Assessment of Toxicogenomic Risk Factors in Etiology of Preterm Delivery

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Abstract

Worldwide, preterm birth accounts for more than 60% of prenatal morbidity and mortality and is the leading cause of neonatal deaths. Globally, every year, around 13 million babies are born preterm, with rates being highest in the low and middle-income group. India is leading all the nations in PTD, as reported by WHO. PTD continues to be a major clinical and public health problem and is regarded as a syndrome with multiple causes. Environmental stressors such as heavy metal, Organochlorine pesticides have a long history of widespread use all around the world. Because of high stability, extremely low biodegradability and long half life, these compounds are typically very persistent in the environment, and are known to accumulate in soil, food, blood, fatty tissue etc. Humans, being at the top of the food chain are most vulnerable to health effects as the level of the toxic chemicals is several folds higher through the process of bio-magnifications. The problem gets compounded in women because of increased adipose tissue in them and high liposolubility of these OCPs. Pesticide toxicity is of utmost concern during pregnancy as studies have revealed that mother and fetus are more vulnerable to their toxic effects. OCPs are metabolized by xenobiotic metabolizing enzymes such as CYP P450 and GSTs. Polymorphism in xenobiotic metabolizing genes may cause improper metabolism of xenobiotics which may cause high free radical generation, increased oxidative stress, improper cytokines release and also increased inflammation. OCPs levels are also correlated significantly with increased expression of COX-2 gene. The mechanisms underlying the etiology and onset of preterm birth is not clearly understood. Still more than 40-50% cases of preterm delivery are ‘idiopathic’. So, it is very essential to assess the etiological factors and the mechanisms by which they result in preterm birth in order to make further research in targeted interventions to prevent preterm labor.

Keywords: Preterm delivery; Organochlorine pesticides; CYP gene polymorphism; GST gene polymorphism; Period of gestation; Inflammation; mRNA expression; Gene-environment interaction

Introduction

Preterm delivery

Preterm Delivery (PTD), period of gestation <37 weeks, is the largest cause of prenatal deaths, neonatal morbidity, mortality, and adult illness [1]. According to a WHO (2012) report, India tops the list of countries, with maximum number of preterm deliveries [2]. PTD complicates between 5-11% of all births and result in 70-80% of neonatal mortality and morbidity [3]. PTD affects approximately 5-7% of live births in developed countries, but its incidence is significantly higher in developing countries [4]. It is regarded as a syndrome with multiple causes. Preterm birth causes a range of neonatal morbidity like respiratory illness, infections, intraventricular hemorrhage, and necrotizing enterocolitis. The long term consequences like neurological disorders, cerebral palsy, developmental delay, hearing and vision impairment, retinopathy of prematurity, bronchopulmonary dysplasia and cognitive disorders, also prolonging monetary burden, emotional and stress related problems [4].

Etiology of PTD

The patho-physiology of preterm labor is not entirely clear, still 40-45% cases are idiopathic. It has been suggested the balance of reproductive hormones, such as progesterone, estrogen, infection, genetic factors, inflammation, and oxidative stress have an important role in PTD. Progesterone promotes the uterine quiescence activity. However, estrogen, may promote myometrial activation with increased receptivity to utoerotic agents by up-regulating membrane receptors and gap junctions. Therefore, it has been suggested that Endocrine Disruptor Chemicals (EDCs), especially those having estrogenic effect, may induce preterm labor [5,6]. The proposed mechanism of action of these exogenous substances is that it can alter function(s) of the endocrine system, alter the homeostasis of sex steroidal hormones and thus cause adverse health effects. EDCs may mimic, block or modulate the synthesis, release, transport, metabolism and binding or elimination of steroidal hormones.

The presence of very small quantity of EDC in environment are potential enough to cause adverse health effects [7]. OCPs such as DDT and HCH may act as EDCs thus causes hormonal imbalance. Several studies have also reported that OCPs may increase the oxidative stress and the high oxidative stress causes the damage to macromolecules such as lipid, DNA, protein etc.

The reproductive effects due to persistent exposure of pesticides especially OCPs are a matter of worldwide concern. Although several studies have reported the reproductive effects of DDT in humans [6], there are very few studies about other OCPs such as β-HCH, aldrin, dieldrin, and endosulfan. Recently, studies from our laboratory have shown OCPs such as isomers of HCH, DDT, aldrin and its metabolites are one of the risk factors for adverse reproductive outcomes such as PTD, FGR, recurrent miscarriage, etc. [8-12].

Pesticides and its Association with Preterm Delivery

Pesticides are substances or mixture of substances intended for
preventing, destroying, repelling or mitigating any pest. Pesticides have contributed greatly to the increase of yields in agriculture to fulfill the need of increasing population by controlling pests and also have been used in public health program for checking the insect-borne diseases such as malaria, dengue, encephalitis, filariasis.

OCPs are chemically stable, lipophilic in nature, and have a long half life and are very slowly degradable in nature. OCPs are still detected in ecosystem i.e., in water, soil, air, food items etc. and are biomagnified through food chain [13]. Increasing OCPs residues have been found in different human samples such as placental tissues, blood, amniotic fluid, semen, breast milk, etc. [8,12,14,15] and have wide range of acute and chronic health effects. The exposure to pesticides both occupationally and environmentally has been found to be associated with several human health related problems like hormone disruption, immune suppression, adverse reproductive outcomes, cancers, neurological disorder etc. [8,16,17].

Pesticides (Global Scenario)

Approximately 5.6 billion pounds of pesticides are used worldwide. As a result, millions of people worldwide experience unintentional pesticide exposure each year. It has been suggested that 80% of all pesticides used in developed countries and two-thirds of the pesticides produced around the world are used in the USA, Canada and Japan. France and China are the fourth and fifth largest pesticide market respectively with annual consumption worth around US$ 2Billion/year. Pesticide use in China is growing at the rate of five percent annually and it is projected to become the fourth largest consumer of pesticides within a decade. Globally herbicides are the leading pesticide of choice followed by insecticides and fungicides [18]. Biological monitoring studies indicate that pesticide exposure is widespread in pregnant women in New York City, Salinas Valley in California, the Netherlands, and Norway [19,20]. The amount of pesticides used internationally has risen fifty-fold since 1950. The recent study by [21,22] has reported the presence of DDE in 100% of the samples, with a median concentration of 19.7 ng/mL (4788.7 ng/g lipid), while DDT was detected in 3 samples (4.3%). Further, they have reported that serum DDE concentrations were associated with time of residence in the study area, personal hygiene after work, and body mass index in adjusted multivariable logistic regression models with tertiles of area, personal hygiene after work, and body mass index as independent variables. Interestingly, in the same study participants who did not take a daily bath after work showed an OR of 3 for the 2nd tertile and an OR of 6.3 for the 3rd tertile of exposure to DDE.

Results have also revealed high levels of exposure to DDE which might be derived from a heavily polluted local environment and past occupational exposure.

Considering the higher percentage of body fat in women, the storage of these toxins is of great in them [23]. Owing to their xenoestrogenic nature, OCPs disturb the normal estrogen–progestrone balance, which is important in the maintaining the pregnancy [5,6,9,24]. Moreover, during pregnancy, concern over OCPs residue levels in maternal and cord blood becomes greater since studies have revealed that mother and fetus are more vulnerable to the toxic effects of these OCPs [9].

Pesticides and their Usage in India

Hexachlorocyclohexane (HCH)

HCH known as Benzene Hexachloride (BHC) is a synthetic chemical that exists in eight chemical forms called isomers. The different isomers are named according to the position of the hydrogen atoms in the structure of the chemical. One of these forms, gamma-

HCH (or γ-HCH, commonly called lindane), is produced and used as an insecticide on fruit, vegetables, and forest crops, and animals and animal premises. Moreover, it is also available as a prescription medicine (as cream, lotion, shampoo) to treat scabies (mites) and head louse in humans. γ-HCH alters the level of thyroid, pituitary and sex hormones in females and also suppresses follicle stimulating hormones and transforming growth factor β-1 stimulated progesterone production. It is also a potent carcinogen. Environmental Protection Agency (EPA) advises that level of γ-HCH in drinking water for adult humans should not be more than 0.0002 mg/litre, for lifetime. WHO recommended permissible levels of γ-HCH are 0.02 mg/mL in whole blood and 0.025 mg/L in serum samples. γ-HCH has also been found to inhibit Cyclodextrins (CD) and CYP enzymes and Steroidogenic Acute Regulatory protein (StAR) expression in cultured rat granulose cells [25]. StAR protein mediates the intra-mitochondrial transfer of cholesterol to the CYP450 enzyme and CYP450 enzyme catalyzes steroid hormone biosynthesis [26]. Impaired level of sex hormone may interfere with the normal pregnancy and can induce fetal abnormalities.

β-HCH is the most persistent isomer of HCH which constitutes 7-12% of technical HCH. β-HCH has been found to be a xenoestrogen in various in vitro and in vivo studies. β-HCH has negligible insecticidal activity but measurable estrogenic effect [27]. β-HCH increases uterine contraction frequency in a concentration-dependent manner in rats [28]. It may be the most toxicologically significant HCH isomer as evidenced by recent reports of its estrogenic effects in mammalian cells, laboratory animals and fish [29,30]. It has been reported that blood levels of β-HCH in ppb (ng/mL) range have the potential of producing estrogenic effects in mice [29]. β-HCH with mixture of other OCPs has the ability to generate an estrogenic microenvironment through Estrogen Receptor (ERα) activation [32]. It produces moderate uterotropic effects in the rodent uterus [29]. Adverse effects of β-HCH were also seen in an in vivo rat bone marrow chromosomal aberration study [31]. These observations lend support to the role of β-HCH in reproductive toxicity and a possible association with preterm labor due to its estrogenicity.

Aldrin and dieldrin

Aldrin and dieldrin are structurally similar OCPs belonging to cyclodiene family and these pesticides are widely used in agriculture and public health program. Under most environmental conditions, aldrin is largely converted via biological and/or abiotic mechanisms to dieldrin, which is significantly more persistent. Because of low water solubility and tendency to bind strongly to soil both aldrin and dieldrin migrate downwards very slowly through soil or into surface or ground water [33].

Data regarding the health effects of dieldrin in humans come from either epidemiological reports of occupational exposure or case reports of accidental poisonings. The proliferative efficiency due to dieldrin at 10 µM was 54.89% that of estradiol, suggesting high estrogenic potential of dieldrin. Earlier it has been reported aldrin and dieldrin are accumulated in the pregnant women on age and dietary habit basis [34].

Endosulfan

Endosulfan is sold as a mixture of two different forms of the same chemical (referred to as α- and β-endosulfan). It has been suggested that exposure to endosulfan is higher for people living near hazardous waste sites. Apart from occupational exposure which has resulted in many poisonings, residues in food and drinking water are widespread globally at sufficiently high levels to constitute a threat to human health [35]. Endosulfan contamination with adipose tissue, placental tissue and...
umbilical cord blood, meaning that the unborn child is exposed, and then re-exposed on birth through breast milk—both exposures taking place at critical periods of development where estrogenic substances can have a profound life-long impact on new born [33].

**Dichlorodiphenyl Trichloroethane (DDT)**

DDT is a pesticide that was once widely used to control insects on agricultural crops and insects that carry diseases like malaria and typhus, but is now used in India to control malaria. Technical-grade DDT is a mixture of three forms, p,p'-DDT (60–80%), o,p'-DDT (15–21%), and p,p'-DDD (trace amounts). Most DDT in the environment is a result of past use. DDT still enters the environment because of its current use in other areas of the world. p,p'-DDE tends to persist for much longer in comparison to the parent compound and is considered a marker of past exposure to DDT.

Earlier studies have reported that p,p'-DDT and/or p,p'-DDE in maternal, umbilical cord blood and serum are associated with PTD [5,6,36,37]. The most comprehensive assessment of the association between exposure to DDT and its metabolite included 2,380 women (361 of whom delivered at preterm) who had participated in the U.S. Collaborative Perinatal Project between 1959 and 1965 [5]. Furthermore, by using a logistic regression analysis of third-trimester maternal serum DDE concentrations, they have found that the odds increased steadily and significantly with increasing concentrations of serum DDE (p<0.0001), with adjusted ORs of 2.5 (95% CI=1.5–4.2) when levels in serum were 45 to 59 µg of DDE/liter and 3.1 (95% CI=1.8–5.4) when levels in serum were ≥60 µg of DDE/liter. The same study failed to find a significant association between either third trimester maternal serum DDT concentrations or the ratios of maternal serum DDT levels to serum DDE levels. In contrast, a logistic regression analysis failed to detect an association between maternal serum DDE levels and/or maternal β-HCH levels (3rd vs.1st tertile, OR=1.8-3.3, p-trend=0.17) [6]. Further, they have also found a dose-response relationship between preterm birth and 1st trimester maternal serum β-HCH levels (3rd vs.1st tertile, OR=1.9, 95% CI 0.9–3.7, p-trend=0.08).

Newborns are exposed to these pesticides through placental transmission as well as breastfeeding [12,35]. In view of the reproductive toxicity of OCPs, their presence in pregnant women and subsequent transfer to the developing fetuses has the potential to hamper growth and development of the baby in the womb [5] have shown that the risk of Preterm Delivery (PTD) increases steadily with increasing concentration of serum DDE levels. Furthermore, [6] have also reported increased risk of PTD among women in the highest tertile of β-HCH values. OCPs are metabolized by Xenobiotic metabolizing enzymes which are divided into two phases, 'Phase I' enzymes, include Cytochrome P-450 and 'Phase II' enzymes mainly include Glutathione S-transferases (GSTs) [41-45]. Earlier studies have reported that polymorphism in xenobiotic metabolizing genes increases the risk of PTD [46,47]. A Review of studies on OCPs and its association with preterm delivery has been summarized in Table 1 and Figure 1.

### Table 1: A review of studies on organochlorine pesticides levels and risk of PTD cases.

| Population/Ethnicity | Sample size | Cases/ Control | Findings of the study | Reference |
|----------------------|-------------|----------------|------------------------|-----------|
| Indian population    | 40          | 15/25          | Higher levels of OCPs were found in PTD cases as compared to controls. | 37        |
| US population        | 2380        | 361/2019       | The adjusted Odds Ratio (OR) of preterm birth increased steadily with increasing concentration of serum DDE. Further, they reported that DDE correlated with increased risk of PTD with the OR of 1.5 to 3.1 for DDE amounts of 15 µg/L or more as compared to less than 15 µg/L. | 5         |
| USA                  | 40          | 20/20          | Median DDE 25 g/L serum Median DDE 1+3 g/L (cases). | 39        |
| Mexican population   | 233         | 100/133        | Dose-response relationship between preterm birth and 1st trimester maternal serum DDE and β-HCH levels (3rd vs. 1st tertile, OR=1.7, 95% CI 0.8–3.3, p-trend=0.17) and (3rd vs. 1st tertile, OR=1.9, 95% CI 0.9–3.7, p-trend=0.08) respectively were reported. | 6         |
| Indian population    | 46          | 23/23          | Maternal and cord blood levels of α-HCH, β-HCH, γ-HCH, total-HCH, p,p′DDE and p,p′DDE were found higher in preterm labor cases than term labor. However, a statistically significant relation was observed between preterm birth and β-HCH levels only. | 9         |
| European population  | 1322        | -              | Shortening of gestational age of -0.2 week per unit increase in p,p′-DDE concentration was observed. | 17        |
| San Francisco popula-| 420         | -              | Binary logistic regression analysis failed to detect an association between preterm birth and maternal serum concentrations of DDE or DDT or the DDT/DDDE ratio among 420 women who had participated in the Child Health and Development Studies of the San Francisco Bay Area from 1959 to 1967. | 38        |
| Indian population    | 307         | 157/151        | After adjustment of the confounding factors education, baby weight, drinking water, residential area and maternal age the levels of α-HCH, γ-HCH, p,p′-DDEE in maternal blood and α,p′-DDT and p,p′-DDEE in cord blood of PTD cases as compared to term delivery. | 85        |
Genetic Susceptibility to Preterm Delivery

Genomes are 99.9% identical in any two unrelated individual. Variation in individuals is brought due to differences in DNA sequences and structure. Specific sequences variation more than 1% which occurs in individual of any population is referred as polymorphisms. Commonly 10% or more is generally observed in any populations. A polymorphism may be functional or silent. In functional polymorphism stability, level of expression and catalytic function are altered in resulting protein. Functional polymorphisms have (1) SNPs in coding regions of genes resulting in amino acid substitutions, which may alter catalytic activity, enzyme stability, and/or substrate specificity (2) SNPs in the non-coding regulatory regions of genes, which affect their transcription and translation, and in turn the amount of protein expression (3) duplicated or multi-duplicated genes, resulting in higher gene product levels (4) completely or partially deleted genes, resulting in no gene product and (5) splice site variants that result in truncated or alternatively spliced protein products [48].

Figure 2: A possible gene environment interaction mediated pathway in the risk development of preterm delivery.

Women who deliver prematurely are at a greater risk of PTD during their subsequent pregnancies. A woman who herself was delivered preterm is more likely to experience spontaneous preterm labor and preterm birth [4]. Several epidemiological evidences indicate that genetic factors play a significant role in the etiology of spontaneous PTD [49-51]. A number of candidate gene studies, almost exclusively using case-control design, have identified some genes that are associated with PTD [52-54]. CYP450-dependent monoxygenases such as CYP1A1 and CYP1B1 genes are major phase-I enzymes, while mu 1 (GSTM1) and 01 (GSTM1) are the major phase II enzymes, which are responsible for oxidative metabolism/detoxification of xenobiotics such as OCPs [55-57]. In our previous studies, we have reported metabolic genes, such as GSTM1, GSTT1, are highly polymorphic in north Indian women and are genetic factors that confer susceptibility to preterm delivery [58].

CYP1A1 polymorphisms cause variation in activity of its enzymes, altering the metabolism of xenobiotics and steroid hormones [59]. The polymorphism in GSTM1 and GSTT1 gene loci is caused by deletion which results in absence of enzyme activity, especially in individuals with null genotypes [60]. These oxidative stress-related genes such as CYP450 and GST genes are good candidate preterm delivery susceptibility genes [46,47,61]. Xenobiotic metabolizing gene polymorphism may cause improper metabolism of OCPs and thus increased free radical generation which may lead to PTD.

Genes encoding enzymes that are involved in the bio-activation and detoxification of environmental chemicals have been reported to have various polymorphic forms. Polymorphism in xenobiotic metabolizing enzyme, e.g., in the CYP 450 and GST enzyme system, is suspected to influence susceptibility to other environmental factors, affecting the risk of PTD [46,62]. Xenobiotics are known to induce changes in enzyme, e.g., in the CYP 450 and GST enzyme system, is suspected to influence susceptibility to other environmental factors, affecting the risk of PTD [46,62]. Xenobiotics are known to induce changes in cell metabolism/ detoxification of xenobiotics such as OCPs [55-57]. In our previous studies, we have reported metabolic genes, such as GSTM1, GSTT1, are highly polymorphic in north Indian women and are genetic factors that confer susceptibility to preterm delivery [58].

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Xenobiotic Metabolizing Enzymes and Risk of PTD

Phase I metabolizing enzymes (Cytochrome P 450 enzymes)

Human cytochrome P450 (abbreviated CYP, P450, CYP450 or infrequently as CYPs) is a diverse family of enzymes. Their ability to serve as terminal oxidase in steroid hormones [63] and xenobiotics metabolism [64]. These observations were followed by the identification of numerous cytochrome P450 enzymes [65]. Currently, around fifty seven human P450 genes (functional) and twenty nine pseudogenes (nonfunctional) have been identified [66]. Cytochromes P450 metabolize a plethora of both exogenous and endogenous compounds.
Cytchrome P450 enzymes are heme-containing enzymes. The heme iron in cytochrome P450 is present in the ferric (Fe³⁺) state which reduces to the ferrous (Fe²⁺) state during ligands binding. Cytochrome P450 enzymes catalyze mono-oxygenation reaction in which one atom of oxygen is incorporated into a substrate (RH). General reaction catalyzed by cytochrome P450 enzymes is:

\[ O_2 + 2e^- + RH + 2H^+ \rightarrow H_2O + ROH \]

The earliest P450s were believed to metabolize steroids (especially steroidal hormone) and fatty acids. The CYP450 enzymes mainly are classified into P450 families, namely CYP1, CYP2 and CYP3, CYP17 and CYP19 (a new family is also reported named as CYP4 and it is encoded in liver microsome), the substrates of which include several fatty acids, eicosanoids and few xenobiotics. Due to the role of CYP450 enzymes in metabolism of several xenobiotics, carcinogens and endogenous hormones any variation in these genes might be associated with health risks. Polymorphisms in these family of genes is well known and leads to variations in enzyme activity hence these polymorphisms can be associated with increased risk of a number of human disorders including preterm delivery. Although this group of xenobiotic-metabolizing enzymes has been extensively studied in the fields of toxicology and pharmacology, their importance in the area of adverse reproductive outcomes needs to be explored. CYP genes polymorphism displays parallelism in racial, ethnic and geographical distribution and the ethnic-specific effect of CYP genes is well known. Indian population is a major distinct ethnic group representing 1/6 of the total world population.

**Cytochrome P 450 1A1 (CYP1A1):** CYP1A1 is a major gene of CYP450 family; gene encoding CYP1A1 enzyme has been localized at chromosome 15 and contains seven exons [66]. Complete gene spans 6311 base pairs [67]. Various studies have reported the CYP1A1 is a candidate gene for preterm delivery [46,47]. CYP1A1 also known as aryl hydrocarbon hydroxylase is an important member of cytochrome P450 enzyme family and expressed in various organs of our body including liver, placenta, kidney, etc. It is involved in the metabolism of relatively large flat structured aromatic hydrocarbons including dimethyl-benz-a-anthracene and nitrosoyrene and a number of persistent organochlorines.

CYP1A1 gene coding region consists of four functional polymorphisms. CYP1A1 m1 3801 T→C at the MSP1 site in the 3’ untranslated region (rs464903) located downstream of exon-7 [68]. It does not exert any effect on CYP1A1 induction but possibly increases the microsomal enzymatic activity [69,70]. CYP1A1 m2 2455 A→G transition leads to an amino-acid substitution of Ile462Val (rs1048943) in exon 7 [68] and is significantly associated with CYP1A1 inducibility [69,70]. CYP1A1 m3,3205T→C located on 3’ non coding region i.e., upstream of polyadenylation site in exon 7 and is reported to be African-American specific and CYP1A1 m4, 2453 C→A, in exon 7 causing Thr461Asn (rs1799814) is located 2 bp upstream of m2 site. However, effect of this polymorphism on enzyme activity is not yet clearly elucidated. All four functional polymorphisms as mentioned above, allele frequency of CYP1A1 m1 and CYP1A1m2 polymorphic variants are eight to eighteen times higher in Asian than in Caucasian population [71].

Polymorphic variants of CYP1A1 enzyme when elicited by persistent organochlorine causes over expression of enzyme and has been shown to be associated with free radical generation resulting in oxidative stress. The high levels of free radicals may cause severe damage to crucial molecules which lead to PTD.

**Phase II metabolizing enzymes (Glutathione S-transferases)**

Phase II enzymes are involved in conjugation of phase-I products with glutathione making them hydrophilic, completing the detoxification cycle leading to excretion of metabolized compounds. Human GSTs (GST-EC 2.5.1.18) are ubiquitous multifunctional enzymes of phase II xenobiotic metabolizing enzymes family [27]. They are involved in the conjugation of phase I metabolites with glutathione, rendering the products more hydrophilic leading to their elimination. For this reason these enzymes are crucially involved in the protection of cellular macromolecules from free radical damage [72]. In Humans GSTs isoenzymes share upto 65% homology, and have been grouped into eight families based on sequence homology, designated as GSTA (alpha), GSTT (theta), GSTM (mu), GSTK (kappa), GSTO (omega), GSTP (pi), GSTS (sigma), and GSTZ (zeta) [73,74].

Two genes encode the cytosolic enzymes GSTM1 (chromosome 1p13.3) and GSTT1 (chromosome 22q11.2). These enzymes catalyze the addition (conjugation) of aliphatic aromatic heterocyclic radicals, epoxides, or arene oxides to glutathione. Epidemiological studies showed that GSTM1 and GSTT1 deficiency caused by homozygous deletion of the respective genes confers an increased risk of PTD [47]. Null genotypes of the GSTM1 and GSTT1 genes have been reported in approximately 50% and 20% of the Caucasian population and other racial groups, respectively [75,76].

**Glutathione S-transferase (GSTM1):** Human GST mu family of enzymes (GSTM) are organized in a 100 kb gene cluster on chromosome 1p13.3 in the order: 5’-GSTM4-GSTM1-GSTM5-GSTM3-3’ and are known to be highly polymorphic. GSTM1 gene is ~6kb long with a total of 8 exons. GSTM1 enzyme is located in the cytoplasm. GSTM1 deletion can modify/afecct an individual's susceptibility to xenobiotics and toxins as well as affect the toxicity and efficacy of certain drugs [77]. This gene has been of special interest in molecular epidemiological studies since up to half of individuals tested in Caucasian population were found to have homozygous gene deletion polymorphism of GSTM1 which leads to the complete lack of the enzyme activity, which can vary from 30-70% depending upon ethnic group [78]. In Asian populations frequency of the null genotype is similar to Caucasians where as lower in African i.e., 30% [79].

The GSTM1 genotype plays an essential role in detoxification metabolism as a phase II enzyme for metabolism of exogenous chemicals including Polycyclic Aromatic Hydrocarbons (PAHs) and chlorinated compounds [62,80]. Due to absence of GSTM1 genotype DNA adduct level being increased. The effect increased tremendously when null genotypes persons were exposed to xenobiotics such PAHs, OCPS and leads to a high risk of toxicity which also increase the risk of toxicity [81,82].

**Glutathione S-transferase (GSTT1):** GSTT1 and GSTT2 is subfamily of human GSTT1, in which GSTT1 produce homodimeric enzyme of 239 amino acids by single gene [60]. It is approximately 8 kb in length and includes 5 exons [83]. Like GSTM1, the GSTT1 locus has a homozygous deleted allele, namely GSTT1 deletion, resulting in the complete lack of enzyme activity [60]. The consequence of the null genotype is involved in reduced conjugation activity and, in most cases, an inability to efficiently eliminate electrophilic and reactive carcinogens [60]. The frequency of the GSTT1 deletion varies among different ethnic group [84]. The prevalence of the GSTT1 deletion is higher among Asians (20-40%) in comparison to Caucasians (10-20%). The GSTT1 gene is located on chromosome number 22 (22q11.23). GSTT1 is claimed to have an important role in adverse reproductive
The disease occurrence in terms of natural history, severity, etiologic factors in preterm birth. This can result in major improvements in determining complex traits like PTD, FGR as well as recurrent miscarriage [8,62,89]. In Korean woman during third trimester CYP1A1-1462V as well as GSTM1 genotype and higher level of PM10 brings significant reduction in POG when causes PTD [62]. Report also shows that there is correlation between low level of exposure to benzene, shortened gestation period and polymorphisms with susceptible gene [46]. There is significant high risk of PTD in women, who smoke and carrying genotype CYP1A1 along with GSTT1 [90]. It has also been suggested that there is increased risk of disease like lung, prostate cancer due to exposure of harmful chemicals like OCP and GST null genotype interactions [17,91]. Recent study, from our laboratory has shown that the Gene environment interaction of GSTM1/GSTT1 gene with OCPs decreased the period of gestation. Mustafa et al. [85] have shown relationship between β-HCH, dieldrin, and GSTM1, CYP1A1m2 genotype which leads to reduction in the POG. In that study when GSTM1 genotype absent, there is increasing level of β-HCH in maternal blood results in a significant interaction between β-HCH, dieldrin, and GSTM1 - and GSTT1 gene

| Genes | Population/ Ethnicity | Sample size | Cases/ Controls | Findings of the studies | Reference |
|-------|-----------------------|-------------|----------------|-------------------------|-----------|
| CYPP450A1m1 | Korian Papulatin | 235 | 117/118 | No significant association was observed with PTD | 62 |
| CYPP450A1m2 | Korian Papulatin | 235 | 117/118 | No significant association was observed with PTD | 62 |
| CYPP450A1m1m2 | Chinese population | 542 | 302/240 | CYP1A1m1 was significantly associated with PTD | 46 |
| CYPP450A1m1 | Korian Papulatin | 265 | 145/120 | Significantly associated with PTD | 62 |
| CYPP450A1m1m2 | Korian Papulatin | 265 | 145/120 | No significant association was observed with PTD | 62 |
| GSTM1/GSTT1 | Korian population | 265 | 145/120 | Significantly associated with GSTM1 but not with GSTT1 | 62 |
| GSTM1/GSTT1 | American population | 955 | - | GSTT1 was significantly associated with PTD | 61 |
| CYP1A1 | Chinese population | - | 198/524 | CYP1A1 and GSTs together, but not by any single genotype | 22 |
| GSTT1 | Chinese population | - | 198/524 | CYP1A1 and GSTs together, but not by any single genotype | 22 |
| GSTM1, GSTT1, CYP1A1, CYP1B1 | Indian population | 307 | 151/157 | GSTM1/GSTT1 null, CYP1B1’2 mutant and CYP1B1’3 and CYP1B1’7 heterozygous genotype was significantly associated with PTD. | 85 |

Table 2: A review of studies on xenobiotics metabolizing gene polymorphism and susceptibility to preterm delivery.
Several studies suggest that gene-environment interactions, such as interactions between inflammatory gene alleles and bacterial infections also influence this disorder. Together, these studies imply that the etiology likely involves genetic as well as environmental factors in complex interactions. Gene environment interaction reflects the complex interaction between an individual genetic makeup and environment agents. This explains why some individuals have fairly low risk of developing diseases as a result of environmental insults while others are much more susceptible. A review of studies on Gene environment interaction and risk of preterm delivery in the different population has been summarized in the Table 3.

### Correlation between OCPs Level, Inflammatory and Antioxidant Gene Expression

OCPs are reported to be associated with the mRNA expression of inflammatory and xenobiotic metabolizing genes [86] reported that o,p′-DDT dose dependently increases the COX-2 gene expression in in-vitro models. Similarly [87], also reported that environmental pollutants, like diesel particles, induces COX-2 gene expression which cause pulmonary inflammation and leads to conditions like asthma [88], have reported that diabetic rat models receiving Hyperbaric Oxygen (HBO) treatment have significantly increased levels of Reactive Oxygen Species (ROS) and decreased mRNA expression of Cu-Zn SOD and CAT gene, when compared to diabetic control group (without HBO treatment). Foreign chemicals like particulate matter have been reported to induce IL-6 secretion via Reactive Oxygen Species (ROS) and decreased mRNA expression of Cu-Zn SOD [88], have reported that diabetic rat models receiving Hyperbaric Oxygen (HBO) treatment. On the other side, DDT and DDE decreased PGE2 secretion from endometrial cells. The opposite effect of DDT and its metabolite on PGF2α secretion 

![Image of gene-environment interaction](image-url)

**Figure 3:** Gene and environment interaction in the development of complex disorders.

![Image of endometrial cells](image-url)

**Figure 4:** Hypothesis of regulation of prostaglandin production by OCPs via COX-gene expression leading to PTD.

| Population/Ethnicity     | Sample size | Cases/ Controls | Findings of the study                                                                 | Reference |
|--------------------------|-------------|-----------------|--------------------------------------------------------------------------------------|-----------|
| Korean population        | 265         | 145/120         | Exposure to high levels of PM10 during the third trimester in the presence of GSTM1 null genotype is significantly associated with the risk of PTD. | 62        |
| Chinese population       | 542         | 302/240         | A significant decrease in POG was reported among mothers exposed with benzene and CYP1A1 AA-GSTT1 absent. | 75        |
| Chinese population       | -           | -               | Significant joint association of maternal smoking and CYP1A1 (Aa/aa) and GSTT1 null genotype, increases the risk of preterm birth by 5.8 times. | 48        |
| Indian population        | 307         | 157/151         | The interaction between high OCPs levels and polymorphism in CYP1A1m2 and GSTM1 null genotypes may magnify the risk of PTD. | 85        |

**Table 3:** A review of studies on gene environment interaction and risk of preterm delivery.
membrane rapture and finally the early delivery. Progesterone is given to such pregnant women to delay the period of gestation. β-methasone is given when the women in under early pregnancy labor to inhibit the COX-2 gene expression as well as lung maturation of the fetus. PG is synthesized from arachidonic acid under the control of COX-2 gene. Studies have shown that OCPs effect the COX-2 gene expression in dose dependently manner. Therefore, high OCPs level may increase the prostaglandin synthesis and ultimately PTD.

Future Prospects and Research Needs

The problem of multiple chemical exposures is not confined to India. People throughout the world are exposed to similar or even worse conditions of environmental contamination. Besides, the rate of PTD remains high throughout the world and most cases PTD occurring in general population cannot be readily explained by any of the known or suspected risk factor. It will be therefore interesting to ascertain whether genetic susceptibility to pesticides increases the risk of PTD and such gene environment interaction studies would help to shed light on the patho-physiology of PTD, possibly leading to better strategies for preventive diagnosis and treatment.

Our review stresses generation of epidemiological data and establishment of relative risk/relationship between the incidence of preterm births and mother’s exposure to pesticides, with special reference to organochlorine pesticides. This review will lead to the identification of specific expression profiles of genes viz. molecular signature, which will help in understanding the mechanism of pesticide induced toxicity in preterm birth. This will help us to extend these molecular expression profiles to screen individuals who are occupationally exposed to pesticides such as farmers, pesticide formulators, sprayers, etc.

One of the major challenges of exploring mechanism and treatment of complex diseases is that neither environment nor purely genetic factors can fully explain the observed estimate of disease incidence and progression. To correctly estimation of risk we must measure genetics and environment together in the same studies. In the present review we have found that the Gene Environment interaction between xenobiotic metabolizing gene and levels of OCPs may lead to reduction the POG and adverse reproductive outcomes especially preterm birth were extensively searched. Further, most of the articles were searched through PubMed, Scopus Authors search, Google Scholar search tools etc.

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