

Research Paper

Black Women's Predisposition to Preterm Birth; Could We Be Near The Answer?

Background

Pre-term Premature Rupture of Membranes (PPROM) is attributable to several causes including asymptomatic bacterial vaginosis among Caucasians and is commoner among black pregnant women. While malaria and high Body Mass Index (BMI) have been reported among Nigerians, the influence of metalloproteinases on PPRM has never been studied in Nigeria.

Methods

Qualitative estimation of active matrixmetalloproteinase-8 (a-MMP-8)for the purpose of determining the influence of chronic periodontitis on time to conception among Nigerian Pregnant women led to the accidental discovery of an almost universally elevated levels of a-MMP-8 among all pregnant women assessed. Values of a-MMP-8 were compared across demographics of participants as well as educational status, BMI and other parameters.

Results

One hundred and seventeen (117, 87.3%) of the 134 participants had elevated a-MMP-8 based on a novel qualitative assessment using salivary diagnostics. Levels were increased across all age groups independent of age, Estimated Gestational Age(EGA), BMI, educational level and trimester of pregnancy.

Conclusion

Prevalence of active matrixmetalloproteinase-8 level was found to be higher among participants in the present study compared with Caucasians independent

of demographic, educational and pregnancy trimester. Reasons for the association need to be further investigated.

Key words

Preterm birth, Matrixmetalloproteinase, aMMP-8, Nigeria, Racial

Background

Time to conception has been reportedly associated with periodontitis¹. In their study, the prevalence of periodontitis was significantly higher in pregnant women who took more than 12 months to conceive compared to those who took less than 12 months to conceive (34.9% vs 25.7%)[1]. Furthermore, in the study, non-Caucasian pregnant women had a higher prevalence of periodontal disease than Caucasian women (41.4% vs 23.8%) having an increased likelihood of TTC > 1 (13.9% vs 6.2%, OR = 2.88). The conclusion of their finding was that the presence of periodontal disease was a modifiable risk factor which affected a woman's time to conceive particularly among non-Caucasian women [1]. In order to investigate this association, we undertook a cross-sectional estimation of periodontitis risk among pregnant women in Nigeria at the University of Abuja Teaching Hospital. The tool we used in our assessment is a novel method in salivary diagnostics which utilizes a bio-marker for periodontitis. [2] The bio-marker performs a qualitative assessment of Neutrophil collagenase-2 also known as active matrix metalloproteinase-8 (aMMP-8) which selectively detects elevated levels of the enzyme as a risk-marker for chronic periodontitis [3] so delineated to differentiate it from the latent MMP-8 which corresponds to gingivitis experience.[4]

However, the study exposed a dimension the authors never conceived at inception-- a shockingly large percentage of study participants had elevated aMMP-8- such percentages of elevated aMMP-8 as has never been reported.

This triggered our interest in discovering the reason for this wide disparity. Since the study was carried out among Nigerians, we suspected a racial connection between our participants and elevated aMMP-8 levels. This prompted us to perform a further search of literature to find explanations for our findings.

The role of metalloproteinases in causing premature rupture of membranes and consequent preterm birth under the influence of bacterial lipopolysaccharide (LPS) is established [5,6] These established positions raise so many questions.

First, are there racial differences in the expression of aMMP-8? If such an association can be validated it might help to explain two things the unexpected results in this study and increased prevalence of preterm birth among blacks compared with Caucasians. [7]

Secondly, since aMMP-8 responds to LPS stimulation [5,6] are there racial differences in the sensitivity of blacks to LPS? A racial difference in such sensitivity will translate to greater expression of aMMP-8 among blacks.

An establishment of racial preferences in aMMP-8 levels will help to explain the relative preponderance of pre-term birth among black pregnant women. Interventions targeted at aMMP-8 reduction will therefore help to reduce the prevalence of pre-term birth among black pregnant women.

Methods

Consent and confidentiality: The study was performed in all aspects in accordance with the World Medical Association Declaration of Helsinki after approval was obtained from the ethics committee of the University of Abuja Teaching Hospitals. Written informed consent (appended signatures) was obtained from all study participants and participation was completely voluntary and no personally identifiable information were recorded.

Study Population, Setting and Instrument: Study participants were all pregnant women within the age range 20-45 years, mean age 30.6 years with a standard deviation of 4.5, modal age of 30 years and Body Mass Index (BMI) of 17.8-46.9.

All patients were systemically healthy non-smokers (except one smoker) with gravidity ranging from 1-8, parity of 0-5, estimated gestational age (EGA) of 4-36 weeks and last child birth duration (LCB) of 0-126 months. Duration of last child birth was computed as the time between last child birth and the time when the present study was conducted-measured in months.

Sampling: Purposive, non-random sampling method which involved sequential recruitment of all new willing patients registering at the antenatal clinic of the obstetrics and gynecological clinic of the hospital was done.

Calibration and Examination: The examination was done by one periodontologist and two dental therapists. All examiners were calibrated to recognize the color change prior to commencing the examination and the percentage agreement was 100 percent due to the clarity of the blue color change(Figure 1). aMMP-8 was qualitatively assessed using lateral flow immunoassay test kit which is a point-of-care chair-side immunoassay kit which selectively detects elevated levels of aMMP-8. Participants had to rinse their mouth with tap water for thirty seconds and then wait for one minute.

This was followed by a 30 seconds rinse with the distilled water that came with the kit and the saliva was collected in a small sterile dish that came with the kit. About 3 ml of the saliva was then drawn up with the syringe and a filter is fitted. Three to four drops of the saliva was then placed in the immunoassay dish and the results read-off within 5 minutes and not beyond 10 minutes from starting time.

The result was read-off as a color change. One blue line indicates a negative test (normal aMMP-8) while two lines indicates a positive test (elevated aMMP-8) (Figure 1). Even a faint line indicated a positive test according to the manufacturer's instructions.

Statistical analysis: Statistical analysis was performed with the PASW-18 (SPSS) statistical software to analyze uni-variate data such as frequencies, means and standard deviation. Chi-square statistic was applied using cross-tabulations to

examine the relationship between the outcome variable (aMMP-8 test result) and several independent (explanatory) variables like age, estimated gestational age, BMI, last child birth etc. The confidence level of the test was set at 95% therefore p-values yielding results ≤ 0.05 were accepted as being statistically significant.

Results

A total of 134 participants were seen in this study. Overall, 117 of 134 (83.7%) participants had elevated aMMP-8 levels. aMMP-8 levels were generally elevated in participants across all age groups with no defined pattern. The participants aged 20-45 years showed no difference in aMMP-8 levels across all age groups--(Table 1).

Based on BMI, 53 of 117(45.3%) were of normal weight, 46(36.8%) were overweight and 18 (15.4%) were obese. aMMP-8 levels were generally elevated in participants across all BMI values with minor differences but there were no significant differences in aMMP-8 levels among participants by BMI-($p= 0.62$) (Table 2).

aMMP-8 levels were generally elevated in participants across all levels of education following a definite pattern: aMMP-8 levels increased with level of educational qualification but the differences failed to attain statistical significance-($p= 0.16$) (Table 3).

aMMP-8 levels were generally elevated in participants across all trimesters of pregnancy following a definite pattern but aMMP-8 levels increase with trimester of pregnancy failed to attain statistical significance-($p= 0.78$) (Table 4).

aMMP-8 levels were generally elevated in participants irrespective of duration of last child birth. The relationship between duration of last child birth and aMMP-8 levels followed no defined pattern--(Table 5).

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141 **Discussion**

142 Fetal membranes are susceptible to the effect of matrix metalloproteinases
143 similar to other extracellular matrices anywhere in the body. Any mechanism
144 leading to a premature degradation of these tissues are likely to lead to preterm
145 birth. Matrix metalloproteinases have been implicated in the chain of events
146 leading to preterm birth [8].

147 Reasons for the higher prevalence of preterm birth among blacks have remained
148 largely speculative. While asymptomatic bacterial vaginosis have been implicated
149 in their etiology [9], it is well-known that the final pathway in the pathogenesis of
150 preterm birth is through the breakdown of the delicate membranes-- an action
151 performed by matrix metalloproteinases-[8] The question is; are blacks
152 genetically predisposed to producing more matrix metalloproteinases than
153 Caucasians?

154 Again, the role of tissue inhibitors of metalloproteinases (TIMPs) is considered as
155 important as that of the matrix metalloproteinases themselves. [10] Now, do the
156 {TIMPs}-play any role in our findings and could their expression also be racially
157 influenced? Are these racial differences completely genetic, environmental or
158 both?

159 While the current report lacks the strength to answer these questions, we believe
160 we have have stumbled upon a probable explanation for the previously
161 unexplained predisposition of black women to preterm birth albeit accidentally.
162 These findings would hopefully provide a template for in intense research into
163 why black women are predisposed to preterm birth.

164 The present study found increased levels of mouthrinse aMMP-8 among
165 pregnant women independent of their age, educational qualification, gestational
166 age, duration of last child birth and BMI. This is different from recent findings
167 among Caucasians. [11]

While BMI had no impact in aMMP-8 assessment in the present study, it appeared to be a major risk factor for preterm birth in a Nigerian Teaching Hospital. [12]

Neither the presence nor history of bacterial vaginosis was assessed in the present study but the role of intrauterine infections in preterm birth through Preterm Premature Rupture of Membranes (PPROM) is well-reported in literature. [13, 14] Equally already established in literature is the mediating role of lipopolysaccharide (LPS) from the gram negative bacteria cell walls. LPS leads to the release of enzymes that remodel the extracellular matrix through activation of the innate immune system. [5,6] This position is easily explained by a report that LPS is recognized by the innate immune system.[15] Since the production of matrix metalloproteinases is a final pathway in the pathogenesis of preterm birth and PPRM, we believe that aMMP-8 estimation is sufficient for the scope of the current study.

The fact that all participants in the present study were Nigerians raised our suspicion of a racial twist in our observations. Only recently, a likely racial tendency in host response to the influence of LPS was reported. [16] While African-Americans were twice more sensitive to E.coli, their Caucasian counterparts showed significantly greater sensitivity to LPS [16] The pathway through which this recognition occurs has been identified as a signal transduction pathway possessing the capacity to increase the production of pro-inflammatory cytokines and matrix metalloproteinases.[13]

Furthermore, Ferdinand and colleagues [17] explored the genetic pathway in an attempt to explain this unexplained predisposition of black women to PPRM through mutations in the human gene CARD15 of the NOD1/APAF1 gene family and the potentially protective TLR4 variant alleles but failed to find a difference between the expression of these mutant and alleles between black Americans and Caucasians. This means the pathogenesis of this differential prevalence is either not genetic or occurs through yet-to-be identified genetic pathways.

Perhaps the answer to the dilemma does not lie in complex genetics but in simple quantitative dynamics of matrix metalloproteinases. By this we propose that the simple reason why black women are selectively predisposed to preterm birth may be due to an increased production of matrix metalloproteinases. The genetic angle could then be considered in an attempt to explain why black women seem to produce more matrix metalloproteinases compared to their Caucasians counterparts.

Observations from recent studies are in favor of our position. Since LPS levels directly correlate with infection, it might possibly imply that black women simply suffer more intrauterine infections than their Caucasian counterparts. The finding of greater prevalence of amniotic fluid markers of inflammation by Guinn and colleagues and Fiscella corroborate this suggestion.[18, 19] The infections angle is further supported by reports of elevated matrix metalloproteinases among women suffering from bacterial vaginosis compared with healthy controls.

In Nigeria, Adesiji and colleagues found no association between bacterial vaginosis and preterm birth [20]. The closest link was the association with malaria in a recent report [21]. A foundational statement by Fiscella “Significantly higher rates of bacterial vaginosis among black women may account for nearly 30% of the racial gap in preterm births” [9] is strong enough reason to further investigate these associations among Nigerian women using well-designed study protocols.

The likelihood that matrix metalloproteinases play a major role in determining the predisposition of black women is further supported by a report which cited a polymorphism in MMP-9 promoter as being positively associated with an increased risk of preterm birth among African-Americans [7]. Their findings were further corroborated by the report by Fujimoto and colleagues which observed an increased risk of preterm birth attributable to a single nucleotide polymorphism in MMP-1.[22]

Moyer reported on the benefit of MMP-8 as a risk marker of intra-amniotic inflammation and a useful tool in identifying the presence of intra-amniotic inflammation in preterm PPROM patients. [8]

Apart from these possible mechanisms, at least two other positions have been proposed in literature. First, a hypothesis that defects in the caspase system could affect the programmed death of inflammatory cells leading to an overactive immune system which in turn potentially causes an increased release of inflammatory mediators. [23] It is well-recognized that African-Americans tend to have higher systemic inflammation than Caucasians [24] and more inflammatory variants of Pelvic inflammatory disease have been reported in Africans compared with Caucasians.[25]

The second position is a microbiological explanation which posits that P. intermedia can up-regulate MMP production.[26] It is however not clear if there are any racial differences in P. intermedia and how this could have affected our findings.

Conclusions

There appears to be a yet-to-be explained racial difference in the differential production of aMMP-8 among black pregnant women compared with their Caucasian counterparts. This racial difference partly explains the differential preponderance of pre-term birth among black pregnant women.

Limitations:

The present study was a qualitative assessment of aMMP-8 levels which simply detected whether mouthrinse levels of aMMP-8 were normal or elevated among black pregnant women. Future studies should involve actual quantitative aMMP-8 measurements through well designed controlled studies using larger sample sizes in order to confirm or refute these findings.

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256 **Competing interests**

257 Nil

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259 **Authors' contributions**

260 All authors were involved in conceptualization, data collection and eventual
261 approval of final draft for submission.

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349 **Tables and figures**

aMMP-8 Level by Age Group

		aMMP-8 Level					
		Normal		Elevated		Total	
		n	%	n	%	n	%
Age Group	20 to 25 Years	2	11.8	15	88.2	17	100
	26 to 31 Years	9	13.8	56	86.2	65	100
	> 31 Years	6	11.5	46	88.5	52	100
Total		17	12.7	87.3	117	134	100

350 Table 1: aMMP-8 levels by age group. aMMP-8 Levels were elevated across all age groups. $X^2=$
 351 0.154, df= 2, p= 0.93

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aMMP-8 Level by Basic Metabolic Index

		aMMP-8 Level					
		Normal		Elevated		Total	
		n	%	n	%	n	%
Basic Metabolic Index	Normalweight	8	15.1	45	84.9	53	100
	Overweight	4	8.7	42	91.3	46	100
	Obese	2	11.1	16	88.9	18	100
Total		14	12.0	103	88.0	117	100

354 Table 2: aMMP-8 levels by BMI. aMMP-8 Levels were elevated across all BMI values.

355 $\chi^2 = 0.972$, df = 2, p = 0.62

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aMMP-8 Level by Educational Qualification

		aMMP-8 Level					
		Normal		Elevated		Total	
		n	%	n	%	n	%
Qualification	Up to Secondary School	10	19.6	41	80.4	51	100
	Diploma	4	9.5	38	90.5	42	100
	Degree	3	7.3	38	92.7	41	100
Total		17	12.7	117	87.3	134	100

357 Table 3: aMMP-8 levels by Qualification. aMMP-8 Levels were directly proportional to
 358 educational level. Differences did not attain statistical significance. $X^2= 3.652$, $df= 2$, $p=$
 359 0.16.

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aMMP-8 Level by Pregnancy Trimester

		aMMP-8 Level					
		Normal		Elevated		Total	
		n	%	n	%	n	%
Trimester	1 st Trimester	4	15.4	22	84.6	26	100
	2 nd Trimester	10	13.2	66	86.8	76	100
	3 rd Trimester	3	9.4	29	90.6	32	100
Total		17	12.7	117	87.3	134	100

362 Table 4: aMMP-8 levels by trimester. aMMP-8 Levels were directly proportional to
 363 trimester. Differences did not attain statistical significance. $X^2 = 0.503$, $df = 2$, $p = 0.78$

364 *2 cells (33.3%) have expected count less than 5 hence table for description only, X^2 not
 365 valid.

aMMP-8 by Last Child Birth Duration

	aMMP-8 Level					
	Normal		Elevated		Total	
	n	%	n	%	n	%
LCB (months) Up to 25 months	2	11.8	15	88.2	17	100
Up to 31 months	9	13.8	56	86.2	65	100
> 31 months	6	11.5	46	88.5	52	100
Total	17	12.7	117	87.3	134	100

366 Table 5: aMMP-8 levels by LCB duration. aMMP-8 Levels were elevated across all LCB
 367 durations. $X^2=0.154, df= 2, p= 0.926$

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371 Figure 1: aMMP-8 Test Kit showing control test lines.