RESEARCH ARTICLE

Lack of Association of Intron 3 16 bp Polymorphism of TP53 with Breast Cancer among Iranian-Azeri Patients

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Abstract

Background: p53 gene is a well-known tumor suppressor gene that has several polymorphisms in both its exons and introns. It has been suggested that intron 3 16 bp duplication polymorphism may affect the gene function resulting in reduction or suppression of p53 anti tumor activity. In most case control studies a duplicated allele has been noticeably more frequent in cases rather than controls but there are also conflicting results. The aim of this study was to assess the association of intron 3 16 bp duplication polymorphism of p53 with breast cancer risk among Iranian-Azeri population. We also analyzed the clinicopathological information of patients as an epidemiological description of breast cancer in the north-west of Iran. Materials and Methods: This case-control study was performed on 221 breast cancer patients and 170 controls. Genomic DNA was extracted from peripheral blood samples and tumor tissues. p53 PIN3 genotype was determined using electrophoresis of PCR products on 8% non-denaturing polyacrylamide gels and silver staining. Results: In the control and case groups, respectively, 62.9% and 61.1% had no 16 bp insertion (A1A1 genotype), 7.1% and 7.7% had insertion in both p53 alleles (A2A2) and 30% and 31.2% were heterozygous (A1A2). There was no significant difference between genotype frequencies as well as allelic frequencies in two case and control groups. Conclusions: According to the result of the present study, the intron 3 16 bp duplication polymorphism of p53 could not be assessed as a marker of risk factor for predisposition to breast cancer in Azeri population. However, a high frequency of A2 allele (22.1%) in our population suggested that intron 3 16 bp duplication polymorphism may be a valuable marker for study in other cancers with well designed large groups.

Keywords: Intron 3 - p53 gene - polymorphism - breast cancer

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Introduction

Among women, breast cancer is the most prevalent cancer, both in the developed and developing countries. Public health schemes in the developed world have led to the stabilization or even decrease in the incidence rates of breast cancer, whilst in most developing countries, the incidence is certainly rising (Pathy et al., 2011; American Cancer Society, 2013).

p53 is a well-known transcription factor that regulates the expression of more than 2500 genes (Steg, 2012). This protein is activated in response to diverse cellular stresses, such as hypoxia, the inappropriate activation of oncogenes and DNA damage, resulting in induction of cell cycle arrest or apoptosis (Hofseth et al., 2004). TP53 gene mutations are responsible for about half of the cancers and seems that the other half are developed by alterations in upstream or downstream signaling pathways of p53 (Machado-Silva et al., 2010; Guleria et al., 2012). p53 is important as a clinical marker, because its status in a tumor could significantly affect the prognosis of patients and response to treatment (Farnebo et al., 2010; Pouladi et al., 2013). Several polymorphisms have been identified in coding and noncoding regions of p53 that some of them have been associated with increased risk of cancer (Whibley et al., 2009). Three p53 well studied functional polymorphisms include Arg/Pro polymorphism in exon 4, 16 bp duplication in intron 3 (PIN3) and intron 6 G13964C alteration (Guleria et al., 2012), although two studies in India showed no link between polymorphisms and breast cancer (Roy et al., 2012; Vijayaraman et al., 2012)

It has been reported that the 16 bp duplication in intron 3 is correlated with a reduced level of p53 mRNA and decreased both DNA repair capacity and apoptotic indices in lymmphoblastoid cell lines (Wu et al., 2013; Gemignani et al., 2004). Recently, several meta-analyses have highlighted the association between PIN3 and increased risk of cancer predisposition (Hu et al., 2010; He et al., 2011; Sagne et al, 2013; Wu et al., 2013). He et al. (2011) indicated that PIN3 is likely an important genetic

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marker, contributing to susceptibility of breast cancer. Although, Sagne et al. (2013) and He et al. (2011) showed that PIN3-related cancer risk is dependent on ethnicity and geographical origin (He et al., 2011; Sagne et al, 2013).

Iran, a country in the center of the Middle East and on the route of the ancient Silk Road, has a surface area of 1,648,000 km^2 and based on a World Health Organization report is a highly diverse country, not only in its ethnic variety, but also in topography and climate. Azeri population is a main Iranian ethnic group living in the north west of Iran. The majority of the molecular aspects of breast cancer are unidentified in this geographically and genetically distinct region and not a great deal is known about its genetic background, epidemiology and clinicopathological features. Therefore, it is crucial that we improve our understanding of this problem among Iranian women, both on the subject of genetic susceptibilities and environmental risk factors. To achieve this mission, we aimed to investigate whether the PIN3 polymorphism is responsible for susceptibility to breast cancer in Iranian-Azeri patients. We also analyzed the clinicopathological information of patients as an epidemiological description of breast cancer in the north-west of Iran.

Materials and Methods

A total of 221 breast cancer patients, without regard to family history, was selected for this study. Tissues were collected immediately after surgery and transported to the laboratory in liquid nitrogen and stored at -80˚C prior to extraction of DNA.

170 women with no previous history of cancer related illness were studied as a control group. All control subjects were selected from the Iranian-Azeri population, with full consent obtained. Our project was approved by the Ethics Committee of Tabriz University of Medical Sciences research center (www.tbzmed.ac.ir/Research).

Genomic DNA was extracted from frozen tumor tissues and peripheral blood lymphocytes by proteinase K digestion and the salting-out method. For PIN3 (rs17878362) genotyping, we used the following primers: forward 5'TGGGACTGACTTTCTGCT CTT3' and reverse 5'TCAAATCATCCATTGCTTGG3'. Polymerase chain reactions were performed in a 25μl, containing 1X PCR buffer, 0.2mM dNTPs, 1.5mM $MgCl_2$, 1U Taq polymerase. PCR was carried out by thermal cycler (Sensoquest, GmbH, Germany) at 35 cycles consisting of steps: denaturation at 95˚C for 5 minutes, annealing at 60˚C for 30 seconds and extension at 72˚C for 30 seconds in each cycle. The amplified fragments were separated on 12% polyacrylamide gel and visualized by silver staining. Wild type alleles (no duplication) resulted in 180bp fragment and variant alleles (duplicated) resulted in 196bp fragment. Both fragments were observed in heterozygote individuals (Figure 1).

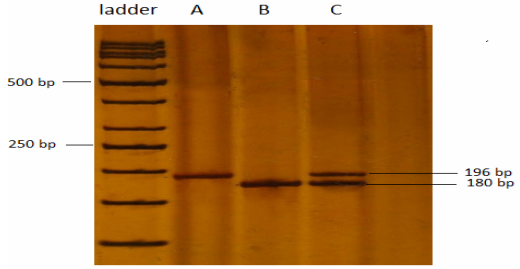

Figure 1. Electrophoresis of PCR Products on 8% Non-denaturing Polyacrylamide Gels and Silver Staining. A: Homozygous genotype for duplicated allele; B: Homozygous genotype for non duplicated allele; C: Heterozygous genotype

The distribution of two alleles in the case and control groups was analyzed by Hardy-Weinberg Equilibrium calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.p1.). The chi-square test was used to find out the possible associations between clinicopathological features (i.e., Stage, size, tumor grade, age, and metastases) and p53 PIN3 genotypes. Significance was established at $p<0.05$. Statistical analyses were performed using the SPSS software (version 16) program.

Results

A total of 221 Azeri breast cancer patients and 170 controls were analyzed for genotyping of the TP53 gene PIN3 polymorphism. The patients age range at diagnosis of their first episode of breast cancer was 12-91 years (47.44±10.19), with that of controls 17-76 (48.06±13.08).

The frequency of the wild-type allele A1was 76.7% (n=339) in cases and 77.9% (n=265) in controls. The frequency of the variant allele A2 was 23.3% (n=103) in cases and 22.1% (n=75) in controls. The distribution of PIN3genotypes frequencies among the controls was in agreement with that expected under HWE. In the control group, the genotype distribution of p53 polymorphism, showed 62.9%, 30 % and 7.1% for the A1/A1, A1/A2, A2/A2 genotypes, respectively. In the cancer group, the frequencies were 61.1% for the A1/A1, 31.2% for A1/A2 and 7.7% for A2/A2 (Table 1). There were no significant differences in the frequency distribution of p53 PIN3 genotype between breast cancer patients and controls ($p<0.05$). In addition, no significant associations between age, lymph node metastasis, side involved, tumor size, tumor stage, and p53 PIN3 genotype was observed (Table 2).

Beside the polymorphism results, our study reflected several facts about breast cancer among Iranian-Azeri women. In this group of Azeri women with breast cancer, the age of diagnosis ranged between 12 and 91 years. Approximately 49.3% of the patients were diagnosed before the age of 46. 23% of patients were aged younger

Table 1. The TP53 PIN3 Genotype Distribution and Allele Frequency Data, Association with Breast Cancer Risk

	Cases	Controls	OR (95% CI)	p value*
Genotypes				
A1/A1	135 (61.1%)	107 (62.9%)	Reference	
A1/A2	69 (31.2%)	51 (30.0%)	1.072 (0.673-1.710)	0.757
A2/A2	17 (7.7%)	12 (7.1%)	1.123 (0.483-2.630)	0.771
Alleles				
A1	339 (76.7%)	265 (77.9%)	Reference	
A2	103 (23.3%)	75 (22.1)	1.074 (0.755-1.527)	0.681

*Chi-square test

Table 2. Relationship between p53 Codon 72 Polymorphism and Clinicopathologic Parameters

	A1/A1	A1/A2	A2/A2	Total	p value*
Tumor size					
<3cm	36 (52.17)	27 (39.13)	6 (8.7)	69	0.374
>3cm	49 (63.64)	23 (29.9)	5 (6.5)	77	
Age					
<45	65 (61.9)	33 (31.43)	7 (8.75)	105	0.775
>45	64 (59.3)	34 (31.5)	10 (9.2)	108	
Tumor stage					
I or II (%)	44 (55)	29 (36.25)	7 (8.75)	80	0.7
III (%)	55 (61.1)	29 (32.2)	6 (6.7)	90	
Lymph node metastasis					
Negative	33 (54.1)	22 (36.1)	6 (9.8)	61	0.523
Positive	68 (62.96)	32 (29.63)	8 (7.4)	108	
Side involved					
Left breast	65 (60.75)	33 (30.84)	9 (8.41)	107	0.555
Right breast	63 (61.76)	32 (31.37)	7 (6.86)	102	
Right and Left	3 (75)	0 (0)	1(25)	4	

*Chi-square test

than 40 at presentation. A great majority of the tumors exhibited invasive ductal histology (81.1%), whilst only 7% of the patients presented with ductal carcinoma in situ (DCIS), invasive lobular cancers made up 5.9%, and another 6% of tumors were of other histological types. In addition, 63.9% of patients had lymph node positive disease.

Discussion

The attainment of accurate breast cancer epidemiological data from more heterogenous communities in the Asian population and the careful monitoring of treatment and survival outcomes among regions and ethnic groups within individual countries, are difficult. Despite many of the problems, such as sociocultural barriers, missing and unavailable of treatment and outcome data and other limitations, some figures and facts were revealed about breast cancer in Asian women by researchers. Compared with western countries, Asian countries have a lower incidence, but significantly higher mortality. Patients are about one decade younger and the proportion of young patients (<35 years) varies from about 10% in developed to up to 25% in developing Asian countries. In addition, early detection is rare and the cancer is detected at advanced stages (Martin and Weber, 2000; Forouzanfar et al., 2011; Pathy et al., 2011). These facts are illustrated in our epidemiological results. Thus 22.39% of these patients were aged 40 or younger at presentation and 53% of the patients presented with advanced stage disease (stage III).

Breast cancer is the first cause of cancer-related death and the first cancer to have been diagnosed in Iranian women (21.4% of all cancers) (Babu et al., 2011), we should improve our understanding of this problem among Iranian women, both on the subject of genetic susceptibilities and environmental risk factors. Molecular changes, including the p53 gene mutation and polymorphism, are related to increasing the risk of developing breast cancer.

Some in vitro studies have been suggested that a duplicated allele of the p53 intron 3 polymorphism has a negative impact on expression and apoptotic function of p53 (Marcel et al., 2011). Also, the 16 bp fragment contributes to the formation of a G-quadruplex structure in p53 intron 3 that affects the splicing and leads to increased production of D40p53 isoform, which has been suggested as a negative regulator of wild type p53 (Marcel et al., 2011). Several meta-analyses have highlighted the association between p53 intron 3 polymorphism and increased risk of cancer predisposition (Hu et al., 2010; He et al., 2011; Sagne et al, 2013; Wu et al., 2013).

Some studies have been reported an association between intron 3 16 bp polymorphism and a variety of cancers, but there are also conflicting results (Osorio et al., 2008; Trifa et al., 2010). Sagne et al showed that the ethnicity and geographical origin are important parameters in PIN3-related cancer risk. This may interpret those inconsistent findings. In Iran, we have several reports that tried to assess the association between the TP53 codon 72 polymorphism and different cancer (Kazemi et al., 2009; Salehi and Hadavi 2009; Doosti et al., 2011). To our knowledge, there is only one report regarding PIN3and breast cancer among Iranian patients. This case-control study performed among breast cancer patients and healthy women from the central part of Iran, Isfahan province (Faghani, 2011). Faghani et al showed a significant difference in the PIN3 variants between the cases and controls (Faghani, 2011). But, in our study, no significant difference was found in genotype distribution between case and control groups. According to the literatures, there are differences in allele frequencies among ethnic or racial groups. The frequency of A2 allele (Minor allele frequency) was 22.1% in our population (Azeri-Iran), 23% in the Turkish population (Akkiprik et al., 2009), 30.8 in Arab (Alawadi et al., 2011), 29% Persian-Iran (Faghani et al., 2011), 19% in India (Sagne et al., 2013), 16% in German (Wang-Gohrke et al., 2002), 15% in Mediterranean countries and Northern Europe and14% in health women from United State (Sagne et al., 2013). A high frequency of A2 allele (22.1%) was found in our population. A high frequency of A2 allele (22.1%) in our population suggested that intron 3 16 bp duplication polymorphism may be a valuable marker for study in other cancers with well designed large groups.

The overall results of this study indicate that there is no correlation between intron 3 genotypes and the risk of breast cancer. However, these results do not tell the whole story; breast cancer is a multifactorial disease with critical interaction between genetic and environmental factors. In the most cases, genetic factors create the potential for cancers or "load the gun", whilst environmental triggers is required to initiate a cascade of cellular activities responsible for cancer initiation, promotion and progression (Olden, 2011). The environmental factors like diet and air pollution are rapidly changing in most developing countries by westernization as the lifestyle (such as the first birth >30 years of age, less breastfeeding, early menarche, late menopause, etc.) has been changed during the last decades in the most Asian peoples (Pathy et al., 2011). Therefore, it is not unthinkable that the environmental changes may alter cancer association results in the future.

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