International Journal of Medical Laboratory 2016;3(2):86-91.

International Journal of Medical Laboratory
Shahid Sadoughi University of Medical Sciences

Original Article

Frequency of p53 Gene Codon 72 Polymorphisms in Women with Breast Cancer in Iran

Shokouh Rajabi Firoozabadi[1] M.Sc., Seyed Mostafa Shiryazdi[2] M.D., Fateme Keshavarz[1] M.Sc., Tahere Nazari[3] M.D., Nasrin Ghasemi[*4] M.D.,Ph.D.

[1] Department of Biology, Science and Research branch, Islamic Azad University, Kurdistan, Iran.
[2] Department of Surgery, Shahid Sadoughi Hospital, Shahid Sadoughi University of Medical Sciences,Yazd, Iran.
[3] Department of General Surgery, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[4] Recurrent Abortion Research Centre, Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

A B S T R A C T

Article history
Received 10 Feb 2016
Accepted 1 May 2016
Available online 21 June 2016

Key words
ARMS-PCR
Breast cancer
Codon 72 Arg/Pro
P53
Polymorphism

Backgrounds and Aims: P53 gene is regarded important in pathogenesis of different cancers. Therefore, this study aimed to investigate the frequency of p53 gene codon 72 Arg/Pro polymorphism in women suffering from breast cancer.

Materials and Methods: A total of 90 patients with breast cancer and 83 matched healthy control women participated in this case-control study. Genomic DNA was extracted from peripheral blood circulation, and codon 72 polymorphism of the p53 gene was examined using the amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method with 4 different primers contemporaneously.

Results: A significant difference was observed between patients and controls ($p<0.05$) in regard with allele frequency of the p53 gene codon 72 Arg/Pro. The distribution of genotypes frequencies was also proved to be significantly different between the cases and controls ($p<0.05$).

Conclusions: The results of the present study revealed that Pro/Pro homozygous and its Pro/Arg heterozygous genotypes of p53 gene codon 72 constitute breast cancer in our community. However, the complex instinct of the breast cancer and polymorphisms needs to be further studied in regard with different polymorphisms along p53 and various genes in this population in order to clarify the role of codon 72 in susceptibility of the breast cancer.

***Corresponding Author:** Recurrent Abortion Research Centre, Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. **Tel:** +98-35 38247085-6, **Email:** nghasemi479@gmail.com

Introduction

Breast cancer is one of the main general health problems in 21th century. Several different factors such as genetics and environment play a considerable role in breast cancer pathogenesis [1]. In fact, it is considered as one of the main causes of death among women worldwide. Nearly, 1.5 million new cases of the breast cancer are annually diagnosed worldwide [2]. In Iran, breast cancer is one of the main causes of the death among women [3].

It is accepted that cancers are the result of the mutation accumulation in the genome. *P53* has a significant role in the formation and progression of breast cancer. Since *p53* gene has a repair responsibility, it is also known as the genome guard [4]. The results of different studies indicate that p53 gene, one of the tumor suppressor genes, is involved at least in various cancers.[5, 6]. P53 has been known as "the guardian of the genome", which aids in DNA stability by preventing genome mutation. There are several different Single nucleotide polymorphisms (SNPs) in p53 gene that the biological effects of most of these polymorphisms have been studied and their clinical results are well known. According to the molecular epidemiological studies, p53 gene mutations are estimated to be identifiable in breast cancer cases [7]. The codon 72 is one of the main SNPs through p53 that has been frequently studied in the breast cancer. This polymorphism should be effective in predicting the disease process. The 72-codon is located on the exon 4 and substitution of the C instead of G (Arg to Pro) is a risk factor for the incidence of such cancers as breast and endometrium [7, 8].

Patients with TP53 gene polymorphism (pro/pro) showed poor prognosis and survival in breast [9, 10], ovarian [11], lung [12], head and neck cancers [12], as well as esophageal squamous cell carcinoma [13]. Several methods such as restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR). Direct DNA sequencing and amplification-refractory mutation system-PCR (ARMS-PCR) have been used to examine the polymorphism of the p53 gene codon 72. In the current study, blood samples from breast cancer patients were collected to determine the prevalence of Arg72pro, rs1042522 polymorphism using ARMS-PCR technique.

Materials and Methods

Study population

This retrospective case-control study was conducted among 173 participants after obtaining permission from the ethical committee at the clinical research from Islamic Azad University of Tehran. The patients were 90 women with breast cancer who referred to the Shahid Sadoughi hospital of Yazd for treatment during 2 years. Their invasive ductal carcinoma tumor was confirmed by the pathological assessments. A group of 83 healthy women without the cancer history were screened as controls from the city population. Moreover, a signed informed consent was obtained from all the participants.

Genotyping

The participants' blood samples were collected on Ethylenediaminetetraacetic acid (EDTA) 0.5 mol for DNA extraction, using the commercial extraction kit (Qiagen, Hilden, Germany), which then were stored at 4°C. Genotyping was performed using ARMS-PCR method on the Gene Amp PCR System- 2000 (Perkin Elmer). Briefly, two sets of the primers were developed for simultaneous detection of the polymorphism at codon 72 of *p53* gene. The ARMS-PCR reaction was performed in the final 25 µl volume containing: 2.5 µl Buffer, 0.5 µl dNTP mix, 0,7 µl MgCl2, 0,5 µl for each primer, 0.2 µl Taq Polymerase, 1 µl DNA and distilled water. PCR initial cycle was performed by denaturation at 94°C for 4 min., followed by 35 cycles (94°C for 30 sec., 55°C for 30 sec. for Pro, 60°C for 30 sec. for Arg, and 72°C for 45 sec.). The primers sequences are summarized in table 1.

Electrophoresis of the PCR products was performed via applying 1% agarose gels that was already prepared using 1gr agarose and 100 ml Tris/Borate/EDTA. The electrophoresis buffer was a 0.5x Tris-acetate/EDTA buffer (20 mmol/L Tris, pH 7.2; 10 mmol/L sodium acetate; 0.5 mol/L EDTA). The DNA staining was performed using EDTA and the bands were visualized under UV light, using gel document system (Gel Digidoc II; Qiagen, Valencia, CA; Tehran, Iran).

Statistical analysis

In the present study, the statistical analysis was performed using SPSS software (version 17.0). Frequency of the genotypes and alleles within and between groups was carried out using Fisher's exact and Chi-square tests. $P<0.05$ was considered statistically significant, and the odds ratio (OR) was calculated with 95% confidence interval (CI).

Table 1. The primers sequences used in p53 codon 72 analyses by ARMS-PCR.

Primers	Sequence	Products size
P1(Arg forward amplifying)	5'-TCCCCCTTGCCGTCCCAA-3'	
P2 (Arg reverse amplifying)	5'-CTGGTGCAGGGGCCACGC-3'	141 bp
P3 (Pro forward amplifying)	5'-GCCAGAGGCTGCTCCCCC-3'	
P4 (Pro reverse amplifying).	5'-CGTGCAAGTCACAGACTT-3'	177 bp

Results

The mean age of two groups was not demonstrated to be significantly different ($p<0.05$, cases 45.35 ±7.53 years, controls 45.28 ±7.59 years). In this study, the genotypic and allelic frequencies for codon 72 of the p53 gene were compatible with the Hardy-Weinberg equilibrium. The study results showed a significant association between Pro allele and breast cancer risk among the patients (Table 1). There was a statistically significant difference in the frequencies of the codon 72 genotypes between the patients and

controls (Table 2). The observed genotypes frequencies for patient group were 39% for Pro/Pro, 45% for Pro/Arg and 11% for Arg/Arg. While in the control group, the genotypes frequencies were identified as Pro/Pro 35%, Pro/Arg 47% and Arg/Arg 25%. Similarly, the study findings revealed that the Pro/Pro genotype was significantly more associated with breast cancer occurrences (p=0.03). In both groups, the Pro allele frequency was higher than Arg. Thus, it can be assumed that there is an apparent correlation between Pro/Pro genotype and breast cancer (p=0.03, OR=1.6, CI=1.04-2.47). Although the present study indicated the statistical significance of the codon 72 with breast cancer, the p53 codon 72 genotypes did not correlate with the cancer grade or stage (p=0.4, p=0.1, respectively).

Table 2. Allelic and genotypic frequencies of polymorphism in codon 72 of p53 gene.

TP53-72Pro/Arg	Patient frequency (No.)	control frequency (No.)	p-value
Total women	N=90	N=83	
Allele frequency			
Pro	64% (115)	52% (87)	
Arg	36% (65)	48% (79)	**0.03**
Genotypic frequency			
Pro/Pro	39% (35)	35% (60)	
Pro/Arg	45% (45)	47% (82)	
Arg/Arg	11% (10)	25% (31)	

Discussion

There are a large number of the literatures, which aimed to examine the relationship between p53 gene codon 72 polymorphism and risk of different cancers such as breast, lung, esophagus, stomach, nasopharynx, liver and prostate [14-21]. Arg allele at codon 72 seems to be associated with higher suppress cellular transformation and apoptosis. Codon 72 of the p53 gene has a complex role in breast cancer risk [22]. Singh et al. showed a protective role for p53 Arg72Pro genotype against breast cancer among Indian postmenopausal women. Moreover, this heterozygous genotype with another polymorphism along the p53 was associated with premenopausal breast cancer risk [1].

The results of several studies demonstrated that the p53 gene codon 72 genotypes were associated with breast cancer susceptibility. In contrary, several studies do not present consistent results to support the association between the p53 gene codon 72 polymorphisms and breast cancer [16,17].

Furthermore, the findings of the present study revealed that codon 72 Pro/Arg genotypes were associated with breast cancer incidence. I Pro homozygote and heterozygote genotypes were observed to be associated with higher breast cancer occurrences. Codon 72

polymorphism does not seem to be sufficient for breast cancer incidence due to the environmental and other genes interactions.

Conclusion

Our findings showed that Pro/Pro homozygous and its Pro/Arg heterozygous genotypes of p53 gene codon 72 constitute breast cancer in Iranian population. Further studies with larger sample size needs to be to clarify the role of p53 gene codon 72 polymorphisms in susceptibility to breast cancer.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to Professor Seyed Mehdi Kalantar for his critical comments during study performance and the paper preparation.

References

[1]. Singh V, Rastogi N, Mathur N, Singh K, Singh M. Association of Polymorphism in MDM-2 and p53 Genes with Breast Cancer Risk in Indian Women. Ann Epidemiol. 2008;18:48–57.

[2]. Borresen AL. TP53 and Breast Cancer. Hum Mut. 2003; 21: 292-300.

[3]. Taghavi A, Fazeli Z, Vahedi M, Baghestani AR, Pourhoseingholi A, Barzegar F, et al. Increased trend of breast cancer mortality in Iran. Asian Pac J Cancer Prev. 2012; 13(1): 367-70.

[4]. Loeb LA, Loeb KR, Anderson JP. Multiple mutations and cancer. Proc Natl Acad Sci. U S A. 2003; 4; 100(3): 776-81.

[5]. Soussi T, Beroud C. Assessing TP53 status in human tumors to evaluate clinical outcome. Nat Rev Cancer 2001; 1: 233-40.

[6]. Ghasemi N, Karimi-Zarchi M, Mortazavi-Zadeh MR, Atash-Afza A. Evaluation of the frequency of TP53 gene codon 72 polymorphisms in Iranian patients with endometrial cancer. Cancer Genet Cytogenet. 2010; 15; 196(2): 167-70.

[7]. Jin MS, Park IA, Kim JY, Chung YR, Im SA, Lee KH, et al. New insight on the biological role of p53 protein as a tumor suppressor: re-evaluation of its clinical significance in triple-negative breast cancer. Tumour Biol. 2016 Feb 19. [Epub ahead of print]

[8]. Ueda M, Hung YC, Terai Y, Saito J, Nunobiki O, Noda S, et al. Glutathione-S-transferase and p53 polymorphisms in cervical carcinogenesis. Gyneco Onco. 2005;96(3): 736-40.

[9]. Sreeja L, Syamala V, Raveendran PB, Santhi S, Madhavan J, Ankathil R. P53 Arg72Pro polymorphism predicts survival outcome in lung cancer patients in Indian population. Cancer Invest. 2008; 26(1): 41-6.

[10]. Gokgoz N, Wunder JS, Mousses S, Eskandarian S, Bell RS, Andrulis IL. Comparison of p53 mutations in patients with localized osteosarcoma and metastatic osteosarcoma. Cancer 2001; 92(8): 2181-189.

[11]. Toyama T, Zhang Z, Nishio M, Hamaguchi M, Kondo N, Iwase H, et al. Association of TP 53codon 72 polymorphism and the outcome of adjuvant therapy in breast cancer patients. Breast Cancer Res. 2007; 9(3): R34.

[12]. Galic V, Willner J, Wollan M, Garg R, Garcia R, Goff BA, et al. Common polymorphisms in TP53and MDM2 and the relationship to TP53mutatio ns and clinical outcomes in women with ovarian and peritoneal carcinomas. Genes Chromosomes Cancer 2007; 46(3): 239-47.

[13]. Yang W, Zhang Y, Tian X, Ning T, Ke Y. p53 codon 72 polymorphism and the risk of esophageal squamous cell carcinoma. Mol Carcinog. 2008; 47: 100-4.

[14]. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Sci.1991; 253(5015): 49-53.

[15]. Ozeki C, Sawai Y, Shibata T, Kohno T, Okamoto K, Yokota J, et al. Cancer susceptibility polymorphism of p53 at codon 72 affects phosphorylation and degradation of p53 protein. J Biol Chem. 2011; 286(20): 18251-8260.

[16]. Xi Chen, Fei Liu, Bo Li, Yong-Gang Wei, Lv-Nan Yan, Tian-Fu Wen. p53 codon 72 polymorphism and liver cancer susceptibility: A meta-analysis of epidemiologic studies. World J Gastroenterol. 2011; 17(9): 1211-218.

[17]. Vietri MT, Riegler G, Ursillo A, Caserta L, Cioffi M, Molinari AM. P53 codon72 polymorphism in patients affected with ulcerative colitis. J Gastroenterol. 2007; 42(6): 456-60.

[18]. Perrone F, Mariani L, Pastore E, Orsenigo M, Suardi S, Marcomini B, et al. P53 codon72 polymorphism in human papilomavirus-negative and human papillomavirus-positive

squamous cell carcinomas of the oropharynx. Cancer 2007; 109(2): 2461-465.
[19]. Stefanaki I, Stratigos AJ, Dimisianos G, Nikolaou V, Papadopoulos O, Polydorou D, et al. P53 codon72 Pro homozygosity increases the risk of cutaneus melanoma in individuals with dark skin complexion and among noncarriers of melanocortin 1 receptor red hair variants. Br J Dermatol. 2007; 156(2): 357-62.
[20]. Shao Y, Tan W, Zhang S. P53 codon72 polymorphism and risk of esophageal squamous cell carcinoma: a case/control study in a Chinese population. Dis Esophagus 2008; 21(2): 139-43.
[21]. Lee yl, Chen W, Tsai WK, Lee JC, Chiou HL, Shih CM, et al. Polymorphism of p53 and p21 genes in chronic obstructive pulmonary disease. J Lab Clin Med. 2006; 147(5): 228-33.
[22]. Gonçalves ML, Borja SM, Cordeiro JA, Saddi VA, Ayres FM, Vilanova-Costa CA, et al. Association of the TP53 codon 72 polymorphism and breast cancer risk: a meta-analysis. Springerplus. 2014; 17; 3: 749.