Chlamydia trachomatis lower genital tract infection and spontaneous preterm birth: a case-control study nested in the BRISA cohort

Infecção genital do trato genital inferior por Chlamydia trachomatis e parto pré-termo espontâneo: um estudo caso-controle aninhado na coorte BRISA

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ABSTRACT

Introduction: *Chlamydia trachomatis* (CT) is a sexually transmitted bacterium that is highly prevalent in young patients. Chlamydial infections during the gestational period have been associated with adverse obstetric outcomes, such as spontaneous preterm birth (sPTB). However, results in the literature are inconclusive. **Objective:** To evaluate the association between CT infection and sPTB. **Methods:** This was a case-control study nested in the cohort of the prospective Brazilian Ribeirão Preto and São Luís birth cohort study. Pregnant patients were recruited in private and public health clinics in São Luís and Ribeirão Preto, Brazil. At the gestational age of 20-25 weeks, cervicovaginal fluid samples were collected for the diagnosis of CT using a polymerase chain reaction. Plasma levels of Transforming Growth Factor-α (TGF- α), Interferon-γ (IFN- γ), Interleukin-10 (IL-10), IL-13, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, Tumor Necrosis Factor-α (TNF- α), and TNF-β were measured using a multiplex assay. **Results:** Of the 561 pregnant patients evaluated, 121 had sPTB and 440 had a full-term delivery (control group). According to our results, CT infection was not associated with sPTB (odds ratio, 1.13; 95% confidence interval, 0.50–2.56); however, it was more frequent among younger patients (p=0.0078), unmarried patients (p=0.0144), and those with multiple sexual partners (p=0.0299). There were no significant differences in the immune mediators between patients with sPTB or full-term deliveries, or between patients with or without a CT infection. **Conclusion:** In conclusion, CT infection was not associated with sPTB in our study. However, its correlation with younger pregnant patients suggests that these patients require careful clinical management.

Keywords: *Chlamydia trachomatis*; premature birth; pregnancy outcome; bacterial infections.

RESUMO

Introdução: *Chlamydia trachomatis* (CT) é uma bactéria sexualmente transmissível com alta prevalência em mulheres jovens. As infecções por CT durante o período gestacional têm sido associadas com desfechos obstétricos adversos como o parto pré-termo espontâneo (PPTe). No entanto, os achados na literatura sobre essa temática ainda são inconclusivos. **Objetivo:** Avaliar a associação entre infecção por CT e PPTe. **Métodos:** Trata-se de um estudo caso-controle aninhado na coorte prospectiva de nascimentos de Ribeirão Preto e São Luís (acrônimo BRISA em inglês). As gestantes incluídas foram recrutadas com idade gestacional entre 20 e 25 semanas em serviços de saúde públicos e privados de São Luis e Ribeirão Preto, Brasil. O diagnóstico de CT foi feito por reação em cadeia da polimerase em amostras de conteúdo cervicovaginal coletadas no momento da inclusão no estudo. Também foram dosados os níveis séricos de fator transformador de crescimento-α (TGF- α), interferon-γ (IFN-γ), interleucina-10 (IL-10), IL-13, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, fator de necrose tumoral-α (TNF-α) e TNF-β por ensaio multiplex. **Resultados:** Das 561 gestantes avaliadas, 121 tiveram PPTe e 440 tiveram parto a termo (grupo controle). De acordo com nossos resultados, a infecção por CT não esteve associada ao PPTe (*odds ratio* 1,13; intervalo de confiança de 95%, 0,50–2,56); no entanto, ela foi mais frequente entre as gestantes mais jovens (p=0,0078), solteiras e divorciadas (p=0,0144) e aquelas com múltiplos parceiros sexuais (p=0,0299). Não houve diferença significativa quanto aos imunomediadores entre o grupo com PPTe e controle nem entre as gestantes com diagnóstico positive para CT e as que não apresentavam a infecção. **Conclusão:** No presente estudo, a infecção por CT não esteve associada ao PPTe, no entanto sua relação com gestantes jovens sugere que essa população requeira maiores cuidados e atenção no manejo clínico.

Keywords: *Chlamydia trachomatis*; nascimento prematuro; resultado da gravidez; infecções bacterianas.

INTRODUCTION

Preterm birth (PTB) and its complications are among the leading causes of neonatal death according to a report from the World Health Organization.[1] In 2014, the PTB global rate was 10.6% of live births,[2] while the PTB rate in Brazil in 2014 was 11.2%.[3]

Despite being a multifactorial syndrome, bacterial infections are associated with 25-40% of PTBs.[4] *Chlamydia trachomatis* (CT) is a bacterial infection of the lower female genital tract (FGT). It is a mandatory intracellular pathogen that is typically asymptomatic and has the potential to trigger a PTB by activating a Th1 inflammatory response.[5-8] However, currently there is no consensus in the literature regarding this causal association.[9-13] Therefore, the Brazilian Public Health System has no CT screening plan, and the prevalence of CT in pregnant patients in Brazil reached 9.8% in 2011.[14]

OBJECTIVE

We aimed to evaluate the association between CT infection and spontaneous PTB (sPTB) in pregnant patients who participated in the study "Etiological factors of preterm birth and consequences of perinatal factors for infant health: birth cohorts from two Brazilian cities" (the BRISA – Brazilian Ribeirão Preto and São Luís birth cohort study). We evaluated the possibility of this association with a case-control study nested in the BRISA cohort.

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This is the most recent study published searching for an association between CT and sPTB in the populations of Ribeirão Preto, SP, Brazil, and São Luís, MA, Brazil. Our results may contribute with new evidence that may aid in the treatment of pregnant women mainly among the Brazilian population.

METHODS

This case-control study was nested in the prospective BRISA cohort study. The BRISA study was approved by the Institutional Review Board of Ribeirão Preto Medical School (Proceeding: 11157/2008) and the Federal University of Maranhão (Proceeding: 4771/2008-30) and followed the principles of the Declaration of Helsinki. All subjects provided written informed consent to participate.

In the base cohort, pregnant patients were recruited during prenatal visits to public and/or private health care clinics in São Luís, MA, Brazil, and Ribeirão Preto, SP, Brazil. The recruitment period was between 2010 and 2011. All pregnant patients with a single fetus and gestational age of less than 20 weeks, according to their obstetric ultrasound, were included. The BRISA protocol was previously reported.[15]

At the time of the study, all pregnant patients were between 20-25 weeks of gestation and were interviewed to collect socio-demographic, behavioral and clinical data. The socio-demographic and behavioral data collected were age (in years), self-reported race/ethnicity (white, black, mixed race or Asian), education (in years of scholarity), marital status (single, divorced, married or living with a partner), lifetime sexual partners (in number), and whether they smoked or not. The clinical history data obtained during the interview were diabetes and/or hypertension before pregnancy period, pre-eclampsia and/or gestational diabetes, number of pregnancies (primigravida or multigravida), history of previous PTB, and previous abortion (yes or no).

The CT infection diagnosis was only accessed after childbirth, although the cervicovaginal lavage (CVL) samples were collected during the prenatal period in a gynecological exam. Besides 1 mL of CVL, 20 mL of blood was collected via venipuncture and centrifuged for plasma separation. The CVL and plasma specimens were stored in cryotubes in a -80°C freezer until processing. Samples from São Luís, MA, Brazil, were processed with samples from Ribeirão Preto at the Multi-User Laboratory of Ribeirão Preto Medical School (FMRP–USP).

All pregnant patients whose babies were born preterm were included as cases in this case-control study. Preterm was defined as less than 37 weeks, according to the World Health Organization.[16] Medically indicated and iatrogenic PTBs were excluded from our analysis. Therefore, the case group consisted of pregnant patients who had spontaneous preterm birth (sPTB).

The controls for this study were randomly selected from the remaining patients who had full-term delivery included in the BRISA cohort. The intended case-control ratio was 1:2; however, several samples were lost on the way from São Luís, MA, Brazil, to Ribeirão Preto, SP, Brazil, and incorrectly identified samples, incorrect storage and insufficient sample volume also led to a significant loss of control samples, as shown in **Figure 1**.

CT was diagnosed on the CVL specimens through a polymerase chain reaction (PCR) using a QIAamp® extraction kit (Cat. Number: 69506, QIAGEN®, Venlo, NL), according to the manufacturer's instructions. The DNA integrity was observed on a 1.5% agarose gel stained with GelRed® (Biotium, San Francisco, USA). All DNA samples were quantified by using NanoDrop® 2000 equipment (Thermo Scientific®, Waltham, USA), according to the manufacturer's instructions. For CT identification, we used two pairs of plasmid DNA specific primers: CtP1: 5′-TAGTAACTGCCACTTCATCA-3′, CtP2: 5′-TTCCCCTTGTAATTCGTTGC-3′ and PL6.1: 5′-AGAGTACATCGGTCAACGA-3', PL6.2:

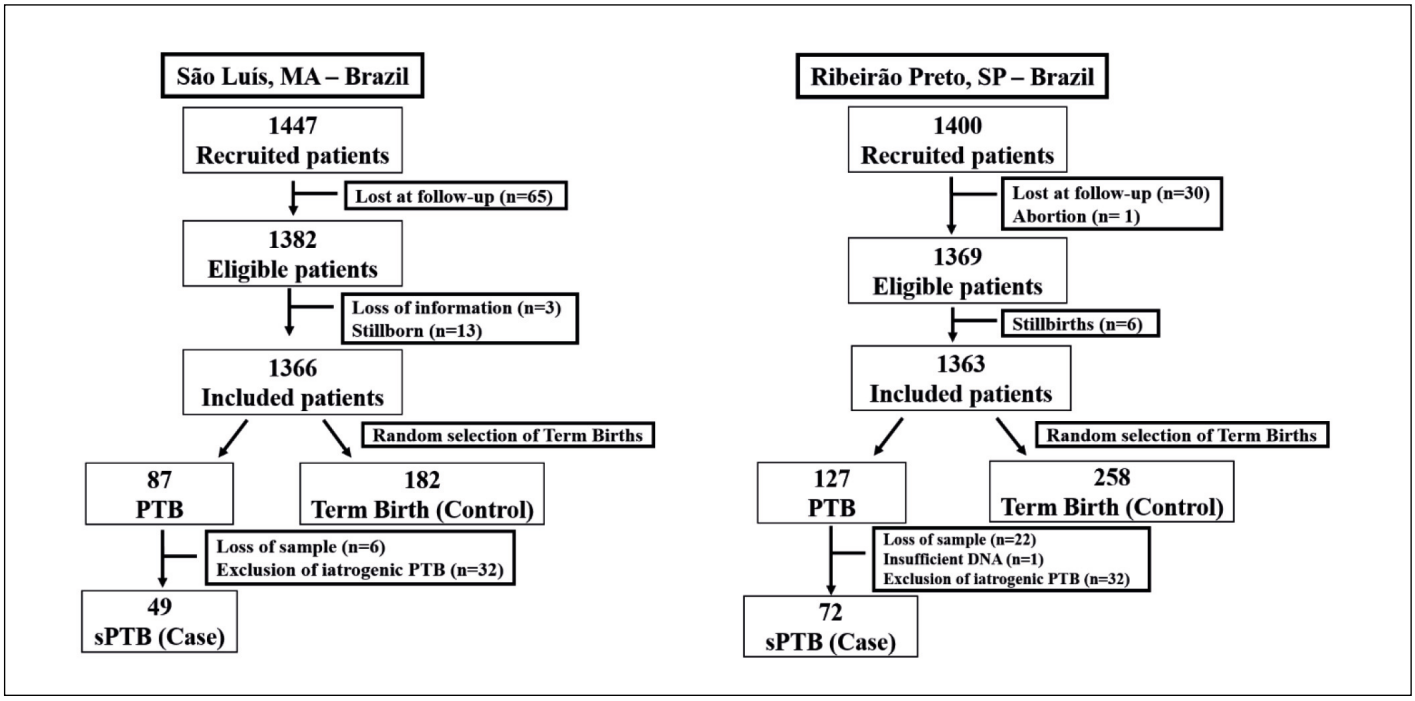

Figure 1 – Flowchart of patients included and specimens analyzed in this case-control study, nested in the BRISA cohort study of 2010–2011.

5'-TCACAGCGGTTGCTCGAAGCA-3′ (IDT – Integrated DNA Technologies, Coralville, USA).

The PCR reactions were performed in a Veriti thermal cycler (Applied Biosystems, Foster City, USA) as follows: initial denaturation at 95°C for 5 minutes, 40 cycles; denaturation at 95°C for 1 minute, annealing at 54°C for 1 minute, extension at 72°C for 1 minute and 30 seconds, followed by a final extension at 72°C for 5 minutes. The fragments generated by the 201 bp CtP and 130 bp PL6 primer pairs were observed on 2.5% agarose gel stained with GelRed® (Biotium, San Francisco, USA), compared with the 100 bp DNA Ladder molecular weight marker (Promega, Part. No. G210A, Madison, USA), after electrophoresis in 1× TBE buffer, to separate the bands. Samples that presented the two bands for 201 and 130 bp were considered positive.

The CVL was not performed immediately after sample collection. Thus, the PCR-based diagnosis of CT was only made after delivery. For this reason, only those patients who were symptomatic (presenting mucopurulent cervicitis) during the specimen collection period (gestational age of 20-25 weeks) received treatment for CT.

We also measured immune mediators, including TGF-α, IFN-γ, IL-10, IL-13, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α and TNF-β, in plasma samples. The immunological mediators were chosen according to their role in labor and in the inflammatory response of the FGT. MILLIPLEX Multiplex® Assay kits using Luminex LX 200® (Cat #:HCYTOMAG-60K, Merck, New York, USA) were used to measure these mediators, following the manufacturer's instructions. The detection limit was 50 beads per set, and the results were calculated with the MilliPlex Analyst program using the Curve Fit 4-parameter.

Statistical analysis

This is a case-control study nested in a cohort. The data were initially evaluated via exploratory analysis using measures of central position and dispersion. Categorical variables, such as CT diagnosis, ethnicity and smoking, were analyzed through the chi-square test. The quantitative variables, such as age and levels of immunological mediators of the plasma samples, were analyzed using Wilcoxon non-parametric tests for independent samples, as they were not normally distributed.

Univariate and multivariate logistic regression models were constructed to identify variables associated with prematurity and to quantify this association via the odds ratio (OR) effect size.

All statistical analyses were performed by using the SAS software version 9.4 (SAS Institute, Cary, USA) to investigate which variables were different between the specimens of patients who underwent sPTB and those who had a full-term delivery. We also investigated pregnant patients according to their CT status.

For our analysis, we considered only statistically significant results with a p value <0.05 (α=5%).

RESULTS

The study flowchart is shown in **Figure 1**. A total of 561 patients from two participating centers were included in this study; sPTBs occurred in 121 patients, and 440 delivered at full term.

There was a selection of pregnant patients from Ribeirão Preto, São Paulo and São Luis, Maranhão – Brazil for the BRISA cohort and a subsequent selection of the patients' samples analyzed for this present case-control nested in the BRISA cohort. Abbreviations: PTB: preterm birth; sPTB: spontaneous PTB.

There was no significant difference in the presence of CT or demographic, behavioral or medical history data between pregnant patients who underwent sPTB and those who delivered at full term (**Table 1**). The mean patient age was 26 years in both groups. In addition, nearly 70% of patients self-declared their race as black and had 9–12 years of schooling. More than 70% were married and had a history of one to four sexual partners in their lifetime.

The diagnosis of CT and variables that showed a statistical difference in the chi-square test was included in the multivariate analysis, as shown in **Table 2**. A history of previous PTB was found to be a risk factor for sPTB (adjusted OR [aOR] 17.442; 95% confidence interval [CI], 8.77–34.68).

The levels of TGF-α, IFN-γ, IL-10, IL-13, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α and TNF-β were very low and were not significantly different between patients with sPTB or full-term deliveries (data not shown).

We further divided the patients by presence (CT+, n=34) or absence (CT-, n=527) of CT (**Table 3**). The mean age of the CT+ group was 23 years, which was significantly lower than that of the patients in the CT- group (26 years) (p=0.0091; data not shown). Significantly more patients in the CT+ group were not married (p=0.0144) and had five or more sexual partners in their lifetime (p=0.0299) compared with the CT- group. As CT infection is a predictor and not the outcome of our study, we did not perform a multivariate analysis to measure the association of CT with age, marital status or number of sexual partners in a patient's lifetime.

The levels of TGF-α, IFN-γ, IL-10, IL-13, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α and TNF-β were not significantly different between the CT+ and CT- groups (data not shown).

DISCUSSION

Our results do not indicate any association between CT infection and sPTB. The CT rate in patients who underwent sPTB was similar to that in patients who delivered at full term. We found a strong association between previous PTB and sPTB in the current pregnancy. In addition, CT infection was associated with younger patients (p=0.0078), patients who were unmarried (p=0.0144) and patients with more than five sexual partners in their lifetime (p=0.0299). We did not find an association of any immunological mediator with the gestational outcome or CT status.

The vaginal microbiota during the gestational period is distinct from the microbiota at other times. Changes in vaginal microbial composition have become an important research issue due to a possible association with adverse obstetrical outcomes, such as PTB, preterm premature rupture of the membranes, low birth weight and stillbirth.[4,10,17] Thus, the presence of pathogenic bacteria, such as CT, has the potential to generate an inflammatory response capable of inducing an sPTB as the maternal immune system is suppressed during pregnancy to allow and promote fetal development.[4,18]

Table 1 – Patient demographic, behavioral and medical history data.

	sPTB	Control	p*
C. trachomatis diagnosis			
CT+	8 (6.6)	26 (5.9)	0.7743
CT-	113 (93.4)	414 (94.1)	
Age			
≤19	20 (16.5)	52 (11.8)	0.2209
20–25	40 (33.1)	177 (40.2)	
≥26	61 (50.4)	211 (47.9)	
Ethnicity			
White	39 (32.2)	149 (34.2)	
Black	81 (66.9)	284 (65.1)	0.9147
Asian	1 (0.8)	3 (0.7)	
Gestational BMI			
Underweight	24 (20)	66 (15.2)	
Normal weight	53 (44.2)	176 (40.5)	0.2794
Overweight	29 (24.2)	116 (26.7)	
Obesity	14 (11.7)	76 (17.5)	
Years of schooling			
9 years	26 (21.5)	91 (20.8)	0.9798
9–12 years	82 (67.8)	301 (68.7)	
>12 years	13 (10.7)	46 (10.5)	
Marital status			
Married/Live with a partner	92 (76)	361 (82.1)	0.1374
Single/Divorced	29 (24)	79 (17.9)	
Lifetime sexual partners			
1–4	93 (76.8)	352 (80)	0.4500
≥5	28 (23.1)	88 (20)	
Smoking			
Yes	19 (15.7)	30 (6.8)	0.0022
No	102 (84.3)	410 (93.2)	
Chronic Hypertension			
Yes	3 (2.5)	15 (3.4)	0.6047
No	118 (97.5)	424 (96.6)	
Gestational Hypertension			
Yes	17 (14.1)	40 (9.1)	0.1136
No	104 (85.9)	398 (90.9)	
Diabetes			
Yes	1 (0.8)	3 (0.7)	0.8670
No	120 (99.2)	437 (99.3)	
Gestational Diabetes			
Yes	6 (5)	22 (5)	0.9853
No	115 (95)	418 (95)	
Pregnancies			
1	49 (40.5)	187 (42.5)	0.4621
2	28 (23.1)	118 (26.8)	
≥3 pregnancies	44 (36.4)	135 (30.7)	
Previous abortion			
Yes	30 (24.8)	99 (22.5)	0.5955
No	91 (75.2)	341 (77.5)	
Previous PTB			
Yes	42 (66.7)	22 (9.9)	<0.0001
No	21 (33.3)	199 (90.1)	

sPTB: spontaneous preterm birth; BMI: body mass index; PTB: preterm birth; *χ^2 test. Data are presented as numbers and percentages.

Table 2 – Factors associated with *C. trachomatis* infection.

	OR (95%CI)*	aOR (95%CI)*
C. trachomatis diagnosis		
CT+	1.127 (0.50–2.56)	-
CT-	Reference	-
Smoking		
Yes	2.546 (1.38–4.71)	1.836 (0.66–5.10)[a]
No	Reference	Reference
Previous PTB		
Yes	18.091 (9.13–35.86)	17.442 (8.77–34.68)
No	Reference	Reference

OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; PTB: preterm birth; CT+, presence of CT; CT-, absence of CT; [a]Adjusted for Previous PTB; *Logistic Regression Model.

However, the role of CT during the gestational period is unclear, and there is no consensus in the literature regarding its effects on gestational outcomes. This inconsistency in the literature is associated with several factors, such as the study design, sample size, population studied, CT diagnostic methods, gestational period of pregnancy and sample source used to investigate CT.[11]

We did not find any association between CT infection and sPTB (p=0.7743), which correlates with the results of previous studies.[12,13] Prior Brazilian studies have reported that the prevalence of CT in pregnant patients varies from 12-18%,[19-21] depending on the city and region. Previous studies have also shown that CT can be vertically transmitted.[22] In addition, CT may cause a series of comorbidities in the newborn, such as chronic obstructive pulmonary disease (COPD),[23] low birth weight,[12] conjunctivitis and pneumonia.[24] However, the present study did not include any vertical transmission analysis in patients with CT.

Although we did not find an association between CT and sPTB, we did find a higher rate of CT among pregnant patients under 19 years of age, which is consistent with the literature. According to previous studies, age <19 years old, sexual behavior beginning at a young age and multiple sexual partners are risk factors for CT infection.[5-7] Therefore, these patients require careful clinical management.

In contrast with our findings, Liu et al. noted a causative association among patients with a prior CT diagnosis and sPTB (aOR 1.17l; 95%CI 1.01–1.37).[10] In addition, in a recent meta-analysis by Olson-Chen et al., CT during pregnancy is associated with PTB (OR=1.27; 95%CI 1.05–1.54).[25] Nevertheless, the lower limit of the CI in these studies is close to one, indicating a potential lack of significance. Therefore, the results of these previous studies may be attributed to the sample sizes. Similar conclusions were reported in previous systematic reviews that found that studies with small sample sizes, low-quality or unadjusted analysis methods reported an association between CT and sPTB.[26,27]

In this study, a history of PTB was the only factor associated with sPTB after adjusting for confounding factors (aOR 17.42; 95%CI 8.77–34.68). Obstetric history is considered one of the most known risk factors for PTB. In clinical practice, according to the Brazilian Ministry of Health, pregnant patients with previous PTB are classified as having high-risk pregnancies.[14]

Table 3 – Patient demographic, behavioral and medical history data according to the presence of *C. trachomatis* in CVL.

	CT +	CT -	p*
Age			
≤19	10 (29.4)	62 (11.8)	0.0078
20–25	13 (38.24)	204 (38.7)	
≥26	11 (32.3)	261 (49.5)	
Ethnicity			
White	8 (23.5)	180 (34.4)	
Black	26 (76.5)	339 (64.8)	0.3601
Asian	0 (0)	4 (0.76)	
Gestational BMI			
Underweight	7 (20.6)	83 (15.9)	
Normal weight	14 (41.2)	215 (41.3)	0.3416
Overweight	11 (32.3)	134 (25.8)	
Obesity	2 (5.9)	88 (16.9)	
Years of schooling			
9 years	11 (33.3)	106 (20.1)	0.1606
9–12 years	18 (54.5)	365 (69.4)	
>12 years	4 (12.1)	55 (10.5)	
Marital status			
Married/Live with a partner	22 (64.7)	431 (81.8)	0.0144
Single/Divorced	12 (35.3)	96 (18.2)	
Lifetime sexual partners			
1–4	22 (64.7)	423 (80.3)	0.0299
≥5	12 (35.3)	104 (19.73)	
Smoking			
Yes	2 (5.9)	47 (8.9)	0.5434
No	32 (94.1)	480 (91.1)	
Chronic Hypertension			
Yes	0 (0)	18 (3.4)	0.2729
No	34 (100)	508 (96.6)	
Gestational Hypertension			
Yes	2 (5.9)	55 (10.5)	0.3910
No	32 (94.1)	470 (89.5)	
Diabetes			
Yes	0 (0)	4 (0.8)	0.6102
No	34 (100)	523 (99.2)	
Gestational Diabetes			
Yes	0 (0)	28 (5.3)	0.1679
No	34 (100)	499 (94.7)	
Pregnancies			
1	18 (52.9)	218 (41.4)	0.4140
2	7 (20.6)	139 (26.4)	
≥3 pregnancies	9 (26.5)	170 (32.2)	
Previous abortion			
Yes	5 (14.7)	124 (23.5)	0.2360
No	29 (85.3)	403 (76.5)	
Previous PTB			
Yes	8 (23.5)	94 (18.1)	0.4301
No	26 (76.5)	425 (81.9)	

BMI: body mass index; CVL, cervicovaginal lavage; PTB: preterm birth; CT+, presence of CT; CT-, absence of CT; *χ^2 test. Data are presented as numbers and percentages.

The immune mediators we evaluated in the plasma have an important role during labor and on the immune response in the FGT. During pregnancy, the immune response profile in the FGT is predominantly Th2. However, considering that the Th2 mediators are related to humoral defense, pregnant patients may be more vulnerable to intracellular pathogens. In addition, in the case of infection, activation of a Th1 response initiates labor.[4,9,28,29]

According to the cytokines reported to be involved in the Th1 and Th2 responses,[29] we expected to find increased levels of TNF-α, IL-1β, IL-8 and IFN-γ, and decreased levels of IL-4, IL-13, IL-10, IL-6 and TGF-β. However, the immune mediator levels were not significantly different between the sPTB and full-term delivery groups. Manning et al. reported similar results regarding IL-2, IL-4, IL-6 and IL-10 levels in the FGT of high-risk pregnant patients between 22–24 gestational weeks.[30]

In addition, systemic alterations in immune mediators do not occur until 25 weeks of gestation.[30] Therefore, alterations of these mediators were not found in the specimen analyzed in this study, which may explain why there was no association between these mediators and sPTB. We also found no significant differences in immune mediators between CT+ and CT- patients, which may suggest that CT did not trigger any inflammatory response in these patients. A more likely explanation is that the samples degraded during storage, and our data may not be accurate.

This study has several strengths. Patients from two different cities were included, which allowed for a larger sample size and greater variability. In addition, molecular biology was used for the diagnosis of CT, which is the most sensitive methodology available at our institution. We excluded medically indicated PTB to reduce possible confounding factors that trigger PTB.

The limitations of the study include additional factors that may influence pregnancy outcomes which were not included in our analysis. In addition, the diagnosis of CT in this study was limited to one moment during the gestational period; it is not possible to state whether CT+ patients continued to be positive throughout their pregnancy, cleared the infection or were re-infected before delivery. Finally, the diagnosis of CT and the Multiplex analyses were not performed immediately after sample collection, and the storage period may have influenced our results. Despite these limitations, our study design adequately confirmed our primary hypothesis.

CONCLUSION

The screening for CT in the prenatal period is not essential to assess the risk of sPTB. However, CT is frequently asymptomatic, commonly found in young patients, and can be vertically transmitted. Thus, a screening program for CT infection in younger pregnant patients is important to prevent reproductive consequences and vertical transmission in pregnant patients, since they are a high-risk population.

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PARTICIPATION OF EACH AUTHOR

All authors contributed equally to this work.

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CONFLICT OF INTERESTS

The authors of this manuscript declare they have no conflicts of interest.

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