

# Periodontal Disease and Salivary Pro-Inflammatory Cytokines Level: A Study of Final Trimester Pregnant Women

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Received: 10.10.20, Revised: 06.11.20, Accepted: 23.12.20

## ABSTRACT

Periodontal disease is one of oral diseases that is experienced by most people in the world, whereas Indonesian basic health survey reported that approximately 75% people suffer from periodontal disease. Pregnant women are more at risk of periodontal disease and it is found that periodontal disease leads to several adverse pregnancy outcomes. This study aimed to compare salivary pro-inflammatory cytokines (IL-6 and TNF- $\alpha$ ) levels between final trimester healthy pregnant women and final trimester pregnant women with periodontal disease. This research is a cross-sectional, analytical observational study that was conducted after passing the ethical aspect. Subjects were 37 final trimester pregnant women divided into 2 groups, healthy group (Group 1/G1) and periodontal disease group (Group 2/G2). All of them were asked to fill out questionnaires and performed an intra oral clinical examination. Unstimulated saliva was collected from each subject using passive drool method. Salivary levels of IL-6 and TNF- $\alpha$  were determined using ELISA method. Data that had been obtained were analyzed using SPSS. Subjects' characteristics were described in number and percentage. The difference in pro-inflammatory cytokines levels between G1 and G2 were analyzed using Independent t test (TNF- $\alpha$ ) dan Mann-Whitney test (IL-6). Salivary levels of both IL-6 and TNF- $\alpha$  were found significantly higher in periodontal disease group compared to the healthy group ( $p=0.026$  and  $p=0.001$ ). Periodontal disease might contribute to the increased level of salivary pro-inflammatory cytokines of pregnant women, especially in TNF- $\alpha$  and IL-6. Thus, periodontal status of pregnant women could be one of important factors that must be considered during pregnancy

**Keywords:** interleukin-6, tumor necrosis factor- $\alpha$ , pregnancy, periodontitis, gingivitis

## INTRODUCTION

Periodontitis is defined as an inflammatory disease of supporting tissues in teeth caused by certain microorganisms or certain groups of microorganisms, causing destructive damage of periodontal ligament and alveolar bone and it is characterized by increased probing depth, recession, or both (Newman et al., 2019). Indonesian basic health survey in 2018 reported that approximately 75% of Indonesians suffer from periodontal disease, occupying second place as the most oral and dental problem. The severity of periodontal disease is varied with 4-5% of Indonesians suffering from advanced periodontal disease will cause tooth mobility and sudden tooth detached (Sriyono, 2009). Periodontal disease can be found in various group of people, including patients with systemic

diseases, elderly patients, and pregnant women. The numerous physical and physiological changes occurred during pregnancy affect every major body system and result in localised physical alterations in many parts of the body, including oral cavity. Several studies reported that hormonal imbalance in pregnant women caused gingivitis. In addition, immune and inflammatory responses are important factors that influence periodontal disease. Periodontitis is reported to increase the risk of pregnant women in experiencing adverse pregnancy outcomes, for example low birth weight and premature birth (Marakoglu et al., 2008). A frequent observation potentially linking subclinical infection and preterm birth is an increased incidence of histologic chorioamnionitis in preterm delivery,

which is usually a result of infection (Uriza et al., 2018).

Saliva secretion and salivary components are important for dental health. Inorganic and organic components in saliva may increase colonization and elimination of microorganisms from oral cavity. Pathogenic bacteria that cause periodontal diseases such as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans plays a role as an antigen for the body (Fujiwara et al., 2018). The invasion of antigens into body will cause immune response to release pro-inflammatory cytokines. Some pro-inflammatory cytokines that can be used as inflammatory biomarkers are interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ). TNF- $\alpha$  is a part of an integral network of interactive signals that orchestrate inflammatory and immunological events that promote inflammatory cell infiltration by leukocyte adhesion molecules on endothelial cells and activate phagocyte killing mechanisms. IL-6 is a multifunctional cytokine playing a central role in inflammation and tissue injury. During an antigen invasion, the body recognizes pathogenic bacteria and gives response by increasing the number of pro-inflammatory cytokines production (Machado et al., 2018). In pregnant women, increased levels of pro-inflammatory cytokines are associated with the occurrence of pregnancy complications (Opeodu and Arowojolu, 2007).

This study aimed to compare the salivary pro-inflammatory cytokines (IL-6 and TNF- $\alpha$ ) levels between final trimester healthy pregnant women and final trimester pregnant women with periodontal disease.

## MATERIALS AND METHODS

### Ethical consideration

This study passed the ethical aspects from The Research Ethic Committee of the Faculty of Dental Medicine, Universitas Airlangga (No. 234/HRECC.FODM/V/2019). This research is a cross-sectional, analytical observational study without medical or dental intervention that was conducted in one of Mother and Child hospitals in Surabaya, Indonesia. Written informed consent was obtained from all subjects prior to appointment. All data were registered on a database specifically created for this purpose, where a coded number was attributed to each subject.

### Research subjects

Subjects of this study are pregnant women who attended regular check-up at Kendangsari Mother and Child Hospital Surabaya, Indonesia between July – October 2019, and were chosen by simple

random sampling technique. The inclusion criteria were final trimester pregnant women and willing to participate in this research. Pregnant women with diabetes or under certain medication, and medically compromised were excluded in this study. Thirty-seven pregnant women were enrolled in this study and divided into two groups, seventeen women with no periodontal disease as healthy group (G1), while the other twenty subjects were women with periodontal disease (G2).

### Questionnaire and clinical examination

After signing the written informed consent, subjects were asked to fill out a questionnaire to obtain the socio-demographic status, general information regarding their pregnancy, and their personal oral hygiene care. Each subject who is willing to participate in this study was assessed by one of the researchers as examiner. Subjects were instructed to sit on a regular chair in Kendangsari Mother and Child Hospital, then clinical examination was performed using headlight. Periodontal disease is defined as redness, with or without swelling in gingiva surrounding tooth, and the presence of calculus in more than two sites in each region. The average time for this examination was 15-20 minutes without radiograph examination.

### Pro-inflammatory cytokines measurement of saliva

Subjects were asked to rinse their mouth for 1 minute with clean and distilled water, and waited for approximately 5 minutes. Saliva samples were collected by passive drooling using Eppendorf tube. The samples were frozen at the collection day and stored at -30°C until further analysis. Measurement of IL-6 and TNF- $\alpha$  concentration was performed using ELISA kit (Bioassay Technology Laboratory, Shanghai, China) under the manufacturer's protocol. IL-6 and TNF- $\alpha$  concentration were expressed in pg/mL.

### Data analysis

The data obtained were analyzed using IBM SPSS statistic version 25.0 for Windows (Armonk, NY: IBM Corp). Descriptive statistic such as frequencies and standard deviation was counted. Subjects' characteristics were described in mean and percentage. Comparison of pro-inflammatory cytokines levels between G1 and G2 was analyzed using independent t test (TNF- $\alpha$ ) dan Mann-Whitney test (IL-6). Level of significant was set at 5%, meaning that p value less than 0.05 ( $p < 0.05$ ) was considered significant.

## RESULTS AND DISCUSSION

**Table 1: Socio-Demographic Characteristic Of The Research Subjects.**

Characteristics	G1 – Control (n=17)		G2 – Periodontal Diseased (n=20)	
	n	%	n	%
<b>Age</b>				
Range (Min-Max)	21-37		23-36	
Mean	28		28	
<b>Socioeconomic Status</b>				
Middle	5	29.4	8	40.0
High	12	70.6	12	60.0
<b>Educational level</b>				
High school	1	5.9	7	35.0
Diploma / Bachelor	16	94.1	13	65.0
<b>OHI-S</b>				
Bad	0	0	1	5.0
Average	2	11.7	18	90.0
Good	15	88.3	1	5.0
<b>Dental Visit During Pregnancy</b>				
No	5	29.4	7	35.0
Yes	12	70.6	13	65.0
<b>Use Of Mouthwash</b>				
No	11	64.7	13	65
Yes	1	5.9	1	5
Rare	5	29.4	6	30
<b>Depth of Probing</b>				
3.0	15	88.2	2	10.0
3.5	2	11.8	6	30.0
4.0	0	0	12	60.0

The subjects' socio-demographic characteristics, according to periodontal diagnosis, are shown in the Table 1. The average age of G1 and G2 is 28 years old. Socioeconomic status percentage did not show significant difference, but periodontal disease group was mostly at high social status (60%) with diploma/bachelor education level (65%). Concerning the attitude and behavior of the subjects, 90% of subjects in periodontal disease group (n = 18) had an average Oral Hygiene Index Simplified (OHI-S) value and 65%

of healthy group (n = 13) had routine dental visits during pregnancy. Most of healthy group (64.7%) and periodontal disease group (65%) didn't use mouthwash routinely. Clinical examination to detect the presence of periodontal disease could be done by probing the gingival sulcus. In healthy group, 15 women (88.2%) had 3.0 mm depths of probing and in periodontal disease group, the majority depths of probing were 4.0 mm (12 people, 60%).

**Table 2: Mean ± SD of salivary pro-inflammatory cytokines level (pg/ml) and statistical difference test between healthy and periodontal diseased subjects.**

	G1 – Control (n=17)	G2 – Periodontal Diseased (n=20)	p value*
	Mean ± SD	Mean ± SD	
IL-6	24.3 ± 12.924	25.5 ± 28.6	0.026 <sup>a</sup>
TNF- α	163.8 ± 75.2	267.6 ± 7.1	0.001 <sup>b</sup>

SD: Standard deviation

\*significant: p<0.05

<sup>a</sup>Mann-Whitney test

<sup>b</sup>Independent t-test

Descriptive statistic of IL-6 and TNF- $\alpha$  was calculated and displayed in the Table 2. Cytokine concentration were significantly different between the healthy (G1) and periodontal disease (G2) group. Salivary levels of IL-6 and TNF- $\alpha$  were significantly increasing ( $p=0.026$  and  $p=0.001$ ) in periodontal disease group compared to healthy group.

The aim of this study is to determine the production comparison of pro-inflammatory cytokines (IL-6 and TNF- $\alpha$ ) between healthy and periodontal disease group of third trimester pregnant women, in order to emphasize the importance of periodontal health during pregnancy and its relation to pregnant women's general health. Thirty-seven final trimester pregnant women were randomly chosen and divided into two groups.

In this study, it was found that pregnant women with periodontal disease were mostly from high social status (60%) with bachelor/diploma education level (65%). This is consistent with previous research that the socioeconomic level does not affect individual awareness of their dental and oral health (Opeodu and Arowojolu, 2007). However, other studies mentioned that social and economic status have an impact on dental and oral health status (Kadtane et al, 2014). People with low socio-economic status have low awareness level of dental and oral examinations thus it is possible that they didn't realize of their periodontal disease. In addition, people with low economic level are unable or difficult to buy dental and oral health support tools such as mouthwash, interdental brush, or good toothpaste due to its high price. In this study 65% of pregnant women with periodontal disease had never used mouthwash.

The results of the OHI-S were obtained from examination of debris index and calculus index. If higher OHI-S values are found, it can be ascertained that there are more periodontal diseases. This study reported that 90% of pregnant women with periodontal disease had an average OHI-S value. This is consistent with previous research which suggests that pregnant women with periodontal disease have the average OHI-S value up to 63% (Bozorgmehr et al., 2018).

During pregnancy the inflammatory response to dental plaque is increased, leading to swollen gingiva which tend to bleed on brushing. Normally gingival sulcus has 2-3 mm of probing depth. If the probing depth is higher, an attachment loss may occur, which is a sign of periodontal disease. This study found that the majority of periodontal disease group had

probing depth of 4.00 mm. This conforms the previous studies that pregnant women with periodontal disease have probing depth more than 3.00 mm (Lieff et al., 2004).

The results of this study indicate that IL-6 and TNF- $\alpha$  levels are increased significantly in periodontal disease group compared to the healthy group. Similar studies examining pregnant women in Portugal also showed that the level of IL-6 and TNF- $\alpha$  were increased significantly in periodontal disease group compared to those without periodontal disease (Machado et al., 2018). Pro-inflammatory cytokines such as IL-6 and TNF- $\alpha$  are often used as biomarkers for marking systemic disorders, such as obesity and metabolic syndrome (Khosravi et al., 2013; Mohammadi et al., 2017). Bacteria that cause periodontal diseases such as *Porphyromonas gingivalis* are gram-negative bacteria that has lipopolysaccharides (LPS) and found in the outer membrane. Bacterial stimulation especially by LPS can be one of the causes of pro-inflammatory cytokines increased production (Vahabi et al., 2011).

The presence of periodontal disease will cause more pro-inflammatory cytokines production in pregnant women. This is consistent with a review which said that TNF- $\alpha$  shows a high correlation with the formation of periodontal disease and can be used as a biomarker to determine progression of periodontal disease (Gomes et al., 2016). Periodontal disease diagnosis is a diagnosis obtained through complex examinations, including the need for radiographic photographs. Radiographic photographs are not recommended for pregnant women because of their association with radiation. Therefore, researchers took saliva from their oral cavity as a safer method. Saliva describes the overall state of the oral cavity, and can determine disease progression (Machado et al., 2018). However, the limitations of this study focused on several factors that did not adequately describe the details of the study. The subjects' visit to the dentist during pregnancy does not describe what dental activities are carried out and during which trimester. In addition, the subjects of this study were all pregnant women. Further research needs to be done to determine pro-inflammatory cytokines level. Thus, it is known that the significant increase is purely due to periodontal disease.

## CONCLUSIONS

Based on this research, it was found that there was a significant difference on pro-inflammatory cytokines between healthy and periodontal disease group, and periodontal disease could

increase the level of pro-inflammatory cytokines. Hence, it could be concluded that periodontal health and the presence of periodontal disease are factors that must be seriously considered during pregnancy. Those involved in obstetric and prenatal care may be the first health professionals who are aware of oral conditions and it is important that they can provide appropriate information, advice and reassurance followed by referral for a dental examination, treatment and monitoring as necessary.

#### ACKNOWLEDGEMENTS

We thank the dean, Prof. Dr. Darmawan Setijanto., Drg., MKes for the support of the "Mandat" Grant from the Faculty of Dental Medicine, Universitas Airlangga, which was given for this research.

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