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Unveiling the Microbial Contribution to Preterm Birth : The Role of Asymptomatic Bacteriuria

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Abstract*. Background: Preterm labor (PTL) remains a major global health concern, contributing significantly to neonatal morbidity and mortality. Asymptomatic bacteriuria (ASB), has been implicated as a predisposing factor to PTL. This research investigates association between ASB and PTL, emphasizing inflammatory mediators such as high-sensitivity C-reactive protein along with tumor necrosis factor- α. Methods: This observational case-control study was conducted at Tikrit Teaching Hospital, Iraq, involving 100 pregnant women (50 PTL cases and 50 full-term controls). Demographic, clinical, and laboratory data were collected. Urine cultures identified bacterial isolates. In addition, levels of TNF-α and hs-CRP in serum were measured using ELISA. Statistical analyses were performed using SPSS. Results: ASB was significantly associated with PTL, with 30% from PTL cases having a positive urine culture compared to 8% in controls (p = 0.009). Escherichia coli was the predominant pathogen (53%). TNF-α and hs-CRP levels were significantly elevated in PTL cases (p < 0.001), suggesting an inflammatory pathway in PTL pathophysiology. Conclusions: ASB is a significant risk factor for PTL, likely mediated by systemic inflammation. Routine ASB screening and targeted antimicrobial therapy may reduce PTL risk. Further study is required to explore the mechanistic connections amongst microbial infection and inflammatory responses in PTL.*

Keywords*: Preterm labor, asymptomatic bacteriuria, inflammation, TNF-α, hs-CRP*

1. INTRODUCTION

Preterm labor (PTL), defined as the onset of regular uterine contractions with cervical changes before 37 weeks of gestation, remains a critical global health challenge, contributing to over 35% of neonatal deaths and long-term disabilities in survivors(Blencowe et al. 2013; Howson et al. 2013). Premature birth is associated with increased risk of disability and mortality, with rising rates worldwide (Liu et al. 2016). Despite advances in obstetric care, the etiological complexity of PTL complicates prevention strategies, with maternal infections implicated in nearly 40% of cases (Nicolle et al. 2019). Among these infections, asymptomatic bacteriuria (ASB) has emerged as a significant yet modifiable risk factor for preterm birth. ASB affects 2–10% of pregnancies globally, with marked disparities in prevalence and outcomes across socioeconomic strata (Romero, Dey, and Fisher 2014).

ASB is a uniquely insidious condition during pregnancy. Physiological adaptations, including urinary stasis due to progesterone-induced ureteral dilation and glucosuria, create an environment conducive to bacterial proliferation (Gilstrap III and Ramin 2001). Common pathogens such as *Escherichia coli* , *Klebsiella pneumonia* , and *Enterococcus*

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faecalis colonize the urinary tract, often ascending undetected to the kidneys. Left untreated, ASB progresses to pyelonephritis in 20–40% of cases, a condition strongly associated with systemic inflammation, preterm contractions, and fetal compromise (Abde et al. 2024). Even in the absence of overt pyelonephritis, ASB has been independently linked to a 1.5- to 2-fold increased preterm birth risk (Kazemier et al. 2012). The mechanisms by which ASB precipitates PTL are multifaceted, involving microbial virulence, host immune responses, and placental dysfunction. Uropathogens such as E. coli express adhesins (e.g., type 1 fimbriae and P pili) that facilitate binding to urothelial cells, enabling biofilm formation and resistance to urinary clearance (Shahzad et al. 2022). Toll-like receptor 4 (TLR4) activation by bacterial lipopolysaccharide (LPS) stimulates decidual cells to release inflammatory mediators, including IL-6, IL-8, and TNF-α (Robertson et al. 2020). These mediators promote prostaglandin E_2 (PGE_2) synthesis, a potent inducer of uterine contractions and cervical ripening (Romero et al. 2014). Robust epidemiological data affirm ASB's correlation with increased risk of pregnancy –related morbidity. Results of 18 cohort studies (n=45,632 pregnancies) revealed that untreated ASB increases preterm birth risk by 60% (RR=1.60; 95% CI: 1.34–1.91), with the highest risk observed in low- and middle-income countries (LMICs) (Smaill and Vazquez 2019). The relationship between the urinary microbiome and pregnancy outcomes is a topic of growing interest. Research suggests that the composition of the urinary microbiome can impact pregnancy outcomes, with Lactobacillus-dominated microbiota associated with reduced inflammation (Rao et al. 2022).

On the other hand, dysbiosis, characterized by an overgrowth of Gardnerella or Streptococcus, has been linked to preterm birth (Fox and Eichelberger 2015). Studies indicate the maternal microbiota exhibits a crucial impact in pregnancy outcomes, and alterations in the microbiome have been associated with adverse outcomes such as preterm birth (Chu et al. 2018).

The cornerstone of ASB management—antibiotic therapy—faces mounting challenges. While treatment with nitrofurantoin or cephalexin effectively eradicates bacteriuria, a Cochrane review found insufficient evidence that antibiotics reduce preterm birth incidence (Smaill and Vazquez 2019). Disparities in ASB screening and treatment exacerbate global inequities in preterm birth incidence. In developed countries, routine prenatal care ensures early detection and treatment, contributing to preterm birth rates below 8% (Chawanpaiboon et al. 2019). The significance of this study lies in its potential to elucidating the mechanisms by which ASB contributes to preterm labor, this study can

inform the development of effective prevention and treatment strategies. Aim of this study is to investigate the relationship between asymptomatic bacteriuria (ASB) and preterm labor, also to explore mechanisms by which inflammatory marker like TNF- α and hsCRP levels contribute to adverse pregnancy outcomes.

2. SUBJECTS AND METHODS

An Observational Case-Control research design was employed in obstetrics and gynecology department of Tikrit Teaching Hospital (Tikrit, Iraq).The study population consisted of pregnant women experiencing preterm labor who presented to the labor ward between December 2023 and August 2024.

3. ETHICS STATEMENT

This observational case-control research protocol was reviewed and approved by the Scientific Research Ethical Committee, pharmacy college, Tikrit university,Iraq (approval no. SREC 9). All participants provided written consent before enrolling in the study.

Participant Selection

The study sample comprised 100 pregnant women, divided into two groups: 50 women with preterm labour (cases) and 50 women at full term (controls). Participants were randomly selected and matched for age and gestational age.

Inclusion Criteria

Age range from 20 to 40 years, Gestational age: 24-36 weeks for cases and 37-40 weeks for controls women with Singleton pregnancy.

Exclusion Criteria

a. Multiple pregnancy (twins, triplets, etc.)
b. Pregnancy >36 weeks
c. Current use of antibiotics or other medications
d. Symptoms of urinary tract infection (dysuria, frequent urination, lower back pain)
e. Polyhydramnios
f. Diabetes mellitus
g. Smoking and Obesity.

Data Collection

A structured questionnaire was used to gather demographic and clinical data, including age, gestational age, educational level, residence, and medical history.

Clinical Evaluation

Each participant underwent a comprehensive clinical evaluation, including a targeted abdominal examination and ultrasonography.

Laboratory Tests

Blood samples (5ml) were collected from participants (cases and controls) through venous puncture. The samples were centrifuged to separate the serum, which was then stored in Eppendorf tubes at -20°C for subsequent measurement levels of TNF-α and hs-CRP via ELISA technique.

Urinary Specimen Collection for Bacteriological Analysis

Participants received standardized instructions on proper midstream urine collection techniques. Approximately 10 mL of midstream urine was aseptically collected into sterile universal containers, following vulvar cleansing with running water and discarding the initial urine flow to minimize contamination. Urine cultures were performed to diagnose asymptomatic bacteriuria (ASB).

Statistical Analysis

SPSS version 25 and Excel were used for the analysis of data. Frequencies and percentages were used to describe categorical variables, whereas numerical data were expressed as mean values accompanied by their standard deviations. Statistical significance was set atp<0.05.

4. RESULTS

Demographics and Clinical Characteristics of Participants

The results indicate a significant relationship between maternal age and the likelihood of preterm labor. Women aged 25-29 years accounted for the largest proportion of preterm deliveries (48%), whereas those in the same age group with full-term deliveries comprised only 24%. In contrast, women aged 35-40 years had a lower proportion of preterm deliveries (4%) compared to full-term deliveries (16%), as illustrated in table 1:

Table 1. The distribution of age among study cohort

Age group	Preterm delivery (cases) N(%)	Full term delivery(control) N(%)	P-value
20-24years	16(32%)	15(30%)	**0.020***
25-29 years	24(48%)	12(24%)	
30-34 years	8(16%)	15(30%)	
35-40 years	2(4%)	8(16%)	

Discrete variables presented as numbers and frequencies.

*The chi-square(X2) statistic is 9. 7627.The result was statistically significant (p <0 .05).

According to table 2, the results didn't reach statistical significance in the distribution of preterm delivery cases and full-term delivery controls by residence location.

Table 2. The distribution of the study population by residence location

Residence location	Preterm delivery (cases) N(%)	Full term delivery(control) N(%)	P-value
Urban	22(44%)	24(48%)	0.688
Rural	28(56%)	26(52%)	

Discrete variables presented as numbers and frequencies.

The chi-square(X^2) statistic is 0.161. Not significant at p <0 .05.

The educational attainment of women with preterm delivery and full-term delivery was compared. The majority of women with preterm delivery (76%, n=38) had a primary education, whereas 84% (n=42) of women with full-term delivery had a primary education. However, the difference was found to be non-significant, as presented in table3.

Table 3. The distribution of study groups according to educational level

Education	Preterm delivery (cases) N(%)	Full term delivery(control) N(%)	P-value
Primary	38(76%)	42(84%)	0.332
Secondary	6(12%)	2(4%)	
High Education	6(12%)	6(12%)	

Discrete variables presented as numbers and frequencies.

The chi-square (X^2) statistic is 2.2.

As illustrated in table 4, resulting data suggests a parity-dependent increase in preterm delivery risk, with multiparous women exhibiting the highest risk (56%), followed by primiparous (32%) and nulliparous women (12%). However, the observed association didn't achieve statistical significance (p=0.167).

Table 4. Parity distribution among study participants

Parity	Preterm delivery (cases) N(%)	Full term delivery(control) N(%)	P-value
0	6(12%)	2 (4%)	0.167
1	16(32%)	12 (24%)	
≥2	28(56%)	36 (72%)	

Discrete variables presented as numbers and frequencies.

The chi-square(X2) statistic is 3.5714.

The table 5 demonstrates a significant association between positive urine culture and preterm delivery (p=0.009). Specifically, 30% of preterm delivery cases had a positive urine culture, whereas only 8% of full-term delivery controls had a positive culture.

Table 5. The association Between Asymptomatic Bacteriuria and Preterm Birth

Urine culture	Preterm delivery (cases) N(%)	Full term delivery(control) N(%)	P-value
Positive	15(30%)	4(8%)	**0.009***
Negative	35(70%)	46(92%)	

Discrete variables presented as numbers and frequencies.

The Fisher exact test yield a p-value less than0.05.

The table 6 shows the distribution of bacterial isolates in preterm delivery cases and full-term delivery controls. *E. coli* was the most prevalent isolate in preterm delivery cases (53%), followed by *Klebsiella* (27%) and *Group B Streptococcus* (20%). However, no significant differences were observed between cases and controls for any of the bacterial isolates (p>0.05).

Table 6. The distribution of bacterial isolates among study groups

Bacterial isolates	Preterm delivery (cases, n=15) N(%)	Full term delivery (control, n=4) N(%)	P-value
Group B Streptococcus	3(20%)	1(25%)	0.976
Klebsiella	4(27%)	1(25%)	
E.coli	8(53%)	2 50%)	

Discrete variables presented as numbers and frequencies.

The chi-square(X^2) statistic = 0.0475.

In table 7, females who gave birth preterm exhibit significantly elevated levels of TNF-α and hs-CRP compared to women who gave birth at term. Additionally, the differences in both TNF-α and hs-CRP levels between the two groups are statistically significant (p-value < 0.001).

Table 7. Descriptive statistics for TNF- α, hs-CRP level among studied groups

Parameters	Preterm delivery (cases) Mean ± SD	Full term delivery(control) Mean ± SD	P-value
TNF-α(pg/ml)	42.5 ± 3.5	21.2 ± 1.8	**< 0.001*a**
hs-CRP(mg/l)	12.3 ± 1.2	5.2 ± 0.6	**< 0.001*a**

Continuous variables presented as mean± SD; *****: The difference between the two groups was significant at p<0.0001; **a**: independent sample T test

5. DISCUSSION

In present cohort, women aged 25–29 years were accounted for 48% of preterm deliveries compared to 24% of full-term deliveries, whereas those aged 35–40 years contributed only 4% of preterm cases. This finding contrasts with several studies that have identified maternal ages less than twenty and more than thirty-five years as risk factors for preterm birth, suggesting a U-shaped relationship (Hochler et al. 2023; Kahveci et al. 2018). For instance, Hochler *et al.* reported that advanced maternal age was linked to increased obstetric complications, including preterm delivery (Hochler et al. 2023). The differences observed in present study may reflect specific demographic characteristics or unmeasured confounding factors unique to our population. Whereas, the lack of significant differences in preterm birth rates based on residence location and educational level in this investigation is in agreement alongside previous research. (Zhurabekova et al. 2024) noted that while sociodemographic factors might indirectly influence outcomes, they are often less predictive than direct clinical or biological markers. Although the present data suggested a tendency toward increased preterm delivery risk amongst multiparous women, the association did not reach statistical significance. This is consistent with previous research by Ananth and Vintzileos (Ananth, Ananth, and Vintzileos 2006) and other epidemiologic investigations that have found parity to be only one of several factors influencing the risk of preterm birth. In many cases, parity interacts with other maternal factors, such as age and socioeconomic status, making it difficult to isolate as an independent predictor.

Notably, this study revealed a significant correlation between positive urine culture and preterm delivery (30% vs. 8% in controls, p = 0.009). The predominance of Escherichia coli (53% among isolates) supports the idea that urinary tract infections can trigger inflammatory responses leading to preterm labor. This is consistent with the Iraqi study done by Farhan (Farhan 2020) and systematic review research implemented by (Wang, Tang, and Chen 2024) who documented that asymptomatic bacteriuria increases the risk of preterm delivery if left untreated. Although the specific distribution of bacterial isolates didn't vary significantly when comparing cases to control, presence of bacteriuria itself remains a critical risk factor.

The study's most compelling findings were significantly elevated TNF-alpha and hs-CRP values in women alongside preterm deliveries. Elevated inflammatory markers are

presumed to have a direct contribution in initiating labor prematurely. The reported results are align with established research findings of numerous studies that have identified systemic inflammation as a crucial intermediary in the pathophysiology of early term labor (Huang et al. 2019; Romero et al. 2014). described preterm labor as "one syndrome many causes" and highlighted that inflammatory processes—whether due to infection or other stressors—play a central role (Romero et al. 2014). Similarly, Huang *et al.* and Riboni et al. described in previous studies that higher maternal serum levels of inflammatory markers are independently associated with an increased risk of premature delivery (Huang et al. 2019; Riboni et al. 2012). By conclusion, the preterm delivery is a multifactorial condition primarily influenced by infection and systemic inflammation. A significant association was found between asymptomatic bacteriuria (especially due to Escherichia coli) and preterm birth. Also, elevated inflammatory markers (hs-CRP in addition to TNF-α) in women with premature deliveries highlight the role of systemic inflammation.

Conflicts of Interests

Regarding the authorship or publication of this research, no conflicts of interest have been disclosed by the authors.

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Author Contributions

Each author contributed substantially to the conception, writing, and revision of this manuscript.

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