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No association between polymorphism MNS16A in the telomerase catalytic subunit gene (hTERT) and breast cancer in Ardabil, Iran

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ABSTRACT

Breast cancer is the most frequent malignancy among women and its mortality is estimated to be about 519,000 deaths per year. The telomerase activity seems to be necessary for the immortality of tumor cells. The association between telomerase reactivation and its catalytic subunit's (hTERT) expression has been reported in several studies. The length polymorphism MNS16A in the hTERT gene's promoter has been recognized to be associated with its expression status. The aim of this study was assaying the role of this polymorphism in conferring susceptibility of gaining breast cancer in women of Ardabil. Among the women affected with breast cancer in Ardabil, 53 paraffin embedded tumor samples were selected. Fifty-one persons with any positive history of cancer in them and their 1st and 2nd degree of relatives have been selected as control group. After DNA extraction by phenol-chloroform based method, the presence of variants of the polymorphism MNS16A was detected by PCR method. The alleles lengthen 302 and 333 bp were nominated as L [Long] allele and S [Short] allele was referred to fragments with 243 and 272 bp lengths. Data was analyzed by statistical methods and P value less than 0.05 was determined as significant difference. The frequency of genotypes SS, SL, and LL among cases were 56.6%, 18.86%, and 24.52%, respectively, and were 50.98%, 24.49%, and 21.56% for genotypes SS, SL, and LL among control group. Any significant relationship was detected between cases and control groups [P>0.05]. In this investigation, it was revealed that the noted polymorphism seems not to be a susceptibility factor for gaining breast cancer in Ardabil province.

Keywords: Breast cancer, polymorphism, hTERT, MNS16A

INTRODUCTION

Breast cancer is the most frequent malignancy among women with the worldwide death rate of 519,000 per year [1]. Previous studies on Iranian women showed breast cancer in 17 out of 100,000 women with high incidence aging 45 to 54 years old [2,3]. They revealed that 15-30% of women who are suffering breast cancer had a family history of breast or ovarian cancers. Studies on the impact of genetic and non-genetic factors and family studies associated

with early onset of disease have shown that breast cancer follows dominant traits. Genetic susceptibility against breast cancer emerges by accumulation of various mutations in some critical genes such as tumor suppressors and the genes involving in DNA repair., These alterations lead uncontrolled cell division, survival, proliferation, and tumor invasion[3,14,26].

Cancer incidence and mortality rates vary by race and geographic location. North Europe, North America, the Mediterranean and South American populations indicates higher, and Asian and Africans showed lowest incidence of breast cancer [4].

Telomerase is a DNA polymerase enzyme that is responsible to build a six-nucleotide replications (TTAGGG) at the ends of chromosomes to form a telomere structure. It creates caps at the end of chromosomes that prevent from the destruction and those annexations.[5,21,23,25]

Since the DNA polymerase basically is unable to replicate the complete chromosome, so in each replication of 50 to 100 nucleotides, shortening of chromosomes occurs which leads to aging the cell [5,12,21].

In embryonic cells as well as some somatic cells, telomerase is responsible to restore telomeres length[6,15]. About 90% of malignant cells show telomerase hyper-activation thus consequences unlimited cell division and immortality [5,18, 22].

Human telomerase enzyme activity is composed of the human telomerase reverse transcriptase (hTERT), human telomerase RNA (hTR) and Dyskryn [6,17,19,27,28].

Several recent studies have provided evidence that polymorphisms in the telomerase reverse transcriptase [TERT] gene sequence are associated with cancer development, but a comprehensive synopsis is not available[6,16,20,29,30].

hTERT is located at chromosome 5p15.33 and has 16 exons[6,16].

Importance of a functional polymorphism of repetitive minisatellite in hTERT gene which is called MNS16A in cancer incidence has been shown previously [7,8,10].

In 2003, Wang et al. first reported in a lung cancer study the identification of a polymorphic tandem repeats minisatellite hTERT termed MNS16A[7] .

This minisatellite is located down-stream of exon 16 of hTERT gene and up-stream in the putative promoter.[8]

MNS16Awas found to have two repeat elements forming a 23 bp core sequence or a 26 bp core sequence with a CAT insertion representing a transcription factor binding site for GATA-1. Two different variable number of tandem repeats [VNTR alleles, VNTR-302 and VNTR-243 were named on basis of their polymerase chain reaction [PCR] fragment size. In addition, two other rare alleles [VNTR-272 and VNTR-333] were discovered in cancer cell lines. For statistical analysis, a classification in short alleles [S alleles: VNTR-243 and VNTR-272] and long alleles [L alleles: VNTR-302 and VNTR-333] was introduced by Wang et al. [8]. Region of an antisense hTERT transcript MNS16A position is as a polymorphism that is classified as a long or short position.[7,8]

Numerous studies on gene expression of hTERT revealed that MNS16A alteration on hTERT gene was associated with its increased transcription and promotor activity [8] as far as it has been considered as a potential marker for glioblastoma [9], breast [10] and lung [11] cancers.

The aim of this study was to investigate the possible relationship of hTERT MNS16A polymorphism and breast cancer risk in the women population of Ardebil province, Northwestern Iran.

MATERIALS AND METHODS

Sampling

53 paraffin-embedded breast tumor samples and 51 normal Breast tissue samples obtained from the pathology laboratories at Ardabil University of Medical Sciences Imam Khomeini Hospital were participated.
All patients signed detailed consent forms before the study was conducted.

The experimental samples were pre-made on standard slides with 5 micron thick FFPE tissues using the standard method by the Department of Pathology, Imam Hospital. 3-5 slices of 5 micron from selected samples placed in the 1.5 ml micro- tube and DNA samples were extracted with phenol-chloroform manually with using xylol, ethanol, Layzyz buffer, proteinase K, phenol saturated, chloroform - isoamyl alcohol, sodium acetate, isopropanol and the finally 50 micro- liter of distilled water added.

DNA fragment analysis

Paraffin removal from paraffin-embedded samples was done by xylene solution, . Genomic DNA was extracted using phenol-chloroform standard procedure. DNA quality was measured by a spectrophotometer Nanodrop, and a mixture of 20 microliter for each sample was prepared. Polymerase chain reaction [PCR] was performed to amplification of target DNA using specific oligonucleotide primers.

PCR protocol was as follow:

10 ml of PCR reaction mixture, plus 1 ml of the primer mixtures [forward; AGGATTCTGATCTCTGAAGGGTG-`3, reverse; 5`- TCTGCCTGAGGAAGGACGTATG -3], 16 ml Taq DNA polymerase enzyme [5 µL/U] and 3 ml of genomic DNA were mixed in a 0.2 ml micro-tubes.

DNA denaturation took place at 95 °C for 30 seconds, annealing was done at 55 ° C for 45 minutes and elongation was performed at 72 °C for 1 minute. All PCR reaction was done in 35 cycles. Final elongation step was taken 10 minutes at 72 °C. PCR product was analyzed by agarose gel electrophoresis.

According to previous researches, four alleles of MNS16A were identified and they were divided into L long allele [333 and 302 bp] and S short alleles [272 and 243 bp][7,8]. Differences in genotype distribution in two groups were studied.

Data analysis

Achieved non-parametric data were analyzed by using the SPSS v. 16 . applying Mann-Whitney test to compare frequency distribution of three different MNS16A genotypes in the hTERT gene, P <0.05 was considered as significant difference. The odds ration and confidence level for the dependent and independent variables were calculated as 95% interval.

RESULTS

All patients participating in the study were female and the mean age was 48.35± 1.3 [25-79] and control group mean age was 39.2± 1.03 [17-56].

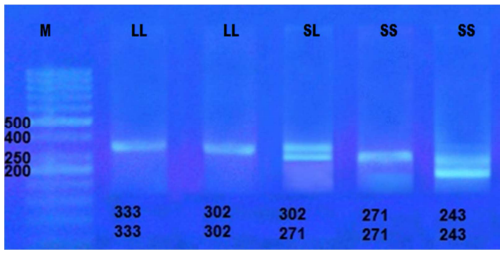

Figure1. PCR result on electrophoresis gel

The Mann-Whitney distribution of genotypes and alleles prevalence in patients and controls group with showed that the distributions of genotypes were not significantly different in two groups [P> 0.05].

The SS, SL and LL polymorphism frequencies in the patients were 56.60. , 18.86 and 24.52 percent respectively. While, the ratios in the controls group were as 50.98, , 24.49 and 21.56 percent respectively. SS genotype frequency in addition to SL in patient group was observed as 75.47% whereas in the control group it was 76.47% [Table 1]. In both patients and control groups, SS genotype percentage was high.

Also no correlation was found between the frequency of genotypes and S and l alleles with breast cancer [P> 0.05].

Table1 :Investigated geneotype and allele frequencies in MNS16A gene in the studied breast cancer cases and healthy controls

	SS	SL	LL	SL	S	L
Cases [53]	30 [56.6%]	10 [18.9%]	13 [24.5%]	40 [75.5%]	66%	34%
Controls [51]	26 [51%]	13 [49.2%]	12 [23.5%]	39 [76.5%]	63.7%	36.3%

In addition, the relationship between SS + SL and LL of MNS16A in hTERT gene with cancer stages [TNM], tumor grade [Figure 2], ALN [metastasis] [Figure 3] and patients' ages [Figure 4] were studied and no significant correlation was found.

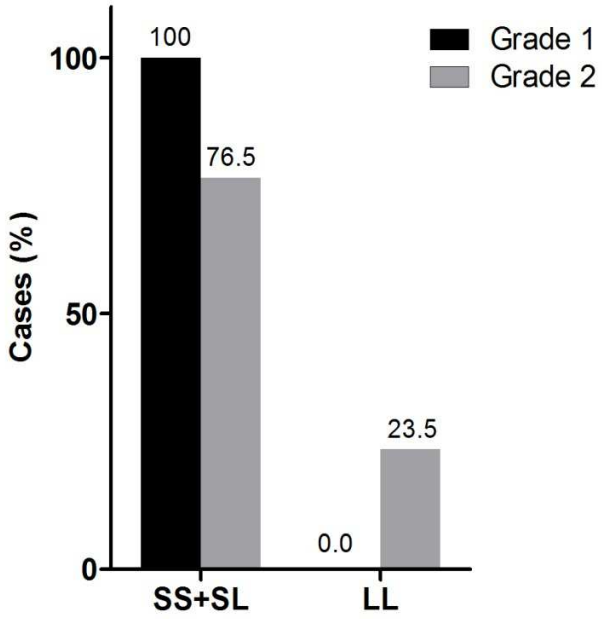

Figure2. The relationship between MNS16A genotypes in hTERT gene and tumor grade

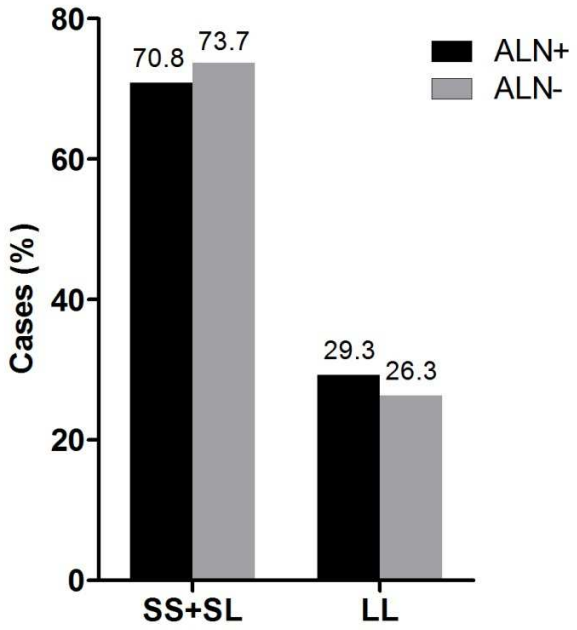

Figure 3: The relationship between MNS16A genotypes in hTERT gene and metastasis [ALN]

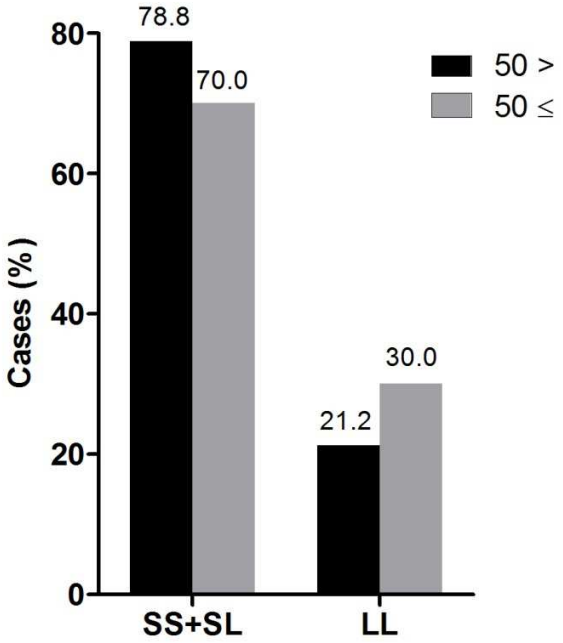

Figure 4: The relationship between MNS16A genotypes in hTERT gene and patients' ages

DISCUSSION

The previous studies suggest that factors such as genetic polymorphism may reflect individual differences in the incidence of cancer. Breast cancer is the leading cause of death among women in Western countries and molecular changes including hTERT gene polymorphism is associated with the development of the disease. MNS16A is a

functional mini satellite polymorphism with repetitions in hTERT gene which has been recognized as a molecular polymorphism in hTERT gene [11].

Breast cancer in three conducted studies showed that only in Chinese carrier with short alleles, the risk of breast cancer increases [10], while in two other studies on whites and Greek Caucasian women no change in the risk of breast cancer was reported. [12 ,13]

This study did not found any significant relationship between L and S alleles and susceptibility to breast cancer either, so the results of this study are consistent with Zagory and et. al in 2012 on Greek Caucasian women [13] and also Chen and et al [2010] results [12], but the results of this study are different from the study of breast cancer among Chinese women in 2008 which was done by Wang and et al [2008] that positively support the relationship between MNS16A S allele of hTERT gene and increased risk of breast cancer and also invasive behavior is correlated with metastatic axillary lymph nodes [10].

Other studies show that racial background may have a significant impact on the genetic diversity of telomere and breast cancer. As new findings of breast cancer studies showed, apart from race, a certain texture is different from positive association with lung cancer[7], nasopharyngeal carcinoma[31], colorectal cancer[8] and malignant glioma[9]. It should be noted that probably there are some essential cofactors in specific functional tissues that needs to be explored in the future. In any case, Hardy-Weinberg inconsistency necessarily invalidates the results of an association study.

CONCLUSION

MNS16 A polymorphism of hTERT gene does not seem to be a risk factor for breast cancer among women in Ardabil province. However, in addition, other studies showed that this polymorphism as a special case needs to be examined more deeply.

As a result, it is suggested that this study be conducted on more individuals to determine whether previous studies that have reported a positive relationship, caused by ethnic differences or intervention factors.

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