Research Square

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Do socio-demographic factors, gravida, and parity influence salivary levels of active Matrix Metalloproteinase 8 in pregnant women?

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Abstract

Matrix metalloproteinases are a group of enzymes associated with the destruction of the extracellular matrix. Specifically, aMMP-8 is a good biomarker of periodontal destruction and preterm birth if its value is high around mid-trimester of pregnancy. However, gaps exist in whether other stressors, such as socioeconomic status, gravity, or parity in pregnant women, affect their salivary levels. This is with a view to validating the rapid chair-side use of aMMP-8 from the oral rinse and to screen for women predisposed to preterm birth and institute preventive treatment earlier.

Objectives: To determine the effects of socio-demographic status and gravida on the levels of aMMP-8 in the saliva of Nigerian pregnant women.

Method: A total of 128 pregnant women who presented at the ante-natal clinics (Eleyele Community Comprehensive Health Center and Enuwa Primary Health Center) for ante-natal care and who met the inclusion criteria and consented to the study were recruited. Patients were grouped based on their **periodontal inflammation caused by oral hygiene conditions** into Group A (Gingival index < 1) and Group B (Gingival index > 1). Detailed history included tribe, socio-economic status, gravida, and parity. Unstimulated salivary sample (5mls each) was collected from all participants. aMMP-8 of each salivary sample was determined using **Enzyme-Linked Immunosorbent Assay (ELISA).**

Results: The majority of the subjects belonged to the SES Group II (51.6%) and are of Yoruba extraction (78.1). Also, most of the women (34.4%) were pregnant for the third time; next were the primigravida women (29.7%). There was no significant variation in the distribution of the participants by tribe ($p = 0.73$), socio-economic status ($p = 0.45$), and gravida (0.84). The salivary levels of aMMP-8 are significantly higher in group B (20.14 ± 18.03) compared to group A (12.03 ± 8.62), irrespective of the tribe, socioeconomic status, gravida, or parity ($p = 0.00$). Analysis of variance shows no significant variation in the values of aMMP-8 in both groups, irrespective of their tribe ($F = 0.44$ and 0.31 for groups A and B, respectively). Similarly, there was no significant variation according to socioeconomic status in both groups ($F = 1.60$ and 0.69 in groups A and B, respectively) and gravida ($F = 0.89$ and 0.56, respectively, for groups A and B)

Conclusion: aMMP-8 as a biomarker to determine pregnancy outcome is not affected by tribe, socio-economic status, or gravida of Nigerian pregnant women.

Introduction

The National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” [1] Also, the World Health Organization (WHO), in collaboration with the International Labor Organization and the United Nations, defined a biomarker as “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease”. [2] A more comprehensive

definition considers the effects of treatments, interventions, and even unintended environmental exposure, such as to chemicals or nutrients, and not just the incidence and outcome of a disease. [3]

Biomarkers are used for diagnosis, staging, treatment monitoring, and/or maintenance. [4] They can be extrinsically or intrinsically generated. [4] Those intrinsically generated can be physical (clinical or radiographic) or laboratory markers that are assessed through physiological, pharmacological, biochemical, haematological, microbiological, histological, and genetic components [4] or through body fluids such as saliva. [5]

Aside from this, they are used to assess local or systemic inflammation,[6, 7,] and the relationship or causality between two or more disease entities or conditions. For example, the interrelationship between periodontal diseases and adverse pregnancy outcomes, diabetes mellitus, and chronic kidney diseases has been variously demonstrated by the use of different biomarkers. [8, 9] A group of such biomarkers that is recently gaining attention in the field of periodontology is the active Matrix Metallo-Proteinaise-8 (aMMP-8). Specifically, the relationship between aMMP8 and poor periodontal condition has been established, with a high value indicating poor periodontal health. [10, 11] In addition, evidence has shown that values of MMP8 above 23ng/ml in or around the second trimester could be a predictor of adverse pregnancy outcomes such as pre-term birth in pregnant mothers. [12] Furthermore, it has been established that pregnant mothers with high aMMP-8 from salivary samples are predisposed to adverse pregnancy outcomes. [12, 13] Also, simple non-surgical scaling and polishing treatment leads to a reduction in the aMMP-8 in salivary samples and consequently better pregnancy outcomes in such pregnant mothers. [13, 14, 15] This has led to the assumption that a relationship exists between poor periodontal condition and preterm birth and that early non-surgical periodontal intervention in pregnant women with poor oral hygiene can reduce the incidence of preterm birth. [16, 17] Similarly, IL-1β, TNF-α, C-Reactive Protein, ESR, etc., have also been used as surrogate markers for the improvement of systemic conditions such as COPD, diabetes mellitus, Alzheimer's disease, and rheumatoid arthritis when oral intervention such as scaling and polishing is done. [18]

As pointed out earlier, these systemic biomarkers are usually products of systemic inflammation and, therefore, in studies where they are used as surrogates, other sources of systemic inflammation that may provoke a rise in the level of such biomarkers are excluded. However, other non-disease sources of systemic inflammation, such as stress, poverty/nutritional status, gravida, and parity, are usually overlooked, especially in studies establishing a relationship between poor oral hygiene and preterm birth.

For a biomarker to be adjudged as reliable and reproducible in studies establishing a causal relationship between periodontitis and preterm birth, it should be sensitive and specific to the parameter to be measured, and it should not vary significantly with other factors such as age, sex, social status, gravida, and parity. The extent to which these biomarkers are influenced by the stressors mentioned above remains to be seen.

A biomarker that is of particular interest to gynaecologists and periodontologists is aMMP-8. The aMMP-8 marker is synthesised by polymorphonuclear neutrophils and is associated with periodontal destruction. [19, 20] The bacterial by-products, such as lipopolysaccharides (LPS), stimulate the cellular components of inflammation, which produce cytokines and enzymes that destroy the host's tissues in a bid to get rid of the bacteria and their by-products. [21, 22] This results in an inflammatory and catabolic process that destroys bone and collagen via the matrix metalloproteinases (MMPs)[22–25] resulting in a periodontal pocket. [21, 26] All of the above stimulate prostaglandin synthesis and release in addition to initiating neutrophil chemotaxis, infiltration, and activation, leading to the synthesis of matrix metalloproteinases (MMPs) and other bioactive substances. [27–32]

Many studies have been done to show the effect of periodontal diseases on pregnancy. [33, 34] However, there is little or no study done to show that this biomarker is not affected by socio-demographic parameters such as socio-economic status, educational attainment, gravida, and parity amongst pregnant women. The results of such an investigation will justify the use of aMMP-8 in periodontal medicine, especially for adverse pregnancy outcomes. It will further validate the rapid chair-side method of evaluating MMP-8 levels from oral rinse with a view to screening for women predisposed to preterm birth and instituting early preventive treatment.

Method

This was a cross-sectional survey of pregnant women in Ile-Ife, South West Nigeria. A total of 128 pregnant women who presented at the ante-natal clinic (Eleyele Community Comprehensive Health Center and Enuwa Primary Health Center) for ante-natal care and consented to the study and met the inclusion criteria were recruited. Approval to collect secondary data (Obstetric and Gynaecological history) from the hospital case notes of recruited participants was obtained from the Heads of the Department of Obstetrics and Gynaecology and Community Health, respectively. Ethical clearance for the study was obtained from the Ethics Committee of Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, with protocol number ERC/2016/11/10, and International Number: IRB/IEC/0004553. All experiments were performed in accordance with relevant guidelines and regulations as contained in the Declaration of Helsinki, 2024. Written informed consent to participate in the study was obtained from all of the study participants. Information extracted from the hospital case notes included tribe, occupation, educational level, and marital status.

The socioeconomic classification of the respondents was based on their occupation (Famuyiwa et al., modified by Opeodu et al.). [35]

Social Class I: executives, professionals (lawyers, doctors, engineers)

Social Class II: civil servants, nurses, secretaries

Social Class III: semi-skilled – fashion designer, caterer, hairdresser, housewives

Social Class IV: unskilled – messengers, auxiliary nurses, labourers

Social Class V: students

A detailed and thorough medical history of the participants was taken. The history of pregnancy, such as gravida and parity, and other history to exclude participants with confounding details (e.g., previous pregnancy history, complications, etc.) were extracted. Extra-oral and intra-oral examinations were performed on each participant on a dental chair under dental light. Previous studies have shown that aMMP-8 varies with periodontal destruction, and to control for this, the participants were grouped into two groups (A and B) based on their periodontal inflammatory status using the Gingival index (GI). Group A includes participants with minimal periodontal inflammation **(good oral hygiene)** (GI <1mm), while Group B comprises those with moderate to poor periodontal inflammation (**poor oral hygiene)** (GI >1mm). Sterile dental mouth mirrors and sterile periodontal probes were used for the intra-oral examination and periodontal examination, respectively. The intra-oral findings were communicated to the participants, and those with poor periodontal inflammation and poor oral hygiene were referred for scaling and polishing, and root planning, while those with minimal periodontal inflammation and good oral hygiene were given oral hygiene instructions. After examination, about 5 mL of an unstimulated salivary sample for all subjects was collected. The samples were then stored in a freezer at 4 degrees Celsius. aMMP-8 of each salivary sample was determined following the standard assay procedure for the determination of aMMP-8 from salivary samples. Enzyme-Linked Immunosorbent Assay (ELISA) was used to assess pre- and post-intervention aMMP-8 levels quantitatively. Data analysis was done using IBM SPSS Version 25, Chicago, USA. Descriptive statistics were used to characterize the sociodemographic variables such as tribe, parity, gravida, and socioeconomic status. For continuous variables such as age and MMP8, the mean, standard deviation, and range were determined. A one-way ANOVA was used to explore any variation in their tribal, pregnancy history, and socio-economic status. A Post hoc (Games-Howell) test was performed, where there was a significant F change. Statistical significance was set at $p < 0.05$.

Results

The majority of the participants were from the Yoruba ethnic group (78.1%), followed by the Igbos (13.3%) and other ethnic groups listed in **Table I**. The educational level attained did not correspond with their occupation. Hence, it may not reflect the socio-economic status (SES) of the participants; however, it reflects the recent societal norm in the country and, thus, justifies the use of occupational status to classify the subjects in the present study. The socio-economic status (SES) of the subjects was classified based on the occupation of the respondents as postulated by Famuyiwa et al. and modified by Opeodu et al. [35] The majority of the subjects belonged to the SES Group II. Also, there were more unskilled respondents in the control group (14.1%) than in the test group (7.8%); however, there was no significant difference in the respondents' socio-economic class according to their group ($p = 0.11$) (**Table I**).

Table I: Socio-demographic characteristics of the subjects according to their group

Socio-demographic Characteristics	Group B		Group A		Total		p
	N	%	N	%	N	%	
TRIBE							
YORUBA	50	82.0	50	74.6	100	78.1	0.73*
IGBO	6	9.8	11	16.4	17	13.3	
HAUSA	1	1.6	1	1.5	2	1.6	
MINORITY	4	6.6	5	7.5	9	7.0	
TOTAL	61	100	67	100	128	100	
SOCIAL -ECONOMIC STATUS							
SES I	5	3.9	4	3.1	9	7.0	0.45*
SES II	36	28.1	30	23.4	66	51.6	
SES III	4	3.1	6	4.7	10	7.8	
SES IV	10	7.8	18	14.1	28	21.9	
SES V	6	4.7	9	7.0	15	11.7	
TOTAL	**61**	**47.7**	**67**	**52.3**	**128**	**100.0**	
EDUCATIONAL LEVEL							
SECONDARY	9	7.3	17	13.7	26	21.0	0.11**
TERTIARY	51	41.1	47	37.9	98	79.0	
TOTAL	**60**	**48.4**	**64**	**51.6**	**124**	**100.0**	
*** Likelihood ratio chi-square**							

** Pearson chi-square. Four participants did not indicate their educational level

The majority (34.4%) of the women under investigation in both the test and the control groups were pregnant for the third time; next were the primi-gravida women (29.7%). However, there was no statistically significant difference in the number of previous pregnancies by group among the women ($p > 0.05$) (**Table II**).

Table II: Gravida of the subjects according to group

Pregnancy history	Group				Total		p
	Group B		Group A				
N	%	N	%	N	%		
GRAVIDA							
1	20	15.6	18	14.1	38	29.7	0.84*
2	12	9.4	17	13.3	29	22.7	
3	21	16.4	23	18.0	44	34.4	
4	8	6.3	9	7.0	17	13.3	
TOTAL	**61**	**47.7**	**67**	**52.3**	**128**	**100**	
*** Likelihood ratio chi-square**							

Table III shows the mean of salivary aMMP-8 in both the test and the control group. A significantly higher mean value for salivary aMMP-8 level was found in the test group compared to the control group (p = 0.00). This reveals that the subjects with high GI above 1 had a significantly higher mean value of salivary aMMP-8 level than those with lower values of GI.

The study explored whether there is a variation in the mean value of salivary aMMP-8 according to the tribe, socio-economic status, and the number of previous pregnancies (gravida) in the pregnant women. One-way ANOVA indicated no significant variation in the aMMP-8 mean values in the salivary samples in both groups according to the tribes. Furthermore, there was no significant variation in the mean difference in aMMP-8 levels across the tribes (**Table IV**). Similarly, there was no variation in mean values of salivary aMMP-8 in both groups according to the socioeconomic status (**Table V**) and the previous number of pregnancies (**Table VI**).

Hence, the results show that the socioeconomic status, tribe, and the number of pregnancies did not influence the mean salivary aMMP-8 levels of the subjects allocated into the two groups.

Table III: Mean value of aMMP-8 AND GI in the test and the control group

aMMP-8 status	Group	N	Mean	SD	t	p
aMMP-8	B	52	20.14	18.03	2.94	0.00
GI	A	55	12.03	8.62		
	B	61	1.10	0.24	3.27	0.00
	A	67	0.94	0.32		

Table IV: One-way ANOVA of the mean values of salivary aMMP-8 according to ethnic group

Group	aMMP-8 status		Sum of Squares	Df	Mean Square	F	p
B	aMMP-8 before delivery	Between Groups	316.07	3	105.36	0.31	0.82
		Within Groups	16255.00	48	338.65		
		Total	16571.061	51			
A	aMMP-8 before delivery	Between Groups	101.98	3	33.99	0.44	0.72
		Within Groups	3910.72	51	76.68		
		Total	4012.71	54			

Table V: One-way ANOVA of the mean aMMP-8 values according to the SES

Group	aMMP-8 status		Sum of Squares	Df	Mean Square	F	p
B	aMMP-8 before delivery	Between Groups	915.74	4	228.94	0.69	0.60
		Within Groups	15655.32	47	333.09		
		Total	16571.06	51			
A	aMMP-8 before delivery	Between Groups	445.05	4	111.26	1.60	0.20
		Within Groups	3567.65	50	71.35		
		Total	4012.71	54			

Table VI: One-way ANOVA of the mean salivary aMMP-8 values by the number of pregnancies.

Group	aMMP-8 status		Sum of Squares	Df	Mean Square	F	p
B	aMMP-8 before delivery	Between Groups	556.52	3	185.51	0.56	0.65
		Within Groups	16014.54	48	333.64		
		Total	16571.06	51			
A	aMMP-8 before delivery	Between Groups	199.91	3	66.64	0.89	0.45
		Within Groups	3812.79	51	74.76		
		Total	4012.71	54			

Discussion

Efforts have been made to incorporate active-matrix metalloprotease-8 (aMMP-8) as a biomarker in the recent grading and staging of periodontal diseases because of its ability to readily identify, predict, and prevent the disease. [36, 37] In addition to its ability to quantify or measure the severity of periodontal destruction, it also predicts the occurrence of adverse pregnancy outcomes when its value is higher than 23ng/ml in or around the second trimester. [12] There is now evidence of a relationship between periodontal inflammation and adverse pregnancy outcome such that non- surgical periodontal treatment in the second trimester reduces the risk of preterm birth by lowering the salivary and systemic levels of aMMP-8 in pregnant mothers with periodontal inflammation caused by poor oral hygiene.

Aside from periodontal diseases, other known confounders to high levels of salivary aMMP-8 are malaria, chronic inflammatory diseases such as arthritis, cancers, and increased parity (especially greater than four). Once the above are controlled for, aMMP-8 may be a good predictor of preterm birth (PTB) in pregnant mothers. Recently, a rapid chair-side technique was developed by Sorsa et al with the aim to diagnose, stage, and monitor disease progression and or treatment in patients with periodontitis. [36] This same method may be a cheaper, safer, and rapid method of predicting pregnant mothers likely to have adverse pregnancy outcomes so that appropriate preventive measures can be instituted. Yet, how the levels of aMMP-8 are influenced by stressors such as socio-economic status and gravida has not been assessed. [37]

This present study controlled for other factors such as adverse pregnancy history, malaria, and other inflammatory conditions by excluding such pregnant women from the study. However, other factors that could induce stress, such as ethnicity/tribe, socio-economic status, and gravida/parity, were not controlled for. Therefore, this study seeks to determine if ethnicity, SES, and gravida contribute to fluctuation in aMMP-8 level with a view to validating the use of rapid chair-side use of the aMMP-8 kit as a method of detecting pregnant women predisposed to preterm birth.

There are variations in the incidence of preterm birth between Blacks, Caucasian and other minority tribes in the USA and European countries. [38, 39] Studies have shown variation in the levels of aMMP-8 between blacks and whites, with the blacks having higher aMMP-8 levels. [40] This probably may explain why the incidence and prevalence of pre-term birth are high even among blacks living in the developed world with access to good maternity care. The reason for the differences is not clear, but it is likely to be due to genetic differences. Apart from racial variations, intra-population variations also exist, but presently, no study has determined the aMMP-8 salivary levels among the different ethnic groups in Nigeria.

This present study found no significant variation in the salivary levels of aMMP-8 among the different ethnic groups studied, irrespective of their periodontal status. One would have expected slight differences because of the different genetic makeup of each ethnic group/tribe. However, the genetic admixture over time due to intertribal marriage might have affected our results.

The socioeconomic status of an individual has been used as a measure of nutrition and social well-being. Those with high socio-economic status are considered to have reasonable high level of education, access to health care, and good nutritional status. On the contrary, low socio-economic status is associated with poverty. Poor nutritional status is a known stressor capable of evoking both systemic and local inflammation. For example, necrotizing ulcerative gingivitis (NUG) and cancrum oris are found mainly in children or adults suffering from severe malnutrition. [41] Up until now, no study has determined any association between socio economic status of an individual and the salivary levels of aMMP-8. Our study showed that socio-economic status, which is a reliable measure of nutritional status, does not affect the aMMP8 salivary levels of pregnant women in both groups. No evidence has been adduced for our findings. A plausible reason may be that the local and systemic effects of malnutrition may not be evident until it is severe, and tissue breakdown is likely to occur, thereby leading to high salivary and or systemic levels of aMMP-8. None of our participants showed any form of severe malnutrition, even though some of them belonged to the lower social class. Further studies are needed to verify our assumption.

Furthermore, the present study explored the effect of gravida (pregnancy history) on aMMP-8 levels but found no significant influence. Though studies have shown that women with a gravida greater than 4 are likely to have preterm birth. [42, 43] These groups of participants were excluded from the study. No reason can be adduced for our result. It may be that greater than gravida 4 is the critical point at which MMP-8 levels reach the critical levels, thus predisposing such women to preterm birth. Previous studies on the association between periodontal status and pregnancy outcome have excluded twining and multiple pregnancies and women with high parity. Presently, no study has assessed the aMMP-8 levels in women with a gravida greater than 4. Such studies are needed to validate our assumptions.

Matrix metallo-proteinase-8, a collagenase, is known as a marker of periodontal destruction[43] and systemic inflammation. [44, 45] The present study showed that those pregnant women with moderate to severe periodontal inflammation had significantly higher levels of aMMP-8 compared to those with good or mild periodontal inflammation. Recent studies have shown that aMMP-8 should be considered an adjunct in the management of periodontal diseases, as it can measure and predict future periodontal destruction in addition to its features as a good biomarker due to its sensitivity to disease activity and management. [37, 46, 47, 48]. Our results further validate these assertions that aMMP-8 can be used to quantify, stage, monitor progression, and treatment outcome of periodontal disease.

It is also known that salivary aMMP-8 correlates with systemic markers. [49] Therefore, in pregnant women, if the oral hygiene is good with high salivary aMMP-8, the systemic MMP-8 level will probably be high with a high propensity for delivering prematurely. The reversal can also occur – a pregnant woman with poor oral hygiene leading to a high level of salivary aMMP-8 will have higher-than-normal MMP-8 (due to increase from the mid-trimester increase from the pregnancy itself), leading to a higher-than-normal amount of systemic aMMP-8, predisposing the woman to preterm birth. Therefore, salivary aMMP-8, aside from its use to quantify periodontal destruction and monitor treatment outcome, could

be used to predict pregnant women who may likely have preterm birth or other pregnancy outcomes. This is even more so because the salivary values of aMMP-8 do not vary by socioeconomic status and gravida (para < 5).

Conclusion

The salivary levels of aMMP-8 in Nigerian pregnant women do not vary by socio-demographic factors and gravida less than 5. We propose that the rapid chair-side kit developed for quantifying periodontal destruction could also be used to screen pregnant women who may be predisposed to pre-term birth to institute early preventive treatment against preterm birth.

Declarations

Ethics approval and consent to participate

Ethical clearance for the study was obtained from the Ethics Committee of Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, with protocol number ERC/2016/11/10, and International Number: IRB/IEC/0004553. All experiments were performed in accordance with relevant guidelines and regulations as contained in the Declaration of Helsinki, 2024. Written informed consent to participate in the study was obtained from all of the study participants.

Consent for publication

Not Applicable

Competing interests

There are no competing interests

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Author Contribution

Contribution to joint authorshipMT wrote the main manuscript text SO reviewed the article and contributed to writing the manuscriptMA reviewed the manuscriptOB reviewed the manuscript.

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Data Availability

The data that support the findings of this study are available from the Department of Preventive and community Dentistry, Faculty of Dentistry, Obafemi Awolowo University, Ile-Ife, but restrictions apply to the availability of these data, which were used under licence for the current study and so are not publicly available. The data are, however, available upon request and with the permission of Ethics Committee of Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife.

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