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ORIGINAL ARTICLE

Pregnancy-induced periodontal inflammation: Influence of salivary cytokines and antimicrobial proteins

Taye J. Lasisi [a,b,*], **Rukiyat A. Abdus-salam** [c]

[a] *Department of Physiology, University of Ibadan, Nigeria*
[b] *Department of Oral Pathology, University of Ibadan and University College Hospital Ibadan, Nigeria*
[c] *Adeoyo Maternity Teaching Hospital, Ibadan, Nigeria*

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KEYWORDS

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Abstract The aim of this study was to evaluate the association between pregnancy-induced periodontal inflammation and levels of some salivary cytokines and antimicrobial proteins (AMPs). The study was a cohort longitudinal study that included pregnant women attending a secondary health facility. Consented participants had oral examination and saliva sampling during pregnancy and post-partum (three months after pregnancy). Saliva samples were used for the analysis of cytokines (TNF-α, IFN-gamma and IL-1β) and AMPs (Lactoferin, Lysozyme, and β defensin-1) using ELISA. Data are presented as median with interquartile range and compared using related sample Wilcoxon signed rank test. Correlations between levels of the salivary factors and indices of periodontal inflammation were determined using Spearman's correlation test. Salivary flow rate, pH, levels of salivary IL-1β and IFN-gamma were significantly lower; while gingival index, periodontal index and level of salivary TNF-α were significantly higher during pregnancy compared with postpartum period. However, salivary lactoferin, lysozyme and β defensin-1 did not show significant difference comparing during pregnancy and postpartum period. Level of salivary IFN-gamma showed negative correlation with gingival index while level of salivary TNF-α showed positive correlation with gingival and periodontal indices. Lower levels of salivary IL-1β and IFN-gamma along with higher TNF-α concentration during pregnancy suggest their contributions to the pathophysiology of pregnancy-induced periodontal inflammation.

* Corresponding author at: Department of Physiology, College of Medicine, University of Ibadan, P.O. Box 22040, University of Ibadan Post Office, Ibadan, Nigeria.
E-mail address: tj.lasisi@ui.edu.ng (T.J. Lasisi).
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1. Introduction

Changes in salivary factors may predispose pregnant women to oral diseases. A variety of physiological changes occurring during pregnancy has been shown to affect the oral health (Chaloupka et al., 2014). Several studies have reported increased prevalence and severity of gingival inflammation during pregnancy occurring more commonly in the 2nd and 3rd trimesters, which usually disappears postpartum (Tilakaratne et al., 2000; Gürsoy et al., 2008; Figuero et al., 2013). However, the correlation between changes in hormone levels during pregnancy and the increase in gingival inflammation remains controversial (Wu et al., 2016). The precise mechanism responsible for the gingival changes is unknown but various hypotheses have been proposed including depression of the immune system (Wu et al., 2016) and changes in oral biofilms (Raber-Durlacher et al., 1993). Hence, pregnancy gingivitis may be an aggravated inflammatory response that marks a host-parasite imbalance.

In assessing the role of oral fluid levels of cytokines in the higher prevalence of gingivitis in pregnancy, Jonsson et al. (1988) reported higher levels of interleukin 1 beta (IL-1β) in the gingival crevicular fluid of pregnant women compared to non-pregnant women. Also, the pregnant women showed a significant reduction in the gingival crevicular fluid levels of IL-1β three months post- partum (Jonsson et al., 1988).

Salivary defense mechanisms are numerous and include locally and systemically produced cytokines, immunoglobulins, lysozyme, mucins, and an array of antimicrobial proteins (AMPs). As part of the immune response, AMPs, a family of antimicrobial agents, have emerged over the last decades as a major component of the innate defense mechanism. AMPs have been identified in most physiological fluids and in an exposed environment, such as the mouth, where they play an essential role in the balance between health and disease (van 't Hof et al., 2014). In assessing the role of salivary antimicrobial defensin in pregnancy, a recent study by Gursoy et al. (2016) reported reduced salivary concentrations of human Beta Defensin -1 and -2 during the third trimester, whereas human Beta Defensin-3 concentrations did not change comparing during pregnancy and post-partum period. In addition, weak associations were observed between salivary defensin and clinical parameters (plaque and gingival indices).

Despite the reported increased periodontal inflammation in pregnant women, the pathophysiology is still poorly understood. More importantly, the role of cytokines and antimicrobial proteins is scantly documented. Although previous studies have reported elevated levels of TNF-α in gingival crevicular fluid, serum, gingival tissues, and plasma of non-pregnant chronic periodontitis patients compared to controls, to the best of our knowledge there is no report on changes in salivary levels of INF- gamma and TNF-α in pregnant women with or without periodontitis. Thus, evaluation of salivary cytokines and antimicrobial proteins in whole saliva can provide a significant tool for assessing the immunological status associated with pregnancy-induced periodontal inflammation. Hence, this study is aimed at assessing changes in the levels of some salivary cytokines and AMPs during pregnancy and postpartum and to evaluate their relationship with periodontal inflammation. The hypothesis was that pregnancy-induced periodontal inflammation is associated with higher levels of salivary pro-inflammatory cytokines along with reduced levels of salivary antimicrobial proteins (AMPs).

The objectives of this study were to determine levels of salivary cytokines (TNF-α, IFN-gamma and IL-1β) as well as AMPs (Lactoferin, Lysozyme, and β defensin-1) in pregnant women; to compare levels of the salivary cytokines and AMPs during and after pregnancy and to evaluate the association between periodontal inflammation and levels of TNF-α, IFN gamma and IL-1β as well as Lactoferin, Lysozyme, and β defensin-1 in pregnant women.

2. Methodology

2.1. Study design

This was a cohort longitudinal study that used purposive sampling. Sample size of 47 (including 20% attrition) was calculated using standard normal values set at 0.05 and a power of 90 and estimated means values for IL-1β in known test and control groups from a previous study (Otenio et al., 2012). Inclusion criteria were clinically confirmed pregnancy in 2nd and 3rd trimester of pregnancy and consent to participate in the study. Exclusion criteria were pregnancy complications like eclampsia, diabetes mellitus, anaemia, HIV/AIDS, malaria and any other known systemic diseases during pregnancy. Also, individuals with history of smoking and use of drugs (e.g. antibiotics and anti-inflammatory drugs) were excluded.

2.2. Study site and population

Following ethical approval from the institution, all consenting eligible pregnant women attending the Ante natal clinic of the Hospital, during the period of the study were included.

2.3. Oral examination

Clinical and Biodata of each participant was obtained through a self-administered proforma. Oral examination was carried out on each participant using dental mirror and periodontal probe. Evaluation of periodontal inflammation was performed using the plaque, gingival and periodontal indices (Löe, 1967; Ainamo et al., 1982).

2.4. Collection of saliva sample

Whole saliva was collected by asking each participant to spit into a graduated universal bottle for a period of 10 min (Lasisi and Ngwuadu, 2014). The samples were stored at −20 °C until the time for laboratory analysis. The flow rate of salivary secretion was determined based on the volume and duration. The pH of each saliva sample was determined using a calibrated pH meter (PH-012 Portable pH Meter, China).

2.5. Follow up

The participants were re-evaluated 3 months after delivery (Tilakaratne et al., 2000; Gürsoy et al., 2008). In addition, saliva sampling was repeated for evaluation of the salivary

parameters. Out of the 47 participants that were included after consent was obtained during pregnancy, only 42 returned for the follow up evaluation.

2.6. Quantification of TNF-alpha, IFN-gamma, IL-1β, Lactoferin, Lysozyme, and β defensin-1

Estimation of salivary levels of the cytokines and AMPs was done using Enzyme Linked Immunosobent Assay (ELISA) following the manufacturer's instructions (ASSAYPRO^{TM} ELISA Kits, USA, for TNF-alpha, IFN-gamma, IL-1β, Lactoferin, Lysozyme; and Elabscience^{R} ELISA Kit, China for β defensin-1) and as previously described (Tilakaratne et al., 2000; Gürsoy et al., 2008; 2016).

2.7. Statistical analysis

The outcome variables were plaque, gingival, and periodontal indices as well as salivary levels of TNF-α, IFN-gamma, IL-1β, Lactoferin, Lysozyme, and β defensin-1. Data are presented as either mean ± standard deviation and compared using paired sample *t* test (for parametric data) or median with interquartile range compared using related sample Wilcoxon signed rank test (for non-parametric data). Correlations between levels of the salivary factors and indices of periodontal inflammation were determined using Spearman's correlation test. All analyses were done using IBM SPSS Statistics (version 22) at 5% level of significance.

3. Results

The clinico-demographic characteristics of the participants are shown in Table 1.

Salivary flow rate and pH were significantly lower during pregnancy (0.46 ± 0.21 mls/min and 7.41 ± 0.31, respectively) when compared with postpartum period (0.55 ± 0.3 mls/min and 7.83 ± 0.47, respectively). Levels of salivary IL-1β and IFN-gamma were significantly lower ($p = 0.03$ and $p < 0.001$ for IL-1β and IFN-gamma, respectively); while the level of salivary TNF-α was significantly higher ($P < 0.001$) during pregnancy when compared with postpartum period. However, salivary lactoferin, lysozyme and β defensin-1 did not show significant difference when comparing levels during pregnancy and postpartum (Table 2). Level of salivary IFN-gamma showed negative correlation with gingival index while level of salivary TNF-α showed positive correlation with gingival and periodontal indices (Table 3). However, levels of salivary lactoferin, lysozyme and β defensin-1 did not show significant correlation with the gingival and periodontal indices (Table 4).

Table 1 Clinicodemographic characteristics of participants.

	During pregnancy	After pregnancy	P value
Age (years)	29 ± 4.38	NA	–
Gestational age at booking (weeks)	19.82 ± 5.26	NA	–
Gestational age at sampling (weeks)	34.74 ± 3.33	NA	–
Plaque index	0.86 ± 0.61	0.52 ± 0.54	<0.001*
Gingival index	1.04 ± 0.66	0.27 ± 0.46	<0.001*
Periodontal index	0.51 ± 0.36	0.28 ± 0.3	<0.001*

Values are mean ± standard deviation. P values with asterisk represent significant difference between means. Mean comparisons were performed using paired sample student *t* test.

Table 2 Levels of salivary cytokines and AMPs during pregnancy and postpartum.

	During pregnancy	After pregnancy	P value
IL-1 beta (pg/ml)	19.4 (45.7)	68.5 (1 0 2)	0.001*
INF gamma (ng/ml)	0.02 (0.01)	0.04 (0.03)	0.001*
TNF α (ng/ml)	0.09 (0.1)	0.03 (0.03)	0.003*
Lactoferin (ng/ml)	21.1 (34.2)	28.3 (38.2)	0.13
Lysozyme (ng/ml)	17.8 (10.3)	16.4 (13.3)	0.64
β defensin-1 (pg/ml)	15.8 (15.2)	16.9 (46.8)	0.83

Values are medians (interquartile ranges). P values with asterisks represent significant difference between medians. Comparisons were performed using related sample Wilcoxon signed rank test.

Table 3 Correlation between gingival/periodontal indices and salivary cytokines.

	Spearman's rho	P value
IL-1 beta and Gingival index	−0.07	0.55
IL-1 beta and Periodontal index	−0.20	0.09
IFN γ and Gingival index	−0.24	0.4
IFN γ and Periodontal index	−0.27	0.02*
TNF α and Gingival index	0.28	0.02*
TNF α and Periodontal index	0.26	0.03*

P values with asterisks represent significant correlation between factors. Comparisons were performed using Spearman correlation.

Table 4 Correlation between gingival/periodontal indices and salivary AMPs.

	Spearman's rho	P value
Lactoferin and Gingival index	0.19	0.11
Lactoferin and Periodontal index	0.17	0.14
Lysozyme and Gingival index	0.1	0.42
Lysozyme and Periodontal index	0.04	0.72
β defensin-1 and Gingival index	0.22	0.06
β defensin-1 and Periodontal index	0.16	0.17

P values with asterisks represent significant correlation between factors. Comparisons were performed using Spearman correlation.

4. Discussion

Despite numerous in vitro studies on the hormonal modulatory effects of cytokines on oral tissues, only a few human studies have investigated the changes in the local inflammatory mediators in pregnant individuals (Jonsson et al., 1988; Bieri et al., 2013; Fiorini et al., 2013; Kaur et al., 2014). The reduced

level of salivary IL-1β and IFN-gamma observed in pregnant women in this study may be attributed to hormonal effects on these cytokines. The reduced salivary levels of IFN-gamma may be explained by the reports that one of the major alterations in the immune system during pregnancy is partial dampening of the mother's cell-mediated immune responses associated with T-helper type 1 (Th1) lymphocytes (Poole and Claman, 2004; Jamieson et al., 2006; Singh and Perfect, 2007). This is accompanied by augmentation of antibody-mediated immune responses by T-helper type 2 (Th2) lymphocytes, which promote replication and stimulation of antibody-producing B cells (Chaouat, 2003; Poole and Claman, 2004; Jamieson et al., 2006; Singh and Perfect, 2007). Stimulated Th2 cells produce an array of cytokines, such as interleukin-4, interleukin-5 and interleukin-10, that suppress cell mediated immune responses by suppressing IFN-gamma production as well as Th1 mediated IFN-gamma responses. These mechanisms are partly dependent on changes in progesterone, estrogen and human chorionic gonadotropin during pregnancy (Ehring et al., 1998; Song et al., 2000; Kanda and Watanabe, 2004).

Likewise, Morishita et al. (1999) documented suppression in IL-1β production by peripheral blood lymphocytes by their stimulation with LPS and incubation with progesterone and estradiol. They also commented that IL-1β was inhibited by estradiol and progesterone in a dose-dependent manner. Also, another in vitro study reported that sex hormones at physiological concentrations had an inhibitory effect on the secretion of some inflammatory cytokines including IL-1β (Polan et al., 1990). In agreement with our study, Polan et al. documented that a lower IL-1β was observed in monocytes isolated during the third trimester of pregnancy compared to those isolated during the luteal phase (Polan et al., 1990). However, a study found no significant differences in the level of IL-1β in the gingival crevicular fluid (GCF) during pregnancy and postpartum (Otenio et al., 2012). This discrepancy may be because of the differences in the sample source (i.e. GCF vs. saliva).

Some studies have suggested that elevated salivary IL-1β is a good indicator of periodontitis and that its measurement can discriminate samples from individuals with periodontal disease and those provided by healthy individuals (Miller et al., 2006; Tobón-Arroyave et al., 2008; Kaushik et al., 2011), but these findings have not been replicated in other studies (Christodoulides et al., 2007; Teles et al., 2009). In the present study, the reduced level of IL-1β in saliva during pregnancy possibly suggests different roles of IL-1β in the pathogenesis of periodontal disease in pregnant and non-pregnant conditions.

The observed elevated salivary level of TNF-α during pregnancy when compared with post-partum in this study suggests that this cytokine is up-regulated and may be involved in the pathophysiology of gingival and periodontal inflammation associated with pregnancy. Previous studies have reported elevated levels of TNF-α in gingival crevicular fluid, serum, gingival tissues, and plasma of chronic periodontitis patients compared to controls. TNF-α is a pro-inflammatory cytokine that causes periodontal tissue destruction through various actions. It causes leukocyte recruitment and vascular permeability which are facilitated by the stimulated expression of selectins and adhesins (Marlin and Springer, 1987). Macrophage-induced angiogenesis is also mediated by TNF-α and has a pivotal role in vascular proliferation in the periodontal granulation tissue formation (Leibovich et al., 1987). In response to bacterial infection, TNF-α triggers osteoclast activation, proliferation and differentiation, which results in bone resorption (Beutler et al., 1985; Mundy, 1989).

Importantly, whereas salivary level of TNF-α showed positive correlation with both gingival and periodontal indices; salivary level of IL-1β showed negative correlation with gingival index. These results indicate that levels of these cytokines could be used as biomarkers of gingival inflammation in pregnancy. Saliva is readily available, non-invasively collected and contains both locally and systemically produced serum markers which are of significant diagnostic importance. Whole, non-stimulated saliva samples were used in this study, because stimulated saliva samples are associated with altered composition (Polland et al., 2003; Nederfors et al., 2004). Whole saliva compared to gland specific saliva is a likely source for identifying unique biomarkers that reflect oral and systemic health changes because it is a mixture of the salivary gland secretion, serum products, GCF, epithelial and immune cells and organisms (Miller et al., 2006). Thus, the analysis of whole saliva holds great promise than gland-specific saliva, especially while considering the development of a diagnostic test for periodontal diseases. The reduced salivary levels of IL-1β and IFN-gamma as well as elevated levels of TNF-α during pregnancy observed in the present study, not only indicate their roles in the pathophysiology of gingival as well as periodontal inflammation but can also explain a possible link to the development of complications of pregnancy.

Lack of changes in the levels of the AMPs (lactoferin, lysozyme and β-defensin 1) observed in this study may suggest that the periodontal inflammation in pregnancy is less related to alterations in the antimicrobial factors studied. Contrary to our finding, Gursoy et al. (2016) reported reduced salivary concentrations of human Beta Defensin -1 and -2 during the third trimester, whereas human Beta Defensin-3 concentrations did not change comparing during pregnancy and postpartum period. However, it was also observed that weak associations occurred between salivary defensin and clinical parameters of periodontal inflammation (plaque and gingival indices) which agrees with our results. Also, the present finding may be explained by the reports that the gingival inflammation in pregnancy is less related to changes in oral microbial pathogens (Adriaens et al., 2009). Some microbiological studies have shown that estrogen and progesterone changes associated with pregnancy have an effect on the composition of the subgingival microbiota (Jensen et al., 1981; Kornman and Loesche, 1982; Yokoyama et al., 2008) which may be associated with changes in the antimicrobial factors in saliva. However, other investigators did not find difference in composition of the subgingival microbiota (subgingival levels of *P. intermedia*) in pregnant individuals when compared with non-pregnant individuals (Raber-Durlacher et al., 1993; Adriaens et al., 2009).

One limitation of this study is that most of the women were in 3rd trimester of pregnancy which hindered the evaluation across all the trimesters of pregnancy. This is due to the fact that majority of the women book in the late second trimester with exception of women with concurrent conditions who are likely to have booked earlier in the pregnancy. The latter group of women falls into the exclusion criteria for our study. Also, our inability to assess more cytokines and AMPs in saliva due to available funds limits the full understanding of the role of cytokines and AMPs in pregnancy-induced periodontal inflammation.

5. Conclusion

Lower levels of salivary IL-1β and IFN-gamma along with higher TNF-α concentration during pregnancy suggest their contributions to the pathophysiology of pregnancy-induced periodontal inflammation. Further studies are needed to elucidate other mechanisms underlying these findings.

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Ethical statement

This work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. In addition, this work obtained ethical approval from the institution Ethics Review Committee (UI/EC/15/0256).

Conflict of interest

None declared.

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Further reading

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