



Association Between Periodontal Health And Sub-Gingival Microbiota In Pregnancy And The Role Of Non-Surgical Periodontal Therapy In It: A Clinico-Microbiological Study

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Abstract

Background: Numerous studies have elaborated upon the relationship of periodontal tissues and pregnancy on both clinical and microbiological levels and the role of treatment in it. The purpose of this study was to analyse the qualitative change in the sub-gingival microbiota during pregnancy and to assess whether non-surgical periodontal intervention alters the sub-gingival environment in pregnant women and improves the clinical status or not.

Materials and Methods: Fifty pregnant (second trimester) and 50 non-pregnant subjects aged 20-35 years, with at least four mm of probing depth in at least two teeth in each quadrant, were selected for the study. For each subjects, all clinical periodontal parameters- plaque index (PI), gingival index (GI), probing depth (PD), clinical attachment loss (CAL) and microbial counts of *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* at baseline, one and three months post-scaling and root planing (where required) (SRP) were recorded. The microbial counts were assessed using culture method. Data analysis was carried out using Statistical package for social sciences (SPSS) 20.0 software for statistics. Comparison within the group for parametric variables and were carried out using repeated measure ANOVA with Bonferroni Post hoc test and between the groups using Independent t-test. Comparison within the group for non-parametric variables was evaluated using Freidman test with Wilcoxon Signed rank test and between the groups using Mann Whitney U test. Pearson's correlation coefficient was use to correlate between clinical and microbiological parameters.

Results: The results indicated that at baseline, microbial counts specifically *P. intermedia* was significantly higher for pregnant subjects ($p < 0.05$). There was a significant reduction in PI, GI, PD and CAL post SRP at all follow up visits within each group ($P < 0.001$). Similarly, microbial counts also reduced significantly for both the groups ($p < 0.001$) post SRP and was maintained low throughout the study period. There was a weak positive correlation between clinical parameters and microbial counts.

Conclusions: The results of the study suggest that during pregnancy there is a qualitative change in sub-gingival microbiota which leads to overt gingival inflammation. However SRP is highly effective in reducing microbial burden and improving the clinical status for entire period of pregnancy.

Keywords: Microbe; Non-surgical; Plaque; Pregnancy gingivitis

Introduction

Periodontal disease is a chronic, poly-microbial infection causing an inflammatory response of the periodontal tissues. It is characterized by the formation of periodontal pockets and irreversible destruction of the tooth supporting tissues. These changes arise due to the interaction between microorganisms and immune, environmental, behavioural and/or hereditary factors [1]. Pregnancy is a physiological condition that is associated with various changes in women. These changes occur in the cardiovascular, hematologic, respiratory, gastrointestinal, genitourinary, endocrine and orofacial systems. These physiological changes occur in order to nurture the developing foetus and prepare the mother for labour and delivery [2].

Studies have shown a relationship between periodontal disease and pregnancy. Periodontal tissue undergoes marked changes throughout pregnancy. It has been demonstrated that pregnancy increases the susceptibility to gingivitis. This is known as 'pregnancy gingivitis' [3]. Pregnancy gingivitis is defined as gingival inflammation initiated by plaque and exacerbated by endogenous sex steroid hormones. It is a common disease that affects 36–100% of pregnant women. The clinical features of pregnancy gingivitis are similar to that of common gingivitis. However, there is a tendency for developing severe signs of gingival inflammation without associated changes in plaque levels [3, 4]. The most frequently affected areas are the anterior sextants of the oral cavity, especially inter-proximal sites. It is characteristically self-limiting and diminishes post-partum with the decrease in hormone production. Another interesting feature of pregnancy gingivitis is that the risk of developing periodontitis is negligible despite the inflammatory status [3].

The gingival inflammation during pregnancy can be explained by four potential mechanisms-

1. Increase in levels of sex hormones.
2. A change to a more susceptible gingival phenotype.
3. Immune system depression and
4. Changes in the sub- or the supragingival biofilm [3, 5, 6].

At present, limited data is available regarding the composition of sub-gingival plaque bacteria during

pregnancy. Kornman and Loesche were the first to report an increase in *Prevotella intermedia* in the sub-gingival biofilm during the second trimester of pregnancy [6]. Jensen et al also reported a 55-fold increase in *P. intermedia* levels in pregnant women when compared to non-pregnant women [3, 7]. *Prevotella intermedia* and *Prevotella nigrescens* are the most prevalent periodontal pathogens found in the sub-gingival plaque of pregnant women due to their capability to use female sex hormones for growth [7]. In addition to these, other periodontal pathogens are also found.

Several studies have shown that periodontal disease during pregnancy is positively correlated with adverse pregnancy outcomes such as pre-term birth and low birth weight infants. These women have high bacterial load and lower maternal immunoglobulin levels against oral microorganisms. Consequently, dissemination of bacteria or bacterial products in the systemic circulation may occur. These bacteria and their by-products may reach the placental membranes hematogenously and might induce preterm labour thus causing complications [8, 9].

Periodontal intervention during pregnancy has shown significant improvement in clinical, biochemical and microbiological parameters. Interventional studies have demonstrated a significant reduction in preterm births (PT) and in low birth weight (LBW) infants in women with chronic periodontitis who received periodontal therapy pre-partum when compared to women who did not receive periodontal intervention. These preliminary studies provide initial evidence that periodontal disease is a risk factor for PT/LBW infants and that periodontal therapy may reduce the risk of PT/LBW [9].

Thus, the purpose of this study is to analyse the qualitative change in the sub-gingival microbiota during pregnancy and to assess whether periodontal intervention alters the sub-gingival environment in pregnant women and improves the clinical status.

Materials And Methods

The present interventional study was carried out on 50 pregnant women who were in second trimester and 50 non-pregnant women aged between 20-35 years with probing depth (PD) of ≥ 4 mm. Subjects with any systemic illness, with any history of intake

of systemic antibiotics and/or anti-inflammatory drugs in last three months, periodontal treatment in the last six months and with a history of tobacco use were excluded from the study. The study was reviewed and approved by the institutional ethical committee. All subjects were explained about the study and written informed consent was taken from them.

All subjects underwent a thorough history taking and clinical examination as per mentioned examination proforma. The clinical parameters assessed were plaque index (PI), gingival index (GI), probing depth (PD) and clinical attachment loss (CAL) using university of North Carolina (UNC) 15 probe according to criteria.

Sub-Gingival Sampling

Each sampling site was isolated with cotton rolls, following which supragingival plaque was removed using a sterile hand scaler and cotton gauge, to prevent any contamination of samples. Sub-gingival plaque samples were obtained using a sterile Gracey curette (Figure 1) and sent for microbiological examination in a sterile container containing RTF (reduced transport fluid) (Figure 2).

Microbiological Examination

Sub-gingival plaque samples were sent for quantification of three periodontopathic bacteria - *P. Gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* by culture method. The microbiologic analysis of the samples was carried out.

Treatment And Follow Up

Each subject underwent thorough scaling and root planing (wherever required) (SRP) and oral hygiene instructions were enforced. Subjects were recalled after one and three months post-SRP for follow up. At each visit clinical parameters and sub-gingival plaque samples were assessed. Periodontal examination, sample collection and SRP were carried out by a single surgeon.

Statistical Analysis

Data was expressed as mean and standard deviation. Data analysis was carried out using Statistical package for social sciences (SPSS) 20.0 software for statistics. Comparison within the group for parametric variables and were carried out using

repeated measure ANOVA with Bonferroni Post hoc test and between the groups using Independent t-test. Comparison within the group for non-parametric variables was evaluated using Freidman test with Wilcoxon Signed rank test and between the groups using Mann Whitney U test. Pearson's correlation coefficient was use to correlate between clinical and microbiological parameters.

Results

The results indicated that at baseline, microbial counts specifically *P. intermedia* was significantly higher for pregnant subjects ($p < 0.05$). There was a significant reduction in PI, GI, PD and CAL post SRP at all follow up visits within each group ($P < 0.001$). Similarly, microbial counts also reduced significantly for both the groups ($p < 0.001$) post SRP and was maintained low throughout the study period. There was a weak positive correlation between clinical parameters and microbial counts. (Tables 1-5)

Discussion

The relationship between periodontal disease and pregnancy has been well established by several studies in the past ^[10]. Pregnant women undergo many physiological changes due to an upsurge in progesterone and estrogen levels ^[2]. This brings about a change in the periodontal status of the pregnant patients which presents as an increase in bleeding, oedema, erythema and hyperplasia of gingiva without a significant increase in the plaque levels. Also there is a concomitant increase in microbiological species specifically *P. intermedia* ^[6]. Periodontal disease is an independent risk factor for PTB/LBW. Non-surgical periodontal therapy is considered to be the gold standard for the treatment of gingivitis and chronic periodontitis. It not only improves the clinical status of the gingiva and periodontium but also changes the microbiologic and immunological profile ^[11]. So, non-surgical periodontal therapy during pregnancy might diminish the level of oral infection and the host inflammatory response that may, in turn, result in a reduction of PTB/LBW.

The findings of the present interventional study showed that there was a significant improvement in clinical (PI, GI, PD, CAL) and microbiological parameters post SRP (Table 1 and Table 3). This could be attributed to the fact that SRP removes

elements that are responsible for the gingival inflammation (i.e., plaque, calculus and endotoxins) in the oral environment. This brings about tissue shrinkage and results in decrease in bleeding on probing and reduction in gingival inflammation [12]. On intergroup comparison, PI and GI were significantly lower for pregnant women whereas PD and CAL were non-significant between pregnant and non-pregnant subjects at baseline (Table 2). Similar results were obtained in a study by Machado F et al (2012) wherein no difference in gingival inflammation between pregnant and non-pregnant women was seen [13]. This variation in the results could possibly be explained by the design of the present study (non-blinded, single operator), sample size and methodological variability. Mean plaque scores were significantly ($p < 0.001$) lower for pregnant women as compared to non-pregnant subjects at baseline, suggesting that gingival inflammation during pregnancy is not related to the amount of plaque levels alone (Table 2). It is also modified by the elevated levels of estrogen and progesterone in the gingival tissue [6, 14]. This is in accordance with study conducted by Tilakaratne et al (2000) [15].

However at one and three months post SRP, on intergroup comparison, the improvement in GI and PI was significantly higher ($p < 0.001$) suggesting that pregnant women showed better compliance to oral hygiene regimen post SRP (Table 2). This improvement could be attributed to extra vigilance of patients during pregnancy. Similar results were obtained in Penova et al (2015), Naik A et al (2014), Fiorini T et al in (2013) [16-18]. On intergroup comparison, PD and CAL showed no significant difference between the two groups at all intervals post SRP (Table 2). Similar results were obtained by Fiorini T et al (2012) [17] and Penova et al (2015) [16, 18]. This suggest that in pregnant patients increased inflammation affects only gingival region rather than periodontal sites, indicating that pregnancy only has reversible effect on the gingiva without inducing periodontal attachment loss. It could be speculated that periodontal attachment loss requires a chronic inflammatory state of the gingiva lasting longer than pregnancy when the gingival changes occur [19]. But this hypothesis still needs to be proved in the future research. Many studies have proved that changes in the subgingival microbiota have been proposed as a

potential mechanism for exacerbated gingival inflammation during pregnancy [2, 3, 5, 6, 7, 20, 21].

Efficacy of non-surgical periodontal therapy is not only related to reduction in severity of clinical inflammation but also to the degree of reduction in perio-pathogens. At baseline, on intergroup comparison it was observed that *P. intermedia* was significantly higher in pregnant group as compared to non-pregnant group ($p < 0.05$). 60% of pregnant subjects as compared to 32% of non-pregnant subjects were detected with *P. intermedia* (Table 5). The marked increase in proportion of *P. intermedia* in pregnant subjects can be attributed to the fact that there is an increase in serum levels of progesterone and oestrogen which substituted for the naphthaquinone requirement of the pathogen and thus act as a growth factor for the bacteria [6]. Furthermore, oestrogen and progesterone are also involved in fumarate reductase system of *P. intermedia* which directly influences the metabolic pathway of the pathogen [22]. The result of our study was in accordance with other studies by Kornman K et al (1980), Carrillo-DeAlbornoz et al (2010), Emmatty R et al (2013) [3, 6, 20]. However, the proportion of *P. gingivalis* and *A. actinomycetemcomitans* in pregnant patients was higher but non-significant ($p > 0.05$) as compared to non-pregnant subjects. 62% and 42% of pregnant subjects were harbouring *P.gingivalis* and *A.actinomycetemcomitans* as compared to 50% and 32% of non-pregnant subjects respectively (Table 5). Similar results were obtained in the studies by Carrillo-De-Albornoz et al (2010), Adriaens L et al (2009) [3, 22].

At one and three months, post SRP there was a significant reduction in the mean counts of three periopathogens in both the groups. However on intergroup comparison, the mean counts of *P. intermedia* was found significantly higher in pregnant group at three months ($p < 0.05$) and mean counts of *A. actinomycetemcomitans* was significantly higher at one month ($p < 0.05$, Table 3). *P. intermedia* was found in 16% of pregnant and 6% of non-pregnant patients at one month which increased to 22% in pregnant and reduced to 4% in non-pregnant at three months respectively. Whereas *A. actinomycetemcomitans* was found in 26% of pregnant and 8% of non-pregnant subjects at one month which reduced to 20% and 10% at three

months respectively (Table 5). An increase in *P. intermedia* at three months follow up post SRP could possibly be explained by the fact that levels of progesterone and estrogen are at peaks during 3rd trimester of pregnancy. This, in turn could increase the chances for an uninterrupted growth of the bacteria. Similar results were obtained by Offenbacher S et al (2006) [23].

Correlation between clinical and microbiological parameters was found to be positively correlated but all were statistically non-significant at all time intervals. This positive correlation clearly indicates that clinical status of the gingiva is directly affected by the subgingival microbial environment which via release of various endotoxins, cytokines and pro-inflammatory mediators evokes a host response in the form of gingival inflammation [24, 25]. The results of our study is in agreement with study conducted by Velitchka Dosseva-Panova et al (2014) and R.P. Teles (2010) [24, 25]. In both the studies a positive significant correlation was found between clinical and microbiological (orange and red complex) parameters. Moreover, as SRP reduces this microbial burden by removing plaque and calculus there is reduction in gingival inflammation.

There are various limitations for this interventional study which would have affected the result of the present study. They are as follows-

1. Culture method was used to detect microbial counts because of its accuracy and ability to simultaneously detect and quantify multiple bacterial species and reveal unexpected bacteria. But it has a higher detection threshold as compared to PCR. So this could have posed a limitation in assessing microbial counts in patients who had lower threshold for bacteria.
2. Correlation of levels of oestrogen and progesterone in the saliva with the present parameters would have provided a better and comprehensive insight of the relationship of pregnancy with periodontal disease.
3. A direct comparison of the present study with other similar studies quoted cannot be done unreservedly, as some of these studies quoted have untreated pregnant women as controls. As such their results can be extrapolated with the results of the present study only with reservation.

Within the limitations of the present interventional study, there was significant improvement in all the clinical parameters post-SRP at all follow up intervals within each group, however PI and GI in pregnant group showed significantly higher improvement at all follow up intervals post-SRP suggesting extra-vigilance of pregnant subjects during pregnancy. The microbial counts of *P. intermedia*, were significantly higher in pregnant subjects at baseline and microbial counts of *P. gingivalis* and *A. actinomycetemcomitans* was found to be higher for pregnant patient but non-significant. However significant reduction in microbiological burden was appreciated post SRP at all follow up visits. Hence the present study suggests that pregnancy alters the sub-gingival environment and this in turn represent as gingival inflammation clinically. However, non-surgical periodontal therapy is effective in improving the clinical status and reducing the microbial burden in pregnant subjects.

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TABLE 1-Intragroup Comparison Of Clinical Parameters From Baseline To 1 And 3 Months Post SRP

Pregnant group	GI	PI	PD	CAL
At baseline	2.449±0.201	2.431±0.244	3.798±0.524	3.934±0.604
At 1 month	0.952±0.460	0.913±0.520	2.736±0.591	2.856±0.689
At 3 month	0.962±0.427	1.496±0.258	2.444±0.544	2.561±0.655
p-value ^(a)	0.000**	0.000**	0.000**	0.000**
Non-pregnant group				
At baseline	2.597±0.212	2.601±0.199	3.725±0.427	3.897±0.437
At 1 month	1.418±0.268	1.472±0.249	2.714±0.550	2.895±0.593
At 3 months	1.014±0.277	1.179±0.056	2.376±0.458	2.569±0.530
p-value ^(a)	0.000**	0.000**	0.000**	0.000**

GI=Gingival index, PI= Plaque index, PD=probing depth, CAL= Clinical attachment loss

*significant (p<0.05), **highly significant (p<0.001), ^{a)}repeated measure analysis of variance

TABLE 2-Intergroup Comparison Of Clinical Parameters At Different Intervals

	PREGNANT GROUP	NON-PREGNANT GROUP	P-value ^(a)
GI	mean± SD	mean± SD	
At baseline	2.449±0.201	2.597±0.212	0.000**
At 1 month	0.952±0.460	1.418±0.268	0.000**
At 3 month	0.962±0.427	1.014±0.277	0.000**
PI			
At baseline	2.431±0.244	2.601±0.199	0.000**
At 1 month	0.913±0.520	1.472±0.249	0.000**
At 3 month	1.496±0.258	1.179±0.056	0.000**

PD			
At baseline	3.798±0.524	3.725±0.427	0.444
At 1 month	2.736±0.591	2.714±0.550	0.850
At 3 month	2.444±0.544	2.376±0.458	0.501
CAL			
At baseline	3.934±0.604	3.897±0.437	0.728
At 1 month	2.856±0.689	2.895±0.593	0.765
At 3 month	2.561±0.655	2.569±0.530	0.949

GI=Gingival index, PI=Plaque index, PD=Probing depth, CAL =Clinical attachment loss
 SD=Standard deviation, **highly significant (p<0.001), ^(a)Independent t-test

TABLE 3-Intragroup comparison of microbiological parameters from baseline to 1 and 3 months post SRP.

PREGNANT GROUP	P.g			P.i			A.a		
	mean± SD	Median ^(a)	IQR	mean± SD	Median ^(a)	IQR	mean± SD	Median ^(a)	IQR
At Baseline	33.75± 48.12	12.50	50	47.88± 61.55	20	95	22.64± 40.04	0.00	26
At 1 month	10.78± 22.83	0.00	10	12.32± 32.82	0.00	0	7.16± 14.43	0.00	4
At 3 month	4.90± 17.24	0.00	0	6.27± 13.99	0.00	0	5.69± 14.43	0.00	0
p-value	0.000**			0.000**			0.000**		
NON-PREGNANT GROUP									
At Baseline	22.10± 33.41	1.00	33	21.40± 48.83	0.00	10	11.60± 24.62	0.00	11
At 1 month	1.30± 4.60	0.00	0	4.00± 17.14	0.00	0	1.90± 7.27	0.00	0
At 3 month	0.88± 3.97	0.00	0	0.40± 2.22	0.00	0	1.10± 3.68	0.00	0
p-value	0.000**			0.000**			0.000**		

P.g-P.gingivalis, P.i-P.intermedia, A.a-A.actinomycetemcomitans

**Highly significant (p<0.001),IQR -Interquartile range, ^(a)Friedman test

TABLE 4-Intergroup comparison of microbiological parameters at baseline, 1 and 3 month post SRP

	PREGNANT GROUP			NON-PREGNANT GROUP			p-value ^(a)
	mean± SD	median	IQR	mean± SD	median	IQR	
P.g							
At baseline	33.75±48.12	12.50	50	22.10±33.41	1.00	33	0.186
At 1 month	10.78±22.83	0.00	10	1.30±4.60	0.00	0	0.005*
At 3 month	4.90±17.24	0.00	0	0.88±3.97	0.00	0	0.412
P.i							
At baseline	47.88±61.55	20	95	21.40±48.83	0.00	10	0.005*
At 1 month	12.32±32.82	0.00	0	4.00±17.14	0.00	0	0.172
At 3 month	6.27±13.99	0.00	0	0.40±2.22	0.00	0	0.011*
A.a							
At baseline	0.40±2.22	0.00	26	11.60± 24.62	0.00	11	0.173
At 1 month	7.16±14.43	0.00	4	1.90± 7.27	0.00	0	0.023*
At 3 month	5.69±14.43	0.00	0	1.10±3.68	0.00	0	0.121

P.g-P.gingivalis, P.i-P.intermedia, A.a-A.actinomycetemcomitans

*significant (p<0.05), IQR-Interquartile range, SD-Standard deviation, ^(a)Mann-whitney U test

TABLE 5-Frequency of detection of perio-pathogens (percentage/absolute number related to group)

Microorganism	Pregnant group			Non-pregnant group		
	Baseline	1 month	3 month	Baseline	1 month	3 month
P.gingivalis	62%(31)	34%(17)	12%(6)	50%(25)	14%(7)	4%(2)
P.intermedia	60%(30)	16%(8)	22%(11)	32%(16)	6%(3)	4%(2)
A.actinomycetemcomitans	42%(21)	26%(13)	20%(10)	32%(16)	8%(4)	10%(5)

FIGURE 1-Collection of subgingival plaque sample using Gracey Curette



Figure 2- Transfer of subgingival plaque sample into RTF

