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Original Research Article

Role of mRNA expression of interleukin-6 and interleukin-10 in idiopathic preterm birth

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ABSTRACT

Background: India is one of the nation's leading in number of preterm births (PTB), as reported by WHO. Despite extensive research the exact cause of PTB remains elusive. The present study was designed to study the effect of inflammatory genes (proinflammatory IL-6 and anti-inflammatory IL-10) in etiopathogenesis of idiopathic PTB.
Methods: Maternal blood and placental tissue samples of PTB cases (n=263) and equal number of term delivery controls (n=263) were collected at the time of delivery. mRNA expression of IL-6 and IL-10 gene was analysed using Real-time PCR.
Results: mRNA expression of IL-6 gene (pro-inflammatory) was 11.73 folds high in maternal blood and 2.60 folds higher in placental tissue in PTB compared to term birth cases. mRNA expression of IL-10 gene (anti-inflammatory) was 25 folds lower in maternal blood and 10 folds lower in placental tissue of PTB compared to term deliveries.
Conclusions: Interleukins have been identified to have a major role in etiopathogenesis of idiopathic preterm birth.

Keywords: IL-6, IL-10, mRNA, Preterm birth, Preterm labour

INTRODUCTION

Every year, an estimated 15 million babies are born preterm, and this number is rising. Preterm birth (PTB) has a major and significant direct and indirect effect on the economy of a nation. Complications of PTB are the single largest direct cause of neonatal deaths, responsible for 35% of the world's 3.1 million neonatal deaths in a year, and the second most common cause of under-5 deaths after pneumonia.[1] India is leading all the nations in preterm births, as reported by WHO.[2] The onset of labor, whether at term or preterm involves the up regulation of numerous inflammatory mediators, including cytokines and prostaglandins.[3] Imbalances in the cytokine response are associated with pregnancy complications, including spontaneous abortion, chorioamnionitis, and preterm birth.[4,5]

Interleukin-6 (IL-6) a proinflammatory cytokine is a major mediator of host response to inflammation and infection. It can be analysed from samples of maternal cervical fluids or serum and to date, IL-6 is one of the most well-studied biomarkers of spontaneous PTB.[6] Devi et al has shown that IL-6 is highly expressed in the decidual cells of placenta obtained from normal term delivery as well as idiopathic preterm delivery but strong expression of IL-6 was seen in the decidual cells of placenta from idiopathic spontaneous preterm labour.[7]

Neurath et al has shown that down regulation of anti-inflammatory cytokine IL-10 causes up-regulation of cycloxygenase-2 which in turn increase the production of prostaglandin E_2.[8]

METHODS

Subjects

In this age-matched, case-control study, blood sample and placental tissue from 263 cases (period of gestation <37

weeks) and equal number of controls (period of gestation >37 weeks) were collected at the time of spontaneous labour. The study was conducted in the departments of Obstetrics and Gynaecology, and Biochemistry, Guru Teg Bahadur (GTB) Hospital associated with University College of Medical Sciences (UCMS). A lifestyle survey of the study subjects was done to collect general demographic information to define the inclusion/exclusion criteria. Socio-economic status of cases and controls was decided by Kuppuswamy's scale. Women with anaemia, hypertension, renal disease, heart disease, diabetes, urinary tract infections, metabolic disorders, tuberculosis, smoking, alcohol consumption or chronic drug intake and having complications during pregnancy and/or labour were excluded from the study. A written informed consent was taken from all the study participants.

Sample collection and storage

Maternal blood (3 ml) and placental tissue was collected in EDTA vials at the time of labour. A volume of 250 µl of whole blood sample was fixed in TRIzol reagent and stored at -80°C for RNA isolation.

RNA isolation and complementary DNA (cDNA) synthesis

RNA was isolated from blood and placenta using TRIzol reagent. Isolated total RNA was quantified using nano drop (Thermo Fisher, USA) by measuring optical density value at 260/280 nm and concentration in ng/µl. Quality of isolated RNA was checked on denatured gel electrophoresis. Total RNA (1000 ng) was converted into first strand cDNA using maxima first strand cDNA synthesis kit (Fermentas, USA) according to manufacturer's protocol. For cDNA synthesis, RNA was first incubated for 30 minutes at 42°C followed by 5 minutes at 95°C.

Quantification of IL-6 and Il-10 genes by real time qPCR

Quantitative real time PCR was performed to measure the expression of IL-6 and IL-10 gene on CFX connect Bio-Rad real time PCR.

In the initial cycles of PCR, there is little change in fluorescence signal (produced from double stranded DNA). This defines the baseline for the amplification plot. An increase in fluorescence above the baseline indicates the detection of accumulated target. In this study, GAPDH gene was used as an endogenous control for normalization of IL-6 and IL-10 gene expression to correct the sample-to-sample variations in RT-PCR efficiency and errors in sample quantification.

ΔCt = Average Ct_target – Average_Ct normalizer.

Again, the difference of mean Ct values of control and cases was determined, which is delta-delta Ct ($\Delta\Delta Ct$).

$\Delta\Delta Ct = \Delta Ct\_control - \Delta Ct\_test$.

After this, true fold change (FC) was represented to compare the expression of genes between cases and controls by the following formula: $FC = 2 - \Delta\Delta Ct$.

Statistical analysis

Microsoft Excel (version 2007) and statistical software SSPS for windows (version 17.0) was used for data presentation and statistical analysis. P value<0.05 was considered as significant. Unpaired Student's t-test and Chi-square/Fisher's exact test was applied to compare all socio-demographic characteristics in preterm and term delivery subjects according to data being quantitative or qualitative. Correlations were tested by Spearman's and Pearson's co-efficient of correlation.

RESULTS

The incidence of preterm birth in the study was 18.19%. The two study groups were well matched for most of the socio-demographic characteristics except for a significant difference in socioeconomic status (Table 1).

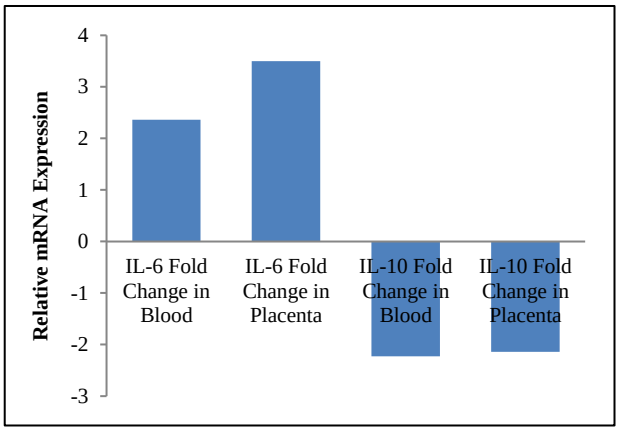

Figure 1: IL-6 and IL-10 gene expression in blood and placenta of cases and mRNA levels in control were considered as 1.

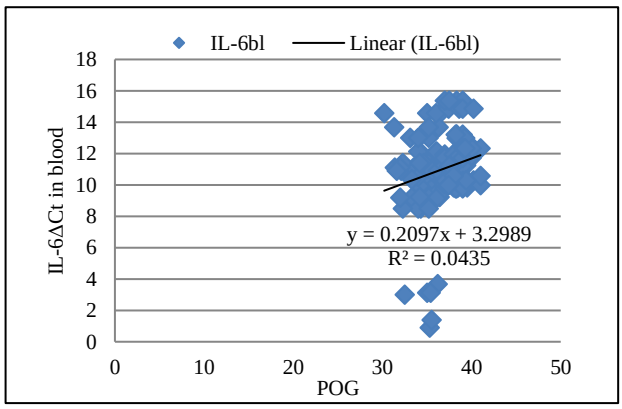

Figure 2: Correlation between IL-6 delta Ct in blood and POG.

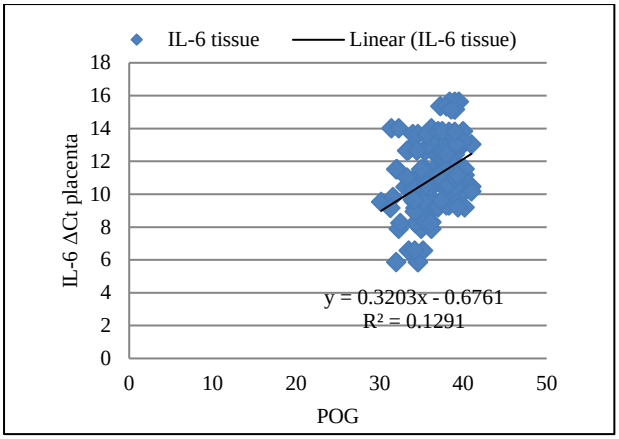

Figure 3: Correlation of IL-6 delta Ct in placenta with POG.

The mean value of IL-6 delta Ct was significantly lower in maternal blood of cases (10.04±1.77) than in controls (mean value of 13.59±1.83) and also in placental tissue of cases (4.40±1.01) than in controls (5.79±1.61) (Table 2). Thus, this signifies that expression of IL-6 gene was 11.73 folds higher in maternal blood and 2.60 folds higher in placental tissue in preterm labor as compared to term labor. A significant linear negative correlation was observed between IL-6 gene expression in maternal blood as well as placental tissue and period of gestation (Figures 2 and 3).

Also, there was a significant correlation between IL-6 delta Ct in placenta with birth weight of babies who delivered preterm (p value 0.002) which means in women in whom IL-6 gene is overexpressed in placental tissue will have reduction in birth weight. The mean value of IL-10 delta Ct was significantly high in maternal blood of cases (12.76±1.39) than in controls (8.42±3.46) and in placental tissue of cases (13.01±1.65) than in controls (9.80±2.17) (Table 2). The expression of IL-10 gene was 25 folds lower in maternal blood and 10 folds lower in placental tissue in preterm labor as compared to term labor. A significant positive correlation was found between IL-10 gene expression in blood and POG (Figure 4 and 5).

Table 1: Demographic characteristics of the subjects.

Characteristics	Controls (n=263) mean±SD	PTB (n=263) mean±SD	P value
Maternal age (years)	24.25±3.43	24.23±3.74	0.935
Baby weight (kg)	2.72±0.37	1.90±0.44	<0.001
POG (weeks)	38.92±1.12	33.60±2.43	<0.001
Baby sex			
Male	147(55.90%)	146 (55.52%)	0.922
Female	116 (44.10%)	117 (44.48%)	
Residential area			
Urban	231 (87.84%)	228 (86.70%)	0.554
Rural	32 (12.16%)	35 (13.30%)	
Socioeconomic status			
Class I (upper)	0 (0%)	0 (0%)	0.039
Class II (upper-middle)	12 (4.56%)	9 (3.42%)	
Class III (lower-middle)	130 (49.42%)	162 (61.59%)	
Class IV (upper-lower)	0 (0%)	0 (0%)	
Class V (lower)	121 (46.02%)	92 (34.99%)	
Education			
Illiterate	31 (11.79%)	31 (11.79%)	0.263
Primary	38 (14.44%)	53 (20.16%)	
Secondary	176 (66.92%)	158 (60.07%)	
Graduate	18 (6.84%)	21 (7.98%)	
Occupation			
Housewife	251 (95.44%)	254 (96.58%)	0.610
Working	12 (4.56%)	9 (3.42%)	
Dietary habits			
Vegetarian	197 (75%)	190 (72.25%)	0.509
Non-vegetarian	66 (25%)	73 (27.75%)	

Data were presented in mean±SD, unpaired 't' test was applied for quantitative variables such as maternal age, POG and baby weight. Chi-square/Fisher's exact test was applied for qualitative data such as area, residential area, religion, socioeconomic status, education, occupation, dietary habits, and source of drinking water, n = number, Kg = kilogram, POG = period of gestation. Figure in parenthesis indicate percent values, *p<0.05 was considered as significant.

Table 2: Comparison of ΔCt value of target genes in PTB cases and controls.

ΔCt of genes	Controls (n=263), mean±SD	PTB (n=263), mean±SD	P value	Fold change
IL6 ΔCt blood	13.59±1.83	10.04±1.77	0.001*	11.73
IL6 ΔCt placenta	5.79±1.61	4.40±1.01	0.001	2.60
IL10 ΔCt placenta	9.80±2.17	13.01±1.65	0.001*	-10
IL10 ΔCt blood	8.42±3.46	12.76±1.39	0.001*	-25

*p<0.005 = significant (after putting Bonferroni correction).

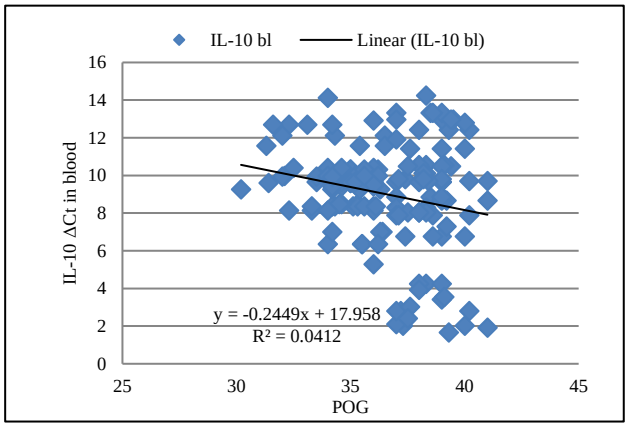

Figure 4: Correlation of IL-10 ΔCt in blood with POG.

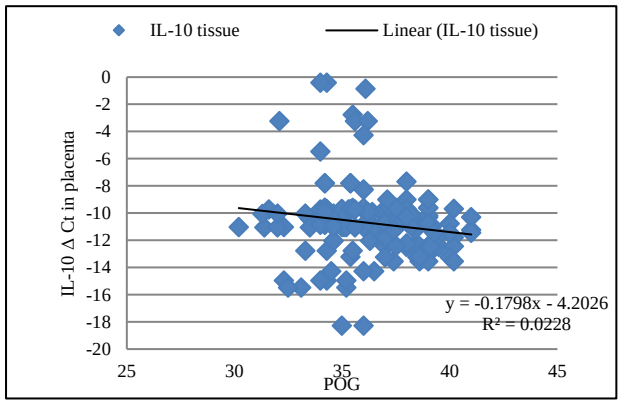

Figure 5: Correlation of IL-10 ΔCt in placenta with POG.

This means that women in whom IL-6 gene is overexpressed, have premature onset of labor and reduction in POG.

Correlation between IL-6 delta Ct in blood with placenta amongst cases (preterm group) did not show any significant correlation (Pearson correlation 0.003 and p value 0.973) indicating that changes in the trends of IL-6 expression in blood and placenta of cases is independent of each other. Significant negative correlation was found between IL-10 delta Ct in blood and POG (p value 0.013) i.e., there is significant positive correlation between IL-10 gene expression and POG. This infers that IL-10 gene downregulation is associated with reduction of POG. No such significant correlation was found between IL-10 delta Ct in blood with birth weight or IL-10 delta Ct in placenta with POG or birth weight.

Table 3: Correlation of IL-6 gene expression in blood and placenta with POG and birth weight of cases.

		POG	Birth weight
IL-6 blood	Pearson correlation	0.208*	0.147
	P value	0.010	0.073
IL-6 placenta	Pearson correlation	0.359**	0.249**
	P value	<0.001	0.002

*Correlation significant at 0. 05 level, **correlation is significant at 0.01level

Table 4: Correlation of IL-10 gene expression in blood and placenta with POG and birth weight of cases.

		POG	Birth weight
IL-10 blood	Pearson correlation	-0.203*	-0.157
	P value	0.013	0.054
IL-10 placenta	Pearson correlation	-0.151	-0.009
	P value	0.013	0.918

*Correlation significant at 0.05 level

Correlation between IL-10 delta Ct in blood with placenta amongst cases (preterm group) did not show any significant correlation (Pearson correlation 0.012 and p value 0.888) indicating that the downregulation of IL-10 gene is independent in the two samples. Also, there is a negative correlation between IL-10 expression in maternal blood as well as placenta and the birth weight (Table 4).

To conclude, there is around 2 folds higher expression of IL-6 gene in maternal blood and 3 folds higher expression in placental tissue in women having preterm birth, thus IL-6 is an important pro-inflammatory cytokine in the mechanism of preterm birth. The expression of IL-10 gene is around 2 folds lower in patients having preterm delivery, this explains the possible protective role of anti-inflammatory cytokine in maintaining normal pregnancy.

DISCUSSION

Preterm birth is a global problem and it is a major cause of neonatal as well as under five morbidity and mortality, and also attributes to long term adult illness and financial burden on our country. It was observed in present study

that IL-6 gene expression was significantly higher in both maternal blood (11.73 folds; p=0.001) and placenta (2.60 folds; p=0.001) of preterm birth cases. Similar findings were observed by Oros et al who showed significant positive correlation between mRNA expression of IL-6 gene with preterm labor.[9] Devi et al have also shown that IL-6 gene is highly expressed in the decidual cells of placenta obtained from normal term delivery as well as idiopathic preterm delivery but strong expression of IL-6 gene was seen in the decidual cells of placenta from idiopathic spontaneous preterm labour.[7] Literature also suggests the positive association between cervical IL-6 levels and adverse neonatal outcomes. It was observed that IL-10 gene expression was significantly lower in both blood (25 folds; p value =0.001) and placenta (10 folds; p value =0.001) of cases as compared to controls. IL-10 is a pregnancy compatible cytokine that plays a vital role in maintaining immune tolerance.[10] Hanna et al observed that IL-10 is expressed in the placenta in a gestational age-dependent manner and its down-regulation at term, may be an important mechanism underlying the subtle changes associated with parturition.[11] Observations made in the present study indicate that IL-10 is an important anti-inflammatory cytokine which is down regulated in PTB suggesting that it is a supportive cytokine in term parturition.

CONCLUSION

The present study was designed to understand the role of IL-6 and IL-10 genes expression in idiopathic preterm birth. This explains the role/mechanism of action of IL-6 and Il-10 gene expression in PTB.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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