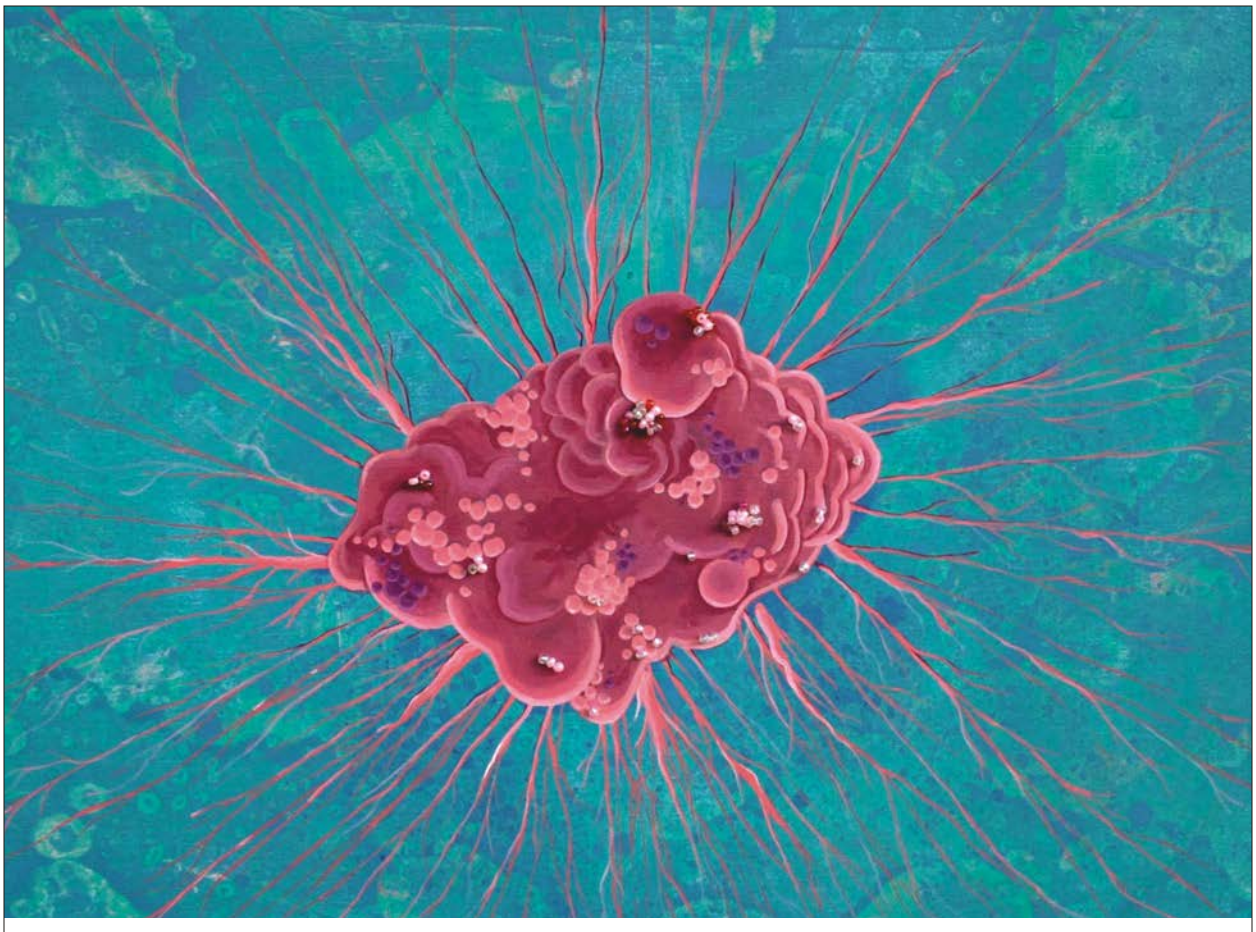

International Journal of Cancer Research

ISSN 1811-9727

Academic Journals Inc.

www.academicjournals.com

International Journal of Cancer Research 2 (4): 415-419, 2006
ISSN 1811-9727

Combination of *XRCC1*, *GSTM1* and *GSTT1* Genetic Polymorphisms and Susceptibility to Male Breast Cancer

[1,2]Leila Kohan, [3]Shahpour Omidvari, [1]Mostafa Saadat
[1]Department of Biology, College of Sciences, Shiraz University, Shiraz 71454, Iran
[2]Department of Biology, Khatam University, Tehran, Iran
[3]Department of Chemotherapy, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract: Male breast cancer is an uncommon disease that has been the focus of limited research. In the present study, we specifically investigated whether combination of common genetic variants in the X-ray repair cross-complementation group 1 gene (*XRCC1*; exon 10, codon 399 *Arg/Gln* and exon 6, codon 194 *Arg/Trp*) and loss-of-function deletion polymorphisms in glutathione S-transferases T1 (*GSTT1*) and M1 (*GSTM1*), were associated with an altered risk of male breast cancer. Blood samples from 10 males with breast cancer and 15 age- and sex-matched healthy persons were collected. The *GSTM1*, *GSTT1* and *XRCC1* genotypes were determined using PCR-based method. The null genotypes of *GSTM1* and *GSTT1* and *399Gln* and *194Trp* alleles of *XRCC1* assumed as high-risk genotypes and alleles. Statistical analysis showed that the χ^2 for linear trend for 0, 1, 2 and 3 putative high-risk genotypes is equal to 5.673 ($p = 0.017$).

Key words: Genetic polymorphism, male breast cancer, *XRCC1*, *GSTM1*, *GSTT1*

Introduction

Male Breast Cancer (MBC) is a rare disease that has been the focus of limited research. It accounts for less than 1% of all cases of cancer in men and about 0.7% of all diagnosed breast cancer cases (Giordano, 2005). Very little is known about the genetic background of MBC. Polymorphisms in a few genes (such as androgen receptor, *BRCA1*, *BRCA2*) have been associated with predisposition to the development of male breast cancer (Basham *et al*., 2002; Csokay *et al*., 1999; MacLean *et al*., 2004). The strongest known risk factors for MBC are mutations in *BRCA1* and particularly *BRCA2* gene (Basham *et al*., 2002; Csokay *et al*., 1999; MacLean *et al*., 2004).

Enzymes involved in the detoxification of carcinogenic compounds as well as DNA repair system may play an important role(s) in the susceptibility to develop some types of cancers. GSTs constitute a super family of ubiquitous, multi functional enzymes, which play a key role in detoxification. The GSTs catalyze the conjugation of the tripeptide glutathione (GSH) to a wide variety of exogenous and endogenous chemicals with electrophilic functional group. Both *GSTM1* (a member of GSTμ) and *GSTT1* (a member of GSTθ) are known to be polymorphic in human and both of them have null alleles resulting in gene deletion and complete absence of enzyme activity. A number of studies have considered *GSTM1* and *GSTT1* polymorphism in relation to female breast cancer risk, with conflicting results (Egan *et al*., 2004; Saadat and Saadat, 2004; Vogl *et al*., 2004).

The X-ray repair cross-complementation group 1 (XRCC1) protein plays an important role in base excision repair (Shu *et al*., 2003). It is reported that the *Arg*194Trp and *Arg*399Gln polymorphisms of *XRCC1* may modulate repair function and are involved in cancer susceptibility (Divine *et al*., 2001; Duell *et al*., 2001; Smith *et al*., 2003; Stren *et al*., 2001; Sturgis *et al*., 1999). The

Corresponding Author: Mostafa Saadat, Department of Biology, College of Sciences, Shiraz University, Shiraz 71454, Iran Tel: +98-711-2273900 Fax: +98-711-2280926

399 Gln and 194*Arg* alleles have been linked to an increased risk of several types of cancers (Divine *et al*., 2001; Stren *et al*., 2001; Sturgis *et al*., 1999). In additional, several studies have reported a positive association between polymorphisms of *XRCC1* at codon 399 and/or codon 194 and female breast cancer but the results are inconsistent (Duell *et al*., 2001; Smith *et al*., 2003).

Because there is no published data on the *GSTM1*, *GSTT1* and polymorphism of *XRCC1* at codons 399 and 194 and susceptibility to male breast cancer, the present study was done.

Materials and Methods

Subjects

This case-control study consisted of 10 males with breast cancer that were recruited from chemotherapy department of Nemazee hospital in Shiraz, Iran, during February 2005 to February 2006. A total of 15 healthy male matched with patients according to age (±5 years), as a control group. The mean age of the patients and the controls was 63.5±11.1 years and 58.13±8.4 years, respectively. Subjects were unrelated Iranian Muslims. Informed consent was obtained from each subject before the study.

DNA Extraction and Genotyping Analysis

Blood sample were collected from the subject. Genomic DNA was extracted from whole blood samples. The PCR condition for determining *GSTM1* and *GSTT1* genotypes were the same as that reported previously (Saadat and Saadat, 2001). To test for contamination, negative controls (tubes containing the PCR mixture without the DNA template) were included in every run. A 1030 bp fragment was amplified by PCR with the *GSTM1* primers and a 480 bp fragment was amplified by PCR with the *GSTT1* primers. The absence of amplified product was consistent with the null genotypes. Successful amplification by albumin specific primers confirmed the proper function of the PCR.

XRCC1 genotypes were examined using PCR-RFLP assays. Genotypic analysis for the *XRCC1* gene was determined in DNA by PCR assay, using the PCR primers described by Lunn *et al*. (1999) for the codons 194 and 399. These primers generate 491 and 615 bp products containing the polymorphic site at codons 194 and 399, respectively. The *XRCC1 Arg399Gln* polymorphism was genotyped by restriction enzyme digestion of the PCR product with the *MspI* enzyme. The *MspI* restricted products, *Arg/Arg*, *Arg/Gln* and *Gln/Gln* genotypes had band sizes of 374/221, 615/374/221 and 615 bp, respectively (Abdel-Rahman and El-Zein, 2000). The *XRCC1 Arg/Trp* polymorphism at codon 194 was genotype by digestion of the PCR fragment with the *PvuII* enzyme. The *PvuII* restricted products, *Arg/Arg*, *Arg/Trp* and *Trp/Trp* genotypes had band sizes of 490, 490/294/196 and 294/196 bp (Hu *et al*., 2001).

Statistical Analysis

Based on the published data the null genotypes of *GSTM1* and *GSTT1* and *399Gln* and *194Trp* alleles of *XRCC1* assumed as high-risk genotypes and alleles, respectively (Divine *et al*., 2001; Duell *et al*., 2001; Egan *et al*., 2004; Saadat and Saadat, 2001; Smith *et al*., 2003; Stren *et al*., 2001; Sturgis *et al*., 1999). In order to count the high-risk genotypes we counted the genotypes as follows:

- Positive genotype of *GSTM1* = 0, Null genotype of *GSTM1* = 1
- Positive genotype of *GSTT1*= 0, Null genotype of *GSTT1* = 1
- Genotypes of *Arg/Arg*, *Arg/Gln* and *Gln/Gln* at codon 399 *XRCC1* counted as 0, 1 and 2, respectively.
- Genotypes of *Arg/Arg* and *Arg/Trp* at codon 194 *XRCC1* counted as 0 and 1, respectively.

The relative associations between the combination of genotypes and breast cancer risk were assessed by calculating Odds Ratios (OR) and 95% Confidence Intervals (CIs). Also the χ^2 for linear trend was calculated. Data analysis was performed using SPSS software version 11.5. A probability of $p<0.05$ considered statistically significant difference.

Results and Discussion

Table 1 shows the genotypes of 25 males comprising of 10 breast cancer cases and 15 healthy subjects as control group. Table 2 shows the number of putative high risk genotypes in patients and control subjects. As shown in Table 2, there is no patient with 0 high risk genotypes, whereas about 13% of control subjects belong to this combination of genotypes. However, 50% of cases and about 6% of controls had 3 putative high risk genotypes. The presence of 3 putative high-risk genotypes results in an almost 20-fold risk of the diseases in comparison with 0 and/or 1 putative high-risk genotypes (OR = 19.99, 95% CI: 1.42-282.4, p = 0.027). Statistical analysis showed that χ^2 for linear trend for 0, 1, 2 and 3 putative high risk genotypes is equal to 5.673 (p = 0.017). This means that the male breast cancer risk increased as a function of the number of high-risk genotypes of *GSTM1*, *GSTT1* and 399Gln and 194*Trp* of *XRCC1*. There are several potential explanations for our findings. First, the high-risk genotypes have additive effect. Same effects were reported previously for developing some types of multifactorial disease (Saadat and Saadat, 2001; Saadat *et al*., 2004). It may be suggested that inability to detoxify many xenobiotics, including an array of environmental carcinogens and endogenously derived reactive oxygen species because of null genotypes of *GSTM1* and/or *GSTT1* (Hayes and Strong 1995; Saadat *et al*., 2004) and decreased DNA repair capacity or fidelity because of the presence 399*Gln* and/or 194*Trp* alleles of *XRCC1* (Abdel-Rahman and El-Zein, 2000), perpetuate the DNA damage and consequently increase male breast cancer risk. Second, we can not rule out chance or random association. Less is known about the genetic influences in male breast cancer

Table 1: Genotypes of *GSTM1*, *GSTT1* and *XRCC1* (at codons 194 and 399) in studied subjects

No.	Subjects	Age	*GSTM1*	*GSTT1*	*XRCC1* (codon 399)	*XRCC1* (Codon 194)
1	Case	76	Null	Positive	*Arg/Arg*	*Arg/Arg*
2	Case	61	Null	Positive	*Arg/Gln*	*Arg/Arg*
3	Case	66	Positive	Positive	*Gln/Gln*	*Arg/Arg*
4	Case	49	Null	Null	*Arg/Arg*	*Arg/Trp*
5	Case	76	Null	Positive	*Gln/Gln*	*Arg/Arg*
6	Case	76	Null	Positive	*Arg/Gln*	*Arg/Trp*
7	Case	65	Positive	Null	*Arg/Arg*	*Arg/Arg*
8	Case	67	Null	Positive	*Arg/Gln*	*Arg/Trp*
9	Case	49	Null	Positive	*Arg/Gln*	*Arg/Trp*
10	Case	50	Null	Positive	*Arg/Gln*	*Arg/Arg*
11	Control	62	Positive	Positive	*Arg/Gln*	*Arg/Arg*
12	Control	61	Null	Positive	*Arg/Gln*	*Arg/Arg*
13	Control	48	Positive	Positive	*Arg/Arg*	*Arg/Arg*
14	Control	49	Null	Positive	*Arg/Gln*	*Arg/Arg*
15	Control	65	Positive	Positive	*Arg/Arg*	*Arg/Arg*
16	Control	64	Positive	Positive	*Arg/Arg*	*Arg/Trp*
17	Control	63	Null	Positive	*Arg/Arg*	*Arg/Arg*
18	Control	64	Null	Positive	*Arg/Arg*	*Arg/Trp*
19	Control	50	Positive	Positive	*Arg/Gln*	*Arg/Arg*
20	Control	64	Null	Positive	*Arg/Gln*	*Arg/Arg*
21	Control	62	Positive	Null	*Arg/Gln*	*Arg/Arg*
22	Control	50	Null	Positive	*Gln/Gln*	*Arg/Arg*
23	Control	48	Null	Positive	*Arg/Arg*	*Arg/Arg*
24	Control	48	Null	Null	*Arg/Arg*	*Arg/Arg*
25	Control	74	Positive	Null	*Arg/Arg*	*Arg/Arg*

Table 2: Association between the combination of *GSTM1, GSTT1* and *XRCC1* (at codons 194 and 399) genotypes and male breast cancer

No. of high risk genotypes	Cases	Controls	OR	95% CI
0	0	2	1.0	-
1	2	6	1.0	-
2	3	6	2.0	0.25-15.9
3	5	1	19.99	1.42-282.4

χ^2 for linear trend for 0, 1, 2 and 3 putative high-risk genotypes is equal to 5.673 ($p = 0.017$)

development. This is due to the fact that this disease is rare and large-scale genetic epidemiologic studies have been difficult to carry out. With limited sample size, present results allow for preliminary conclusions and future large studies are warranted to further test DNA repair genetic variants and polymorphisms of genes involved in detoxification in male breast cancer susceptibility.

Acknowledgments

The authors are indebted to the participants for their close cooperation. The authors are indebted to Dr. Maryam Ansari-Lari for critical reading of the manuscript and for her contribution in discussion. This study was supported by Shiraz University.

References

Abdel-Rahman, S.Z. and R.A. El-Zein, 2000. The 399Gln polymorphism in the DNA repair gene XRCC1 modulates the genotoxic response induced in human lymphocytes by the tobacco-specific nitrosamine NNK. Cancer Lett., 159: 63-71.

Basham, V.M., J.M. Lipscombe, J.M. Ward, S.A. Gayther, B.A.J. Ponder, D.F. Easton and P.D. Pharoagh, 2002. BRCA1 and BRCA2 mutations in a population-based study of male breast cancer. Breast Cancer Res., 4: R2.

Csokay, B., N. Udvarhelyi, Z. Sulyok, I. Besznyak, S. Ramus, B. Ponder and E. Olah, 1999. High frequency of germ-line BRCA2 mutations among Hungarian male breast cancer patients without family history. Cancer Res., 59: 995-998.

Divine, K.K., F.D. Gilliland, R.E. Crowell, C.A. Stidley, T.J. Bocklage, D.L. Cook and S.A. Belinsky, 2001. The XRCC1 399 glutamine allele is a risk factor for adenocarcinoma of the lung. Mutat. Res., 461: 273-278.

Duell, E.J., R.C. Millikan, G.S. Pittman, S. Winkel, R.M. Lunn, C.K.J. Tse, A. Eaton, H.W. Mohrenweiser, B. Newman and D.A. Bell, 2001. Polymorphisms in the DNA repair gene XRCC1 and breast cancer. Cancer Epidemiol. Biomarkers Prev., 10: 217-222.

Egan, K.M., Q. Cai, X.O. Shu, F. Jin, T.L. Zhu, Q. Dai, Y.T. Gao and W. Zheng, 2004. Genetic polymorphisms in GSTM1, GSTP1 and GSTT1 and the risk for breast cancer: Results from the Shanghai breast cancer study and meta-analysis. Cancer Epidemiol. Biomarkers Prev., 13: 197-204.

Giordano, S.H., 2005. A review of the diagnosis and management of male breast cancer. The Oncologist, 10: 471-479.

Hayes, J.D. and R.C. Strong, 1995. Potential contribution of the glutathione S-transferase supergene family to resistance to oxidative stress. Free Radic. Res. Commun., 22: 193-207.

Hu, J.J., T.R. Smith, M.S. Miller, H.W. Mohrenweiser and A.L.D. Golden, 2001. Case, Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. Carcinogenesis, 22: 917-922.

Lunn, R.M., R.G. Langlois, L.L. Hsieh, C.L. Thompson and D.A. Bell, 1999. XRCC1 polymorphisms: Effects on aflatoxin B-DNA adducts and glycophorin A variant frequency. Cancer Res., 59: 2557-2561.

MacLean, H.E., R.W. Brown, J. Beilin, G.L. Warne and J.D. Zajiac, 2004. Increased frequency of long androgen receptor CAG repeats in male breast cancers. Breast Cancer Res. Treat., 88: 239-246.

Saadat, I. and M. Saadat, 2001. Glutathione S-transferase M1 and T1 null genotypes and the risk of gastric and colorectal cancers. Cancer Lett., 169: 21-26.

Saadat, I. and M. Saadat, 2004. Glutathione S-transferase M1 and T1 null genotypes and risk of breast cancer. Arch. Iran. Med., 6: 273-277.

Saadat, M., Z. Saboori, A. Emad and I. Saadat, 2004. Combiantion of CC16, GSTM1 and GSTT1 polymorphisms is associated with asthma. J. Allergy Clin. Immunol., 113: 996-998.

Shu, X.O., Q. Cai, Y.T. Gao, W. Wen, F. Jin and W. Zheng, 2003. A population-based case-control study of the *Arg*399*Gln* polymorphism in DNA repair gene XRCC1 and risk of breast cancer. Cancer Epidemiol. Biomarkers Prev., 12: 1462-1467.

Smith, T.R., E.A. Levine, N.D. Perrier, M.S. Miller, R.I. Freimanis, K. Lohman, L.D. Case, J. Xu, H.W. Mohrenweiser and J.J. Hu, 2003. DNA-repair genetic polymorphisms and breast cancer risk. Cancer Epidemiol. Biomarkers Prev., 12: 1200-1204.

Stern, M.C., D.M. Umbach, C.H. van Gils, R.M. Lunn and J.A. Taylor, 2001. DNA repair gene XRCC1 polymorphisms, smoking and bladder cancer risk. Cancer Epidemiol. Biomarkers Prev., 10: 125-131.

Sturgis, E.M., E.J. Castillo, L. Li, R. Zheng, S.A. Eicher, G.L. Clayman, S.S. Strom, M.R. Spitz and Q. Wei, 1999. Polymorphisms of DNA repair gene XRCC1 in squamouse cell of the head and neck. Carcinogenesis, 20: 2125-2129.

Vogl, F.D., E. Taioli, C. Maugard, W. Zheng, L.F.R. Pinto, C. Ambrosone, F.F. Parl, V. Nedelcheva-Kristensen, T.R. Rebbeck, P. Bernnan and P. Boffetta, 2004. Glutathione S-transferases M1, T1 and P1 and breast cancer: A pooled analysis. Cancer Epidemiol. Biomarkers Prev., 13: 1473-1479.