Interleukin-6 gene polymorphisms and its relation to spontaneous preterm labour among Egyptian women

Original Article

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ABSTRACT

Objective: to verify the possible relationship between interleukin-6 (IL-6) promoter -174 polymorphisms and spontaneous preterm birth between 28 and 37 weeks of gestation among Egyptian women.

Methods: This is a multicenter case control study comprising 184 pregnant women between 28 and 36+6 weeks of gestational age (GA) at termination, divided into a study group including sixty one women with established preterm labor, and another 123 pregnant women at full term represented the control group. Venous blood samples were taken for DNA extraction using QIAamp DNA Blood Mini Kit for IL-6 promoter polymorphism status.

Results: Among the preterm delivering women, 65.6%, 26.2% and 8.2% had C/C, G/C and G/G variants, respectively. On the other hand, within the 123 women representing the control group, we found 84.6%, 15.4% who showed C/C and G/C variants. There was no single woman showing the G/G variant. This was statistically significant ($P < 0.05$).

Conclusion: The genetic make-up of interleukin-6 production may have a role in the etiology of preterm labour among Egyptian women of Middle Eastern ethnicity. PCR for IL-6 polymorphism could be proposed as one of the predictors for preterm labor, however, in a multi-factorial problem like preterm labor it is not the only predictor tool.

Key Words: egyptian women, gene polymorphisms, interleukin -6, preterm labor.

Received: 12 March 2017, **Accepted:** 3 May 2017

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ISSN: 2090-7265, August 2017, Vol. 7, No. 3

INTRODUCTION

Prematurity or preterm birth (PTB) is one of the major causes of neonatal mortality and birth related morbidity without congenital anomalies or chromosomal abnormalities[1,2]. It is responsible of fifty six percent of neonatal deaths and fifty percent of neonatal neurological disability[3]. In spite of this high neonatal mortality and morbidity 5- 11% of deliveries are still premature4. This rate did not decrease in the past 40 years, in spite of advances in obstetric care[5]. The etiology of preterm labour is complex and multifactorial6 .There is compelling evidence that both maternal and fetal genomes contribute to risk[7].

African-American ancestry is consistently associated with an increased risk of preterm birth even after considering the epidemiologic risk factors, such as income, education, lack of prenatal care, and other socioeconomic factors[8].

Expression studies of IL-6 with allelic variants of the rs1800795 polymorphism have produced different results in different tissues[9]. However, there is no strong evidence of natural selection near rs180079510. Activated lymphocytes and macrophages are the primary source of the biologically active glycoproteins (Interleukins). The progress in recombinant DNA technology, cell culture techniques and protein purification have led to study of the biological and biochemical properties of interleukins[11].

Activated T lymphocytes, blood monocytes, fibroblasts and tissue macrophages secrete 26 kD glycoprotein which is coded as Interleukin-6 (IL-6)12. Interleukin-6 activates

 DOI: 10.21608/ebwhj.2017.3992

the acute phase response and induces C reactive protein production and terminal differentiation of B lymphocytes, therefore, it is a crucial cytokine in the response of the host to infection[13]. Amniotic fluid concentration of IL-6 increases more than that of other pro-inflammatory products in cases of intrauterine infection and preterm labor[14]. This increase in IL-6 is also seen in amniotic fluid, maternal serum and cervix in cases of preterm labor[15]. The risk of preterm labor approximately doubled in the study done by Romero and his colleagues when a single nucleotide polymorphism involved in controlling fetal inflammation (IL-6 receptor 1) and DNA variants in maternal genes encoding for proteins involved in extracellular matrix metabolism occurred[16].

Many human cytokine genes polymorphisms have been described[17]. Polymorphisms are normal allelic variation present within the regulatory region of cytokine genes. Increased severity of certain autoimmune diseases and increased susceptibility to certain infections are associated with specific polymorphisms[18]. Polymorphism in the promoter region of IL-6 (At position 174) on chromosome 7 is associated with the production of IL-6. Decrease in the promoter activity is due to substitution of cytosine (C) for guanine (G) at this position. Lower production of IL-6 is present in individuals who have cytosine at both positions (homozygous C/C) as compared to individuals who have guanine at both positions (homozygous G/G) or at one position (heterozygous G/C) who have normal IL-6 production[19].

The aim of this study was to verify the possible relationship between IL-6 promoter -174 polymorphisms and spontaneous preterm birth between 28 and 36 +6 weeks of gestation in a trial to find a good predictor to preterm birth which is a major problem in Egypt.

PATIENTS AND METHODS

In this multicenter case control study, two hundred and thirty four were recruited all of them had Middle Eastern ethnicity and Arabic speaking language. Inclusion criteria included healthy women with singleton living fetus, while, exclusion criteria were women with medical or obstetric problems other than preterm labor, those taking any form of medications except multi-vitamins, any form of infection, fever, multiple pregnancies or a fetus with congenital anomalies. One hundred and eighty four women met the inclusion criteria were enrolled in the study. Fifty women were excluded due to premature rupture of the membranes (34/ 50), evident vaginal infection (11/ 50) by clinical and high vaginal swab results, three women had urinary tract infection (3/ 50) by mid stream urine test and two women had babies with major congenital malformation (2 /50). The cases were recruited from the antenatal care clinic and casualty unit of Obstetrics and Gynecology department, Kasr El Eini hospital, Cairo University, Benisuef university hospital and the Obstetrics and Gynecology Department 6th October University as well as the antenatal clinic of Reproductive Health and Family Planning department, Medical Research Centre of Excellence, National Research Centre, Cairo. This was carried out during the period from October 2011 to May 2012. Informed written consents of all participants were taken. The Ethical Committees of the National research Centre, Kasr El Eini University Hospital and 6th October University Hospital also endorsed their approval for the study. All women within the study group were pregnant between 28- 36^{6+} weeks, while controls were pregnant 37 weeks or more.

The study group included 61 pregnant women with established preterm labor confirmed by regular uterine contractions (3- 5 contractions/10minutes each lasted for 45- 60 seconds) increasing strength, frequency and duration associated with cervical changes (cervical dilation >3cm). Contractions were monitored using continuous cardiotocography. The control group represented the one hundred and twenty three women who were at full-term (>37^{0+} weeks) with no symptoms or signs of labor.

All women were subjected to detailed history taking including nutritional history, educational history & information about the socio-economic status. Examination by sterile speculum prior to digital vaginal examination to rule out rupture of membranes and high vaginal swabs were taken. Two-dimensional ultrasound was performed for fetal biometry, placental localization and detection of possible gross fetal anomalies. Urine samples were taken for analysis. Venous blood samples were taken for DNA extraction using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's "Blood and Body Fluid Spin Protocol". Real time PCR was used to determine IL-6 promoter polymorphism status using the following primers and probes;

5'- GACGACCTAAGCTGCACTTTTC-3',

5'- GGGCTGAT-TGGAAACCTTATTAAGATTG-3',

5'- CTTTAGCAT(C) GCAAGAC-3',

5'- CTTTAGCAT (G) GCAAGAC-3'.

Statistical analysis

Data was analysed using IBM SPSS Advanced Statistics version 20.0. Numerical data were expressed as mean and standard deviation and range. Qualitative data were expressed as frequency and percentage. Fisher's exact test was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using independent sample t-test. *P* value < 0.05 was considered significant.

RESULTS

In this study the mean age of the case and control groups is 27.8 ±3.7 and 28.0± 3.8, respectively (ranging from 21.0 to 37.0 years in both). Moreover, the mean gestational age of the case groups is 33.3± 1.6 (ranging from 28.0 to 36.0+ 6), while that of the control group is 38.8 ±1.4 (ranging from 37.0 to 42.0).

Among the preterm delivering women, there were 40 women (65.6%) with C/C variant, 16 women (26.2%) with G/C variant and five women (8.2%) with G/G variants. On the other hand, within the one hundred and twenty three women representing the control group, there were 104 women (84.6%) who showed C/C variant and 19 women (15.4%) with G/C variant, while no single woman showing the G/G variant. This difference between the two groups was statistically significant (*P* =0.001) (Table 1). The odds ratio for C/C genotype was 0.35 (95% confidence interval=0.17–0.79)

Sub-analysis of the groups according to gestational age at delivery among case group was 33.9 ±1.0, 32.8 ±1.6 and 29.2± 0.8 for C/C, G/C and G/G respectively with significant difference for G/G than both C/C and G/C that showed no significant difference between them (Figure 1), while gestational age at pregnancy termination among control group was 38.8 ±1.3 and 38.4 ±1.7 for C/C and G/C respectively with no significant difference between them.

Table 1: Comparison between cases and controls as regard interleukin-6 gene polymorphisms

	Genotype	Study group	Control group	*P*
IL-6 gene promoter- 147 polymorphisms	C/C	65.6% (40/61)	86.2% (106/123)	
	G/C	26.2% (16/61)	13.8% (17/123)	0.001
	G/G	8.2% (5/61)	0% (0/123)	

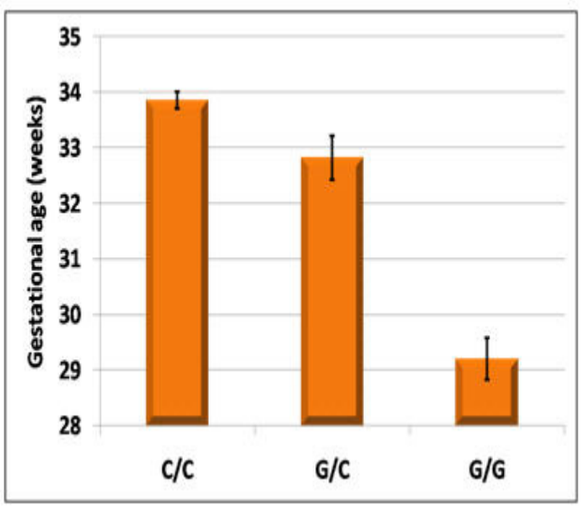

Fig. 1: Comparison between interleukin-6 gene polymorphisms as regard gestational age at delivery case group, C: cytosine, G: guanine.

DISCUSSION

IL-6 is a critical element of the host response to infection and genetic variation in its production may influence the nature and degree of inflammatory activity. Furthermore, infections or inflammatory diseases are also influenced by the genetic regulation of IL-6 production. Cox and colleagues have demonstrated that lower frequency of acute rejection in renal transplant is seen in patients who have genetic predisposition towards lower production of IL-6 20.

Our results have shown that women with G/G variant of IL-6 gene have almost doubled the risk for preterm birth (OR 1.5, 95% CI 0.593.83-). On the other hand, women carrying the C/C variant are less likely to deliver before term (OR 0.43, 95% CI 0.21 -0.89). We believe that the C/C variant of IL-6 gene is a protective one against spontaneous preterm delivery. In a previous study by Simhan and his team in (2003), they studied a group of Afro-American and white women. They revealed that

the IL-6 – 174 allelic variant C/C is significantly less frequent among women with spontaneous preterm birth less than 34 weeks[21]. This was endorsed by the results of the meta-analysis carried out by Wu *et al*. (2013), who studied preterm labor phenotype and single nucleotide polymorphism (SNP) rs1800795 in both mothers and babies separately then stratified the results by population. They concluded that IL-6 SNP rs1800795 genotype C/C was protective against preterm labor in women of European descent[22].

We also noted that primigravid women whether carrying the homozygous or the heterozygous form of the G variant are more likely to have give birth at less than 34 weeks gestation than their counterparts. This may partly be explained by the better antenatal care and medical steps taken to avoid preterm delivery and the medication received as a result of the previous preterm birth. Moreover, epigenetic and other environmental factors may play a role in affecting the expression and action of IL-6 gene. This was shown in our study as we noticed that most cases of preterm birth lived in industrial areas which have high rates of pollution suggesting a possible role of pollution in this genetic polymorphism. We also noticed that most cases of preterm labor were among women with lower level of education, the latter may have been a cause for delayed seeking of medical advice.

CONCLUSION

The genetic make-up of interleukin-6 production may have a role in the etiology of preterm labour among Egyptian women. PCR for IL-6 polymorphism could be proposed as one of the predictors for preterm labor, however, in a multi-factorial problem like preterm labor it is not the only predictor tool. The level of education also plays a role in early detection of preterm labor among Egyptian women of Middle Eastern ethnicity which may increase the chances of success of tocolytic therapy in early cases. Accurate tools for detection of preterm labor will assist in the development of targeted treatments to alleviate this major public health problem.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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