

Periodontal diseases among pregnant women attending an antenatal clinic at Dhaka, Bangladesh.

Enfermedades periodontales entre mujeres embarazadas que asisten a una clínica prenatal en Dhaka, Bangladesh.

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Abstract: **Background:** The most common maternal oral diseases that potentially could bring adverse pregnancy outcome are periodontal diseases. So periodontal diseases during pregnancy require special attention. Oral health education is often not addressed with importance during antenatal counseling. **Objective:** The purpose of the study was to assess the periodontal status, treatment needs and factors that influence periodontal diseases among pregnant women attending to antenatal clinic situated at Dhaka, Bangladesh. **Material and Methods:** A descriptive cross sectional study was conducted including 170 pregnant women selected by convenience sampling technique. Data were collected by pretested semi-structured questionnaire. Periodontal assessment was done by Community Periodontal Index of Treatment Need (CPITN) and oral hygiene status was assessed by the Simplified Oral Hygiene Index (OHI-S). The data analysis was done by SPSS software. Chi-square test was used for different variables. A probability value of <0.05 was taken as statistically significant. **Results:** The prevalence of periodontal diseases among pregnant women was found 95.3% where it was 52.4% for gingivitis and 43% for periodontitis. Age, employment status, income, oral hygiene status, frequency of tooth brushing and dental visit, previous pregnancy and pregnancy trimester were found statistically associated with periodontal condition of pregnant women. 75.3% of the respondents had never gone for dental checkup and only 4.7% were found to receive oral hygiene instructions during pregnancy. **Conclusion:** Policy should be formulated to include oral health education and periodontal assessment in antenatal checklist to improve oral hygiene awareness and ensure healthy periodontium during pregnancy.

Keywords: *periodontal disease; oral hygiene index; pregnancy; oral health; prenatal care; needs assessment.*

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Resumen: **Antecedentes:** Las enfermedades orales maternas más comunes que potencialmente podrían traer resultados adversos en el embarazo son las enfermedades periodontales. Por lo que las enfermedades periodontales durante el embarazo requieren una atención especial. La

educación en salud bucodental a menudo no se aborda con importancia durante el asesoramiento prenatal. **Objetivo:** El propósito del estudio fue evaluar el estado periodontal, las necesidades de tratamiento y los factores que influyen en las enfermedades periodontales entre las mujeres embarazadas que asisten a una clínica prenatal situada en Dhaka, Bangladesh. **Material y Métodos:** Se realizó un estudio descriptivo de corte transversal con 170 gestantes seleccionadas mediante la técnica de muestreo por conveniencia. Los datos fueron recolectados mediante un cuestionario semiestructurado previamente validado. La evaluación periodontal se realizó mediante el Índice Periodontal Comunitario de Necesidad de Tratamiento (CPITN) y el estado de higiene oral se evaluó mediante el Índice de Higiene Oral Simplificado (OHI-S). El análisis de datos se realizó mediante el software SPSS. Se utilizó la prueba de chi-cuadrado para diferentes variables. Se tomó como estadísticamente significativo un valor de probabilidad

<0,05. **Resultados:** La prevalencia de enfermedades periodontales entre las gestantes fue de 95,3%, donde fue de 52,4% para gingivitis y 43% para periodontitis. La edad, el estado laboral, los ingresos, el estado de higiene bucal, la frecuencia del cepillado dental y la visita al dentista, el embarazo anterior y el trimestre del embarazo se encontraron asociados estadísticamente con la condición periodontal de las madres embarazadas. El 75,3% de las encuestadas nunca había ido a revisión odontológica y solo el 4,7% recibió instrucciones de higiene bucal durante el embarazo. **Conclusion:** Se debe formular una política para incluir la educación sobre salud bucal y la evaluación periodontal en la lista de verificación prenatal para mejorar la conciencia sobre la higiene bucal y garantizar un periodonto saludable durante el embarazo.

Palabras Clave: enfermedades periodontales; índice de higiene oral; embarazo; salud bucal; atención prenatal; evaluación de necesidades.

INTRODUCTION.

Pregnancy is a very important period of a woman's life. Pregnancy affects nearly every aspect of life of a woman including her oral health. Healthy periodontal health ensures healthy pregnancy. Periodontal diseases are however the most common oral diseases during pregnancy. These could be simply gingivitis or progressing to severe periodontitis, even tooth mobility and loss.¹

Prevalence of periodontal diseases was found ranging from 14% to 95% among pregnant women in different populations.²⁻⁷ The frequency of gingivitis was found to range from 94% to 100% and frequency of periodontitis was found to be 27% among pregnant women in Bangladesh.^{8,9} Periodontal diseases are multifactorial. The development of periodontal diseases during pregnancy can be influenced by hormonal changes as well as other factors such as lack of dental care, poor oral hygiene, low-educational level, low-employment status, increased gestational age,

frequency of tooth brushing, pregnancy trimester and negligence in dental visits.^{4,5,10-12}

The disease does not only affect the mother, but may also have detrimental effects on the fetus if left untreated. Research has indicated that women with periodontal diseases may be at risk of adverse pregnancy outcomes, such as giving birth to a premature, low-birth weight baby or even developing pre-eclampsia.^{11,13,14}

The American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP) recommends pregnant women to maintain periodontal health. In addition, the American College of Obstetricians and Gynecologists encourages pregnant women to sustain their oral health and recommended regular dental cleanings during pregnancy.¹

Despite such recommendations, a very low number of pregnant women are encouraged to arrange for dental checkups during pregnancy. The antenatal

clinic (ANC)'s checklist doesn't include oral health screening during pregnancy in Bangladesh. Only a very small portion of pregnant women are referred to dentists for oral check ups during pregnancy.⁸

The purpose of the study was to assess the periodontal status, treatment needs of pregnant women and to find out the relationship between the different variables that influence the periodontal health of pregnant women.

MATERIALS AND METHODS.

Ethical issues

Before conducting the research, approval was obtained from the Research Review Committee (RRC) and Ethical Review Committee (ERC) of American International University, Bangladesh (AIUB) and consent of the authority of Spreeha Bangladesh. Informed consent was taken before interview and clinical examinations from the respondents.

Privacy, confidentiality and anonymity were strictly maintained. Nature and purpose of the study was explained to both respondents and to the authority of Spreeha Bangladesh.

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975.

Study Design

This study was a cross sectional descriptive study.

Study Setting

The study was conducted at the health center of 'Spreeha, Bangladesh' – a non-profit organization, located at Mohammadpur, Dhaka city, Bangladesh, where pregnant women have access to diagnostics, curative and ANC services. The study period was 6 months from March 2018 to August 2018.

Study population

The study population was pregnant women aged between 16 to 45 years who were interested to participate and able to understand the nature and purpose of the study. Non-pregnant women and pregnant women who refused to participate, were unable to understand the nature and purpose of the

study or with history of use of steroids, a diagnosis of asthma, diabetes and hypertension, epilepsy, or other chronic diseases were excluded from the study.

Study sampling technique

Convenience sampling technique was used as a sampling method for selecting a sample for the study.

The sample size was calculated using the formula at 95% confidence interval.

For sample size

$$\text{Sample size (N)} = n = Z^2pq/d^2$$

Where,

Z = Normal deviate 1.96 which corresponds to 95% confidence interval

p = Proportion of the target population estimated to have desired characteristics

Here, $p = 0.852$ (Prevalence of gingivitis among pregnant women was found 85.2% on a previously conducted study).⁶

q: When p is in percentage terms: $(1-p) = (1-0.852) = 0.0.148$

d: degrees of freedom = 0.05

Using the formula, we needed a 194 sample for the study. Due to time constraint and limited resources, 170 samples were obtained for the study.

Research instruments and data collection

A pre-tested semi-structured Interview Questionnaire including a clinical checklist was developed. The English questionnaire was translated into the Bengali language.

Face to face interview was conducted by using the pretested semi-structured questionnaire. Clinical examination was carried out in daylight and records were written down in paper forms. Dental mirror and CPITN explorer was used for clinical examination.

Periodontal status and treatment need was assessed with Community Periodontal Index of Treatment Needs (CPITN)¹⁵ and oral hygiene status of pregnant women was assessed using the Simplified Oral Hygiene Index OHI-S.¹⁶

From CPI scores:

Code 0 (Healthy periodontium),

Code 1 (Mild gingivitis) and

Code 2 (Established gingivitis) were considered as gingivitis;

Code 3 (Mild periodontitis) and Code 1 (Oral hygiene needs improvement);
 Code 4 (Established periodontitis) was considered Code 2 (Oral hygiene needs improvement and
 as periodontitis. According to CPI Scores, treatment Professional scaling needed);
 need was categorized as: Code 3 (Oral hygiene improvement, professional
 Code 0 (No treatment needed); scaling and root planning needed) and

Table 1. Sample distribution in relation to different variables (n=170).

Variables		Frequency (%)	
Age group	16 -20 yrs	51(30.0)	
	21-25 yrs	44(25.9)	
	26-30yrs	62(36.5)	
	31-36 yrs	13(7.6)	
Religion	Islam	160 (94.1)	
	Hinduism	8 (4.7)	
	Christianity	2 (1.2)	
Residence	Urban Slum area	160 (94.1)	
	Urban Regulated area	10(5.9)	
Educational qualification	Illiterate	38 (22.4)	
	Primary level	62 (36.5)	
	Secondary level	44(25.9)	
	Higher secondary level/ Equivalent	25 (14.7)	
	University/ Equivalent	1 (0.6)	
Employment Status	Unemployed (149)	Homemaker	149 (87.6)
	Employed(21)	Teacher	2 (1.2)
		House Maid	15 (8.8)
		Garment worker	4 (2.4)
Personal Income	Not Employed	149 (87.6)	
	less than 5000	15 (8.8)	
	5000 up to less than 10000	5 (2.9)	
	10000 up to less than 15000	1(0.6)	
Frequency of teeth brushing	Not regularly	8 (4.7)	
	Once daily	79 (46.5)	
	Twice daily	76 (44.7)	
	More than two times daily	7 (4.1)	
Frequency of dental visit	Never	128 (75.3)	
	Once yearly for oral health check up	2 (1.2)	
	Whenever in need of treatment	40 (23.5)	
First pregnancy	Yes	81 (47.6)	
	No	89 (52.4)	
Previous pregnancy	One	55 (32.4)	
	Two	25 (14.7)	
	More than Two	9 (5.3)	
Pregnancy trimester	1 st trimester	31 (18.2)	
	2 nd trimester	81 (47.6)	
	3 rd trimester	58(34.1)	
Oral hygiene instructions during Antenatal Care visit	Yes	8 (4.7)	
	No	162 (95.3)	
Referral to dentist during pregnancy	Yes	14 (8.2)	
	No	156 (91.8)	
Total		170 (100)	

Code 4 (Oral hygiene improvement, professional scaling and Complex specialized surgical treatment is needed).¹⁵

Before data collection, the nature and purpose of the study was explained to respondents. Consent was obtained from all respondents.

Statistical Methods and Data analysis

Collected data were analyzed by SPSS 16 software (Statistical Package for Social Sciences, version 16) and descriptive statistics were used. Chi-square test was used for different variables. A probability value of <0.05 was taken as statistically significant.

Data presentation and interpretation and Data quality management

The data are presented here by Tables. The data quality was maintained by supervision, matching

raw and printed data and checking the consistency of data.

RESULTS.

The study covered a population of 170 respondents who attended the health center for antenatal care (ANC) services; 170 questionnaires were distributed to the respondents to obtain data. For easy analysis and interpretation of collected data different responses are categorized into different tabulations and then they are calculated as demonstrated in the following sections of the chapter.

Table 1 shows that most of the pregnant women belonged to the 26-30 years age group which is about 36.5% of the total respondents; mean age (\pm standard deviation, SD) for the respondents was

Table 2. Oral hygiene status and CPITN score of the respondents (n=170).

Variables		Frequency (%)
Oral Hygiene Status	Good	17 (10.0)
	Fair	86 (50.6)
	Poor	67 (39.4)
Periodontal Status (CPITN index)	Code 0 (Healthy Periodontium)	8(4.7)
	Code 1 (Mild Gingivitis)	38 (22.3)
	Code 2 (Established Gingivitis)	51 (30.0)
	Code 3 (Mild Periodontitis)	61 (35.9)
	Code 4 (Established Periodontitis)	12 (7.1)
Periodontal diseases (any form)	Present	162 (95.3)
	Absent	8 (4.7)

*CPITN: Community periodontal index of treatment needs.

Table 3. Distribution of the respondents by periodontal treatment need of the pregnant women (using CPITN Index) (n=170)

CPI Scor	Treatment Needs	Frequency (%)
Code 0	No Treatment Needed (0)	8 (4.7)
Code 1	Oral hygiene needs improvement (I)	38 (22.3)
Code 2	(I)+Professional scaling needed (II)	51 (30.0)
Code 3	(I)+(II) and root planning needed	61 (35.9)
Code 4	(I)+(II)+Complex specialized surgical treatment is needed	12 (7.1)

Table 4. Association of periodontal status (CPITN) in relation to variables (n=170).

Variables	Periodontal Status according to CPITN score					p-value	
	Code 0 (f) %	Code 1 (f) %	Code 2 (f)%	Code 3 (f)%	Code 4 (f)%		
Age group	16 -20 yrs	2(3.9)	17(33.3)	19(37.3)	11(21.6)	2(3.9)	0.000
	21-25 yrs	2(4.5)	5(11.4)	15(34.1)	18(40.9)	4(9.1)	
	26-30yrs	1(1.6)	16(25.8)	15(24.2)	27(43.5)	3(4.8)	
	31-36 yrs	3(23.1)	0(0.0)	2(15.4)	5(38.5)	3(23.1)	
Residence	Urban Slum area	0(0.0)	3(30)	1(10)	4(40)	2(20)	0.527
	Urban Regulated area	22(13.8)	29(18.1)	77(48.1)	21(13.1)	2(20)	
Educational qualification	Illiterate	3(7.9)	8(21.1)	5(13.2)	14(36.8)	8(21.1)	0.514
	Primary level	2(3.2)	6(9.7)	12(19.4)	33(53.2)	9(14.5)	
	Secondary level	5(11.4)	7(15.9)	8(18.2)	24(54.5)	0(0.0)	
	Higher secondary level/Equivalent	1(4)	4(16)	5(20)	9(36)	6(24)	
	University/Equivalent	0(0.0)	0(0.0)	0(0.0)	1(100)	0(0.0)	
Employment status	Unemployed : Homemaker	9(6)	22(14.8)	30(20.1)	68(45.6)	20(13.4)	0.023
	Employed : Teacher	0(0.0)	1(50)	1(50)	0(0.0)	0(0.0)	
	House Maid	0(0.0)	2(13.3)	0(0.0)	10(66.7)	3(20)	
	Garments worker	2(50)	0(0.0)	0(0.0)	2(50)	0(0.0)	
Personal income	Not Employed	9(6)	22(14)	30(20)	68(46.7)	20(13.3)	0.001
	less than 5000	0(0.0)	0(0.0)	0(0.0)	12(76.9)	3(23.1)	
	5000 up to less than 10000	2(40)	3(60)	0(0.0)	0(0.0)	0(0.0)	
	10000 up to less than 15000	0(0.0)	0(0.0)	1(100)	0(0.0)	0(0.0)	
Frequency of teeth brushing	Not regularly	1(12.5)	2(25.0)	1(12.5)	4(50)	0(0.0)	0.009
	Once daily	0(0.0)	7(8.9)	17(21.5)	41(51.9)	14(17.7)	
	Twice daily	7(9.2)	16(21.1)	12(15.8)	35(46.1)	6(7.9)	
	More than two times daily	0(0.0)	2(28.6)	2(28.6)	0(0.0)	3(42.9)	
Frequency of dental visit	Never	4(3.1)	17(13.3)	16(12.5)	16(40)	6(15)	0.000
	Once yearly for oral health check up	0(0.0)	0(0.0)	0(0.0)	72(56.2)	19(14.8)	
	Whenever in need of treatment	4(10)	10(25)	0(0.0)	2(100)	0(0.0)	
First pregnancy	Yes	5(6.2)	19(23.5)	15(18.5)	32(39.5)	10(12.3)	0.08
	No	3(3.4)	8(9)	17(19.1)	48(53.9)	13(14.6)	
Previous pregnancy (Parity)	One	0(0.0)	6(10.9)	8(14.5)	35(63.6)	6(10.9)	0.011
	Two	1(4)	2(8)	7(28)	11(44)	4(16)	
	More than Two	2(22.2)	0(0.0)	2(22.2)	2(22.2)	3(33.3)	
Pregnancy trimester	1 st trimester	2(6.5)	8(25.8)	6(19.4)	14(45.2)	1(3.2)	0.013
	2 nd trimester	2(2.5)	24(29.6)	30(37)	22(27.2)	3(3.7)	
	3 rd trimester	4(6.9)	6(10.3)	15(25.9)	25(43.1)	8(13.8)	
Referral for dental check up	Yes	2(14.3)	4(28.6)	8(57.1)	0(0.0)	0(0.0)	0.009
	No	6(3.8)	34(21.8)	43(27.6)	61(39.1)	12(7.7)	
Oral hygiene status	Good	6(35.3)	7(41.2)	4(23.5)	0(0.0)	0(0.0)	0.000
	Fair	2(2.3)	27(31.4)	28(32.6)	29(33.7)	0(0.0)	
	Poor	0(0.0)	4(6)	19(28.4)	32(47.8)	12(17.9)	

Chi-Square test *A Probability value of <0.05 was taken as statistically significant. *%: Within age in group.

24.22 (± 5.07) years (ranging between 16 and 35 years). About 94.1% of the pregnant women were Muslim and the majority of the respondents lived in the urban slum areas.

Only 14.7% of the total respondents had higher secondary or equivalent level education and 0.6% were university graduates. Among the total respondents, 87.6% of the pregnant women were homemakers (unemployed) with no income, and among the employed, the majority had their personal income below 5000 BDT. About 46.5% of the women were found to brush their teeth only once in a day, 4.7% of pregnant women did not even brush their teeth regularly and 75.3% of the respondents had never visited any dental clinic or hospital.

For 47.6% of the respondents, it was their first pregnancy; the number of previous pregnancies was more than two for 5.3% of the respondents and 47.6% were in their second pregnancy trimester. Among the pregnant women attending the health center, only 4.7% were found to receive oral hygiene Instructions and 8.2% had been referred for dental checkups during their ANC visit. Table 2 shows only 10% had good oral hygiene.

Only 4.7% had CPITN score 0 (Healthy periodontium). The majority of the respondents, 35.9%, had a CPITN score 3 (Mild periodontitis: pocket depth 4mm-5mm). The overall prevalence of periodontal diseases among pregnant women who attended the health center was found to be 95.3%, consisting of 52.4% for gingivitis and 43% for periodontitis.

Table 3 shows that the majority of the respondents (95.3%) were in need of periodontal treatment. The community periodontal index of treatment needs (CPITN) score was compared with different variables for association.

Table 4 shows that pregnant women's age, employment status, personal income, frequency of tooth brushing; frequency of dental visits, previous pregnancy (parity), pregnancy trimester, being referred for dental checkups and oral hygiene status were found statistically associated with periodontal status of pregnant women.

DISCUSSION.

The key finding of the study is that the majority of the respondents had periodontal diseases and were in need of periodontal intervention, representing 95.3% of all respondents. This is consistent with the study of Gupta *et al.*,⁴ who found prevalence of periodontal diseases to be 95% among pregnant women of Raichur District, India.

Age is an important factor associated with periodontal status of pregnant women; Severity of periodontal disease increases with age. This study found statistical association between maternal age and periodontal status. Code 2 (Established gingivitis); Code 3 (Mild periodontitis) and Code 4 (Established periodontitis) were more frequent among the age group 26-30 years. Though this was consistent with some studies,^{17,18} age was not significantly related to CPITN score of pregnant women according to another study⁷ (Table 4).

The low socio-economic status recorded in this study could have occurred because the study site was located within a non-profit organization in an urban slum setting. In a previous study, there was statistical association between periodontal status, educational qualifications and employment status of pregnant women.¹²

In our study there was a statistical relation between periodontal status and employment status ($p=0.023$) but no relationship was found with educational qualification ($p=0.527$) of the pregnant women. Pregnant women were divided into two groups of employed (according to their profession) and homemaker (unemployed).

The employed group had healthy periodontium or mild form of periodontal disease (Code 0, Healthy periodontium) and (Code 2, Established gingivitis), whereas advanced form of periodontal diseases (Code 3, Mild periodontitis) and (Code 4, Established periodontitis) were found among unemployed group in comparison with employed group. This was also consistent with the results of a previous study.¹⁷ In the present study, a statistical association was found regarding the pregnant woman's personal income ($p=0.001$).

Severity of periodontal diseases was found more often among pregnant women with no income or income below 5000 BDT in comparison to the others (Table 4). There was statistical association between periodontal status, frequency of tooth brushing ($p=0.009$) and frequency of dental visit ($p=0.000$). It is expected that brushing twice daily should improve oral hygiene; this was reflected in this study.

Good periodontal health and milder form of periodontal diseases (Code 0 and Code 1) were observed more often in respondents who brush twice daily, while worsened periodontal conditions (Code 3 and Code 4) were observed more often in respondents who brush once daily only or who do not brush regularly. Among the respondents 75.3% had never visited any dental clinic or hospital.

These findings were consistent with several other studies.^{7,17,18} Moderate to Severe periodontal conditions Code 3 and Code 4 were more frequent among the pregnant women who had never had any dental visit. This indicates that lack of a dental visit could lead to increased plaque accumulation that could result in worsened periodontal condition in the pregnant women (Table 4).

This finding reflects lack of awareness regarding tooth brushing and regular dental visits among pregnant women. Though some studies^{4,7,12} have not found any association between periodontal disease of pregnant women and pregnancy parity, in our study the association of periodontal disease with parity was statistically significant ($p=0.011$). Code 1 and Code 2 were more frequent in women with two or more previous pregnancies, while Code 3 and Code 4 were more frequent in pregnant women with one previous pregnancy (Table 4).

Some authors¹⁷ have also observed the relationship between parity and CPITN. This may happen due to occurrence of periodontal disease during first pregnancy and then negligence due to getting busier with children or to an increase in maternal age at further pregnancies.

Majority of the respondents (47.6%) were at their second trimester of pregnancy while for 34.1%, it was their third trimester and for 18.2%, it was their first

trimester of pregnancy. We found that pregnancy trimester was associated with periodontal status of pregnant women ($p=0.013$) and CPITN score increases with pregnancy trimester (Table 4).

Our finding is consistent with other study.^{4,7} However a study in South Brazil reported higher CPITN scores in the first trimester of pregnancy.²⁰ The women's oral hygiene status was scored good, fair and average. Surprisingly only 10% of respondents were found with good oral hygiene status. Lack of awareness, infrequent tooth brushing, low income status, and lack of dental visits may also have contributed to the poor oral hygiene conditions of the pregnant women. There was a statistical association between periodontal status and oral hygiene status of pregnant women ($p=.000$). As expected, advanced stages of periodontal condition (Code 3 and Code 4) were frequent in respondents with poor oral hygiene status.

Though this finding is not consistent with the finding of one previous study,⁴ but is consistent with another study⁵ where an association between poor periodontal condition with poor oral hygiene was reported. Oral health education programs need to be organized targeting pregnant women to increase their knowledge on frequency of tooth brushing, importance of dental visits and to improve oral hygiene status. In a study conducted in Bangladesh it was found that only 3% women were advised to have a dental checkup during pregnancy by their prenatal care providers.⁸

In that study, 8.2 percent of the total respondents were referred by their doctor for a dental checkup during their pregnancy. The CPITN score was compared with respondents being referred for dental checkups during their ANC visits and statistical association was found by Chi-square test ($p=.0009$).

Moderate to severe form of gingivitis and periodontitis (Code 2, Code 3 and Code 4) were frequent in respondents who were never referred by their doctor or health professionals (Table 4). This implies failure in timely referral and appropriate management, which leads to worsened periodontal

condition of pregnant women. Again, the majority of the pregnant women were in need of periodontal treatment according to severity. So attention should be given to early detection and timely management of periodontal diseases during pregnancy.

Limitation of the study

Limited funding has limited the location of data collection. We acknowledge the finding can-not be generalized due to limited sample size using convenience sampling technique. Further nationwide exploration is needed.

CONCLUSION.

The higher percentage of periodontal diseases among the pregnant respondents urges that 'Oral Health Education' and 'Periodontal Health Assessment' should be included as an integral part of antenatal clinic's checklists.

Physicians should be encouraged to motivate pregnant women to visit the dentist for a periodontal checkup during pregnancy, especially at the very beginning of the pregnancy.

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Chowdhury SF: Statistical analysis.

Chowdhury SF and Islam MdN: data interpretation .

Islam MdN: Supervision.

Chowdhury SF and Islam MdN: Drafting, editing and revision of the manuscript. Read and approved the final version of the manuscript.

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