Original Research

Polymorphism of xenobiotic metabolizing gene and susceptibility of epithelial ovarian cancer with reference to organochlorine pesticides exposure

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Impact statement

Among pervasive environmental toxins, OCPs are one of the largest and most hazardous classes of contaminants in use around the world. Because these compounds possess the estrogen mimicking properties, the accumulation of these compounds in the human body may be a risk for several hormone-dependent diseases. EOC is hormonally dependent cancer and the mortality rate due to this disease is increasingly prevalent, and it has become imperative to explore the role of OCPs in the disease pathogenesis. The present study highlights the significant association of high OCPs level in the disease pathogenesis. It is also observed that the polymorphism in xenobiotic metabolism enzymes alters the predisposition of OCPs. The synergistic effect of gene polymorphism (CYP1A1, GSTM1, and GSTT1) and non-occupational exposure to OCPs was also assessed considering CA125 level as dependent variable with the risk of EOC and concluded that there exist a potential role of genotypic/environmental interaction in the etiology of EOC.

Abstract

The transformation of ovarian surface epithelial cells, stromal cells, sex cord, or germ cells initiates ovarian malignancy. Epithelial ovarian cancer (EOC) is clinically silent with vague, non-specific symptomatology and is generally diagnosed at an advanced stage, resulting in a high mortality rate. The known main etiological factors are: age at menarche and menopause (early menarche or late menopause), use of oral contraception (estrogen and/or progesterone), family history, genetic factors, inflammation, occupational and environmental exposure. The study is intended to assess the association between blood organochlorine pesticide (OCP) levels and polymorphic status of phase I and phase II metabolizing enzymes (CYP1A1, GSTM1, and GSTT1) in the pathogenesis of epithelial ovarian cancer. The study included 200 subjects in total, of which 100 were epithelial ovarian cancer cases and 100 were controls. Estimation of blood organochlorine pesticide levels was carried out using gas chromatography and significantly high levels of beta-hexachlorocyclohexane (β-HCH), endosulfan-I, endosulfan-II, dichlorodiphenyltrichloroethane (p′p′-DDT), dichlorodiphenyldichloroethylene (p′p′-DDE) were observed in cases as compared to controls (P-value = 0.029, 0.042, 0.044, 0.039 and 0.037 respectively). For studying the polymorphism of CYP1A1, GSTM1/T1, PCR-RFLP, AS-PCR and multiplexing were performed and the frequency of null deletion of GSTM1/T1 was significantly higher in epithelial ovarian cancer cases. Regression model testing was also performed to check the interactive effect of organochlorine pesticide levels and polymorphic variant of genes keeping CA-125 as the dependent variable and observed a statistically significant role of genotypic/environmental interaction in epithelial ovarian cancer cases in the North Indian population.

Keywords: Epithelial ovarian cancer, organochlorine pesticide, risk assessment, xenobiotic metabolizing enzymes, CYP1A1, GSTM1, GSTT1

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Introduction

The transformation of ovarian surface epithelial cells, stromal cells, sex cord, or germ cells initiates ovarian malignancy. EOC is increasingly prevalent in pre-and post-menopausal women and is more prevalent than germinal or embryonal ovarian malignancies commonly

observed in young age women.[1] EOC is clinically silent with vague, non-specific symptomatology and is generally diagnosed at an advanced stage, resulting in a high mortality rate. The known main etiological factors are: age at menarche and menopause (early menarche or late menopause), use of oral contraception (estrogen and/or progesterone), family history, genetic factors, inflammation, occupational and environmental exposure. The numbers of man-made environmental toxins are increasing and many of them pose credible health risk. Among pervasive organic environmental toxins, pesticides are one of the largest and most hazardous classes of contaminants in use worldwide.[2] It is estimated that around 85–90% of all the pesticides used in agriculture never reach their target organism, but instead are dispersed throughout the environment, affecting off-target species, including humans.[3]

Evidence indicates that the genes whose polymorphic variants are responsible for impaired ability to metabolize environmental carcinogens could affect an individual's cancer susceptibility.[4] Individual susceptibility to cancer due to environment toxin exposure may be influenced by polymorphism of metabolic susceptible enzymes gene families and identification of these risk modifier genes is critical to define high-risk genotypes.[5] These enzymes are responsible for the metabolism of diverse range of xenobiotics, carcinogens, drugs, steroidal hormone, and toxins. Phase I includes Cytochrome P-450 (CYP450) system and Phase II includes glutathione S-transferase (GST).[5–8]

CYP450 is a diverse superfamily of monooxygenase hemoenzymes responsible for oxidative metabolism by oxidation of its numerous exogenous and endogenous substrates. CYP1A1 is one of the Phase I xenobiotic metabolizing enzymes amenable for the metabolism of various carcinogens and steroidal hormones including estradiol.[8,9] Polymorphism in this gene may alter its activity and could affect the metabolism of carcinogens and steroidal hormones. In contrast, GSTs are the important phase II xenobiotic metabolizing enzymes involved in the conjugation of reactive chemical intermediates activated by phase I CYP450 enzymes and perform a significant role in detoxification and subsequently in the excretion of these compounds. GSTM1 and GSTT1 are two important genes in the GST family and are known to be polymorphically distributed in humans. These enzymes are involved in conjugation of electrophilic intermediates of xenobiotics and a variety of other substrates with glutathione, rendering the products more water-soluble and therefore, protect cellular macromolecules (DNA and protein) from free radical damage. Homozygous deletions in both of these genes are responsible for phenotypic absence of enzyme activity.[10,11]Variation/deletion amongst these genes may subject individuals to increased cancer susceptibility throughout their lifetime.[4,12] A numbers of studies have analyzed the role of GSTM1 and GSTT1 genes in increased risk of numerous cancers including ovarian cancer but the results are inconsistent. In some populations/ethnic groups, deletions of these GSTs confer an increased risk, while in others no profound risk has been reported.[4,12–15] Keeping in view the above facts, the present study is intended to assess correlation between gene polymorphism (CYP1A1, GSTM1, GSTT1), OCP levels in Epithelial Ovarian Cancer cases and controls. To estimate the impact of gene polymorphism and OCP levels upon CA-125 blood levels, linear regression modeling will be utilized.

Materials and methods

The study was conducted at Environmental Biochemistry and Molecular Biology Laboratory, Department of Biochemistry in collaboration with Department of Obstetrics and Gynaecology, University College of Medical Sciences associated with GTB Hospital (University of Delhi), Delhi, India. The study was conducted with the approval of Institutional Ethical Committee – Human Research (IEC-HR). In the present study, 100 histological/cytological confirmed cases of EOC with equal number of controls were recruited. For each case, an otherwise healthy woman with non-specific peri-menopausal complaints were taken as control and matched for age, BMI, and other factors. Normal bilateral ovaries were confirmed on trans-vaginal ultrasonography. To reduce the biases, case and control subjects were recruited from the same geographical/residential area.

A venous blood sample (3 mL) was collected in an EDTA vial and kept at 4°C; 1 mL was used for OCP estimation and the remainder of the sample was used for genomic analysis.

Estimation of organochlorine pesticides residue levels

Pesticide residue was extracted by the following methodology.[16] A blood sample (1 mL) was placed in a 100 mL conical flask. Hexane and acetone were added in 2:1 ratio and the flask was kept on the mechanical shaker for 3 to 4 h at room temperature. The clear top hexane layer was collected in a clean flask and the same procedure was repeated twice. The cleanup of the collected solvent was done by USEPA method using florisil (Sigma) column. Sample was allowed to pass through the florisil column and 20 mL of hexane was further added for complete elution. The process was repeated thrice for remaining residues. The entire extract was transferred in a round bottom flask and the hexane was evaporated to concentrate the sample upto 1 mL for further analysis. Quantification of OCP residue was done by Perkin Elmer gas Chromatograph equipped with ^{63}Ni Electron capture detector (Perkin Elmer Pvt. Ltd Singapore).

Genetic polymorphism study

DNA was extracted from whole blood using commercially available Quick gDNA isolation kit (Zymo research, USA). Agarose gel electrophoresis was done to check the quality of genomic DNA. Electrophoretic movement of genomic DNA was analyzed in 0.8% agarose gel at 100 V for 1 h using 0.5X TBE buffer (Tris borate EDTA buffer). For genetic polymorphism of CYP1A1 gene, two polymorphic sites CYP1A1m1 (T6235C) and CYP1A1m2 (−1462 V) were analyzed using PCR-RFLP and AS-PCR as described by Kumar *et al.*[9] Genotyping of GSTM1/GSTT1 deletion was done by the method described by Sharma *et al.*[17]

Statistical analysis

Independent *t*-test was performed to compare the quantitative date and Chi square was used for qualitative data. Binary logistic regression was applied for comparing the genotypic variation of gene. Multiple linear regression was done for assessing the combined effect of genetic and environmental factor on disease risk.

Results

The two groups of the study were age matched which is reflected by the mean age of both the groups which were 45.28 ± 12.23 for cases and 45.64 ± 12.35 for control group. The other demographic factors which were compared (area of residence, socio economic status, education, religion, dietary habit) but none were found to be significantly associated except the source of drinking water. Cases and control patients recruited lived in similar residential areas and have similar lifestyle. Other potential risk factors considered to be relevant for EOC (parity, menstrual history, family history, BMI, and use of oral contraceptive pills) were significantly different between cases and control patients (Table 1). As CA-125 is an established diagnostic as well as prognostic marker for EOC, its level was also taken into consideration for cases and it was observed that serum CA-125 level was significantly elevated among cases with the mean value of 965.71 U/mL as compared to the reference range 0–35 U/mL. Significantly high levels of β-HCH, endosulfan-I, endosulfan-II, p′p′-DDT, p′p′-DDE and heptachlor were found in cases as compared to the controls with the *P*-values = 0.029, 0.042, 0.044, 0.039, 0.037, and 0.001, respectively (Table 2). While comparing the genotypic variation of CYP1A1 m1 and m2, no differences were observed. In contrast, the frequency of null deletion of GSTM1/GSTT1 was statistically significantly in EOC cases compared to controls (Table 3).

To assess the role of gene-environment interaction, multiple linear regression model testing was performed considering CA-125 level as the dependent variable and interaction between gene polymorphism and OCP levels was independent variables. While performing regression model testing, various confounding factors including age, family history, parity, and drinking water source were adjusted. B is unstandardized coefficient and this represents the mean CA125 difference. For this analysis, $P < 0.005$ was considered as statistically significant as the Bonferroni correction was performed due to multiple comparison tests (nine times) and the corrected *P*-value was $0.05/9 = 0.005$. The calculation was done by assuming adjusted co-variants as constant: $CA125 = B1 \times CYP1A1m1 + B2 \times \beta\text{-HCH} + B3 \times CYP1A1 \times \beta\text{-HCH}$, considering β-HCH = 1, CYP1A1m1 = 1; taking B1 = 26.107, B2 = 2.022 and B3 = −5.223 (Table 4). The interaction of CYP1A1m1 genotype and level of β-HCH among case group correlated with mean increase of 22.90 U/mL in CA-125 level. However, no significant increase in CA-125 was observed with CYP1A1m2 (Table 5). The interaction of GSTM1 genotype and level of β-HCH and DDT among cases group correlated with mean increase of 20.98 U/mL and 11.04 U/mL in CA-125 levels, respectively (Table 6). The interaction of GSTT1 genotype and level of β-HCH and DDT among case group correlated with mean increase

Table1. Comparison of various socio-demographic features, risk/protective factors in the two groups.

Characteristics	Cases (*n*=100)	Controls (*n*=100)	*P*-value
Age (in years)	45.28±12.23	45.64±12.35	0.851
Residential area			
Urban	88 (88%)	98 (98%)	0.674
Rural	12 (12%)	2 (2%)	
Socioeconomic status			
Lower	0 (0%)	0 (0%)	0.668
Middle	91 (91%)	93 (93%)	
Higher	9 (9%)	7 (7%)	
Occupation			
Unemployed	100 (100%)	95 (95%)	0.742
Employed	0 (0%)	5 (5%)	
Education			
Illiterate	67 (67%)	71 (71%)	0.538
Literate	33 (33%)	29 (29%)	
Religion			
Hindu	90 (90%)	90 (90%)	0.975
Muslim	10 (10%)	10 (10%)	
Dietary habit			
Vegetarian	68 (68%)	61 (61%)	0.526
Non-vegetarian	32 (32%)	39 (39%)	
Water resource			
Govt. supply	60 (60%)	85 (85%)	**0.040***
Ground water	40 (40%)	15 (15%)	
Living style			
Colony	96 (96%)	95 (95%)	0.786
Industrial area	4 (4%)	5 (5%)	
Parity			
Nullipara	9 (9%)	9 (9%)	0.987
Multipara	91 (91%)	91 (91%)	
Menstrual history			
Early menarche	5 (5%)	3 (3%)	0.813
Normal onset menarche	95(95%)	97 (97%)	
Family history			
Present	4 (4%)	3 (3%)	0.893
Absent	96 (96%)	97 (97%)	
BMI (kg/m^2)	24.78±3.69	24.01±4.53	0.805
Oral contraceptive intake			
Taken	5 (5%)	10 (10%)	0.600
Not taken	95 (95%)	90 (90%)	

*P-value ≤ 0.05 is considered as significant.

Table 2. Estimation of organochlorine pesticide (OCP) levels in cases and controls.

OCPs (ng/mL)	Cases (*n*=100) mean ± S.D	Controls (*n*=100) mean ± S.D	*P*-value
α-HCH	6.89±3.66	6.46±2.54	0.289
β-HCH	5.47±2.34	3.65±1.72	**0.029***
γ-HCH	4.44±2.32	4.31±2.55	0.356
Dieldrin	1.96±0.76	1.85±1.33	0.329
Endosulfan-I	2.01±1.52	1.52±0.91	**0.044***
Endosulfan-II	2.78±1.21	1.32±0.77	**0.042***
p′p′-DDT	1.79±1.22	0.98±0.32	**0.039***
p′p′-DDE	1.89±1.22	0.82±0.36	**0.037***
Heptachlor	5.99±2.41	3.10±1.21	**0.001***

*P-value ≤ 0.05 is considered as significant.

Table 3. Genotypic distribution of CYP1A1 m1 and m2 and GSTM1/GSTT1 genotypes among EOC cases and controls.

Genotypes	Cases ($n=100$)	Controls ($n=100$)	Odd ratio (OR)	95% CI	*P*-value
CYP1A1m1					
wt/wt	26 (26%)	34 (34%)	Ref.		
wt/mt	56 (56%)	52 (52%)	1.408	0.746–2.657	0.291
mt/mt	18 (18%)	14 (14%)	1.681	0.708–3.994	0.239
CYP1A1m2					
wt/wt	27 (27%)	35 (35%)	Ref.		
wt/mt	55 (55%)	50 (50%)	1.426	0.758–2.681	0.271
mt/mt	18 (18%)	15 (15%)	1.556	0.665–3.637	0.308
GSTM1/GSTT1					
GSTM1+/GSTT1+	33 (33%)	47 (47%)	Ref.		
GSTM1+/GSTT1−	26 (26%)	23 (23%)	1.610	0.787–3.295	0.192
GSTM1−/GSTT1+	22 (22%)	21 (21%)	1.492	0.708–3.144	0.293
GSTM1−/GSTT1−	19 (19%)	9 (9%)	3.007	1.211–7.466	**0.018***

Ref.: Reference, $*P < 0.05$ significant.

of 22.12 U/mL and 11.05 U/mL in CA-125 levels, respectively (Table 7).

Discussion

It has been observed that the prevalence of EOC is significantly higher in that group who were using ground water as a direct source of their drinking water (Table 1) which is one of the possible routes of pesticide exposure to the population. This finding is in consistent with the other reports in which ground water of Delhi and nearby region was tested and found the presence of pesticide contaminant in significant level.[16,18]

The results show that there is significant high levels of pesticide (β-HCH, endosulfan-I, endosulfan-II, p′p′-DDT, p′p′-DDE and heptachlor) among cases group as compared to controls (Table 2) which is in consistent with one of our earlier published study in which high levels of pesticides were reported among EOC subjects.[16] The possible reason for this finding may explain by the earlier study in which it has been found that there is maximum traceability of OCPs in water samples of Yamuna river and its canals in Delhi and nearby states. Moreover, these compounds are strongly lipophilic in nature with slow degradation rate and due to this property these become persistent in environment once entered.[16,19,20]

CYP450 and GST are xenobiotic metabolizing enzymes which have a key role in the vital detoxification process. These genes have reported to be polymorphically distributed amongst distinct human ethnic population. Functional polymorphisms affect enzymatic activity of the corresponding enzyme and can potentially alter the metabolism of harmful substances which may cumulatively promote disease throughout a person lifetime. Moreover, polymorphisms show ethnicity-dependent frequency distribution; therefore, risk of developing disorders/diseases in a population might depend upon frequencies of some of these high-risk alleles. There is growing evidence that among the risk factors that have been hypothesized to be associated with EOC, genetic predisposition plays a significant role. In Table 3, the genotypic variations were compared statistically. For CYP1A1 m1 and m2, the frequency of heterozygous and homozygous mutant type was found to be higher among EOC cases compared to that of controls. These observations are in agreement with earlier studies[21,22] where the authors have mentioned about the risk of EOC due to the polymorphic variation in CYP1A1 gene but no statistically significant results were demonstrable when polymorphic variants of CYP1A1 m1 and m2 (CYP1A1 MspI and Ile/Val) were checked. In contrast, another study[23] has shown a positive significant association between the polymorphic variant of CYP1A1 (Ile/Val) with increased risk of EOC. One of the possible reasons for this finding may be the ethnicity-related differences among the different population which may determine the susceptibility of an individual. As per the earlier studies, genes which are associated with metabolic detoxification pathway (CYP1A1, GSTM1 and GSTT1) are found to be associated as risk factors for EOC.[24] Polymorphic variation in CYP genes may be associated with increased toxification process, whereas GST genes found to be associated with impaired detoxification process.[25]

While comparing the GST genotypes, it was observed that the frequency of GSTM1/GSTT1 null deletion was significantly higher among EOC cases and found to be associated with increased disease risk (Table 3). It is an enzyme family which is considered to play a crucial role in cellular protection and oxidative stress due to foreign toxic compounds.[26] Null deletion of GTM1/T1 is an indicator of high risk for disease as null deletion signifies the phenotypic absence of enzyme activity or homozygous deletion of the whole gene. Null deletion of these genes will lead to the impaired catalysis of the conjugate of glutathione and detoxification process of organic peroxidases.[27,28] Moreover, the presence of null deletion may impair the detoxification process for OCPs and may promote oxidative stress and DNA damage contributing to the pathogenesis of EOC.[29,30]

The present study identifies the high-risk allele of CYP1A1, GSTM1, and GSTT1 and their possible association with epithelial ovarian cancer. Moreover, this study also highlights that the presence of variant type of allele may promote the deposition of OCPs throughout the body, thereby promoting OCP-associated disease risks. Here we

Table 4. Regression model testing an interactive effect of OCPs (ppb) and CYP1A1m1 genotype on CA125 in EOC cases.

	Blood sample of EOC cases	
CYP1A1 m1 interaction	**B-value**	***P*-value**
CYP1A1m1 (variants vs. wild type)	3.459	0.627
α-HCH	0.353	0.695
Interaction term of CYP1A1m1-α-HCH levels	−0.301	0.759
CYP1A1m1 (variants vs. wild type)	26.107	0.001
β-HCH	2.022	0.095
Interaction term of CYP1A1m1-β-HCH levels	−5.223	**0.001***
CYP1A1m1 (variants vs. wild type)	−5.868	0.234
Υ-HCH	−1.208	0.179
Interaction term of CYP1A1m1-Υ-HCH levels	1.828	0.096
CYP1A1m1 (variants vs. wild type)	−3.435	0.544
Dieldrin	−3.562	0.172
Interaction term of CYP1A1m1-dieldrin levels	3.046	0.301
CYP1A1m1 (variants vs. wild type)	−6.362	0.135
Endosulfan-I	−0.917	0.546
Interaction term of CYP1A1m1-Endosulfan-I levels	3.671	0.057
CYP1A1m1 (variants vs. wild type)	−7.594	0.335
Endosulfan-II	−1.273	0.740
Interaction term of CYP1A1m1-Endosulfan-II levels	3.344	0.393
CYP1A1m1 (variants vs. wild type)	−4.980	0.322
pp-DDT	−1.696	0.610
Interaction term of CYP1A1m1-ppDDT levels	3.849	0.264
CYP1A1m1 (variants vs. wild type)	−3.932	0.433
ppDDE	3.727	0.116
Interaction term of CYP1A1m1-ppDDE levels	1.395	0.608
CYP1A1m1 (variants vs. wild type)	−7.039	0.246
Heptachlor	−0.507	0.615
Interaction term of CYP1A1m1-Heptachlor levels	1.402	0.204

*P value <0.005 is significant; B: regression coefficient.

Table 5. Regression model testing an interactive effect of OCPs (ppb) and CYP1A1m2 genotype on CA125 in EOC cases.

	Blood sample of EOC cases	
CYP1A1 m2 interaction	**B-value**	***P*-value**
CYP1A1m2 (variants vs. wild type)	−1.00	0.988
α-HCH	−0.220	0.800
Interaction term of CYP1A1m2-α-HCH levels	0.371	0.702
CYP1A1m2 (variants vs. wild type)	−15.229	0.008
β-HCH	−0.356	0.705
Interaction term of CYP1A1m2-β-HCH levels	3.127	0.005
CYP1A1m2 (variants vs. wild type)	1.031	0.825
Υ-HCH	−0.205	0.802
Interaction term of CYP1A1m2-Υ-HCH levels	0.340	0.738
CYP1A1m2 (variants vs. wild type)	−3.650	0.541
Dieldrin	−3.793	0.158
Interaction term of CYP1A1m2-dieldrin levels	3.353	0.263
CYP1A1m2 (variants vs. wild type)	−7.469	0.058
Endosulfan-I	−1.529	0.253
Interaction term of CYP1A1m2-Endosulfan-I levels	4.862	0.006
CYP1A1m2 (variants vs. wild type)	0.205	0.961
Endosulfan-II	1.402	0.234
Interaction term of CYP1A1m2-Endosulfan-II levels	0.466	0.746
CYP1A1m2 (variants vs. wild type)	0.169	0.969
pp-DDT	1.002	0.703
Interaction term of CYP1A1m2-ppDDT levels	0.886	0.747
CYP1A1m2 (variants vs. wild type)	−1.204	0.805
ppDDE	4.318	0.058
Interaction term of CYP1A1m2-ppDDE levels	0.374	0.887
CYP1A1m2 (variants vs. wild type)	−1.652	0.751
Heptachlor	0.149	0.846
Interaction term of CYP1A1m2-Heptachlor levels	0.633	0.464

B: regression coefficient.

Table 6. Regression model testing an interactive effect of OCPs (ppb) and GSTM1 genotype on CA125 in EOC cases.

	Blood sample of EOC cases	
GSTM1 interaction	**B-value**	***P*-value**
GSTM1 (null vs. present type)	−3.692	0.432
α-HCH	0.419	0.329
Interaction term of GSTM1-α-HCH levels	−0.737	0.248
GSTM1 (null vs. present type)	23.449	0.001
β-HCH	3.091	0.001
Interaction term of GSTM1-β-HCH levels	−5.552	**0.001***
GSTM1 (null vs. present type)	1.159	0.784
ϒ-HCH	1.035	0.101
Interaction term of GSTM1-ϒ-HCH levels	−2.172	0.011
GSTM1 (null vs. present type)	2.451	0.575
Dieldrin	1.794	0.221
Interaction term of GSTM1-dieldrin levels	−5.648	0.006
GSTM1 (null vs. present type)	0.034	0.993
Endosulfan-I	2.333	0.060
Interaction term of GSTM1-Endosulfan-I levels	−4.039	0.015
GSTM1 (null vs. present type)	−1.644	0.697
Endosulfan-II	1.627	0.040
Interaction term of GSTM1-Endosulfan-II levels	−2.276	0.101
GSTM1 (null vs. present type)	12.398	0.001
pp-DDT	8.833	0.001
Interaction term of GSTM1-ppDDT levels	−10.187	**0.001***
GSTM1 (null vs. present type)	4.623	0.418
ppDDE	5.991	0.005
Interaction term of GSTM1-ppDDE levels	−5.367	0.033
GSTM1 (null vs. present type)	0.735	0.871
Heptachlor	0.822	0.085
Interaction term of GSTM1-Heptachlor levels	−1.516	0.026

**P* value <0.005 is significant; B: regression coefficient.

Table 7. Regression model testing an interactive effect of OCPs (ppb) and GSTT1 genotype on CA125 in EOC cases.

	Blood sample of EOC cases	
GSTT1 interaction	**B-value**	***P*-value**
GSTT1 (null vs. present type)	−4.257	0.373
α-HCH	0.391	0.359
Interaction term of GSTT1-α-HCH levels	−0.599	0.354
GSTT1 (null vs. present type)	24.871	0.001
β-HCH	3.127	0.001
Interaction term of GSTT1-β-HCH levels	−5.875	**0.001***
GSTT1 (null vs. present type)	1.889	0.661
ϒ-HCH	0.753	0.214
Interaction term of GSTT1-ϒ-HCH levels	−2.331	0.008
GSTT1 (null vs. present type)	3.173	0.468
Dieldrin	1.644	0.258
Interaction term of GSTT1-dieldrin levels	−5.868	0.005
GSTT1 (null vs. present type)	0.877	0.822
Endosulfan-I	2.527	0.040
Interaction term of GSTT1-Endosulfan-I levels	−4.311	0.010
GSTT1 (null vs. present type)	0.197	0.963
Endosulfan-II	1.497	0.052
Interaction term of GSTT1-Endosulfan-II levels	−2.978	0.046
GSTT1 (null vs. present type)	12.433	0.001
pp-DDT	8.869	0.001
Interaction term of GSTT1-ppDDT levels	−10.248	**0.001***
GSTT1 (null vs. present type)	5.376	0.321
ppDDE	6.256	0.002
Interaction term of GSTT1-ppDDE levels	−5.677	0.020
GSTT1 (null vs. present type)	1.107	0.810
Heptachlor	0.711	0.131
Interaction term of GSTT1-Heptachlor levels	−1.581	0.025

**P* value <0.005 is significant; B: regression coefficient.

have taken EOC as our study model as it is one of the hormonally (estrogen) dependent cancers for which OCPs may contribute estrogen mimicking properties.

In the present study, regression model testing was performed to assess the role of gene–environment interaction in the etiology of EOC. For this model testing, CA125 which is a diagnostic as well as prognostic marker for EOC was taken as dependent variable and OCP levels and genotypic variation in genes were taken as independent variables. In other words, the effect on the level of CA125 was checked when variant type of gene is present and high level of OCP was detected.

A significant increase in CA125 level was observed when interaction term was assessed between β-HCH and CYP1A1m1 (Table 4). In other words, when CYP1A1m1 gene is polymorphic, increased level of β-HCH resulted in an estimated increase of 20.90 U/mL in CA125 levels. No significant interaction was observed with CYP1A1 m2 and pesticide levels (Table 5). Significant interaction is also observed between GSTM1 and GSTT1 null genotypes and increasing level of β-HCH and DDT resulting in the increase in CA-125 level. In association with presence of GSTM1 and GSTT1 null genotype and increased level of β-HCH and DDT, mean elevated in CA125 by 20.98 U/mL, 11.04 U/mL, 22.12 U/mL, and 11.05 U/mL, respectively was observed (Tables 6 and 7). These findings are supported by a previously published study in which it was observed that the presence of null genotypes for GSTM1 and T1 and increased levels of pesticides are associated with increased risk of bladder cancer.[17] Other published studies have observed the null genotype of GSTM1 being associated with increased DNA adduct formation when exposure of carcinogens such as polycyclic aromatic hydrocarbons (PAHs) is present.[31,32] The role of gene–environment interaction in the etiopathogenesis of various types of cancer including colon, oro-pharyngeal, and laryngeal cancer in other studies which have noted that the presence of variant type of GSTM1, GSTT1 and ADH (aldehyde dehydrogenase) may increase the risk of developing cancer when the exposure to certain environmental stimuli including PAHs or alcohol is present.[33–35] The present study further supports that there is a plausible role of gene–environment interaction in the etiology of EOC. Moreover, further mechanistic-based studies using *in vitro* and/or animal models should be carried out to validate the present findings. The regression model testing is one of the relevant statistical tools to assess the role of genotype-environmental OCP interaction to date had not been carried out for EOC. The present study is first in its kind where the role of gene–environment interaction is explored for the etiology of EOC using statistical tool which can be used for drug targeting and therapeutic intervention.

Authors' contributions: TS has carried out the experimental work, data interpretation and manuscript writing, GT was involved in experimentation, BDB and KG were involved in concept formation and editing of the manuscript, DM was involved in sample collection.

DECLARATION OF CONFLICTING INTERESTS

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