PULMONOLOGY

Influence of Maternal Ethnic Background on Cervical Length in Pregnancy: A Perinatal Ultrasound Study

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Abstract

Short cervical length is a predictor of preterm birth. We evaluated if there were racial differences in variables associated with cervical length in pregnant Brazilian women. Cervical length was determined by vaginal ultrasound in 414 women seen at 21 weeks gestation. All women were seen at the same clinic and analyzed by the same investigators. Women found to have a short cervix (≤25 mm) received vaginal progesterone throughout gestation. Composition of the vaginal microbiome was determined by analysis of the V1-V3 region of the gene coding for bacterial 16S ribosomal RNA. Demographic, clinical and outcome variables were determined by chart review. Subjects were 53.4% White, 37.2% mixed race and 9.4% Black. Pregnancy and medical history and education level were similar in all groups. Mean cervical length was shorter in Black women (28.4 mm) than in White (32.4 mm) or mixed race (32.8 mm) women ($p \leq 0.016$) as was the percentage of women with a short cervix (23.1%, 12.2%, 7.8% in Black, White, mixed race respectively) ($p \leq 0.026$). Mean cervical length increased with maternal age in White ($p=0.001$) and mixed race ($p=0.045$) women but not in Black women. There were no differences in bacterial dominance in the vaginal microbiota between groups. Most women with a short cervix delivered at term. We conclude that Black women in Brazil have a

shorter cervical length than do White or mixed race women independent of maternal age, pregnancy and demographic history or composition of the vaginal microbiome.

Keywords: cervical length; race; vaginal microbiome

Introduction

The identification of women at elevated risk for a premature delivery and utilization of risk-specific protocols to prevent or reduce their susceptibility remain major unresolved problems in obstetrics. There has been no significant reduction in the rate of premature delivery in the past several decades and preterm birth continues to be the predominant cause of neonatal morbidity and mortality.[1] In women who were previously pregnant a prior preterm birth, spontaneous abortion and short inter-pregnancy interval are recognized risks for a subsequent premature delivery. In primiparous women a range of factors such as multifetal gestation, uterine abnormality, prior uterine surgery, young age, low maternal weight, poor maternal weight gain, smoking, the absence of a *Lactobacillus*-dominated vaginal microbiome and dietary factors have all been shown to increase the likelihood of spontaneous preterm birth in subpopulations of pregnant women.[2,3]

However, most cases of preterm birth occur in women with none of the above identifiable risk factors. A major advance in the ability to predict elevated risk for this outcome was the finding of an association between cervical length and preterm birth. Women with a cervical length $\leq$ 25 mm at 24 gestational weeks detected by vaginal ultrasound were found to have a higher rate of preterm birth than did women with a longer cervix.[4] Subsequently it was discovered that treatment with vaginal progesterone reduced the risk of short cervix-related preterm birth.[5,6]

It remains controversial and a matter of debate whether all pregnant women should be screened for cervical length or if this testing be reserved for those women with an identified risk factor for preterm delivery. A recent analysis concluded that

while universal screening for cervical length coupled with progesterone intervention may be cost effective and therefore justified, its application did not appreciably reduce the incidence of preterm birth.[7] Furthermore, the costs, effort and training associated with obtaining an accurate cervical length measurement by ultrasound is considerable.

The influence of race as a variable that may differentially influence cervical length has not received much attention. Three studies of populations at low risk for preterm birth have concluded that Black women have an increased likelihood of having a shorter cervical length than do women of other racial groups.[8-10] To further assess racial differences in cervical length and to explore possible reasons for any observed disparity we performed a prospective study of racial differences in factors associated with cervical length and subsequent pregnancy outcome in a large population of women in Sao Paulo, Brazil.

Material and Methods

Subjects The study population in this prospective study consisted of 414 mid-trimester pregnant women who were undergoing a routine vaginal ultrasound to assess cervical length at the obstetrical outpatient clinic at The Federal University of Sao Paulo. Exclusion criteria were the presence of a multifetal gestation, signs or symptoms suggestive of a vaginal infection, antibiotic usage in the previous two weeks, presence of an immune or endocrine disorder or the inability to give informed consent. Race was self-defined by the subjects. Mixed race indicates parents self-identified by the subject as one White and one Black. The study was approved by the Institutional Review Board at The Federal University of Sao Paulo and all subjects gave written informed consent.

Cervical length measurement Cervical length evaluation by transvaginal sonography was performed with a 5-9MHz probe (Accuvix XQ and V10, Medison, S.Korea; Voluson Expert 730, USA) according to standard technique. Women were asked to empty their bladder, placed in the dorsal lithotomy position and a transducer was inserted into the anterior vaginal fornix. The cervix was visualized in the longitudinal plane, the endocervical mucosa was identified and the cervical length was measured as the distance from the internal to the external os. The shortest cervical length measurement was recorded after 3–5 min of transvaginal sonography. Transfundal pressure was applied in order to note adverse dynamics and funneling of the cervix. The same group of experienced physicians with fetal medicine training background performed all ultrasound evaluations.

All women with a short cervical length, defined as ≤25 mm, received 200 mg vaginal progesterone (Utrogestan®) daily up to 36 weeks gestation or until the time of delivery.

Vaginal microbiome determination Just prior to the cervical length assessment samples were obtained from the posterior vagina for the analysis of the vaginal microbiome. Samples were collected using the Copan ESwab sample collection system (Fisher Scientific, Pittsburgh, PA) and stored at -80^{o}C. The microbiome samples were shipped on dry ice to the Forney lab at the University of Idaho for analysis. The assays were performed by staff blinded to all clinical information. The microbiome analysis was as reported previously.[11] Briefly, bacterial cells in vaginal samples were lysed using an enzyme cocktail and bead beating, and genomic DNA was isolated using a QIAamp DNA Mini kit. The yield of DNA was determined by fluorometry and the DNA size and integrity were verified using an Agilent Bioanalyzer. The V1 to V3 regions of bacterial 16S rRNA genes were amplified using primers that flanked the variable regions and amplicons were produced by two consecutive rounds of PCR that attached sample barcodes and sequencing adapters. The concentrations of amplicons were determined by fluorometry. DADA2 software was used to identify distinct sequence variants and remove sequence chimeras. The remaining sequences were classified to the genus level using the RDP naive Bayesian classifier in combination with the Silva reference database, then assigned to species using SPINGO software. Data was cleaned to include only samples with ≥ 3,000 reads and those taxa that occurred with a relative abundance of at least 0.01 (1%).

Dominant is defined as the bacterium being present at a level >50% of the total bacteria detected.

Statistics Comparisons of normally distributed values were analyzed by the Student t test; values without a normal distribution were compared by the Mann-Whitney test and the Kruskal-Wallis test, as appropriate. A p value <0.05 was considered significant.

Results

Characteristics of the study population are shown in Table 1. The racial composition was 221 (53.4%) White, 154 (37.2%) mixed race and 39 (9.4%) Black. Women in the three groups were comparable in age, educational level, gravidity, parity and body mass index. A similar percentage of women in each racial group (<10%) were at a potentially elevated risk for a preterm birth due to a prior premature delivery, a history of spontaneous abortion at > 12 weeks gestation, a Mullerian defect or being a smoker.

Data showing the association between race and cervical length is presented in Table 2. In all cases cervical length was measured at a mean of 21 weeks gestation. Black women had a lower mean cervical length (28.4 mm) compared to White (32.4 mm, $p = 0.016$) or mixed race (32.8 mm, $p = 0.005$) women. Similarly, Black women had the highest percentage of women with a short cervix (23.1%) as compared to White (12.2%, $p = 0.026$) or mixed race (7.8%, $p = 0.017$) women. Only one woman (White) with a history of preterm birth, two women (one White, one Black) with a prior late miscarriage and 2 women (One White, one Black) who smoked had a cervical length $\leq$ 25 mm.

The association between maternal age and cervical length is shown in Table 3. For both White ($p = 0.001$) and mixed race ($p = 0.045$) women mean cervical length increased as the women became older. In contrast, there was no association between age and mean cervical length in the Black subjects ($p = 0.700$). In addition, at every age range examined the mean cervical length was lowest in Black women.

Lactobacillus crispatus, Lactobacillus iners and *Gardnerella vaginalis* were the three most numerically dominant bacteria in vaginal communities and there was no difference in their occurrence based on race (Table 4). There was no association between specific bacterial dominance and cervical length in each group (data not shown).

The majority of women with a short cervix in each racial group had a term delivery (>36 weeks gestation). A preterm birth occurred in 33.3% of White, 11.1% of mixed race and 40% of the Black women with a cervical length ≤ 25 mm. There was no association between maternal age and percent of women with a short cervix who delivered preterm.

Discussion

The mean cervical length was shorter and the percent of women with a cervix ≤ 25 mm was higher in pregnant Black Brazilian women than in those who self identified as White or mixed race. This finding parallels earlier reports on different populations[8-10] and thus highlights that race must be considered as a variable in investigations related to cervical length in pregnant women. The present study also reveals that in both White and mixed race women, but not in Blacks, mean cervical length increased with maternal age. Genetic factors, possibly combined with differences in environmental exposures, may influence the rate of cervical maturation and maintenance between races over time.

The numerically dominant bacteria in the vaginal microbiome was similar among women of different races in our pregnant population. This is contrary to studies demonstrating differences in the composition of the vaginal microbiome based on race.[12,13] A possible explanation for this divergence is the apparent unique racial make-up of the Brazilian population. It has been suggested that Brazilians are a highly genetically admixed population and that determination of race by evaluation of skin color might be inaccurate.[14,15]

Despite progesterone treatment 33-40% of women in our study with a short cervical length delivered preterm. The factors influencing efficacy of progesterone-related responses remain to be determined. Variations between individual women in gene expression by vaginal microorganisms and/or host epithelial or immune cells may account for differences in progesterone activity. A prior investigation concluded that vaginal progesterone did not impact composition of the pregnancy vaginal

microbiota[16] but additional studies are needed to verify this observation and to assess possible variations in microbial activity..

An advantage of the present study is that all subjects were seen at the same institution by the same team of investigators. This assures uniformity in cervical measurement as well as progesterone treatment strategy. A limitation of the present study is the relatively low number of self-identified Black women in our population. , In addition, as noted above, the Brazilian population is characterized by extensive racial mixing. Without performing a genetic analysis we cannot be certain that an unspecified proportion of women who self-identified as belonging to a specific racial group were, in fact, members to a variable extent of a different group. Therefore, the classification of our study population into the three different racial categories may not be entirely accurate. In addition, our designation of $\leq$25 mm as the cut-off value for a short cervix may not be optimal for different populations.

A genetic basis for race-related variations in immune responses has been reported.[17,18] Genetic-related variations in recognition, magnitude and/or direction of the immune response to specific bacteria or their products may contribute to differences in cervical length in women with comparable vaginal microbiomes. For example, qualitative and/or quantitative differences in the local up-regulation of extracellular matrix metalloproteinase levels which stimulate hyaluronan breakdown in the cervical extracellular matrix would influence cervical length.[19-21]

Based on our findings, and acknowledging limitations of the present study and the need for further investigation, it seems reasonable to propose that an alternative to universal mid-trimester screening for cervical length might be to preferentially offer

this screening to all Black women and to those White and mixed race women who are under the age of 25. Replication of the present study in first trimester pregnant women to identify racial variations in cervical length at an earlier stage of gestation may further enhance the efficacy of strategies to improve pregnancy outcome.

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Table 1. Characteristics of the study population

Characteristic	White N = 221	Mixed race N = 154	Black N = 39
Age (years)	30.2 (7.1)	27.7 (7.9)	27.7 (7.2)
Years education	10.8 (3.2)	10.3 (2.4)	10.8 (2.0)
Gravidity	2.4 (1.5)	2.5 (1.6)	2.5 (1.6)
Parity	1.0 (1.1)	1.0 (1.2)	1.0 (1.3)
Body mass index (kg/m^2)	27.1 (5.1)	27.9 (7.0)	26.3 (6.1)
Gestational age (weeks)	21.3 (1.4)	21.4 (1.5)	21.1 (1.1)
History of preterm birth	5.9%	8.4%	2.6%
History of late miscarriage	2.7%	1.3%	2.6%
Mullerian abnormality	1.8%	1.3%	0
Smoker	5.0%	5.8%	7.6%

Values are mean (standard deviation). Late miscarriage is defined as a spontaneous pregnancy loss at > 12 weeks gestation.

Table 2. Association between race and cervical length

Race	No. tested	Mean CL (mm)	% with short cervix
White	221	32.4	12.2%
Mixed race	154	32.8	7.8%
Black	39	28.4[a]	23.1%[b]

Cervical length (CL) was measured by transvaginal ultrasound at 21 weeks gestation.

Short cervix is defined as a length ≤25 mm.

[a]p = 0.016 vs. White, 0.005 vs. mixed race; [b]p = 0.026 vs. White, 0.017 vs. mixed race

Table 3. Association between maternal age and cervical length

Race	Mean cervical length (mm)				
	14-18	19-24	25-35	>35 years old	p value
White	25.9	30.4	32.8	34.6	0.001
Mixed race	27.6	31.8	33.4	35.6	0.045
Black	20.7	27.2	28.1	31.2	0.700

Differences between age groups for each race were determined by the Kruskal-Wallis test.

Table 4. Association between race and numerically dominant bacterium in the vagina

Race	Percentage of community			
	L. crispatus	*L. iners*	*G. vaginalis*	Other
White	46.0%	30.7%	9.0%	14.3%
Mixed race	40.9%	31.5%	14.3%	13.2%
Black	41.0%	41.0%	10.3%	7.6%

Dominant is defined as the bacterium being present at a level >50% of the total bacteria detected.