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Research Article
Role of Human *PTEN* and *TP53* Sequence Mutations in the Etiology of Breast Cancer in Saudi Patients

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Abstract

Background and Objective: According to the Saudi cancer registry reports, breast cancer is the first cause of cancer-related death in women and the eleventh cancer incidence in male of Saudi Arabia. Phosphatase and tensin homolog and tumor protein 53 *PTEN* and *TP53* mutations in codons are relatively common in increasing prevalence of the most types of cancer incidence as tumor suppresser genes. The primary objective of this study was to investigate the mutations in *PTEN* and *TP53* in both male and female patients with breast cancer in Saudi Arabia and the role of these mutations on the protein expression of *PTEN* and *TP53* antibodies using immunohistochemical method. **Materials and Methods:** The present study encompasses 342 Saudi breast cancer patients that were carrier of either *PTEN* or *TP53* gene mutations. The analysis was conducted using sanger sequence and immunohistochemical staining for protein expression. **Results:** The frequency of *PTEN* mutation in male and female was 22.0 and 78.0%, respectively. Similarly, the frequency of *TP53* mutation among male and female was 19.2 and 80.8%, respectively. Seven distinct mutations were identified in *PTEN* and *TP53* gene. The *PTEN* and *TP53* mutations involving transitions, transversions and additions or deletions were identified in the present investigation. Inactivation of *PTEN* and overexpression of *p53* by mutation has been detected in number of breast cancer samples as screened through immunohistochemical staining. Breast cancer samples were tested with anti-*PTEN* monoclonal antibody 6H2.1 and anti-*p53* monoclonal antibody DO 7 for estimation of immunohistochemical expression. **Conclusion:** Overall 26.8% of the analyzed breast cancer tumors displayed loss of *PTEN* expression. The *TP53* over expression was observed in 65.7% of the tumors.

Key words: Breast cancer, tumor suppressor gene, *TP53* mutation, *PTEN* mutation and Immunohistochemistry

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the last few decades genetic testing for mutations in cancer susceptibility genes has been highly emphasized. However, owing to the expensive testing procedure and technical limitations, most of the mutations still remain unidentified or poorly understood. Several studies have linked genetic predispositions of cancer with mutations of tumor suppressor genes. Of the several genes, genetic testing of inherited mutations in *PTEN* and *TP53* tumor suppressor genes have been extensively carried out to explore the detail mechanism underlying its role in tumorigenesis and tumor progression.

The *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) and *TP53* (in chromosome 17) are the most frequently mutated tumor suppressor genes have been implicated in the pathogenesis of breast cancer[1]. The *PTEN* encodes for a dual specific phosphatase protein. The *TP53* gene encodes a 53-kd transcription factor that mediates tumor suppressor activity. These genes are known to regulate each other's activity at both transcription as well as translational level[2]. Though inherited mutations of *TP53* and *PTEN* are reported to be infrequent they are often found associated with early onset of breast cancer. The tumour suppressor activity of *PTEN* is mediated by protein phosphatase activity and lipid phosphatase activity. Lipid phosphatase activity is manifested by dephosphorylation of 3-phosphoinositide products (PIP3) of the phosphatidylinositol-3-kinase (PI3K), antagonizing AKT (also known as protein kinase B, PKB) signaling. In normal condition *PTEN* negatively regulates PI3K pathway responsible for cell proliferation, survival and increase in cell size. When *PTEN* is mutated AKT is activated leading to initiation of tumorigenesis[3].

The tumor suppressor activity of *TP53* is mediated by protein *p53* through many auto-regulatory pathways with the involvement of several modulator proteins. The *p53* protein is a multifunctional transcription factor and regulates either by inducing or repressing genes involved in regulation of cell cycle, DNA repair, differentiation, senescence and apoptosis[4,5]. Mutations in the *TP53* tumor suppressor gene have been reported in15-30% of breast cancer patients[6] and are thought to be associated with poor clinical prognosis[7]. Mutations alter p53 activity by activating several stress-induced regulatory pathways.

A comprehensive knowledge of *PTEN* and *TP53* mutations in breast cancer is still insufficient in Saudi population. Present study addresses the mutation frequency of *PTEN* and *TP53* in breast carcinoma tissues among Saudi male and female patients with breast cancer and to determine the level of gene expression patterns using immunohistochemistry method for archived formalin-fixed paraffin-embedded blocks and histomorphological portraits.

MATERIALS AND METHODS

Ethics statement: The current study was approved by the deanship of scientific research of Princess Nourah Bint Abdul Rahman University. Informed consent was taken from all of the breast cancer patients before collection of tumor samples from them.

Study population and samples collection: A total 342 samples of malignant breast tumors diagnosed between November, 2017 to January, 2018 were collected during surgeries from King Fahad Medical City (KFMC), Riyadh, Saudi Arabia. Only Saudi patients with breast cancer diagnosed within the age of 40 years were included in the study. Approximately 7-8 mm of breast tumors' biopsies were immersed immediately in 10% buffered formalin in labeled tubes for one hour then these fixed biopsies were embedded in paraffin wax. All the specimens used in the study were coded and the patient's confidentiality was preserved in accordance with the guidelines for studies of human subjects.

DNA extraction: The DNAs were isolated from formalin fixed, paraffin embedded (FFPE) breast cancer tumor tissues (malignant) of 342 samples and 20 randomly selected non tumor tissues (benign) using Qiagen DNA isolation kit (Cat No./ID: 69506, Quigen, Hilden, Germany) according to the manufacturer's recommendations. The ratio of DNA concentration and quality were estimated by using NanoDrop Spectrophotometer (Thermo Scientific, USA).

Targeted sequencing of *PTEN* and *TP53* mutations: Mutational analysis for all coding exons of the *PTEN* and *TP53* genes were assessed in 342 breast cancer samples (212 samples of female patients and 130 samples of male patients) through heteroduplex analysis[8] or single-stranded conformation analysis[9].

PCR and sanger sequencing for detection of *PTEN* and *P53* mutations: Conventional Sanger sequencing was performed for the entire coding regions of *PTEN* and *TP53* from 342 FFPE blocks. Primer 3 software was used to design the primers for all coding exons of *PTEN* and *TP53* genes, the sequences of

Table 1: *PTEN* and *TP53* primers sequence

PTEN primers		Sequence
Exon 1	Forward	5'-TTCTGCCATCTCTCTCCTCC-3'
	Reverse	5'-ATCCGTCTACTCCCACGTTC-3'
Exon 2	Forward	5'-GTTTGATTGCTGCATATTTCA-3'
	Reverse	5'-TCTAAATGAAAACACAACATGAA-3'
Exon 3	Forward	5'-AGCTCATTTTTGTTAATGGTGG-3'
	Reverse	5'-CCTCACTCTAACAAGCAGATAACTTTC-3'
Exon 4	Forward	5'-CCTCACTCTAACAAGCAGATAACTTTC-3'
	Reverse	5'-AAAGATTCAGGCAATGTTTGTTAG-3'
Exon 5	Forward	5'-TGACAGTAAGATACAGTCTATCGGG-3'
	Reverse	5'-TCATGTTGCAGCAATTCAC-3'
Exon 6	Forward	5'-ATGGCTACGACCCAGTTACC-3'
	Reverse	5'-AAGAAAACTGTTCCAATACATGG-3'
Exon 7	Forward	5'-CAGTTAAAGGCATTTCCTGTG-3'
	Reverse	5'-GCTTTTAATCTGTCCTTATTTTGG-3'
Exon 8	Forward	5'-GCATTTGCAGTATAGAGCGTG-3'
	Reverse	5'-TCAAGCAAGTTCTTCATCAGC-3'
Exon 9	Forward	5'-AATCCAGAGGCTAGCAGTTC-3'
	Reverse	AAGGTCCATTTTCAGTTTATTC-3'
***TP53* primers**		
Exon 1	Forward	5'-GAGAATCCTGACTCTGCACC-3'
	Reverse	5'-AGCCGAGAGCCCGTGACTCA-3'
Exon 2-3	Forward	5'-CCAGGGTTGGAAGCGTCTCATGC-3'
	Reverse	5'-GAGCAGTCAGAGGACCAGGTCC-3'
Exon 4	Forward	5'-GACCTGGTCCTCTGACTGCT-3'
	Reverse	5'-GCATTGAAGTCTCATGGAAG-3'
Exon 5	Forward	5'-ACTTGTGCCCTGACTTTCAACT-3'
	Reverse	5'-CAATCAGTGAGGAATCAGAGGC-3'
Exon 6	Forward	5'-TCAGATAGCGATGGTGAGCAG-3'
	Reverse	5'-GCCACTGACAACCACCCTTA-3'
Exon 7	Forward	5'-AGGCGCACTGGCCTCATCTT-3'
	Reverse	5'-GAAATCGGTAAGAGGTGGGC-3'
Exon 8	Forward	5'-GGAGTAGATGGAGCCTGGTTT-3'
	Reverse	5'-GGTGATAAAAGTGAATCTGAGGC-3'
Exon 9	Forward	5'-GGAGACCAAGGGTGCAGTTAT-3'
	Reverse	5'-GTTAGTTAGCTACAACCAGGAGCC-3'
Exon 10	Forward	5'-CAATTGTAACTTGAACCATC-3'
	Reverse	5'-ATGAGAATGGAATCCTATGG-3'
Exon 11	Forward	5'-GCACAGACCCTCTCACTCATGT-3'
	Reverse	5'-CAAGGGTTCAAAGACCCAAAAC-3'

Table 2: Anti-*p53* and anti-*PTEN* antibodies used for immunohistochemistry profile

Antibodies	Clone	Class	Dilution	Antigen retrieval
Anti-*p53*	DO7	MM	1:100	PC
Anti-*PTEN*	mAb 6H2.1	MM	1:800	PC

MM-mouse monoclonal, PC-pressure cooker

primers for *PTEN* and *TP53* are provided in Table 1, respectively. The 50 ng of tumor DNA was used for each PCR analysis. Sequencing reactions were performed using BigDye® terminator v1.1 Cycle Sequencing kit for each purified PCR product. The products of sequencing reaction were purified using MontageTM SEQ96 Sequencing Reaction kit, followed by electrophoresis through Applied Biosystems 3130 Genetic Analyzer. Mutation peak reaching 20% height of the normal peak were estimated.

Tumor tissue preparation and histopathological examination: The histological features of malignant breast tissues were stained with hematoxylin and eosin stains (H and E). Different lesions from malignant tumor's from male and female breast tissues (10 each) were selected randomly from the stained section. Similarly, 10 benign breast tumour tissues were selected as controls. The H and E sections were arrayed based on standard procedures[10]. The histological types investigated in the study were infiltrative ductal carcinoma, lobular ductal carcinoma and *in situ* ductal carcinoma.

Immunohistochemical stating for *PTEN* and *TP53* antibodies: Immunohistochemical studies were performed in 114 breast cancer samples (41 with *PTEN* mutation and 73 with *TP53* mutation) obtained from paraffin-embedded blocks. 10 benign breast tumour tissues were selected as controls. The 3 µm sections cut from paraffin wax blocks were mounted in saline-glass slides and air-dried overnight at 37°C, deparafinized in xylene and then rehydrated in graded alcohol (100 and 70%). Slides were incubated in 3% H_2O_2/methanol for 10 min, followed by washing in phosphate-buffered saline (PBS). The antigen retrieval procedure is mentioned in Table 2. The sections were incubated overnight in primary antibodies at 4°C. After washing in PBS, sections were incubated with biotinylated secondary antibody, followed by the streptavidin-biotin-peroxidase complex reagent. Immunostaining was visualized with 4,6-diamidino-2-phenylindole (DAPI). Negative control slides were obtained by omitting the primary antibody. The percentage of stained nuclei was scored as per standard procedure[11]. Briefly, expression of antigen in stained cellular components were estimated and scored based on positive or negative reaction with respective antibody. For *p53* immunoreactions, the percentage of positive tumour nuclei were evaluated based on following score level: None (0%), weak (<10%), moderate (10-30%) or strong staining intensity (>50%). Tumors exhibiting more than 15% (cutoff values) positive reactions were considered p53 positive. For evaluation of *PTEN* expression a histoscore (H-score) was used. The intensity of staining score used in the study were 0, 1, 2 and 3 for none, weak, moderate and strong staining intensity. The proportion of positive cells was divided as follows: 1-10% (score 1), 11-50% (score 2) and more than 50% (score 3). The final numerical H-score for each case was obtained by multiplication of scores obtained in above categories.

RESULTS

Deleterious *PTEN* and *TP53* gene mutation in Saudi breast cancer patients: The study included 342 samples with 130 male and 212 female breast cancer samples. The age at diagnoses of breast cancer for *PTEN* and *TP53* mutation carriers were within 40 years. The complete coding region was screened for deleterious mutations in each sample, using both heteroduplex DNA analysis (HAD) and single strand conformation analysis (SSCA). Deleterious gene mutations were found in 114 of the 342 malignant Saudi studied (33.3%). Of the mutations identified, 36.0% were located in *PTEN* gene and 64.0% in *TP53* gene. The frequency of *PTEN* mutation in male and female was 22.0 and 78.0%, respectively. Similarly, the frequency of *TP53*, mutation among male and female was 19.2 and 80.8%, respectively. Most of the mutations identified were missense or frame shift mutations. Interestingly, most of the mutations were detected within exon 8 of *PTEN* gene and exon 9 of *TP53* gene. Seven distinct mutations of *PTEN* gene were identified, out of which 68T>C and 697C>G (exon 8) and 567AC>TG (exon 5) substitution mutation occurred in maximum number of individuals under study. The frequency of mutations ranged from 4.9-29.3% with most frequent mutation (29.3%) being the 68T>C substitution in exon 8. Similarly, in *TP53* gene seven mutations were identified. The frequencies of these mutations varied from 2.7-32.9% among the *TP53* gene mutations (Fig.1, Table 3). In *TP53* gene the most frequent mutation being the 308 AC> GG substitution (32.9%) and 311 ins T (23.4%) in exon 9 and 119 del C (23.4%) in exon 4. The SSCA shift in exon 3 was detected in 2 breast carcinoma samples for 113 del C mutation, in 15 samples in exon 4 for 119 del C mutation and in 17 samples in exon 9 for 311 ins C mutation. None of these mutations were detected from 20 randomly selected benign samples. No frameshift mutation was detected in *PTEN* gene. No family history of carcinoma had been reported by the individuals included under study.

Table 3: The *PTEN* and *TP53* sequence mutations with clinicopathological types in breast cancer

Gene	Exon	Mutation	Mutation type	Clinical type	Gender
PTEN	8	68 T>C	Substitution	Malignant	Male
	8	70 G>A	Substitution	Malignant	Male
	8	700 T>C	Substitution	Malignant	Male/Female
	8	697 C>G	Substitution	Malignant	Male
	5	567 AC>TG	Substitution	Malignant	Female
	3	329 GT>CA	Substitution	Malignant	Female
	7	1005 T>C	Substitution	Malignant	Female
TP53	9	308 AC>GG	Substitution	Malignant	Female
	9	314 A>G	Substitution	Malignant	Male/Female
	9	321 G>A	Substitution	Malignant	Female
	9	324 GG>AC	Substitution	Malignant	Female
	3	113 C	Deletion	Malignant	Male
	4	119 C	Deletion	Malignant	Male/Female
	9	311 T	Insertion	Malignant	Female

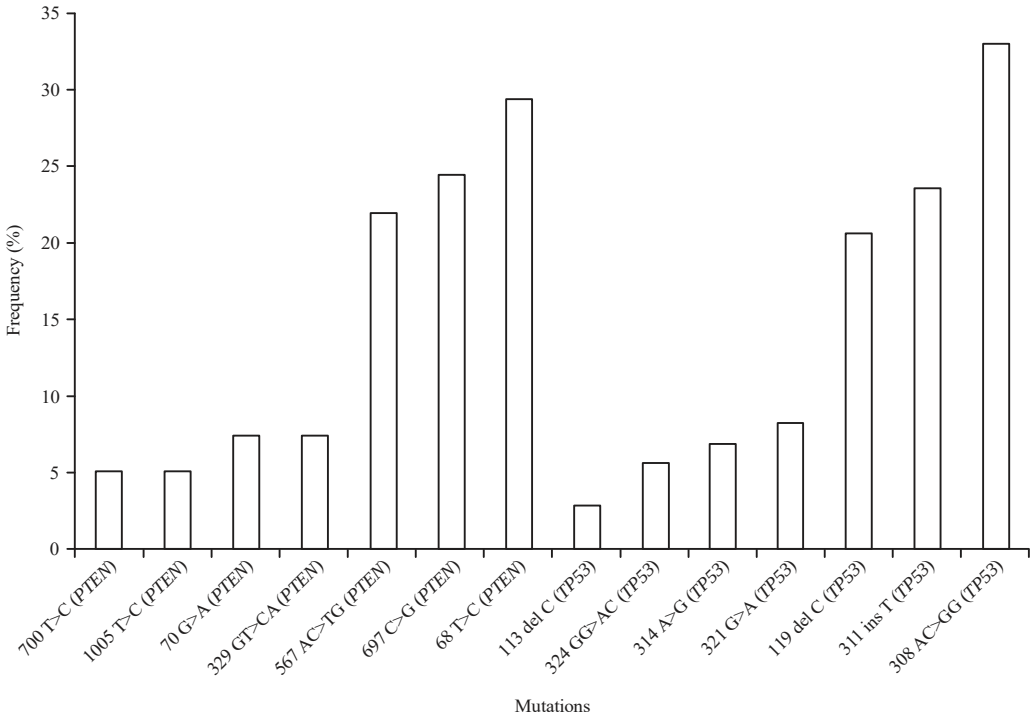

Fig. 1: Frequencies of different mutations in *PTEN* and *TP53* genes in Saudi breast cancer patients

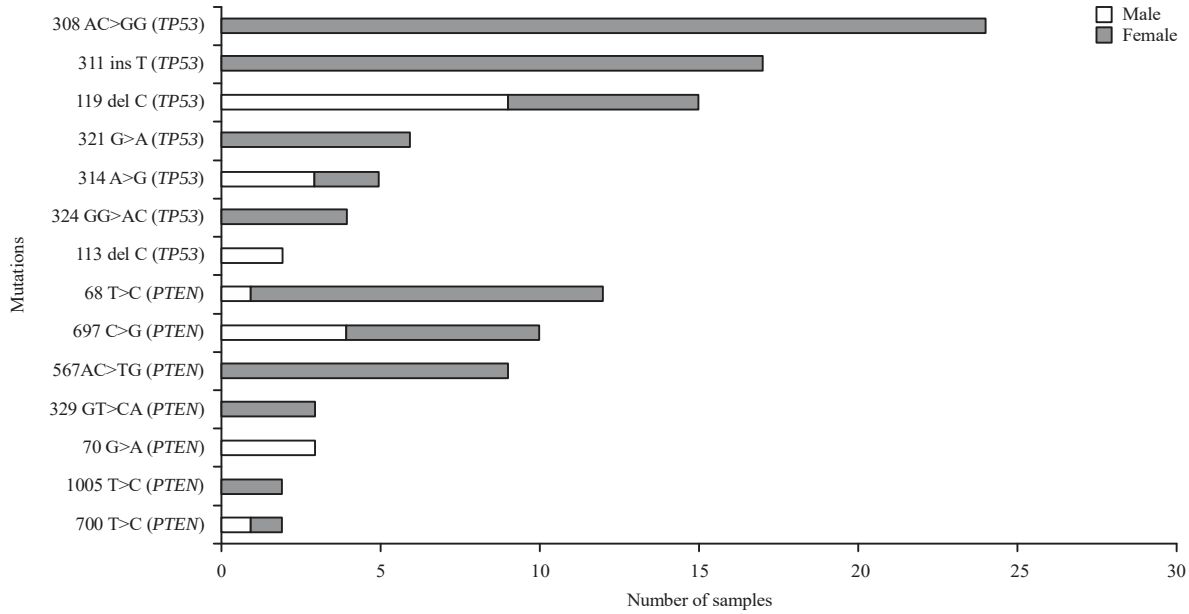

Fig. 2: Distribution of PTEN and TP53 mutations in Saudi male and female breast cancer patients

Figure 2 shows distribution of *PTEN* and *TP53* mutations in Saudi male and female breast cancer patients. Of the seven *PTEN* mutations detected, 697 C>G substitution mutations were predominantly found in male breast cancer patients. Similarly, out of seven *TP53* mutations 113 del C mutations were found to be predominant in males. The 700 T>C, 697 C>G and 68 T>C substitution mutations of *PTEN* gene were detected in both male and female. In *TP53* gene the 314 A>G substitution and119 del C mutations were found in both the sexes.

All *PTEN* mutations occurring between exons 1 and 9 and *TP53* mutations occurring between exons 4 and 19 from breast cancer tumors were screened for mutational analysis. Mutations identified were transitions, transversions and additions or deletions (Fig. 3). The frequencies of these mutations were compared between male and female. Transitions occurred in 55.6% and transversions in 44.6% of male *PTEN* mutation carriers. Conversely, in female *PTEN* mutation carriers, transitions and transversions accounted for 43.8 and 56.2%, respectively (Fig. 4). No, addition or deletion was noted in either male or female *PTEN* mutation carriers. In males with *TP53* mutation carriers only transitions and deletions were recorded. The frequency of transitions in male *TP53* mutation carriers was 21.4%. Complex mutation like deletion accounted for 78.6%. In females with *TP53* mutation carriers, the frequency of transitions and transversions accounts for 20.3 and 40.7%, respectively. Conversely, complex mutations like insertion and deletions accounted for 28.9 and 10.1%, respectively.

Histopathology and immunohistochemistry: The *PTEN* mutation carriers showed poorly differentiated infiltrating ductal carcinomas, higher mitotic counts and pleomorphism, few tubule formations compared to non *PTEN* mutation carrier or control patient (benign). Moreover, carriers of *PTEN* exhibited typical or atypical medullary carcinomas and are high-grade tumours. The H and E stained sections from the breast cancer patients showed increase in mitotic activity combined with dysregulation of proliferation in surrounded epithelial cells, nuclear pleomorphism, disruption in histological patterns involving invasive marked in the ductal breast carcinoma with irregular patterns of breast tumors and cytological atypia (Fig. 5a,b). The carrier of *TP53* germline mutation showed intense and widespread staining and exhibited invasive lobular and tubular carcinomas. Strong nuclear staining was observed on majority of the tumor cells.

The expression of *PTEN* and *TP53* was assessed using immunohistochemistry (Fig. 6, 7). Overall 41% of the analyzed breast cancer tumors displayed loss of *PTEN* expression. The *PTEN* immunohistochemistry revealed strong staining intensities in 21% and moderate staining in 10% of the tumor tissues. The *TP53* over expression was observed in 73% of the tumors (Table 4).

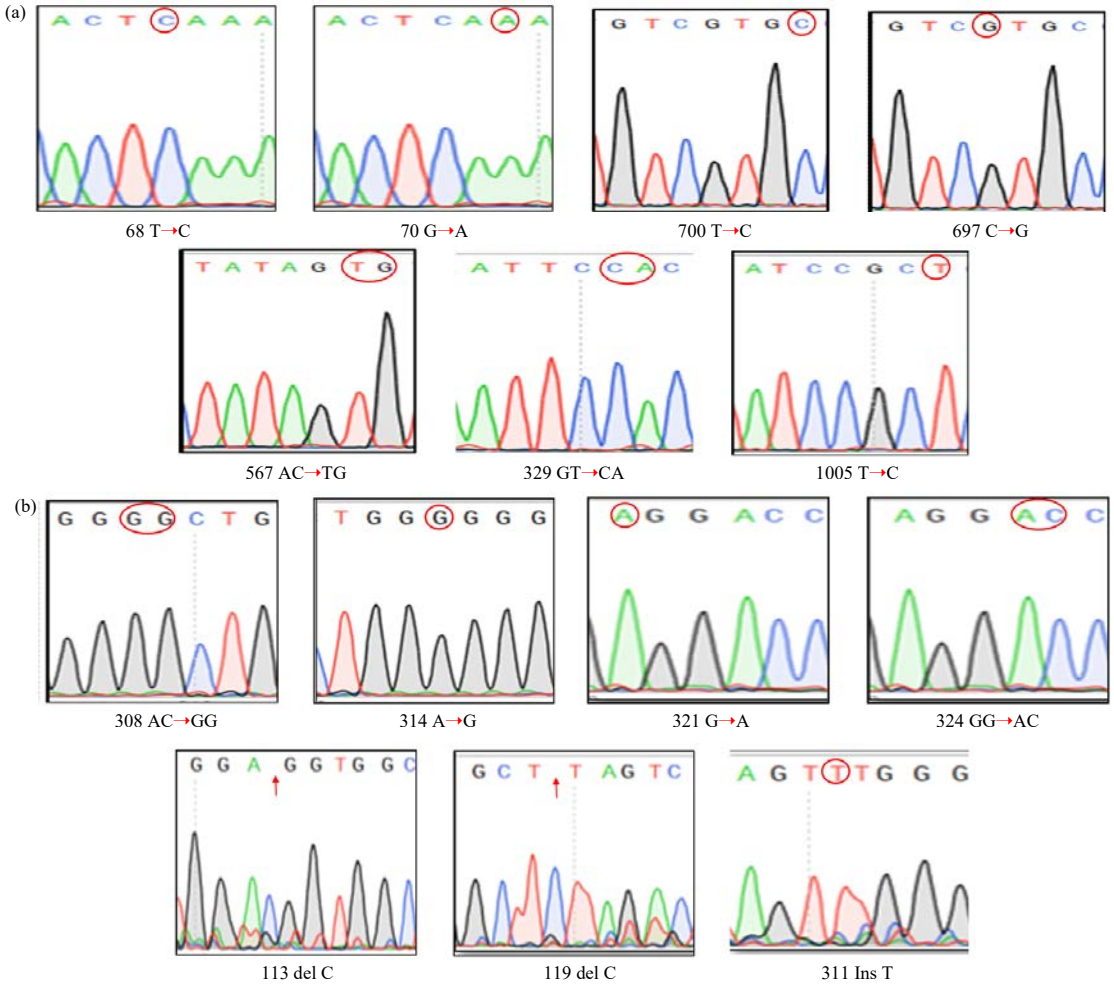

Fig. 3(a-b): Sanger sequencing electropherograms of (a) *PTEN* and (b) *TP53* gene sequence mutations from genomic DNA samples taken from male and female Saudi patients with breast cancer

Respective exons are presented in Table 3

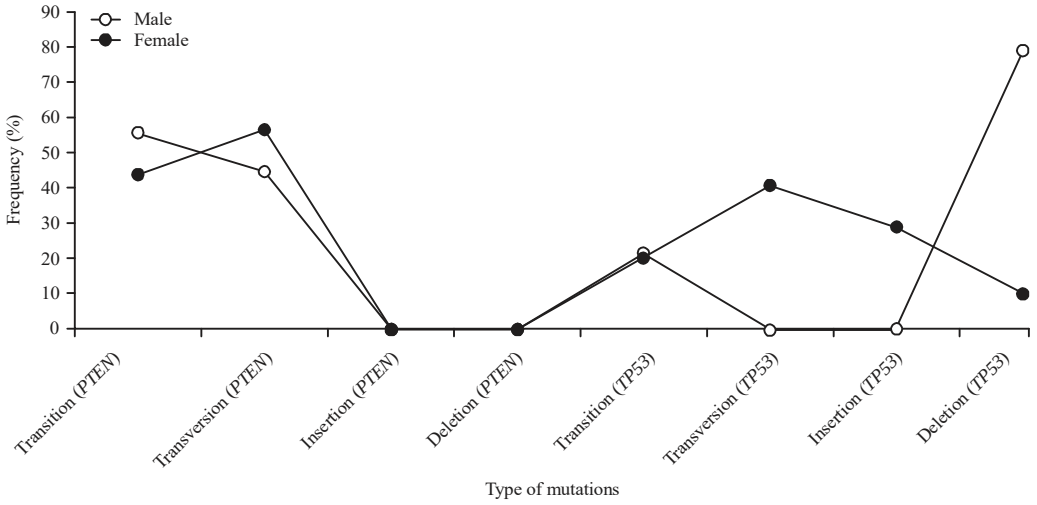

Fig. 4: Characterization of *PTEN* and *TP53* mutations identified in Saudi male and female breast cancer patients

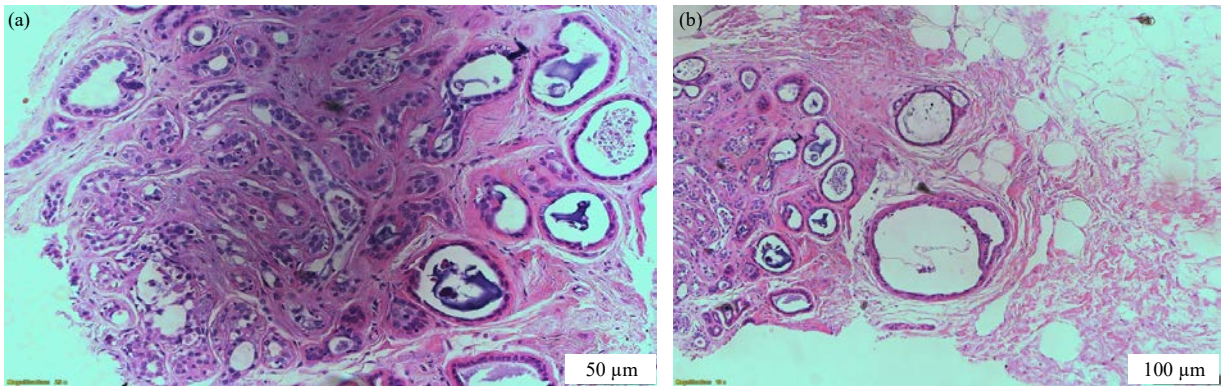

Fig. 5(a-b): Photomicrographs of H and E stained sections from breast cancer patients carrying, (a) *PTEN* mutation and (b) *TP53* mutation, showing increased mitotic activity combined with dysregulation of proliferation in surrounded epithelial cells, nuclear pleomorphism, disruption in histological patterns involving invasive marked in the ductal breast carcinoma (arrows) with irregular patterns of breast tumors and cytological atypia is lacking

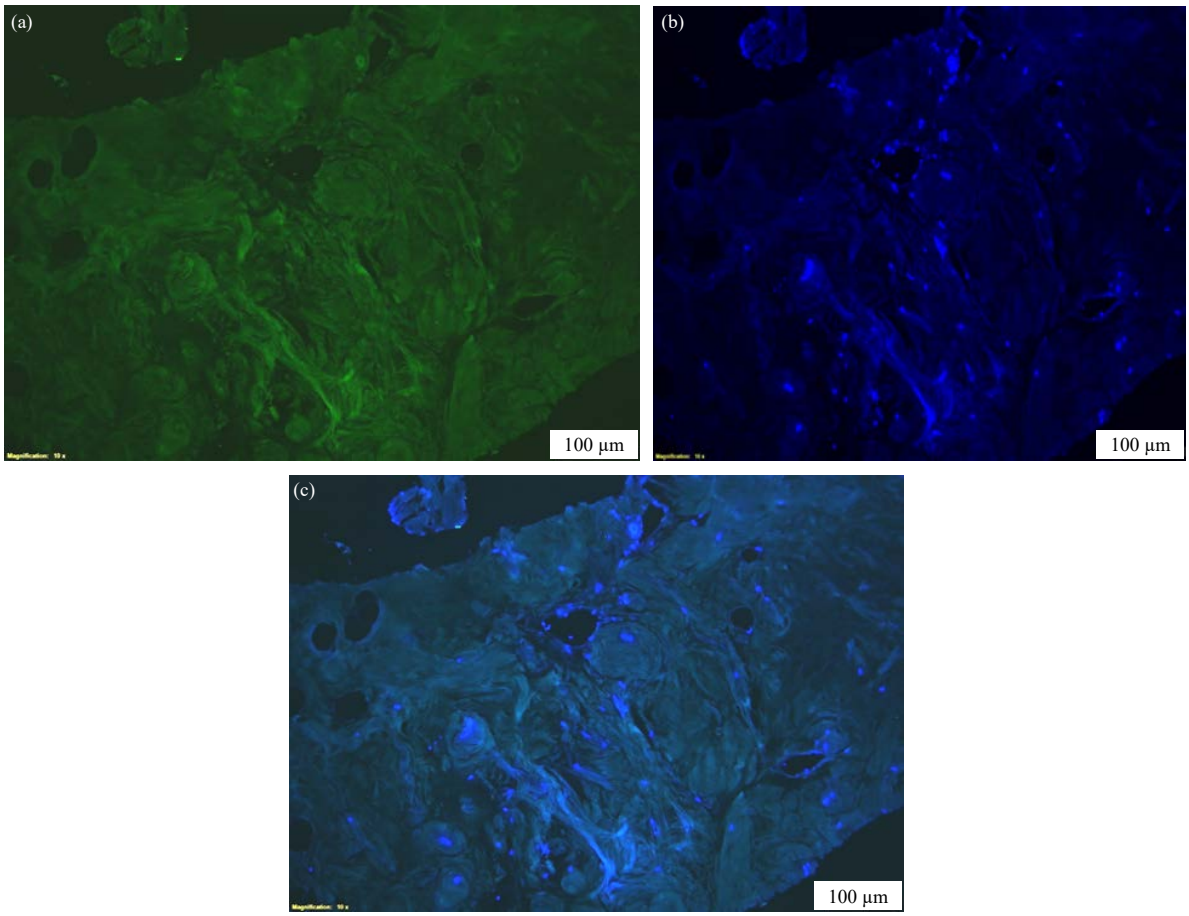

Fig. 6(a-c): Photomicrographs of immunostained labeling sections with DAPI dye from the breast cancer patients of p53 antibody were taken by confocal microscope with scale bar: 100 µm, (a) Immunohistochemical staining slide with p53 mutant (green) revealed overexpression of p53 protein in breast cancer tissue, (b) Slide stained with DAPI nuclear fluorescent staining dye (blue) and (c) combined slide of a and b slides

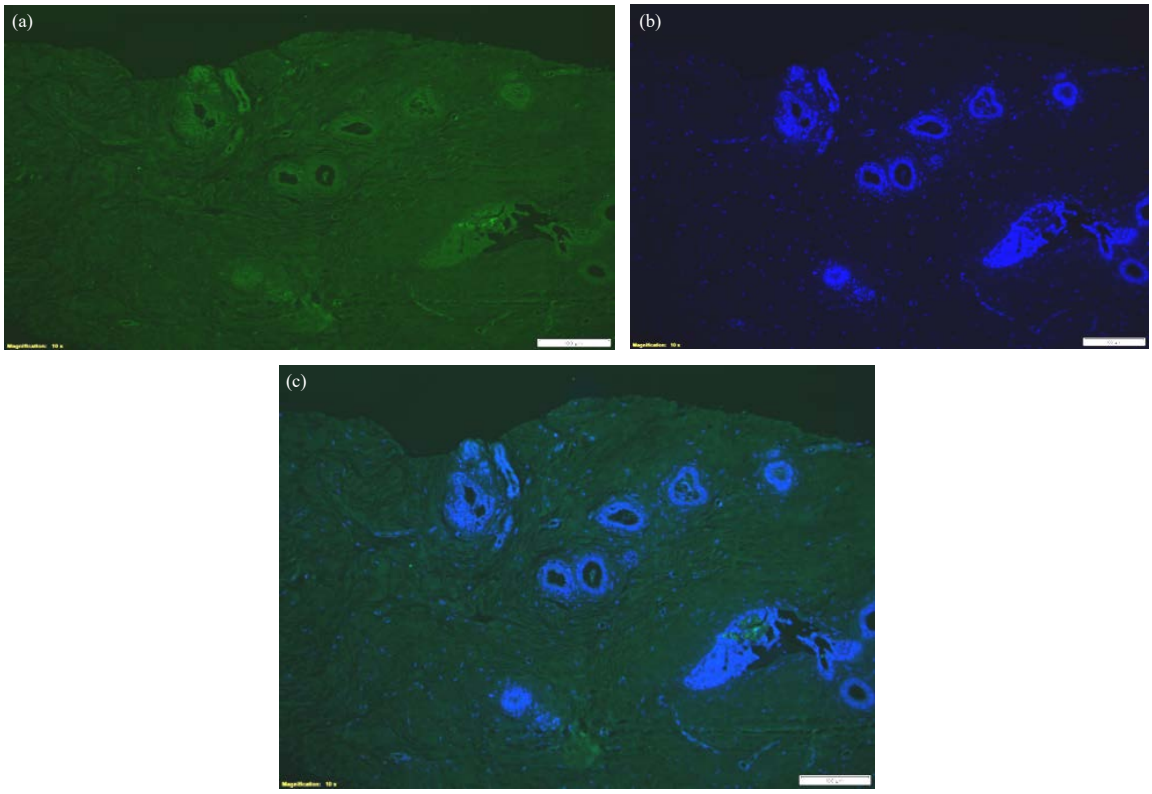

Fig. 7(a-c): Photomicrographs of immunostained labeling sections with DAPI dye from the breast cancer patients of PTEN antibody were taken by confocal microscope with scale bar: 100 µm, (a) Immunohistochemical staining slide with *PTEN* mutant (green) revealed overexpression of *PTEN* protein in breast cancer tissue, (b) Slide stained with DAPI nuclear fluorescent staining dye (blue) and (c) Combined slide of a and b slides

Table 4: Proportion of cancers showing greater than 75% of cells positive by immunohistochemistry (IHC)

p53 IHC	*TP53* mutation carrier
Strong	41
Moderate	23
Weak	7
None	2
Total	73
PTEN IHC	*PTEN* mutation carrier
Strong	21
Moderate	10
Weak	9
None	1
Total	41

DISCUSSION

In the present study, the frequency of *PTEN* and *TP53* gene in Saudis breast cancer patient was strongly positive. In normal conditions these genes maintain balance of the cell population thereby preventing tumor formation exhibiting its tumor suppressor activities. Mutation in these genes are known to predispose early-onset breast cancer[1]. The *PTEN* functions are down regulated due to mutations leading to stoppage or reduction of apoptosis and induction of cell cycle, both of which are involved in development of carcinoma[12]. The association of germline mutations of *TP53* with early onset of breast cancer has been well eastablished[13]. Women with *TP53* mutations were reported to have 50-90% life time risk of developing breast cancer and that with *PTEN* mutations to have 25-85% risk[14].

Traditional Sanger DNA sequencing was used to characterize the mutational status of the *PTEN* and *TP53* genes and deleterious gene mutations accounts for 33.3%. Seven distinct mutations of *PTEN* gene were identified, out of which 68T>C and 697C>G in exon 8) and 567AC>TG in exon 5 occurred in maximum number of individuals under study. Similarly, 7 distinct mutations of *TP53* gene were identified, the most frequent mutation being the 308 AC>GG and 311 ins T in exon 9 and 119 del C (23.4%) in exon 4. The results corroborate with one of the earlier findings[15]. The *TP53* is considered to be one of the frequently mutated gene in Arab breast cancer patients[16]. The prevalence rate of *TP53*

mutations being 29% which is at par to the present result. Mutational analysis of *PTEN* and *TP53* revealed transitions, transversions and additions or deletions. Most of the mutations were single base pair substitutions. The finding is in consistence with some of the previous reports[17].

Besides molecular analyses for tumor suppressor gene (TSG) mutation, histopathological phenotypes are successfully used to determine the carrier of *PTEN* and *TP53* germline mutation. Breast cancer patients with germline mutation in TSG often exhibit different histological phenotypes with variable proliferation rates. In the present study breast cancer samples with *PTEN* and *TP53* mutation displayed different histological features suggesting an alternative mechanism of molecular pathogenesis. Thus, the impact of germline mutation in variability of histological phenotype cannot be ruled out[18].

The histological features of malignant breast tissues were stained with hematoxylin and eosin stains (H and E). The histological types investigated in the study were infiltrative ductal carcinoma, lobular ductal carcinoma and *in situ* ductal carcinoma. The malignant tissues were tested with anti-*PTEN* monoclonal antibody 6.H2.1 and anti-p53 monoclonal antibody DO7 to assess immunohistochemical expression. Patient with *TP53* mutation carrier were found to exhibit higher level of *p53* expression and high proliferation rate of histological features. Thus, overexpression of *p53* protein is an indicative of *TP53* alterations[18]. Over expression of *p53* has been attributed to early-onset of breast cancers compared to later-onset breast cancers. Previous studies on *TP53* mutation also identified increment in p53 expressions in *TP53* mutation carriers. Majority of the *TP53* mutations were IHC positive. Moderate to strong p53 expression was observed in 65.7% of tumors, while weak to complete absence of expression was observed in 34.2% of tumors. The present result is in concordance to one of the earlier studies[19]. However, expression of p53 was absent in benign control. Contrary to this, absence or decrement of *PTEN* expression was an indicative of PI3K-AKT pathway activation [20]. The loss of *PTEN* expression is often invariably linked to breast cancers[21]. The monoclonal antibody 6H2.1 exhibited presence of molecular alteration of *PTEN* as manifested by variable immunostaining reactions. In contrast variable histological features were exhibited by *PTEN* and *TP53* mutation carriers. More lobular and intraductal carcinomas were noted in patient with *TP53* mutation carriers. No such variations were observed in tumours from control patients. Thus, it has been established from the present study that breast cancer tumours in carriers of *PTEN* and *TP53* gene mutations differ morphologically and histopathologically. The association between *PTEN* loss or p53 over expression with pathogenesis of breast cancer has been well attributed.

SIGNIFICANCE STATEMENT

This study discovers tumor suppressor genes like *PTEN* and *TP53* have been identified in breast cancer samples, their contribution to breast cancer in Saudi population is not well established. This study will help the researcher to use the molecular analysis of TSG mutations to be exploited as molecular biomarkers for effective management of breast cancer patients through surgical or chemotherapeutic interventions.

CONCLUSION

Although germline mutations of tumor suppressor genes (TSG) like *PTEN* and *TP53* have been identified in breast cancer samples, their contribution to breast cancer in Saudi population is not well established. The present study revealed association of *PTEN* down regulation and positive *p53* expression with the pathogenesis of breast cancer in Saudi population. The findings may help in the development of sensitive diagnostic tests for identification and screening of *PTEN* and *TP53* mutation in breast cancer patients. The specific tests may be used in the early diagnosis and efficient prognosis of breast cancer. Moreover, molecular analysis of TSG mutations can be exploited as molecular biomarkers for effective management of breast cancer patients through surgical or chemotherapeutic interventions.

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