Interleukin-6 Gene Polymorphism and its Relation to Spontaneous Preterm Labor

A thesis

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List of Abbreviations

AA	: Arachidonic Acid
AF	: Amniotic fluid
AFI	: Amniotic fluid index
AFP	: Alpha feto-protein
BMI	: Body mass index
BV	: Bacterial vaginosis
C.S	: Cesarean Section
C.trachomatis	: Chlamydia trachomatis
CI	: Confidence interval
CIN	: Cervical intraepithelial neoplasia
COX	: Cyclooxygenase
CREB	: Cyclic AMP response element binding protein
CRH	: Corticotrophin releasing hormone
CRP	: C-reactive protein
CS	: Cesarean section
CTG	: Cardiotocography
D&E	: Dilatation & Evacuation
DBD	: DNA binding domain
DC	: Dendritic cell
DD	: Differential diagnosis
DDT	: Dichlorodiphenyltrichloroethane
DES	: Diethyl Stilbestrol
DNA	: Deoxyribonucleic acid
fFN	: Fetal fibronectin
GBS	: Group B streptococcus
HBD	: Hormone binding domain
HCG	: Human chorionic gonadotrophin
HLOD	: Heterogoneity logarithm of odds
IBS	: Identical by state
IFNG	: Interferon gamma
IGF-1R	: Insulin Like Growth Factor Receptor 1
IGFBP-1	: Insulin-like growth factor binding protein-1
IL	: Interleukin

List of Abbreviations *(Cont...)*

IL-1B	: Interleukin-1 B
IQR	: Interquartile range
IUGR	: Intra uterine growth Restriction
LEEP	: Loop electrosurgical excision procedures
LPS	: Lipopolysaccharide
MOI	: Mode of inheritance
NF-KB	: Nuclear Factor Kappa B
NST	: Non-stress test
OR	: Odds ratio
P	: Probability
PCR	: Polymerase chain reaction
PG D2	: Prostaglandin D2
PG DH	: 15 Hydroxy Prostaglandin dehydrogenase
PG E2	: Prostaglandin E2
PG F2a	: Prostaglandin F2a
PG H2	: Prostaglandin H2
PG I2	: Prostaglandin I2
PGE2	: Prostaglandin E2
PGF2 alpha	: Prostaglandin F2 alpha
PGS	: Prostaglandins
PKC	: Protein kinase C
PL	: Phospholipase
PPRES	: Peroxisome proliferator response elements
PPROM	: Preterm prelabor rupture of membranes
PRA	: Progesterone receptor A
PROM	: Prelabor rupture of the fetal membranes
PTB	: Preterm Birth
PTD	: Preterm delivery
PTL	: Preterm labor
RDS	: Respiratory distress syndrome
ROM	: Rupture of membranes

List of Abbreviations *(Cont...)*

RRR	: Relative risk Ratio
RT-PCR	: Reverse transcriptase PCR
SD	: Standard deviation
SLE	: Systemic lupus erythematosus
STD	: Sexually transmitted disease
T. vaginalis	: Trichomonas vaginalis
TNF alpha	: Tumor necrosis alpha
TNFA	: Tumor recrosis factor
TPROM	: Term prelabor rupture of membranes
TX	: Thromboxane
vAF	: Vaginal amniotic fluid

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Abstract

Spontaneous preterm labor and delivery accounts for approximately one-third of preterm births, which is the predominant cause of perinatal mortality and morbidity. In the setting of intrauterine infection and preterm labor, the amniotic fluid concentration of interleukin 6 (IL-6) is increased in excess of that of other proinflammatory products. A polymorphism in the promoter region of IL-6 at position 174 on chromosome 7 is associated with the production of IL-6.

In this study, the aim was to establish a relationship between IL-6 promoter region at position 174 on chromosome 7 polymorphism and preterm labor.

The study was conducted at the Maternity Hospital of Ain Shams University. Patients were recruited from the casualty unit of the hospital. The study started on mid of October 2013 till the end of April 2014.

It was a prospective case-control study involving 156 patients divided into 2 groups. The case group included 78 patients presenting with preterm labor. The control group included 78 term patients in labor. Determination of IL-6 gene polymorphism status was done using conventional Polymerase chain reaction (PCR) and agarose gel electrophoresis.

Of the 78 case patients, 67 were homozygous for cytosine at the promoter region of IL-6 at position 174 on chromosome 7 Of the 78 control patients, 52 were homozygous for cytosine at the same site (OR 0.33, 95% CI: 0.15-0.73). This result supports the hypothesis of this study that homozygosity for cytosine at the promoter region of IL-6 at position 174 on chromosome 7 protects against preterm labor.

This study paves the way for using IL-6 gene polymorphism status determination as a tool for assessment of women at risk of preterm labor.

Key Words:

Preterm labor, Interleukin 6, polymorphism, Polymerase chain reaction, chromosome 7, electrophoresis.

Introduction

Spontaneous preterm labor and delivery accounts for approximately one-third of preterm births, which is the predominant cause of perinatal mortality and morbidity***(Tan et al., 2006).***

Preterm delivery accounts for 65% of neonatal deaths and 50% of neurological disability in childhood. Spontaneous preterm labor or prelabor rupture of membranes account for 80% of preterm deliveries. Prematurity rates have not changed in recent decades ***(Shennan, 2003).***

A preterm delivery, as defined by the World Health Organization, is one that occurs at less than 37 and more than 20 weeks gestational age. In the United States, the preterm delivery rate is approximately 11%, whereas in Europe it varies between 5% and 7%. In spite of advances in obstetric care, the rate of prematurity has not decreased over the past 40 years***(Goldenberg et al., 2002).***

Preterm labor is associated with preterm rupture of membranes, cervical incompetence, polyhydramnion, fetal and uterine anomalies, infections, social factors, stress, smoking, heavy work and other risk factors. The diagnosis is made on the patients presenting symptoms, clinical findings and of progressive effacement and dilatation of the cervix ***(Haram et al., 2003).***

A short cervix identified on vaginal ultrasound has a good predictive value for early delivery, even in a low risk group, and a cervical length of less than 15 mm. will result in a 50% chance of delivery before 32 weeks' gestation. The predictive value in multiple pregnancies is similar. Identifying fetal fibronectin in vaginal secretions after 22 weeks gestation also has a strong association with preterm delivery. Both these tests perform far better than previous history as predictors of preterm delivery, which has traditionally been the way to identify a group at risk, as most women with recurrent prematurity will achieve a term pregnancy in their subsequent pregnancy. Although it is impractical to screen all women, populations at risk could be targeted with these tests to identify groups for intervention ***(Shennan, 2003).***

Most preterm birth prevention programs have attempted to identify women who are likely to deliver prematurely based on the presence of numerous risk factors, including low socioeconomic status, maternal age less than 18 or greater than 40 years, increasing parity, race, previous preterm labor and delivery, multiple gestation, uterine malformations, and bacterial vaginosis. The most important of these factors is a history of preterm delivery, which may be influenced by genetic and environmental determinants ***(Porter et al., 1997).***

Over the past several years, an important concept known as *fetal inflammatory response syndrome* has been developed. This implies that most cases of idiopathic preterm labor are due

to an inflammatory response in fetal and maternal tissues that is generated against intrauterine infection, which is often subacute. This inflammatory process is mediated by cytokine production that results in prostaglandin production, onset and propagation of myometrial contractility, and subsequent labor and birth ***(Genc et al., 2002).***

Interleukin-6 (IL-6) is a critical cytokine in the cascade of host response to infection. IL-6 activates the acute phase response, stimulates T lymphocytes, induces the terminal differentiation of B lymphocytes, and induces C-reactive protein production. In the setting of intrauterine infection and preterm labor, the amniotic fluid concentration of IL-6 is increased in excess of that of other proinflammatory products. Increases in IL-6 concentration are seen in maternal serum, cervix, and amniotic fluid in preterm labor. In large part, the production of IL-6 is under genetic regulation ***(Simhan et al., 2003).***

Polymorphisms have been described for many human cytokine genes. These polymorphisms represent normal allelic variation, frequently within the regulatory region of cytokine genes. Specific polymorphisms are associated with increased susceptibility to certain infectious diseases and increased severity of autoimmune disease. A polymorphism in the promoter region of IL-6(at position -174) on chromosome 7 is associated with production of IL-6. The substitution of cytosine (C) for guanine (G) at this position is responsible for a decrease in promoter activity. The individual with guanine at both

positions (homozygous G/G) or one position (heterozygous G/C) displays normal production of IL-6. Those individuals who are homozygous C/C display lower production ofIL-6. The C/C variant of this polymorphism is related to IL-6 production and to the incidence and severity of inflammatory conditions such as juvenile chronic arthritis and end-stage renal disease ***(Simhan et al., 2003).***

Aim of the Work

To establish the relationship between IL-6 promoter-174 polymorphism and spontaneous preterm birth (more than 28 weeks according to the accepted age of viability in Egypt and less than 37 weeks).

Chapter (1)

The Epidemiology of Preterm Labor and Delivery

Defining the problem

The true incidence of preterm delivery and preterm labor can only be ascertained if a consistent definition is used, and if the data are population based. The reported incidence of preterm delivery is affected by the method of gestational age assessment, and by the differing definitions of viability used and therefore the registration of every preterm delivery. Further problems occur in the measurement of outcome because of the heterogeneity of preterm birth – delivery may occur near to the 37-week upper limit of gestation where there may be no pathological cause and the baby has relatively few if any problems, or it may occur at the extreme of prematurity at around 24 weeks' gestation, where survival rates are poor, and the risk of severe morbidity in those survivors is high. The birth may be spontaneous or elective (iatrogenic); the spontaneous delivery may be uncomplicated (and the outcome usually better ***(Chng, 1981)***) or complicated, for example by prelabor rupture of the membranes. The outcomes of such wide variations in aetiology and gestational age will obviously be dissimilar, and so comparisons are difficult and often of little clinical relevance ***(Danielin et al., 2010).***