF protein level obtained from inflamed gingiva is much higher and similar to concentration of proteins in serum.[27,28]

After an extensive pursuit for the similar literature involving the identification of IL-35 in patients with type 2 Diabetes Mellitus with generalized moderate chronic periodontitis.we have found that the IL-35 has been investigated and assesssed by using samples like GCF[29] ,saliva[30] and serum. [31]

A similar kind of study was carried out by *Thakare S Kaustubh et al*, to evaluate and compare the levels of IL-35 in gingival crevicular fluid (GCF) in patients with chronic gingivitis and chronic periodontitis.Gingival crevicular fluid samples were obtained from chronic gingivitis patients (n = 15) and patients with chronic periodontitis (n = 15). Clinical measurements like probing pocket depth, bleeding on probing, papillary bleeding index, and modified plaque index were recorded. Enzyme-linked immunosorbent assay was used for the determination of GCF IL-35 levels in samples.The Clinical parameters were significantly higher in the chronic periodontitis group than the chronic gingivitis group. The GCF IL-35 levels were significantly higher in the chronic gingivitis group than the chronic periodontitis group. The IL-35 levels were higher in chronic gingivitis group as compared with chronic periodontitis group, indicating that the levels of IL-35 decrease with increase in the inflammatory status, so it might play a role in suppressing gingival inflammation and maintaining periodontal health.[29]

A comparable invivo study was conducted by *Moslemi et al,* to determine the level of the salivary IL-35 level cytokine in patients with type 2 Diabetes Mellitus with generalized moderate chronic periodontitis.Entirely, 88 patients (44 female, 44 males) with a mean age of 42. 5± 10. 5 years old participated in this case control study. The patients were divided into four groups and each group included 22 patients: Group 1: generalized moderate chronic periodontitis patients with type 2 diabetes, Group 2: generalized moderate chronic periodontitis patients without diabetes, Group 3: diabetic patients with normal periodontium, Group 4: healthy periodontium and non-diabetic group (control) Then saliva was collected and centrifuged, the amount of IL-35 was determined with commercial ELISA kit.Later the Data were analyzed.The salivary IL-35 level is decreased in both periodontitis and type 2 diabetes. However, Diabetes Mellitus does not exacerbate this deterioration in patients with periodontitis.[30]

A cross-sectional examination was led by Avideh Maboudi,et al.,to explore the serum levels of IL-23 and IL-35 in individuals with type 2 Diabetes Mellitus (DM) and chronic periodontitis (CP).Further the selected 72 patients were separated into four equivalent groups: group A, patients without type 2 Diabetes Mellitus and chronic periodontitis; group B, patients with type 2 DM without CP; group C, patients with CP and without type 2 DM; and group D, patients with type 2 DM and CP. Demographic data were obtained and periodontal conditions including clinical attachment loss, bleeding on probing, plaque index, gingival index, and probing depth was evaluated on all existing teeth. Fasting blood sugar (FBS) levels, hemoglobin (Hb) A1c, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were assessed. Likewise, serum levels of IL-23 and 35 were estimated utilizing enzyme-linked immunosorbent assay. The serum levels of IL-23 and 35 indicated no huge contrasts between all groups (P>0.05). A huge positive connection between the serum concentration of IL-23 and clinical connection misfortune in the benchmark group (r: 0.548, P=0.019) was distinguished. A critical negative connection between IL-35 and the plaque list in group B (r: - 0.578, P=0.012), in addition to huge negative relationships between IL-23 with ESR (r: - 0.487, P=0.040) and CRP (r: - 0.498, P=0.035) in group C and D were additionally recognized. Notwithstanding the significant associations of serum concentration of IL-23 and 35 with certain periodontal and inflammatory indices, neither type 2 DM nor CP differentially affects serum levels of these two cytokines.[31]

In the present study, clinical parameters like plaque index (PI), gingival index (GI), probing depth (PD) and clinical attachment level (CAL) were assessed in all the three groups. The values of PI, GI, PD and CAL were higher in Group II and Group III compared to Group I. There was no significant difference in PI scores and CAL between Group II and Group III. However, after 6 weeks of SRP, there was significant reduction in all the clinical parameters in Group II and III. This can be attributed to resolution of inflammation following phase-I therapy.

In the present study, the baseline IL-35 values were higher in experimental groups such as group II and group III compared to the (control group) group I. Similar observations were made by Kalburgi et al[14], Mitani et al[15], Koseoglu et al[16] in their respective studies. Kalburgi et al analyzed the expression profile of IL-35 mRNA in gingiva of chronic periodontitis and aggressive periodontitis patients by semiquantitative real time PCR. They observed that IL-35 levels were higher in chronic periodontitis group compared to the aggressive periodontitis group and healthy group. The investigators stated that the increased expression of IL-35 in chronic and aggressive periodontitis suggests its possible role in pathogenesis of periodontitis. In the present study also, higher level of IL-35 levels was recorded in CP group compared to healthy group, thus supporting the findings in the previous studies.[14,15,16]Increase in IL-35 level in chronic periodontitis patients in our study attributed to its property of anti-inflammatory effect. Another study conducted by Jin and co-workers concluded that increased level of IL-35 plays a protective role in periodontal disease by maintaining immune system homeostasis and dampening the inflammatory response.[26] However, our findings were in contrast to those by *Thakre and co-workers*, who observed that IL-35 levels were higher in chronic gingivitis as compared with chronic periodontitis group.[13]

In the present study, IL-35 levels were found to be increased with the severity of chronic periodontitis and the values were decreased with the resolution of inflammation after the periodontal therapy (SRP). Our results are in agreement to those observed by Raj & co-workers who observed that GCF and serum IL-35 concentration was highest in chronic periodontitis patients compared to healthy and gingivitis group which was reduced significantly after receiving non-surgical periodontal therapy.[17]*Shindo and co-workers* have suggested that IL-35 produced from regulatory T cells might inhibit progression of periodontitis by decreasing IL-17A-induced levels of IL-6 and IL-8.[32,33,34]

At baseline, in group III (periodontitis with diabetes) patients, the IL-35 levels were higher compared to the those having periodontitis only. Also, there was significant decrease in IL-35 levels in both the groups after Phase-I therapy. However, the reduction in group II was significantly higher than that

observed in group III. In order to deal with with the inflammatory component, IL-35 levels were elevated in group III in contrast to other groups. Hence even after Phase-I therapy, IL-35 levels were not significantly reduced in group III compared to group II.

5 | CONCLUSION

Hence, within the limitations of this study it can be concluded that the GCF IL-35 levels are directly corelated with the CAL values & are also increased in periodontitis and periodontitis with Diabetes Mellitus as compared to healthy patients. Also, phase-I periodontal therapy results in a considerable reduction in the levels of IL-35 for both groups.

FUTURE PROSPECTIVE

Further studies with larger sample size and long term follow up are required to substantiate the results obtained in this study. As IL-35 is relatively a newest cytokine identified,which has not been premeditated in many disease models, hereafter further studies are required to assess the exact role of IL-35 in the pathophysiology of periodontal disease.

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Nil

Conflict of interest:

There are no conflicts of interest

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