UNIVERSITAS AIRLANGGA
FAKULTAS KEDOKTERAN GIGI
DEPARTEMEN ILMU KEDOKTERAN GIGI ANAK
Jalan Mayjen.Prof.Dr.Moestopo 47 Surabaya 60132 Telp. (031) 5030255, Fax (031) 5020256 Website : http://www.fkg.unair.ac.id – E-mail : fkg@unair.ac.id

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B. Kategori Publikasi Jurnal Ilmiah (beri ☐pada kategori yang tepat)

- ☑ Jurnal Ilmiah Internasional Bereputasi (SJR > 0,10)
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A		Identitas Karya Ilmiah	
	1	Judul: **Quantification of Porphyromonas gingivalis Bacteria in Final Trimester of Pregnant Women According to Their Oral Health Status**	
	2	Nama Penulis : **1. Retno I Roestamadji; 2. Udijanto Tedjosasongko; 3. Nuraini Indrastie; 4. Indeswati Diyatri; 5. Meircurius D.C. Surboyo; 6. Subijanto M. Sudarmo; 7. Budi Santoso; 8. Nobuhiro Takahashi**	
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B		**Peng-index :** SCOPUS, EBSCO, ProQuest, Google Scholar **SCOPUS Q4, SJR: 0,15**	
C	Relevansi kompetensi dosen dengan substansi karya ilmiah	1. Artikel ini untuk menganalisis hubungan status kebersihan mulut (OHI-S) dengan ekspresi Porphyromonas gingivalis pada ibu hamil trimester III. Ibu hamil lebih berisiko mengalami infeksi gigi. Infeksi gigi yang tidak diobati selama kehamilan dapat menyebabkan masalah yang lebih serius bagi ibu dan anaknya, seperti kelahiran prematur dan berat lahir rendah. 2. Artikel sesuai dengan bidang keahlian pengusul yaitu **Cariology.** **3.** Tidak ada keterkaitan dengan naskah Disertasi pengusul yang berjudul: **Initial acquisition and transmission of Mutans Streptococci in children at day nursery.**	
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Quantification of *Porphyromonas gingivalis* Bacteria in Final Trimester of Pregnant Women According to Their Oral Health Status

Retno I. Roestamadji[1] Udijanto Tedjosasongko[2] Nuraini Indrastie[3] Indeswati Diyatri[1] Meircurius D. C. Surboyo[4] Subijanto M. Sudarmo[5] Budi Santoso[6] Nobuhiro Takahashi[7]

[1] Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[2] Department of Pediatric Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[3] Magister of Dental Health Science, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[4] Department of Oral Medicine, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[5] Department of Pediatric, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[6] Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[7] Graduate School of Dentistry, Tohoku University, Sendai, Japan

Address for correspondence Retno I. Roestamadji, DDS., MDS, Department of Oral Biology. Faculty of Dental Medicine, Universitas Airlangga, Jln. Prof. Dr. Moestopo No. 47, Surabaya 60132, Indonesia (e-mail: retnoindrawati@fkg.unair.ac.id).

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Abstract

Objective Pregnant women are more at risk to suffer dental infection. Untreated dental infection during pregnancy can lead to more serious problems to mothers and their children, such as premature birth and low birth weight. This study aims to analyzed the relationship between oral hygiene status (OHI-S) and the expression of *Porphyromonas gingivalis* in third trimester pregnant women.

Materials and Methods This was an observational analytic study with a cross-sectional study design. Patients consisted of 37 final trimester pregnant women, divided into good OHI-S and fair OHI-S. The *P. gingivalis* expression was measured using real-time qPCR from the mucosal swab.

Results The *P. gingivalis* expression found no differences between good OHI-s and fair OHI-S ($p = 0.557$).

Conclusion Based on this study, although there was no significant difference in *P. gingivalis* expression in the final trimester based on their oral health status, oral health is considered important to be taken care of during pregnancy.

Keywords
- OHI-S
- qPCR
- pregnant women
- *Porphyromonas gingivalis*
- human health

Introduction

Oral hygiene and health are important aspects of human life since the World Health Organization (WHO) expanded the definition of health. Since then, oral health has also been seen contributing to general health and not simply the absence of disease.[1] The decline in oral health and hygiene is marked by problems in the oral cavity, with 57.6% of Indonesians having dental and oral health problems.[2] Pregnant women are a population that is vulnerable to dental and oral health problems. A study conducted in 2019 on 150 pregnant

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women in Iran showed that more than 50% of patients had moderate and poor OHI-S rates.[3] Periodontal disease is a problem in the oral cavity that is often suffered by pregnant women. Research in 2010 shows that pregnant women have 2.2 times higher risk of developing gingivitis compared with women who are not pregnant.[4]

For pregnant women, many considerations are needed to determine periodontal disease therapy because the condition of pregnant women is prone to invasive procedure. Therapy for periodontal disease, which has been commonly used, is also somehow insufficient.[5] There have been some failures despite the treatment of periodontal tissue and this is a disadvantage of current periodontal therapy. The failure of periodontal therapy can be caused by periodontal-pathogenic bacteria that remain in the oral mucosa and can recolonize at any time.[6,7] In pregnant women, levels of pathogenic bacteria *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* in subgingival plaques in the early and mid-pregnancy stages were higher than those in nonpregnant women.[8] Periodontal disease is reported to increase the risk of pregnant women experiencing pregnancy complications, for example low birth weight[9] and preterm birth.[10]

The aim of this study is to quantify the amount of *P. gingivalis* on final trimester pregnant women bacteria based on their oral health status.

Materials and Methods

Ethical Research

This was an observational analytical with cross-sectional study design that has passed the ethical aspect from the Health Research Ethical Clearance Commission, Faculty of Dental Medicine Universitas Airlangga (identifier: 234/HRECC.FODM/V/2019). This study was conducted without any dental intervention. Patients were chosen by simple random sampling technique.

Patient Participant

Patients were 37 women in the final trimester pregnancy who attended regular check-up in Mother and Children Hospital in Surabaya, Indonesia, between July and October 2019. Pregnant women with under certain medication and medically compromised were excluded in this study.

Before the sample was collected, each participant was given questionnaires on their pregnancies and their personal oral hygiene care. The questionnaires including the ages, academic degree, height and weight body, pregnancy tatus, habit for maintaining oral hygiene, and duration visiting dentist during pregnancy.

Oral Health Assessment

Patients were instructed to sit on a regular chair and clinical examination was performed.

The oral hygiene index (OHI-S) is the sum of the debris index (DI) and calculus index (CI). DI and CI examinations were performed using an explorer that was placed on the tooth surface horizontally. The sum of the debris assessment then divided by the number of teeth examined will get the DI score. The sum of the calculus scores then divided by the number of teeth examined will get a CI score and then divided into good OHI-S (0–1.2) and fair OHI-S (>1.3).

Mucosal Swab

Patients were asked to rinse their mouth using clean and sterile water, and waited for approximately 5 minutes. Swab samples were taken using sterile cotton swabs. The samples were frozen on the collection day and stored at −30°C until further analysis.

DNA Extraction

DNA extraction was performed using the manufacturer's protocol (QIAamp DNA Stool Mini Kit, Qiagen, USA). Samples were taken from the tube and added to the 1.5 mL micro centrifuge tube. In total, 180 µL of buffer ATL and 20 µL of proteinase K were added to the same tube. The tube was vortexed for 15 seconds and then incubated at 60°C for 24 hours. Buffer AL (200 µL) was added to the tube using a micropipette. The tube was vortexed for 15 seconds and then incubated for 10 minutes at 70°C. Next, 200 µL of 96% ethanol was added to the tube for 15 seconds and then spined down. This mixture was placed in the QIAamp Mini spin column (found in a 2 mL collection tube). The mixture was centrifuged at 8000 rpm for 1 minute. The collection tube containing the filtrate was discarded and replaced with a new collection tube. Then, 500 µL of the AW1 buffer was added and centrifuged for 1 minute at 8000 rpm. The collection tube containing the filtrate was discarded and replaced with a new collection tube. Next, 500 µL of the AW2 buffer was added and centrifuged for 3 minutes at 13,000 rpm. The collection tube containing the filtrate was discarded and replaced with a new collection tube, and then centrifuged for 1 minute at 13,000 rpm. The spin column was transferred to a 1.5 mL microcentrifuge tube. Next, 50 µL of Buffer AE or distilled water was added and incubated at room temperature for 1 minute, and then centrifuged for 1 minute at 8000 rpm. At the end of the extraction, 50 µL of DNA was obtained from the sample. This DNA was used for qPCR.

Quantification of *Porphyromonas gingivalis*

Primers were optimized using the manufacturer's protocol (GoTaq, Promega Corporation, United States). Specific primers to detect *P. gingivalis* was used (*P. gingivalis* ATCC 33277 specific forward primer 5'-ATAGTAGCGTGTCCGGCTTC-3' and *P. gingivalis* ATCC 33277-specific reverse primer 5'-ATCGTAGGCGGATTGGAGA-3', (Macrogen Asia Pacific Pte. Ltd., Singapore).[11] The master mix was removed from the storage until it reached room temperature and then vortexed. As much as 12.5 µL of master mix was mixed with 10 µL of NFW in a 0.2 mL microcentrifuge tube. In the same tube, forward and reverse primers were added, 1 µL each. The tube was transferred to a qPCR machine. The primary optimization was performed to find a curve with one peak as the melting temperature (Tm = 82°C). The qPCR procedure was performed using the same steps with 5 µL of DNA samples added. The qPCR in samples was performed at a

denaturation temperature of 95°C for 2 minutes, annealing temperature of 60 to 72°C for 50 cycles, and an extension temperature of 60 to 97°C for 1 minute. The qPCR process was performed until the amplification curve and Cq/Ct value for each sample appeared.

P. gingivalis expression was calculated using the relative quantification method with one sample as a comparison (reference). The reference sample was calculated first before calculating the entire sample. The calculation used the ΔCt Eq.[12] The NFW expression was read as percentage.

Statistical Analysis

The data that had been obtained were analyzed using SPSS statistic. After homogeneity and normality test were performed, the differences in *P. gingivalis* between good OHI-S and fair OHI-S were analyzed using the Mann–Whitney test with significant consider as $p < 0.05$.

Results

Sociodemographic Characteristic

Most patient participants were more than 27 years old (62.2%), with a minimum educational background of diploma or bachelor or equivalent (78.4%). More than half of the patients had a BMI (≤ 30) during pregnancy (62.1%) and were in the first pregnancy (56.7%). All patients had the habit of brushing teeth more than twice a day (100%) and visited dentist during pregnancy (67.5%) (►**Table 1**).

Oral Health Profile

The oral health profile was assessed through DI, CI, and OHI-S. The lowest DI and CI on score 0 and the highest DI and CI on score 1.5 (►**Fig. 1A** and **1B**). The lowest OHI-S has score 0 and the highest has score 3 (►**Fig. 1C**). The OHI-S in patient participant was distributed into two categories. In all, 17 participants were classified as a good OHI-S (0–1.2) and 20 participants classified as fair OHI-S (> 1.3; ►**Fig. 1D**).

Quantification of *Porphyromonas gingivalis*

The *P. gingivalis* expression was observed in the lowest quantification on 0% and the highest quantification on 364.56% (►**Fig. 2A**). The mean *P. gingivalis* expression in the good OHI-S around 26.186% and in the fair OHI-S around 20.513% (►**Fig. 2C**). Both groups showed nonsignificant different ($p = 0.577$; ►**Table 2**). There were four samples not detected the *P. gingivalis* expression, three samples from fair OHI-S group and 1 sample from good OHI-S group (►**Fig. 2D**).

Discussion

This study aimed to identify the expression of *P. gingivalis* in the oral cavity of final trimester pregnant women based on the oral hygiene status (OHI-S). The results showed that the number of *P. gingivalis* expression found no significant difference between the good OHI-S group and the fair OHI-S group ($p = 0.557$). In pregnant women, a good condition of the oral cavity can have the potential to reduce vertical transmission of pathogenic bacteria. The existence of

Table 1 Social-demographic characteristics

	Good OHI-S		Fair OHI-S		Total	
	n	%	*n*	%	*n*	%
Age (y)						
≤27	6	35.3	8	40	14	37.8
≥27	11	64.7	12	60	23	62.2
Academic degree						
High school/equals	2	11.8	6	30	8	21.6
Diploma/bachelor/equals	15	88.2	14	70	29	78.4
BMI (kg/m^2)						
< 30	11	64.7	12	60	23	62.1
≥30	6	35.3	8	40	14	37.8
Pregnancy time						
1	11	64.7	10	50	21	56.7
>1	6	35.3	10	50	16	43.3
Tooth brushing habit						
≥2 times	17	100	20	100	37	100
<2 times	0	0	0	0	0	0
Dental treatment during pregnancy						
Yes	13	76.5	12	60	25	67.5
No	4	23.5	8	40	12	32.5

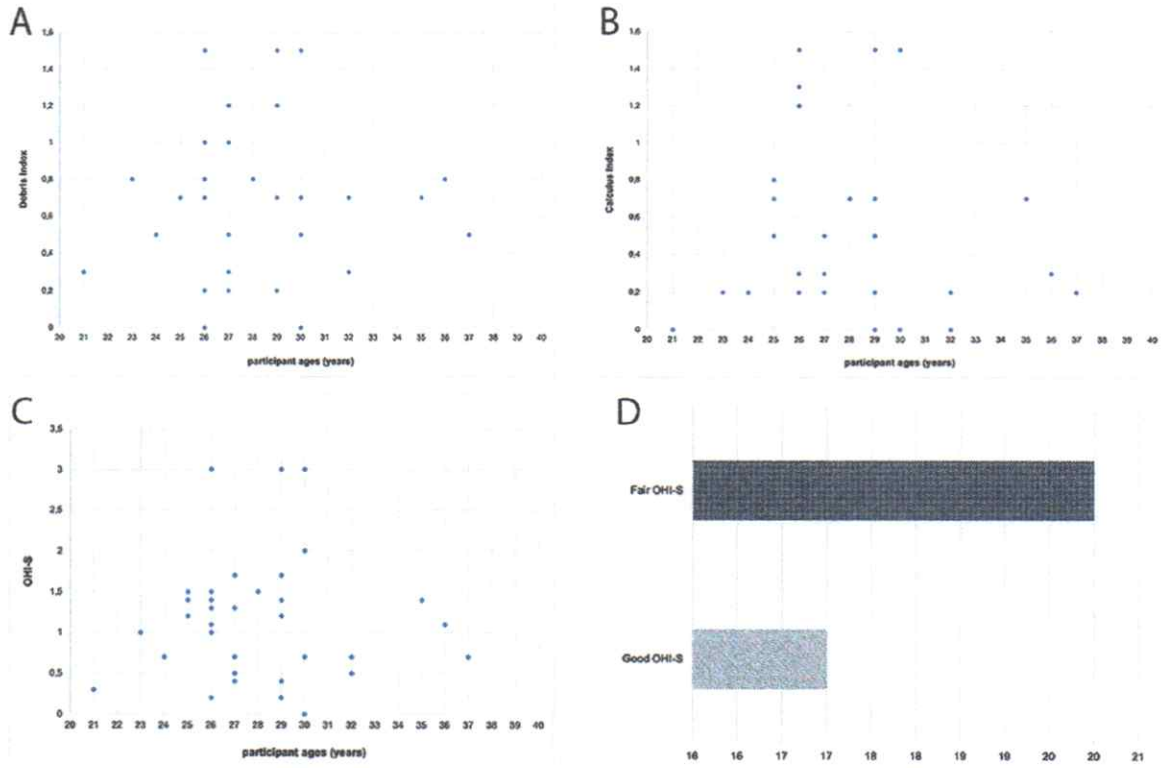

Fig. 1 The scatter plot of oral health for all participant. (A) Debris index; (B) Calculus index; (C) OHI-S and (D) the calssification of OHI-S.

physiological changes during pregnancy and the lack of individual awareness to seek information about dental health services during pregnancy make pregnant women more susceptible to oral cavity infections. One of the disorders that can occur during pregnancy is periodontal disease. A study by Uriza et al in 2018 stated that in pregnant women, periodontal disease causes an inflammatory response.[10] Not only periodontitis, dental caries is also a concern for pregnant women. Dental treatments such as prevention of the development of caries-causing bacteria do not show changes in these bacteria in the microbiome.[13]

In the results of this study, it was found that the mean expression of *P. gingivalis* in the fair OHI-S group was lower than that of the good OHI-S group with no significant differences ($p = 0.557$). Research with pregnant women patients in 2015 stated that the average number of *P. gingivalis* in pregnant women tended to be higher than in those who were not pregnant, but the number was not higher than other periodontal-pathogenic bacteria from the genus *Fusobacterium* and *Prevotella*.[8] This is in accordance with research which states that *P. gingivalis* was found in more than half of patients with pregnancy conditions but did not show a relationship with clinical diagnosis.[14] In another study, it was also found that the oral microbiome did not change significantly, especially in orthodontic users. Most bacteria dominated by *Streptococcus* sp., *Gemella* strain, *Lactobacillus fermentum*, and *Abiotrophia defective*.[15]

No significant difference result in this study is likely to be influenced by pregnancy hormones. Pregnancy hormones, namely estrogen and progesterone in the third trimester of pregnancy increase at week 3 to 6 times compared with before pregnancy, and reach their peak in third trimester. This increase in estrogen and progesterone hormones is one of the many aspects of pregnant women that may play a role in modulating the immune system of pregnant women. This is in accordance with the results of research that states that oral bacteria, including *P. gingivalis*, are associated with pregnancy hormones in pregnant women.[16]

Limitations

There are several limitations to this study. The existence of a large standard deviation (SD) in the study data may have influenced the results of the study. SD shows how big the variation of the data is from the mean.[17] The larger the SD, the more varied the data in one group. The amount of SD in this study could be caused by the lack of controllable variables from the patients, because the researcher wanted to get an overall picture of the examined patients. The existence of research with pregnant mother patients is still very much needed. Oral health during pregnancy is very important to know, understand, and apply even before pregnancy. Pregnant women are a population with limited access to dental and oral health services due to their pregnancy conditions, with invasive oral and dental health procedures as contraindications. Other approaches such as promotive and preventive measures are needed to maintain oral health during pregnancy. This is necessary to avoid

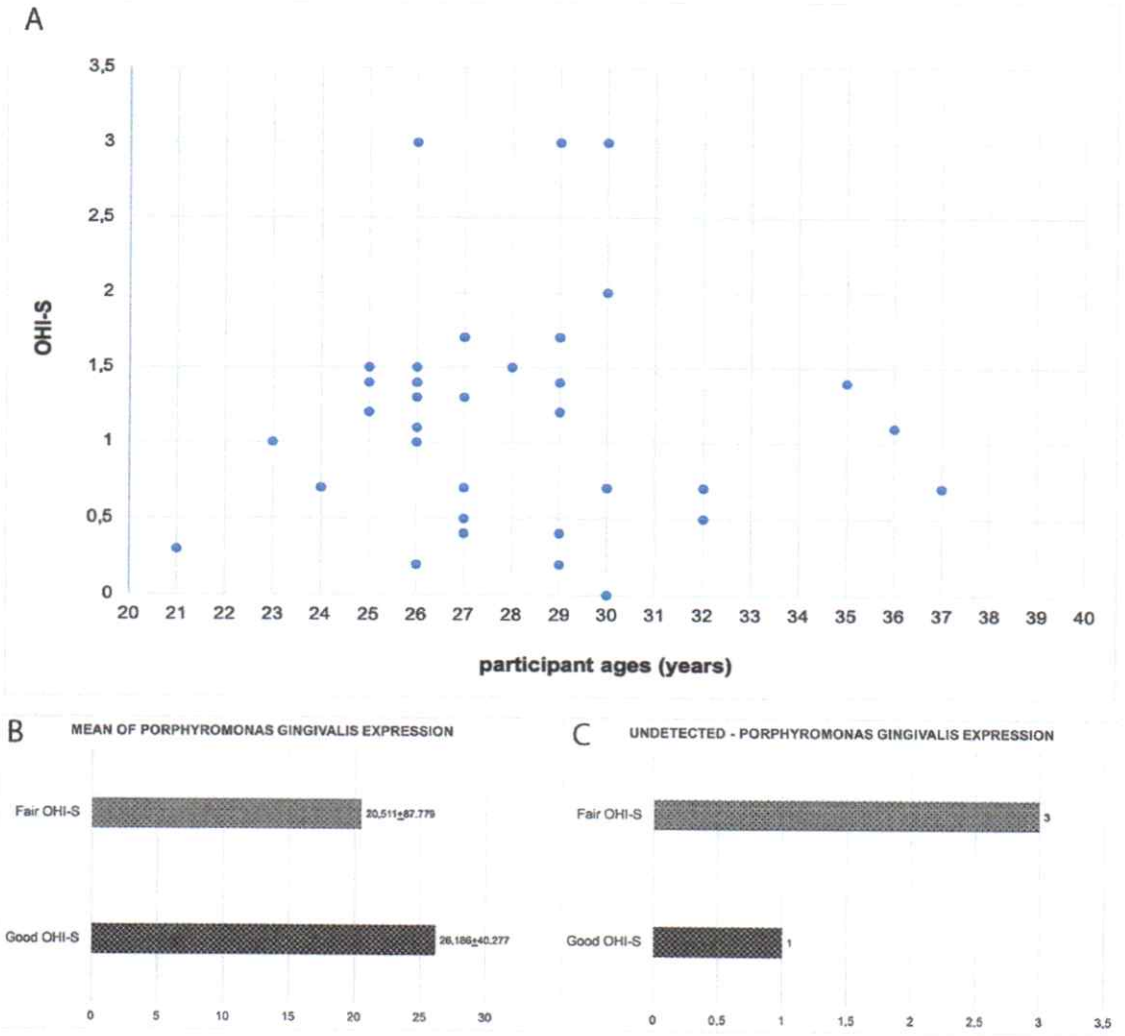

Fig. 2 The scatter plot of Porphyromonas gingivalis expression in the oral cavity of final trimester pregnant women (A), The Porphyromonas. gingivalis expresion based on OHI-S (B) and The undetect Porphyromonas. gingivalis expresion based on OHI-S (C).

Table 2 Differences of *P. gingivalis* expression in the oral cavity of final trimester pregnant women based on OHI-S

Groups	Mean ± SD	*p*-Value*
Good OHI-S	26.186 ± 87.779	0.557
Fair OHI-S	20.511 ± 40.227	

*p-Value with Mann–Whitney test (significant at $p < 0.05$).

adverse pregnancy outcomes associated with oral health. With this research, it can be the basis and the beginning for other research for other therapeutic approaches in pregnant women.

Conclusion

Based on this study, although there were no significant difference in *P. gingivalis* expression in the final trimester based on their oral health status, oral health is considered important to be taken care of during pregnancy. It is necessary to do socialization related to the importance of oral health on the health of pregnant women and visits to the dentist are necessary to maintain the health of both mother and baby.

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Conflict of Interest
None declared.

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