Polymorphisms in Phase I (CYP450) Genes CYP1A1 (rs4646421), CYP1B1 (rs1056836), CYP19A1 (rs749292) and CYP2C8 (rs1058930) and Their Relation to Risk of Breast Cancer: A Case-Control Study in Mazandaran Province in North of Iran

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Abstract

BACKGROUND: The second leading cause of cancer-related death in women is breast cancer. Xenobiotic Metabolizing Enzymes (XMEs) contribute to the detoxification of numerous cancer therapy-induced products. In the metabolism of xenobiotic, cytochrome P450s or monooxygenases perform an important function by catalysing the hydroxylation reaction. In this study, the susceptibility and genetic polymorphisms of CYP450 isoenzymes was investigated that may have an etiological role in breast cancer.

AIM: The main purpose of this study was to evaluate the association of CYP1A1 (rs4646421), CYP1B1 (rs1056836), CYP2C8 (rs1058930), and CYP19A1 (rs749292) polymorphisms with the risk of breast cancer in Mazandaran province.

MATERIAL AND METHODS: This cross-sectional case-control study were recruited 72 patients and 51 healthy individuals and was performed between March 2018 to May 2018 in the Oncology Department at Imam Hospital in Sari city, Iran. Peripheral blood samples were collected in EDTA tube, and DNA extraction was performed using the salting-out method and WizPrep extraction kits. Breast cancer patients with known clinicopathological characters and healthy women as control group were genotyped for genes polymorphisms by PCR-RFLP technique, using restriction enzymes. Chi-square, Fisher exact test and Logistic regression model, were applied for statistical analysis.

RESULTS: The results of the experiments showed that there was a significant relationship between two groups and the age of the patients is significantly higher than the control group (p = 0.044). According to the chi-square and Fisher exact test, education, pregnancy, menopause status and oppose were significant between the two groups. Based on using a logistic regression model in two normalized and age-adjusted models to finding relationship between the genotypes of each gene and breast cancer risk, it was determined that in the CYP2C8 genotype, those who have the CG allele have a 7.47 degree increased risk of breast cancer (CI = 95% 0.95-62.5) and in the CYP1A1 gene, individuals with GA genotype, increased risk of breast cancer (CI = 95% 1.52-27.21), about the CYP1B1 gene, people with two genotypes of GG and higher risk of breast cancer (CI = 95% 1.19-5.71) and allele G has decreased risk of breast cancer in this gene (P = 0.0271), also allele G in CYP2C8 gene had the protective effect (P = 0.02). In the age-adjusted model, for the CYP2C8 gene, GG genotype increased risk of breast cancer (CI = 95% 1.11-75.84) as well as, the GG and GG genotype in CYP1B1 gene (CI = 95% 1.31-6.57).

CONCLUSION: Our results confirm the association between CYP2C8 (rs1058930), CYP19A1 (rs749292) and CYP1B1 (rs1056836) gene polymorphisms and increased risk of breast cancer in women in Mazandaran province.

Introduction

Breast cancer is the most frequent malignancy in women in all races and a growing number in advanced and underdeveloped countries in Asia. According to a report of Iran’s Ministry of Health and Medical Education, breast cancer is the most common primary cancer in Iranian women [1]. Today, this disease accounts for about one-third of all cancers in women, and it represents the second leading cause of cancer death among women (15% of cancer deaths) after lung cancer. According to WHO statistics, one in every 8 to 10 women have breast cancer [2]. According to the latest statistics from the Iranian Cancer Research Center, breast cancer cases
in Iran are one decade younger than their western counterparts [3]. About 8500 new cases of breast cancer are reported annually in the country that 1400 cases are reported died from breast cancer; it is also estimated in 2014, about 40,000 people were living with this disease [4].

Breast cancer disease is defined as uncontrolled growth of cells in breast tissue, which is caused by abnormal growth in the glands which have role of producing milk (lobules) or in the ducts that connect lobules to the nipple duct [5] and also is a heterogeneous disease [6] that is caused by environmental and genetic factors [7]. The genetic damage caused by endogenous and exogenous metabolites could be a cause of breast cancer. Clinical and epidemiological studies have demonstrated that estrogen and progesterone play a significant role in the growth and differentiation of normal tissue in the breast [8].

The aetiology of breast cancer is complex and still poorly understood. A small proportion of breast cancer cases can be attributed purely to genetic reasons whereas risk factors such as age, reproductive events (menarche, menopause, pregnancy, breastfeeding), estrogens, exogenous hormones (hormone replacement therapy and oral contraceptives), lifestyle and exposure to environmental carcinogens (pollution, alcohol, diet, obesity), ionizing radiation, chemopreventive agents, as well as genetic factors, breast cancer susceptibility genes high penetrance genes (BRCA1, BRCA2, PTEN) and low penetrance genes (CYP450, GSH, UGTA) that encodexenobiotic metabolizing enzymes in phase I and II of metabolism [9]. As mentioned above, one of the main risk factors for breast cancer is estrogen, and there are two forms of estrogen in our body: endogenous estrogen that biosynthesises from cholesterol in the body, then undergoes metabolic processes and exogenous form (hormonal drugs, etc.). The main effect of estrogens is stimulating of the breast cells and increases the chance of errors during multiple DNA divisions and the possibility of mutations [10]. Also, it has been identified that Xeno-estrogens, which include pesticides, paints, contaminants, plastics and food preservatives, would have similar effects with estrogen, and contributed to the causes of breast cancer. They have endocrine disruption effects [11].

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 catalysate the conjugation of glutathione (GSH) to a wide variety of xenobiotic. This detoxification ability plays an important role in cellular protection from environmental and oxidative stress. Hence, the toxic effects of exposure, absorption and detoxification of carcinogens depend on a delicate balance between the phase I and phase II enzymes [12], [13]. The cytochrome P450 and GSTs, UGTA and etc.that phase II enzymes increase its solubility in such a way as to facilitate its excretion and detoxification. These genes involved in the metabolism of these toxins (cytochrome p450), and also contribute to the pathway of biosynthesis and estrogen metabolism, and also contribute to the progression of breast cancer Polymorphisms in both phase I and phase II enzyme genes may result in alteration of their expression, function and activity. These genes are regulated at the transcriptional level, and their expression is influenced by genetic factors, polymorphism in the structural and regulatory gene and by environmental factors [14], [15]. Polymorphisms and expression pattern of these genes are believed to be key factors in determining cancer susceptibility to toxic or environmental chemicals [16]. Polymorphisms are including single nucleotide polymorphisms (SNPs). The most common form of human genetic differences is single Nucleotide Polymorphisms (SNPs) which may play an important role in individual allergies. Single-nucleotide polymorphism is a change in only one open DNA. These single-nucleotide variations cause different phenotypes, predisposing to or susceptibility to certain diseases.

SNPs in genes involved in xenobiotic metabolism and estrogen biosynthesis and metabolism might affect circulating estrogen levels and modulate the individual susceptibility to environmental carcinogens about developing breast cancer [10]. Candidate genes for this study are low-penetrance breast carcinoma susceptibility include those encoding for Xenobiotic Metabolizing Enzymes (XMEs) involved in carcinogen metabolism and detoxification [17]. These XMEs can be divided into phase I (Cytochrome P450 family) and phase II (GST, UGTA, etc.) enzymes that metabolically activate potentially carcinogenic forms. In this study, polymorphisms of phase I enzymes CYPA1 (rs4646421C/T), CYP2C8 (rs1058930C/G), CYP1B1 (rs1056836 C/G) and CYP19A1 (rs749292A/G) in breast cancer patients who referred to Mazandaran province clinics, during March 2018 to May 2018 were investigated. DNA extracted from peripheral blood leukocytes of patients with breast cancer and healthy patients. Identifying the genetic polymorphisms of breast cancer patients, in order to recognizing the mechanism of the disease is effective on diagnosis and screening patients who are prone to the disease and, therefore, preventing them. So, position, structure and function of studied genes were briefly explained, then polymorphisms of them were studied.

CYP1A1 is one of the “phase I” enzymes located on chromosome 15q22-q24 and is a 5987-bp long gene. It has 7 exons and 6 introns that encodes for a 512 amino acid protein. This is one of the major components of the detoxification pathway that is highly expressed in non-hepatic cells, such as breast tissue [18]. It is a polymorphic gene involved in the
metabolism of steroids and several potentially genotoxic chemicals. In estrogen metabolism, it plays a main role in producing 2-hydroxy estrogen. Four single nucleotide polymorphisms (SNP) were identified in CYP1A1 gene including M1, T/C transition at nucleotide 3801; M2, A/G transition at position 2455 resulting in change of Ile to Val at codon 462; M3T/C transition at nucleotide 3205; and M4 C/A transition at position 2453 resulting in 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 [19], [20]. CYP1B1 is located on chromosome 2 at position 2p22.2. The gene is 42930-fold-wide and 60846 Molecular weight. It has 3 exons and 2 introns and was considered as the key enzyme of P450, which is exogenous in metabolism and endogenous substrate.

CYP1B1 plays the main role in the metabolism of androgens and estrogen substrates. CYP1B1 can catalyse 4-hydroxyl-estrogens, which is a key reaction to hormonal carcinogenesis. CYP1B1, 5 different SNPs have been identified that can play a vital role in replacing amino acids A119S, R48G, L432V, A443G and N453S. Va32Leu polymorphism has recently been identified as the most influential allele on cytotoxicity in breast cancer. The main effect of this enzyme is in the biosynthesis and the final stages of estrogen metabolism of androgens and estrogen substrates. The aromatase is coded by the CYP19A1 gene, which is one of the first human cytochromes and plays a vital role in drug metabolism in drug metabolism in endogenous in metabolism and endogenous substrate.

Material and Methods

Subjects

A total of 123 unrelated subjects (51 controls and 72 patients), living in Mazandaran province were enrolled in this study. The cases were all new incident breast cancer patients histologically diagnosed at the Oncology Department of Cancer Research Center at Imam Hospital in Sari city in Iran, during the period of March 2018 to May 2018. Controls were 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. A detailed description of the clinical-pathological characteristics of this study was summarized in (Table 1). Control subjects having years ranging from 23 to 66 years. Informed consent was obtained from all participants, and a structured questionnaire was administered by trained interviewers to collect information on demographic and anthropometric data, reproductive and medical history, residential history, and occupation as well, lifestyle, exposure parameters were reported in Table 1. Tobacco smoking and alcohol consumption also were asked from the subject, 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 stored at -20°C.

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

| Clinic pathological Variables | Cases | Controls | P value |
|---|---|---|---|
| Age (mean ± SD) | 48.08 ± 10.3 | 43.69 ± 13.3 | 0.044* |
| Age at menarche (mean ± SD) | 1.2 ± 1.15 | 1.2 ± 1.31 | 0.485 |
| Age at menopause (mean ± SD) | 4.4 ± 2.13 | 5.0 ± 2.25 | 0.274 |
| Age at 1st pregnancy (mean ± SD) | 4.4 ± 4.93 | 8.7 ± 4.73 | 0.21 |
| Age < 45, n (%) | 31 (43%) | 29 (36.9%) | 0.13 |
| Age > 45, n (%) | 41 (56.9%) | 22 (43.1%) | |
| Pregnancy, n (%) | | | 0.036* |
| NO | 6 (8.3%) | - | |
| YES | 66 (91.7%) | - | |
| Oral contraceptive use, n (%) | | | 0.047* |
| NO | 38 (52.8%) | 36 (70.6%) | |
| YES | 34 (47.2%) | 15 (29.4%) | |
| Menopause Status, n (%) | | | 0.043* |
| Premenopausal | 29 (40.3%) | 30 (58.8%) | |
| Past menopausal | 43 (59.7%) | 21 (41.2%) | |
| Body mass index, n (mean±SD kg/m²) | | | 0.9 |
| BMI < 20 kg/m² | 3 (4.2%) | 3 (5.9%) | |
| 20 ≤ BMI < 25 | 19 (26.4%) | 14 (27.5%) | |
| BMI ≥ 25 | 50 (69.4%) | 34 (66.6%) | |
| Family history of breast cancer in first-degree relatives n (%) | | | < 0.001* |
| No | 53 (73.6%) | - | |
| Yes | 19 (26.4%) | - | |
| Education, n (%) | | | 0.001* |
| ≤ 12 years | 41 (56.9%) | 12 (23.5%) | |
| > 12 years | 31 (43.1%) | 39 (76.5%) | |
| Occupational exposure to pesticides (Agricultural), n (%) | | | < 0.001* |
| NO | 52 (72.2%) | - | |
| YES | 20 (27.8%) | - | |
| Grade, n (%) | | | < 0.01* |
| I | 13 (18%) | - | |
| II | 47 (65.3%) | - | |
| III | 12 (16.7%) | - | |
| Stage, n (%) | | | < 0.001* |
| I | 5 (6.9%) | - | |
| II | 42 (58.3%) | - | |
| III | 18 (25%) | - | |
| IV | 7 (9.7%) | - | |
DNA extraction

Blood samples were collected in EDTA-containing tubes, and genomic DNA was isolated from buffy coats using a WizPrep DNA blood kit and salting-out method [25]. In the method by using extraction kit, in the presence of strong anionic detergents, the white blood cells are lysed, then proteins are removed with dehydration and prophyaxis. Briefly, 200 μl of blood were mixed with 20 μl protein kinase K, then 200 μl GB buffer was added then the mixture was incubated (10 min 56°C), 200 μl EIOH %100 was added. Then, washing buffer 1 and 2 was added. In the final step, 50 μl Elution Buffer was added. Reciprocated proteins were removed by centrifugation. The DNA in the supernatant fluid was precipitated with ethanol. In every step, we centrifuged the mixture based on the protocol of the kit. The DNA pellet was dissolved in 400 μl of sterile distilled water. After extraction, the quality and quantity of the extracted DNA were measured by the spectrophotometer. Then, DNA samples were stored at -20°C, and its purity was checked through agarose following the protocol of the manufacturer. SNPs were genotyped.

Genotyping

Polymorphic sites of genes were genotyped by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay. Amplification was performed using a specific primer. Pair of Primers were designed using Gene Runner software. The sequence of primers is listed in (Table 2) reaction contain. 25 μl Polymerase Chain Reaction (PCR) containing 2 μl genomic DNA (100 ng/μl), 12.5 μl Master mix PCR, and 1 μl (10 picomols) of each primer which ultimately reached 25 μl with distilled water. The PCR reaction program was set for each gen. Then the product of the enzyme was digested. Digestion was electrophoresed on 2% agarose gel and photos were taken by Gel Doc [26].

Polymorphisms analysis

Previously reported primers and restricted enzymes in RFLP-PCR are listed in (Table 2). All PCR reactions were performed in an independent blinded duplicate manner, and for each polymorphism, some samples were 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 bp fragment 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 the (Table 2). CYP1B1 (rs1056836) (C-Gval432leucin) also simultaneously revealed by Restriction Fragment Length Polymorphism (PCR-RFLP) PCR reaction was performed in a total volume of 50 μl containing 100 ng of genomic DNA, 200 μmol dNTPs, 2 mM MgCl2, 1 x Taq polymerase buffer, 100 pmol of CYP1A1 and CYP1B1 (primers and 1 U of Taq DNA polymerase). There action conditions used with the thermal cycler were as follows for CYP1A1: initial denaturation at 95°C for 5 min, 32 cycles of denaturation at 95°C for 30 sec, 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 μl of PCR product were analysed on a 2% agarose gel and stained with ethidium bromide, the amplification of 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 Fast Digest RsaI (37°C, 5 min) and BsrI (65°C, 16 h) restriction enzymes. In CYP1A1 the homozygote wild-type CC genotype produces two 183 and 36 bp fragments, homozygote mutated TT genotype results in single band of 183 bp length, and heterozygote TG genotype produces three fragments of 519, 183 and 336 bp fragments and in CYP1B1 homozygote wild-type CC genotype produce single band of 44 bp length, homozygote mutated GG genotype results from two 44 and 171 bp fragments and heterozygote GC genotype produce three fragments of 215, 171, and 44 bp fragments.

To verify proper amplification conditions, 10 μl of PCR product were analysed on a 2% agarose gel and stained with ethidium bromide, the amplification of was revealed by the presence of bands. Homozygote wild-type CC genotype produce single band of 44 bp length, homozygote mutated GG genotype results from two 44 and 171 bp fragments and heterozygote GC genotype produces three fragments of 215, 171 and 44 bp fragments. Digestion conditions were performed according to the manufacturer’s instructions and summarized for each gen in (Table 3).
Digestion products were separated at the appropriate concentrations on a 2, 3 or 4% Low melting point agarose gel and stained with ethidium bromide. The splice-site mutation of CYP19A1 (rs749292) (A-G intron) and CYP2C8*4 (rs1058930) (C-G exon5) was also analysed by PCR-RFLP, PCR amplification also was performed the same as the above genes. The cycling conditions for both of genes including one pre-treatment cycle denaturation at 94°C for 5 min, 32 cycles of denaturation at 94°C for 30 sec, followed by annealing at 66°C for 30 sec and elongation at 72°C for 25 sec and a final elongation at 72°C for 10 min. Products were analysed by electrophoresis at 2% agarose gel.

Table 3: Restriction enzymes used for Genes polymorphism genotyping

| SNPS   | Restriction Enzymes | Temperature and Incubation time | Fragment size (bp) |
|--------|---------------------|---------------------------------|--------------------|
| rs4646421 | FastDigestBsuI (10 µ) | 37°C-5 min 519, 183 + 336 |
| rs1058930 | TaqI (10 µ) | 65°C-1h16 358, 144 + 214 |
| rs1058930 | TaqI (10 µ) | 65°C-1h16 303, 84 + 219 |

**Statistical analysis**

The genotype and allele frequency of the genes were tested for both patient and control group using chi-square test, Fisher exact test, quantitative (numerical) parameters analyzed by t-student test. Odds ratio (OR), confidence intervals (CI) and P-values were calculated using unconditional logistic regression and adjusted estimate the association between genotypes or some other clinicopathological data and the risk of breast cancer. In this research, statistical analyses were performed using SPSS software ver. 21.

**Results**

**Demographic and clinicopathological data**

This study was performed in 72 breast cancer patients and 51 healthy controls with known demographic and clinicopathological data in Mazandaran province in the north of Iran. Characteristics of the study population were compared by case-control status, as shown in Table 1. Student's t-test showed significant relationships between the two groups and the mean age of controls (43.69 ± 13.5 years) was significantly lower (P = 0.044) than that of breast cancer patients (48.08 ± 10.3 years). Chi-square and Fisher exact test showed that Pregnancy, menopausal status, family history of breast cancer and education, stage of cancer and grade of the tumour were significantly different between cases and controls. However, no significant differences were found between them regarding BMI, Age at menarche, age at menopause and Age at 1 St pregnancy. In the case group, the frequency of level education is lower than diploma and difference is significant (p < 0.001).

The majority of cases have a pregnancy in the patient group, and the difference between the two groups was significant (p = 0.036). Regarding the menopause status, the majority of cases were not at the menopause status (P = 0.044). In the case groups, the majority of people have 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 tumours 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 distribution of genotypes in two groups in CYP2C8 (P = 0.11), CYP19A1 (P = 0.019), CYP1B1 (P = 0.026) Had a significant difference, and only in CYP1A1 (P = 0.416) is homogeneous (Table 4).

Table 4: Genotype frequencies of genes among cases and controls, and risk of breast cancer

| Gene name | Genotype | Groups  | Chi-square test |
|-----------|----------|---------|-----------------|
|            |          | Cases   | Controls        | Test statistic | Fisher's exact test | P value |
| CYP2C8     | GG       | 82      | 48             | 48.41          | -                 | 7.4     | 0.011*          |
|            | CG       | 0       | 2              | (3.9)          | -                 | 7.7     | 0.019*          |
|            | GC       | 14      | 25             | 42.33          | -                 | -       | -               |
| CYP19A1    | GA       | 42      | 52             | (52.99)        | -                 | -       | -               |
|            | GG       | 27      | 14             | (27.5)         | 0.011             | -       | -               |
|            | CC       | 16      | 30             | (17.45)        | 0.019*            | -       | -               |
| CYP1A1     | TC       | 16      | 13             | (22.22)        | 0.17              | -       | 0.416           |
|            | TT       | 0       | 0              | 0              | -                 | -       | -               |
|            | CC       | 50      | 74             | (52.99)        | 0.026*            | -       | -               |
| CYP1B1     | GG       | 20      | 5              | (51.98)        | 7.26              | -       | -               |
|            | CC       | 27      | 30             | 14(18.4)        | 8(15.7)           | -       | -               |

Also, there was no significant correlation between age and breast cancer risk (P = 0.13). But there was a significant relationship between the risk of cancer and menopause status, and the chance of having cancer is 0.466 times lower than those who did not have menopause status (p = 0.048). Besides, there was a significant relationship between the risk of cancer and LD Consumption, and these people have 0.47 times higher risk of breast cancer (P = 0.043) (Table 5).
Table 5: Relation between demographic characteristic and risk of breast cancer

| Group          | Case | Control | P-value | OR (95% CI) |
|----------------|------|---------|---------|-------------|
| Age < 40       | 31 (25.2%) | 29 (22.6%) | 0.57 | 0.13 (0.28,1.19) |
| Age 41-50      | 41 (33.3%) | 22 (17.2%) |       |              |
| Age > 50       | 27 (21.8%) | 12 (9.1%)  |       |              |
| Agriculture    | YES  | 25 (20.4%) |       |              |
|                | NO   | 36 (29.3%) |       |              |
| Menopause status | YES | 18 (15.2%) | 0.466 | 0.048* (0.22,0.99) |
|                | NO   | 38 (30.9%) |       |              |
| Family history | NO   | 34 (27.1%) |       |              |
|                | YES  | 29 (23.5%) |       |              |
| DCP USE        | YES  | 30 (24.4%) |       |              |
|                | NO   | 34 (27.6%) |       |              |
| Grade          | I, II | 21 (17.1%) | 0.47 | 0.043* (0.23,0.98) |
|                | III, IV |            |       |              |
| Stage          | I-IV |         |         |              |

Regarding the relationship between the genotypes of each gene and the demographic characteristics, it can be concluded that there is no significant difference between the genotype and the demographic characteristics such as the age of the patients, education, pregnancy history, menopause status, agricultural occupation, LD consumption, stage of disease, grade of tumours and BMI. There was only a 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, 72 breast cancer patients and 51 healthy controls were studied. The results of the experiments showed in (Table 6). The frequency of genotypes CYP1B1 gene among of 72 patients was CC (52.8%), CG (27.8%) and GG (19.4%), and in healthy subjects 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 healthy controls were CC (80%) and G allele (20%). Regarding the CYP1A1 gene, the frequency of genotypes in patients was CC (77.8%), CT (22.2%) and TT (0%) and in healthy subjects were CC (74.5%), CT (25.5%) and TT (0%), and the frequency of allele C was determined in patients (89%) and G allele (11%) and allele C (87%) and G (13%) in controls have frequency. 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 GG (27.5%), GA (52.9%) and AA (19.6%). The prevalence of alleles in patients with allele Cis (66%) and T (34%) and the healthy subjects, allele C (54%) and T (46%). Regarding the CYP2C8 gene, the frequency of CC (86.1%), CG (0%) and GG (13.9%) genotypes in patients and 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 (Table 6). 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 that in the CYP2C8 genotype, those who have the CC allele have a 7.44 degree increased risk of breast cancer compared to those who have the CC genotype and in the CYP19A1 gene of individuals with GA genotype, the risk of breast cancer was 6.42 compared to those of genotype AA (CI=%95 1.52–27.21) (Table 6), about the CYP1B1 gene, people with two genotypes of CG + GG is associated with a higher risk of breast cancer 2.61 times higher compared to the CC genotype (CI = %95 1.19-5.71) and allele G has protective effect in breast cancer and decreased risk of breast cancer (P = 0.0271) and CYP2C8 gene allele G decreased risk of breast cancer (P = 0.02). In the age-adjusted model, for the CYP2C8 gene, GG genotype compared to CC has an increased risk of breast cancer about 9.17 compared to other genotypes (CL = %95 1.11-75.84). Regarding CYP1B1, the CG + GG genotype is 2.93 times higher than CC genotype increased risk of breast cancer (CL=95% 1.31-6.57).

Table 6: Relationship between the genotypes and breast cancer risk

| Genes name | Genotype and Alleles | Cases (%) | Controls (%) | Non-Adjusted OR (95% CI) | Adjusted OR (95% CI) |
|------------|----------------------|-----------|--------------|--------------------------|----------------------|
|          | CC vs GG              |           |              |                          |                      |
|          | C                  | 96%       | 98%         | Ref                      | Ref                  |
|          | G                  | 14%       | 2%          |                          |                      |
| CYP2C8    | AA vs GG             | 72 (58.3%)| 51 (41.4%)  | 0.167                    | 0.012                |
|          | CC                  | 96%       | 98%         | Ref                      | Ref                  |
|          | CG + GG             | 4%        | 2%          |                          |                      |
|          | G                  | 14%       | 2%          |                          |                      |
|          | T                  | 86%       | 98%         | Ref                      | Ref                  |
|          | Ref                 |           |              |                          |                      |
| CYP19A1   | AA vs GG             | 39 (29.3%)| 47 (37.2%)  | 0.42                     | 0.22                 |
|          | CC                  | 73%       | 60%         | Ref                      | Ref                  |
|          | CG + GG             | 27%       | 40%         |                          |                      |
|          | G                  | 54%       | 32%         |                          |                      |
|          | Ref                 |           |              |                          |                      |
| CYP1A1    | AA vs GG             | 72 (58.3%)| 51 (41.4%)  | 0.642                    | 0.421                |
|          | CC                  | 96%       | 98%         | Ref                      | Ref                  |
|          | CG + GG             | 4%        | 2%          |                          |                      |
|          | G                  | 14%       | 2%          |                          |                      |
|          | Ref                 |           |              |                          |                      |
| CYP1B1    | CC vs GG             | 72 (58.3%)| 51 (41.4%)  | 0.835                    | 0.661                |
|          | C                  | 96%       | 98%         | Ref                      | Ref                  |
|          | G                  | 14%       | 2%          |                          |                      |
|          | Ref                 |           |              |                          |                      |

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 CYP1B1 gene (P-value = 0.015, OR = 0.38, CI = 0.18, 0.84), and no significant results were obtained for other (Table 7).

It's noteworthy that CC genotype of CYP1B1 decreases about 0.38-time risk of breast cancer in comparison the CC + GG genotype. In addition, for 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 significant relationship between two groups (Table 7).
Breast cancer is by far the most common female cancer and comprising about 21% of all new cancers in women. The highest age-adjusted incidence rate is reported for North America, is 87 per 100 thousand women per year, while the lowest rate 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 are reported 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 [27]. The aetiology of breast cancer could not be described by allelic variability at a single locus. Instead, the main burden of breast cancer in the population probably results from complex interactions between many genetic and environmental factors over time. 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 for breast cancer. The genetic polymorphisms that may be linked to breast cancer are multiple. Cumulative lifetime exposure to estrogen, estrogen metabolites, and other physiological factors, as well as environmental exposures, could play an important role in the aetiology of breast cancer in genetically predisposed women. Carcinogenesis, determining response to drugs and cell signalling. In the metabolism of xenobiotic (foreign chemicals), cytochrome P450s or monooxygenases perform an important function by catalysing the hydroxylation reaction [28]. CYP450 enzymes associated with the development of breast cancer which involved in biosynthesis and metabolism of estrogens and other CYP enzymes can involve in the development of breast cancer risks like CYP19, CYP21, CYP17, CYP1A2, CYP1D1, CYP2D6, CYP2C19, CYP3A4/5, CYP1A1, CYP1B1, CYP2C8/9. In this study, distribution of CYP450 isoenzymes CYP1A1 (rs4646421), CYP1B1 (rs1056836), CYP19A1 (rs749292) and CYP2C8 (rs1056836) and gene polymorphisms in patients with breast cancer in Mazandaran province was investigated by the PCR-RFLP method using restriction enzyme activity.

In a study by Mandana Gheyasar et al., they determined that through PCR-RFLP reaction, 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 CYP19A1 are not associated with breast cancer risk [29]. Joanna Trubicka et al. genotyped 597 cancer patients and 597 controls for three CYP1B1 SNPs. 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 CYP1B1 that are associated with altered function appear to influence susceptibility to colorectal cancer in Poland (30). In another study, Marc T Goodman et al., 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 showed that the A allele of rs749292 was positively associated with ovarian cancer risk in a codominant model for all races combined (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); P trend = 0.002. Similar significant associations of the rs749292 A allele on the 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 suggest CYP19A1 variants may influence susceptibility to ovarian cancer [31].

Jernstrometal. Investigated CYP2C8 and CYP2C9 polymorphisms about tumour characteristics and early breast cancer. In a prospective series of 652 breast cancer patients from southern Sweden was 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 (P=0.002) and fewer PR tumours (P=0.012) were associated with CYP2C8*4. Median follow-up was 25 months, and 52 breast cancer-related events were reported. In a multivariate model,
CYP2C8*9/3/*1/*2/*1 was the only factor associated with increased risk for early events in 297 tamoxifen-treated, ER-positive patients, adjusted HR 2.54 (CI = 95% 1.11–5.79). The effect appeared to be driven by CYP2C8*3, adjusted HR 8.56 (95%CI 1.53-51.1). They found that polymorphic variants of CYP2C8/9 may influence breast tumour characteristics and disease-free survival in tamoxifen-treated patients [32].

In conclusion, the results of this study indicate that CYP2C8 (rs1058930), CYP19A1 (rs7492929) and CYP1B1 (rs1056836) gene polymorphisms are associated with breast cancer, and screening for these genes polymorphisms can be used to prognosticate disease, prevent disease progression, and to use appropriate therapeutic.

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