

Study of Cytochrome P450 1A1 (T3801C) Single Nucleotide Polymorphism in Patients with Breast Cancer in Mazandaran Province-Northern Iran

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Abstract

Background: Breast cancer is the first leading cause of cancer-related death in women. Pesticides which are excessively used in northern Iran are one of the most important risk factors for breast cancer incidence. The cytochrome P450 1A1 (cyp1A1) is a key enzyme in xenobiotics metabolism and SNPs of its coding gene has been verified to be important in cancer susceptibility. The aim of this study was to evaluate the association of cyp1A1 M1 polymorphism with the risk of breast cancer in Mazandaran province.

Materials and Methods: Ninety six breast cancer patients with known clinopathological characters and 110 healthy women as control were genotyped for cyp1A1 M1 polymorphisms by PCR-RFLP technique using MspI restriction enzymes. Logistic regression model was applied for statistical analysis.

Results: The frequency of TT and TC genotypes of M1 polymorphism was calculated 86, 14% for cases and 79 and 21% for control group, respectively. Surprisingly, the mutant CC genotype was not found in any subjects. Statistical analysis showed no significant correlation between allelic variants and breast cancer risk (p value; 0.42, OR; 0.66, CI; 0.24-1.81). No significant correlation was also found between genotypic frequency and clinopathological characters.

Conclusion: Only TT and TC genotypes were found in the studied subjects. The M1 allelic variants were significantly associated neither with breast cancer risk nor with clinopathological characteristics.

Keywords: Breast cancer; cyp1A1 gene; Single nucleotide polymorphism; Pesticide

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Introduction

Breast cancer is the most prevalent malignancy of women worldwide (1, 2). According to a report of the Iran's Ministry of Health and Medical Education, breast cancer has become the most common primary cancer in Iranian women (3). Iranian Breast cancer cases are one decade younger than their western counterparts (4). The etiology of breast cancer is complex; a small proportion of breast cancer cases can be attributed purely to genetic reasons whereas for a vast number of cases there is compelling evidence for the role of other factors, such as family and reproductive history, diet, alcohol consumption, and exposure to environmental carcinogens (5, 6, 7). Pesticides are among the most carcinogenic

compounds. According to statistics released by the Plant Protection Organization of Mazandaran province high amounts of organophosphorus pesticides are being used for protection of over 600,000 hectares under cultivation of crops. Pesticides can easily enter surface water through irrigation water and rain leading to water pollution and can either directly or indirectly threat human health (8). Many carcinogenic compounds are oxidized by phase I enzymes, represented by cytochrome P450 family, into reactive metabolites that are detoxified by phase II enzymes. Hence, the toxic effects of exposure, absorption and detoxification of carcinogens depends on a delicate

balance between the phase I and phase II enzymes (9, 10). The cytochrome P450 enzymes are regulated at the transcriptional level and its expression is influenced by genetic factors, polymorphism in the structural and regulatory gene and by environmental factors (11, 12). Polymorphisms and expression pattern of these genes are believed to be key factors in determining cancer susceptibility to toxic or environmental chemicals (13). The cytochrome P4501A1 (*cyp1A1*) enzyme is one of the major components of detoxification pathway that is highly expressed in non-hepatic cells such as breast tissue (14). The *cyp1A1*, located on chromosome 15q22–q24, is a 5987-bp long gene that encodes for a 512 amino acid protein (15). It is a polymorphic gene involved in metabolism of steroids and several potentially genotoxic chemicals (16). 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; M3 T/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 (17, 18). In a population-based, case-control study we analyzed the association of the *cyp1A1* M1 genotypes with breast cancer risk in Mazandaran province.

Materials and Methods

Cases and controls

The study involved 96 patients who underwent surgery in some referenced hospitals in Mazandaran province from September 2012 to December 2014. Patients' age ranged from 29 to 72 years. Demographic and clinopathological data of patients were extracted from their records in hospitals. A group of 110 healthy females ranging from 23 to 83 years were also included in this study to investigate whether certain M1 genotype is a susceptible marker. Five ml peripheral blood was collected from both patients and control group and stored at -80 °C.

DNA extraction

Blood samples were collected in EDTA-containing tubes and genomic DNA was extracted from blood lymphocytes by proteinase K/SDS digestion and phenol-chloroform extraction method (19). DNA concentration was measured spectrophotometrically and its purity was checked through agarose gel electrophoresis.

cyp1A1 MspI genotyping (*cyp1AIM1*)

The *cyp1A1* M1 single nucleotide polymorphism

involves a T>C substitution at position 3801 of the 3' untranslated region (3'-UTR) which creates a MspI restriction site. The genotype of this region was determined by polymerase chain reaction-restriction fragments length polymorphism (PCR-RFLP). A 340 bp fragments containing T/C allele was amplified using forward: 5'- CAGTGAAGAGGTGTAGCC GCT -3' and reverse: 5'- TAGGAGTCTTGTCTGAT GCCT -3' primers. PCR amplification was performed in a 25 µl reaction containing 1X PCR buffer, 100 ng genomic DNA, 1.5 mM MgCl₂, 0.3 mM each forward and reverse primers, 0.2 µM dNTPs and 2.5 U taq DNA polymerase (10 u/µl). The cycling conditions including an initial denaturation at 94°C for 4 min, 32 cycles of denaturation at 94°C for 40 sec, annealing at 55 °C for 35 sec and extension at 72 °C for 37 sec and a final extension at 72 °C for 7 min. Products were analyzed by electrophoresis at 1.5% agarose gel and visualized by ethidium bromide staining. The amplified fragment was digested with 1 U MspI restriction enzyme at 37 °C for 2 hr and was analyzed on 2% agarose gel. When digested with MspI the homozygote wild-type TT genotype produce a single band of 340 bp length, the homozygote mutated CC genotype results in two 200 and 140 bp fragments and heterozygote TC genotype produce three fragments of 340, 200 and 140 bp fragments (figure 1). A sample of each genotype was sequenced in Bioneer company (south Korea) to verify the results of genotyping.

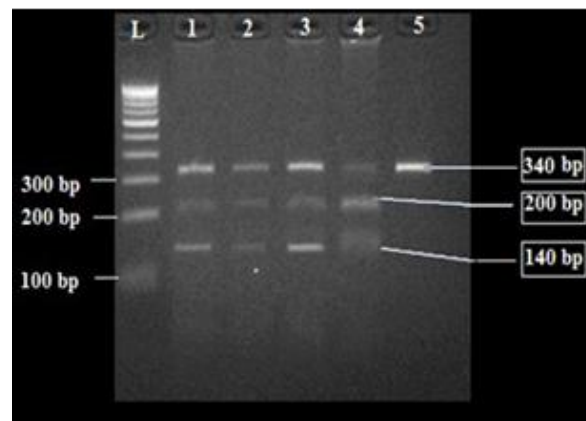


Figure 1. Agarose gel electrophoresis of PCR-RFLP patterns of m1 allelic variant of *cyp1A1* gene. L: 100 bp DNA ladder (Fermentas); 1- 4: Heterozygous TC genotype; 5: Wild-type homozygous TT genotype.

Ethics Statement

All patients and healthy controls provided their verbal informed consent to participate in this study.

Statistical analysis

The genotype and allele frequency of *cyp1A1* M1 genotype were tested for Hardy-Weinberg

equilibrium (HWE) for both patient and control group using χ^2 test. Odds ratio (OR), confidence intervals (CI) and P-values were calculated using unconditional logistic regression and adjusted for age to estimate the association between genotypes or some other clinopathological data and the risk of breast cancer (20). All data were analyzed by SAS 9.1 statistics software and $P < 0.05$ was considered as statistical significance.

Results

Demographic and clinopathological data

This study was performed in 96 breast cancer patients and 110 healthy controls with known demographic and clinopathological data (table 1) in Mazandaran province, northern Iran.

Table 1. Demographic and clinopathological characteristics of patientsa.

Clinopathological Variables	No. of Patient (%)
Age	
≤45	42 (45.6)
>45	50 (54.4)
Menopause	
Positive	38 (42)
Negative	53 (58)
Grade	
I	3 (5)
II	53 (60)
III	31 (35)
Family history	
Positive	7 (10)
Negative	78 (90)
Smoking	
Positive	6 (7)
Negative	80 (93)

The mean ages of patients and healthy individuals were 48.21 ± 8.2 and 46.27 ± 6.1 years, respectively. Student's t-test showed no significant relationships between the two groups ($p > 0.365$). Chi-square test showed that the genotype frequency of case and control groups did not significantly diverge from HWE (both $p > 0.05$). Despite the importance of family history in disease occurrence only 10% of patients had a positive history. Results of pathologic information showed that 55% of cases were diagnosed at advanced stages.

Table 2. Distribution of *cyp1A1* gene polymorphisms and breast cancer risk

Polymorphism	Genotype	No of subjects (%)		Non-adjusted ^a		Adjusted ^b		CI
		Case	Control	P value	OR	P value	OR	
M1	TT	83 (86)	87 (79)	-	1	-	1	-
	TC	13 (14)	23 (21)	0.30	0.59	0.42	0.66	0.24-1.81

^a Logistic regression model, non-adjusted.

^b Logistic regression model, adjusted for diagnostic age.

The cyp1A1 genotype distribution and its association to known clinopathological data

The *cyp1A1* m1 allelic variants were determined using PCR-RFLP and resulting genotype distribution is presented in table 2. The allelic frequency of TT and TC genotypes was 89% and 14% for the case and 79% and 21% in control group, respectively (table 2). Surprisingly, the homozygote CC genotype was not present in any case and control groups. As shown in table 2, in the logistic regression model no significant association was found between M1 allelic frequencies of subjects and breast cancer risk (P value; 0.42, OR; 0.66, CI; 0.24-1.81). The TT and TT+TC genotypes were not significantly correlated with demographic and clinopathological characteristics including age at diagnosis ($p = 0.94$), menopause ($p = 0.49$), grade ($p = 0.22$), smoking ($p = 0.96$), and family history ($p = 0.64$) (Table 3).

Discussion

Breast cancer is one of the most common female malignant tumors in Iran (21). 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 (5). Cytochrome P450 (CYP) 1A1, a member of human CYP1 family, is generally expressed in extrahepatic tissues for bioactivation of xenobiotics such as pesticides and drugs (22). Some single nucleotide polymorphisms occurring in genes coding for these xenobiotic-metabolizing enzymes are known to influence their functional properties and their association to cancer incidence have been stated in several reports (23). In this study, we investigated M1 polymorphisms of *cyp1A1* in 96 breast cancer patients along with 110 healthy controls in an attempt to investigate its association to breast cancer risk in Mazandaran province. The mean age of breast cancer patients (48.21 ± 8.2 years) in this study is consistent with the results of other researches in Iran, verifying again the younger age of breast cancer development for Iranian women (24, 25). Most patients were diagnosed to be at grades II and III. Similar results were also reported in a research conducted on a group of women with breast cancer ($n = 3085$) in Iran (25).

These reports emphasize that cultural and social issues, lifestyle change and reproductive behaviors are amongst the key factors for late diagnosis of breast cancer in Iranian women. So, breast self-examination and regular evaluations may be helpful to screen for the disease at primary stage (26). PCR-RFLP analysis of M1 polymorphism in this study

showed that just wild-type homozygote TT and heterozygote TC genotypes were appeared in our subjects. But, none of the cases and healthy controls showed mutant CC genotype. Statistical analysis by logistic regression model indicated no significant relationship between M1 genotype frequency and breast cancer risk ($p=0.42$ and $OR=0.66$).

Table 3. Relationship between *cyp1A1* (M1) polymorphism and known clinopathological variables.

clinopathological Variables	Genotype (%)		P value	Adjusted ^a	
	TT	TT+TC		OR	CI
Age					
≤ 45	34 (82)	8 (18)	-	1	-
>45	41 (83)	9 (17)	0.94	1.05	0.21-5.29
Menopause					
Negative	35 (91)	3 (9)	-	1	-
Positive	43 (82)	10 (18)	0.49	0.31	0.01-5.66
Grade					
I – II	48 (82)	10 (18)	-	1	-
III	28 (90)	3 (10)	0.22	0.25	0.002 – 2.30
Family history					
Negative	66 (87)	12 (13)	-	1	-
Positive	5 (67)	2 (33)	0.64	0.51	0.04-6.37
Smoking					
Negative	70 (88)	10 (12)	-	1	-
Positive	6 (100)	0 (0)	0.96	>999.999	<0.001- > 999.999

^aLogistic regression model adjusted for diagnostic age. All statistical tests were two-sided with a significance of $p<0.05$

Several genetic polymorphisms have been identified in *cyp1A1* gene. But, no agreement has been made on the association of these SNP and breast cancer risk. Although, similar results indicating no association between *cyp1A1* gene variants and breast cancer risk was presented by Bailey et al., (27) some other reports conversely pointed out positive associations (28,29). The study of relationship between M1 genotypes and clinopathological characteristics such as age, menopause, smoking, family history and stage of the cancer demonstrated no significant correlation. Study of the association between allelic variants and clinopathological features did not create consistent results. Wang et al. found an association between M1 genotype and stage of cancer (30). Overall, the discrepancy between the results of the association between SNP and breast cancer risk may be due to several factors including difference in ethnicity, diet, geographical variation and environmental exposures. Further researches with larger groups, are needed to clarify these points.

Conclusion

This case-control population-based study was performed in 96 breast cancer patients with known clinopathological characters and 110 healthy women

as control to determine genotypic variants of *cyp1A1* M1 polymorphisms in Mazandaran province, north of Iran. Only TT and TC genotypes were found in the studied subjects and Logistic regression model applied for statistical analysis showed significant correlation of M1 allelic variants neither with breast cancer risk nor with clinopathological characteristics.

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Authors' Contributions

AB and GK participated in the whole process of the study including design of research work, data analysis and drafting manuscript. NN performed surgery and provided samples. ZAP helped in providing some samples and equipments.

Conflicts of Interest

The authors have no conflict of interest to declare.

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References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics. *CA Cancer J Clin.* 2005; 55(2): 74–108. PMID: 15761078
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics. *CA Cancer J Clin.* 2008; 58(2): 71-96. PMID: 18287387
3. Mehrabani D, Ahrari Z, Rezaianzadeh A, Mehrabani G, Talei AR. Incidence of Breast Cancer in Fars Province, Southern Iran: A Hospital-Based study. *Word J Plast Surg.* 2012; 1(1): 16-21. PMID: 257340
4. Khadivi R, Harrirchi I, Akbari ME. Ten year breast cancer screening and follow up in 52200 women in Shahre-Kord, Iran (1997-2006). *Iran J Cancer Prev.* 2012; 1(2): 73-77.
5. Collins LC, Baer HJ, Tamimi RM, Connolly JL, Colditz GA, Schnitt SJ. The influence of family history on breast cancer risk in women with biopsy-confirmed benign breast disease: results from the Nurses' Health Study. *Cancer.* 2006; 107: 1240–1247
6. Faraglia B, Chen SY, Gammon MD, et al: Evaluation of 4-aminobiphenyl DNA adducts in human breast cancer: The influence of tobacco smoke. *Carcinogenesis.* 2003; 24: 719-725.
7. Pineda B, Garcia-Perez MA, Cano A, Lluch A, Eroles P. Associations between aromatase CYP19 rs10046 polymorphism and breast cancer risk: from a case-control to a meta-analysis of 20,098 subjects. *PLoS one.* 2013; 8(1): 53902. PMID: 23342035
8. Shokrzadeh M, Karami M, Ghadi MAE. Measuring Organophosphorus Insecticide Residue in Rice Produced in Amol, North of Iran. *J Mazandaran Univ Med Sci.* 2013; 23(1): 215-21.
9. Bartsch H, Nair U, Risch A, Rojas M, Wikman H, Alexandrov K. Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiol Biomarkers Prev.* 2000; 9(1): 3-28. PMID: 10667460.
10. Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nat Rev Cancer.* 2006; 6(12): 947-60. PMID: 17128211
11. Pavek P, and Dvora Z. Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochrome p450 superfamily in human extrahepatic tissues. *Current Drug Metabolism.* 2008; 9(2): 129-43.
12. Goth-Goldstein R, Stampfer MR, Erdmann CA, Russell M. Interindividual variation in CYP1A1 expression in breast tissue and the role of genetic polymorphism. *Carcinogenesis.* 2000; 21(11): 2119-22. PMID: 1106217.
13. Safarinejad MR, Dadkhah F, Asgari M, Hosseini SY, Kolahi AA, Iran-Pour E. Glutathione S-Transferase Polymorphisms (GSTM1, GSTT1, GSTP1) and Male Factor Infertility Risk: A Pooled Analysis of Studies. *Urol J.* 2012; 9(3): 541-8. PMID: 22903474
14. Zhou SF, Liu JP, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. *Drug Metab Rev.* 2009; 41(2): 89-295. PMID: 19514967
15. Lu N, Wu B, Xia Y, Wang W, Gu A, Liang J, et al.

Polymorphisms in CYP1A1 gene are associated with male infertility in a Chinese population. *Int J Androl.* 2008; 31(5): 527-33. PMID: 17651397

16. Schwarz D, Kisselev P, Cascorbi I, Schunck WH, Roots I. Differential metabolism of benzo [a] pyrene and benzo [a] pyrene-7, 8-dihydrodiol by human CYP1A1 variants. *Carcinogenesis.* 2001; 22(3): 453-9. PMID: 11238186
17. Srivastava DS, Mandhani A, Mittal RD. Genetic polymorphisms of cytochrome P450 CYP1A1 (*2A) and microsomal epoxide hydrolase gene, interactions with tobacco users, and susceptibility to bladder cancer: a study from North India. *Arch Toxicol.* 2008; 82: 633-9. PMID: 18200441
18. Ding X, Kaminsky LS. Human extrahepatic cytochrome P450: Function in Xenobiotic Metabolism and Tissue-Selective Chemical Toxicity in the Respiratory and Gastrointestinal Tracts. *Annu Rev Pharmacol Toxicol.* 2003; 43(1): 149-73. PMID: 12171978
19. Green MR, Sambrook J. Molecular cloning: a laboratory manual. Vol.1. New York: Cold Spring Harbor Laboratory Press, 2012.
20. Huang B, Guo J, Charnigo R. Statistical Methods for Population-Based Cancer Survival in Registry Data. *J Biomet Biostat.* 2014; 5: e129.
21. Sadjadi A, Malekzadeh R, Derakhshan MH, Sepehr A, Nouraei M, Sotoudeh M, Alimohammadian M. Cancer occurrence in Ardabil: Results of a population-based Cancer Registry from Iran. *Int J Cancer.* 2003; 107(1): 113-8. PMID: 12925965
22. Roddam PL, Rollinson S, Kane E, Roman E, Moorman A, Cartwright R, Morgan GJ. Poor metabolizers at the cytochrome P450 2D6 and 2C19 loci are at increased risk of developing adult acute leukaemia. *Pharmacogenetics,* 2000; 10(7): 605-15. PMID: 11037802.
23. Gra OA, Glotov AS, Nikitin EA, Glotov OS, Kuznetsova VE, Chudinov AV, Nasedkina TV. Polymorphisms in xenobiotic-metabolizing genes and the risk of chronic lymphocytic leukemia and non-Hodgkin's lymphoma in adult Russian patients. *Am J Hematol.* 2008; 83(4): 279-87. PMID: 18061941.
24. Harirchi I, Kolahdoozan S, Karbakhsh M, Chegini N, Mohseni SM, Montazeri A, Ebrahimi M. Twenty years of breast cancer in Iran: downstaging without a formal screening program. *Ann Oncol.* 2011; 22(1): 93-7. PMID: 20534622.
25. Sadjadi A, Nouraei M, Ghorbani A, Alimohammadian M, Malekzadeh R. Epidemiology of breast cancer in the Islamic Republic of Iran: first results from a population-based cancer registry. *East Mediterr Health J.* 2009; 15(6): 1426-31. PMID: 20218134.
26. Harirchi I, Ebrahimi M, Zamani N, Jarvandi S, Montazeri A. Breast cancer in Iran: a review of 903 case records. *Public Health.* 2000; 114(2): 143-5. PMID: 10800155.
27. Gaspar PA, Moreira J, Kvitko K, Torres MR, Moreira A, Weimer TA. CYP1A1, CYP2E1, GSTM1, GSTT1, GSTP1, and TP53 polymorphisms: Do they indicate susceptibility to chronic obstructive pulmonary disease and non-small-cell lung cancer? *Genet Mol Biol.* 2004; 27(2): 133-8.
28. Shin A, Kang D, Choi JY, Lee KM, Park SK, Noh DY, et al. Cytochrome P450 1A1 (CYP1A1) polymorphisms and breast cancer risk in Korean women. *Exp Mol Med.* 2007; 39(3): 361-6. PMID: 17603290

29. Masson LF, Sharp L, Cotton SC, Little J. Cytochrome P-450 1A1 gene polymorphisms and risk of breast cancer: A HuGE Review. *Am J Epidemiol.* 2005; 161 (10): 901-15. PMID: 15870154

30. Wang H, Wang WJ. Relationship between CYP1A1 polymorphisms and invasion and metastasis of breast cancer. *Asian Pac J of Trop Med.* 2013; 6(10): 835-8. PMID: 23870476.