Interleukin-1β and interleukin-1 receptor antagonist genes polymorphisms and the risk of spontaneous preterm delivery in the population of Polish women

JAROSŁAW KALINKA¹, ADAM BITNER²

Abstract

Objectives: Cytokines are involved in the patomechanism of preterm delivery. It is sought that preterm birth due to preterm uterus contractions and due to preterm premature rupture of membranes (PPROM) is having different etiology. The aim of this study was to evaluate the relationship between the occurrence of interleukin-1β [IL-1β (+3953C>T)] and interleukin-1 receptor antagonist genes polymorphisms and the risk of preterm birth caused exclusively by preterm uterus contractions in the population of Polish women. Materials and methods: A case-control study, 93 Caucasian women were examined including 30 cases and 63 controls. Case subjects experienced a delivery at less than 36 weeks of gestation due to preterm uterus contractions, not preceded by PPROM while control subjects gave birth at term. Polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism analysis. Logistic regression model were used to compute odds ratios and its 95% confidence intervals. Results: Maternal carriage of IL-1RN*2 was associated with increased risk of preterm delivery due to preterm contractions (OR = 3.12; 95% CI: 1.14-9.00). No association between duration of pregnancy and the IL-1β polymorphism was stated (OR = 1.23; 95% CI: 0.50-3.04). Conclusions: Maternal carriage of IL-1RN allele 2 increases the risk of preterm delivery due to preterm contractions in the population of Polish women.

Key words: interleukin-1β, interleukin-1 receptor antagonist, gene polymorphisms, preterm delivery, preterm contractions not preceded by PPROM

Introduction

The substantial body of evidence suggests that clinical and subclinical infections of the lower genital tract play a major role in many cases of spontaneous preterm birth [1]. The inflammatory process is being mediated by cytokines and results in production of prostaglandin by chorion, amnion and decidua [2]. Enhanced production of prostaglandins is responsible for onset and propagation of myometrial contractility, and subsequent preterm delivery. It seems that interleukin-1β (IL-1β) produced mainly by activated monocytes and macrophages in response to bacterial products such as lipopolysacharide is a key proinflammatory cytokine [3]. In pregnant rhesus monkeys intraamniotic infusion of IL-1β resulted in the production of tumor necrosis factor-α and prostaglandins with subsequent uterine contractions [4]. Moreover elevated levels of IL-1β were observed in the cervicovaginal fluid of women in labor, both preterm and at term [5].

IL-1 receptor antagonist (IL-1ra) is a natural competitive inhibitor of IL-1β activity. It competes with IL-1β for binding to its receptors on target cells but is devoid of signal transducing activity. IL-1 ra terminates IL-1β induced inflammation [6]. In mice models pretreatment with interleukin-1 receptor antagonist prevented interleukin-1-induced preterm parturition [7]. In in vitro studies it also reduces interleukin-1-induced prostaglandin production by amnion and chorion [8].

The genes encoding for both IL-1β and IL-1 ra are polymorphic and result in variations in their specific nucleotide sequences among individuals. These variations result in phenotypic differences. A single nucleotide polymorphism [IL-1β (+3953C>T)] of the IL-1β gene containing a C by T substitution at position +3953 (allele 2 – IL-1β*+3953C>T) is associated with increased production of the protein in vitro [9]. A region within intron 2 of IL-1RN (gene encoding for IL-1 ra) contains a variable number of 86-base pair tandem repeats, which results in 5 alleles. The alleles 1, 2, 3, 4 and 5 represent four, two, five, and six repeats of 86-bp tandem repeats, respectively [10]. Carriers of IL-1RN allele 2 (IL-1RN*2) have higher levels of IL-1 ra and IL-1β than noncarriers [11].

Theoretically, carriers of IL-1β allele 2 should be more susceptible to preterm delivery because of the higher levels of “contraction inducing” IL-1β. On the contrary, maternal carriage of IL-1RN allele 2 related to increased levels of IL-1ra should be associated with lower risk of preterm delivery. We undertook this study to examine the associations between maternal carriage of polymorphic alleles of IL-1β and IL-1RN genes and the risk of preterm delivery in the population of Polish women. However, because recent data suggests that preterm birth due to spontaneous preterm contractions not preceded by PPROM and preterm birth in consequence of PPROM might have different etiology we restricted our case subjects only to women who gave a preterm birth after spontaneous preterm labor without PPROM [12, 13]. We suspected that in these two etiologically distinct groups of preterm delivery the impact of analyzed genes polymorphism on the pregnancy duration might be different. In this case if we considered these groups of preterm delivery together we could have achieved incorrect results.

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Material
The study was approved by the Committee for Bioethics of Medical University of Łódź (number RNN/29/04/KE). Written informed consent was obtained from all patients.

93 Caucasian women who delivered a child at Department of Perinatology, Medical University of Łódź from 2006 to 2007 were examined including 30 cases and 63 controls. Case subjects were defined as those who experience a delivery at less than 36⁶ weeks of gestation due to spontaneous preterm uterine contractions not preceded by PPROM while control subjects gave birth at term. In all cases gestational age was estimated based on the date of last period and confirmed by the ultrasound evaluation performed between 11 and 13⁶ weeks of pregnancy. Mothers with incompetent cervix, congenital anomalies of uterus, fetal malformations, iatrogenic preterm delivery and preterm delivery as a result of PPROM were excluded from the study. Detailed demographic, medical and obstetric data were collected based on structured questionnaire and medical record.

Methods
Maternal genomic DNA was isolated from white cells of peripheral blood obtained after delivery according to Higuchi. The polymerase chain reaction (PCR) amplification was carried out with the use of GeneAmp PCR System 2007 and GeneAmp PCR System 2400 (Applied Biosystems, USA) following the manufacturer’s recommendations. In order to analyze IL-1β+3953 allelic variants the polymorphic region that contained Taq I restriction site was amplified with oligonucleotides

5'-GTTGTCATCAGACTTTGACC-3',
5'-TTCAGTTCATATGGACCAGA-3'
as PCR primers. The PCR conditions were as followed: 95°C for 5 minutes, followed by 30 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute. 8 μl of PCR product was digested with Taq I (Fermentas, Canada) at 65°C for 24 hours. Fragments were analyzed on 10% polyacrylamide gels with the use of Mini Protein II and Mini Protein III equipment (Bio-Rad, USA) and stained with ethidium bromide. Taq I digestion of the 249-bp fragments results in products that either are cut into 2 fragments of 135 bp and 114 bp (IL-1β+3953*1) or remain intact (IL-1β+3953*2). Oligonucleotides

5’-CTCAGCAACACTCCTAT-3’,
5’-TCTGTTGTCAGGTAA-3’
were used as PCR primers in order to analyze allelic variants in intron 2 of IL-1RN. The PCR conditions were as followed: 1 cycle at 95°C for 5 minutes, followed by 40 cycles of 94°C for 1 minute, 63°C for 1 minute and 72°C for 1 minute. The PCR products were analyzed on 10% polyacrylamide gels and stained with ethidium bromide. PCR produces products that are 410 bp (IL-1RN*1), 240 bp (IL-1RN*2) and 500 bp (IL-1RN*3) in size.

Genotype frequencies in case and control groups were compared by using exact logistic regression model. Analysis was used to estimate the OR and its 95% confidence intervals. Analysis was performed with the statistical analysis package R version 2.7.2 (http://www.r-project.org).

Results
The analyzed population consist of 30 women with preterm delivery due to preterm contractions of uterus not preceded with PPROM (cases) and 63 women who delivered after 36⁶ weeks’ gestation (control group).

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<th>Table 1. Demographic characteristics</th>
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<td><strong>Maternal age at delivery (y)</strong></td>
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<td>Percentage of cesarean section (%)</td>
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<th>Table 2. Relationship between IL-1RN and IL-1β genes polymorphisms and the risk of preterm delivery due to preterm contractions in the population of Polish women</th>
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<td>Analized gene</td>
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The mean gestational age at delivery and the mean birth weight were significantly lower among preterm subjects (30.5 weeks vs. 39.2 weeks and 1506 g vs. 3469 g). 25.9% of case subjects had a history of preterm delivery compared with 4.6% of control subjects. 29.6% of preterm subjects declared smoking during pregnancy compared with 17.5% of term subjects. We found no statistically significant differences in mean maternal age at delivery (29.0 years in both groups) and the percentage of cesarean sections performed (31.7%, 29.6% respectively) among term and preterm subjects. Above-mentioned data are depicted in table 1.

Analysis of the distribution of IL-1β genotypes have shown that in both groups the most prevalent genotype was IL-1β*1/IL-1β*1 (allele 1/allele 1). It was diagnosed in 46.7% of case subjects and in 51.9% of control subjects. We have found no statistically significant impact of maternal carriage of allele 2 of IL-1β (genotypes IL-1β*1/IL-1β*2 or IL-1β*2/IL-1β*2) on the risk of preterm delivery due to preterm labor in the population of Polish women (OR = 1.23; 95% CI: 0.50-3.04). The evaluation of the distribution of IL-1RN genotypes have revealed that in case subject the most prevalent genotype was IL-1RN*1/IL-1RN*2, whereas in control subject IL-1RN*1/IL-1RN*1. These genotypes were diagnosed in 50.0% and 56.6%, respectively.

Maternal carriage of one copy of IL-1RN*2 was associated with increased risk of preterm delivery due to preterm labor (OR = 3.12; 95% CI: 1.14-9.00). Maternal carriage of two copies of allele IL-1RN*2 was associated with increased risk of preterm delivery, however because of relatively small numbers in this subgroup this did not reach statistical significance (OR = 2.86; 95% CI: 0.75-10.95). In the whole examined group allele 3 was stated only two times (genotypes IL-1RN*1/IL-1RN*3 and IL-1RN*2/IL-1RN*3). No alleles 4 and 5 were found. The impact of IL-1RN and IL-1β genotypes on the risk of preterm delivery due to preterm labor are depicted in table 2 and in figure 1 and 2.

Discussion

This is the first study that has revealed that maternal carriage of IL-1RN allele 2 is associated with increased risk of PD due to spontaneous preterm uterine contractions in the population of Polish women. These results seem a bit surprising because theoretically that allelic variant is connected with higher IL-1 ra level which is fought to suppress the IL-1β induced inflammation. However, this data are consistent with other authors’ results. Murtha et al. have studied an influence of maternal carriage of IL-1RN*2 on the risk of giving birth to premature infant in the white and black population of New Carolina, USA [14]. Authors have demonstrated that the presence of at least one copy of IL-1RN allele 2 increases the risk of PD more than 3 times (OR = 3.2; 95% CI: 1.68-6.14).

In a few studies an influence of fetal carriage of IL-1RN*2 on the duration of pregnancy was also examined. Genç et al. have shown that fetal possession of allele 2 of IL-1RN gene is associated with higher chance of prematurity [11]. Similarly, the carriage of this allele by Israeli fetuses resulted in a higher rate of preterm delivery [10]. An interesting paper, examining a relationship between fetal and maternal carriage of polymorphic alleles of IL-1RN and the outcome of multifetal pregnancy in New York, USA was published by Kalish el al. [15]. Authors have demonstrated that carriage of IL-1RN*2 by both fetuses is associated with higher risk of preterm delivery in comparison with pregnancies in which only one or no fetus carry this polymorphic allele. In their study genotype IL-1RN*1 was protective against preterm delivery. The authors have also revealed that fetal carriage of IL-1RN*2 was associated with neonatal morbidity. What is more, when they have compared within a given pregnancy only the neonates which were discordant for IL-1ra genotype they noticed a trend towards significance for worse outcomes in neonates carrying IL-1RN*2. In this study no correlation between maternal carriage of IL-1RN*2 and the duration of pregnancy was noticed.

Several authors tried to explain the relationship between the carriage of IL-1RN*2 and the increased risk of prematurity. Murtha et al. and Kalish et al. suggest that carriage of allele 2 of IL-1ra is associated with significantly increased production not only of IL-1ra but also of IL-1β [14, 15]. The interactions between these two cytokines determine the severity
of inflammatory response. Thus, the relative inability of IL-1RN*2 carriers to terminate IL-1β-mediated proinflammatory activity may influence the risk of preterm delivery [14,15].

The study of Genç et al. yields different results [16]. In their research carriage of IL1RN*2 was associated with an elevated vaginal pH, a reduced IL-1β response to anaerobic Gram-negative rods and/or Gardnerella vaginalis and a decreased rate of spontaneous preterm delivery. They conclude that possession of this allele is related to blunted proinflammatory IL-1β response to abnormal vaginal flora and thus may decrease susceptibility to infection-related preterm delivery. In our study maternal carriage of polymorphic alleles of IL-1β seemed to have no impact on the duration of pregnancy. These results are consistent with the results achieved by Edwards and by Kalish [3, 15]. Both authors have examined the impact of fetal and maternal carriage of polymorphic alleles of IL-1β gene on the risk of preterm delivery. None of them have found the relationship between fetal or maternal IL-1β genotype and the pregnancy outcome. Knowing the fact that IL-1β*2 carriers have higher levels of IL-1β and that IL-1β acting by enhancing prostaglandins production, is one of the major agents that stimulates contraction of the uterus in response to inflammation, we have expected different results [4, 9]. In fact we have found only one study by Genç et al. in which authors revealed the impact of IL-1B*2 possession on the risk of preterm delivery and what is interesting this impact was restricted only to African populations [11]. These divergences might be related to racial differences.

There are a lot of evidences that the presence of polymorphism of certain genes might increase the risk of preterm delivery. Therefore, it seems to be very important to examine the impact of polymorphism of particular genes in various populations on the duration of pregnancy. This study indicates, for the first time, a profile of genetic polymorphisms that increase the susceptibility to preterm birth in population of Polish women. This knowledge, might in future allow early detection and treatment of women at particularly high risk of PD before preterm labor appears.

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