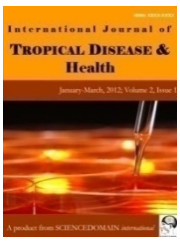

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Black Women's Predisposition to Preterm Birth; Could We Be Near The Answer?

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Authors' contributions

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

Original Research Article

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ABSTRACT

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 PPROM has never been studied in Nigeria.
Methods: A qualitative estimation of active matrixmetalloproteinase-8 (a-MMP-8) to assess the effect of chronic periodontitis on time to conception led to an accidental discovery of widespread elevation of a-MMP-8 among pregnant participants. Values of a-MMP-8 were compared across demographics of participants as well as educational

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status, BMI and other parameters.
Results : One hundred and seventeen of 134 participants (117, 87.3%) had elevated a-MMP-8 based on a novel qualitative assessment using salivary diagnostics. Levels were increased across independent of age, Estimated Gestational Age(EGA), BMI, educational level and trimester.
Conclusion: This population of black pregnant women exhibited higher a-MMP-8 levels than reported among pregnant Caucasians independent of demographics, educational level and trimester of pregnancy. Reasons for the association need to be further investigated.

Keywords: Preterm birth; Matrix metalloproteinase; aMMP-8; Nigeria; Racial.

1. BACKGROUND

An association between periodontitis and increased time to conception has been reported. When increased time to conception was taken as conception occurring after 12 months, a study found greater prevalence of increased TTC among pregnant women suffering from periodontitis compared with periodontally-healthy controls (34.9% vs 25.7%)[1]. This observation was significantly pronounced among non-Caucasian women. (13.9% vs 6.2%, OR = 2.88). The workers therefore concluded that presence of periodontal disease may be a modifiable risk factor for increased time to conception particularly among non-Caucasian women.[1]

The current study --a cross-sectional estimation of periodontitis risk among black pregnant women at the University of Abuja Teaching Hospital- Nigeria, utilized a novel salivary bio-marker for periodontitis [2] to qualitatively assess levels of Neutrophil collagenase-2 (active matrix metalloproteinase-8) (aMMP-8). aMMP-8 selectively detects elevated levels of the metalloproteinase-8 and therefore qualifies as a selective risk-marker for chronic periodontitis [3,4]. The study however exposed a new finding-- a shockingly large percentage of study participants had elevated aMMP-8 levels than has ever been reported. Authors suspected a racial connection because all participants were black women.

It is established that bacterial lipopolysaccaride upregulates metalloproteinases which in turn induce premature rupture of membranes and consequent preterm birth labor.[5,6] This mechanism however raises several questions. First, are there racial differences in the expression of aMMP-8 which might explain reported increased prevalence of preterm birth among black women? [7]

Secondly, since aMMP-8 responds to LPS stimulation [5,6], are there racial differences in the sensitivity to LPS? A racial difference in such sensitivity will translate to greater expression of aMMP-8 among blacks. If racial differences in sensitivity to LPS is indeed established from larger controlled studies, a major leap would have been made in addressing the question of greater prevalence of PPROM among black pregnant women. This would also make a case for aMMP-8 reduction interventions among this susceptible population.

2. METHODS

2.1 Consent and Confidentiality

The study was performed in accordance with the World Medical Association Declaration of Helsinki after ethical clearance from the University of Abuja Teaching Hospital. Written informed consent (appended signatures) was obtained from participants. Participation was completely voluntary personally identifiable information excluded.

2.2 Study Population, Setting and Instrument

Participants were pregnant women age range 20-45 years, mean age 30.6 years (standard deviation of 4.5), modal age of 30 years and Body Mass Index (BMI) of 17.8-46.9. Participants 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.

2.3 Sampling

Purposive, non-random sampling method involving sequential recruitment of new willing patients registering at the antenatal clinic of the University of Abuja Teaching Hospital.

2.4 Calibration and Examination

One periodontologist and two dental therapists calibrated to recognize the color change performed all examinations. The percentage agreement was 100 percent due to the clarity of the blue color change (Fig. 1). aMMP-8 was qualitatively assessed using lateral flow immunoassay test kit --a point-of-care chair-side immunoassay kit which selectively detects elevated levels of aMMP-8. Participants mouth-rinsed with clean water for thirty seconds and waited for one minute.

A 30 second rinse with the distilled water followed and saliva expectorated into a small sterile dish that came with the kit. About 3 ml of the saliva was drawn up with the syringe with a filter fitted. Three to four drops of the saliva was placed in the immunoassay dish 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 indicated a negative test (normal aMMP-8) while two lines indicated a positive test (elevated aMMP-8) (Fig 1). Even a faint line indicated a positive test according to the manufacturer's instructions.

2.5 Statistical Analysis

This was performed with the PASW-18 (SPSS) statistical software. Uni-variate data such as frequencies, means and standard deviation were evaluated. Chi-square statistic was applied using cross-tabulations to examine the relationship between the outcome variable (aMMP-8 test result) and explanatory variables --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.

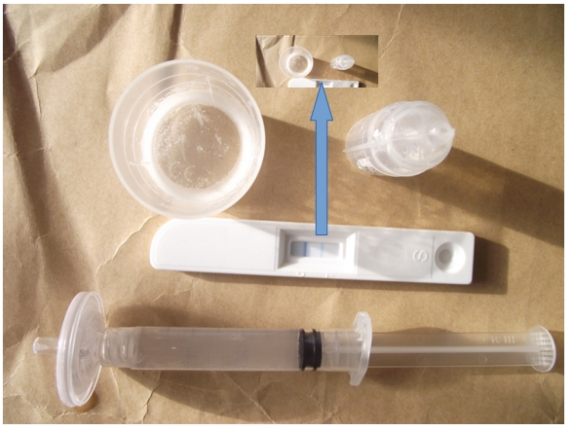

Fig. 1. aMMP-8 Test Kit showing blue control and test lines.

3. RESULTS

One hundred and thirty four participants participated in this study of which 117 (83.7%) had elevated aMMP-8 levels. aMMP-8 levels were generally elevated in participants across all age groups with no defined pattern. Participants aged 20-45 years showed no difference in aMMP-8 levels across all age groups. (Table 1).

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

aMMP-8 Level by Age Group								
	aMMP-8 Level				Total			
	Normal		Elevated					
	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		

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 across all BMI values with minor insignificant differences (p= 0.62) (Table 2).

Table 2. aMMP-8 levels by BMI. aMMP-8 Levels were elevated across all BMI values. X^2= 0.972, df= 2, p= 0.62

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		

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

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

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	

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

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

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

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

aMMP-8 levels were also 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).

Table 1. aMMP-8 levels by LCB duration. aMMP-8 Levels were elevated across all LCB durations. X^2=0.154,df= 2,p= 0.926

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

4. DISCUSSION

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

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

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

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

Contrary to recent findings among Caucasians [11], the present study found increased levels of mouthrinse aMMP-8 among pregnant women independent of their age, educational qualification, gestational age, duration of last child birth and BMI. 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]

The role of intrauterine infections in PPROM is well-reported in literature [13,14] but was not assessed in the current study. Equally established in literature is the mediating role of lipopolysaccaride (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] The production of matrix metalloproteinases is a final pathway in the pathogenesis of preterm birth and PPROM. Authors therefore 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 the authors' suspicion of a racial twist in our observations. Only recently, a likely racial tendency in host responses to 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 unravel this unexplained predisposition of black women to PPROM. A possible explanation was 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] warrants further investigation among Nigerian women.

The likelihood that matrix metalloproteinases play a major role in determining the predisposition of black women to PPROM 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.

5. 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.

CONSENT

The study was performed in accordance with the World Medical Association Declaration of Helsinki after ethical clearance from the University of Abuja Teaching Hospital. Written informed consent (appended signatures) was obtained from participants. Participation was completely voluntary personally identifiable information excluded.

ETHICAL APPROVAL

The study was performed in accordance with the World Medical Association Declaration of Helsinki after ethical clearance from the University of Abuja Teaching Hospital. Written informed consent (appended signatures) was obtained from participants. Participation was completely voluntary personally identifiable information excluded.

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.

COMPETING INTERESTS

Authors have declared that there are no competing interests.

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