Original Article

Salivary Interleukin-6 Levels among Polycystic Ovary Syndrome Patients with and Without Chronic Periodontitis – A Comparative Study

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Abstract

Background: Periodontitis is associated with various systemic diseases one of which is poly cystic ovarian syndrome (PCOS). PCOS is a genetically complex endocrinopathy of uncertain etiology affecting women of the reproductive age group which results in the most common cause of anovulatory infertility, menstrual dysfunction, and hirsutism. PCOS has a close association with cardiometabolic risk profile, insulin resistance (IR), hyperinsulinemia, central obesity, dyslipidemia, and increasing the prevalence of cardiovascular risk factors. The common pathway is the chronic low-grade inflammation which is constituted by pro-inflammatory cytokine interleukin (IL)-6. **Aim:** The aim of the study was to compare salivary IL-6 levels among polycystic ovary syndrome (PCOS) patients with and without chronic periodontitis. **Materials and Methods:** Newly diagnosed PCOS patients were selected for the study, and the periodontal parameters were recorded. Group A consists of 42 patients of PCOS with periodontitis and Group B consists of 42 patients of PCOS without periodontitis. Salivary levels of IL-6 were compared between the two groups and were assessed by enzyme-linked immunosorbent assay kit (bioassay). **Results:** The mean pocket depth in Group A was 4.23 ± 0.134 and that of Group B was 1.30 ± 0.06. The mean bleeding on probing in Group A was 1.40 ± 0.40 and in Group B it was 0.91 ± 0.18. The mean clinical attachment level in Group A was 4.87 ± 0.124 and that of Group B was 1.30 ± 0.06. The mean difference in clinical parameters was statistically significant between the groups ($P \leq 0.001$). IL-6 level in group A is 102.59 ± 18.2 and in Group B it was 51.3 ± 25.3. **Conclusion:** Salivary IL-6 levels show a double-fold increase in PCOS with periodontitis than in PCOS without periodontitis. This study reflects the importance of periodontal health and the prevention of periodontal disease so as to minimize IR in PCOS patients with periodontitis.

Keywords: *Chronic periodontitis, inflammatory cytokine, insulin resistance, polycystic ovarian syndrome, salivary interleukin-6*

Introduction

Chronic periodontitis is a risk factor in the development of cardiovascular diseases, diabetes mellitus (DM), occlusive respiratory diseases, rheumatoid arthritis, and polycystic ovary syndrome (PCOS).[1] Dursun *et al*. reported for the first time an association between periodontal disease and PCOS. PCOS is the main cause of anovulatory infertility and the most common gynecologic and endocrine condition among women in the reproductive age.[2,3]

The pathogenomic state of chronic systemic inflammation and insulin resistance (IR) in both periodontitis and PCOS can be hypothesized as a converging channel associating the disorders.[4] IR and hyperinsulinemia play a vital role in women with PCOS.[5] Interleukin (IL)-6 is an inflammatory mediator that stimulates osteoclast activity and bone resorption in periodontitis.[6] Chronic periodontitis patients exhibit increased levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), IL-1, IL-6 in serum, and/or in Gingival Crevicular Fluid (GCF).[7] This chronic inflammatory phase will result in IR. The levels of pro-inflammatory cytokines can possibly be a marker or mediator of the association between periodontitis and PCOS.[8] Among the various cytokines implicated in the pathology of periodontal inflammation and PCOS, IL-6, TNF-α, and hs C-reactive proteins have gained greater attention as they are markers of systemic inflammation.[9]

Association between periodontal disease and PCOS has been analyzed in patients with gingivitis, and very few studies have explored this relationship in

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periodontitis.[4,10,11] To the best of our knowledge, there are no studies correlating IL-6 levels in patients with PCOS and periodontitis. In lieu with the above, the present study aims to compare salivary IL-6 levels among PCOS patients with and without chronic periodontitis in an attempt to explore the link between the two disease entities.

Materials and Methods

Study protocol and population

The study was initiated after approval by the Institutional Ethics Committee and Institutional Review Board of Indira Gandhi Institute of Dental Sciences, Sri Balaji Vidyapeeth, Deemed to be University, Pondicherry (IGIDSIRB2015 NDP13PGATPAI). The study was conducted during the period from August 2015 to May 2017. The sample size was calculated using G Power software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), and accordingly 42 participants in each group were Selected.

Inclusion criteria

Systemically healthy women of reproductive age (15–35 years) newly diagnosed with PCOS with ≥16 natural teeth were included for the study.

Disease definitions

PCOS diagnosis was established by a gynecologist according to the Rotterdam criteria. The diagnostic criteria are as follows: two of the following three criteria are required, oligo/anovulation, hyperandrogenism, or polycystic ovaries on ultrasound.[12] Periodontitis was defined as ≥2 interproximal sites with attachment loss ≥4 mm or ≥2 interproximal sites with probing depth ≥5 mm, not on the same tooth.[13]

Exclusion criteria

Patients with a history of thyroid dysfunction, hyperprolactinemia, and androgen-secreting tumors; patients with chronic inflammatory disease such as nephritic syndrome, chronic renal failure, significant cardiovascular disease, and known Type I/II DM; and patients with adverse habits such as smoking and drinking and history of systemic antibiotic within 3 months were excluded from the study. Patients who have undergone periodontal treatment within 6 months were also excluded from the study.

Periodontal parameters

The following periodontal parameters were recorded: bleeding on probing (BOP), pocket depth (PD), and clinical attachment level (CAL). Both the groups were examined by a single examiner with UNC 15 probe (Marked to the nearest 0.5 mm) and no. 5 mouth mirror. BOP was recorded by modified sulcus bleeding index.[14] Probing PD was calculated from marginal gingiva to the base of the pocket, at six sites per tooth. CAL was calculated from cementoenamel junction to the base of the pocket, at six sites per tooth.

Based on clinical periodontal parameters, the participants were divided into two groups: Group A (PCOS patients with periodontitis) and Group B (PCOS patients without periodontitis).

Saliva sampling

For estimation of IL 6 levels, unstimulated saliva sample was collected in 5 ml sterile container and stored in −40°C and centrifuged at 3000 rpm for 15 min. The supernatant saliva was collected in 1.5 ml sterile container, and the IL level was analyzed using enzyme-linked immunosorbent assay kit.

Statistical analysis

The statistical analysis was performed using the SPSS software(IBM Corporation, New York.). The periodontal parameters and salivary IL levels between the two groups were analyzed using independent *t*-test and the level of significance was set at $P < 0.05$.

Results

In our study, the mean age of Group A was 24.88 ± 3.2 years and that of Group B was 24.09 ± 2.9 years. On comparing age between Group A and Group B, the values were not statistically significant ($P = 0.24$), indicating that the groups were age matched.

The mean BOP in Group A was 1.40 ± 0.40 and in Group B it was 0.91 ± 0.18. On comparing BOP between the groups, the values were statistically significant ($P < 0.001$). The mean PD value for Group A was 4.23 ± 0.134 and for Group B it was 1.30 ± 0.06. On comparing statistically, the values were significant ($P < 0.001$). The mean value for CAL for Group A was 4.87 ± 0.124 and for Group B was 1.30 ± 0.06. On comparing between groups, the values were statistically significant ($P < 0.001$) [Table 1]. All the values in Group A were statistically higher than that of Group B.

The mean IL-6 level in Group A was 102.59 ± 18.2 and in Group B it was 51.3 ± 25.3. On comparing IL-6 levels between groups, there was statistically significant increase in Group A ($P < 0.001$).

Table 1: Comparison of age, bleeding on probing, probing pocket depth, clinical attachment level, and levels of interleukin-6 between Group A and Group B

Variable	Mean±SD		*t*	*P*
	Group A (*n*=43)	Group B (*n*=43)		
Age (years)	24.88±3.2	24.09±2.9	1.17	0.24
BOP	1.40±0.40	0.91±0.18	7.325	<0.001
PPD (mm)	4.23±0.134	1.30±0.06	164.6	<0.001
CAL (mm)	4.87±0.124	1.30±0.06	169.9	<0.001
IL-6 (pg/dl)	102.59±18.2	51.3±25.3	10.79	<0.001

Independent *t*-test used; $P<0.05$ is statistically significant. BOP: Bleeding on probing; PPD: Probing pocket depth; CAL: Clinical attachment level; IL-6: Interleukin-6; SD: Standard deviation

Discussion

The chronic nature of periodontal infection, inflammation, and pathogenesis can cause effects elsewhere in the body, and the most studied and linked diseases with periodontitis are DM, cardiovascular disease, and adverse pregnancy outcomes.[15] In PCOS patients, increase production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-17 results in a state of constant low-grade inflammatory response. This, in turn, can have adverse effects on the periodontal tissue.[16,17]

Frodge *et al*. in 2008 and Taba *et al*. in 2005 stated that several inflammatory and immune mediators which are responsible for periodontal destruction can be detected in saliva.[18,19] Pellegrini *et al*., 2012, stated that quantifying biomarkers in saliva serve as a useful tool to predict an individual's susceptibility to periodontitis, to provide information for periodontal activity, and to monitor the effectiveness of periodontal therapy.[20] Hence, the present study used saliva as a marker for IL6 levels.

Salivary IL-6 for Group A was 102.59 ± 18.2 and that of Group B was 51.3 ± 25.3. This reveals that IL-6 levels had two-fold increase in PCOS patients with periodontitis than in PCOS patients without periodontitis.

Noh *et al*. in 2013 showed increased IL-6 level in periodontitis patients.[21] Gabriela Teixeira *et al*. in 2014 stated that there is an association between IL-6 and periodontitis, and IL-6 is an important biomarker for periodontitis.[22] Costa *et al*. in 2010 suggested that there is an increase in salivary IL-6 level in periodontitis patient with diabetes than periodontitis without diabetes.[23] Ozcaka *et al*., 2012, reported that PCOS and gingival inflammation appear to act synergistically on the pro-inflammatory cytokines IL-6 and TNF-α, and thus PCOS may have an impact on gingival inflammation or vice versa.[11] Tarkun *et al*. in 2006 in a case–control study to evaluate the levels of IL-6 in PCOS patients found that PCOS group had elevated IL-6 levels than the controls.[24] Küçük *et al*., 2014, compared IL-6 levels in patients with and without PCOS and found increased levels of IL-6 in PCOS group.[25] Peng *et al*., 2016, in a meta-analysis found that IL-6 levels were increased in the PCOS group as compared with the control group.[26]

Our study reveals that IL-6 levels increased with an increase in periodontal inflammation in patients with PCOS. Since periodontitis is a chronic inflammatory condition, this can also lead to increased levels of IL-6. IL-6 is one of the very important cytokines associated with IR.[27] IL-6 induces IR by decreased tyrosine phosphorylation of IR substrate-1 and decreased association of the p85 subunit of phosphatidylinositol 3-kinase.

This study reflects the importance of periodontal health and the prevention of periodontal disease to avoid an increase in IR in PCOS patients. Hyperinsulinaemia promotes hyperandrogenism in PCOS by two distinct and independent mechanisms: (1) by increasing circulating ovarian androgens and (2) by directly reducing serum sex hormone-binding globulin concentrations.[28] The net result of these actions is to increase the circulating free testosterone concentrations.

Limited evidence suggests that hyperinsulinemia might also promote ovarian androgen production by influencing the pituitary release of gonadotrophins.[29-31] This hyperandrogenism has direct effects on development, progression, and treatment resistance in PCOS patients.[32] Treatment aimed in bringing down hyperinsulinemia, such as the use of drugs like metformin resulted in decreased levels of IL-6 post treatment.[33] This also resulted in the decreased levels of circulating androgens. The clinical implication of these findings is that amelioration of hyperandrogenism in women with PCOS may be achieved by interventions which improve insulin sensitivity and reduce circulating insulin. Such measures might include, but are not limited to weight loss, dietary modification, and insulin-sensitizing medications.[30]

Vidal *et al*. in 2009 reported that nonsurgical periodontal therapy was effective in improving the periodontal clinical data and in reducing the plasma levels of IL-6 in patients with severe periodontitis.[34] Mammen *et al*. in 2017 described that effective periodontal therapy reduced IR and improved periodontal health status and insulin sensitivity in patients with type II DM and chronic periodontitis.[35] This proves that periodontal therapy is an important means to decrease levels of IL-6 and improve insulin sensitivity in such patients. Hence, comprehensive periodontal screening and therapy are very essential in patients with PCOS to improve insulin sensitivity and thereby decreasing hyperandrogenism.[11]

The limitations in our study are that chronic periodontitis without PCOS was not included and lack of interventional periodontal therapy to check for reduction in levels of IL-6. This study does not allow to establish a causal relationship between both diseases. In addition, the analysis was only limited to one marker and serum samples were not analyzed. Further studies with a larger sample size are required to confirm the association between IL-6 levels in PCOS patients with periodontitis.

Conclusion

Results show that there is an increase in the salivary IL-6 levels in PCOS patient with periodontitis by twofold as compared to those without periodontitis. Periodontal therapy is an important means to decrease levels of IL-6 and improve insulin sensitivity in such patients. Hence, comprehensive periodontal screening and therapy is essential in patients with PCOS to improve insulin sensitivity, thereby decreasing hyperandrogenism.

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Conflicts of interest

There are no conflicts of interest.

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