Polymorphisms and diazinon and malathion levels in the etiology of breastcancer: a case-controlstudy in Mazandaran Province, North of Iran

Polimorfismos y niveles de diazinón y malatión en la etiología del cáncer de mama: un estudio de casos y controles en la provincia de Mazandaran, al norte de Irán

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Abstract

Breast cancer is the first leading cause of cancer-related death inwomen. Xenobiotic-Metabolizing Enzymes (XMEs) contribute to the detoxification of numerous cancer therapy-induced products. In the metabolism of xenobiotics, cytochrome P450s or monooxygenases and Glutathione –s transferase (GSTs) perform an important function by catalyzing the hydroxylation reaction andconjugationofglutathione(GSH) to a wide variety of xenobiotics. Pesticides, which are excessively used in northern Iran, are one of the most-important risk factors for breast cancer incidence. They detoxify by phase I and II enzymes. The aim of this study was to evaluate the association of CYP1A1(rs4646421),CYP1B1(rs1056836),CYP2C8(rs1058930),CYP19A1(rs749292) and GSTP1(rs 1695) polymorphisms and serum levels of pesticides (Diazinon and Malathion) with the risk of breast cancer in Mazandaran province. This cross-sectional case-control study included 72 patients and 51 healthy individuals who were recruited.It wasperformed betweenOctober 2017 to May 2018 in Oncology department at Imam Hospital inSari City.Breast cancer patients with knownclinicopathological characters and healthy women, as control, were genotyped for genes polymorphisms by PCR-RFLP and GC-MS method used for quantification of poisons.Chisquare test, Fisher exact test,and logistic regression model were applied for statistical analysis. The results of the experiments showed that there weresignificant relationships between two groups and the age of the patients wassignificantly higher than the control group(p = 0.044).Regarding the relationship between the genotypes of each gene and breast cancer risk, using a logistic regression model in two normalized and age-adjusted models, it was determined that in CYP2C8 genotype, those havingtheCGallele, increased the risk of breast cancer in adjusted model (CI=95%1.11,75.84). In the CYP19A1gene of individuals with GA genotype, the risk of breast cancer increasedinnon-adjusted model (CI95%=1/52-27/21) about the CYP1B1 gene, people with two genotypes of CG + GGwereassociated with a higher risk of breast in non-adjusted and adjusted model (CI71/5 – 19/1 95% =)(CI=95% 1.31,6.57). In CYP2C8gene,the G allelehada protective effect on breast cancer and decreased the risk of breast cancer (P = 0.02) and in GSTP1gene, theGallele increased the riskof breast cancer(P=0.0480). Moreover,in CYP1B1 gene, G alleledecreased the riskof breast cancer (P=0.0271).Regardingthe serum levels of OPs, Diazinon in the case group hada much lower level than the control groupbut(p<0.001) there was a significant relationship between serum levels of Diazinon and risk of breast cancer (p<0.001). The results of the current studyconfirmed the association between CYP2C8(rs1058930),CYP19A1(rs749292) and CYP1B1(rs1056836)gene polymorphisms and increased the risk of breast cancer.Also,there was a significant relationship between serum levels of Diazinon and risk of breast cancerin women in Mazandaran province.

Keywords: CYP1A1 (rs4646421), CYP1B1 (rs1056836), CYP19A1 (rs749292), CYP2C8(rs1058930), GSTP1 (rs 1695) genes, Diazinon, Malathion, Breast Cancer, Polymorphism, Mazandaran province.

Resumen

Breast cancer is the first leading cause of cancer-related death inwomen. Xenobiotic-Metabolizing Enzymes (XMEs) contribute to the detoxification of numerous cancer therapy-induced products. In the metabolism of xenobiotics, cytochrome P450s or monooxygenases and Glutathione –s transferase (GSTs) perform an important function by catalyzing the hydroxylation reaction andconjugationofglutathione(GSH) to a wide variety of xenobiotics. Pesticides, which are excessively used in northern Iran, are one of the mostimportant risk factors for breast cancer incidence. They detoxify by phase I and II enzymes. The aim of this study was to evaluate the association of CYP1A1(rs4646421),CYP1B1(rs1056836),CYP2C8(rs1058930),CYP19A1(rs749292) and GSTP1(rs 1695) polymorphisms and serum levels of pesticides (Diazinon and Malathion) with the risk of breast cancer in Mazandaran province. This cross-sectional case-control study included 72 patients and 51 healthy individuals who were recruited.It wasperformed betweenOctober 2017 to May 2018 in Oncology department at Imam Hospital inSari City.Breast cancer patients with knownclinicopathological characters and healthy women, as control, were genotyped for genes polymorphisms by PCR-RFLP and GC-MS method used for quantification of poisons.Chisquare test, Fisher exact test,and logistic regression model were applied for statistical analysis. The results of the experiments showed that there weresignificant relationships between two groups and the age of the patients wassignificantly higher than the control group($p = 0.044$).Regarding the relationship between the genotypes of each gene and breast cancer risk, using a logistic regression model in two normalized and age-adjusted models, it was determined that in CYP2C8 genotype, those havingtheCGallele, increased the risk of breast cancer in adjusted model (CI=95%1.11,75.84). In the CYP19A1gene of individuals with GA genotype, the risk of breast cancer increasedinnon-adjusted model (CI95%=1/52-27/21) about the CYP1B1 gene, people with two genotypes of CG + GGwereassociated with a higher risk of breast in non-adjusted and adjusted model (CI71/5 – 19/1 95% =) (CI=95% 1.31,6.57). In CYP2C8gene,the G allelehada protective effect on breast cancer and decreased the risk of breast cancer ($P = 0.02$) and in GSTP1gene, theGallele increased the riskof breast cancer($P=0.0480$).Moreover,in CYP1B1 gene, G alleledecreased the riskof breast cancer ($P=0.0271$).Regardingthe serum levels of OPs, Diazinon in the case group hada much lower level than the control groupbut($p<0.001$) there was a significant relationship between serum levels of Diazinon and risk of breast cancer ($p<0.001$). The results of the current studyconfirmed the association between CYP2C8(rs1058930),CYP19A1(rs749292) and CYP1B1(rs1056836)gene polymorphisms and increased the risk of breast cancer.Also,there was a significant relationship between serum levels of Diazinon and risk of breast cancerin women in Mazandaran province.

Palabras clave: CYP1A1 (rs4646421), CYP1B1 (rs1056836), CYP19A1 (rs749292), CYP2C8 (rs1058930), genes GSTP1 (rs 1695), diazinón, malatión, cáncer de mama, polimorfismo, provincia de Mazandaran.

Introduction

Breast cancer is one of the most common cancers among females worldwideso far and is the leading causeof cancer-related mortality for almost 14% of all cancerdeaths[1]. It is a heterogeneous diseasecaused by interactions of environmental and geneticfactors. About 8500 new cases of breast cancer are reported annually in the country, and 1,400 are reported to die of breast cancer.It is also estimated By 2014, there were about 4 0,000 people living with this disease according to the latest statistics from the Cancer Research Center in Iran[2]. Iranian Breast cancer cases are one decade younger than their western counterparts[3].The etiology of breast cancer iscomplex; a small proportion of breast cancer casescan be attributed purely to genetic reasons whereas for a vast number of cases there is acompelling evidence for the role of other factors, such as familyand reproductive history, diet, alcohol consumption,and exposure to environmental carcinogensas well as genetic factors,breast cancer susceptibility genes,highpenetrance genesBRCA1,BRCA2,PTEN,…) and lowpenetrance genes (CYP450, GSH, UGTA,..) thatencode, xenobiotic metabolizing enzymes in phase I and II of metabolism

Many carcinogenic compounds are oxidized by phase I enzymes, represented by cytochrome P450 family, into reactive metabolites that are detoxified by phase II enzymes. GSTs are a family of Phase II detoxification enzymes that catalyze the conjugation ofglutathione (GSH) to a wide variety of xenobiotics. Thisdetoxification ability plays a role in cellular protectionfrom environmental and oxidative stress, Hence, the toxic effects of exposure, absorption, and detoxification of carcinogens depends on a delicate balance between the phase I and phase II enzyme polymorphisms and expression pattern of these genes are believed to be key factorsin determining cancer susceptibility to toxic orenvironmental chemicals[4]. The term Gene-environment interaction (GEI) refers to the joint influence of genetic and environmental factors on health and disease. Environmental exposures affect gene regulation and/or act asadditive risk factors in conjunction with a particular allelic formof a gene (genetic polymorphism).Such interactions may be important determinantsin cancer[5].

Ophosphate pesticides (OPs), triesters of phosphoric acid, are a widely used group of pesticides. Besides their use in agriculture, disease control and as therapeutic agents, OPs are also used inindustry as solvents, plasticizers, flame retardants and in defenseforces as nerve agents[6,7,44]. OPs inhibit acetylcholinesterase, resulting in chronic harmful effects on human health e.g., neuropsychological disorders, disruption of the endocrine system, developmental anom-

alies, disorders of the immune system andhypersensitivity[8]. Diazinon and Malathion are organophosphate insecticide that arewidely used in gardens and orchards, to control various pests including the Mediterranean fruit, which arecurrently used in agriculture[9]. OPs are metabolized by cytochrome P450s (CYPs) through either a dearylation reaction to form an inactive metabolite (Phosphorothioate), or through a desulfuration reaction to form an active oxon metabolite[10,11,43] which is a potent cholinesterase inhibitor, causing the accumulation of acetylcholine within synapses and the consequent over-stimulation of postsynaptic receptors. Along withphase II enzymes like GST, UGTA,… they excrete[12]. Diazoxon and Malaoxone are the activated forms of diazinon, and malathion respectively, whereas p-nitrophenol (PNP) and pyrimidinol(IMP) represent the detoxified metabolites such asdimethylthiophosphate(DMTP), a detoxication product indeed, mammalian carboxylesterases catalyze rapiddegradation of malathion to mono-acid and di-acid derivatives[13].

Pesticides can significantly damage cell structuresand can interfere with metabolic processes or transport mechanisms. In addition, the poisonous effects of them include the damage to DNA such as changes or losses of nucleotic bases and double and single-strandbreakageof DNA(14).

Cytogenetic alterations in subjects occupationallyexposed to pesticides could be used to obtain informationon genotoxic exposure during the use of pesticides[15] Candidate genes for this study are of low-penetrance breast carcinoma susceptibilityincluding those encoding for Xenobiotic metabolizing enzymes (XMEs) involved in carcinogenmetabolism and detoxification(16).These XMEs can be divided into phase I(Cytochrome P450 family) and phaseII(GST,UGTA,…) enzymes that metabolically activate potentially carcinogenic forms. In this study, polymorphisms of phase I enzymesCYP1A1(rs4646421C/T), CYP2C8(rs1058930C/G),CYP1B1(rs1056836 C/G), CYP19A1(rs749292A/G) and phase II enzyme GSTP1 (rs1695 A/G) were investigated in breast cancer patients who referred to Mazandaran province clinics during October 2017 to May 2018.Moreover,the association between serum levels of the Ops (Diazinon and Malathion) with breast cancer risks in these patients have been analyzed.

CYP1A1 is one of the “phase I” enzymes located on chromosome 15q22–q24, which isa 5987-bp long gene, having 7 exonsand 6 intronsthat encodes for a 512amino acid protein. This is one of the majorcomponents of detoxification pathway that is highlyexpressed in non-hepatic cells such as breast tissue[17]. It is a polymorphic genes being involvedin the metabolism of steroids and severalpotentiallygenotoxicchemicals. In estrogen metabolism, it plays a role in producing 2-hydroxyestrogens.Four single nucleotide polymorphisms (SNP) were identified inCYP1A1 gene including M1, T/C transition atnucleotide 3801; M2, A/G transition at position 2455 resulting in the change of Ile to Val at codon 462; M3T/C transition at nucleotide 3205; and M4 C/Atransition at position 2453 resulting in the change of Thr to Asn at codon 461. The M1 polymorphism at 3’-flanking region (T3801C) was verified to be associated with increased activation of carcinogens[18,19,40]. CYP1B1is located on chromosome 2 at position 2p22.2. The gene is 42930-fold-wide and 60846Damolecular weight. It has 3 exons and 2introns. It is considered as the key enzyme of P450, which is exogenous in metabolism and endogenous substrate. CYP1B1 plays a role in the metabolism of androgens and estrogen substrates. CYP1B1can catalyze 4-hydroxy estrogens, a key reaction to hormonal carcinogenesis.CYP1B1, 5 different SNPs have been identified that can play a role in replacing amino acids A119S, R48G, L432V, A443G, and N453S. Val432Leu polymorphism has recently been identified as the most influential agent on the catalytic properties of CYP1B1 and will also affect the function of 4-hydroxy in the Val32 allele, which indicates a 3-fold increase in activity relative to the Leu432 allele, for the specific CYP1B1 gene rs 163077, 163086 and 162556 involved with invasive breast cancer[20,21,42]. CYP19A1is located on chromosome 15, q21.2, consisting of 18 exons and 17 introns and has a molecular weight of 57883 Da. The enzyme is stagnant, which plays an important role in biosynthesis and the final stages of estrogen biosynthesis. The aromatase is coded by the CYP19A1gene, a key estrogen biosynthesis enzyme and plays an important role in the development of breast cancer. The main effect of this enzyme is in catalyzing of the final stage of estrogen biosynthesis that convertsrostenedione and testosterone into estrogen and estradiol. The direct effect of aromatase on cytotoxicity in the breast is completely reported[22]. A high level of aromatase expression has been reported in breast tumors, which is also visible in the normal breast.CYP2C8 is located on chromosome 10 at position10q23.33consisting of 10 exons and 9 introns. This gene has55825Da molecular weight. CYP2C8 is one of the first human cytochromes, whichplays a role in drug metabolism in cytochrome P450, which can also be used in response to chemotherapy and survival chances. Patients with breast cancer have been shown to be more closely related to the metabolism of CYP2C8 * 2 and CYP2C8 * 3[23] Glutathione S-transferase P1 (GSTP1) is a member of phase II enzymes that catalyzes the glutathione conjugation of a variety of electrophilicxenobiotics, including substrates that range from environmental toxins and carcinogens todrugs used in the treatment of cancer. This gene is located on chromosome 11, atposition11q13.2, consisting of 7 exons and 8 introns, and has a molecular weight of 23356 Da. One of polymorphisms of the GSTP1, A313G (Ile 105 Met) gene have been identified. Two different alleles, GSTP1 * B, * C, in addition to the wild allele of the GSTP1 * A type have been identified. GSTP1*A has been reported to play a role in the acquisition of resistance to cisplatin via the formation of platinum–GSH conjugation. The GSTP1 is the major GST expressed consistently in both normal and tumor breast tissue. It has been speculated that the absence or decrease of the dexpression of GSTp results in reduced detoxification of possible carcinogens that may be causal to malignant transformation and disease progression[24,25,41].

Hence, the present study was designed to evaluate the role of these genes polymorphisms and OPs levels in breast cancer.

Subjects

A total of 123 unrelated subjects (51 controls and 72patients), living in Mazandaran province were enrolled in this study. The cases were all new incident breast cancer patients histologically diagnosed at Oncology department of Cancer Research Center at Imam hospital in Sari, Iran, during the period of March to May 2018. Control group individualswere randomly selected from healthy women who visited patients admitted to the same hospitals and were healthy blood donors having no evidence of any personal or family history of cancer or other illnesses. Patients' age ranged from 20 to 75 years. Detaileddescription of the clinical-pathological characteristicsofthis study was summarized in (Table 1).Controlgroup`s individuals` ageranged from 23 to 66years old. Informed consent was obtained from all participants and a structured questionnairewas administered by trained interviewers to collect informationon demographic and anthropometric data, reproductiveand medical history, residential history, andoccupation as well aslifestyle,exposure parametersof which have been reported in Table 1.Tobacco smokingand alcohol consumption also were asked from the subjects but there was no case.To investigate whether certain genotypes are a susceptible marker, 5 ml peripheral blood was collected in the EDTA tube from both patients and control group and were stored at -20 °C.

Table 1. Comparison of cases and controls by selected demographic factors and major risk factors for breast cancer

Clinicopathological Variables	Cases(n=72)	Controls (n=51)	*P value*
Age (mean ± SD)	48.08±10.3	43.69±13.5	*0.044**
Age at menarche (mean±SD)	1.2±13.15	1.2±13.31	*0.485*
Age at menopause (mean±SD)	4.4±21.52	5.0±22.55	*0.274*
Age at 1 St pregnancy (mean±SD)	4.4±49.53	8.7±47.13	*0.21*
Age<45 ,n (%)	31(43%.1)	29(56%.9)	*0.13*
Age>45, n (%)	41(56%.9)	22(43%.1)	
Pregnancy, n (%)			
NO	6(8%.3)	-	*0.036**
YES	66(91%.7)	-	
Oral contraceptive use, n (%)			
NO	29(40%.3)	30(58%.8)	*0.047**
YES	43(59%.7)	21(41%.2)	
Menopause Status, n(%)			
Premenopausal	38(52%.8)	36(70%.6)	*0.043**
Post menopausal	34(47%.2)	15(29%.4)	
Body mass index, n (mean±SD kg/m2)			
BMI <20 kg/m2	3(4%.2)	3(5%.9)	
20 □BMI<25	19(26%.4)	14(27%.5)	*0.9*
BMI □25	50(69%.4)	34(66%.6)	
Family history of breast cancer in first-degree relatives n(%)			
No	53(73%.6)	-	*<001**
yes	19(26%.4)	-	
Education, n(%)			
□12 years	41(56%.9)	12(23%.5)	*`<001**
>12 years	31(43%.1)	39(76%.5)	
Occupational expouser to pesiticedes (Agriculturist), n(%)			
NO	52(72%.2)	-	*<001**
YES	20(27%.8)	-	
Grade, n(%)			
I	13(18%.1)	-	
II	47(65%.3)	-	*<001**
III	12(16%.7)	-	
Stage, n(%)			
I	5(6%.9)	-	*<001**
II	42(58%.3)	-	
III	18(25%.0)	-	
IV	*7(9%.7)*	-	

DNA extraction

Blood samples were collected in EDTA-containingtubes and genomic Genomic DNA was isolated from buffycoats using a WizPrep DNA blood kitand Salting-out method[26]. In the method, using extraction kit in the presence of strong anionic detergents, the white blood cells werelysed; then, proteins were removed with dehydration and prophylaxis. Briefly, 200 ul of blood were mixed with 20 ulproteinkinase k, then200ul GB Buffer was added then the mixture was incubated (10 min 56c), and 200ul EtOH %100 was added. Washing buffer 1 and 2 were added next.In the final step, 50ul Elution Buffer was added. Precipitated proteins were removed by centrifugation. The DNA in the supernatant fluid was precipitatedwith ethanol. In every step, the mixturewas centrifuged basedon the protocol of the kit. The DNA pellet was dissolved in 400 µlofsterile distilled water. After extraction, the qualityand quantity of the extracted DNA weremeasured by the spectrophotometer. Then, DNAsamples were stored at -20°C.and its purity was checked through agarose following the protocol of the manufacturer. SNPs were genotyped.

OPs residue extraction and quantification

OPs extraction was done using hexane andacetone (1:1) according to the method of Bush et al. (1984).Serum of Blood (2 mL) was taken in a 15 mL falcon tubes and (2 ml) of Hexane and acetone mixture (1:1) were added.The contents were shaken at room temp for 24 hr in a mechanical shaker.100 ul HCL 5Nwas added to the mixture and was centrifuged for 10 min at 3800 rpm and the clear top upper layer (layer of hexane) was collected. The remaining portion was re-dissolved in hexane for further analysis and ineach step, the mixturewas shaken for 10 min. The new extraction get to volume 5mlwithhexane. After evaporating the mixture with N2 gas to concentrate Residue of the OPs remain in the bottom of the tube. Detection and Quantification of OPs levels were done by Gas Chromatograph (GC) equipped with Mass Detector. GC device model was Agilent. Oven Program was set On 50 °C for 1 min and then 20 °C/min to 120 °C for 0 min and then 10 °C/min to 280 °C, for 5 min run time of 25.5 min per sample. Theused column wasHP-5MS 5% Phenyl Methyl Silox with samples injected from Front Injector. The carrier and makeup gas was Helium, device was set at Pressure 7.6522psi witha flow rate of 1 mL minemployingthe splitless mode, with Holdup Time36.445 cm/sec. Final extract (1 uL) was injected at a temperature of column set at 250 C. Total run time of each sample was25.5 min.

Quantitative analysis of OPs residues of eachsample was done by comparing the peak area with those obtained from a chromatogram of a mixed OPs standard (Supelco, Sigma–Aldrich) of known concentration. Analyses were confirmed by spiking with the known standards of pesticides (Supelco, Sigma–Aldrich). Standard curve of poisons was drawn. The case and control samples were run in the same analyticalbatches and for quality check, a sample was always run with eachset of samples for pesticide analysis to maintain accuracy for the internal control of our measurements, pesticide identification wasconfirmed byAGILENT TECHNOLOGIES,5975 GC–MS at Mazandaran university research center[27].

Genotyping

Polymorphic sites of genes were genotyped by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay. Amplification was performed using aspecific primer. A pairof primerswas designed using Gene Runner software. The sequence of primers has beenlisted in (Table2) reaction contain. 25 µl Polymerase Chain Reaction (PCR) containing 2 µl genomic DNA (100ng/µl), 12.5µl Master mix PCR, and 1 µl (10 picomols) ofeach primer which ultimately reached25 µl with distilled water. The PCR reaction program was setfor each gene. Then, the product of enzyme digestion was electrophoresed on 2% agarose gel and photos were taken by Gel Doc[28].

Table 2. Primers and restriction enzymes used for polymorphism genotyping

Genes	SNP	Polymorphisms	Primers	*Restriction enzymes*
CYP1A1	rs4646421	Intron/splicemutation (m1) 3'uR T→C (T56392)(+303C>T)	Forward CCATTTATTCTCTG:CTCTCTGGTA Reverse CCCACCACACTTAGGAAAATCA	*RsaI*
CYP1B1	rs1056836	CYP1B1*3 Exon3/missensc C251G(Val432 Leu)	Forward CTGTGGTTTTTGTCAACAAGTGGTC Reverse TGAGCCAGGATGGAGATGAAGAGA	*BsrI*
CYP19A1	rs749292	Intron / Exon1	Forward CCAAGGTCCCACAGCTAATTAGTGA Reverse TAAAAGGGCAAGAGCAGAGATGAGC	*TaqI*
CYP2C8	rs1058930	CYP2C8*4 Exon5/missense C792G (ILe 264 Met)	Forward AATCAGGGCTTGGTGTAAGATA Reverse CGATGAATCACAAAATGGACAAG	*TaqI*
GSTP1	*rs1695*	*missense* *A313G (Ile 105 Met)*	*Forward* *TCACAGACAGCCCCCTGGTT* *Reverse* *TCTCTGTCCTTGCACTCGCC*	*AIW261*

Polymorphisms analysis

Previously reported primers andrestricted enzymes in RFLP-PCR are listed in Table 2. All PCR reactions were performed in an independent blinded duplicate manner and for each polymorphism, somesampleswere confirmed by sequencing the PCR products.

The polymorphic site of the CYP1A1(rs 4646421)(C—T intron)was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). A 519 bpfragment containing C/T allele was amplified using forward and reverse primers that the specifications of primers and size of product and restriction enzymes of genes have been explained in Table2 .CYPP1B1 (rs1056836) (C-G Val 432 lucin) also simultaneously revealed by -Restriction Fragment Length Polymorphism (PCR-RFLP) PCR reaction was performed in a total volume of 50 μlcontaining 100 ng of genomic DNA, 200 μmoldNTPs, 2mM MgCl2, 1 × Taq polymerase buffer, 100 pmol ofCYP1A1 and CYP1B1 (primers and 1 U ofTaq DNA polymerase). There action conditions used with the thermal cycler were as follows for CYP1A1: initial denaturation at 95°Cfor 5min, 32 cycles of denaturation at 95 °C for 30sec, annealing at 62°C for 30 sec and extension at 72°C for 30 sec and a final extension at 72 °C for 10 min for CYP1B1 annealing set at 66.5 °C for 30 sec.To verify proper amplification conditions, 10 μlof PCR product was analyzed on a 2% agarose gel andstained with ethidium bromide, the amplification of which was revealed by the presence of bands. To detect CYP1A1 (C-T) and CYP1B1 (C-G) polymorphisms, amplified DNA was digested with 10 U of FastDigestRsaI(37°C, 5 min) and BsrI(65°C, 16h) restriction enzymes. In CYP1A1, the homozygote wild-type CCgenotypeproduce a two183 and 36bpfragments,homozygote mutated TT genotype results insingle band of 183bp length and heterozygote TC genotypeproduces three fragments of 519, 183and 336bpfragments (figure 1)and in CYP1B1, homozygote wild-type CC genotypeproducessingle band of 44bplength, homozygote mutated GG genotype results intwo44 and 171bp fragments and heterozygote GC genotypeproduces three fragments of 215, 171and 44bp fragments.

To verify proper amplification conditions, 10 μlof PCR product was analyzed on a 2% agarose gel andstained with ethidium bromide, the amplification of which was revealed by the presence of bands.Homozygote wild-type CC genotypeproducessingle band of 44bplength, homozygote mutated GG genotype resultsin two44 and 171bp fragments and heterozygote GC genotypeproduces three fragments of 215, 171 and 44bp fragments. Digestion conditions have beenperformed according to the manufacturer's instructions and have been summarized for each gene inTable3.Homozygotewild-type CCgenotyperesults in two 84 and 219bp fragments, homozygote mutated GG genotype produces single band.

Digestion products were sep arated at the appropriate concentrations on a 2, 3 or 4% Low-melting point agarose gel and were stained with ethidiumbromide.The splice-site mutation of CYP19A1(rs749292)(A-G intron) and CYP2C8*4 (rs1058930)(C-G exon5) was also analyzed by PCR-RFLP.PCR amplification also was performed the same as the above genes. The cycling conditions for both of genes including one pretreatment cycle denaturation at 94°Cfor 5min, 32 cycles of denaturation at 94 °C for 30sec, followed by annealing at 66 °C for 30 sec and elongation at 72°C for 25 sec and final elongation at 72 °C for 10 min.Products were analyzed by electrophoresis at 2%agarose gel and visualized by ethidium bromide staining. This amplified fragment was digested TaqI restriction enzyme at 65°C overnight and was analyzed on 2% agarose gel. CYP19A1,when digested with TaqI, the homozygote wild-type GGgenotyresultsin two144 and 214bp fragments, homozygote mutated AA genotype producesa single band of 144bpand heterozygote GA genotypeproduces three fragments of 358, 144and 214bp fragments(figure 2) whereas Digestion of CYP2C8yeilded band of 84bpand heterozygote GC genotypeproducesthree fragments of 303, 84 and 219bp.GSTP1gene was digested by AlW261 at 37°C overnight and we observed homozygote wild-type AA genotypeproducingsingle band of 173 bplength, homozygote mutated GG genotype resultsin two173 and 264 bp fragments and heterozygote AG genotypeproduces three fragments of 437, 173and 264 bp.Annealing time for this gene was 66 °C for30sec(Table 3)

Figure 1. Agarose gel 2% of PCR-RFLP pattern of CYP1A1 gene

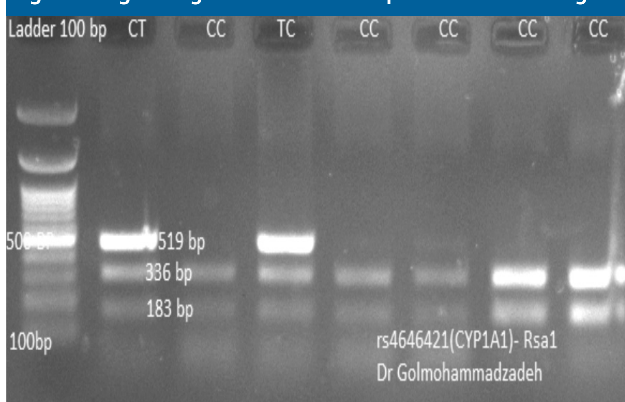

Figure 2. Agarose gel 2% of PCR-RFLP patter CYP19A1 gene

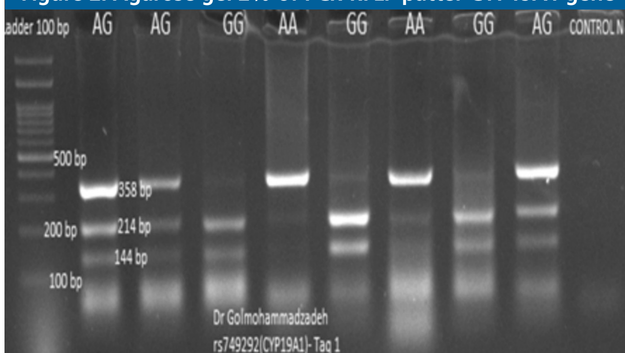

Table 3. Restriction enzymes` conditions used for Genes polymorphism genotyping

SNPS	Restriction Enzymes	Temperature and Incubation time	*Fragment size (pb)*
rs4646421	FastDigestRsaI(10u)	37ºC-5min	*183-336*
rs1056836	BSrI (10u)	65ºC-1h16	*44+171*
rs749292	TaqI(10u)	65ºC-1h16	*144+214*
rs1058930	TaqI(10u)	65ºC-1h16	*84+219*
rs1695	*AlW261(10u)*	*37ºC-1h16*	*173+264*

Statistical Analysis: The genotype and allele frequency of the genes were tested for both patient and control groups.

Chi-squaretest, Fisher exact test, quantitative (numerical) parameters analyzed by t-student test,odds ratio (OR), confidence intervals (CI) and P-values were calculated usingunconditional logistic regression and adjusted estimate the association between genotypes orsome other clinicopathological data and the risk ofbreast cancer. In this research, statistical analyseswere performed using SPSS, version 21 software. For analyzing the poisons, we testedlogistic regression, koroscal Valis.

Results

Demographic and clinicopathological data

This study was performed on72 breast cancer patients and 51 healthy controls with known demographic and clinicopathologicaldatainMazandaranprovince, northern Iran.

Characteristics of the study population were compared by case-control status, as shown in (Table1). Student's t-test showed significant relationships between the two groups and the mean age of controls (43.69±13.5years) was significantly lowerP(044/0=) than that ofbreast cancer patients (48.08±10.3years). Chi-square and Fisher exacttest showed that pregnancy, menopausal status, family history of breast cancer and education, stage of cancer and grade of the tumor were significantly different between cases and controls. However, no significant differences were found between them in regard to BMI, Age at menarche, Age at menopause and Age at 1 St pregnancy.

In the case group, the frequency of level education waslower than diploma and difference wassignificant (p <0.001). The majority of cases hada pregnancy in the patient group and the difference between two groups was significant (p=0.036).Regarding the menopause status,the majority of cases werenot at the menopausestatus(p(047/0=. In cases groups, the majority of people used LD tablets and the difference between the two groups was significant (p=0.043). About the stage of cancer, the majority of patients were in stage 2 (p <0.001)and Grades of tumors were mostly reported in Grade 2 (p <0.001). Regarding the agricultural occupation, few people were in cases group (p <0.001). About the family history of cancer, the majority of patients in the first-degree subjects had no history of cancer in their family (p <0.001).

Regarding the distribution of genotypes, it can be said that the distribution of genotypes in two groups in CYP2C8 (P =0.11), CYP19A1 (P =0.019), CYP1B (P =0.026)) had a significant difference, and they were homogeneous only in CYP1A1(p =0.416) (Table4).

In addition, there was no significant correlation between age and breast cancer risk (P =0.13). However, therewas a significant relationship between the risk of cancer and menopausestatus, and the chance of having cancer was0.466 times lower than those who did not have menopausestatus(p=0.048).In addition, there was a significant relationship between the risk of cancer and LD consumption, andthese people had0.47 times higher risk of breast cancer(=0.043) (Table 5)

Table 4. Associationbetween genesand risk of breast cancer

Gene	Genotype	group		Chi-square test		
		Group of patients	control group	Test statistic	Fisher's exact test	*P value*
CYP2C8	CC	62(%86.1)	48(%94.1)	-	7.4	*0.011**
	CG	0(%0.0)	2(%3.9)			
	GG	10(%13.9)	1(%2.0)			
CYP19A1	AA	3(%4.2)	10(%19.6)	7.7	-	*0.019**
	GA	42(%58.3)	27(%52.9)			
	GG	27(%37.5)	14(%27/5)			
GSTP1	AA	49(%68.1)	39(%76.5)	-	10.034	*0.006**
	GA	12(%16.7)	12(%23.5)			
	GG	11(%15.2)	0(%0.0)			
CYP1A1	CC	56(%77.8)	38(%74.5)	0.17	-	*0.416*
	TC	16(%22.2)	13(%25.5)			
	TT	0(%0.0)	0(%0.0)			
CYP1B1	CC	38(%52.8)	38(%74.5)	7.26	-	*0.026**
	GC	20(%27.8)	5(%9.8)			
	GG	*14(%19.4)*	*8(%15.7)*			

Table 5. Relationship between demographic characteristic and risk of breast cancer

		group		Result		
				OR	P-value	CI
Age	<45	31 (25%.2)	29(22%.6)	0.57	0.13	(0.28,1.18)
	>45	41 (33%.3)	22(17%.9)			
Agriculture	NO	52 (72%.2)	-	-	-	-
	YES	20 (27%.8)	-			
Menopausestatus	NO	36 (29%.3)	36(29%.3)	0.466	0.048*	(0.22,0.99)
	YES	15(12%.2)	15(12%.2)			
Family history in first degree	NO	-	-	-	-	-
	YES	-	-			
OCP USE	NO	30 (24%.4)	30(24%.4)	0.47	0.043*	(0.23,0.98)
	YES	21 (17%.1)	21(17%.1)			
Grade	I, II	-	-	-	-	-
	III	-	-			
Stage	I, II	-	-	-	-	-
	III, IV	-	-			

Regarding the relationship between the genotypes of each gene and the demographic characteristics, it can be concluded that there wasno significant difference between the genotype and the demographic characteristics such as the age of the patients, education, pregnancy history, menopausestatus, agricultural occupation, LD consumption, stage of disease, Gradeoftumors, BMI. There was onlya significant relationship between genotypes of CYP1A1 gene and education (p =0.004), and the group with under diploma education had more CC genotype, and the group with diploma education and higher, had the highest TC genotype.

Frequency of Genotypes and Alleles

In this case-control study, 156 participants(72 patients and 51 healthy individuals) were studied.The results of the experiments have been representedin (table 6).The frequency of genotypes CYP1B1 gene among 72 patients, was CC(52.8%), CG (27.8%) and GG (19.4%), and in healthy subjects, it was CC(74.5%) CG(9.8%) and GG (15.7%). The frequency of alleles in this genotype, allele C (66%) and G allele (34%) in patients and in control subjects were C (80%) and G allele (20%). Regarding the CYP1A1 gene, the frequency of genotypes in patients CC (77.8%), CT (22.2%) and TT (0%) and in healthy subjects were CC (74.5%), CT (25.5%) and TT (0%).Moreover,the frequency of allele C was determined in patients (89%) and G allele (11%) and allele C (87%) and G (13%) in controls had thefrequency.about.CYP19A1 gene, the frequency of genotypes was determined in patients with GG (37.5%), GA (58.3%) and AA (4.2%), and in healthy subjects, it was GG (27.5%), GA (52.9%) and AA (19.6%). The prevalence of alleles in patients with allele C was 66%and with allele T was 34% and in the healthy subjects, allele C was 54% and allele T was 46%.Regarding the CYP2C8 gene, the frequency of genotypes in patients were CC (86.1%), CG (0%) and GG (13.9%) and in healthy subjects were CC (94.1%), CG (3.9%) and GG (2%), and frequency of alleles Patients were assigned C allele (86%) and T (14%), and in the control group, allele C (94%) and T (6%) were determined. (Table6) Regarding the relationship between the genotypes of each gene and breast cancer risk using a logistic regression model (Table 6)in two normalized and age-adjusted models, it was determined thatin the CYP2C8 genotype, those who hadthe CG allele,hada 9.17 degree increased risk of breast cancer compared to those havingthe CC genotype in adjusted model (CI=95%1.11,75.84) .In the CYP19A1gene of individuals with GA genotype, the risk of breast cancer was 6.42 compared to those of genotype AA in non-adjusted model (CI95%=1/52-27/21)(Table 6).Regardingthe CYP1B1 gene , people with two genotypes of CG + GG were associated with a higher risk of breast cancer, it means that their cancer risk was2.61times higher thanCC genotype in non-adjusted model and was more than 2.93 degrees in adjusted model(CI71/5 – 19/1 95% =)(CI=95% 1.31,6.57). About alleles, inCYP2C8gene, the G allelehada protective effectonbreast cancer and decreased risk of breast cancer (P =0.02) and in GSTP1 gene, the Galleleincreasedthe risk of breast cancer. (P=0.0480) also in CYP1B1 gene, the G alleledecreasedthe risk of breast cancer.(P=0.0271)

Table 6. The relationship between the genotypes and Alleles and breast cancer risk

Genes name	Genotype and Alleles	groups		Non Adjusted		Adjusted	
		cases **(%)**	controls **(%)**	P	OR(95% CI)	P	OR(95% CI)
CYP2C8	CC	62(86%.1)	48(94%.1)	0.16	-	0.12	-
	CG	0(0%.0)	2(3%.9)	0.06	7.74(0.95,62.5)	0.04	9.17(1.11,75.84)
	GG	10(13%.9)	1(2%.0)	0.99	1.615E+10	0.99	1.560E+10
	CC vs GG+CG	72(58%.53)	51(41%.47)	0.167	2.58(0.67,9.89)	0.112	3.027(0.77,11.88)
	C (%)	86%	%96	-	Ref	-	-
	G(%)	14%	%4	P=0.02	0.25(0.08,0.81)	-	-
CYP19A1	AA	3(4%.2)	10(19%.6)	0.038	-	0.015	-
	GA	42(58%.3)	27(52%.9)	0.011	6.42(1.52,27.21)	0.61	6.21(1.43,26.86)
	GG	27(37%.5)	14(27%.5)	0.601	1.24(0.55,2.77)	0.066	1.24(0.54,2.8)
	AA+GA vs GG	72(58%.53)	51(41%.47)	0.246	1.58(0.73,3.45)	0.263	1.57(0.71,3.46)
	G(%)	%66	%54	-	Ref	-	-
	A(%)	%34	%46	P=0.084	1.65(0.93,2.92)	-	-
GSTP1	AA	43(68%.1)	39(76%.5)	0.885	-	0.848	-
	GA	12(16%.7)	12(23%.5)	0.999	1285788459(0,.) (0,.)	0.99	1136050164(0,.)
	GG	11(15%.2)	0(0%.0)	0.999	16154778807	0.99	1487441293(0,.)
	CC vs TT+CT	72(58%.53)	51(41%.47)	0.99	1350641208(0,.)	0.99	1205664754(0,.)
	A(%)	%76	%87	-	Ref	-	-
	G(%)	%24	%13	P=0.0480	2.1134 (1.0065-4.4375)	-	-
CYP1A1	CC	56(77%.8)	38(74%.5)	-	-	-	-
	TC	16(22%.2)	13(25%.5)	0.67	0.835(0.36,1.93)	0.63	0.81(0.34,1.9)
	TT	0(0%.0)	0(0%.0)	-	-	-	-
	CC vs GG+CG	72(58%.53)	51(41%.47)	0.67	0.835(0.36,1.93)	0.63	0.81(0.34,1.9)
	C(%)	%89	%87	-	Ref	-	-
	T(%)	%11	%13	P=0.66	1.21(0.51,2.84)	-	-
CYP1B1	CC	38(52%.8)	38(74%.5)	0.034	-	0.024	-
	GC	20(27%.8)	5(9%.8)	0.26	1.75(0.66,4.65)	0.165	2.045(0.74,5.6)
	GG	14(19%.4)	8(15%.7)	0.22	0.44(0.118,1.62)	0.278	0.479(0.127,1.8)
	CC vs CG+GG	72(58%.53)	51(41%.47)	0.016	2.61(1.197,5.71)	0.009	2.93(1.31,6.57)
	C(%)	%66	%80	-	Ref	-	-
	G(%)	%34	%20	P=0.0271	0.48(0.25,0.92)	-	-

The results of logistic regression model regarding the frequency of genotypes of each gene and the incidence of breast cancer indicated that there was a significant relationship between the frequency of genotypes and the risk of breast cancer only in CYP1B1gene (P-value =0.015, OR=0.38, CI,0.18 0.84), and no significant results were obtained for other genes.(Table 7)

It can be said that CC genotype of CYP1B1decreasedthe risk of breast cancer about 0.38 time in comparison to the CG + GG genotype. In addition, about the correlation between CC and CG + GG genotypes with demographic and clinical variables such as age (P-value = 0.25), agriculture (P-value = 0.2), menopause (P = 0.16) (P-value = 0.29), LDL consumption (P-value = 0.86), tumor grade (P-value = 0.83), and stage of cancer(P-value 0.16) =), there was no significan trelationshipamong two groups (Table 7).

Table 7. Relationship between the frequency of CYP1B1 genotypes and the risk of breast cancer and demographic and clinical variables

		CYP1B1		Result		
		CC	GC+GG	OR	P-value	CI
Group	Case	38(30%.9)	34(27.6)	0.38	0.015*	(0.18,0.84)
	Control	38(30%.9)	13(10%.6)			
Age	<45	34(27%.6)	26(21%.1)	0.65	0.25	(0.32,1.36)
	>45	42(34%.1)	21(17%.1)			
Agriculture	NO	25(34%.7)	27(37%.5)	0.5	0.2	(0.17,1.45)
	YES	13(18%.1)	7(9%.7)			
Menopause status	NO	42(34%.1)	32(26%.0)	0.58	0.16	(0.27,1.24)
	YES	34(27%.6)	15(12%.2)			
Family history in first degree	NO	26(36%.1)	27(37%.5)	0.56	0.29	(0.19,1.65)
	YES	12(16%.7)	7(9%.7)			
OCP USE	NO	36(29%.3)	23(18%.7)	0.94	0.86	(0.45,1.94)
	YES	40(32%.5)	24(19%.5)			
Grade	I, II	32(44%.4)	28(38%.9)	1.14	0.83	(0.33,3.94)
	III	6(8%.3)	6(8%.3)			
Stage	I,II	22(30%.6)	25(34%.7)	0.49	0.16	(0.18,1.34)
	III, IV	16(22%.2)	9(12%.5)			

According to the results of serum levels of Diazinon and Malathion, it can be concluded that the mean serum level of diazinon in both case and control groups was statistically significant (p <0.001). According to the mean+_SD serum level of this poison, it can be concluded that diazinon in the case group hada much lower level than the control group(Table 8).

Table 8. Serum levels of Diazinon and Malathion in Case and Control groups

Serum levels	Group	Mean±SD	Statical value	DF	P-value	95% Confidence Interval of the Difference	
						Lower	Upper
	Case						
Diazinon	Control	35.4±38.9	-4.1	36.5	<0.001	-97.3	-32.5
		100.4±73.2					
Malathion	Case	79.6±101.0	0.02	55	0.98	-45.8	46.6
	Control	65.7±12.8					

According to the results of the logistic regression model, there was a significant relationship between serum level of diazinon andthe risk of breast cancer (P <0.001).Results indicated that, for each unit of increase in serum level of diazinon, the chance of a breast cancer risk increased 1.019 degree. No significant correlation was found between serum levels of malathion and the risk of cancer in this study (Table 9).

Table 9. The relationshipbetween the risk of cancer and serum level of Diazinon and Malathion

OPs	Mean±SD	β	P-value	Exp(B)	95% C.I. for EXP (B)	
Diazinon	65.1±65.35	0.019	0.001	1.019	1.01	1.03
Malathion	79.4±85.98	0	0.98	1	0.99	1.01

Regarding the results, after adjusting the regression model based on the serum level of diazinon and malathion, no double and triple of genotypes were found to have a significant relationship with the incidence of disease (Table 10).

Table 10. The relationship between the Risk of Cancer and Type of Genotype by Adjusting the Level of Diazinon and Malathion

Gene	Genotype	Group		Adjusted for Diazinon		Adjusted for Malathion	
		Case (%)	Control(%)	P	OR(95% CI)	P	OR(95%CI)
CYP2C8	CC	62(86%0.1)	48(94%0.1)	0.22	-	0.74	-
	CG	0(0%0.0)	2(3%0.9)	0.08	22.04(0.67,719.2)	0.44	2.49(0.24,25.66)
	GG	10(13%0.9)	1(2%0.0)	0.99	4.56E+10	0.99	4.99E+10
	CC vs GG+CG	72(58%0.53)	51(41%0.47)	0.65	1.67(0.17,15.9)	0.82	0.82(0.15,4.46)
CYP19A1	AA	3(4%0.2)	10(19%0.6)	0.24	-	0.21	-
	GA	42(58%0.3)	27(52%0.9)	0.099	6.16(0.71,53.35)	0.09	5.12(0.78,33.65)
	GG	27(37%0.5)	14(27%0.5)	0.317	2.02(0.51,7.9)	0.27	1.94(0.58,6.4)
	AA+GA vs GG	72(58%0.53)	51(41%0.47)	0.18	2.43(0.65,9.2)	0.14	2.33(0.74,7.37)
GSTP1	AA	49(68%0.1)	39(76%0.5)	0.23	-	0.13	-
	GA	12(16%0.7)	12(23%0.5)	0.99	760099542(0,.)	0.99	1179022271(0,.)
	GG	11(15%0.2)	0(0%0.0)	0.99	5767349012(0,.)	0.99	1.130E+10(0,.)
	AA+GA vs GG	72(58%0.53)	51(41%0.47)	0.99	946807232(0,.)	0.99	1610795464(0,.)
CYP1A1	CC	56(77%0.8)	38(74%0.5)	-	-	-	-
	TC	16(22%0.2)	13(25%0.5)	0.22	2.66(0.56,12.75)	0.39	1.72(049,6.01)
	TT	0(0%0.0)	0(0%0.0)	-	-	-	-
	CC vs TT+CT	72(58%0.53)	51(41%0.47)	0.22	2.66(0.55,12.75)	0.63	0.81(0.34,1.9)
CYP1B1	CC	38(52%0.8)	38(74%0.5)	0.11	-	0.15	-
	GC	20(27%0.8)	5(9%0.8)	0.71	1.37(0.27,7.25)	0.84	1.15(0.28,4.76)
	GG	14(19%0.4)	8(15%0.7)	0.09	0.12(0.01,1.48)	0.13	0.22(0.03,1.59)
	CC vs CG+GG	72(58%0.53)	51(41%0.47)	0.09	3.23(0.81,12.98)	0.15	2.31(0.74,7.22)

According to the results, the serum level of organophosphate diazinon and malathion did not differ significantly in different stages of the disease (Table11).

Table 11. Serum Levels of Organophosphate Diazinon and Malathion in Different Stages of breast cancer

Serum levels	stage	Descriptive values		Kruskal–Wallis		
		Average rating	Mean±SD	Statical value	DF	P-value
Diazinon	1	10.5	17.22±6.4	1.99	3	0.57
	2	16.67	38.2±39.1			
	3	18	42.9±53.1			
	4	14	20.6±13.8			
Malathion	1	16	59.3±58.1	4.31	3	0.23
	2	18	105.6±120.6			
	3	12.14	42.9±52.3			
	4	7.5	14.5±20.5			

Discussion: Breast cancer is one of the most common female malignant tumors in Iran[29]. Pesticides are one of the most important risk factors for cancer, which are excessively used in Mazandaran province located in south coast of Caspian Sea[30].

Breast cancer comprises about 21% of all new cancers in women. The highest age-adjusted incidence rate is reported for North America, being 87 per 100 thousand women per year, while the lowest rate has been reported in China. Breast cancer follows a steeply increasing age gradient up to 40 years of age, after which the rate of increase slows down. Even though, there are three times as many new cases diagnosed annually as in the late 1980s, breast cancer mortality has remained largely unchanged. This may at least partly be explained by earlier detection of the disease due to effective screening programs and availability of improved therapies. The highest annual mortality rates for breast cancer have beenreported for the UK, the Netherlands, and Denmark, being over 25 per 100 thousand in these countries. So far, conflicting results have been reported from association studies[30]. The etiology of breast cancer could not be described by allelic variability at a single locus.Instead,an improved understanding of the interplay of xenobiotic exposures,endogenous physiology, and genetic variability at multiple loci may help to identify women who are at increased risk of breast cancer. The genetic polymorphisms that may be linked to breast cancer are numerous. Cumulative lifetime exposure to estrogen, estrogen metabolites, and other physiological factors, as well as environmental exposures, could play an important role in the etiology of breast cancer in genetically predisposed women;carcinogenesis,determining response to drugs and cell signaling. Many carcinogenic compounds are oxidized by phase I enzymes, represented by cytochrome P450 family, into reactive metabolites that are detoxified by phase II enzymes. GSTs are a family of Phase II detoxification enzymes that catalyze the conjugation ofglutathione (GSH) to a wide variety of xenobiotics. Thisdetoxification ability plays a role in cellular protectionfrom environmental and oxidative stress[31]. CYP450 and GSH enzymes associated with the development of breast cancer[32] which involved in biosynthesis and metabolism of estrogens and other CYP enzymes can involve in the development of breast cancer risks like CYP19,CYP2,CYP17,CYP1A2,CYP11A1,CYP2D6,CYP2C19,CYP3A4/5,CYP1A1,CYP1B1,CYP2C8/9. In this study, distribution ofCYP450 and GSTisoenzymesCYP1A1(rs4646421), CYP1B1(rs1056836), CYP19A1(rs749292) and CYP2C8(rs1058930) and GSTP1(rs1695) gene polymorphisms in patients with breast cancer in Mazandaran province and association with serum levels of Diazinon and Malathion was investigated by the PCR-RFLP using restriction enzyme activityand GC-MS methods.

Vivek Kumar etal. searched about CYP 1A1 polymorphism and organochlorine pesticides levels in the etiologyof prostate cancer. They studied 70 newly diagnosedprostate cancer patients and 61 age-matched healthy male controls. OCP levels in blood were determinedusing gas chromatography-mass spectrometry (GC–MS) and CYP1A1 polymorphisms were analyzed by allele-specific PCR and RFLP–PCR methods. Significantly higher levels of β -HCH, γ-HCH and ρ,ρ′ -DDE were found in cases as compared to controls (p-values = 0.04, 0.008, and 0.01, respectively). Higherlevels of γ-HCH were observed in advanced stages of prostate cancer cases (<T2 vs.> T3), (p-value =0.04).Dieldrin was found significantly higher in cases with initial stages (p-value =0.03). They did not observe a correlation between prostate cancer and CYP1A1 polymorphisms. Hence, a higher level of OCPs, especially β-HCH, γ-HCH and ρ,ρ′ -DDE might be associated with prostatecancer risk.

In a study byMandanaGhisaretal, they determinedusing-PCR-RFLP reaction that polymorphisms in genes involved in xenobiotic metabolism and estrogen biosynthesis, like CYP1A1 (Ile462Val;rs1048943), CYP1B1(Leu432Val; rs1056836) and CYP19A1 (C> T; rs10046) and they found an independent association of CYP1A1 (Val) with BC risk.CYP1B1 and CYP19A1were not associated with breast cancer risk[32]. Joanna Trubicka etal. genotyped 597 cancer patients and 597 controls for three CYP1B1SNPs. They found that the three SNPs rs10012, rs1056827, and rs1056836 alone did not provide any significant evidence of association with colorectal cancer risk. Haplotypes of rs1056827 and rs10012 or rs1056827 and rs1056836 revealed an association with colorectal cancer which was significantly stronger in the homozygous carriers. Genetic variants within the CYP1B1associated with altered function appeared to influence susceptibility the colorectal cancer in Poland[33].

Ghazaleh Khalili etal, studied about Cytochrome P450 1A1 (T3801C) Single Nucleotide Polymorphism inPatients with Breast Cancer in Mazandaran Province-Northern Iran. Ninety six patients with breast cancerand known-clinicopathological characters and 110 healthy women as control were genotypedforCYP1A1 M1 polymorphisms by PCR-RFLP technique using Msp1 restrictionenzymes. Logistic regression was applied for statistical analysis. They resulted thatthe frequency of TT and TC genotypes of M1 polymorphism wascalculated 86, 14% for cases and

79 and 21% for the control group, respectively.Surprisingly, the mutant CC genotype was not found in any subjects. Statisticalanalysis showed no significant correlation between allelic variants and breastcancer risk (p-value; 0.42, OR; 0.66, CI; 0.24-1.81). No significant correlation wasalso found between genotypic frequency and clinicopathological characters. Only TT and TC genotypes were found in the studied subjects. TheM1 allelic variants were significantly associated neither with breast cancer risk norwithclinicopathological characteristics[34].

In another study, Marc T Goodman etal. Determined Genetic variation in two CYP19A1 single-nucleotide polymorphisms (SNPs), rs749292 and rs727479, by PCR-RFLP method and association with the risk of ovarian cancer. Results showedthat A allele of rs749292 was positively associated with ovarian cancer risk in a codominant model for all combined races (AG versus AA genotype: odds ratio (OR), 1.48 and 95% confidence interval (CI, 1.07–2.04); GG versus AA: OR, 1.87 (CI, 1.24–2.82); Ptrend=0.002). Similar significant associations of the rs749292 Aalleleon risk of ovarian cancer were found among Caucasian and Japanese women. No relation of the rs727479 SNP to ovarian cancer risk was observed overall, although Caucasian women carrying the variant A allele compared with women with a CC genotype had an OR of 2.91 (CI, 1.15–7.37).These data suggested thatCYP19A1 variants may influence susceptibility to ovarian cancer(35).

H Jernstro¨metal investigated CYP2C8and CYP2C9 polymorphisms in relation to tumor characteristics and early breast cancerin a prospective series of 652 breast cancer patients from southern Sweden which were genotyped for CYP2C8*3, CYP2C8*4,CYP2C9*2, and CYP2C9*3. Frequencies of CYP2C8/9 polymorphisms were similar to healthy European populations. Significantly less node involvement (P¼0.002) and fewer PRþtumors (P¼0.012) were associated with CYP2C8*4. Median follow-up was 25 months and 52 breastcancer-related events were reported. In a multivariate model, CYP2C8/9*3/*1*/*2/*1 was the only factor associated with the increase drisk for early events in 297 tamoxifen-treated, ER-positive patients, adjusted HR 2.54 (95%CI 1.11–5.79). The effect appeared to bedriven by CYP2C8*3, adjusted HR 8.56 (95%CI 1.53–51.1). They found that Polymorphic variants of CYP2C8/9 may influence breast tumor characteristics and disease-free survival in tamoxifen-treated patients[36].

In a study, Catherine Duggan etal. measured the presence of the null mutation in GSTT1 and GSTM1, and the rs1695 polymorphism inGSTP1 by a polymerase chain reaction and assessed associations between breast-cancer specific and all-causemortality using Cox proportional hazards models.Results showed that participants with ER-negative tumors were more likely to be GSTT1 null ($\chi 2$=4.52; P=0.03) and AfricanAmerican women were more likely to be GSTM1 null ($\chi 2$=34.36; P<0.0001). NeitherGSTM1 nor GSTT1 null mutationswere associated with breast cancer-specific or all-cause mortality. In a model adjusted for body mass index,race/ethnicity, tumor stage, and treatment received at diagnosis, the variant Val allele of rs1695 was associated withthe increased risk of all-cause (HR=1.81, 95% CI 1.16-2.82, P=0.008), but not breast cancer-specific mortality. The GSTT1nullmutation was associated with significantly higher levels of C-reactive protein. Theyconcluded that GSTM1and GSTT1 null genotypes had no effect on outcome; however, the variant allele of rs1695 appearedto confer the increased risk for all-cause mortality in breastcancer survivors[37-39].

Conclusions

The results of this study indicated that CYP2C8 (rs1058930), CYP19A1 (rs749292) and CYP1B1 (rs1056836) gene polymorphisms were associated with breast cancer and there was a significant relationship between serum level of Diazinon and risk of breast cancer. Screening these genes, polymorphisms can be used to prognosticate disease, prevent disease progression, and be sued asappropriate therapeutic intervention.

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