Original Article

Impact of Sociodemographic, Clinical, and Genetic Factors and *Fusobacterium nucleatum* on Premature Birth Outcomes in Women from Kazakhstan: A Case-Control Study

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What's Known

- Preterm birth is defined as delivery before 37 weeks of gestation.
- It is a leading global public health issue, contributing significantly to neonatal morbidity and mortality.

What's New

- A history of preterm birth and specific genetic polymorphisms might be associated with an increased risk of premature delivery.
- Both clinical factors, such as gestational age, maternal age, and obstetric history; and genetic factors, including polymorphisms in parturition-related genes were implicated in preterm birth risk.

Abstract

Background: Premature birth (delivery before 37 weeks of gestation) is a leading cause of neonatal morbidity and mortality worldwide. Specific genetic polymorphisms were associated with immune and inflammatory pathways that might contribute to its pathogenesis. This study investigated the associations between preterm birth and sociodemographic indicators, clinical outcomes, genetic polymorphisms, and microbial factors in Kazakh women.

Methods: This case-control study was conducted from September 2022 to September 2023. It enrolled women with preterm and full-term births in Kyzylorda, Kazakhstan. Data included sociodemographic, clinical variables, genotyping (*TLR2 rs4986790 and MBL2 rs11003125*), and microbial data. The quantification of *Fusobacterium nucleatum* (*F. nucleatum)* in saliva samples was performed using TaqMan real-time PCR. Statistical analysis was conducted using SPSS software (version 26), employing independent sample *t* tests, Chi square tests, Mann-Whitney U tests, and logistic regression. The level of significance was set at $P<0.05$.

Results: Sociodemographic analysis showed no significant differences between the studied groups, except for a higher prevalence of previous preterm birth in the case group. Clinical comparisons revealed significantly lower gestational age at delivery, reduced newborn weight and height, decreased placental weight and dimensions, lower hemoglobin levels, and erythrocyte counts in preterm cases. Genetic analysis demonstrated that all women with preterm labor carried the homozygous AA genotype of *TLR299* rs4986790, while the GG genotype and the G allele of the *MBL2* rs11003125 gene were predominant in this group. Furthermore, the quantitative analysis identified significantly higher *F. nucleatum* levels associated with premature birth, highlighting a potential microbial role in its pathogenesis.

Conclusion: These findings suggested that a history of preterm birth, specific genetic polymorphisms, and microbial factors collectively were associated with an increased risk of preterm birth.

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Keywords ● Premature birth ● Sociodemographic factors ● Clinical study ● Polymorphism, Genetic ● *Fusobacterium nucleatum*

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Introduction

Preterm birth is defined as birth before 37 weeks of gestation.[1] It is a major global public health concern and a leading contributor to neonatal morbidity and mortality.[1] A comprehensive understanding of its multifactorial etiology, encompassing clinical, genetic, and microbial determinants, is essential for developing effective preventive strategies.[2] Established clinical risk factors including shortened gestational age, advanced maternal age, and obstetric history play a significant role in determining the risk of preterm birth.[3] Genetic factors, including genetic susceptibility, particularly polymorphisms in immune-related genes (e.g., *TLR* and *MBL* families), may modulate inflammatory pathways implicated in preterm birth.[2] Additionally, microbial factors, such as infections with bacteria such as *Fusobacterium nucleatum (F. nucleatum)*, were associated with an increased risk of preterm birth.[4] A comprehensive investigation of these interconnected factors provided critical insights into the multifactorial etiology of preterm birth, enabling the development of targeted preventive strategies and personalized interventions to reduce incidence and improve perinatal outcomes.[5]

The Kyzylorda region, located in Central Asia, faces unique environmental and socioeconomic challenges that may adversely affect maternal health.[6] Understanding the clinical, genetic, and microbial factors associated with preterm birth in women from the Kyzylorda region is crucial for developing effective prevention and management strategies.[7] The Kyzylorda, once the world's fourth-largest lake, has experienced significant environmental degradation, leading to the formation of the Kyzylorda region, which encompasses parts of Kazakhstan and Uzbekistan.[6] The resulting environmental degradation has profoundly affected the health and well-being of the local population, with potential impacts on pregnancy outcomes.[8]

Several factors contribute to the elevated preterm birth rates in the Kyzylorda region, including environmental pollutants, limited access to healthcare services, and socioeconomic deprivation.[9] However, comprehensive studies investigating the genetic and microbial factors associated with preterm birth in this region are limited.[10] This knowledge gap underscored the need for integrated research examining both environmental and biological contributors to preterm birth in this environmentally vulnerable region.

Previous studies reported that variations in Toll-like receptor 4 *(TLR4)* and mannose-binding lectin *(MBL2)* genes could influence susceptibility to infections and inflammatory conditions, which were recognized as critical risk factors for preterm delivery.[11, 12] Therefore, investigation of *TLR4* and *MBL2* polymorphisms in the Kyzylorda population would provide elucidate region-specific genetic risk factors, and facilitate early identification of high-risk pregnancies through biomarker discovery. This study aimed to investigate the role of genetic polymorphisms, specifically the *TLR4* and *MBL2* genes, in the pathogenesis of preterm birth among women in the Kyzylorda region.[13] Additionally, this study explored the molecular biology of *F. nucleatum*, a bacterium associated with adverse pregnancy outcomes, in the context of preterm birth.

Materials and Methods

Study Design

This case-control study was approved by the Ethics Committee of Al-Farabi Kazakh National University (Protocol No.IRB-A432, dated 04/28/2022), and adhered to the ethical principles of the Declaration of Helsinki. All participants provided written informed consent, including explicit permission for placental donation.

Study Population and Recruitment

From September 2022 to September 2023, women delivering at the Kyzylorda Regional Perinatal Center were enrolled, comprising cases (preterm birth, <37 weeks) and controls (term birth, ≥37 weeks). Eligible women were aged 19-44 years and had resided continuously in the Kyzylorda region for the past 10 years prior to the time of the study.

Participants

Pregnant women were divided into two groups: the control group (n=41) comprising women with uncomplicated term deliveries (37-40 weeks gestation), and the case group (n=41) consisting of women experiencing spontaneous preterm birth (≤36+6 weeks) (figure 1).

Inclusion and Exclusion Criteria

This study included women with singleton pregnancies who delivered vaginally within 72 hours of membrane rupture and provided signed informed consent.

The exclusion criteria were iatrogenic preterm delivery, multiple gestations, stillbirths, or prolonged rupture of membranes (>72 hours). Women with chronic maternal conditions, such as diabetes mellitus, hypertension, or thyroid pathology, and pregnancy complications affecting fetal development (e.g., preeclampsia, fetal growth restriction) were also excluded to minimize confounding factors.

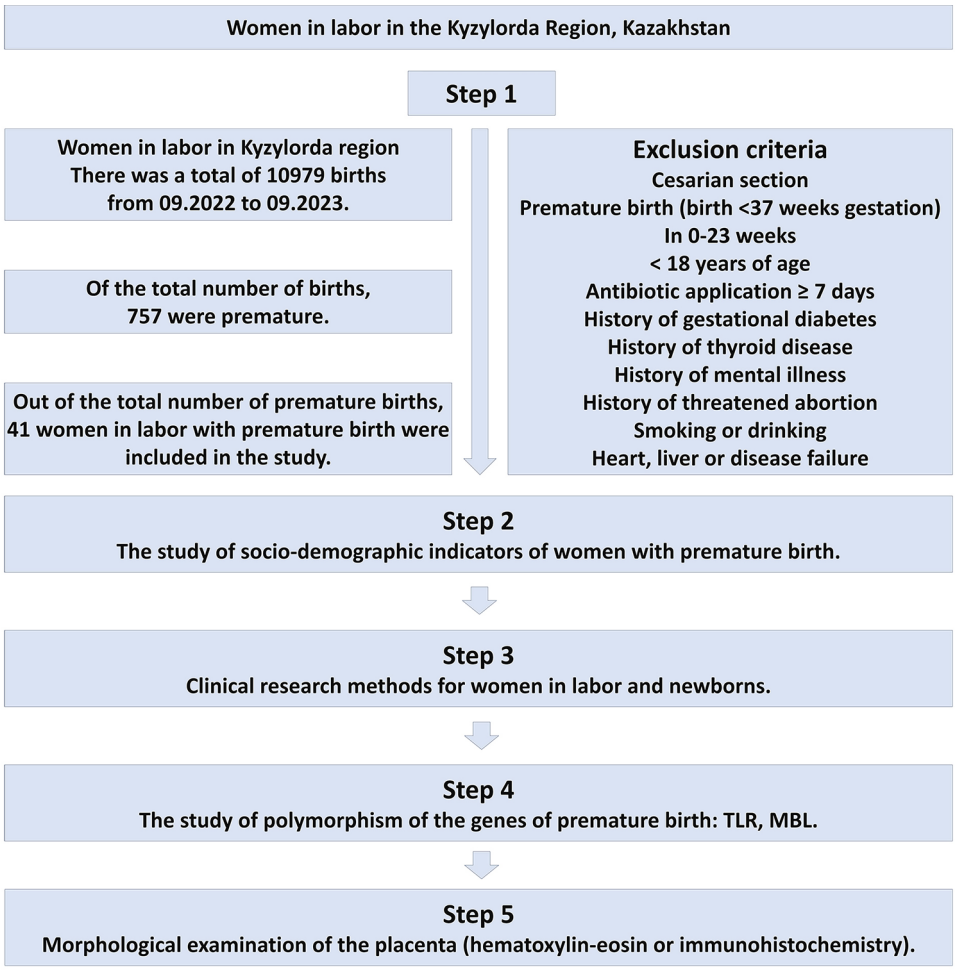

Figure 1: The figure illustrates the study design that examines the sociodemographic, clinical, and genetic factors associated with premature birth in women from Kazakhstan.

Data Gathering Tools

This study employed a multi-modal approach to data acquisition. Structured surveys and validated questionnaires[14-19] were utilized to collect sociodemographic and clinical data from participants. These instruments included questions on education, occupation, income, smoking status, and medical history, ensuring comprehensive coverage of relevant factors associated with premature birth.

Clinical parameters were extracted through systematic medical record review, with particular attention to gestational age at delivery, neonatal anthropometrics (birth weight and height), placental characteristics (weight and dimensions), and maternal hematologic indices (hemoglobin levels, and erythrocyte counts).

For genetic analyses, we collected biological specimens under standardized conditions and performed polymerase chain reaction (PCR)-based genotyping coupled with DNA sequencing to characterize target polymorphisms (*TLR2 rs4986790 and MBL2 rs11003125*), adhering to strict quality control protocols throughout laboratory procedures. Specimens for genetic analysis were collected following established protocols to ensure reliability and accuracy.

Sociodemographic Data Collection

Sociodemographic indicators of women who experienced premature birth were obtained through a survey conducted among women in labor on the first day after delivery. The questionnaire[14-19] included questions based on research No. AP14972889 “Study of gene-infectious interaction in the genesis of spontaneous premature birth”. The sociodemographic data collection protocol encompassed comprehensive maternal characteristics, including age at delivery, educational level, and occupational status, lifestyle factors (smoking and alcohol consumption). Additionally, data on the pregnancy history of each woman, including complete obstetric history, including gravidity and parity, history of preterm deliveries (including gestational timing), previous abortions, and occurrence of fetal growth restriction in prior pregnancies were recorded.

Clinical Research Methods

Clinical parameters were obtained through a dual approach: (1) structured questionnaires[14-19] and (2) systematic extraction from the Damumed electronic medical record system. Maternal assessments included assessing their body mass index (BMI) and recording the trimester of pregnancy. Information on comorbidities, as well as their use of antibiotic therapy within 7 days prior to delivery was collected. Comprehensive obstetric data, including delivery complications and pregnancy-related laboratory values, were documented. Oral health during gestation was assessed through dental visit frequency and documented. Neonatal outcomes comprised anthropometrics (birth weight, length, and sex), vital status at delivery, and exact gestational age determination. Placental morphology was quantitatively analyzed through standardized measurements of weight, maximum diameter, and thickness at the umbilical cord insertion site.

Morphological Examination of the Placenta

For morphological examination, the placental examination process was initiated immediately following the completion of the delivery process and was promptly retrieved after delivery. The Standard Operating Procedures (SOPs) were developed to ensure rigorous standardization across all participating study locations. This documentation guaranteed methodological consistency throughout the research project. These procedures were copyrighted under Certificate No. 29852 on November 1, 2022.

The collection of placental tissue for subsequent histological examination represents a routine and straightforward procedure that was performed immediately after childbirth (figure 2). The placenta, which is naturally expelled during the third stage of labor, was carefully collected by the attending midwife or obstetrician-gynecologist and placed in a sterile tray. The collection process was entirely safe for both the newborn and the postpartum mother, as the placenta tissue no longer served any physiological function following delivery, and its removal did not interfere with normal postpartum recovery.

The placental collection and preparation process utilized the following sterile materials and equipment: sterile disposable scalpel, sterile surgical scissors, histological specimen containers with secure locking lids, precision measuring rulers, disposable alcohol-based antiseptic wipes, Latex-free disposable examination gloves, and 10% neutral buffered formalin solution.

Placental tissue sampling was conducted only after the attending obstetrician-gynecologist had completed the standard procedure of umbilical cord severance. Tissue fragments were meticulously excised using sharp surgical instruments, with particular attention to avoiding any compression artifacts that might result from improper handling with forceps or clamps, which could potentially distort tissue architecture and compromise histological evaluation.

For comprehensive histological assessment, full-thickness placental samples were obtained according to the following specifications: triangular tissue sections incorporating both the maternal (basal plate) and fetal (chorionic plate) surfaces, representative samples from both central and peripheral placental regions, tissue sections carefully selected from areas outside major vascular networks, standardized sample dimensions of approximately 1-2 cm in diameter with a consistent depth of 1 cm.[20]

Immediately following excision, each histological specimen was promptly placed in a dedicated histological container filled with 10% buffered formalin solution. The container was securely sealed to ensure complete

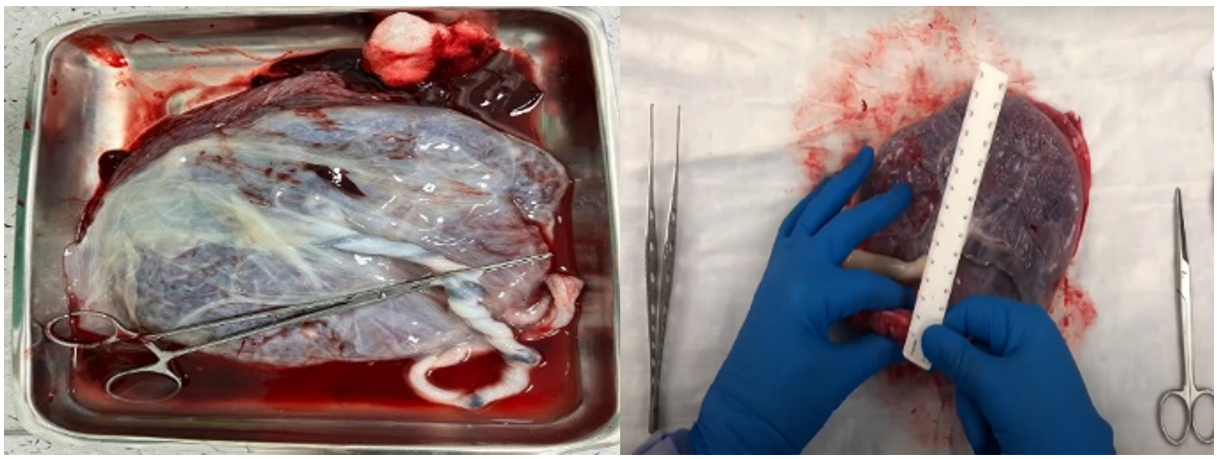

Figure 2: The figure demonstrates the procedure used for the collection and histological examination of placental tissue after birth, detailing the steps involved in the analysis.

immersion of the specimen in a fixative solution, prevention of tissue desiccation, inhibition of autolytic processes, and maintenance of tissue architecture during transport.

In the histopathology laboratory, the fixed placental specimens underwent systematic processing through various stages, including initial gross examination and photographic documentation, standardized tissue processing using automated tissue processors, paraffin embedding for microtome sectioning, preparation of 4-5 µm thick sections using precision microtomes, routine staining with hematoxylin and eosin (H&E), comprehensive microscopic evaluation by board-certified pathologists. All laboratory procedures strictly adhered to internationally recognized histopathological guidelines,[20] ensuring the highest quality of specimen preparation and analysis.

Molecular Biological Study of F. nucleatum

Sample Collection and Processing Protocol: A standardized protocol for periodontal assessment and saliva collection was developed and formally registered under Certificate No. 30427 (November 18, 2022), entitled "Procedure for Periodontal Screening and Saliva Collection for Molecular Analysis of Preterm Birth Biomarkers During Second Trimester Pregnancy." Saliva samples were collected antepartum using this validated protocol, while placental tissues were obtained immediately postpartum.

DNA Extraction Methodology: Genomic DNA isolation was performed using:

- Saliva samples: QIAamp DNA Blood Kit (Cat. 51104, Qiagen, Germany)
- Placental samples: AllPrep DNA/RNA/miRNA Universal Kit (Cat. 80224, Qiagen, Germany)

All specimens were maintained at -80°C until processing. Placental tissues underwent mechanical homogenization using an MT-13K-L Mini Handheld Homogenizer according to manufacturer specifications. Cellular lysis was achieved using a guanidine isothiocyanate-containing buffer, which simultaneously denatured cellular proteins, inactivated nucleases, and preserved DNA integrity.

Quality Control Measures: DNA purity was quantitatively assessed using a BioTek Synergy LX Multi-Mode Microplate Reader.

F. nucleatum in samples was detected following established guidelines.[21] The *NusG F. nucleatum* gene and the reference human gene, *SLCO2A1*, were amplified using custom TaqMan primer/probe sets (DNA synthesis, Russia). The primer and probe sequences for TaqMan gene expression analysis were as follows: *F. nucleatum* direct primer 5'-TGGTGTCATTCTTCCAAAAATATCA-3', reverse primer 5'-AGATCAAGAAGGACAAGT TGCTGAA-3', and probe *FAM* 5'-ACTTTA ACTCTACCATGTTCA-3'; *SLCO2A1* direct primer 5'-ATCCCCAAAGCACCTGGTTT-3', reverse primer 5'-AGAGGCCAAGATAGTCCTGGTAA-3', and probe *VIC* 5'-CCATCCATGTCCTCATCTC-3'.

PCR mixtures were prepared, and analyses were performed on a 96-well optical PCR plate (DNA Technology, Russia), using a DTprime real-time PCR instrument (DNA Technology, Russia) with the following reaction conditions: initial denaturation at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 15 sec and extension at 60 °C for 60 sec.

Genetic Polymorphism Analysis

Venous blood samples collected in EDTA tubes were processed using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany) following manufacturer specifications. PCR and DNA sequencing were employed to determine polymorphisms of the *TLR4* Asp299Gly and *TLR4* Thr399Ile genes, as well as *MBL2* rs11003125 and *rs7096206* genes.

For *TLR4* DNA sequence analysis, the following primers were used: for *TLR4* Asp299Gly, direct (F: 5'-GATTAGCATACTTAGACTACTACCTCCATG) and reverse (R: 5'-GATTCAACTTTCTGAAAA AGCATTCCCAC); for *TLR4* Thr399Ile, direct (F: 5'-GGTTGCTGTTCTCAAAGTGATTTTGG GAGAA) and reverse (R: 5'-CCTGAAGACTGGAGAGTGAGTTAA ATGCT). *MBL2* genotyping utilized the following primers: for promoter polymorphisms, direct (5'-CCAGGGCCAACGTAGTAAAG-3') and reverse (5'-GAGGGGTTCCATCTGTGCC-3'); for exon 1 polymorphisms, direct (5'-GGGC ATGCTCGGTTAAATAG-3') and reverse (5'-TGCCAGAGAATCAGAGCTGA-3').

The extraction protocol involved:

1. Lysis with proteinase K and proprietary buffer (56°C for 10 min)
2. Selective DNA binding to silica-gel membranes
3. Sequential wash steps to remove contaminants
4. Elution in 50-200 µL of AE buffer

Custom TaqMan assays (DNA Synthesis, Russia) were performed in 96-well optical plates (DNA Technology, Russia) using a DT-Prime real-time PCR system under optimized conditions: initial denaturation at 95 °C for 2 min, followed by 45 cycles of 95 °C for 15 sec, annealing at 56 °C for 30 sec, extension at 60 °C for 30 sec, and final extension at 60 °C for 3 min, with a final storage at 10 °C.

Sanger DNA sequencing was performed using PCR products purified from non-bound

primers using Exonuclease I (Fermentas/Thermo Fisher Scientific, USA) and alkaline phosphatase (FastAP, Fermentas/Thermo Fisher Scientific, USA). Sequencing reactions were performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and analyzed on an automatic 3730xl DNA Analyzer (Applied Biosystems, USA). Chromatograms were processed using DNASTAR Lasergene 6.0. Bidirectional sequencing was performed using the same primers as in the PCR amplification.

Sample Size Calculation

The minimum required sample size for this case-control study was calculated using G*Power software (version 3.1.9.2, Kiel University, Germany). The statistical power was set at 0.95, which is typically sufficient to detect significant differences between groups. The α-error probability was set at 0.05 (two-tailed), corresponding to a 5% Type I error rate.

The effect size (w) was set as 0.3, reflecting a medium effect based on previous studies of factors associated with premature birth. Using these parameters (α=0.05, power=0.95), G*Power calculation yielded a required total sample size of n=82.

The present study successfully enrolled 82 women, evenly distributed between cases and controls, ensuring adequate statistical power to detect significant associations among sociodemographic, clinical, and genetic factors associated with premature birth.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (version 26; IBM Corp., Armonk, NY, USA). Continuous variables were compared using independent sample *t* tests or Mann-Whitney U tests (for non-normally distributed data), while categorical variables were analyzed using Chi square tests. Sociodemographic and clinical characteristics between women with premature and term births were compared using these appropriate tests based on data distribution. For further analysis, logistic regression was employed to calculate relative risk (RR) and odds ratio (OR), with statistical significance set at P<0.05. Genetic associations between case and control groups were assessed using Chi square tests for genotype distributions, supplemented by logistic regression analysis to determine ORs and 95% CIs. All tests were two-tailed, with statistical significance set at P<0.05.

Results

Sociodemographic Indicators

The sociodemographic indicators were compared between the control group and the case group of women with premature birth. The results are summarized in table 1.

Table 1: Comparison of sociodemographic indicators between control and case groups of women with premature birth

Variable		Control group (n=41)	Case group (n=41)	P value
Education	School	5 (12.2%)	6 (14.6%)	0.68
	College	23 (56.1%)	19 (46.3%)	
	University	13 (31.7%)	16 (39.0%)	
Occupation	Housewife	20 (48.8%)	14 (34.1%)	0.27
	Employer	21 (51.2%)	26 (63.4%)	
	Student	0 (0.0%)	1 (2.4%)	
Income	High	8 (19.5%)	8 (19.5%)	0.97
	Average	20 (48.8%)	21 (51.2%)	
	Low	13 (31.7%)	12 (29.3%)	
Smoking	Non-smoker	39 (95.1%)	40 (97.6%)	0.56
	Smoker	2 (4.9%)	1 (2.4%)	
Passive smoker	Non-smoker	35 (85.4%)	38 (92.7%)	0.29
	Smoker	6 (14.6%)	3 (7.3%)	
Alcohol consumer	Non-consumer	41 (100%)	40 (97.6%)	0.31
	Consumer	0 (0.0%)	1 (2.4%)	
History of previous preterm birth	No	39 (95.1%)	32 (78.0%)	0.02*
	Yes	2 (4.9%)	9 (22.0%)	
History of miscarriage	No	32 (78.0%)	35 (85.4%)	0.39
	Yes	9 (22.0%)	6 (14.6%)	
History of abortion	No	37 (90.2%)	39 (95.1%)	0.40
	Yes	4 (9.8%)	2 (4.9%)	
Retardation	No	41 (100%)	37 (90.8%)	0.40*
	Yes	0 (0.0%)	4 (9.8%)	

*The Chi square test was used.

Predictive Model For Premature Birth

A predictive model for assessing premature birth probability was developed through binary logistic regression, with the following equation:

$P=(1/(1+e^{-z}))\times100\%$

$z=-0.198+1.702\times X_{prb}$

Where P represents the probability of premature birth in women (%), and X_{prb} represents the history of premature birth (0=absence, 1=present).

The final model demonstrated strong statistical significance (P<0.001), with a Nagelkerke R-squared value of 8.7%, indicating that 8.7% of the variance in premature birth probability was explained by the included predictors.

Regression coefficients revealed a significant positive association between prior premature birth history (X□r□) and subsequent premature birth probability (P<0.001). Complete model characteristics are presented in table 2.

A history of premature birth increased the odds of premature birth in women. The model demonstrated a sensitivity of 22.3% (9 patients out of 41) and a specificity of 95.1% (2 patients out of 41) at this threshold value. These results are shown in table 3.

Clinical Results of the Study of Women in Labor and Newborns

The case group included 41 women with premature birth (23-36 weeks gestation), and the control group consisted of 41 women with term deliveries. The age of the women ranged from 19 to 44 years, with a mean age of 31.5 years (table 4).

Gene Polymorphisms in Preterm Birth

The study examined polymorphisms in *TLR299* (rs4986790 and *TLR399* rs4986791) genes, as well as the *MBL2* (rs11003125 and rs7096206) genes using Sanger sequencing. All women with preterm labor showed a homozygous AA genotype, while one woman with normal labor

Table 2: Characteristics of the relationship between the predictors of the model and the probability of premature birth in women

Predictors	Unadjusted OR; 95% CI	P value
Order of child's birth	5.48; 1.10-27.21	0.04*

*P<0.05 was considered statistically significant.

Table 3: Logistic regression analysis predicting preterm birth (yes=1) based on history of previous preterm birth

Variable	B	SE	Wald	P value	Exp(B)	95% CI for Exp(B)
History of previous preterm (Yes=1)	1.70	0.82	4.34	0.04	5.48	1.11-27.22
Constant	-0.20	0.24	0.69	0.41	0.82	-

B: Regression coefficient; SE: Standard Error of the coefficient; Wald: Wald Chi square test; Exp (B): Odds Ratio; 95% CI for Exp(B): 95% Confidence Interval for the Odds Ratio; P<0.05 was considered statistically significant.

Table 4: Comparison of sociodemographic and clinical characteristics between women with premature birth and those with term birth

Variable	Control group (n=41)		Case group (n=41)		P value
	mean±SD	Q1-Q3	mean±SD	Q1-Q3	
Age	29.20±5.68	24.0-34.0	27.56±6.53	22.0-32.50	0.12
Gestation	39.54±1.22	38.50-41.0	33.02±3.11	32.0-35.0	0.001*
Newborn weight	3816.02±501.80	340.0-4135.0	2054.66±623.78	1685.0-2450.0	0.001*
Newborn height	54.12±2.43	52.0-55.50	41.71±5.20	40.0-44.0	0.001*
Placental weight	706.71±102.51	642.50-755.0	496.41±104.84	450.0-570.0	0.001*
Placenta length D1	18.54±1.14	18.0-19.0	16.55±1.64	15.0-18.0	0.001*
Placenta length D2	18.22±1.06	17.50-19.0	16.28±1.55	15.0-18.0	0.001*
Placental Thickness	2.988±0.208	3.0-3.0	2.21±0.49	2.0-2.50	0.001*
Hemoglobin	106.88±10.60	97.0-114.50	100.31±15.51	90.25-111.0	0.04*
Erythrocyte count	3.68±0.29	3.40-3.90	3.53±0.52	3.20-3.80	0.04*
Platelet count	247.54±59.48	196.50-298.0	254.48±62.38	203.0-314.50	0.58
Erythrocyte sedimentation rate	34.15±8.34	30.0-40.0	33.72±10.77	30.0-40.0	0.62
Leukocyte count	8.82±2.21	7.20-10.05	9.19±3.52	6.85-11.33	0.96

*Mann-Whitney U test was used.

Table 5: Frequencies of the rs4986790 genotypes of the *TLR299* gene in the studied groups

Alleles	Cases (n=24)	Controls (n=26)	χ^2	P value	OR	95% CI
The allele A	1.00	0.98	0.93	0.33	2.83	0.11–71.04
The allele G	0.0	0.02			0.35	0.01–8.90

had a heterozygous AG genotype (figure S1). However, no statistically significant differences were observed (table 5). *TLR299* rs4986791 sequencing results are shown in figure S2.

Besides, Sanger sequencing was performed to analyze the *MBL2* rs11003125 variant (figure 3). Genotype distribution analysis revealed that the GG genotype (58%) and G allele (70%) of the *MBL2* rs11003125 were significantly more prevalent among women with preterm labor than the controls (table 6). Notably, women carrying the GG genotype had a 1.6-fold higher risk of preterm labor than those with other genotypes. In the present study, sequencing analysis of the *MBL2* rs7096206 polymorphism showed complete homogeneity, with all participants exhibiting the CC genotype (figure S3).

Fusobacterium nucleatum Detection In Preterm Birth

A total of 41 pregnant women in their second trimester were randomly selected to assess *F. nucleatum* presence in saliva and placenta samples using the qPCR method. As the women did not exhibit obvious signs of periodontitis, 12 women including both controls and preterm birth cases—were analyzed for comparative evaluation. Saliva and placenta samples were collected from all participants. Genomic DNA was successfully extracted from all specimens and rigorously evaluated, confirming the high purity and integrity of the gDNA. Appropriate control samples were utilized to ensure the reliability of the qPCR analyses. The analysis detected *F. nucleatum* in all 12 (100%) saliva samples from the experimental group and 1 placenta sample. In the control group, *F. nucleatum* was identified in 8 saliva samples and 2 placenta samples. All quality control measures confirmed the accuracy and specificity of the conducted analyses and validated the specificity of the obtained results (figure 4).

Morphological Results of the Placenta

Morphological changes in placental tissue were assessed according to three assessment categories (figure 5). The first category included circulatory in the mother, specifically lesions associated with insufficient transformation of the spiral arteries, such as persistent alteration of the basal plate of the arteries, acute atherosclerosis of the basal plates of the arteries, hypertrophy of the walls of the decidual arteries, fibrinoid necrosis of spiral arteries, persistence of endovascular trophoblast, and spiral artery thrombosis.

The second category involved vascular malperfusion of the fetus, comprising lesions related to blockage of large fetal vessels (chorionic plate and stem villi), such as blood clots in large vessels of the fetus, deposition of fibrin in intima in large vessels of the fetus, and fibromuscular sclerosis in medium-sized vessels.

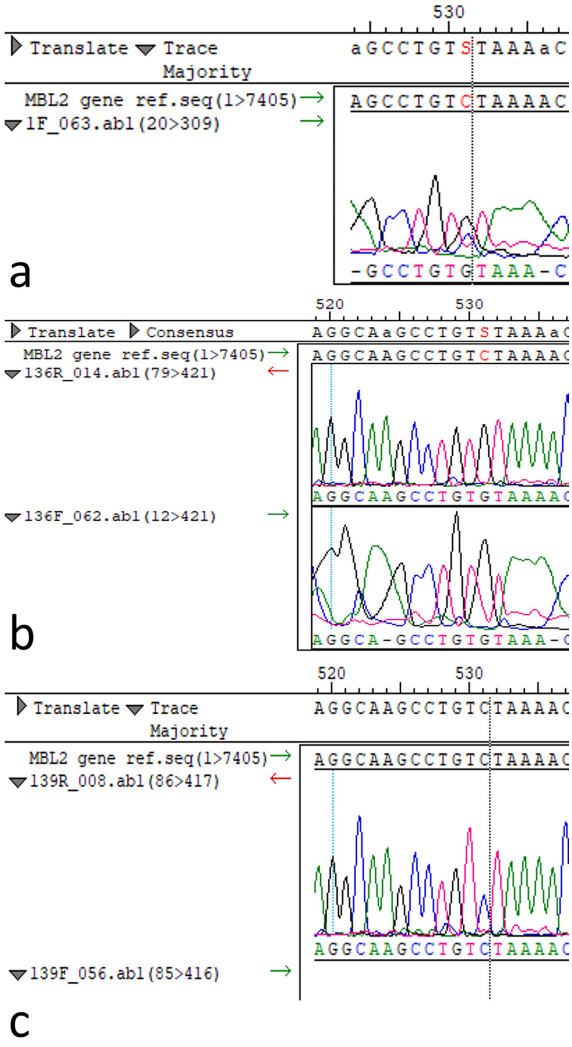

Figure 3: The figure shows the genotype frequency distribution of the *MBL2* rs11003125 polymorphism in women with preterm labor versus controls. a) The panel displays the distribution of the GC genotype. b) The panel shows the GG genotype distribution, and c) the panel represents the CC genotype distribution.

Table 6: Frequency distribution of genotypes and alleles of the *MBL2* rs11003125 gene polymorphism

Genotypes	Cases (n=12)	Controls (n=23)	χ2	P value	OR	95% CI
Allele G	0.71	0.61	0.68	0.41	1.56	0.54-4.51
Allele C	0.29	0.39			0.64	0.22-1.85
Genotype G/G	0.58	0.44	0.70	0.7	1.82	0.44-7.48
Genotype G/C	0.25	0.35			0.63	0.13-2.98
Genotype C/C	0.17	0.22			0.72	0.12-4.41

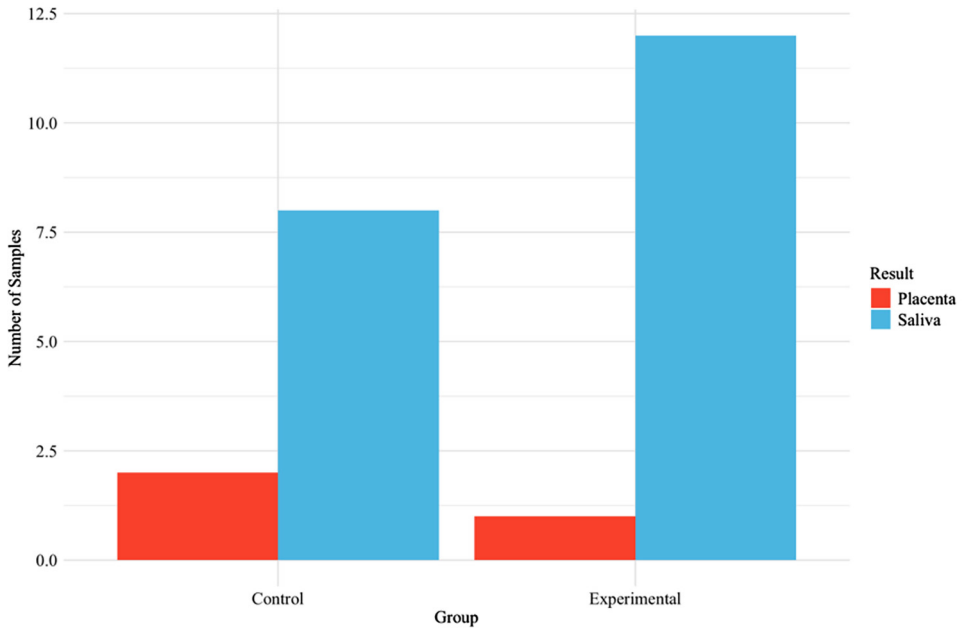

Figure 4: *Fusobacterium nucleatum* was detected in placental and salivary samples from both control and case groups of pregnant women in Kyzylorda. The bar chart displays the distribution of *F. nucleatum* detection in placenta (red bars) and saliva (blue bars) samples. The experimental group showed a significantly higher number of saliva samples with *F. nucleatum* than the control group, while placental detection rates were relatively low in both groups.

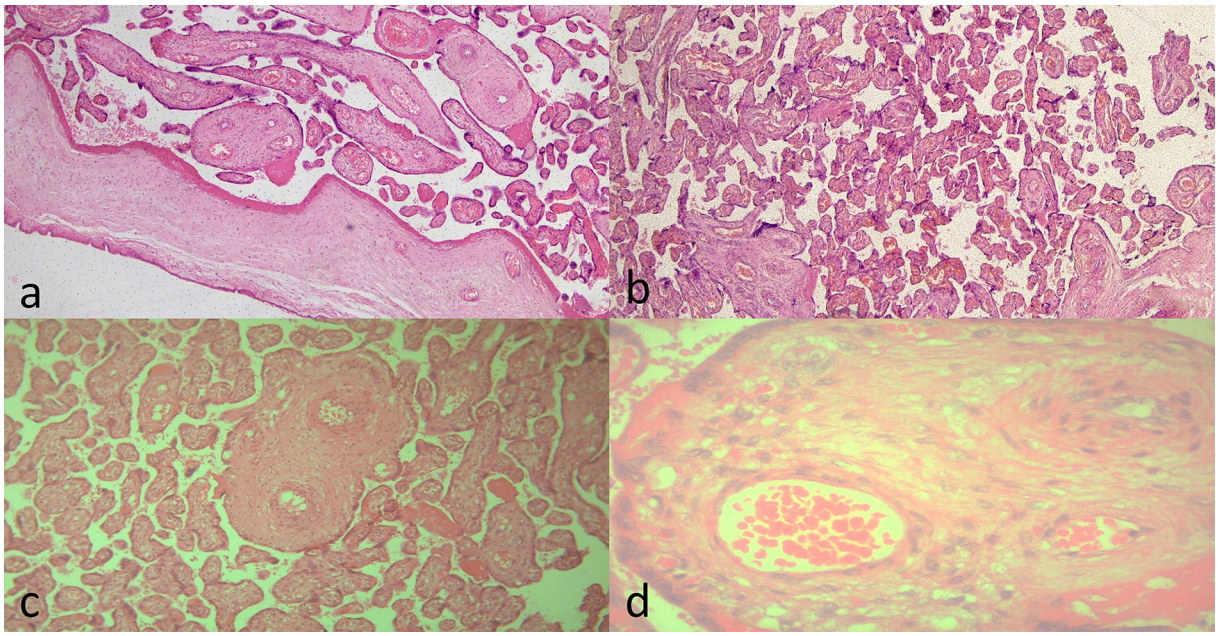

Figure 5: This figure presents the morphological changes in placental tissue across different magnifications. A: The panel shows the control group histology at 100x magnification, B: The panel shows the control group at 40x magnification, C: The panel depicts the case group findings at 100x, and D: The panel shows the case group features at 400x magnification.

The third assessment category focused on placental microvasculopathy, including lesions of the maternal villi, such as distal villi hypoplasia, villous infarcts, karyorexis of the villi of the vascular stroma, hyalinized avascular villi, and thrombotic vasculopathy of the fetus (on average 15 non-vascular villi per slide).

During the macroscopic examination of the placenta, attention was paid to the condition of the fetal membranes, the length of the umbilical cord was measured, and the type of attachment was documented. The uterine surface of the placenta was systematically assessed. Precise placental weight measurements were recorded in grams. Three-dimensional measurements included: the smallest radius of the uterine surface, the largest radius of the uterine surface, and the placenta thickness.

Microscopic examination of the placentas in the control group revealed relatively uniform

vascular network development throughout the placenta tissue. In the marginal zone, although the number of capillaries was reduced, their width and cross-sectional area were increased. The chorionic plate demonstrated normal thickness and was represented by ordered fibers of connective tissue. In thickness, it had full-blooded vessels and focal areas of amniotic epithelial proliferation. Beneath the chorionic plate were narrow bands of fibrinoids. The chorionic villi displayed proper differentiation, predominantly intermediate villi with fewer stem villi and single terminal villi anchoring to the basal decidual plate. Mature chorionic villi contained 5-7 vessels per villus displaying uneven congestion, with occasional syncytiotrophoblastic buds observed in the villous epithelium. The basal lamina stroma consisted of fibrous connective tissue housing clusters of large decidual cells exhibiting oval morphology, distinct cell borders, central round nuclei, and eosinophilic cytoplasm. In general, in the central zone of the placenta, there was an increase in the thickness of the hematoplacental barrier due to the height of the chorionic epithelium, with elements responsible for the trophic, protective, and permeability functions through the hematoplacental barrier prevailing.

In the case group, the mean weight of the placenta was 496.41±104.84 g. Microscopic examination of the placentas revealed various pathological changes in the villous chorion, including sclerosis, fibrinoid deposition secondary to terminal villi hyperplasia, and increased deposition of maternal fibrinoid. The chorionic plate maintained normal thickness with organized connective tissue fibers containing engorged vessels and a single layer of cuboidal amniotic epithelium. Under the chorionic plate, fibrinoid islands were observed engulfing the chorionic villi. The chorionic villi showed complete differentiations, comprising stem, intermediate, and terminal villi with anchoring ends embedded in the decidual basal plate. Most villi demonstrated mature morphology (premature ripening). Stromal changes included fibrous connective tissue hyperplasia in some intermediate villi and edema in others. Vascularization ranged from 5-7 vessels per villus, displaying uneven congestion, with occasional avascular villi noted.

The chorionic villous epithelium had multiple buds of syncytiotrophoblasts in individual villi. The intervillous space contained foci of parenchymal collapse. The basal plate displayed irregular decidual tissue stratification with neutrophil infiltration and hemorrhage foci.

Discussion

The overall results of this study highlighted significant associations between sociodemographic factors, clinical outcomes, and genetic polymorphisms with premature birth. While sociodemographic factors, such as education, occupation, and income, showed no statistically significant differences between the control and case groups, obstetric history revealed a history of previous preterm birth as a prominent risk factor. Clinical outcomes, including gestational age, newborn weight and height, placental characteristics, hemoglobin levels, and erythrocyte count, were significantly different between women with preterm and full-term births. Genetic analysis further implicated specific polymorphisms in the *TLR299 and MBL2* genes and an increased risk of preterm birth, suggesting potential molecular contributors to preterm birth susceptibility. These collective findings underscored the multifactorial nature of preterm birth, involving the interplay between genetic predisposition and clinical manifestations.

In this study, various aspects related to premature birth were investigated, which revealed several important findings. The analysis revealed no statistically significant differences in sociodemographic characteristics, including education level, occupation, income status, smoking habits (both active and passive), and alcohol consumption, between women who experienced preterm delivery and those with term births. However, a significant difference was observed in the history of previous preterm births, with a higher prevalence in the case group than in the controls. This finding suggested that a history of preterm delivery might represent an important risk factor for future preterm birth.[22]

Clinical outcomes demonstrated significant differences between the control and case groups in multiple parameters, such as gestational age, newborn weight and height, placental weight and dimensions, hemoglobin levels, and erythrocyte count. These findings indicated distinct clinical profiles between women with preterm and term births.[23]

Regarding genetic polymorphisms, the homozygous AA genotype of the *TLR299* rs4986790 gene was detected in all women with preterm labor, while the heterozygous AG genotype was detected in one woman with normal labor. Similarly, the GG genotype and the G allele of the *MBL2* rs11003125 gene were most prevalent among women with preterm labor, suggesting a potential association between these genetic variants and preterm birth.[24]

Disorders in the TLR system might arise from single nucleotide polymorphisms (SNPs) in *TLR* genes, altering susceptibility to infections or inflammatory diseases.[25] Specific polymorphisms in the *TLR2* gene, such as Arg753Gln and T597C, were associated with infections caused by various pathogens, including *Candida albicans*, *Mycobacterium tuberculosis*, cytomegalovirus, and herpes simplex virus type 2.[26-29] Similarly, the SNPs in the *TLR4* gene were associated with inflammatory risk, with the Asp299Gly SNP showing linkage to the Thr399Ile polymorphism.[30, 31] This findings suggested that heterozygous CT genotype and C8993T T-allele polymorphism in the *TLR4* might confer protective effects in premature rupture of fetal membranes in the control group.[32]

Genome-wide studies of women with preterm birth have identified rare mutations in genes involved in the negative regulation of the intrinsic immune response and genes encoding antimicrobial peptides. However, these findings remain inconclusive and contradictory, primarily due to limitations in sample sizes and the high costs associated with these methodologies. Future genome-wide studies are exposed to expand the evidence base and improve the interpretation of results.

These findings highlighted several key implications: the importance of early identification of high-risk women during pregnancy to enable appropriate monitoring and care, the need to better understand the biological mechanisms underlying the observed clinical outcome differences, and the potential for developing targeted interventions to improve outcomes in preterm birth cases. Genetic screening might also be a valuable tool for risk stratification, potentially enabling personalized care strategies for women at elevated risk of preterm delivery.

This study provided valuable insights into the multifactorial nature of preterm birth, highlighting the complex interplay between sociodemographic factors, clinical outcomes, and genetic polymorphisms. The findings underscored the need for further research to enhance our understanding of these relationships and develop effective strategies to address the challenges associated with preterm delivery.

This study provided valuable insights into the sociodemographic, clinical, and genetic factors associated with premature birth among women in Kazakhstan, addressing a significant gap in the existing literature. Through a case-control design, the present study compared women with premature births against those with full-term deliveries, enabling a robust analysis of risk factors. The inclusion of genetic polymorphisms, specifically *TLR4* and *MBL2*, offered novel perspectives on genetic contributions to premature birth, potentially informing future maternal health research and interventions.

A key limitation of this study was the focus on only two genetic polymorphisms, which might overlook other relevant genetic factors in preterm birth. Future studies should incorporate larger, more diverse populations and investigate broader genetic markers to yield a more comprehensive understanding of preterm birth risk factors.

Conclusion

The study revealed several key findings concerning premature birth among women in the Kyzylorda region. Our analysis demonstrated associations between premature birth risk and various sociodemographic factors including education level, occupation, income status, smoking habits, and particularly history of previous preterm delivery. The developed logistic regression predictive model confirmed that prior preterm birth significantly increased the probability of subsequent premature deliveries. Clinical parameters such as gestational age, newborn weight, placental characteristics, and hematological values showed significant differences between women with premature births and those with full-term deliveries. The genetic analysis identified polymorphisms in the *TLR2* and *TLR4* genes, potentially implicating these variants in premature birth pathogenesis. However, further research is required to elucidate the full genetic contribution to premature delivery in this population.

In conclusion, the findings of the present study offered critical insights into the clinical, sociodemographic, and genetic factors associated with premature birth among women in Kyzylorda. These findings might guide targeted interventions and future research to mitigate preterm birth rates in this region.

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Authors’ Contribution

G.Z: Study concepts, study design, data acquisition, quality control of data and algorithms, data analysis and interpretation,

statistical analysis, and drafting; A.B: Data analysis and interpretation, editing, and reviewing the manuscript; Zh.O: Study design, data acquisition, quality control of data and algorithms, editing, and reviewing the manuscript; M.S: Statistical analysis, editing, and reviewing the manuscript; G.T: Data analysis and interpretation, editing, and reviewing the manuscript; N.A: Data acquisition, and drafting; A.M: Data analysis and interpretation, editing, and reviewing the manuscript; K.T: Study concepts, editing, and reviewing the manuscript; Ak.B: Data analysis and interpretation, editing, and reviewing the manuscript; ; All authors read and approved the final manuscript and provided an agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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