RESEARCH ARTICLE

PIK3CA mutations in HER2-positive Breast Cancer Patients; Frequency and Clinicopathological Perspective in Egyptian Patients

Fatma Elwy[1], Reham Helwa[1]*, Asmaa A El Leithy[2], Zeinab Shehab El din[3], Magda M Assem[2], Nagwa H A Hassan[1]

Abstract

Missense mutations in *PIK3CA* are common in breast cancers. They mostly involve exons 9 and 20 which encode kinase and helical domains of the protein and may result in its activation. *PIK3CA* activating mutations were previously shown to predict lower pathologic complete response (pCR) in HER2-positive breast cancer cases undergoing neoadjuvant human epidermal growth factor receptor 2-targeting therapy. Hence, the present work was conducted to estimate the mutation frequency in *PIK3CA* in 51 HER2-positive patients by direct sequencing. Our results showed 8 out of 51 (15.7%) to harbor *PIK3CA* mutations in either exon 9 or 20, or both. Three patients had mutations in both exons 9 and 20. Seven (13.7%) possess missense mutations in exon 20 which changed the amino acid sequence of the protein (H1047R, M1040I, and G1049G). Only four cases harbored mutations in exon 9, changing the codon sequences (E545K E545A, and R524K). Taking the clinicopathological data to account, the mutation frequency was greater in ductal than lobular carcinomas, in grade II rather than III and in lymph node positive lesions, with a higher HER2 score and which are ER/PR negative. However, none of the correlations proved statistically significant. In conclusion, to the best of our knowledge, the *PIK3CA* mutation frequency in this study is the first report regarding HER2-positive breast cancer patients in Egypt. Hereby, we highlight a moderate frequency which could be useful in the future as a predictive marker for anti-HER2 therapy.

Keywords: *PIK3CA*- HER2-positive- breast cancer- mutations- Egypt

Asian Pac J Cancer Prev, **18 (1),** 57-64

Introduction

HER2-positive Breast Cancer (BC) is the most aggressive subtype of BC, where the patient has a decreased overall survival and differential responses to chemotherapeutic and hormonal agents (Berry et al., 2000). HER2-positive cancers are characterized by overexpression and/or amplification of ERBB2 gene (HER2) (Slamon et al., 1987; Slamon et al., 1989). Human epidermal growth factor receptor 2 (Her2) is a tyrosine kinase receptor. In invasive breast carcinomas, it is overexpressed or amplified in 20 to 25% of patients (Slamon et al., 1987). Her2 overexpression may lead to increased receptor homo/heterodimerization which induces phosphorylation of the intracellular domain and leading to activation of many downstream signaling molecules, including class A phosphoinositide 3-kinases (PI3K)/AKT. Activated PI3K catalyzes the phosphorylation of inositol lipids to produce phosphatidylinositol-3,4,5-trisphosphate (PIP3), which is dephosphorylated to PIP2 by phosphatase and tensin homolog (PTEN). PTEN is a lipid phosphatase which is acting directly as an inhibitory of PI3K pathway. In sporadic breast cancer, it is infrequently mutated (5%). Approximately 25% of breast cancer cases have decreased expression of PTEN (Saal et al., 2005; Saal et al., 2008). In the downstream signaling, PIP3 activates the serine/threonine kinase AKT which in turn regulates the mammalian target of rapamycin (mTOR) (Barbareschi et al., 2012).

Activating mutations in *PIK3CA* have been found in several cancers, including breast cancer (Slamon et al., 1987; Berry et al., 2000; Zhao and Vogt, 2008). 80% of *PIK3CA* mutations occurs in exons 9 (helical domain) and 20 (kinase domain) which lead to changing in the amino acids sequence and increasing the protein activity (Engelman et al., 2006; Zhao and Vogt, 2008). Mutant *PIK3CA* encodes the p110α catalytic subunit of PI3K enzyme leads to activation of PI3K/AKT signaling pathway which is frequently involved in several cellular processes required for breast cancer development (Isakoff

*[1]Zoology Department, Faculty of Science, [3]Department of Pathology, Faculty of Medicine, Ain Shams University, [2]National Cancer Institute, Cairo University Cairo, Egypt. *For Correspondence: reham.helwa@sci.asu.edu.eg*

et al., 2005; Engelman et al., 2006). Of note, a previous study found that patients with *PIK3CA* mutation and amplified HER2 are less responsive to HER2 inhibitors, reflecting prognostic and therapeutics importance of mutation testing (Berns et al., 2007; Cizkova et al., 2012; Goel and Krop, 2015).

In Egypt, so far, there is no data available about *PIK3CA* mutations frequency in HER2-positive breast cancer patients. Since the *PIK3CA* mutation status could be useful potential prognostic and chemotherapy detection marker, we analyzed *PIK3CA* mutations in a series of 51 patients with Her2-positive BC to investigating the mutations frequencies and their correlation to clinical and pathological parameters.

Materials and methods

Patients and samples

Formalin fixed paraffin embedded (FFPE) sections were collected from 51 female Egyptian patients, who were diagnosed with HER2 positive breast cancer between 2007 and 2013. Clinical and pathological information, including age, tumor type, tumor grade, marital and menopausal status, lymph nodes, HER2 score and ER and PR status, were collected.

The samples were recruited from the National Cancer Institute in cooperation with the Early Cancer Detection Unit of Ain Shams University Maternity Hospital. The study was approved by the ethics committee of the National Cancer Institute.

DNA extraction

Genomic DNA was extracted using QIAamp DNA extraction kit for FFPE tissues (Qiagen, Hilden, Germany) according to the manufacturer's protocol with slight modifications. DNA yield and purity were quantitated and assessed using the Nanodrop.

PCR and Sequencing for mutation screening

Polymerase chain reaction (PCR) was performed for all DNA samples using primers designed to amplify *PIK3CA* (exons 9 and 20). Primers sequence were as the following:

PIK3CA exon 9 F:(ATTAGCAATGTAAAATTTATT GAAAATGTATTT GCTTTTTC)

PIK3CA exon 9 R:(TAAATTCTGCTTTATTTATTC CAATATGGT)

PIK3CA exon 20: (CTCAATGATGCTTGGCTCTG)

PIK3CA exon 20: (TGGAATCCAGAGTGAGCTTTC)

Amplification reactions were carried out using Taq polymerase (Thermoscientific, MA, USA) in accordance to the manufacturer's protocol. Thermocycling condiotions were an initial denaturation at 95°C for 5 minutes (1 cycle), then 40 cycles of denaturation at 95°C for 1 minute, annealing at 51°C for 90 seconds, elongation at 68°C for 2 minute, followed by final elongation at 68°C for 12 minutes. Nontemplate (DNA) control represented the negative control and was included in every PCR run. The PCR product was then analyzed by gel electrophoresis performed on 2% agarose gel stained with ethidium bromide.

All sequencing reactions were accomplished using the same primers. The PCR products were sequenced using Big Dye terminator mixture. Capillary electrophoresis, sequence analysis, and data collection were done using an automated DNA sequencer. The resulted sequences were compared to human genomic DNA sequence using blast and the mutations numbers were identified using ENSEMBL and Cosmic database.

Statistical analysis

Chi-square (Fisher's exact) test was used to examine the relation between qualitative variables as appropriate. Survival analysis was made using Kaplan-Meier method. Comparison between two survival curves used log rank test, P-value ≤0.05 was considered significant. All tests were two tailed. Overall survival (OS) was calculated from date of diagnosis till date of death or last follow up.

Results

Clinical characteristics of the study cohort

In the present study, tumor tissues from 51 breast carcinoma patients with HER-2 positive were analyzed for *PIK3CA* and PTEN mutations. The details of the clinicopathological data of all patients are shown in Table 1. The median age of the cohort was 48 years ranging from 28 to 71 years. According to the histopathological data, the majority of the cases were ductal carcinoma (n = 44, 86.3 %), followed by lobular (n = 2, 3.9%) and others (n = 5, 9.8 %). 84.3 % of the tumors were classified as grade II and 15.7 % as grade III. Most of the tumors were found to be node positivite (58.8 %) while about (25%) were negative. According to tumor subtyping with immunohistochemical analysis for ER and PR, 45.1% and 41.2% of the cases showed positive expression respectively.

PIK3CA mutations type and frequency

By the mean of direct sequencing, 51 tumor samples from HER2-positive breast cancer were analyzed. In our

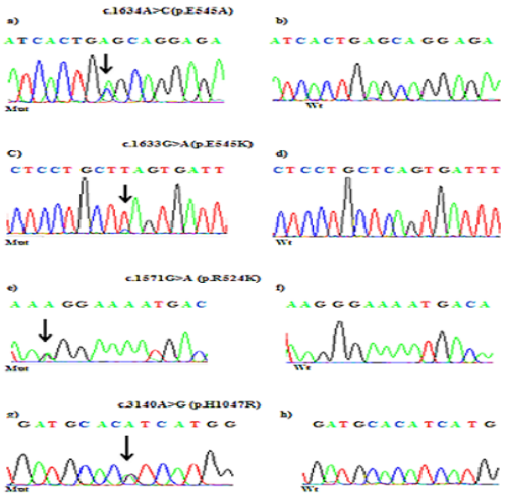

Figure 1. Partial Chromatograms of *PIK3CA* Mutations: a, c and e, *PIK3CA* Exon 9 Mutations (E545A, E545K and R524K); b, d, and f, Wild-Type sequences. g, *PIK3CA* exon 20 Mutation (H1047R); h, Wild-Type Sequence. Arrows, Position of the Missense Mutationas

Table 1. Clonicopathological Data of the Study Cohort (n=51)

Variables	n	%
Age (years)		
Median (range)	48 (28-71)	-
No. of offspring		
Median (range)	3(0-7)	-
Marital status		
Married	50	98.0
Widow	1	2.0
Menopausal status		
Premenopausal	38	74.5
Postmenopausal	13	23.5
Histology		
Ductal	44	86.3
Lobular	2	3.9
Other	5	9.8
Tumor grade		
II	43	84.3
III	8	13.7
Lymph node status		
Negative	13	25.5
Positive		
3	8	15.7
> 3	22	43.1
Missed	8	15.7
HER2 score		
2	10	19.6
3	41	80.4
ER status		
Negative	28	54.9
Positive	23	45.1
PR status		
Negative	30	58.8
Positive	21	41.2

study cohort, we found that the mutations are hitting both helical and kinase domain of the encoded protein. Eight out of 51 patients (15.7%) harbored *PIK3CA* mutations in either exon 9 or 20, or both. Three out of the eight patients with aberrant *PIK3CA* have mutations in exon 9 and 20. As shown in table 2, most of the mutations were missense mutations. Accordingly, the nucleotide

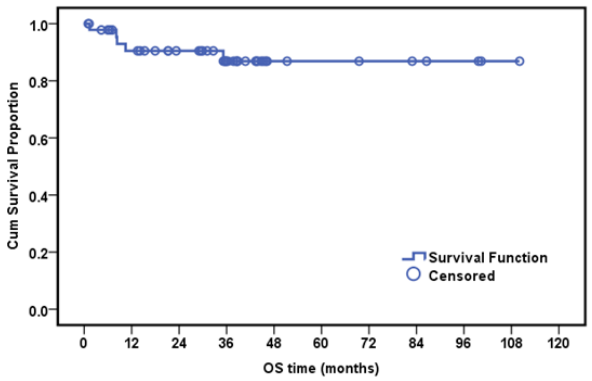

Figure 2. Overall Survival of the Investigated Breast Cancer Cases

substitution is predicted to change the amino acid sequence and consequently the protein function.

Among the 51 analyzed samples, four patients (7.8%) harbored a missense mutation in exon 9. The detected mutations are changing the codon sequences as the following: (two patients possess E545K, one patients with E545A, and the 4th patient harbored R524K). The three mutations are changing the amino acid sequence of the helical domain of the protein.

Three different mutations in exon 20 were also identified in the present study that change the codon sequence in seven patients (13.7%). These mutations hit the kinase domain. Three amino acids substitution (H1047R, M1040I, and G1049G) were identified.

Mutations correlation to clinicopathological data

Table 3 displays all correlations between *PIK3CA* mutation status, exon9 and 20 mutation status, and every mutation found and the clinical, pathological and biological parameters of the study cohort. As noted, there is no statistical significant correlation between all *PIK3CA* mutations and any parameter of the clinicopathological data and also for exon 9 or 20. At the histological level, it was observed that *PIK3CA* mutation was frequently found in ductal carcinomas comparable to lobular carcinomas (15.9 vs. 0 %, p = 0.801). The correlation between *PIK3CA* mutations and tumor grade shows that patients with grade II are more frequent than grade III (16.3 vs. 1.3 %, p = 1.00). In addition, there were more cases with *PIK3CA* mutation in lymph node-positive tumors than negative cases (16.7 vs. 15.4 %). The opposite is for the biomarker parameters (ER and PR), The patients with ER/PR negative pattern have *PIK3CA* mutations more than the patients with positive ER/PR (17.9 vs. 13%, p

Table 2. Frequency and Mutation Types of *PIK3CA*

		cosmic number	Nucleotide change	amino acids	type of mutation	No. of patient	%	Domain
PIK3CA	exon9	COSM763	c.1633G>A	p.E545K	missense	2	3.9	Helical
		COSM297145	c.1634A>C	p.E545A	missense	1	2.0	Helical
		COSM53245	c.1571G>A	p.R524K	missense	1	2.0	Helical
	exon 20	COSM25085	c.3120G>A	p.M1040I	missense	1	2.0	Kinase
		COSM775	c.3140A>G	p.H1047R	missense	5	9.8	Kinase
		COSM87299	c.3147T>C	p.G1049G	coding silent	1	2.0	Kinase

Table 3. Correlation between the Different Types of Mutations and ClinicoPathological Data

		PIK3CA Exons 9 and 20		EX9 total			c.1633 G>A			c.1634 A>C			c.1571 G>A		
	n	wt	mt	p value	wt	mt	p	wt	mt	p	wt	mt	p	wt	mt	p
Age (years)																
≤50	29	25 (86.8)	4 (13.2)	0.713	27 (93.1)	2 (6.9)	1	28 (96.6)	1 (3.4)	1	29 (100)	0 (0)	0.431	28 (96.6)	1 (3.4)	1
>50	22	18 (81.8)	4 (18.2)		20 (90.9)	2 (9.1)		21 (95.5)	1 (4.5)		`21 (95.5)	1 (4.5)		22 (100)	0 (0)	
Menopausal status																
Premenopausal	38	33 (86.8)	5 (13.2)	0.404	36 (94.7)	2 (5.3)	0.695	37 (97.4)	1 (2.6)	0.449	37 (97.4)	1 (2.6)	0.255	37 (97.4)	1 (2.6)	1
Postmenopausal	13	10 (76.9)	3 (23.1)		11 (84.6)	2 (15.4)		12 (92.3)	1 (7.7)		13 (100)	0 (0)		13 (100)	0 (0)	
Histology																
Ductal	44	37 (84.1)	7 (15.9)	0.801	40 (90.9)	4 (9.1)	0.708	42 (95.5)	2 (4.5)	0.847	43 (97.7)	1 (2.3)	0.922	43 (97.7)	1 (2.3)	0.922
lobular	2	2 (100)	0 (0)		2 (100)	0 (0)		2 (100)	0 (0)		2 (100)	0 (0)		2 (100)	0 (0)	
Other	5	4 (80)	1 (20)		5 (100)	0 (0)		5 (100)	0 (0)		5 (100)	0 (0)		5 (100)	0 (0)	
Tumor grade																
II	43	36 (83.7)	7 (16.3)	1	40 (93)	3 (7)	0.506	41 (95.3)	2 (4.7)	1	42 (97.7)	1 (2.3)	1	43 (100)	0 (0)	0.157
III	8	7 (87.5)	1 (12.5)		7 (87.5)	1 (12.5)		8 (100)	0 (0)		8 (100)	0 (0)		7(87.5)	1 (12.5)	
Lymph node status																
Negative	13	11 (84.6)	2 (15.4)	0.928	11 (84.6)	2 (15.4)	0.284	12 (92.3)	1 (7.7)	0.718	13 (100)	0 (0)	-	12 (92.3)	1 (7.7)	0.307
Positive 1(3	8	7 (87.5)	1 (12.5		8 (100)	0 (0)		8 (100)	0 (0)		8 (100)	0 (0)		8 (100)	0 (0)	
Positive > 3	22	18 (81.8)	4 (18.8)		21 (95.5)	1 (4.5)		21 (95.5)	1 (4.5)		22 (100)	0 (0)		22 (100)	0 (0)	
HER2 score																
2	10	10 (100)	0 (0)	0.329	10 (100)	0 (0)	0.069	10 (100)	0 (0)	1	10 (100)	0 (0)	1	10 (100)	0 (0)	1
3	41	33 (80.5)	8 (19.5)		37 (90.2)	4 (9.8)		39 (95.1)	2 (4.9)		40 (97.6)	1 (2.4)		40 (97.6)	1 (2.4)	
ER																
positive	23	20 (97)	3 (3)	0.715	22 (95.7)	1 (4.3)	1	23 (100)	0	0.495	22 (95.7)	1 (4.3)	1	22 (95.7)	1 (4.3)	0.451
negative	28	23 (82.1)	5 (17.9)		25 (89.3)	3 (10.7)		26 (82.9)	2 (17.1)		28 (100)	0 (0)		28 (100)	0 (0)	
PR																
positive	21	18 (85.7)	3 (14.3)	1	20 (95.2)	1 (4.8)	1	21 (100)	0 (0)	0.506	21 (100)	0 (0)	1	20 (95.2)	1 (4.8)	0.412
negative	30	25 (83.3)	5 (16.7)		27 (90)	3 (10)		28 (93.3)	2 (6.7)		29 (96.7)	1 (3.3)		30 (100)	0 (0)	

Table 3. Continued

		EX20 total			c.3140 A>G			c.3140 A>G			c.3147 T>C	
	n	wt	mt	p value	wt	mt	p value	wt	mt	p value	wt	mt	p value
Age (years)													
≤50	29	25 (86.8)	4 (13.2)	1.000	25 (86.8)	4 (13.2)	0.375	29 (100)	0 (0)	0.431	29 (100)	0	0.431
>50	22	19 (86.4)	3 (13.6)		21 (95.5)	1 (4.5)		21 (95.5)	1 (4.5)		21 (95.5)	1 (4.5)	
Menopausal status													
Premenopausal	38	34 (89.5)	4 (10.5)	0.352	34 (89.5)	4 (10.5)	1.000	37 (97.4)	1 (2.6)	1.000	38 (100)	0	0.255
Postmenopausal	13	10 (76.9)	3 (23.1)		12 (92.3)	1 (7.7)		13 (100)	0		12 (92.3)	1 (7.7)	
Histology													
Ductal	44	38	6	0.785	40 (90.9)	4 (9.1)	0.66	43 (97.7)	1 (2.3)	0.922	43 (97.7)	1 (2.3)	0.922
lobular	2	2 (100)	0 (0)		2 (100)	0 (0)		2 (100)	0 (0)		2 (100)	0 (0)	
Other	5	4 (80)	1 (20)		4 (80)	1 (20)		5 (100)	0 (0)		5 (100)	0 (0)	
Tumor grade													
II	43	37 (86)	6 (14)	1.000	39 (90.6)	4 (9.4)	1.000	42 (97.7)	1 (2.3)	1.000	42 (97.7)	1 (2.3)	1.000
III	8	7 (87.5)	1 (12.5)		7 (87.5)	1 (-12.5)		8 (-100)	0		8 (-100)	0	
Lymph node status													
Negative	13	11 (84.6)	2 (15.4)	0.928	11 (84.6)	2 (15.4)	0.851	13 (100)	0 (0)	0.613	13 (100)	0 (0)	0.613
Positive 1(3	18	7 (87.5)	1 (12.5)		7 (87.5)	1 (12.5)		8 (100)	0 (0)		8 (100)	0 (0)	
Positive > 3	22	18 (81.8)	4 (18.8)		20 (90.9)	2 (9.1)		21 (95.5)	1 (4.5)		21 (95.5)	1 (4.5)	
HER2 score													
2	10	10 (100)	0 (0)	0.32	10 (100)	0	0.569	10 (100)	0 (0)	1.000	10 (100)	0 (0)	1.000
3	41	34 (82.9)	7 (17.1)		36 (87.8)	5 (12.2)		40 (-97.6)	1 (-2.4)		40 (-97.6)	1 (-2.4)	
ER													
positive	23	20 (97)	3 (3)	1.000	20 (97)	3 (3)	0.647	23 (100)	0	1.000	23 (100)	0	1.000
negative	28	24 (85.7)	4 (14.3)		26 (82.9)	2 (17.1)		27 (94.4)	1 (5.6)		27 (94.4)	1 (5.6)	
PR													
positive	21	18 (85.7)	3 (14.3)	1.000	18 (85.7)	3 (14.3)	0.637	21 (100)	0	1.000	21 (100)	0	1.000
negative	30	26 (86.7)	4 (13.3)		28 (93.3)	2 (6.7)		29 (96.7)	1 (3.3)		29 (96.7)	1 (3.3)	

Table 4. Correlation of Overall Survival (OS) with PIK3CA Mutation Status and with Clinicopathological Data

Variable	n	Cumulative survival at 36 months	P-value
All	48	86.9%	_
PIK3CA			
Wild	40	87.4%	0.818
Mutant	8	85.7%	
PIK3CA exon 9			
Wild	46	85.4%	*
Mutant	2	100.0%	
c.1633 G>A			
Wild	46	100.0%	*
Mutant	2	86.1%	
c.1634 A>C			
Wild	47	86.6%	*
Mutant	1	100.0%	
c.1571 G>A			
Wild	47	86.5%	*
Mutant	1	100.0%	
PIK3CA exon 20			
Wild	41	87.6%	0.712
Mutant	7	83.3%	
c.3147 T>C			
Wild	47	86.6%	*
Mutant	1	100.0%	
c.3140 A>G			
Wild	43	88.2%	0.423
Mutant	5	75.0%	
c.3120 G>A			
Wild	47	86.5%	*
Mutant	1	100.0%	
Relation of overall survival (OS) and clinicopathological data			
Age			
≤50	28	84.9%	0.404
>50	20	88.9%	
Menopausal status			
Premenopausal	36	87.4%	0.827
Postmenopausal	12	80.0%	
Histology			
IDC	42	89.9%	0.083
Others	6	66.7%	
Tumor grade			
II	40	83.3%	0.242
III	8	100.0%	
Lymph node			
Negative	12	91.7%	0.823
Positive 1-3	8	85.7%	
Positive >3	22	79.0%	
HER2 score			
2	9	100.0%	0.277
3	39	83.9%	
ER			
Positive	20	89.2%	0.828
Negative	28	83.7%	
PR			
Positive	20	94.7%	0.274
Negative	28	79.5	

"*", no P-value because of small no of cases within subgroups; No median survival because more than half of patients were still alive till end of the study

= 1.00) and (14.3 vs. 16.7, p = 1.00) respectively. For HER-2 score we found that there is a slightly significant correlation between the higher score and exon 9 mutations (9.7 vs. 0 %, p = 0.069).

Survival analysis

The median follow-up time was 35.2 months (ranging from 1.0 to 110.1months); OS of BC patients is shown in Fig (2). There is no statistically significant association between BC patients' OS and clinicopathological data of studied cohort or between OS and PIK3 mutation as shown in Table (4).

Discussion

According to GLOBOCAN 2012, breast cancer is the second most common malignancy worldwide and the most frequent cancer in women. A slight majority of cases were in less developed countries rather than developed world. Worldwide, breast cancer is the fifth cause of malignancy death overall. In the less developed region, it is the most frequent cause of cancer death in women (Ferlay et al., 2015). Breast carcinomas are heterogeneous group of tumors which differ morphologically and at the molecular level (Samuels et al., 2005; Engelman, 2009). HER2-positive BC is the most aggressive subtype of BC, where the patient has a decreased overall survival and may have differential responses to a variety of chemotherapeutic and hormonal agents (Berry et al., 2000). In the HER2-positive patients, using therapeutic reagents that target the human epidermal growth factor receptor-2 (HER2) has improved the outcomes in combination with chemotherapy (Romond et al., 2005; Swain et al., 2015). PI3K pathway is the downstream signaling for HER2 and is frequently activated in cancer (Vivanco and Sawyers, 2002). Recently, Loibl et al., (2016) carried out a meta-analysis in which they studied whether *PIK3CA* activating mutations are linked to

lower sensitivity to neoadjuvant therapy. They found that overall *PIK3CA* mutations in HER2-positive significantly decreased the pathological complete response (pCR) compared to the wild type. These findings point out that *PIK3CA* mutations status could potentially act as a predictive marker for HER2-positive cancer.

In the present study, we carried out a sequence analysis for most common mutated exons of *PIK3CA* (exons 9 and 20) in 51 patients. So, this study was executed to calculate the mutation frequency and correlation to clinicopathological data in Egyptian HER2-positive breast cancer patients. To our knowledge, our study is the first study to evaluate *PIK3CA* mutations in HER2-positive patients in Egypt. The only available study (on Pubmed) in Egypt was done in HER2-negative breast cancer patients and *PIK3CA* mutation status was tested in 24 patients. In this previous study, they found 7 out of 24 HER2-negative breast cancer patients (29.2%) in Egypt (Azim et al., 2016). In the present study, we found less frequent overall *PIK3CA* mutations (eight out of 51 patients) were detected in HER2-positive breast cancer (15.7%). However, it is difficult to conclude that the frequency of *PIK3CA* mutations is higher in HER2-negative in the Egyptian patients due to the small number of patients in both cohorts. Nevertheless, Arsenic et al. (Arsenic et al., 2014) found that the *PIK3CA* mutations rate was higher in HR(+)/HER2(-), whereas the lowest mutation rate was observed in HR(+)/HER2(+) in their study. In contrary, another study found that the *PIK3CA* somatic mutations in hormonal positive/HER2-positive are more common, though the data are not significant (Ahmad et al., 2016). The peer studies showed that *PIK3CA* mutations frequency is very variable across the world (Li et al., 2006; Kalinsky et al., 2009; Loibl et al., 2014; Ross et al., 2015; Ahmad et al., 2016; Sudhakar et al., 2016). The percentage of the detected mutations in our cohort is comparable to HER2-positive subtype in a recent Indian study (Ahmad et al., 2016).

As reviewed previously (Dirican et al., 2016), around 80% of *PIK3CA* mutations occur in exons 9 and 20. These two exons have mostly the "hot spot" of *PIK3CA* gene. Exons 9 and 20 encode the helical and kinase domains of the protein. the mutations of this region activate the gene with constant auto-phosphorylation. In the present data, mutations in exon 20 which encodes the kinase domain are more frequent than those in exon 9 (encodes the helical domain). These data are in alignment with several previous studies (Li et al., 2006; Loibl et al., 2014; Arsenic et al., 2015; Ahmad et al., 2016). From the detected mutations, three missense mutations were considered as hot spots which are at amino acid number 524, 545, and 1047 as described previously in the results section.

Considering the clinicopathological data, the *PIK3CA* mutations didn't show any significant association with clinical and pathological data which was shown in many previous studies (Harle et al., 2013; Loibl et al., 2014; Ahmad et al., 2016). However, our results show tendency of having *PIK3CA* mutation in higher HER2 score (data are not significant) with higher mutations rate in exon 9 (with trend to be significant; $p = 0.069$). Consistently with previous studies (Dunlap et al., 2010; Kandula et al., 2013; Arsenic et al., 2015; Ahmad et al., 2016), *PIK3CA* mutations were more frequent in grade II tumors than patients with grade III which could be referred to involvement of the mutations at the onset of the carcinogenesis process. The survival data are not significant in our study which could be related to the limited number of patients in the investigated cohort.

In summary, the present study is the first one which investigating *PIK3CA* mutations in HER2-positive breast cancer patients in Egypt. The frequency reported in this study was comparable to the international frequencies. Interestingly, the frequency found in HER2-positive patients was less than HER2-negative patient reported in another study (Azim et al., 2016). However, the number of patients contributed in this study is not enough for solid conclusion. Even though, we are highlighting the frequency which could be useful in the nearby future research and clinical application of *PIK3CA* as a predictive marker for anti-HER2 therapy.

Compliance with ethical standard

Conflict of interest

None of the authors have any conflict of interest.

Funding

The study was funded by Ain Shams University. Also, Fatma Elwy has received a MSC funding support from BioKMT society.

Informed consent and Ethical approval

The paraffin sections were obtained from the Egyptian National Cancer Institute. A written informed consent was obtained from all patients. The study was approved by the ethics committee of the National Cancer Institute.

References

Ahmad F, Badwe A, Verma G, et al (2016). Molecular evaluation of *PIK3CA* gene mutation in breast cancer: determination of frequency, distribution pattern and its association with clinicopathological findings in Indian patients. *Med Oncol*, **33**, 74.

Arsenic R, Lehmann A, Budczies J, et al (2014). Analysis of *PIK3CA* mutations in breast cancer subtypes. *Appl Immunohistochem Mol Morphol*, **22**, 50-6.

Arsenic R, Treue D, Lehmann A, et al (2015). Comparison of targeted next-generation sequencing and Sanger sequencing for the detection of *PIK3CA* mutations in breast cancer. *BMC Clin Pathol*, **15**, 20.

Azim HA, Kassem L, Treilleux I, et al (2016). Analysis of PI3K/mTOR pathway biomarkers and their prognostic value in women with hormone receptor-positive, HER2-negative early breast cancer. *Transl Oncol*, **9**, 114-23.

Barbareschi M, Cuorvo LV, Girlando S, et al (2012). PI3KCA mutations and/or PTEN loss in Her2-positive breast carcinomas treated with trastuzumab are not related to resistance to anti-Her2 therapy. *Virchows Arch*, **461**, 129-39.

Berns K, Horlings HM, Hennessy BT, et al (2007). A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell*, **12**, 395-402.

Berry DA, Muss HB, Thor AD, et al (2000). HER-2/neu and p53 expression versus tamoxifen resistance in estrogen

receptor-positive, node-positive breast cancer. *J Clin Oncol*, **18**, 3471-9.
Cizkova M, Susini A, Vacher S, et al (2012). *PIK3CA* mutation impact on survival in breast cancer patients and in ERalpha, PR and ERBB2-based subgroups. *Breast Cancer Res*, **14**, R28.
Dirican E, Akkiprik M, Ozer A (2016). Mutation distributions and clinical correlations of *PIK3CA* gene mutations in breast cancer. *Tumour Biol*, **37**, 7033-45.
Dunlap J, Le C, Shukla A, et al (2010). Phosphatidylinositol-3-kinase and AKT1 mutations occur early in breast carcinoma. *Breast Cancer Res Treat*, **120**, 409-18.
Engelman JA (2009). Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer*, **9**, 550-62.
Engelman JA, Luo J, Cantley LC (2006). The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet*, **7**, 606-19.
Ferlay J, Soerjomataram I, Dikshit R, et al (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*, **136**, 359-86.
Goel S, Krop IE (2015). Deciphering the role of phosphatidylinositol 3-kinase mutations in human epidermal growth factor receptor 2-positive breast cancer. *J Clin Oncol*, **33**, 1407-9.
Harle A, Lion M, Lozano N, et al (2013). Analysis of *PIK3CA* exon 9 and 20 mutations in breast cancers using PCR-HRM and PCR-ARMS: correlation with clinicopathological criteria. *Oncol Rep*, **29**, 1043-52.
Isakoff SJ, Engelman JA, Irie HY, et al (2005). Breast cancer-associated *PIK3CA* mutations are oncogenic in mammary epithelial cells. *Cancer Res*, **65**, 10992-1000.
Kalinsky K, Jacks LM, Heguy A, et al (2009). *PIK3CA* mutation associates with improved outcome in breast cancer. *Clin Cancer Res*, **15**, 5049-59.
Kandula M, Chennaboina KK, Ys AR, et al (2013). Phosphatidylinositol 3-kinase (PI3KCA) oncogene mutation analysis and gene expression profiling in primary breast cancer patients. *Asian Pac J Cancer Prev*, **14**, 5067-72.
Li SY, Rong M, Grieu F, et al (2006). *PIK3CA* mutations in breast cancer are associated with poor outcome. *Breast Cancer Res Treat*, **96**, 91-5.
Loibl S, Majewski I, Guarneri V, et al (2016). *PIK3CA* mutations are associated with reduced pathological complete response rates in primary HER2-positive breast cancer: pooled analysis of 967 patients from five prospective trials investigating lapatinib and trastuzumab. *Ann Oncol*, **27**, 1519-25.
Loibl S, von Minckwitz G, Schneeweiss A, et al (2014). *PIK3CA* mutations are associated with lower rates of pathologic complete response to anti-human epidermal growth factor receptor 2 (her2) therapy in primary HER2-overexpressing breast cancer. *J Clin Oncol*, **32**, 3212-20.
Romond EH, Perez EA, Bryant J, et al (2005). Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med*, **353**, 1673-84.
Ross JS, Ali SM, Wang K, et al (2015). Comprehensive genomic profiling of inflammatory breast cancer cases reveals a high frequency of clinically relevant genomic alterations. *Breast Cancer Res Treat*, **154**, 155-62.
Saal LH, Gruvberger-Saal SK, Persson C, et al (2008). Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. *Nat Genet*, **40**, 102-7.
Saal LH, Holm K, Maurer M, et al (2005). *PIK3CA* mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res*, **65**, 2554-9.
Samuels Y, Diaz LA Jr, Schmidt-Kittler O, et al (2005). Mutant *PIK3CA* promotes cell growth and invasion of human cancer cells. *Cancer Cell*, **7**, 561-73.
Slamon DJ, Clark GM, Wong SG, et al (1987). Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*, **235**, 177-82.
Slamon DJ, Godolphin W, Jones LA, et al (1989). Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science*, **244**, 707-12.
Sudhakar N, Priya Doss CG, Thirumal Kumar D, et al (2016). Deciphering the impact of somatic mutations in exon 20 and exon 9 of *PIK3CA* gene in breast tumors among Indian women through molecular dynamics approach. *J Biomol Struct Dyn*, **34**, 29-41.
Swain SM, Baselga J, Kim SB, et al (2015). Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *N Engl J Med*, **372**, 724-34.
Vivanco I, Sawyers CL (2002). The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer*, **2**, 489-501.
Zhao L, Vogt PK (2008). Class I PI3K in oncogenic cellular transformation. *Oncogene*, **27**, 5486-96.