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Original Article

NO SIGNIFICANT ASSOCIATION BETWEEN 40BP INS/DEL PROMOTER POLYMORPHISM OF MDM2 AND BREAST CANCER SUSCEPTIBILITY IN PAKISTANI WOMEN

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ABSTRACT:

Breast cancer remains the most prevalent malignancy among women, and is the second leading cause of mortality worldwide. Genetic heterogeneity in the MDM2 promoter region has been associated with increased cancer susceptibility. The study was designed to assess the impact of a 40-bp deletion/insertion mutation of the MDM2 gene (at position 1518, which has a putative TATA pattern) in both breast cancer patients and healthy participants. A total of 200 female participants were included in the present study, comprising 100 histologically confirmed breast cancer patients and 100 age- and sex-matched healthy controls. Patient samples were obtained from Bolan Medical Complex (BMC) and the Center for Nuclear Medicine and Radiotherapy (CENAR), Quetta, Pakistan. Along with blood sample collection, information regarding demographic, reproductive, and clinical characteristics was recorded. Genotyping of the MDM2 gene 40-bp insertion/deletion (ins/del) polymorphism was carried out using specific forward and reverse oligonucleotide primers. The genotype frequencies of the MDM2 polymorphism among breast cancer patients were 57% for ins/ins, 36% for ins/del, and 7% for del/del, whereas in the control group they were 59%, 35%, and 6%, respectively. The calculated odds ratio for the deletion allele was 1.208, with a 95% CI ranging from 0.383 to 3.812 ($p = 0.939$). No statistically significant associations were observed between breast cancer and menopausal status, age at menopause, parity, number of children, use of oral contraceptives, or history of breastfeeding ($p > 0.05$). These results suggest that the 40-bp ins/del polymorphism in the promoter region of the MDM2 gene is not significantly associated with the development of breast cancer in the studied population.

Keywords: Breast cancer, insertion/deletion polymorphism, MDM2, Oligonucleotide primers, Menopause, breastfeeding

INTRODUCTION

Breast cancer (BC) is a term used to describe the abnormal growth and multiplication of cells originating from breast tissue. A breast tumor is a serious and common disease that affects women's health and is one of the leading causes of cancer-related death. According to a study, 23% of all cancer patients are diagnosed with breast cancer, which also accounts for 14% of cancer-related deaths. Breast cancer affects women 100 times more often than men. Although men are less likely to develop breast cancer, they can still be affected. Different ethnic groups have varying rates of cancer incidence (1). Breast cancer is classified into distinct categories based on its stage, aggressiveness, and genetic composition. Advancing age, female gender, nulliparity, less breastfeeding, heredity, increased hormone levels, and individual lifestyle are the key risk factors linked with the development of breast cancer. Although the cause of breast cancer is uncertain, genetic factors have been demonstrated to have a significant influence on its pathogenesis and progression (2). Among genetic changes, the tumor suppressor protein p53 (tumor suppressor protein) is the main governing factor in a variety of cell lines (3).

In most multicellular organisms, it plays an important function in cancer suppression. It protects genes against mutation and plays a role in maintaining stability. It promotes a transcriptional pathway that induces apoptosis, cell cycle arrest, and autophagy, among other cellular damages, in response to genotoxic stress and oncogenic signals (3). Overexpression of MDM2, a cellular antagonist, can inhibit p53 action in some situations. MDM2 is a key component of the p53 pathway. MDM2 is a p53-specific E3 ubiquitin enzyme that promotes proteasome degradation of p53. MDM2 was discovered to be one of three genes (MDM1, MDM2, and MDM3). This gene was identified on an extrachromosomal acentromeric nuclear region (4). The human MDM2 gene has 11 exons, two promoter regions, and a p53 intronic promoter. It is situated on chromosome 12q14.3–q15.1 (5), present on the q-arm. MDM2

expression may be affected by genetic changes within either of the promoters. MDM2 regulates p53 activity in a variety of ways, and even small changes in MDM2 levels can have an impact on the p53 pathway. MDM2 binds directly to the transactivation domain of p53, which decreases p53 transcriptional activity. Second, it acts as an E3 ubiquitin ligase, facilitating p53 ubiquitination and degradation. Finally, it attaches to p53 inside the nucleus and transports it to the cytoplasm, causing it to degrade (6).

MDM2 is a 40-bp Ins/Del mutation with a putative TATA motif in the promoter region, one of the most studied polymorphisms (7, 8). Because of MDM2's tumorigenic role, researchers may presume that people who have the 40bp deletion allele have an increased chance of developing breast cancer during their lifespan (9). However, there is limited data available from Pakistani population thus this study was conducted.

METHODS

A total of 200 women participated, including 100 patients diagnosed with breast cancer, along with 100 healthy women with no history of cancer as a control group, having the same age range. After obtaining informed consent, all the participants were recruited from CENAR and Bolan Medical Complex (BMC) hospitals Quetta, Pakistan.

Assessment of Demographic and Reproductive Factor

Several demographic and reproductive factors relevant to breast cancer, such as age, ethnicity, gender, smoking status, marital status, family history, menopause and menarche, number of children, nulliparity, breastfeeding, age at first childbirth, age at cancer diagnosis, and other factors, were assessed through interviews and structured questionnaires. Under strict aseptic conditions, 5 ml of blood was drawn from each participant for DNA extraction and subsequent mutational analysis.

DNA Extraction

Genomic DNA was extracted from whole blood samples collected in EDTA tubes employing the standard Phenol-Chloroform method. The extracted DNA was diluted with TE buffer, and its purity was checked using a NanoDrop spectrophotometer, and quantified using a 0.8% agarose gel. The extracted DNA was stored at -20°C for future analysis.

Polymerase Chain Reaction (PCR)

PCR was carried out for amplification of the MDM2 gene promoter region, associated with breast cancer mutations. Gene-specific primers, both forward (5`-GACCACTATGTTTAAGGAAG-3`) and reverse (5`-TGACTCACCTACTTTCCCAC-3`), were employed, producing fragments of 287 bp and 247 bp for the ins allele and del allele, respectively. The 25µl reaction mixture contained 2 µl of genomic DNA, 2.5 µl of dNTPs, 2.5 µl of MgCl2, 1 µl of each primer, 0.3 µl of Taq DNA polymerase, and 2.5 µl of PCR buffer. Amplification was achieved under standard PCR conditions, and the resulting PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized under UV light.

Statistical Methods

Statistical analyses were carried out using Statistical Package for Social Sciences (SPSS), with categorical variables expressed as frequencies and percentages. The association of demographic as well as reproductive factors with breast cancer risk was evaluated by chi-square tests, and a p-value ($p<0.05$) was considered statistically significant.

RESULTS

Genotypic Distribution of MDM2 40-bp Insertion/Deletion (I/D) Polymorphism

To assess the correlation between the 40-bp I/D polymorphism in the MDM2 gene and breast cancer susceptibility, genotypic distribution was analyzed in 100 breast cancer patients and 100 age- and sex-matched healthy controls. The genotype and allele frequencies, along with the corresponding odds ratios (OR) and 95% confidence intervals (CI), are summarized in Table 1. No significant difference in genotype distribution was observed between patients and controls. The deletion allele showed no significant association with breast cancer risk ($p = 0.726$, OR = 1.085; 95% CI: 0.687–1.715). Similarly, co-dominant, dominant, and recessive genetic models did not reveal any statistically significant association between the polymorphism and disease occurrence.

Demographic and Clinical Characteristics

Demographic characteristics of patients and their association with the 40-bp I/D polymorphism are presented in Table 2. No significant association was found between age and genotype distribution ($p = 0.647$).

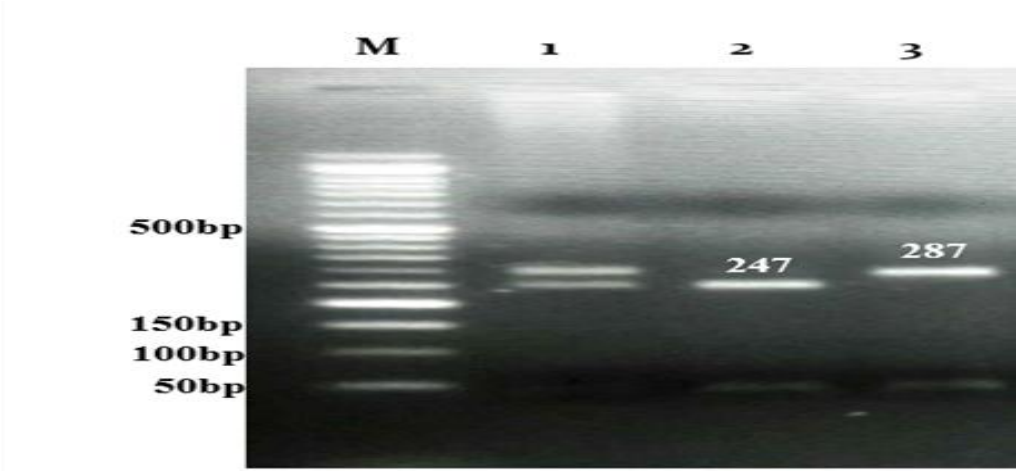

Figure 1: Gel image of the PCR product of the 40-bp I/D polymorphism of MDM2. Well 1: 50 bp DNA marker; Well 2, heterozygote; Well 3, deletion; Wells 4, insertion

Table 1. Distribution of allele frequencies of the 40bp I/D mutation

40bp ins/del	Patients (%)	Control (%)	Co-dominant	P-value
Co-dominant				
Ins/ins	57	59	1	
Ins/del	36	35	1.065(0.590-1.922)	0.939
Del/del	7	6	1.208(0.386-3.812)	
Dominant				
Del/del	7	6	1	
Del/Ins +Ins/Ins	93	94	0.848(0.275-2.619)	0.774
Recessive				
Del/Del + Ins/Del	44	42	1	
Ins/ins	56	58	1.085(0.620-1.900)	0.775
Allele				
Ins	150	153	1	
Del	50	47	1.085(0.687-1.715)	0.726

Among the patients, 96% were married, and 92% had given birth. Approximately 15% of patients reported having their first pregnancy before the age of 25 years, and 88% had a history of breastfeeding. Early menarche (≤12 years) and late menopause (≥50 years) were reported in 67% and 8% of cases, respectively. The use of oral contraceptive pills was noted in 60% of participants, while 85% were non-smokers. Family history of breast cancer was positive in 8% of patients. In total, 42% of women were premenopausal, while 58% were postmenopausal. Menstrual status did not show any significant correlation with the presence of the polymorphism. No significant associations were observed between breast cancer occurrence and family history, smoking status, or inheritance pattern.

Table 2. Association of 40-bp I/D polymorphisms with demographic information

Variables	Genotype			Chi value	P-value
Age	Del	Ins	Ins/Del		
21-40	8(7%)	60(52.6%)	46(40.4%)	2.485	0.647
41-60	2(2.7%)	40(54.1%)	32(43.2%)		
61-80	2(16.7%)	4(33.3%)	6(50.0%)		
Ethnicity					
Baloch	0(0%)	32(53.3%)	28(46.7%)	6.189	0.402
Pathan	4(5.8%)	54(51.9%)	44(42.3%)		
Persian	4(18.2%)	12(54.5%)	6(27.3%)		
Punjabi	2(14.3%)	6(42.9%)	6(42.9%)		
Smoking status					
No	12(7.3%)	82(50%)	70(42.7%)	1.718	0.424
Yes	0(0%)	22(61.1%)	14(38.9%)		
Inheritance					
From mother	0(0%)	6(75%)	2(25%)	0.961	0.619
None	12(6.2%)	98(51.0%)	82(42.7%)		

Hormonal and Reproductive Factors

The relationship between the 40-bp I/D polymorphism and hormonal or reproductive characteristics is summarized in Table 3. No significant association was observed between the polymorphism and age at menarche, menopausal age, number of children, breastfeeding status, or oral contraceptive use. Additionally, radiation exposure showed

a borderline association (p = 0.051), although it did not reach statistical significance. Overall, the 40-bp I/D polymorphism in MDM2 did not show a statistically significant association with breast cancer risk or with demographic, reproductive, or hormonal factors in the studied population.

Table 3. Association between 40-bp I/D mutation with hormonal factors in case and control.

Variable	Del	Ins	Ins/del	Chi value	P value
Menarche(Years)					
11	0(0%)	4(50.0%)	4(50.0%)	3.048	0.931
12	10(8.2%)	64(52.5%)	48(39.3%)		
13	2(3.8%)	24(46.2%)	26(50.0%)		
14	0(0%)	10(71.4%)	4(28.6%)		
15	0(0%)	2(50.0%)	2(50.0%)		
Menopause age					
No menopause	6(4.8%)	70(55.6%)	50(39.7%)	3.093	0.797
30-39	2(11.1%)	6(33.3%)	10(55.6%)		
40-49	4(9.1%)	20(45.5%)	20(45.5%)		
50-59	0(0%)	8(66.7%)	4(33.3%)		
Number of children					
0-4	8(7.5%)	58(54.7%)	40(37.7%)	1.515	0.824
5-9	4(5.4%)	36(48.6%)	34(45.9%)		
10-14	0(0%)	10(50.0%)			
Breastfeeding					
No	0(0%)	18(52.9%)	16(47.1%)	1.357	0.507
Yes	12(7.2%)	86(51.8%)	68(41.0%)		
Oral contraception					
No	8(5.5%)	78(53.4%)	60(41.1%)	0.280	0.869
Yes	4(7.4%)	26(48.1%)	24(44.4%)		
Radio exposure					
No	2(3.3%)	56(45.9%)	62(50.8%)	5.946	0.051
Yes	8(10.3%)	48(61.5%)	22(28.2%)		

DISCUSSION

Globally, among all types of cancer cases, the contribution of BC is 23% (11). Breast cancer starts from breast tissue, most commonly from the inner lining of ducts (ductal carcinoma) or the lobules (lobular carcinoma)(12). Hazard variables for breast cancer may be hereditary qualities, the need for childbearing or need of breast bolstering, increase the amount of few hormones. For breast carcinoma, female gender and increase of age are the important risk factors. Alter in dietary habits, exposure to light contamination, tobacco, high intake of fats, use of liquor also increase risk of development of breast cancer (13). Early menarche, menopause at a late age, first pregnancy at a late age, being pregnant at least three times and the presence of this cancer within first degree relatives are some factors that increased the hazards of BC. A strong association of breast cancer risk with increased age of menopause was observed in many studies (14).

The present study investigated the potential influence of the MDM2 40-bp insertion/deletion (I/D) polymorphism on the risk of developing breast cancer. The findings demonstrated no significant association between this genetic variant and breast cancer susceptibility in the studied population. In contrast, research conducted among Iranian women reported that the MDM2 40-bp I/D polymorphism was linked to an increased risk of breast cancer (2). Similarly, studies involving Chinese cohorts have revealed a significant correlation between this polymorphism and the development of lung cancer (9) as well as hepatocellular carcinoma(15). However, other investigations carried out within Chinese populations found no relationship between the MDM2 40-bp I/D variant and breast cancer risk (8). The MDM2 is linked with p53 and bcl2 pathway that repairs the damaged DNA and also relates to the initiation of apoptosis in case of failure to DNA repair. The MDM2 actually degardes p53 protein once its function is over. In this study when there is no apparent association of MDM2 found in the study population could be linked with a single gene study as this works in a pathway, thus other genes and also epigenetics have its role to play. Therefore it is considered as a limitation of the study. The clinical outcome was not evaluated in this study, though MDM2 might have strong link with it. It is also considered as a limitation. However, the large sample size from a single centre with standard protocols for evaluation of MDM2 susciptability is considered as strength of the study.

CONCLUSION

In the Pakistani female population low levels of conventional risk factors are observed since they show high fertility, numerous births, expanded period of nourishing their child, and early age pregnancy. The MDM2 did not show significant association with breast cancer susceptibility in studied population. Large scale cohort studies with inclusion of other related genes of p53 pathway are recommended.

Conflict of Interest

Authors declare no conflict of interest.

Ethical consideration

The study was approved by local research ethics committee.

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