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THE RELEVANCE OF RESIDENCY ON THE TUMOR SUPPRESSOR GENES MUTATION OF BREAST CANCER WOMEN AND THEIR RELATIVES

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ABSTRACT

Background

Breast cancer is characterized by various malignant tumors arise from breast tissues, Genetic factors, age, reproductive and hormonal factors, diet and environmental factors, socioeconomic factors and some types of benign breast disease are factors related to an increased risk of breast cancer. Tumor suppressor genes are genes whose loss of function results in the promotion of malignancy.

Aim of the Study

The present study was conducted to verify the relevance of residency on the tumor suppressor gene mutations in breast cancer women and their relatives

Material and Methods

Three groups of samples were included. The first consisted of 100 blocks of formalin-fixed, paraffin-embedded (FFPE) tissues of breast cancer women (group 1). The second contained blood samples of 46 breast cancer women (group 2). The third comprised blood samples of46 apparently healthy women who were relatives of the breast cancer patients of group two (group 3). The ages of patients of tissue samples were 46.78±11.5 years, while of blood samples patients were 47.34±11.18 years. The ages of the healthy relatives were 40.79±9.84 years. Five tumor suppressor gene mutations were examined. BRCA1 185delAG, BRCA1 5382insC and BRCA2 6174delT mutations were evaluated by mutagenically separated polymerase chain reaction, while CHEK2 1100delC and Tp53 exon 7 mutations were analyzed by RFLP.

Results

The amplification of *BRCA1*gene for185 delAG mutation revealed amplicons of 335 bp for the wild type and 354 bp for the mutant gene, whereas for 5382 insC mutation exhibited 2 amplicons of sizes 271 bp for the wild type and 295 bp for the mutant gene.

The analysis of *BRCA2*6174 delT mutation indicated 2 amplicons of 151 bp for the wild type and 171 bp for the mutant gene. *Tp53* exon 7 mutation analysis highlighted an amplicon of 125 bp size. Digestion of thisamplicon with the Hae III enzyme resulted in 2 fragments with sizes of 83 bp and 42 bp in the wild type. *CHEK2* 1100 delC mutation evaluation showed an amplicon of 116 bp size. Digestion of this applicant with Sca I enzyme exhibited 2 fragments with sizes of 92 bp and 24 before the wild type. Data analysis demonstrated that 27 (27%) out of the 100 enrolled breast cancer

patients of tissue samples have mutations in tumor suppressor genes, 11 (23.9%) out of 46enrolled breast cancer patients of blood samples have such mutations, while 8 (17.4%) out of 46 relatives have these mutations. Nine (9%) patients of group1 were indicated to have *BRCA1* 185delAGmutation, while 4 (8.7%) and 3 (6.5%) women from groups 2 and3 exhibited the same mutationrespectively. *BRCA1*5382insC mutation was identified in 7 (7%), 3 (6.5%) and 1 (2.2%) women of groups 1, 2 and 3 respectively. The *BRCA2* 6174delT mutation was observed in 5 (6%), 2 (4.3%) and 2 (4.3%) women of groups 1,2 and 3 respectively. Three (3%) patients of group1 and 1 (2.2%) and 1 (2.2%) women from groups 2 and 3 were found to have *CHEK2* 1100delC respectively. *Tp53*exon 7 mutation was evident in 2 (2%), 1 (2.2%) and 1 (2.2%) women of groups 1, 2 and 3 respectively. No patient had more than one mutation. Residency of breast cancer patients appeared to be independent of the distribution of tumor suppressor gene mutations.

Conclusions

About one quarter of the investigated breast cancer patients have mutations in their tumor suppressor genes and 17% of their relatives have such mutations. Residency of breast cancer patients and the relatives are independent on the tumor suppressor gene mutations.

KEYWORDS: Residency, Tumor Suppressor Genes Mutation, Breast Cancer

INTRODUCTION

The breastconsist of glands and fatty tissue, after pregnancy the glands making milk. The glands are responsible for hormones of a female such as progesterone & estrogen [Wiseman et al., 2002].

The milk secreted through a special duct, which is open up in the nipple. Breast fluid normally drains into the lymph nodes in the armpit or axilla by the lymphatic system, therefore it is returned into the blood stream. Important muscles lie under the breasts that permit the movement of the arm, as well as breathing, muscle involvement [Macéa et al., 2006].

Breast cancer is when cancer arises from breast tissue [American Cancer Society. 2014]. Breast cancer signs may involve a breast lump, a breast shape changes, skin dimpling, nipple fluid coming, or a skin becomes red scaly patch [Breast Cancer Treatment (PDQ®), 2014]. With thread, scaly spread [bone pain may be involvement, lymph nodes swollen, breath shortness, or yellow skin participation [Saunders et al., 2009].

In Europe and the United States the commonest malignancy found in Women is breast cancer and the incidence rises slowly [Cancer Research UK. 2008]. About 232,670 females; and 2,360 males were estimated as new cases in the United States in 2014 and deaths from breast cancer were 40,000 females; and 430 males [Breast Cancer. 2014].

Genetic factors, age, reproductive and hormonal factors, diet and environmental factors, socioeconomic factors and some types of benign breast disease are factors related to an increased risk of breast cancer [Begg et al., 2008].

Breast carcinomas are classified as ductal and lobular or invasive and noninvasive (carcinoma in situ), seventy percent of breast cancers are invasive ductal carcinomas that have no special histological features and are designated "not otherwise specified" (NOS). Most tumors arise in the terminal duct section of the breast; carcinoma in situ arises from the epithelium and is confined within the lumen of the ducts or lobules whereas invasive or infiltrating cancers breach the basement membrane [Vani, 2006].

Other fewer public kinds of breast carcinoma contain invasive lobular breast carcinoma that grows in the cells that padded the milk-generating labels, inflammatory breast carcinoma and Paget's disease of the breast. It is probable for breast carcinoma to extend to further parts of the body, frequently through the lymph nodes [Kirby et al., 2005].

In order to match results in clinical research and to determine the most suitable treatment choices, a system of staging according to the extent of disease spread is needed. The tumor, node metastases (TNM) system is used by the Union Internationale Contre Le Cancer (UICC) and the American Joint Committee on Cancer (AJCC) [Greene et al., 2002].

Breast cancer is treated using a mixture of surgery, chemotherapy and radiotherapy. Hormonal treatments can be used also in some cases of breast cancer. One of nine women is affected through breast cancer in their lifetime. There is a good opportunity of recovery if it is noticed in its early times. For this purpose, it is vital that women check their breasts commonly for any alterations and continually get any changes examined by their GP [Howard Hughes Medical Institute. 2012].

A probable genetic involvement to breast cancer hazard is shown via the enlarged rate of these carcinomas among females of a family history, and by the examination of some families in which numerous family members are influenced with breast carcinoma, in a design corresponding with an inheritance of autosomal dominant carcinoma liability. Official researches of families (linkage analysis) have then recognized the presence of autosomal dominant tendencies to breast carcinoma and have directed to the identification of several greatly penetrantgenes as the reason of inherited carcinoma hazard in numerous families [Lindor et al., 2008].

Breast cancer is a component of some autosomal dominant carcinoma syndromes. The syndromes greatest powerfully related with both cancers are the BRCA1 or BRCA2 transmutation syndromes. Breast carcinoma is also a usual aspect of Li-Fraumeni syndrome due to *Tp53* transmutations and of Cowden syndrome due to *PTEN*transmutations [Lindor et al., 2008]. Additional genetic syndromes that may contain breast cancer as a related aspect comprise heterozygous like a variant of harmful *CHEK2* alleles create a considerable involvement to breast carcinoma liability and their recognition might assistance in the medical managing of patients carrying a *CHEK2* transmutation [Alexis et al., 2011].

PATIENTS AND RELATIVE GROUPS

Study samples were obtained from AL-Sader Medical City in AL-Najaf city from September 2012 to December 2013. Three groups of samples were included. The first consisted of 100 blocks of formalin-fixed, paraffin-embedded(FFPE) tissuesof breast cancer women (group 1). The second contained blood samples obtained from 46women who were diagnosed as cases of confirmedbreast cancer(group 2). The third comprised blood samplesobtained from 46 apparently healthy women who were relatives to the breast cancer patients of group two(group 3).

The ages of tissue samples patients were 46.78±11.5 years with a range of 20 –70 years while that for blood samples patients were 47.34±11.18 with a range of 24-65 years. The ages of the relatives group were 40.79±9.84 with a range of 28–54 years.

A questionnaire was designed to obtain the information of breast cancer patients and their relatives. It contained the name, age, sex, address, and marital state, number of children, breast fed, and laterality of disease.

The practical part of the study was performed at the Laboratory of Biochemistry Department / College of Medicine / University of Kufa and Umbilical Cord Stem Cell Research Center (UCSRC), in Tabriz University of Medical Sciences, Tabriz - Iran.

COLLECTION OF SAMPLES

Small sections (up to 25 mg) from blocks of paraffin-embedded tissuewere slicedand transfered to a 1.5 ml microcentrifuge tube. Two aliquots tubes at least were done and stored at refrigerator until isolation of DNA.

Blood samples were obtained from breast cancer patients and their relativesby vein puncture. Four milliliters of blood were collected in two aliquot EDTA tube and stored at (–20 C) until isolation of DNA.

METHODS

The DNA extracted from FFPE by ReliaPrep™ FFPE gDNA Miniprep System while from frozen blood the DNA extracted by ReliaPrep™ Blood gDNAMiniprep System both kits fromPromega™.

Amplification was performed in a programmable thermal cycler gradient PCR system. For detection of 185delAG and 5382insC in *BRCA*1and 6174delT in *BRCA*2 a PCR techniques called mutagenically separated PCR (MS-PCR) polymerase chain reaction (PCR), was performed with allele-specific oligonucleotide primers as described by Chan et al [Chan et al., 1999; Fattahi et al., 2009]. In this method, three primers (one common, one specific for the mutant, and one specific for the wild-type allele) were designed for a mutation. While for *Tp53* a Single strand conformation polymorphism (SSCP) polymorphism was usedthe GT transversion at third position of codon 249 (AGG to AGT) in exon 7 of the *Tp53* gene was detected by PCR-RFLP.

Amplification was performed in a programmable thermal cycler gradient PCR system. Primers as described by Naina and Alpana [Naina and Alpana, 2009; Pallavi et al., 2010 and Sanjay et al., 2000]. In this method, two primers (forward and reverse) were designed, thePCR products were digested with FastDigest HaeIII (BsuRI) (Thermo Scientific #FD0154), the*Tp53* exon 7 amplimer (125 bp) show two distinct bands, 83 bp and 42 bp, indicating absence of a 249 codon mutation.

But *CHEK2* gene amplification was performed in a programmable thermal cycler gradient PCR system. Two sets of PCR primers were used to screen for the *CHEK2* 1100delC mutation (Table 1). Specific primers for the *CHEK2* exon 10 on chromosome 22 were designed to avoid all other homologous sequences in the genome, (Table 1), P12 and P13. The PCR products were then re-amplified with nested primers (Table 1), P14 and P15, which were designed to amplify the region encompassing the site of *CHEK2* 1100delC. The forward primer contained one base substitution (a T to G substitution at position 1097) to generate a restriction site for restriction enzyme ScaI within the wild-type allele after PCR amplification. In the presence of the 1100delC mutation, the 116 bp product was cleaved by ScaI to fragments of 92 and 24 bp, whereas the mutant product remained uncut [Shiyu et al., 2008; Hazal et al., 2013]. The PCR products were digested with Thermo Scientific FastDigest ScaI(Thermo Scientific #FD0434). The wild-type allele of 116 bp product was cleaved by ScaI to fragments of 92 and 24 bp, (the 24-bp band usually runs out off the gel). Whereas the mutant product remained uncut. All the primers are in Table 1.

Table 1: The Primers of Tumor Suppressor Genes

Gene	Primer	Sequence	Length	Tm	Size of Amplified Fragment
BRCA1 185delAGmutation	Common forward (P1)	5'-GGTTGGCAGCAATATGTGAA-3'	20 mer	58.0°C	
	Wild-type reverse (P2)	5'-GCTGACTTACCAGATGGGACTCTC-3'	24 mer	74.0°C	335 bp
	Mutant reverse (P3)	5'-CCCAAATTAATACACTCTTGTCGTGACTTACCAGATGGGACAGTA -3'	45 mer		354 bp
BRCA1 5382insC mutation	Common reverse (P4)	5'-GACGGGAATCCAAATTACACAG-3'	22 mer	64.0°C	
	Wild-type forward (P5)	5'-AAAGCGAGCAAGAGAATCGCA-3'	21 mer	62.0°C	271 bp
	Mutant forward (P6)	5'-AATCGAAGAAACCACCAAAGTCCTTAGCGAGCAAGAGAATCACC -3'	44 mer		295 bp
BRCA2 6174delT mutation	Common reverse (P7)	5'-AGCTGGTCTGAATGTTCGTTACT-3'	23 mer	66.0°C	
	Wild-type forward (P8)	5'-GTGGGATTTTTAGCACAGCTAGT-3'	23 mer	66.0°C	151 bp
	Mutant forward (P9)	5'-CAGTCTCATCTGCAAATACTTCAGGGATTTTTAGCACAGCATGG-3'	44 mer		171 bp
*Tp53*gene exon 7	Forward (P10)	5'-TCTCCTAGGTTGGCTCTGACT -3'	21 mer	64.0°C	125 bp
	Revers (P11)	5'-TCCTGACCTGGAGTCTTCCAG-3'	21 mer	66.0°C	
CHEK2 1100delC mutation	forward (P12)	5'-TTAATTTAAGCAAAATTAAATGTC-3'	24 mer	46.0°C	
	reverse (P13)	5'-GGCATGGTGGTGTGCATC-3'	18 mer	54.0°C	
	forward (P14)	5'-CCCTTTTGTACTGAATTTTAGAGTA-3'	25 mer	51.0°C	116pb
	reverse (P15)	5'-ACAAGAACTTCAGGCGCCAAGTAG-3'	24 mer	59.0°C	

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS v. 20.0 software (SPSS Inc., Chicago, IL). Distribution of genotypes was compared by chi-square test. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Statistical significance was defined as $P< 0.05$.

RESULTS AND DISCUSSIONS

Data analysis demonstrated that 27 (27%) out of the 100 enrolled breast cancer patients of tissue samples have mutations in tumor suppressor genes, 11 (23.9%) out of 46 enrolled breast cancer patients of blood samples have such mutations, while 8 (17.4%) out of 46 relatives have these mutations. Nine (9%) patients of group1 were indicated to have *BRCA1* 185delAG mutation, while 4 (8.7%) and 3 (6.5%) women from groups 2 and 3 exhibited the same mutation respectively (Figure 1). *BRCA1*5382insC mutation was identified in 7 (7%), 3 (6.5%) and 1 (2.2%) women of groups 1, 2 and 3 respectively(Figure 2). The *BRCA2* 6174delT mutation was observed in 5 (6%), 2 (4.3%) and 2 (4.3%) women of groups 1,2 and 3 respectively(Figure 3). Three (3%) patients of group1 and 1 (2.2%) and 1 (2.2%) women from groups 2 and 3 were found to have *CHEK2* 1100delC respectively(Figure 4). *Tp53* exon 7 mutation was evident in 2 (2%), 1 (2.2%) and 1 (2.2%) women of groups 1, 2 and 3 respectively(Figure 5). No patient had more than one mutation.

To perceive the relationship of gene mutations with residency of patients and relatives, women were classified into 2 groups, i.e, those of urban and rural area. Table 2 demonstrates the analysis of the data. There were no significant variations related to tumour suppressor genes mutation among patientsand relatives of the rural and urban areas, also between the patients (group 2) and their relatives (group 3) (Figure 6).

The current results are consistent with those of Inumaruet al., (2012), but they were inconsistent with the finding of Vijayaraman et al., (2012) who have pointed out significant relationship between the *Tp53* exon 7 codon 249 mutation with the residency of investigated patients.

The independency of the mutation that observed on the residency of the enrolled women could be recognized as worldwide phenomenon. Populations have changed rural areas for cities, becoming more sedentary and consuming diet similar to those of city population. Thus obesity and chronic diseases may become similar in rural and urban areas, one of the diseases is cancer. Patterns of physical activity, body composition, and breastfeeding are determined by environmental (urbanization and urban planning), economic (social status), and social (education level) factors, will influence several types of cancer.

Mutations that are related to such disease will be directed by the effective factor [Ministerio et al., 2009; World Cancer Research Fund (WCRF), 2007].

The results of this study are also varying with Jussawalla and Jain (1977) who are showed that interestingly, there is also a significant difference in the incidenceof breast cancer between urban and rural population of India and it also varies among variousreligious groups, the highest (1.5–2.1 times) beingin Parsi population as compared to either Hindu, Muslim or Christian population.

But also correspondence with Suresh Hedau et al. (2004) who interpreted their results as the spectrum of mutations observed in the present study demonstrates no major role ofBRCAgene mutation in familial breast cancer. Also, there is no or negligible role ofBRCA1, BRCA2orp53gene mutation in sporadic breastcancer. However, other factors may play animportant role in India, which is a vast countrywith a varied ethnic population and diverse socioeconomic, cultural, sexual and dietary habits andbreast cancer is fast emerging as a major cancer, the incidence of which differs significantly betweenurban and rural population, and between pre andpost-menopausal women.

One the other hand the outcomes are similarly to the finding of Mufeed J Ewadh et al. (2009) they verify that urban areassuffer from air pollution from exhaust of the oil refineries and car engines. In the rural there is much more

chance for the availability of pure air by the presence of trees, palms and other different types of plants. On contrary to these good conditions and natural facilities to face the cancerous agents a problem ariseshere which is the use organophosphate agents (insecticide and pesticides) to protect their farms. From harmful insects or pests, but the side effectsof these agents are very harmful which inducescancer, especially due to the wrong way of applying these agents to the plants by making solution by their hands on the plant.

CONCLUSIONS

Residency of breast cancer patients appeared to be independent on the distribution of tumor suppressor gene mutations.

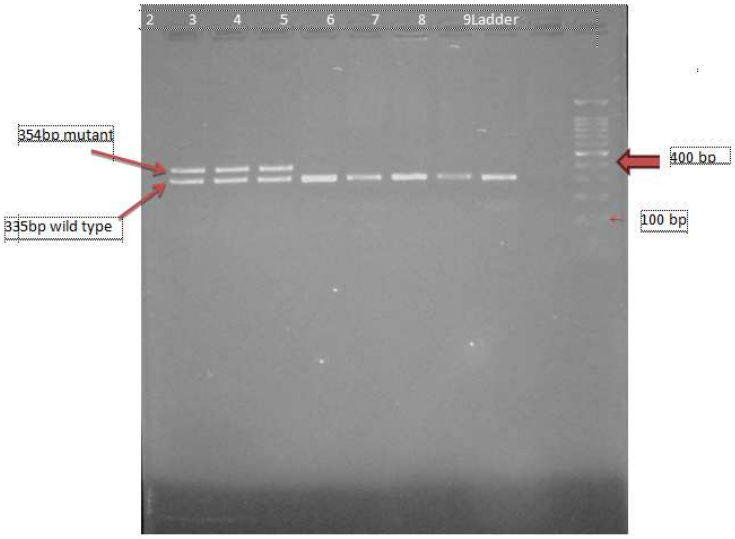

Figure 1: Gel Electrophoresis of PCR Product for BRCA1 185delAG Detection on 3%Agarose Gel by10X TAE Buffer for 35 Minutes, at 140 V, with 100bp DNA Ladder and Ethidium Bromide Stained. Lane 1-3 Show Genotype Mutations. Lane 9 Negative Control

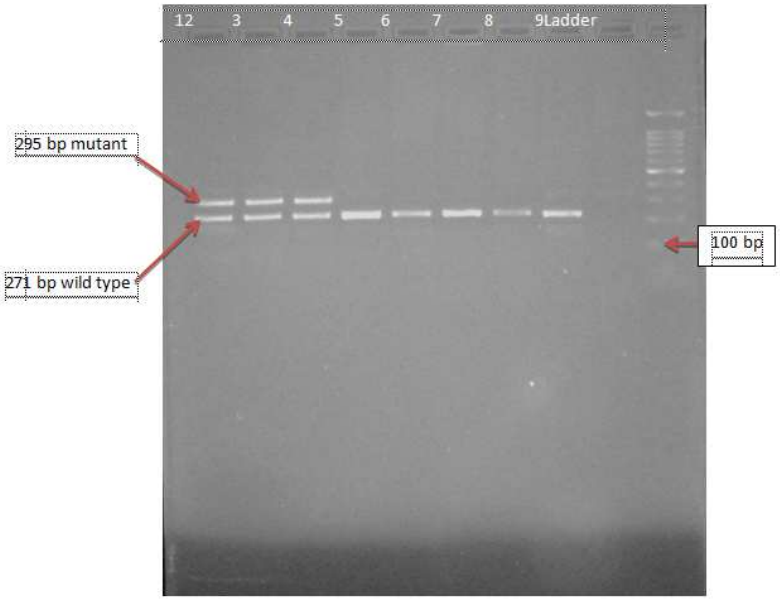

Figure 2: Gel electrophoresis of PCR Product for BRCA1 5382insC Mutation Detection on 3% Agarose Gel by10X TAE Buffer for 35 Minutes, at 140 V, with 100bp DNA Ladder and Ethidium Bromide Stained. Lane 1-3 show Genotype Mutations, Lane 9 Negative Control

Figure 3: Gel electrophoresis of PCR Product for BRCA2 6174delT Mutation Detection on 3% Agarose Gel by10X TAE Buffer for 35 Minutes, at 140 V, with 100bp DNA Ladder and Ethidium Bromide Stained. Lane 1-3 Shows Genotype Mutations, Lane 9 Negative Control

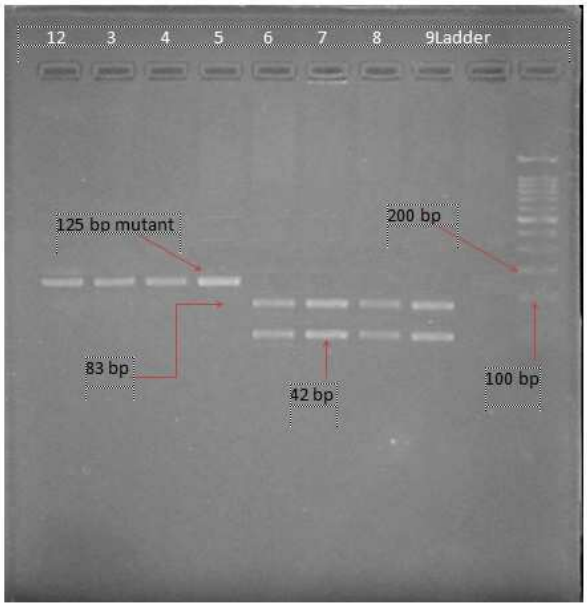

Figure 4: Restriction Enzyme (Hae III) Digestions of Tp53 Polymerase Chain Reaction Products of Tp53 exon 7 Amplimer (125 bp) Show two Distinct Bands, 83 bp and 42 bp, Indicating Absence of a 249 Codon Mutation (lane 5-8)
Lane (1-4) Shows a 249 Codon Mutation of Tp53 Exon 7.
Lane 9 Negative Control
Electrophoresis on 1.5% Agarose gel, for 30 Minutes, at 120 Volts Used 10X TAE Buffer with 100bp DNA Ladder and Stained with Ethidium Bromide

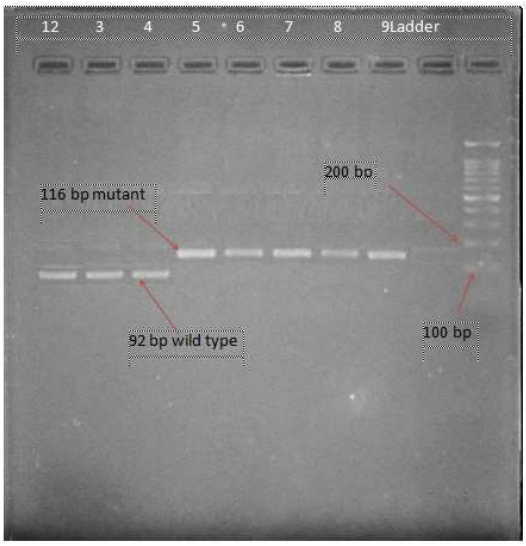

Figure 5: Restriction Enzyme (Sca I) Digestions of CHEK2 PCR Products were Separated by Electrophoresis on 3% Agarose gel, for 35 minutes, at 140 Volts Used 10X TAE Buffer with the 100bp DNA Ladder and Stained with Ethidium Bromide. The Wild-Type Allele (lane 1-3) of 116 bp Product was Cleaved by ScaI to Fragments of 92 and 24 bp, (the 24-bp Band Usually Runs Out off the Gel). Whereas the Mutant Product Remained Uncut (lane 4-8).
Lane 9 Negative Control

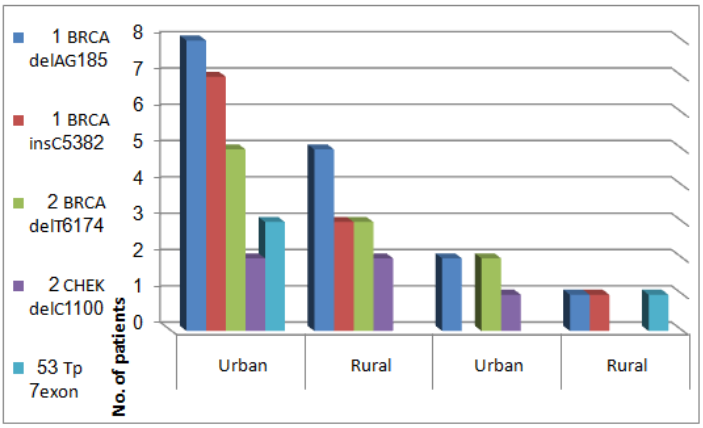

Figure 6: Gene Mutations Affected by Residency

Table 2: Distribution of Gene Mutations in Patients and their Relatives of Urban and Rural Areas

Group	Residency (No)	BRCA1 185delAG Positive % Negative %	BRCA1 5382insC Positive % Negative %	BRCA2 6174delT Positive % Negative %	CHEK2 1100delC Positive % Negative %	Tp53 exon 7 Positive % Negative %	Total
Breast cancer patients	**Urban** (98)	8 8.2 90 91.8	7 7.1 91 92.9	5 5.1 93 94.9	2 2 96 98	3 3.1 95 96.9	25 25.5 71 74.5
	Rural (48)	5 10.4 43 89.6	3 6.3 45 93.7	3 6.3 45 93.7	2 4.2 46 95.8	0 0 48 100	13 27.1 35 72.9
Total of Breast cancer patients (146)		13 8.9 133 91.1	10 6.8 136 93.2	8 5.5 138 94.5	4 2.7 142 97.3	3 2.1 143 97.9	38 26 108 74
Relative group	**Urban** (25)	2 8 23 92	0 0 25 100	2 8 23 92	1 4 24 96	0 0 25 100	5 20 20 80
	Rural (21)	1 4.8 20 95.2	1 4.8 20 95.2	0 0 21 100	0 0 21 100	1 4.8 20 95.2	3 14.3 18 85.7
Total of Relative (46)		3 6.5 43 93.5	1 2.2 45 97.8	2 4.3 44 95.7	1 2.2 45 97.8	1 2.2 45 97.8	8 17.4 38 82.6
Total (192)		**16 8.3 176 91.7**	**11 5.7 181 94.3**	**10 5.2 182 94.8**	**5 2.6 187 97.4**	**4 2.1 188 97.9**	**46 24 146 76**

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