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ORIGINAL ARTICLE

IL-17, A Possible Salivary Biomarker for Preterm Birth in Females with Periodontitis

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Abstract

Background Previous studies have suggested that chronic periodontal infection may be associate with preterm births and low birth weight. The present study was conducted to evaluate the levels of interleukin-17 (IL-17) in saliva samples of pregnant females as a possible marker in determining whether or not an association exists between chronic periodontitis and preterm labor.

Aim The aim of the study is to assess the relation between the periodontal health status and preterm low birth weight of the new born on the basis of salivary IL-17 levels.

Materials and Methods This case–control study included a random sample of 40 female patients, aged 18 to 35 years, who were in their second trimester, assigned to two groups, Group 1 consisted of 20 pregnant females without periodontitis, Group 2 included 20 pregnant females with periodontitis. Saliva samples were obtained in the second trimester and postpartum. Saliva samples were measured by using ELISA for IL-17 levels.

Results IL-17 levels in saliva were significantly higher in Group 2 than that of Group 1($p < 0.001$). There was no significant difference found between the preterm and low birth weight cases and periodontitis.

Conclusion This study did not find any association between the periodontitis and preterm deliveries and low birth weight cases on the basis of IL-17 levels in saliva.

Keywords Periodontitis · Preterm low birth weight · Interleukin-17 · Salivary biomarker

Introduction

Periodontal disease is a chronic inflammatory disease occurs due to the infection by periopathogens which result in the destruction of gingival and periodontal tissues and cause progressive loss of alveolar bone.[1]

These microbial pathogens increase inflammatory infiltrate, i.e., polymorpho-nuclear neutrophils, macrophages cells and B cells with increase in the inflammatory cytokines like IL-1, IL-11, IL-6, TNF-α, TNF-β. In chronic inflammation, pro-inflammatory cytokines like IL-1, TNF-y and IL-6 play significant role in resorption by activating osteoclasts. [2, 3] IL-17 acts on many different cells' types, causing them to secrete cytokines, such as granulocyte/macrophage colony-stimulating factor and IL-6. IL-17 also synergizes potently with TNF-a, increasing its activity by orders of magnitude. [4]

Preterm low birth weight is major predictor of perinatal mortality and morbidity. There are approximately 33% of the infant mortality is recorded to be due to preterm and low

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birth weight, whereas surviving infants are reported to be at higher risk of developing congenital and neurological disabilities and developmental defects. World Health Organization (WHO,1995) has defined low birth weight (LBW) as any live birth of < 2500 gms and very low birth weight to be < 1500 gms. WHO defines preterm birth as any live birth at < 37 weeks of gestation period. [5, 6]

In India, the prevalence of LBW has significantly declined from 20.4% to 16.4% in the last decade. The prevalence of LBW remained high in girl children whose mothers were adolescent and were stunted. Prevalence of LBW declined among second or higher birth order, whose mothers educated up to secondary level and above, belonged to rich wealth quintiles, were from rural area, received better nutrition and adequate antenatal care and were from eastern, northeastern and southern regions of India. [7, 8]

Many studies have explored the role of periodontal status in preterm low birth weight, on the basis of the data collected retrospectively, while the number of the prospective studies, at present, is very few. Similarly, many studies have evaluated the levels of biomarkers associated with periodontitis and adverse pregnancy outcome, in serum and amniotic fluid samples, the invasive collection methods of which might be stressful for the pregnant women along with other associated complications, whereas saliva reflect body's normal and diseased state and its use as diagnostic fluids meets the demand for an inexpensive, non-invasive and accessible diagnostic tools. [9, 10].

Materials and Method

The study has been conducted in the Durg District Hospital, Chhattisgarh, between the periods of November 2019 to May 2020. The study population group included pregnant females between the age 18–35 years. [11].

From a previous study to assess and correlate the maternal periodontal status with their pregnancy outcome, the power calculation was done for the sample size required for this study. [6] According to which, 40 pregnant females were needed in this study.

72 pregnant females of the age 18–35 were screened for the study for the study who attended the outpatient department of the department of gynecologist and obstetrics in District Hospital, Durg, Chhattisgarh, India. The screening was performed until the desired number of participants who fulfilled the inclusion criteria for both the groups were obtained. Two groups were formed: Group I/control group included systemically healthy pregnant females (term/healthy) and Group II/case group included pregnant females with periodontitis(term/periodontitis).

Inclusion criteria: Pregnant females in their 24–34 weeks of gestation period. Age 18–35 years, diagnosed with periodontal disease. Patients who are willing to get enrolled in the procedure with consent. Patient with history of any systemic diseases affecting pregnancy outcome, patient with infectious disease, HIV/AIDS, bacterial vaginosis, use of tobacco in any form, alcoholism, drug abuse, patients on hormone replacement therapy (HRT) and patient undergone in vitro fertilization (IVF) were excluded from the study.

After obtaining an informed consent, the demographic data on the socioeconomic status of the participants were collected by means of a questionnaire. Height and weight were measured to calculate the body mass index of the participants.

Periodontal Examination

CPI index was used to evaluate the periodontal status of the participants and screen them for periodontitis. A single calibrated examiner did the periodontal examination of all the participants. Each patient was diagnosed according to CPI criteria from Code 0 to Code 4.

Saliva Collection Procedure

Saliva collection was done on a different day after the periodontal examination. This was done to avoid collecting the blood contained saliva after bleeding on probing during periodontal examination. Unstimulated whole saliva was collected by drooling method. Briefly, subjects were asked to refrain from eating or drinking for 2 h prior to saliva collection. 2 ml of unstimulated whole saliva was collected by passively drooling into a chilled centrifuge tube for 5–10 min. The tubes were coded and transferred on ice to the laboratory for processing. [12].

Saliva Sample Processing

All samples were centrifuged at 2500 rpm for 10 min at 4 °C. The supernatant constituting clarified saliva was separated and stored at – 80 °C until further analysis. [11].

Sample analysis was done using Biolegend, LEGEND MAX™ IL-17A 433,917/433,918 kit.

Statistical Methods

The statistical analysis was done using SPSS Version 16.0 software. Independent t-test was applied to test two means of independent groups. Chi-square test was used to compare the categorical variables.

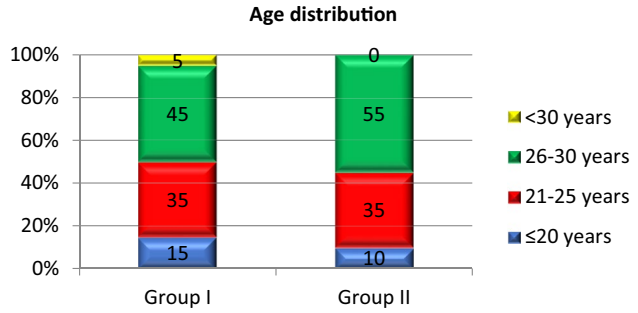

Fig. 1 Age distribution of the participants

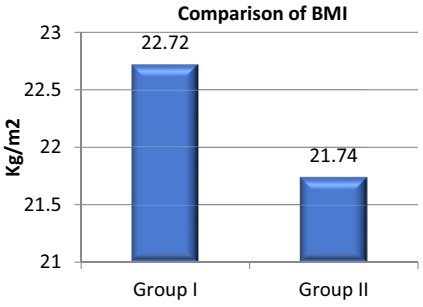

Fig. 3 Intergroup comparison of BMI in control and case group

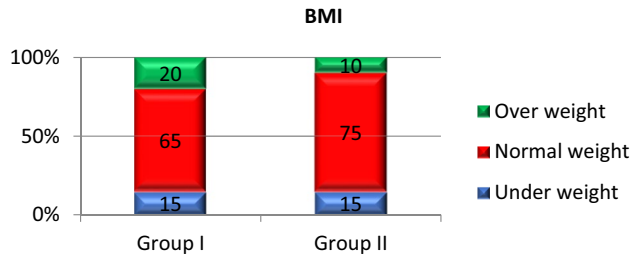

Fig. 2 Percentage distribution of BMI between the groups

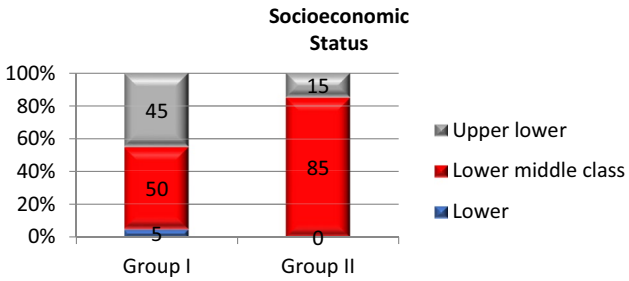

Fig. 4 Percentage distribution of socioeconomic categories

Results

Age distribution of the participants in Group I and Group II. There was $n=3(15\%)$ in Group I and $n=2(10\%)$ participants, in Group II, were less than 20 years. 21–25 years group range participants were equal, i.e., n = 7(35%) in both the groups, while $n=9(45\%)$ and $n=11(55\%)$ were in 26–30 years in Group I and Group II, respectively. There was only 1 participant in the Group I of age more than 30 years while none in the Group II (Fig. 1).The difference between the group was not statistically significant $p=0.70$.

Body Mass Index

In the Group I, the $n=3(15\%)$ participants were found to be underweight, $n=13(65\%)$ participants were in normal weight and $n=4(20\%)$ participants were overweight. In Group II, $n=3(15\%)$, $n=15(75\%)$ and $n=2(10\%)$ were underweight, normal weight and overweight, respectively (Fig. 2). Intergroup comparison showed that participants had similar BMI in both the groups. $X^2=0.81$,P = 0.66 NS (Fig. 3).

Socioeconomic Status of the participants

In Group I, $n=1(5\%)$ belonged to the lower class, $n=10(50\%)$ belonged to the lower middle class and $n=9(45\%)$ belonged to the upper lower class. In Group II, there were no participants in the lower class category. N = 17(85%) belonged to the lower middle class and $n=3(15\%)$ belonged to the upper lower class (Fig. 4). Intergroup comparison showed that participants belonged to similar socioeconomic strata in both the groups. $X^2=5.81$, P = 0.054.

Term-Preterm Births in Study Groups

In Group I, $n=18$ (97.44%) of the participants had normal delivery, $n=1(2.56\%)$ had preterm delivery and $n=1(2.56\%)$ had miscarriage in her second trimester due to accidental injury. In Group II, $n=17(85\%)$ had normal delivery and $n=3(15\%)$ had preterm delivery as shown in Table 1. In Group I, there were a greater number of participants had preterm delivery, but the difference between both the groups was statistically non-significant. $X^2=3.78$, P = 0.058. (Fig. 5).

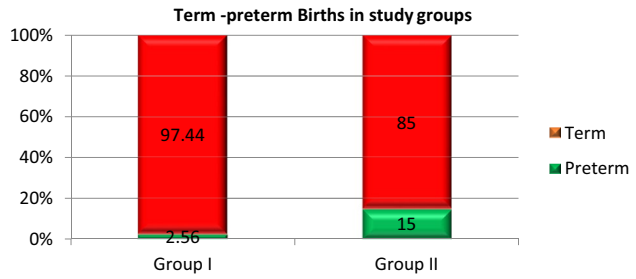

Fig. 5 Term and preterm births in study groups

Weight of Infants at the Time of Birth

In Group I, out of 19 infants born, $n = 18(94.87\%)$ were of normal weight, and $n = 1(5.13\%)$ had low weight, while in Group II, $n = 17(85\%)$ had normal and $n = 3(15\%)$ had low weight. The difference between both the groups was not statistically significant. (Chi-square value $= X^2 = 2.11$, $P = 0.14$).

Interleukin-17 Level

IL-17 level in saliva of participants in Group I in second trimester was 5.52 ± 3.54 pg/ml which after delivery was increased to 12.66 ± 4.78 pg.ml (highly significant $p < 0.001$). In Group II, the IL-17 level was 17.56 ± 7.4 pg/ml in second trimester and was 17.89 ± 5.3 pg/ml (highly significant p 0.0022). The IL-17 level in Group II was increased in the saliva when compared to that of Group I. (Table 1).

Discussion

Associations between maternal periodontal disease and the delivery of preterm or low birth weight babies have been described in the literature for more than 10 years using case–control or cohort observational studies. [13–15].

The present study attempted to assess the interleukin-17 levels in the saliva of the pregnant females with periodontitis and assess its association with preterm and low birth weight cases.

Preterm birth is one of the most prevailing complications of pregnancy causing neonatal mortality and morbidity. Research on many biomarkers in maternal serum, amniotic fluid and cervicovaginal fluid (CVF) has been accomplished such as fetal fibronectin (FFN), α-fetoprotein, C-reactive protein (CRP), multiple members of the interleukin family (interleukin-6, interleukin 8 and interleukin 10), matrix metalloproteinases, plasma proteins and tumor necrosis factor α[66]. Interleukin-17 is relatively new biomarker which is key regulatory cytokine which is known to induce IL 1β, TNF-α,IL-6 and IL-8 production. [17].

According to a study by Fraser et al.,1995, young maternal age of 13–17 years had a significantly higher risk of adverse pregnancy outcome. [18].

While according to another study, advanced maternal age was also found to be associated with an increased risk of preterm birth, irrespective of parity, especially very preterm birth. Women aged 35 years and older, expecting their first, second or third births, should be regarded as a risk group for very preterm birth. [19].

In current study, the 18–34 years of age range of the participants are included to minimize the risk of the preterm births due to much younger or much older maternal age. Also, in this study, the comparison of the age difference in Group I and Group II was statistically non-significant which minimized the age of the participants to act as confounding factor in this study.

The effects of area-level socioeconomic characteristics suggest that aspects of neighborhoods—including housing, safety and social cohesion—may play an important role in socioeconomic effects on birth outcomes. According to a systematic review by Blumenshine et al., socioeconomic disadvantage was consistently associated with increased risk across socioeconomic measures, birth outcomes and countries. [20].

In the present study, the majority of the participants belonged to the similar socioeconomic strata. And the difference between the socioeconomic status in both the groups was not statistically significant.

Underweight (a BMI of < 19. kg/m^2) has been shown to be associated with an increased risk of preterm deliveries, low birth weight and anemia and a decreased risk of pre-eclampsia, gestational diabetes, obstetric intervention and postpartum hemorrhage. [21].

Table 1 Inter- and intra-group comparison of IL-17 levels before and after delivery

	Group I (pg/ml)	Group II (pg/ml)	*t* value	*p* value
IL-17 in second trimester	5.52 ± 3.54	17.56 ± 7.4	6.56	< 0.001 HS
IL-17 after delivery	12.66 ± 4.78	17.89 ± 5.3	3.27	0.0022 HS
BMI	22.72 ± 3.9	21.7 ± 4.03	0.81	0.42 NS
Weight of infant (kgs)	3.21 ± 0.41	3.19 ± 0.56	0.13	0.89 NS

A study which was done by Hendler et al., evaluated the relationship between the pre-pregnancy BMI and the spontaneous preterm birth and the indicated preterm birth and they found a significant occurrence of the preterm birth among the lean and the obese pregnant women. [22].

In the present study, the difference in the BMI of the participants of both groups was statistically non-significant so the bias due to BMI between the groups is minimized. The present study has included the women from the low socioeconomic strata, but the body mass index of these women was in the normal range which indicates good nutritional status of the participants which is mainly due to constant supervision of the Anganwadi workers—the community based voluntary frontline worker of the Integrated Child Development Services program by Government of India, who are required to organize and provide supplementary nutrition to the pregnant women, nursing mothers and children and also educate mothers to look after their nutritional needs during pregnancy and postpartum.

The current study explores the possibility of using saliva as a body fluid to measure the presence of biomarker for preterm birth as the collection of maternal saliva is a non-invasive method unlike collection of other body fluids like maternal serum, amniotic fluid which may subject the pregnant women to unnecessary stress during collection procedure.

Present study demonstrated a statistically significant difference between the Group I (term/healthy) and Group II (term/periodontitis) regarding the IL-17 level in salivary sample, with the higher level in the Group II individuals. A statistically significant difference was also found ($p = 0.001$) between mean IL-17 levels in the saliva samples of the Group I and Group II, with levels being considerably higher in the Groups II in the second trimester. These findings conform to those of Beklen et al. and Vernal et al., both of them reported elevated IL-17 levels in individuals with periodontitis. [17, 23].

In the present study, there was no significant correlation found between the periodontitis and preterm and low birth weight cases as there was no significant difference found between both the groups in preterm ($p = 0.15$) as well as low birth weight($p = 0.63$) was detected. This result is in accordance to the study done by Agueda et al.,2008 and Moore et al.,2004, where they did not find any association between the periodontitis and preterm cases. [24, 25].

The present study found level of interleukin-17 in saliva was in Group 1 (term/healthy individual) and was 5.52 ± 3.54 pg/ml in second trimester which was significantly increased up to 12.66 ± 4.78 pg/ml postpartum. This result is consistent with the study done by Garcia et al. 2010, where they found increase in the serum concentration of the IL-17 from the second trimester to third trimester in the healthy pregnant females. [26].

Current study did not find any association between periodontitis and preterm low birth weight outcome based on the interleukin-17 levels in saliva, the result is consistent with the study done by Gamal et al.,2017 where they also did not find any association between periodontitis and preterm low birth weight based on the interleukin-17 levels in GCF and placental tissue samples. [11].

Conclusion

It can be concluded that there is increase in the IL-17 level in saliva in patients with periodontal conditions. The IL-17 might be related to the preterm deliveries. But this study did not find any association between the periodontitis and preterm deliveries and low birth weight cases.

The role of IL-17 in periodontitis and/or during pregnancy is still not very clear, but it can prove to be a reliable biomarker in predicting the adverse pregnancy outcomes. A study with a larger sample size and interventional studies is needed to assess this issue.

Some limitations should be considered before interpreting the result of this study. Though this study failed to establish the association between the periodontitis and preterm labor and low birth weight cases based on the measurement of the IL-17 levels in the saliva, this might be due to small sample size and lack of intervention. The postpartum saliva collection schedule was disturbed in some of the individuals due to the COVID pandemic which might have resulted in bias in the mean IL-17 levels in saliva postpartum.

Ethical Approval

All procedures performed in these studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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