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Association of mRNA expression of interleukin-6 and interleukin-10 with organochlorine pesticides in idiopathic preterm birth

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Abstract

Objectives: 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 gain insight into inflammatory genes (proinflammatory IL-6 and anti-inflammatory IL-10) and environment (organochlorine pesticides-OCPs) interaction in etiopathogenesis of 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 and OCP levels by gas chromatography.

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. Significant association was found between the higher levels of beta-hexachlorocyclohexane (β-HCH) and ortho, para-dichlorodiphenyldichloroethane (o'p'-DDD) in maternal blood with PTB (OR 1.27 and 5.45 respectively). Also, significant association was found

between higher levels of para, para-dichlorodiphenyl-dichloroethylene (p'p'-DDE) in placental tissue with PTB (OR 1.16). Interaction between IL-6 and high levels of β-HCH, dieldrin and DD resulted in significant reduction in period of gestation (POG) by 7-12 days and interaction between IL-10 and β-HCH by 12 days.

Conclusions: The gene (IL)-environment (OCPs) interaction resulted in significant reduction of POG ranging from 1-2 weeks. Thus, the present study identified the gene-environment interaction as a potential risk for PTB and emerges as a molecular tool in etiopathogesis of PTB.

Summary: Preterm birth (PTB) is responsible for significant neonatal morbidity, mortality and long-term sequelae besides being economic and social burden. There is an urgent need to identify predictors leading to spontaneous preterm birth. Primary prevention of PTB continues to be a challenge despite recent advances in diagnosis and management. This is due to multifactorial etiology of preterm labor, which still eludes the obstetricians. The role of environment and genetic susceptibility in PTB has largely been unexplored. Exposure to environmental chemicals like organochlorine pesticides (OCPs) has shown to cause inflammation and alter mRNA expression of inflammatory cytokines. According to some studies, OCPs can also induce preterm labor by themselves but these associations are not well established. The present study aims to evaluate the impact of OCPs in etiology of PTB by altering mRNA expression of interleukin-6 (IL-6) and interleukin-10 (IL-10).

Keyword: IL-6, IL-10, Organochlorine pesticides, preterm birth, mRNA.

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, chorioaminionitis, 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).

Organochlorine pesticides (OCPs) are widely distributed in our environment, in our food, water, air, almost everywhere. They reach the human system through skin, inhalation, oral and placental routes(9).

OCPs are lipophilic in nature and tend to accumulate in fatty tissue. They are non-biodegradable, and have long half-life and also are biomagnified through the food chain(10). Being xenoestrogenic in nature, they may act as endocrine disruptors and lead to disturbance of normal estrogen-progesterone balance of pregnancy and adverse reproductive outcomes like preterm birth(11). Tyagi et al showed significantly higher levels of α-HCH, β-HCH, DDD, and DDE in maternal blood of PTB cases as compared to term deliveries. Also significantly higher levels of DDE and DDT were found in placental tissue of PTB cases compared to term deliveries in north Indian population(12).

The present study was designed to gain insight into inflammatory genes (IL-6 and IL-10) and environmental (OCPs) interaction in etiopathogenesis of PTB.

Material and Methods

Subjects: In this age-matched, case-control study, blood sample and placental tissue from 263 cases (period of gestation <37 week) and equal number of controls (period of gestation >37 week) 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. There was no occupational or environmental exposure to the agricultural pollutants. 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 and remaining was stored at 4°C for the pesticides residue analysis.

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 min at 42°C followed by 5 min 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 & 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 (ΔΔCt).

ΔΔCt = ΔCt_control – ΔCt_test.

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

OCPs extraction and clean up: Extraction of OCPs was done using hexane and acetone (2:1 v/v). Clean up of the samples was done by column chromatography following US environmental protection agency (USEPA) method. Elutant was collected and hexane was evaporated to concentrate the sample using rotary evaporator. The concentrated residues were dissolved using one ml of hexane for further analysis. Ten pesticides were estimated namely alpha-hexachlorocyclohexane (α-HCH), beta-hexachlorocyclohexane (β-HCH), gamma-hexachlorocyclohexane (γ-HCH), aldrin, dieldrin, α-endosulfan, β-endosulfan, ortho, para-dichlorodiphenyltrichloroethane (o'p'-DDT), para, para-dichlorodiphenyl-dichloroethylene (p'p'-DDE), and ortho, para-dichlorodiphenyldichloroethane (o'p'-DDD). OCP residue levels were estimated by a Perkin Elmer gas chromatography system equipped with a 63Ni selective electron capture detector (Perkin Elmer, USA).Quantitative analysis of OCP residues in each sample was done by comparing the peak areas with those obtained from a standard chromatogram of blended OCPs of known concentration. A quality check sample was always run for each set of sample for pesticide analysis to maintain accuracy.

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. As pesticides and gene expression data were not normally distributed, non-parametric Mann-Whitney test was used to compare pesticide levels in cases and controls. Correlations were tested by Spearman's and Pearson's co-efficient of correlation. Logistic Regression model was created to assess the odds of PTB due to rising pesticide levels in maternal blood of cases (preterm group). Multiple linear regression analysis was done to see the simultaneous effect of OCPs and IL-6 and IL-10 gene expression on outcome of delivery and also to examine if any interaction exists between the two predictor variables taking period of gestation as dependent variable

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 charateristics except for a significant difference in socioeconomic status and source of water supply. As a consequence of recruitment, birth weight and period of gestation among PTB cases was significantly less compared to controls. (Table 1)

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, 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.(fig 2,3) (table 2)

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(fig.4,5) (table 2).

In maternal blood pesticides: β- HCH, o'p'-DDD were significantly increased in preterm delivery cases (Table3) and in placental tissue pesticides: p'p'-DDE was significantly increased in preterm delivery cases (table 4). A significant association was found between the higher levels of β-HCH and DDE in maternal blood and preterm birth with odds ratio of 1.148 and 1.248 respectively and a significant association was found between the higher levels of DDT and DDE in placental tissue with preterm birth with odds ratio of 1.094 and 2.771 respectively.

The interaction of IL-6 gene with high levels of β-HCH and dieldrin in maternal blood resulted in significant reduction in POG by 11.53 and 11.48 days respectively. Similarly,the interaction of IL-6 gene with high levels of DDD in placenta resulted in significant reduction of POGby 7.98 days. On the other hand, the interaction of IL-10 ΔCt and β-HCH levels in maternal blood resulted in significant reduction in POG by 9.14 days and the interaction of IL-10 ΔCt and β-HCH in placental tissue resulted in significant reduction in POG by 11.47 days respectively.

So, preterm birth continues to be a significant health problem in our population. There is 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 2 folds lower in patients having preterm delivery, this explains the possible protective role of anti-inflammatory cytokine in maintaining normal pregnancy. Increased level of pesticides especially HCH isomers and metabolites of DDT appear to be strong attributable risk factors for so called 'idiopathic' preterm birth. The rising levels of some pesticides appear to proportionately enhance the expression of IL-6 gene and dampen the expression of IL-10 gene thus, resulting in preterm labor and birth. A strong interaction of rising levels of certain pesticides with IL-6 gene upregulation and IL-10 gene downregulation resulted in significant shortening of gestational length.

The present study has identified the gene-environment interaction between inflammatory genes and OCPs as a potential risk for PTB. It also reminds us that even after being banned / restricted use, pesticides are still contributing significantly to adverse reproductive effects on our population.

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			0.922
Male	147(55.90%)	146 (55.52%)	
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%)	
Class II (Upper-middle)	12 (4.56%)	9 (3.42%)	
Class III (Lower-middle)	130 (49.42%)	162 (61.59%)	0.039
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%)	
Primary	38 (14.44%)	53 (20.16%)	0.263
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%)	
Water source			
Government supply	249 (94.68%)	231 (87.84%)	0.010
Ground water	14 (5.32%)	32 (12.16%)	

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.

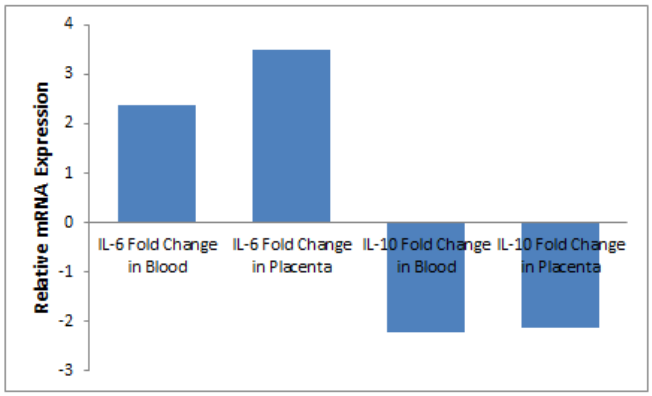

Figure 1: bar diagram showing IL-6 and IL-10 gene expression in blood and placenta of cases and mRNA levels in control were considered as 1.

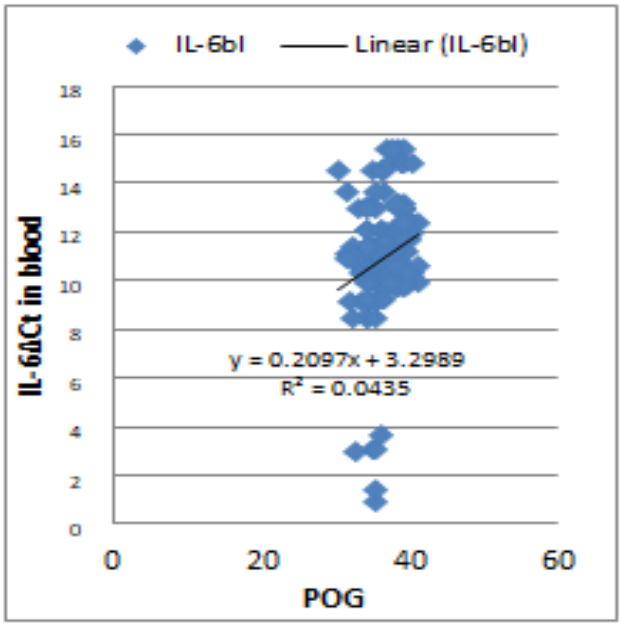

Fig. 2: Scatter diagram showing correlation between IL-6 delta Ct in blood and POG

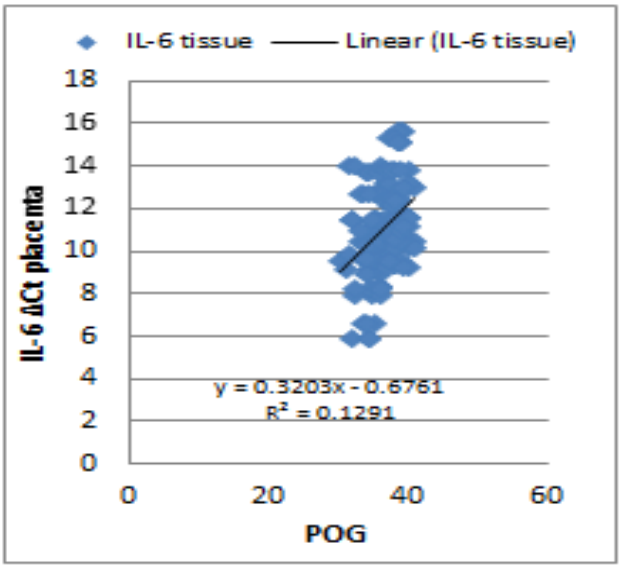

Fig. 3: Scatter diagram showing correlation of IL-6 delta Ct in placenta with POG

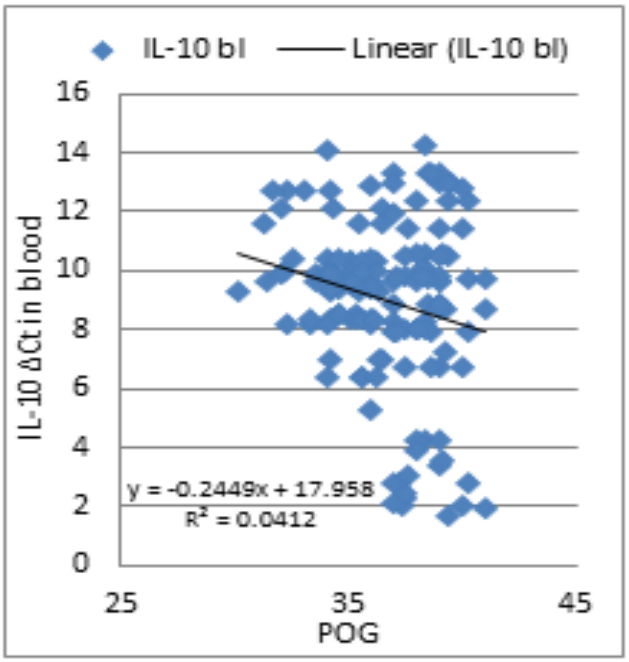

Fig. 4: Scatter diagram showing correlation of IL-10 ΔCt in blood with POG

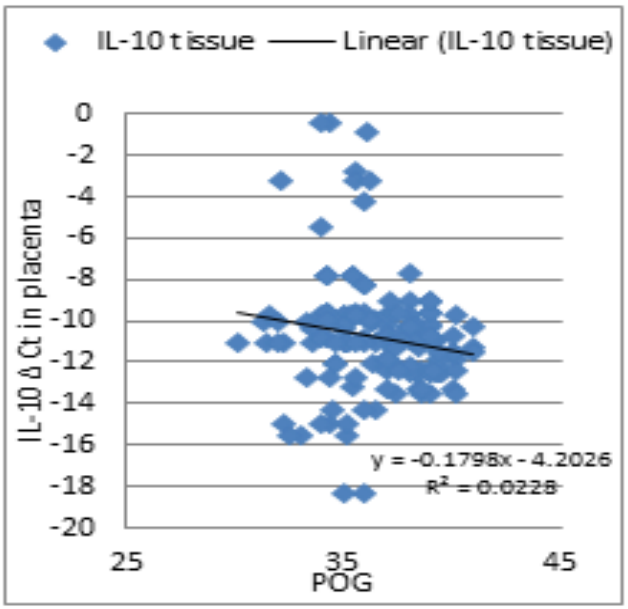

Fig. 5: Scatter diagram showing correlation of IL-10 ΔCt in placenta with POG

Table2: 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).

Table 3: Comparison of OCP levels (ng/mL) in maternal blood of term controls and PTB cases.

OCPs (ng/mL)	Controls (n = 263) (Mean ± SD)	PTB cases (n = 263) (Mean ± SD)	Adjusted OR	95% CI	P-Value
α- HCH	2.96±2.10	3.52±2.45	1.194	1.013-1.407	0.035
β- HCH	3.37±2.97	4.92±3.30	1.2747	1.134-1.432	**<0.001**
γ- HCH	1.02±1.58	2.01±2.29	1.210	0.998-1.468	0.053
Aldrin	1.84±1.69	2.02±1.75	0.744	0.579-0.955	0.020
Dieldrin	1.25±1.15	1.73±1.80	1.325	0.928-1.892	0.122
α-Endo	1.60±1.80	2.18±2.56	0.915	0.758-1.104	0.354
β-Endo	1.25±1.51	1.70±2.05	0.996	0.815-1.217	0.969
o′p′-DDT	0.79±1.06	1.43±1.57	1.168	0.851-1.604	0.337

p′p′-DDE	2.31±1.49	2.68±2.09	1.131	0.913-1.401	0.259
o′p′-DDD	0.05±0.24	0.47±0.91	5.454	1.859-16.000	0.002

After adjustment of confounding factors education, Birth weight, drinking water, residential area and maternal age, Data were presented in Mean ± SD, Unpaired t –student test was applied to analyze the data, n = number, *p < 0.006 was considered statistically significant (after Bonferroni correction).

Table 4: Comparison of OCP levels (ng/mL) in placenta of term controls and PTB cases.

OCPs (ng/mL)	Controls (n = 263) (Mean ± SD)	PTB cases (n = 263) (Mean ± SD)	Adjusted OR	95% CI	p-value
α- HCH	4.29±3.37	4.74±3.96	1.048	0.96-1.14	0.285
β- HCH	9.34±14.95	11.17±23.54	1.005	0.99-1.02	0.511
γ- HCH	4.00±8.01	5.19±8.75	1.007	0.96-1.04	0.754
Aldrin	13.57±16.98	12.98±18.65	1.000	0.97-1.02	0.989
Dieldrin	3.94±7.04	4.30±5.50	0.995	0.92-1.06	0.895
α-Endo	445±4.77	5.61±7.18	1.060	0.99-1.12	0.070
β-Endo	3.85±4.65	4.53±5.51	1.001	0.93-1.07	0.973
o′p′-DDT	4.72±6.25	6.24±8.17	1.079	1.02-1.13	0.006
p′p′-DDE	1.96±3.36	3.41±4.14	1.166	1.05-1.28	**0.003**
o′p′-DDD	0.86±1.77	1.09±1.86	0.887	0.73-1.06g	0.209

After adjustment of confounding factors education, baby weight, drinking water, residential area and maternal age, Data were presented in Mean ± SD, Unpaired t –student test was applied to analyze the data, n = number, *p < 0.006 was considered statistically significant (after Bonferroni correction).

Table: 5 Regression model testing mains and an interactive effect of OCPs (ppb) and IL-6 gene expression in maternal blood on period of gestation in all subjects

	Maternal blood	
IL-6 Interaction	B Value	p-value
IL-6ΔCt	-0.070	0.334
α-HCH	-0.182	0.283
Interaction term of IL-6ΔCt-α-HCHlevels	0.001	0.856
IL-6ΔCt	-0.078	0.486
β-HCH	-10.198	0.018

Interaction term of IL-6ΔCt-β-HCHlevels	-1.26	0.000
IL-6ΔCt	-0.089	0.214
γ-HCH	-0.416	0.000
Interaction term of IL-6ΔCt-γ-HCHlevels	0.013	0.611
IL-6ΔCt	-0.049	0.567
Aldrin	-0.069	0.674
Interaction term of IL-6ΔCt-aldrin levels	-0.008	0.807
IL-6ΔCt	-0.130	0.093
Dieldrin	-10.543	0.001
Interaction term of IL-6ΔCt- dieldrin levels	-0.808	0.003
IL-6ΔCt	-0.037	0.710
α-Endosulphan	-0.158	0.150
Interaction term of IL-6ΔCt-α-Endosulphan levels	-0.023	0.314
IL-6ΔCt	-0.069	0.431
β-Endosulphan	-0.085	0.508
Interaction term of IL-6ΔCt-β-Endosulphan levels	-0.007	0.798
IL-6ΔCt	-0.050	0.494
DDT	-0.450	0.016
Interaction term of IL-6ΔCt-DDT levels	-0.010	0.809
IL-6ΔCt	-0.110	0.232
DDE	-0.240	0.093
Interaction term of IL-6ΔCt-DDE levels	0.019	0.517
IL-6ΔCt	-0.062	0.295
DDD	-1.435	0.000
Interaction term of IL-6ΔCt-DDD levels	0.016	0.835

*p value is significant at <0.001 level

Table: 6 Regression model testing mains and an interactive effect of OCPs (ppb) and IL-6 gene expression in placental tissue on period of gestation in all subjects

	Placental tissue	
IL-6 Interaction	B Value	P-Value
IL-6ΔCt	-3.201	0.000
α-HCH	-0.146	0.052
Interaction term of IL-6ΔCt-α-HCHlevels	0.088	0.430
IL-6ΔCt	-2.580	0.000
β-HCH	-0.098	0.076
Interaction term of IL-6ΔCt-β-HCHlevels	-0.055	0.493
IL-6ΔCt	-2.787	0.000
γ-HCH	-0.258	0.009
Interaction term of IL-6ΔCt-γ-HCHlevels	0.011	0.930
IL-6ΔCt	-2.977	0.000
Aldrin	-0.137	0.143
Interaction term of IL-6ΔCt-aldrin levels	0.013	0.932
IL-6ΔCt	-3.058	0.000
Dieldrin	-0.348	0.006
Interaction term of IL-6ΔCt- dieldrin levels	0.139	0.413
IL-6ΔCt	-2.814	0.000
α-Endosulphan	-0.165	0.050
Interaction term of IL-6ΔCt-α-Endosulphan levels	-0.026	0.822
IL-6ΔCt	-3.112	0.000
β-Endosulphan	-0.079	0.466
Interaction term of IL-6ΔCt-β-Endosulphan levels	0.119	0.396
IL-6ΔCt	-2.852	0.000
DDT	-0.359	0.010
Interaction term of IL-6ΔCt-DDT levels	0.045	0.813
IL-6ΔCt	-3.596	0.000
DDE	-0.284	0.003
Interaction term of IL-6ΔCt-DDE levels	0.272	0.046
IL-6ΔCt	-3.221	0.000
DDD	-2.460	0.000
Interaction term of IL-6ΔCt-DDD levels	-2.299	0.000*

*p value is significant at <0.001 level

Table: 7 Regression model testing mains and an interactive effect of OCPs (ppb) and IL-10 gene expression in maternal blood on period of gestation in all subjects

	Maternal blood	
IL-10 Interaction	B Value	p-value
IL-10ΔCt	-0.127	0.486
α-HCH	-0.078	0.629
Interaction term of IL-10ΔCt-α-HCHlevels	0.017	0.705
IL-10ΔCt	-0.082	0.615
β-HCH	-10.096	0.421
Interaction term of IL-10ΔCt-β-HCHlevels	1.032	0.000*
IL-10ΔCt	-0.132	0.270
γ-HCH	-0.232	0.235
Interaction term of IL-10ΔCt-γ-HCHlevels	-0.039	0.467
IL-10ΔCt	-0.190	0.223
Aldrin	-0.092	0.702
Interaction term of IL-10ΔCt-aldrin levels	-0.003	0.961
IL-10ΔCt	-0.167	0.246
Dieldrin	-0.302	0.290
Interaction term of IL-10ΔCt- dieldrin levels	-0.020	0.803
IL-10ΔCt	-0.357	0.010
α-Endosulphan	-0.509	0.004
Interaction term of IL-10ΔCt-α-Endosulphan levels	0.079	0.109
IL-10ΔCt	-0.119	0.363
β-Endosulphan	0.105	0.638
Interaction term of IL-10ΔCt-β-Endosulphan levels	-0.065	0.303
IL-10ΔCt	-0.094	0.448
DDT	-0.139	0.636
Interaction term of IL-10ΔCt-DDT levels	-0.102	0.208
IL-10ΔCt	-0.237	0.154
DDE	-0.221	0.280
Interaction term of IL-10ΔCt-DDE levels	0.017	0.757
IL-10ΔCt	-0.151	0.139
DDD	-0.931	0.227
Interaction term of IL-10ΔCt-DDD levels	-0.121	0.556

*p value is significant at <0.001 level

Table: 8 Regression model testing mains and an interactive effect of OCPs (ppb) and IL-10 gene expression in placental tissue on period of gestation in all subjects

	Placental tissue	
IL-10 Interaction	B Value	P-Value
IL-10ΔCt	-0.201	0.025
α-HCH	-0.015	0.937
Interaction term of IL-10ΔCt-α-HCHlevels	-0.016	0.491
IL-10ΔCt	-0.140	0.095
β-HCH	-0.534	0.001
Interaction term of IL-10ΔCt-β-HCHlevels	-10.802	0.004*
IL-10ΔCt	-0.323	0.000
γ-HCH	-0.778	0.000
Interaction term of IL-10ΔCt-γ-HCHlevels	0.056	0.044
IL-10ΔCt	-0.302	0.000
Aldrin	-0.298	0.219
Interaction term of IL-10ΔCt-aldrin levels	0.025	0.402
IL-10ΔCt	-0.258	0.000
Dieldrin	-0.447	0.130
Interaction term of IL-10ΔCt- dieldrin levels	0.012	0.735
IL-10ΔCt	-0.246	0.001
α-Endosulphan	-0.247	0.203
Interaction term of IL-10ΔCt-α-Endosulphan levels	0.003	0.910
IL-10ΔCt	-0.300	0.000
β-Endosulphan	-0.330	0.138
Interaction term of IL-10ΔCt-β-Endosulphan levels	0.032	0.240
IL-10ΔCt	-0.298	0.000
DDT	-0.879	0.005
Interaction term of IL-10ΔCt-DDT levels	0.053	0.173
IL-10ΔCt	-0.315	0.001
DDE	-0.380	0.151
Interaction term of IL-10ΔCt-DDE levels	0.027	0.397
IL-10ΔCt	-0.252	0.000
DDD	-2.441	0.002
Interaction term of IL-10ΔCt-DDD levels	0.143	0.128

*p value is significant at <0.05 level

**p value is significant at <0.001 level

Discussion

Preterm birth is a global problem, 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 (13). 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(14). 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 (15). 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. OCPs are ubiquitous in nature, they are Persistent Organic Pollutants (POPs) that persist in the environment, and they accumulate in high concentrations in fatty tissues and are bio-magnified through the food-chain. OCPs have been widely used in public health and agriculture programs in developed as well as developing countries, including India. Indian researchers found considerably higher amounts of organochlorine pesticide residues in blood and placental tissue of the women undergoing premature labor as compared with women in labor at term (16). Tyagi et al showed significantly higher levels of α-HCH, β-HCH, DDD, and DDE in maternal blood of PTB cases as compared to control and significantly higher levels of DDE and DDT were also found in placental tissue of PTB cases as compared to control group (12). In an another study, Mustafa et al showed significantly high levels of α-HCH, γ-HCH and DDE in maternal blood of women delivering preterm (17). Tyagi et al observed significantly higher levels of β-HCH,(95% CI=2.08-4.633, p0 =0.001), DDE (95% CI=0.546-2.551, p=0.003), and DDD (95% CI=0.004-0.690, P=0.047) in maternal blood of PTB cases as compared to term delivery(18). Results from our study also show a significantly high levels of β- HCH (95% CI=1.34-1.432, p <0.001) and DDD (95% CI=1.859-16.000, p=0.002) in maternal blood and DDE (95% CI=1.05-1.28, p=0.003) in placental tissue of women having preterm delivery. The present study was designed to understand the role of IL-6 and IL-10 genes expression, and their association with OCPs levels to find out possible gene-environment interaction in mechanism of PTB. A significant positive correlation was found between IL-6 gene expression with β-HCH

(p-value=0.000) and dieldrin (p-value=0.003) in maternal blood. Also significant positive correlation was seen between IL-6 gene and DDE (p-value=0.046) and DDD (p-value=0.000) in placental tissue. Similarly a significant negative correlation was found between IL-10 gene expression and β-HCH (p-value=0.000) residue in maternal blood. A significant negative correlation between IL-10 gene expression with β-HCH(p value=0.004) and γ-HCH(p value=0.044) residue in placental tissues of preterm delivery subjects was found compared to term delivery subjects. The interaction of IL-6 genewith high levels of β-HCH and dieldrin in maternal blood resulted in significant reduction in POG by 11.53 and 11.48 days respectively. Similarly,the interaction of IL-6 gene with high levels of DDD in placenta resulted in significant reduction of POGby 7.98 days. On the other hand, the interaction of IL-10 ΔCt and β-HCH levels in maternal blood resulted in significant reduction in POG by 9.14 days and the interaction of IL-10 ΔCt and β-HCH in placental tissue resulted in significant reduction in POG by 11.47 days respectively.. This explains the role/ mechanism of action of IL-6 and Il-10 gene expression in PTB in association with OCPs. Thus, inflammatory gene expression and organochlorine pesticide exposure in a pregnant woman do interact to reduce gestational length, hence preterm birth. Our study supports the gene-environment interaction study by Vipin et al, who observed that elevated maternal blood concentrations of β-HCH and p'p'-DDE were significantly associated with reduction in POG leading to PTB. Also, increased mRNA expression of TNF-α was found to be significantly associated with PTB (20). A very few similar studies of an adverse outcome originating due to pesticide and inflammatory genes interaction are available in literature. The present study does prove the hypothesis that alteration in immune response occurs due to exposure to organochlorine pesticides, leading to preterm birth.

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