Association of PON1-L55M Genetic Variation and Breast Cancer Risk: A Case-Control Trial

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Abstract

Background: Paraoxonase 1 (PON1), a multifactorial antioxidant enzyme, has a defensive role against oxidative stress, which is believed to contribute to cancer development. This study aimed to investigate the association of PON1-L55M functional polymorphism with breast cancer risk. Material and methods: In the experimental study, blood samples were collected from 150 healthy women controls and 150 breast cancer subjects. The L55M genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism. Results: Our analysis showed that the genotypes distribution is in Hardy-Weinberg equilibrium for both case and control groups. Our data revealed that there are significant associations between PON1-L55M polymorphism and breast cancer risk in homozygote (OR= 2.13, 95%CI= 1.14-4.00, p= 0.018), dominant (OR= 1.72, 95%CI= 1.07-2.76, p= 0.024), and allelic (OR= 1.55, 95%CI= 1.12-2.15, p= 0.008) models. Conclusions: Our results suggest that the PON1-L55M genetic variation could be a genetic risk factor for breast cancer risk and it could be considered as a molecular biomarker for screening of susceptible women.

Keywords: Breast cancer- paraoxonase 1- genetic polymorphism- PCR-RFLP

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Introduction

Breast cancer (BC) in women is the most common reason for cancer mortality around the world. The rate of this malignancy is varying worldwide, but it is increasing in areas that until recently had a low prevalence of the disease (Key et al., 2001). Many etiologic factors, including nutritional, lifestyle, environmental carcinogenicity, as well as oxidative stress have been considered to increase the risk of breast cancer (Calaf et al., 2018; Wu et al., 2018). The role of paraaxonase 1 (PON1) in detoxification of cancer-causing oxidative stress encourages scientists to evaluate PON1 gene variations in susceptibility to breast cancer (Pan et al., 2019). PON1 binds to HDL and helps to detoxify organophosphorus compounds such as paraoxon and lipid peroxidation-soluble radicals (Shih et al., 1998).

Humans PON1 gene is located on the long arm of chromosome 7 (7q21.3) which is contained 9 exons and encodes a protein with 355 residues (Deakin et al., 2002). Although several pharmaceutical, dietary, and life-style modulators of PON1 are identified, by far the main influence on the activity of PON1, which could differ by above 40 times between persons, is through PON1 genetic variations (Costa et al., 2005; Mackness and Mackness, 2015). There are several main polymorphisms in PON1 gene that could affect the gene function. The PON1-Q192R (rs662) missense single nucleotide polymorphism (SNP) regulates a substrate-dependent influence on activity. The 192R isoform could hydrolyze some substrates such as paraoxon 192Q isoform could hydrolyze some other substrates such as lipid-peroxides and diazoxon (Mackness et al., 1998). Both the missense L55M (rs854560) and the upstream T-108C (rs705379) SNPs are correlated with various activities and serum levels of PON1. The PON1-L55M isoform leads to a lower level of mRNA and then its activity compared to 55L isoform. Besides, the -108C genotype of rs705379 variation could result in higher promoter activity compared to the -108T genotype (Costa et al., 2005; Schrader et al., 2012). There are several studies investigating the role of three mentioned SNPs with various common malignancies e.g. breast cancer. Regarding to the rs854560 polymorphism, there are some reports. For example, Stevens et al., (2006) reported that this polymorphism might be correlated with elevated susceptibility of breast cancer in USA. Naidu et al., (2010) suggested that the L55M SNP may be a genetic biomarker for breast cancer in Malaysian population. However, there is no identified study investigating the
association of this polymorphism with breast cancer in Iranian population. This study aimed to investigate the association of PON1-L55M genetic polymorphism with breast cancer susceptibility in a case-control trial.

Materials and Methods

Subjects

In a hospital-based case-control study, 300 participants including 150 subjects with breast cancer and 150 age-matched healthy controls were enrolled from Pasteur pathobiology and genetics laboratory and also Rohani hospital (Babol, Iran). The breast cancer diagnosis was approved histologically for all breast cancer subjects. Healthy controls individuals without oncological history were referring to the same hospital for routine tests, and after completing the questionnaire and oral interview, it became clear that they did not display positive outcomes. Lastly, 2 mL peripheral blood was obtained from all participants. Besides, the current study was performed according to the criteria outlined in the Helsinki Declaration.

SNP genotyping

After, blood sample collection, genomic DNA was extracted from whole blood by a commercial Kit (Bioneer, Korea) and the PON1-L55M SNP genotyping was performed by polymerase chain-reaction-restriction fragment length polymorphism (PCR-RFLP) method according to the previous study (Kocakap et al., 2016). In brief, PCR was done in a 20 µL overall volume. About 50 ng of genomic DNA, 10 µL of 2X premix, and 0.1 mM of the primers (forward primer: 5’-GAAGAGTAGTGATAGCATGGCCAGCAG-3’ and reverse primer: 5’-TTTAATCCAGAGCTAATGAAAGCC-3’) were used to PCR. After initial denaturation at 95°C for 5 min, the PCR mixture was subjected to 30 repetitive cycles of denaturation (at 94°C for 1 min), annealing (at 61°C 45 sec), and extension (at 72°C for 1 min). The PCR products with length of 171-bp was treated with the NlaIII restriction endonuclease (Fermentas, Germany) which were estimated by logistic regression. A p-value by odds ratios (ORs) and 95% confidence intervals (CIs) were computed and data examination showed that genotypes distribution met the HWE criteria in patient (χ²= 2.54; p = 0.111) and control (χ²= 3.37; p = 0.067) groups. Table 2 displays frequencies of allele and genotype for the rs854560 SNP in our case-control study. Frequencies of genotypes LL, LM, and MM were 44.00%, 39.33%, and 16.67%, respectively for the healthy control group and 31.33%, 43.33%, and 25.34% respectively for the breast cancer group. Alleles L and M rates were estimated by logistic regression. A p-value of less than 0.05 was considered statistically significant. These statistical analyses were performed by SPSS Statistical software version 19 (SPSS Inc., Chicago, IL, USA).

Results

Some demographic and demographic features of included patients in our study are presented in table 1. The average of age and body mass index (BMI) were estimated 45.50±7.26 years and 25.10±4.15 kg/m², respectively. Additionally, 41.33% of patients were postmenopausal. The highest percentage of histologic grade was related to grade I (52.00%). The tumor type for 67.33% of patients was detected as invasive ductal carcinoma (IDC) and 32.00% was detected as invasive lobular carcinoma (ILC). While one of the patients was considered with a mixed tumor (IDC+ILC). Tumor size in 65.33% of patients was less than 2 cm while 34.67% of patients have a tumor with size ≥2 cm. Finally, lymph node metastasis was observed in 52.00% of patients (Table 1).
Table 2. Genotype and Allele Frequencies of rs854560

| Genotype/Allele | Control (n= 150) | Case (n= 150) | Chi-square | OR (95% CI) | p-value |
|-----------------|-----------------|---------------|------------|-------------|---------|
| LL              | 66 (44.00%)     | 47 (31.33%)   | -          | -           | -       |
| LM              | 59 (39.33%)     | 65 (43.33%)   | 2.78       | 1.55 (0.93-2.59) | 0.096   |
| MM              | 25 (16.67%)     | 38 (25.34%)   | 5.68       | 2.13 (1.14-4.00) | 0.018   |
| LM+MM           | 84 (56.00%)     | 103 (68.67%)  | 5.13       | 1.72 (1.07-2.76) | 0.024   |
| L               | 191 (63.67%)    | 159 (53.00%)  | -          | -           | -       |
| M               | 109 (36.33%)    | 141 (47.00%)  | 7.02       | 1.55 (1.12-2.15) | 0.008   |

OR, odds ratio; CI, confidence interval; Significant differences between the case and control groups are bolded.

carriers of allele M were at a high risk for breast cancer (OR= 1.72, 95% CI= 1.07-2.76, p= 0.024). Besides, allele analysis revealed that there is a significant association between allele M and breast cancer risk (OR= 1.55, 95% CI= 1.12-2.15, p= 0.008).

Discussion

Breast cancer is the most common cancer in women that the etiology of this malignancy is not fully understood. It seems that oxidative stress and free radicals play an important role in the pathogenesis and progression of breast cancer (Valko et al., 2006; Hecht et al., 2016). In this study, we investigated the association of the common polymorphism L55M in the PON1 antioxidant gene in a case-control study. The data from our experimental study revealed that there is a significant association between MM, LM+MM, M genotypes and allele and breast cancer risk in our studied population. This shows the main role of this gene in the development of breast cancer. In a meta-analysis published in 2019, it was reported that there are significant associations between PON1-L55M genetic variation and other cancers such as hematologic cancer and prostate cancer (Pan et al., 2019). However, there is another key polymorphism in the PON1 gene which should be noted. It is Q192R missense variation which is associated with decreased risk of breast cancer (Pan et al., 2019).

Oxidative stress could result in impaired in biological membranes, intracellular organelles, and macromolecules such as proteins and DNA (Essick and Sam, 2010). One of the main damage is the oxidation of lipids by free radicals resulting from these stresses, which results in the production of active compounds such as aldehydes, ketones and hydroxyl acids (Valko et al., 2006). These radicals may have an external source or may be due to oxidation-reduction reactions in the body. The imbalance in the formation and removal of these free radicals, including reactive oxygen species (ROS), has been shown to cause genetic dysfunction, interference with cellular signaling, metastasis, neurodegenerative diseases, and aging (Allen, 1998). The human body has many enzymatic systems for the protection of genotoxic damage that activate, indirectly, through the reduction of substrates having the potential to produce free radicals, such as cytochrome P450c17a or directly via or indirectly, through free radical scavenging, such as PON1 (Yu, 1994; Salama et al., 2008). Therefore, it is accepted that the polymorphisms of these genes are key determinants of cancer susceptibility to toxic or environmental toxic chemicals (Shih et al., 1998; de Jong et al., 2002). Single nucleotide polymorphisms based on their gene locations could affect the function of the gene (Salimi et al., 2017; Nejati et al., 2018). For example, genetic variations in promoter regions could affect gene expression level and non-coding variation could alter gene expression via interfering with splicing procedure (Mobasseri et al., 2019; Zamani-Badi et al., 2019). But, the genetic variations in coding sequences (exon variations) could affect the protein structure and function (Noureddini et al., 2018; Bafrani et al., 2019). So based on what was mentioned, the L55M polymorphism as an exon variation, could affect the protein PON1 structure and/or function. Investigation of molecular effects of genetic variation through in vitro and in vivo is too cost- and time-consuming. However, the computational analysis could be useful to evaluate these effects and other molecular evaluations (Tameh et al., 2018; Zamani-Badi et al., 2018). Therefore, assessment of L55M polymorphism on the PON1 protein function by using bioinformatics tools could be a helpful approach.

Our findings suggest that the PON1-L55M polymorphism is a genetic risk factor for breast cancer risk and it could be considered as a possible molecular biomarker for screening of susceptible women. However, there are some limitations in case-control study which should be mentioned. For example, we did not evaluate the gene-gene and gene-environmental factors and also our sample size was somewhat small. Besides, combination effects of PON1-L55M gene polymorphism and some oxidative stress markers such as total antioxidant capacity, malondialdehyde with breast cancer could be useful.

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Conflict of interest
The authors declare no conflict of interests.

Authors’ contributions
AF and RR designed the current project. AF and RR contributed to the statistical analysis, laboratory works, and also preparation of the manuscript draft. HG contributed to the edition of initial manuscript. All of authors revised and confirmed the final manuscript.
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