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Expression and Clinical Significance of *PIK3CA*, *c-MET* and *c-KIT* Mutations in Saudi Breast Cancer Patients

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Abstract

Objective: *PIK3CA* is the most common pathway affected by mutations in breast cancer. *PIK3CA*/PTEN pathway is under intense investigation as a possible target for molecular therapy. Dysregulation *PIK3CA*/PTEN pathway is a substantial mechanism for the development of resistance to anti-HER2 therapy. Therefore, we aimed to study the *PIK3CA*/PTEN in breast cancer patients in Saudi population. **Methods:** We applied PTEN immunohistochemistry on 98 patients. Then, we applied next-generation sequencing to determine the genetic variations associated with the development of breast cancer and their correlations with clinicopathological variables. **Results:** PTEN expression was significantly correlated with lymph node metastasis (LNM), tumor stage, lymphovascular invasion (LVI) and triple negative breast cancer (TNBC). The prevalence of the *PIK3CA* mutation was 33.3% of cases and it was significantly associated with LNM, tumor stage, and with PTEN expression. *c-MET* mutation was identified in 41.7% of cases and it was associated with tumor stage and with TNBC, while *c-KIT* mutation was detected in 20.8% of cases, and it was significantly associated with TNBC only. Patients with positive PTEN expression had a significantly better overall survival (OS); on the contrary, patients with *PIK3CA* and *c-MET* had a significantly worse OS. **Conclusion:** Our study confirms the importance of *PIK3CA/PTEN* pathway in breast cancer patients. A high frequency of *PIK3CA* and *c-MET* mutations was detected and was associated with poor prognosis. Both *c-MET* and *c-KIT* genes have significant roles in developing TNBC. These findings should be expanded to a larger group study to improve the clinical outcomes

and individualizing treatment.

Keywords

PTEN, *PIK3CA*, *c-MET*, *c-KIT*, Breast Cancer

1. Introduction

Breast cancer is one of the most common tumors that intimidate women's health. It accounts alone for 30% of all new cancer cases in women. Although the death rate for breast cancer decreased by 40% from 1989 to 2016, breast cancer leads to cancer deaths among women aged 20 to 59 years. In Saudi Arabia, breast cancer is one of the most frequent malignancies among women between the age of 20 and 60. According to the statistics by the Saudi Health council in 2015, breast cancer ranked the 1st among female which accounted 16.7% from all cancer reports among Saudis [1] [2] [3]. Triple negative breast cancer (TNBC) is marked by bad prognosis and low survival rate regardless of the effectiveness of hormonal therapies. In Saudi population, TNBC patients who are HER2 positive have a high grade and large tumor size at presentation especially for women who are under the age of 50 years [4].

Tumor heterogeneity in breast cancer is due to the wide range of genetic variations which led to phenotypical and functional diversity in the role of developing breast cancer. Breast cancer is characterized by elevated genomic instability confirmed by abundant somatic gene mutations, rearrangements of the chromosome structures and wide range of copy number variations [5]. Recently, the use of the whole-genome sequencing in breast cancer patients revealed these diversities and its frequency in many genes [6].

The phosphoinositide 3-kinase *PIK3CA* gene is present on chromosome 3 (3q26.32) that codes for catalytic subunit which plays an essential role in cellular function such as proliferation, survival, motility and growth. Mutation in the *PIK3CA* gene code for protein plays a significant role in development of breast cancer [7]. This coded protein of a muted *PIK3CA* gene converts the phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3) which leads to downstream signaling through a PI3K/AKT/mTOR pathway [8]. The phosphatase and Tensin-Homolog (PTEN) gene, which is present on chromosome 10 (10q23), encodes for protein that plays a role in DNA repair, apoptosis and cell cycle proliferation. Indeed, PTEN protein inhibits the activation of *PIK3CA* gene. PTEN gene is a tumor suppressor gene and its mutation may lead to tumor development and regression by escaping from the cell cycle arrest and apoptosis process [9].

c-MET, also named hepatocyte growth factor (HGF) receptor, is a cell surface tyrosine kinase receptor which encodes by met gene that is located on chromosome 7q21-31. It increases cell survival after binding to its substrate hepatocyte

growth factor. Receptor activation facilitates the downstream signaling that activates the kinase cascade. This pathway regulates the cellular proliferation, cell growth, migration and invasion [10]. A wide range of genetic variations has been frequently identified in breast cancer patient with different ethnicity. Gene amplification is reported as well in many studies that reported a signification association. These clinical findings confirmed the prognostic importance of *c-MET* mutation and highlighted its potential in developing breast cancer [11] [12].

c-KIT (or CD117) is a transmembrane tyrosine-kinase receptor. It has a fundamental role in regulating proliferating mast cells and stimulates the angiogenesis. There is a significant association between the overexpression of *c-KIT* and high-grade breast cancer with poor prognosis [13]. Mast cells stimulate *c-KIT* activation which in turn stimulates the angiogenic factors such as tryptase. Tryptase has a proteolytic activity that stimulates angiogenic activity in vascular endothelial and tumor cell proliferation, which helps the tumor cell for metastasis [14] [15].

To the best of our knowledge, no data has been published before concerning the mutation of *PIK3CA*, *c-MET* and *c-KIT* genes and their correlations with the clinicopathological variables in Saudi patients with breast cancer. Furthermore, we also aimed in this study to explore the relationship between PTEN expression, *PIK3CA*, *c-MET* and *c-KIT* mutations and overall survival.

2. Material and Methods

Patients and Tissue Samples

The study included 98 female patients diagnosed with breast cancer from local hospital in the western region of Saudi Arabia between 2015 and 2019. This study was approved and conducted under the ethics regulation by the ethical committee at the school of medicine at Umm Al-Qura University. All tissue sections were evaluated after hematoxylin/eosin (H & E) staining by histopathologist. We collected the related demographic and clinical data of the current study including: age, tumor size, tumor stage, axillary lymph nodes, regional and distant metastasis and tumor recurrences from the final pathology reports as shown in **Table 1**.

Any patient who had had radiotherapy, chemotherapy, targeted therapy, or adjuvant endocrine treatment before surgery was excluded from the study. Invasive duct carcinoma (IDC) was graded based on the modification of Elston and Ellis on Bloom and Richardson grading system [16]. A case was considered to be positive for hormone receptors (ER and PR) if has >15% of the tumor cells demonstrate positive nuclear staining. HER2 expression is scored as stated by the American Society of Clinical Oncology [17].

Immunohistochemistry

The paraffin embedded tissue sections were cut at 4 μm thickness and were deparaffinized in xylene and rehydrated in graded alcohols. The slides were incubated with peroxidase-blocking reagent and after rinsing in wash buffer, the

Table 1. The clinicopathological features of breast cancer patients.

Clinicopathological Features		N	%
Age	≤50	46	46.9%
	>50	52	53.1%
Tumor Size	<2 cm	27	27.6%
	2 - 5 cm	51	52.0%
	>5 cm	20	20.4%
Necrosis	Absent	53	54.1%
	Present	45	45.9%
Tumor Grade	Grade 1	20	20.4%
	Grade 2	47	48.0%
	Grade 3	31	31.6%
LVI**	Absent	48	49.0%
	Present	50	51.0%
LNM**	Negative	19	19.4%
	Positive < 3	48	49.0%
	Positive > 3	31	31.6%
Tumor Stage	Stage 1	11	11.2%
	Stage 2	53	54.1%
	Stage 3	24	24.5%
	Stage 4	10	10.2%
PTEN	Negative	53	54.1%
	Positive	45	45.9%
TNBC**	TNBC	31	31.6%
	Non-TNBC	67	68.4%

*LVI: lymphovascular invasion, **LNM: Lymph Node Metastasis, ***TNBC: triple negative breast cancer.

slides were incubated with PTEN antibody (dilution 1:100, clone 6H2.1, Dako) for half an hour at room temperature. After then, the slides were rinsed again and incubated for another half an hour with peroxidase-labeled polymer. Then the slides were incubated with DAB substrate-chromagen solution and counterstained with hematoxylin. Lastly, the slides were dehydrated, mounted and cover slipped.

PTEN positive tumor cells demonstrated either cytoplasmic or nuclear staining or both. The immunohistochemical staining was Semi-quantitative scored according to the intensity as follows: 2 = positive (similar in intensity to normal epithelial cells), 1 = weak (decreased intensity compared to normal epithelial cells), and 0 = negative (no staining in tumor cells, however, it is detected in surrounding normal ductal epithelial cells) [18].

Tumor Dissection and DNA Extraction

After evaluating the cases to be eligible for the current study, we included 46

cases qualified for further molecular analysis. These cases were selected based on positive immunohistochemical expression for PTEN. From each selected case, 10-nm thick tissue section obtained from paraffin-embedded samples was collected in the Eppendorf safe-lock tube to be prepared for DNA extraction.

From each selected case, 10-nm-thick tissue sections obtained from paraffin-embedded samples were collected in the Eppendorf safe-lock tube to be prepared for DNA extraction. The DNA extracted from the collected tissue by using the QIAamp DNA FFPE Tissue Kit (Qiagen®, Hilden, Germany) following the protocol of the manufacture. The extracted DNA was eluted into 40 nL buffer then quantified using two methods: NanoDrop microvolume sample retention system (Thermo Fisher Scientific NanoDrop Products, Hanover Park, IL, USA). The second method of quantification was using Qubit, DS (Thermo Fisher Scientific®, Waltham, MA, USA) and stored at 4˚C. All the 46 cases were fulfilled the recommended DNA quality required for the molecular analysis.

Next-Generation Sequencing

For all the 46 cases, 20 ng of the extracted DNA was prepared for sequencing. The assessment of 15 genes *(TP*53, *PIK*3*CA*, *c-KIT*, *c-MET*, *EGFR*, *PDGFRA*, *KRAS*, *NRAS*, *BRAF*, *AKT*1, *GNA*11, *RET*, *GNAQ*, *ERBB2 and FOXL2)* was applied using TruSight Tumor 15 (Illumina®, San Diego, CA, USA). The resulting pooled libraries were quality controlled using The Qubit® dsDNA High Sensitivity Assay. Sequencing was applied with paired-end reads on MiSeq Platform (Illumina®).

Determination of the Variations

All the reads of the sequenced DNA were arranged and compared to the hg19/GRCh37 reference sequence then analyzed by applying the MiSeq reporter (Illumina®). To specifically identify the variants in breast tissue samples, BaseSpcae Variant Interpreter (Illumina®) was applied. The called variants were considered somatic malignant tumors of the breast (SNOMEDCT) version 4.0.7.6. To decrease the false-positive rate in our study group, we set the values for the cutoff as the following: genotype quality > 30, read depth > 100, Indel repeat length < 8 and for the allele frequency of mutant reads >1%.

Statistical Analysis

The association between PTEN protein expression, *PIK*3*CA*, *c-MET* and *c-KIT* mutations and the clinicopathological parameters were assessed by Chi-square statistical test and Pearson correlation. Survival analyses were estimated using the Kaplan-Meier method. Cox proportional hazards model was used to estimate the hazard ratio (HR) of each clinicopathological variable for OS. P-values were 2-tailed and considered significant when <0.05. Data analyses were carried out using SPSS statistics 22.0 software.

3. Results

Patients Characteristics

Table 1 presents the mean and the proportion of the clinicopathological cha-

racteristics of the patients who are included in the current study. Briefly, the mean age was 53.5 ± 1.3 years (range 29 - 87 years). Twenty-nine patients (29.6%) developed recurrence and twenty-five cases (25.5%) died by the end of the follow up. Nineteen patients (19.4%) had negative lymph nodes and slightly more than half of the cases (54.1%) had stage II disease. Triple-negative breast cancer (TNBC) were 31 cases (31.6%) while 67 (68.4%) were non-triple-negative breast cancer patients.

Correlation of PTEN with Clinicopathological Variables

The correlation of PTEN with different clinicopathological variables is demonstrated in Table 2.

Malignant cells expressed positive PTEN staining either in the cytoplasm or the nucleus or both in 45 cases (45.9%). Loss of PTEN expression was correlated

Table 2. Association between PTEN expression and clinicopathological characteristics in breast cancer.

Clinicopathological Features		PTEN				Sig.
		Negative		Positive		
		N	%	N	%	
Age	≤50 years-old	25	47.2%	21	46.7%	0.96
	>50 years-old	28	52.8%	24	53.3%	
Tumor Size	<2 cm	15	28.3%	12	26.7%	0.348
	2 - 5 cm	31	58.5%	22	48.9%	
	>5 cm	7	13.2%	11	24.4%	
Necrosis	Absent	31	58.5%	21	46.7%	0.242
	Present	22	41.5%	24	53.3%	
Tumor Grade	Grade 1	11	20.8%	9	20.0%	0.306
	Grade 2	22	41.5%	25	55.6%	
	Grade 3	20	37.7%	11	24.4%	
LVI*	Absent	21	39.6%	27	60.0%	**0.044**
	Present	32	60.4%	18	40.0%	
LNM**	Negative	7	13.2%	12	26.7%	**0.018**
	Positive < 3	23	43.4%	25	55.6%	
	Positive > 3	23	43.4%	8	17.8%	
Stage	Stage 1	5	9.4%	6	13.3%	**0.042**
	Stage 2	23	43.4%	30	66.7%	
	Stage 3	17	32.1%	7	15.6%	
	Stage4	8	15.1%	2	4.4%	
TNBC***	TNBC	23	43.4%	8	17.8%	**0.007**
	Non-TNBC	30	56.6%	37	82.2%	

*LVI: lymphovascular invasion, **LNM: Lymph Node Metastasis, ***TNBC: triple negative breast cancer.

significantly with lymph node involvement (p = 0.01), high tumor stage (p = 0.04), lymphovascular invasion (p = 0.04) and TNBC (p = 0.007).

Different variations in PIK3CA, c-MET and c-KIT detected using Next Generation sequencing profile

We purified DNA form 46 breast cancer patients from contiguous area of the tumor tissues to be sequenced by the next-generation sequencing method. These 46 samples were sequenced, and of which there was either complete failure or very low quantity in 22 samples due to poor DNA quality (read depth < 30; alternative variant frequency < 5). We believe the DNA has been degraded in the FFPE material and therefore these cases were excluded. The remaining 24 samples were sequenced successfully. 15 samples showed mutations in these three genes (*PIK3CA*, *c-MET* and *c-KIT)* that we are interested in the current study.

The mean of the total aligned reads for each sample was 2.7 million (it ranges between 1.9 to 4.2 million reads) and the minimum sequencing depth was 484.5X. Several steps were applied to filter the variations that are identified in the run. Any variants that are located in the intron region and have no reported pathogenic relevance were removed. The total of 1368 variants was removed from the study due to the fact that they were known polymorphisms. These polymorphisms are not related to the breast cancer, such as non-pathogenic, or did not pass our quality criteria. The number of mutations in the genes that we were interested in per sample ranged from one to nine mutations with a median of six.

From these 15 samples that showed mutations in the three genes, a total of 78 genetic variants were identified that have been reported to have a pathogenic effect. These 78 variants consisted of 46 synonymous mutations (58.9%), 16 insertion/deletion mutation (20.5%), 10 deletion mutation (12.8%), and 4 missense mutation (5.1%). In the current study, most of the sequenced samples showed multi-variations and each with unique molecular profiles.

Correlation of PIK3CA, c-MET, and c-KIT Mutations with Clinicopathological Variables

The results of the association between *PIK3CA*, *c-MET* and *c-KIT* mutations were summarized in Table 3.

The expression ratio for *PIK3CA*, *c-MET* and *c-KIT* mutation in 24 cases was (33.3%, 41.7% and 20.8%) respectively. *PIK3CA* mutations was associated with metastasis in lymph node (p = 0.018), tumor stage (p = 0.03), and with positive PTEN immunohistochemical staining (p = 0.046). *c-MET* mutations were associated with tumor stage (p = 0.014) and with TNBC (p = 0.003). For *c-KIT*, mutations were only associated with TNBC (p = 0.015).

Correlation of PTEN Immunohistochemical Expression, PIK3CA, c-MET, and c-KIT Mutations with Overall Survival

The patients were followed up for 5 years with a mean of 3.5 ± 1.3 years. Univariate and multivariate analyses of overall survival were summarized in Table 4.

Table 3. The association between *PIK3CA*, *c-MET* and *c-KIT* mutations and the clinico-pathological characteristics in breast cancer.

		*PIK3CA**			*c-MET**			*c-KIT**		
		n	**%**	**Sig**	**n**	**%**	**Sig**	**n**	**%**	**Sig**
Age	<50	3	37.5%	0.247	4	40.0%	0.239	1	20.0%	0.085
	≥50	5	62.5%		6	60.0%		4	80.0%	
Tumor Size	<2 cm	4	50.0%	0.214	4	40.0%	0.344	3	60.0%	0.213
	2 - 5 cm	4	50.0%		6	60.0%		2	40.0%	
	>5 cm	0	0.0%		0	0.0%		0	0.0%	
Necrosis	Absent	5	62.5%	0.770	5	50.0%	0.484	2	40.0%	0.350
	Present	3	37.5%		5	50.0%		3	60.0%	
Grade	Grade 1	1	12.5%	0.741	1	10.0%	0.527	0	0.0%	0.131
	Grade 2	3	37.5%		4	40.0%		1	20.0%	
	Grade 3	4	50.0%		5	50.0%		4	80.0%	
LVI	Absent	5	62.5%	0.770	5	50.0%	0.484	2	40.0%	0.350
	Present	3	37.5%		5	50.0%		3	60.0%	
LNM	Negative	0	0.0%	**0.018**	2	20.0%	0.565	2	40.0%	0.150
	Positive < 3	2	25.0%		3	30.0%		0	0.0%	
	Positive ≥ 3	6	75.0%		5	50.0%		3	60.0%	
Stage	Stage 1	0	0.0%	**0.034**	0	0.0%	**0.014**	2	40.0%	0.092
	Stage 2	1	12.5 %		2	20.0%		0	0 %	
	Stage 3	3	37.5 %		3	30.0%		2	40.0%	
	Stage 4	4	50.0%		5	50.0%		1	20.0%	
PTEN	Negative	8	100.0%	**0.046**	8	80.0%	0.633	4	80.0%	0.772
	Positive	0	0.0%		2	20.0%		1	20.0%	
TNBC	TNBC	3	37.5%	0.155	5	50.0%	**0.003**	3	60.0%	**0.015**
	Non-TNBC	5	62.5%		5	50.0%		2	40.0%	

*Only cases with positive *PIK3CA*, *c-MET* and *c-KIT* mutations were included within the table.

Table 4. Univariate and multivariate analyses for overall survival according to PTEN immunohistochemical expression and *PIK3CA*, MET, and KIT mutations.

	Univariate				**Multivariate**			
	Sig.	**EXP (B)**	**95% CI for Exp (B)**		**Sig.**	**EXP (B)**	**95% CI for Exp (B)**	
			Lower	**Upper**			**Lower**	**Upper**
PTEN	0.011	0.278	0.104	0.742	0.001	0.176	0.061	0.507
PIK3CA	0.001	0.089	0.023	0.350	0.002	0.082	0.018	0.385
c-MET	0.009	0.165	0.042	0.639	0.055	0.215	0.045	1.033
c-KIT	0.577	0.685	0.181	2.590	0.133	3.571	0.678	18.811

In univariate survival analysis, patients with positive PTEN immunohistochemical expression had a significantly better overall survival; (HR 0.278; 95% CI [0.104 - 0.742], p = 0.011)) (Figure 1(a)).

Moreover, PTEN expression was an independent prognostic factor in multivariate analysis; (HR = 0.176; 95% CI [0.061 - 0.507], p = 0.001).

Patients with *PIK3CA* mutation had a significantly worse overall survival in univariate survival analysis; (HR 0.089; 95% CI [0.023 - 0.350], p = 0.001) (Figure 1(b)). Furthermore, *PIK3CA* mutation was an independent prognostic factor in multivariate analysis (HR = 0.82; 95% CI [0.018 - 0.385], p = 0.002).

Regarding *c-MET*, *patients* had *c-MET* mutation exhibited significant worse overall survival in a univariate analysis; (HR 0.165; 95% CI [0.042 - 0.639], p = 0.009) (Figure 1(c))). However, in multivariate analysis; (HR 0.215; 95% CI [0.045 - 1.033], p = 0.055), *c-MET* mutation was not significant.

No significant association has been detected between *c-KIT* mutation and overall survival in both univariate and multivariate analysis (HR 0.685; 95% CI

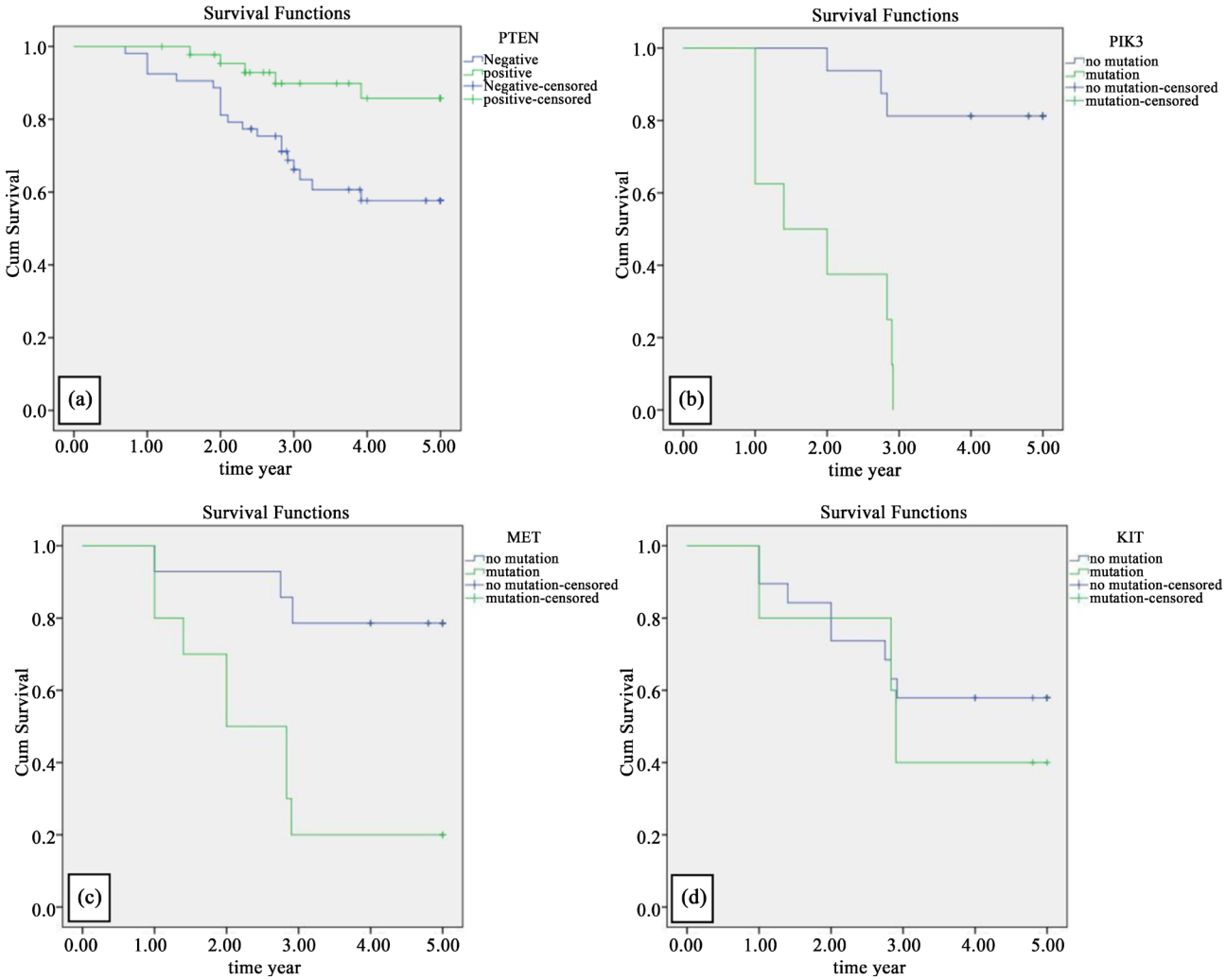

Figure 1. Kaplan-Meier survival curves for overall survival according to the expression of PTEN, *PIK3CA*, and *c-MET* and *c-KIT* in tumor cells. (a) PTEN expression, (b) *PIK3CA* expression, (c) *c-MET* expression and (d) *c-KIT* expression.

[0.181 - 2.590], p = 0.577 and HR 3.571; 95% CI [0.678 - 18.811], p = 0.133, respectively) (**Figure 1(d)**).

4. Discussion

PIK3CA is the most common pathway affected by mutation in breast cancer signaling pathway and *PIK3CA*/PTEN pathway is under intense investigation as a possible target for molecular therapy. PTEN inhibits the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) pathway and activates the expression of pro-apoptotic factors which prevent cellular proliferation and survival. Absence of PTEN efficacy has been detected in many primary and metastatic tumors including breast cancer [19]. Our results are the first to demonstrate the associations between PTEN expression using immunohistochemistry and *PIK3CA*, *c-MET* and *c-KIT* genes mutations using next generation sequencing technology with the clinicopathological characteristics of breast cancer among Saudi women.

Saudi Arabia has a distinctive social pattern, with an increased incidence of consanguineous marriages; therefore, a unique epidemiological profile for breast cancer has been proposed in such population. In the current study, the mean age of patients with breast cancer is 53.5 years (range 29 - 87 years), and most were younger than 50 years old. Our data are in agreement with the regional and national studies which reported that breast cancer in Saudi Arabia is diagnosed at an earlier age comparing to the western population [3] [20] [21] [22].

Results from the current study showed that almost half of the cases had lymphovascular invasion and lymph nodes metastasis. Furthermore, about two thirds of the cases had tumor size larger than 2 cm [23].

PTEN is one of the most frequently mutated tumor suppressor genes [24] [25]. The immunohistochemical expression of PTEN in the current study was successfully performed and positively scored for 45 out of 98 cases (45.9%). Moreover, in the present study, a statistically significant association has been detected between PTEN expression and lymph node metastasis, advanced tumor stage, lymphovascular invasion and TNBC. These findings are consistent with those of Constantinou *et al.* [26]. Nearly all TNBC breast cancers have one or more PTEN/*PIK3CA* pathway modulations opposed to the rate in non-TNBC [27] [28].

In our study, we have found that the overall survival of patients with breast cancer exhibiting positive PTEN staining was higher than those with negative PTEN staining and PTEN expression was an independent prognostic factor for OS. These findings are similar to previous studies done by Li *et al.* and Wang *et al. [7]* [29]. By contrast, other studies have reported that PTEN has no statistically significant correlation with overall survival [30].

PI3KCA mutations have been identified with encouraging results in 33.3% of cases in this study and it was associated with lymph node invasion, tumor stage and PTEN immunohistochemical expression which is in consistence with the

other published studies. Indeed, the majority of breast cancer patients have PTEN/*PIK3CA* pathway alterations that are associated with the poor prognosis. This emphasized the tremendous need for targeting this pathway thorough applying newly developed *PIK3CA* inhibitors [27] [29].

Over the last two decades, many studies have investigated the fundamental role of *c-MET* signaling pathways in breast cancer development [12]. Our findings demonstrate that *c-MET* mutations occur in 41.7% of breast cancer cases and these mutations were significantly associated with advanced tumor stage and TNBC. These findings are supported by those from a previously published study showed that *c-MET* expression levels were associated with the prognosis of breast cancer and this could be used as an independent prognostic biomarker [31]. Several studies showed that *c-MET*/HGF signaling is an important pathway in breast cancer development and it elucidates an appealing target for antitumor molecular therapy [32] [33] [34].

In the current study, we detected *c-KIT* mutation in 20.8% of cases and it was significantly associated with TNBC. However, no significant association has been found between *c-KIT* and the other clinicopathological variables. Abbaspour *et al.* also found *c-KIT* mutation was correlated with lymph node involvement only [15] [35] [36].

The association between *PIK3CA* mutations and overall survival has been investigated in our study and we found that patients with a tumor mutational *PIK3CA* had worse overall survival. Furthermore, *PIK3CA* was identified as an independent prognostic factor. Similar results have been previously reported by Deng *et al.*, who also found that patients and PTEN loss had worse overall survival [37].

Furthermore, we found that *c-MET* mutation had a significant association with overall survival in univariate analysis only. Similar result has been previously reported [38]. On the other hand, no significant association has been detected between *c-KIT* mutation and overall survival in the current study. There are inconsistent data regarding the prognostic role of *c-KIT* in breast cancer, several studies have found that *c-KIT* does not affect the survival of patients with TNBC. Other reports, however, demonstrated that overexpression of *c-KIT* was associated with poor prognosis in TNBC and with tumor recurrence as well [39].

5. Conclusion

In summary, we found novel associations between PTEN/*PIK3CA* pathway and the clinicopathological parameters in Saudi breast cancer patients. Furthermore, we found that *PIK3CA* pathway activation (defined as PTEN loss and *PIK3CA* mutation) contributes to significantly short overall survival in Saudi patients. Our results have substantial clinical implications for managing patients with breast cancer and planning clinical trials that target the PTEN/*PIK3CA* pathway using recently developed *PIK3CA* inhibitors. The current findings need more investigations and should be expanded and regularly screened to enable to indi-

vidualize treatment that improves the clinical outcomes in the future practice.

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Ethical Approval

This work was approved from the Biomedical Ethics Committee at the school of medicine at Umm Al-Qura University (Registration No. in National Committee of Bio Ethics: HAPO-02-K-012).

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Authors Contributions

RN, GE, HMA, AA and GN designed and conducted the research, gathered the research materials and collected the data. RN, GE, HMA analyzed and interpreted the data. RN and GE wrote the initial and final draft of the manuscript, conducted the statistical analysis and provided logistic support. RN and GE have reviewed the final draft and are in charge of the content and similarity index of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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