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PREVALENCE OF BRCA1/BRCA2 MUTATIONS IN THE PAKISTANI BREAST CANCER PATIENTS.

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ABSTRACT… Objectives: Breast cancer is the most common cause of death in women. 20% of breast cancer cases are due to the variations in the BRCA1/BRCA2 genes. Various variants of BRCA1/2 genes have been reported worldwide, which cause breast cancer by forming truncated proteins. In the current study, we aimed to find out the prevalence of mutations 1294DEL40, E1250X and 5382insC (BRCA1) and 2157DELG (BRCA2) in a case control cohort of Pakistani origin. **Study Design:** Case Control Study. **Setting:** Microbiology and Molecular Genetics University of Punjab, Lahore. **Period:** December 2017 to June 2018. **Material & Methods:** Blood samples from 80 female breast cancer patients and 80 healthy females were taken. DNA was isolated from blood by manual method and genotyping was done by PCR-RFLP. **Results:** We found that for the 2157DELG mutation of the BRCA2 gene, homozygous mutant phenotype is present only in the cases at a frequency of 25% while the BRCA1 gene mutations were less common i.e., the E1250X mutation showed 6.6% prevalence in patients, and the 1294del40 and 5382insC mutations were absent in the current cohort. **Conclusion:** Despite of sample size limitation, this study provides information about the prevalence of some common mutations. In addition, clinical risk factors were also noted which are essential in risk assessment. We foresee that targeting of specific genetic variants restricted to a particular ethnic group would be more effective in the future therapeutic approaches for the prevention and cure of breast cancer.

Key words: Breast Cancer, BRCA1 Gene, Mutation, Pakistan.

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INTRODUCTION

Breast cancer is characterized by the development of lesions of the breast that make palpable painful nodules or masses. Fortunately, such lesions are mostly harmless, but lumps produced from the uncontrolled proliferation of breast cells are tumors that may arise from connective tissue or epithelial structure. This malignancy of the breast tissues originates mostly from the inner lining of the milk ducts or from the lobules that provide milk to the ducts. Survival rate and prognosis for breast cancer depend on the stage, cancer type, geographical location and treatment of the patient. It is a multifactorial disease and both genders can get breast cancer, but women are more susceptible to it as compared to males.[1-3]

The susceptibility to breast cancer is normally inherited in an autosomal dominant pattern. Breast cancer is a serious health issue and is the second in number among all cancers in terms of prevalence. The women of any race, ethnic group, area or country can acquire cancer. Breast cancer may be due to environmental and genetic factors causing mutations in the essential genes. Breast cancer frequency varies largely by the socioeconomic status, race/ethnicity and geographic region. Certain mutations can occur at a high proportion in a particular area or ethnic group.[4,5] According to the recent research, there is an important relation between the genetic instability and the progression of cancer. Some of the mechanisms involved are amplification of centrosomes, deterioration of telomeres, DNA damage and epigenetic modifications. Alteration in genetic makeup can cause apoptosis, mutation induction, necrosis and aneuploidy.[6,7]

The causes of breast cancer remain partially understood. However, three sets of influences seem to be considerable: genetic changes, hormonal fluctuations and environmental influences. Some of the risk factors include age, age at menarche and menopause, use of oral contraceptives, obesity, exposure to radiations, cessation of breast feeding, use of alcohol, family history of breast cancer, etc.[8-12] The most important genes which comprises about 20% of breast cancer cases are the BRCA1 and BRCA2 breast cancer susceptible genes.[13] Carriers of p53 mutation are more susceptible to early onset of breast cancer, but it rarely causes cancer syndromes. Low to intermediate risk genes include CHEK2, PALB2, RAD50, BRIPI, NBSI and mismatched repair genes MLH and MSH2.[14,15]

Prevalence of the selected variants 1294DEL40, 2157DELG, E1250X and 5382insC have been checked in the breast cancer patients in many ethnicities but there is no data available for Pakistani population. 1294DEL40 is a variant of exon 11 of BRCA1 gene. Exon 11 spans 3426bp and regarded as largest and significant exon in terms of the number of variants. Variant 1294DEL40 involves deletion of 40bp at nucleotide 1294 (codon 392). It leads to premature translation termination and results in truncated BRCA1 protein.[16,17] 2157DELG variant is located at the exon16 and E1250X variant on exon 11 of BRCA1 gene. 5382insC variant is present on the exon 20 of BRCA1 gene and is caused by the insertion of nucleotide 'C' at 1756 position.[18] Therefore, in the current study, we aimed to find out the spectrum of these selected mutations in Pakistani population. The Pakistani population represents a unique ethnic group with high risk/prevalence of many genetic diseases due to restricted religious, social and cultural norms as well as a high rate of consanguineous marriages.

MATERIAL & METHODS

Study Population

160 study subjects were taken with different age groups. Sampling was done from December 2017 to June 2018 from female's breast cancer patients and healthy individuals. Inclusion criteria for breast cancer patients included they should have well examined breast cancer and should belong to Pakistan. While controls should be healthy with no family history of breast cancer and have no infectious disease. Consent was taken from everyone prior to sampling of blood. Ethical approval was obtained from the ethics committee of University of the Punjab.

Blood Sampling

1ml blood was drawn from the study subjects under aseptic conditions and the blood was poured in the EDTA vials. Then it was stored at 4□ to proceed further for DNA isolation.

Genotyping

DNA from blood samples was isolated by manual method followed by 1% gel electrophoresis. Genotyping was done by PCR (polymerase chain reaction), followed by RFLP (restriction fragment length polymorphism). Allele specific PCR was done to check the presence of 1294del-40 variant using three primers. First primer was common forward: 5'TAAGCAGAAACTGCCATGCT'3, second primer was wild type reverse: 5'CAGCTACTTTGGCATTTGA'3 and third primer was mutant reverse: 5'CAGCTACTTTGGCATTTGT'3. The PCR products were resolved by PAGE. For 2157DELG variant, the sequences of the primers are; forward primer: 5'AATTCTTAACAGAGACAGAAC'3, reverse primer: 5'AAAACTCTTTCCAGAATGTTGT'3. For E1250X, primer sequences are; forward: 5'GATGACCTGTTAGATGATGGTGA'3, and reverse primer: 5'CTCTGTGTTCTTAGACAGACCCT'3. For 5382insC, primer sequences are; forward: 5' GGGAATCCAAATTACACAGC'3, reverse: 5' CCAAAGCGAGCAAGAGAATCTC'3.

An initial denaturation temperature was 95□ for 5 min, followed by 40 cycles at 94□ denaturation temperature for 1min. Extension at 72□ for 1 min and final extension at 72□ for 5 min. Annealing temperature was different for these four variants, 55□ for 1294del40, 58□ for 2157DELG and 57□ for both E1250X and 5382insC.

The PCR product 450bp of 2157DELG was digested by BSuRI restriction enzyme. The digested fragments were of 342bp and 108bp. The PCR product of 252bp of E1250X variant was digested with the help of MspI restriction enzyme into two fragments of 202bp and 50bp. For the 5382insC variant the PCR product 270bp was digested with the help of HindII restriction enzyme and the digested products were of 186bp and 84bp.

RESULTS

Various stratification factors were measured among patients and are shown in table-I 20% of our samples had family history of breast cancer while 80% were negative for any reported family background of breast cancer. 52% sample subjects had metastatic tumour while 48% patients had benign breast tumour. The majority of study subjects were married, housewives and had post-menopausal status.

Body mass index is considered a strong risk factor for breast cancer. BMI characterization given in Table-I, II 55% patients were obese, 27% overweight, 11% patient's fell into the normal category of BMI while 5% study subjects were underweight.

Different parameters including age, weight, BMI, and menopause status was recorded. Table-III summarizes anthropometric parameters i.e. age, weight, and BMI of the study subjects. The mean age (years) of breast cancer patients was 42±7.2 years and mean age of healthy controls was 43.96±8.7 years. Mean weight (Kg) in cases was 68.72±11.79 while mean weight in controls was 63.32±2.5. Mean value of BMI (Kg/m^2) in cases was 29.31±5.03 while in controls was 26.5±4.2.

In the present study, four variants were mutations were genotyped, three by PCR-RFLP and one by allele specific PCR to reveal their association with breast cancer. For 2157DELG, 55% of the cases were the homozygous wild genotype, 25% were the homozygous mutant and 20% were the heterozygous genotype, this mutation was absent in the controls (Table-IV). The two mutations 1294del40 and 5382insC were totally absent while E1250X showed 6.66% prevalence in the current cohort (Table-V).

Sr. No.	Variables		Percentage of Patients
1	Marital status	Married	88%
		Not married	12%
2	Profession	Working lady	19.2%
		House wife	80.7%
3	Menstrual status	Premenopausal	46.1%
		Post menopause	53.8%
4	Family history	Yes	20%
		No	80%
5	Metastatic stage	Yes	52%
		No	48%
6	Age of onset	Below 30 years	7.6%
		Above 30 years	92.3%
7	Disease status	Bilateral	3.8%
		Unilateral left	33.2%
		Unilateral right	62.9%

Table-I. Distribution of various stratification factors in patients.

BMI Categories	BMI (Kg/m^2)	Percentage of Patients
Underweight	<18.5	5%
Normal weight	18.5-24.9	11%
Over weight	25-29.9	27%
Obesity	30 or greater	55%

Table-II. Distribution of study subjects based on BMI.

Parameters	Case	Control	P-Value
Age(years)	42±7.2	43.96±8.5	0.3
Weight(kg)	68.72±11.79	63.32±2.5	0.02
BMI(kg/m^2)	29.31±5.03	26.5±4.2	0.03

Table-III. Comparison of anthropometric parameters in the study subjects.

Sr. No.	Genotype	Percentage
1	Homozygous wild	55%
2	Homozygous mutant	25%
3	Heterozygous	20%

Table-IV. Genotype frequency of 2157DELG.

	Variant	Controls	Cases	Percentage of Prevalence Among Cases
BRCA1 Gene	1294del40	80	80	0%
	5382insC			0%
	E1250X			6.6%

Table-V. Prevalence of BRCA1 mutations among cases.

DISCUSSION

Approximately 5-10% of breast malignancy is due to the genetic predisposition of mutated genes.[7,19] Beside alterations in certain genes, several factors such as family history, marital status and lifestyle play significant roles in the cancer development.[20,21] Most importantly, mutations in tumour suppressor genes such as BRCA1 and BRCA2 play a notable part in the progression to breast cancer. BRCA1 is a nuclear phosphoprotein encoding tumour suppressor gene that play a part in conserving genomic stability.[22] This gene is present on chromosome 17, a mutation in this gene elevates the probability to developing breast cancer.[7,23] Probability of progression of breast tumour increases up to 65% in BRCA1 mutation carriers.[24] Therefore in the present study four variants of BRCA1 phosphoprotein were selected and genotyped to check the prevalence of breast cancer in Pakistani population due to these variants.

In the present study, the prevalence of variant 1294del40, 5382insC, E1250X and 2157DELG was checked in the Pakistani population. The involvement of these variants and various other non-genetic factors was analyzed. Various non-genetic factors such as history of the menstrual cycle, menopausal status, body mass index, physical exercise and hormonal fluctuations are considered to be significant.[5,25]

Metastasis status of patients was also observed and breast cancer patients with carcinoma spreading to other organs were in majority. As indicated in Table-I, most patients have advanced stage of tumor i.e. III and IV. Stage IIIC and IV include invasive breast tumor that affects nearby lymph nodes and organs like liver, lung etc. The reasons may have been the lack of awareness regarding development and risk factors in the public. According to various studies, in Pakistan women are identified at later stages of breast cancer due to lack of screening facilities in rural areas as well as expensive screening mammogram.[26]

An unhealthy diet that leads to excessive body weight is also a significant risk factor that enhances cancer risk. A higher BMI has been reported to predispose to disease. Studies suggest that in both pre-or post-menopausal breast cancer the mechanism of body weight and obesity to increase risk is related to the estrogenic activity. The risk of BC increases significantly due to increased BMI and excessive body fat at post menopause.[27] In the present study, 55% of breast cancer patients were obese while 27% were overweight (Table-II). Out of 55% of obese patients, 38% were of post menopause status. Majority of study subjects were married, housewife and had post-menopausal status. Physical activity and environment of the patient, excluding genetic alterations, also have an association with breast cancer progress in females. Women with an active lifestyle are less likely to develop breast cancer as compared to women with a sedentary lifestyle.[28] Hormonal fluctuations are the important risk factor for developing breast cancer. Studies suggest that post-menopausal women with the sedentary lifestyle and unhealthy BMI i.e. > 25 were at more risk of breast cancer progression.[5]

We compared various anthropometric parameters among cases and controls of the local population as shown in Table-III. Three parameters i.e. age, weight, and BMI were selected. Mean age is higher among control females 42 ± 7.2, while weight and BMI are higher among cases females. The association of age (p-value $= 0.3$) with the disease in cases and controls is not significant as p-value is >0.05. While there is a significant association of weight and BMI with the disease as p-value < 0.05 i.e. p-value 0.02 for weight and 0.03 in case of BMI.

In the present study four variants of BRCA1 gene were genotyped to check its prevalence among Pakistani population. Variant 1294del40 and 5382insC were absent in current cohort. Both variants are well studied in Canadians, Nepalese and Romanian population but not in Pakistan. Variant E1250X was present in 6.6% breast cancer patients the important finding of the study was that, the marker SNP which was used in order to find its association with the breast cancer risk in the Pakistan, it does not have a strong significant association with the Breast cancer in the Pakistan.

Despite of the shortcomings of the used marker, it might open up the gateways to target the more specific types of the treatment strategies, on the basis of the combined outcome of the clinical characteristics of the tumorous growth and the genetic makeup.

According to our results, in case of variant 2157DELG homozygous wild were found more in breast cancer patients than homozygous mutant genotype because the results should be replicated in a large sample size in future. Despite of having some limitations, the findings of the present study are important. 2 out of 4 variants were successfully identified in our population that gives the goals of future research of creating multiplex PCR for highly prevalent genetic variants screening.

CONCLUSION

As breast cancer is the leading cause of deaths in females of Pakistan, it is a necessity of present age to do research to understand the genetic causes of this disease and to evaluate environmental and hormonal risk factors to increase awareness among the local population. Our research focuses not only on prevalence of four variants 1294DEL40, 2157DELG, E1250X and 5382insC in Pakistani population, but also determine the association of breast cancer with BMI metastatic stage, family history, lifestyle, clinical markers and demographic features. To overcome the limitations of this study, furthermore, to properly investigate the prevalence of this variant in Pakistani population, research at a broader level with large sample size is required. Also, the samples should be collected from different hospitals all over the country to better investigate prevalence.

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2	Zainab Liaqat	Experimental work writing the intial draft, Data analysis.	
3	Faiqa Maqsood	Experimental work, initial drafting, data analysis,	
4	Tehmeena Akhtar	Experimental work, initial drafting, data analysis,	
5	M. Farhan Nasir	Sample collection, lab work, Initial drafting.	
6	Sumbal Sarwar	Study design, Lab work, data analysis, paper review.	
7	Warda Fatima	Sample collection, Data analysis, Logistic support.	