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Association between interleukin-19 gene polymorphisms and maternal puerperal infection

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ABSTRACT

Any bacterial infection of the genital tract after childbirth is called maternal puerperal infection. This infection accounts for 13% of pregnancy-related deaths and is the fifth leading cause of maternal mortality. Endometritis (postpartum uterine infection) has been associated with preeclampsia and maternal lethal bleeding in recent decades. In some studies, the presence of meconium in the amniotic fluid has been implicated in the development of endometritis. The study aimed to evaluate the association between interleukin-19 gene polymorphisms and maternal puerperal infection. In this study, 300 pregnant women with a gestational age of at least 37 weeks were studied. Patients were divided into two groups of 150 controls and cases. In the case group, amniotic fluid was impregnated with meconium, and in the control group, it was clear fluid. Both groups underwent cesarean section, and all received prophylactic antibiotics before surgery. Patients were evaluated for purpura infection in the first 40 days after delivery. Five ml of venous blood was taken from each patient and transferred to a tube containing EDTA anticoagulant. Genomic DNA was isolated using a particular kit. Then, the polymerase chain reaction was performed by the ARMS method. Data were analyzed using the chi-square test and SPSS software version 19 in case and control groups. This study's results indicate no significant difference in the frequency of AG, GG, and AA genotypes at position rs2243191 and rs1028181 IL-19 gene polymorphism between patients with puerperal infection and the control group (P>0.05). Also, no significant difference was observed in the frequency of both G and A alleles in the mentioned situations between patients and the control group (P>0.05). Based on the results of this study, no significant relationship was observed between IL-19 gene polymorphism at rs2243191 and rs1028181 locus and puerperal infection.

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Introduction

Postpartum infection (puerperal infection) is any bacterial infection of the genital tract after childbirth (1). Over the past decades, endometritis has been associated with preeclampsia and hemorrhage, lethal triad, and maternal death due to pregnancy problems. Infection is responsible for 13% of pregnancy-related deaths and is the fifth leading cause of maternal mortality (2). Factors associated with increased risk of infection include young mother's age, neoplasia, long-term rupture of the amniotic sac (rupture of the amniotic sac more than 6 hours), long-term induction of labor, and increased frequency of vaginal examinations, internal monitoring, double-barreled, CPD cesarean section, more than 4 kg weight at birth, and no antibiotics (3). Some recent studies have suggested that the presence of meconium in amniotic fluid is effective in developing postpartum endometritis (1-3).

The prevalence of meconium in amniotic fluid is between 7-22% in different studies (4). Maternal complications include meconium excretion, decreased antimicrobial effect of fluid, decreased neutrophil phagocytic activity, decreased host immune resistance, increased chance of microbial growth, and increased endometriosis (5). The different research results contradictorily suggested meconium's existence with endometritis. For example, Liabsuetrakul's study (6) rejects this relationship, and the Javaid *et al*. (7) study shows the presence of the relationship.

Invasive factors of puerperal infection include binding agents, enzyme production, and secretion of specific proteins. Binding agents facilitate the binding

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of infectious agents to vaginal tissue (8, 9). Studies have shown that immune responses against puerperal infection involve a set of cellular and nonspecific natural immune mechanisms (10). Neutrophils and macrophages play a significant role in the fight against puerperal infection through oxidative and non-oxidative mechanisms (production of active oxygen and nitrogen mediators by myeloperoxidase, hydrogen peroxidase, and nitric oxide)(11). The increased number of activated lymphocytes and T_{CD4}^{+} cells in the vaginal mucus indicates their involvement in acquired immunity against this infection. Vaginal dendritic cells induce the proliferation of T cells, which release large amounts of IL-2, IL-6, and IFNγ, and to a lesser extent, IL-10. The innate immune system with phagocytes kills the microorganism and provides it to the acquired immune system (8). Activation of acquired immunity occurs through the presentation of antigens and the secretion of proinflammatory cytokines (12).

The innate immune system with phagocytes kills the microorganism and provides it to the acquired immune system. Activation of acquired immunity occurs through the presentation of antigens and the secretion of proinflammatory cytokines (13). The IL-19 gene is located next to the IL-10 telomere region on chromosome 1q32 and is produced by monocytes and, to a lesser extent, by B cells. The role of IL-19 as an anti-inflammatory agent in various disorders such as respiratory, liver, and autoimmune diseases has been investigated (14). On the other hand, the role of IL-19 in causing the inflammatory response in infections has also been proven. IL-19 induces the production of IL-6 and TNF-α by stimulating monocytes. On the other hand, it induces the production of IL-17, in which the TH17 response plays a protective role against infection, thereby contributing to the inflammatory response to puerperal infection (15).

Cytokines are helical molecules made up of several polypeptide subunits linked together by disulfide bonds and play an essential role in cellular and humoral safety. Changes in the sequence of protein subunits can affect the shape and function of the final molecule (16). Most of these changes result from genetic DNA polymorphisms, and approximately 90% of them are single-nucleotide polymorphisms (SNPs) caused by the replacement of only one organic base in a gene. Also, some of them are related to people's susceptibility or resistance to diseases (17). Since no research has been done on puerperal infection and its relationship with different polymorphisms of the Interleukin-19 gene, this study aimed to determine the association between polymorphisms of the IL-19 gene (rs2243191 T/C and rs1028181-513 T/C) and infection of puerperal.

Materials and methods

In this study, 300 pregnant women with a gestational age of at least 37 weeks were selected and divided into two groups of 150 people. The case group consisted of 150 pregnant women with meconium-stained amniotic fluid, and the control group had 150 cases with clear fluid. Patients in both groups underwent cesarean section based on the obstetric indication. It should be noted that since the cesarean section is a risk factor for endometritis, patients were selected for the study whose method of termination of pregnancy was a cesarean section, so both groups are similar in this regard.

Both groups received prophylactic antibiotics half an hour before surgery and three doses after surgery. Both groups were matched entirely in amniotic sac rupture time of fewer than 18 hours, labor length of fewer than 15 hours, and frequency of vaginal examinations less than seven times. All cases of cesarean section were excluded due to twin pregnancy, pelvic misalignment of the head (CPD), intrauterine death (IUFD), and elective cesarean section. The follow-up period was 40 days after the diagnosis of purpura infection.

Evaluation of puerperal infection

Puerperal infection is any bacterial infection of the genital tract after childbirth with a fever greater than or equal to 38°C or 100.4°F that occurs 24 hours after delivery for the first ten days and lasts for at least 48 hours (2). The degree of fever was determined orally and measured four times a day. The data collection technique was observational and based on physical examinations and paraclinical findings such as CBC, U/A -U/C, and CXR. The minimum sample size was estimated to be 150 people to compare the infection ratio in the two groups, taking into account the prevalence of 0.5% and the error rate of 0.05.

Evaluation of interleukin-19 gene polymorphisms

About 5ml of venous blood was taken from each volunteer and transferred to a tube containing anticoagulant (K2-EDTA). Genomic DNA using a special kit (QIAGEN, Germany) was isolated according to the kit protocol. Amplification Refractory Mutation System (ARMS-PCR) was used to amplify the DNA. The specific primers used in the study of the IL-19 gene in rs1028181 and rs2243191 were designed by Aligo software (Table 1).

Table 1. Specific primers for IL-19 gene amplification

	rs 1028181		rs 2243191
Reverse (allele A)	GGAACATCTCTGCT TATA AGAAA	Reverse (allele A)	GTTCCTTGTCATCA AGCTGAGA
Reverse (allele G)	GGAACATCTCTGCT TATA AGAAG	Reverse (allele G)	GTTCCTTGTCATCA AGCTGAGG
Forward (common)	GCAAATGTGCTCA GTACTTG	Forward (common)	AGCACCTCAGGGA CAAAGAT
Forward (control)	CCTCTGCACAGTTT GGAC	Forward (control)	CCTCTGCACAGTTT GGAC
Reverse (control)	TCTGTCCAGCAATC CAGG	Reverse (control)	TCTGTCCAGCAATC CAGG

The PCR reaction mixture composition with a final volume of 22µl was added as follows: 11µl Master Mix (Amplicon, Denmark), 0.7µl of reverse and forward of A primers, 0.9µl of F common and R common primer, 9.9µl of distilled water, and 0.8µL of DNA were added. The PCR program consisted of three stages with optimal conditions set for 33 cycles. For the rs1028181 position, the initial denaturation process was at 94°C for 5 minutes, and the denaturation process was per cycle for 40 seconds. The primer annealing step was at 26°C for 40 seconds, the extension step was at 72°C for 40 seconds, and the final extension step was at 72°C for 5 minutes. In the case of rs2243191, except for the annealing temperature (58°C), the other steps were similar to rs1028181. Amplified products were observed by electrophoresis on 1.5% agarose gel by staining SMO Blo, Taiwan (SAFE STAIN) with a gel dock device (Syngenta, UK).

Statistical analysis

A Chi-square test was used to analyze the data compared with qualitative variables, and a t-test or analysis of variance was used to compare the quantitatively independent variables with the dependent variables.

The studied polymorphisms in patients and controls were compared using the chi-square test and SPSS software, and for all tests, a significance level of less than 0.05 was considered. The algorithm of the Harley Quinn program was also used to check whether the studied genotypes in the study site follow the Hardy-Weinberg equilibrium (18).

Results and discussion

The case group included 150 patients, 140 patients (93%) were under 35 years old, and ten patients (7%) were over 35 years old. In the control group, 110 (73%) were under 35, and 40 (37%) were over 35 years old. Of all patients, about 300 patients, 260 patients (82.9%) were under 35 years old, and 50 patients (17.1%) were over 35 years old. There was a statistically significant difference between the two groups regarding age between the case and control groups (P <0.001).

Only two postoperative fevers occurred in 1.3% of the case group, and 148 patients (7.98%) had no fever. No case of postoperative fever was reported in the control group. There was no statistically significant difference between the two groups that did not confirm the increased risk of postoperative endometritis with meconium-stained amniotic fluid (P <0.05). About 300 patients had a fever of 0.7%, and 93.3% did not have a fever. None of the patients had uterine tenderness, purulent leukemia, or leukocytosis. In the case group, 100 (66%) were nulliparity, and 50 (34%) were multiparity. In the control group, 65 (43%) were nulliparity, and 85 (57%) were multiparity. One hundred sixty-five patients (55%) were nulliparity, and 135 patients (45%) were multiparity. From the parity point of view, the difference between the two groups was statistically significant (P <0.001). In the case group, 135 neonates (90% of birth weight were under 4kg, 15 (10%) were over 4kg in the control group, 123 (82%) were under 4kg, and 15 (10%) were over 4kg. In the control group, 123 patients (82%) were less than 4kg, and 27 patients (180%) were over 4kg. 87% were less than 4kg, and 13% were over 4 kg. Statistically, it was not significant (P>0.05) (Table 2).

In the control group, the cause of cesarean section was 49 cases (30.8%) repeat II, 13 cases (8.9%) repeat III, 51 cases (34.9%) fetal distress, 16 cases (11%) High risk, 18 cases (6.8%) Breech, one case (0.7%) was Transverse, and 2 cases (1.4%) were IUGR.

After electrophoresis, three genotypes appeared for these polymorphisms, and based on the length of the pieces obtained, the genotype of each individual was determined. The expected lengths for the rs1028181 position SNPs were 169 bp as control and 372bp as a test (Figure 1).

Table 2. Comparison of maternal age, parity, BMI, and neonatal weight in case and control groups

Variation	Maternal age (year)		Parity		Body Mass Index (BMI)				Neonatal weight	
	<35	≥35	nulliparity	multiparity	<19	19-25	25-30	>30	<4	≥4
Case	140 (93%)	10 (7%)	100 (66%)	50 (34%)	1 (0.6%)	132 (88%)	11 (7.3%)	6 (4%)	135 (90%)	15 (10%)
Control	110 (73%)	40 (37%)	65 (43%)	85 (57%)	0 (0%)	120 (80%)	30 (20%)	0 (0%)	123 (82%)	27 (18%)

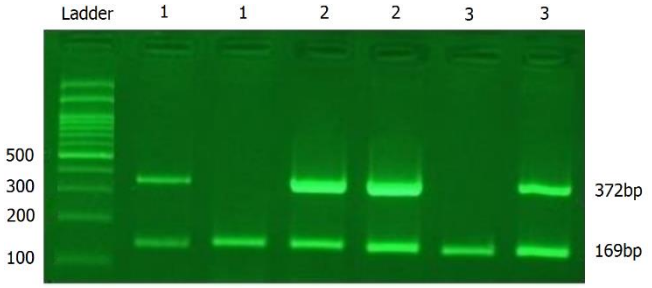

Figure 1. Different genotypes of rs1028181 polymorphism on gel electrophoresis; 1: AA homozygous band, 2: AG Heterozygous band, and 3: GG Homozygous band

Also, for rs2243191 position SNPs, the length of the control part and the tested part were 169 and 492bp, respectively (Figure 2).

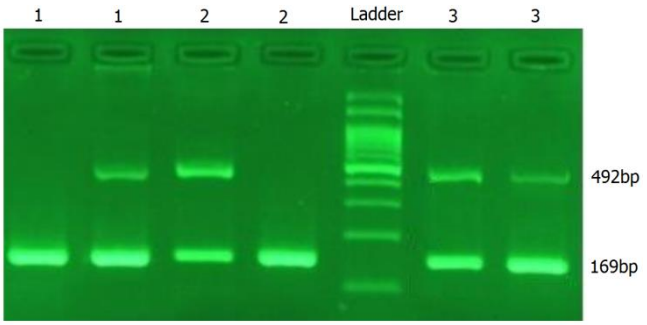

Figure 2. Different genotypes of rs2243191 polymorphism on gel electrophoresis; 1: GG Homozygous band, 2: AA Homozygous band, and 3: AG Heterozygous band

In electrophoresis, if a person has a homozygous genotype, only part 6 or part A is observed, and if the person is heterozygous, both parts A are visible in electrophoresis. Genotypes among patients with puerperal infection at rs2243191 position included GG genotype (10%), AG genotype (81%), and AA genotype (9%). The ratio of the above genotypes in control individuals was 12.7%, 78.2%, and 9.1%, respectively (Table 3).

Table 3. Comparison of IL-19 gene polymorphism at rs2243191 and rs1028181 loci between case and control group

Genotype	Case group (%)	Control group (%)	Alleles	P-value
rs2243191	9	9.1	AA	0.821
	81	78.2	AG	
	10	12.7	GG	
rs1028181	3	9.1	AA	0.102
	87	85.4	AG	
	10	5.5	GG	

According to the chi-square test, there was no significant difference between patients and controls. Also, 19-IL gene alleles at rs2243191 showed that 49.5% of patients have an A allele and 50.5% have a G allele, while 11.8% of control individuals have a G allele 48.2%. Percentages had allele A. There was no significant difference in the frequency of alleles A between patients and controls (Table 4).

Table 4. Frequency of A and G alleles in IL-19 gene at rs2243191 and rs1028181 locus

Polymorphism	Allele	Case group (%)	Control group (%)	P-value	Odd Ratio (OR)	Confidence Interval (CI)
rs2243191	A	49.5	48.2	0.787	1.054	2.265
	G	50.5	51.8			
rs1028181	A	51.8	46.5	0.276	0.808	1.737
	G	48.2	53.5			

In recent decades, endometritis has been associated with preeclampsia and hemorrhage, fatal triad, and causes of maternal death (19). Some recent studies have suggested that the presence of meconium in the amniotic fluid is effective in developing endometritis after childbirth (19, 20). In the present study, 300 patients were studied in two groups (with amniotic fluid impregnated with meconium) and the control group (with clear amniotic fluid). In terms of the incidence of infection, only in 2 patients with febrile fever (in the case group) was observed that the difference was not statistically significant (P> 0.05). In other words, Meconium Stained Amniotic Fluid (MSAF) was not associated with an increased chance of endometritis. In a study conducted by Tran *et al*. (21) in 43,200 pregnant women, 9.18% of cases were

associated with MSAF, and the risk of endometritis was higher in this group (P <0.0001). The researchers concluded that MSAF was associated with an increased risk of STIs. In Tran's study (21), intervention factors such as delivery method, long-term amniotic sac rupture, and use of internal monitoring, birth weight, age, and maternal parity were not considered.

Numerous studies have been performed on the association of polymorphisms of some proinflammatory cytokines with maternal puerperal infection. For example, in one study, Johnson and Jefferys (22) examined the association between IL-128 (rs17860508) and L-128 (rs41292470) with systemic puerperal infection. According to the results, mutations in the above polymorphisms led to infection.

In a study by Velazquez-Hernandez *et al*. (23), the MBL gene polymorphism decreased MBL levels in women with puerperal infection and thus increased the risk of women becoming infected. Babula *et al*. (24) Also investigated the association between the IL-4 polymorphism and puerperal infection and the replacement of nucleotide C with T in the promoter of the IL-4 gene at amino acid position 579 along with increased production of IL-4 and suppression of innate immune system and increased incidence of was a puerperal infection. Examining the IL-1Ra gene polymorphism in 100 people with puerperal infection and 100 healthy people, Maes *et al.* (25) found that puerperal infection was not significantly associated with this polymorphism.

As the first attempt to investigate interleukin 19 gene polymorphisms with maternal puerperal infection, the effect of IL-19 polymorphisms at rs1028181-513 T/C and in the present study, rs2243191 in patients with this disease was investigated. This study showed that the frequency of patients' genotypes in the rs2243191 position included 10% GG, 81% AG, and 9% AA and in the control group was 12.7% GG, 78.2% AG, and 9.1% AA, did not show a significant difference. The frequency of patients' genotypes in rs1028181-513 T/C position included 10% GG genotype, 87% AG genotype, and 3% AA genotype, and the frequency of genotypes in control subjects included 5.5% GG, 85.4% AG, and 9.1% AA. . Statistical analyzes also did not show significant differences between patients and controls. In other words, in the presence of AA, AG, and GG genotypes, the incidence of infection does not change, and it seems that these genotypes may not play an essential role in puerperal infection. Also, statistical analysis of IL-19 polymorphism at rs1028181 and rs2243191 shows no significant difference between the frequency of alleles A in the patient and control groups.

Although millions of SNPs have been identified throughout the human genome and most individual differences, including disease susceptibility, are determined by them (26), the results of this study on IL-19 polymorphisms at rs1028181-513 T/C and rs2243191 T/C showed that the frequency of T/C genotype in people with puerperal infection is not significantly different from healthy people. Also, the C allele does not offer a statistically higher frequency in patients than in controls. Therefore, allele change from T to C of IL-19 gene polymorphisms in the studied loci does not affect the incidence of puerperal infection. Therefore, the change of allele from T to C of IL-19 gene polymorphisms in the studied loci does not affect the incidence of puerperal infection. However, the available data suggest that the immunopathology of puerperal infection is very complex. A complete understanding of the mechanisms involved in the host vaginal mucosal defense system is needed to develop a specific immunological treatment strategy to prevent and control puerperal infection. Also, the lack of a significant relationship between gene polymorphism and disease risk factors will not be unexpected, so it is better to examine the status of other polymorphisms of other genes to achieve a more reliable assessment.

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Authors' contribution

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Interest conflict

The authors declare that they have no conflict of interest.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Statement and Declarations

The author declares that no conflict of interest is associated with this study.

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