Saudi Journal of Biological Sciences 29 (2022) 1808–1812

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Implementation of Next-Generation Sequencing in Saudi Arabia for HER2-Positive Breast Cancer

Rami Nassir [a,*], Ghada Esheba [a,b]

[a] Department of Pathology, School of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia
[b] Department of Pathology, Faculty of Medicine, Tanta University, Egypt

ARTICLE INFO

Article history:
Received 18 September 2021
Revised 14 October 2021
Accepted 16 October 2021
Available online 22 October 2021

Keywords:
HER2 positive
C-*MET*
c-KIT
PIK3CA

ABSTRACT

Breast cancer is a common malignancy that poses a hazard to women's health. In 2021, around 2.3 million new cases are predicted to be discovered, with a mortality rate of 6.9% on average. Breast cancer accounts for 14.8% of malignancies among the Saudis with an 8.5% fatality rate. Breast cancers that are HER2 positive account for 15 to 20% of all breast cancers. We intended to investigate the genetic mutations and the clinicopathological aspects of HER2 positive breast cancer patients. We used TruSight Tumor 15 using Next-Generation Sequencing (NGS) to look at genetic changes in 126 Saudi women with stage I to IV breast cancer. c-MET ($p = 0.001$), c-KIT ($p = 0.001$), and PIK3CA ($p = 0.0001$), were shown to be substantially linked with HER2 positive patients. We also detected mutations in other genes, including BRAF, EGFR, and KRAS. Tumor size, grade, stage, and nodal status were all associated with increased levels of HER2 expression. Our results recommend that patients with HER2 positive breast cancer in Saudi Arabia have a high mutational burden, which may be related to trastuzumab resistance. We expect that in the future, targeting these mutations will be a promising therapeutic method for the treatment of breast cancer.

1. Introduction

One of the most frequent malignancies that threatens women's health is breast cancer. According to the Global Cancer Statistics 2020, 2.3 million new cases of breast cancer are expected to be identified in 2021, with a 6.9% fatality rate. Although the mortality rate for breast cancer is lower than for other types of cancers such as lung cancer and colorectal cancer, it is still quite high (Sung et al., 2021). In 2018, the rate of newly diagnosed breast cancer in Saudi Arabia was 14.8%, with an 8.5% death rate (Alqahtani et al., 2020). Many variables, including changing lifestyles and expanded services in offering screening programs, are thought to be contributing to an increase in the incidence of breast cancer. Variations in incidence and mortality, according to various studies, are attributed to a variety of factors including family history and age (DeSantis et al., 2019). In many generations, family history plays an important influence in the development of breast cancer. It suggests that those who have a high percentage of consanguinity are more likely to develop a genetic disease like breast cancer. The high proportion of consanguinity in Saudi Arabia's population raises the risk of various hereditary illnesses, including the development of breast cancer. As a result, it's critical to evaluate genetic differences linked to the development of breast cancer (El-Mouzan et al., 2007). Another key risk factor for breast cancer is one's age. The risk increases significantly with age. Furthermore, ovarian hormones have an effect on the development of breast cancer from the adolescent to menopausal period, as well as during and after menopause (Colditz & Rosner, 2000; Thakur et al., 2017).

Breast cancer has been divided into up to ten molecular relevant subgroups, according to recent reports. Four subtypes, however, are the most clinically important, and they can also be diagnosed by immunohistochemistry. The four subtypes are luminal A, luminal B, HER2-enriched, and basal-like. In this study, only the HER2-positive or HER2-enriched subtype will be examined (Perou et al., 2000; Sørlie et al., 2001). HER2-positive breast cancer accounts for 15–20% of all breast cancers. Multiple copies of the HER2 gene are present, which can be discovered by Next-

* Corresponding author at: Department of Pathology, School of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia.
E-mail address: rmnassir@uqu.edu.sa (R. Nassir).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.10.044
1319-562X/

Generation Sequencing (NGS) or Fluorescence In-Situ Hybridization (FISH). It can also be detected through immunohistochemistry due to the overexpression of similar kinase receptor proteins (Cronin et al., 2010; Slamon et al., 1987). HER2 receptors are present on the plasma membrane of the ErbB family of receptor tyrosine kinases. Once HER2 has assembled into a dimer, the phosphorylation of the tyrosine in the cytoplasmic domain is initiated. As a result, it activates numerous genes, including PIK3CA, c-MET, and c-KIT, which promote cell proliferation, migration, adhesion, and survival. As a result, overexpression of HER2 raises the growth factor's reactivity and accelerates the physiological process beyond what is normal (Rubin & Yarden, 2001; Wolff et al., 2018). These amplifications are linked to higher pathological grades and a more widespread form of ductal carcinoma in situ (DCIS), which has a poor prognosis. All of these HER2 status indicators are regarded as predictive biomarkers that aid in the selection of the most successful treatment option (Meijnen et al., 2008; Ross et al., 2009).

Many genes have been linked to HER2-positive breast cancer in recent years, according to research. PIK3CA is one of the genes that has been reported. According to the literature, a mutation in the PIK3CA gene increases protein kinase B phosphorylation and overactivates AKT and mTOR pathways, which involve PIK3CA and AKT/mTOR, promote the growth, proliferation, and survival of breast cells as well as chemotherapy resistance (Freelander et al., 2021; Karakas et al., 2013; Thorpe et al., 2015; Zardavas et al., 2018). The c-MET oncogene, which codes for a proliferation and survival-promoting tyrosine kinase receptor is another gene that has been discovered. According to recent findings on the c-MET gene, there is a link between mutations in the c-MET gene and the development of numerous types of cancer, including breast cancer and lung cancer. The high level of protein expression caused by a mutation in the c-MET gene in breast cancer promotes tumor growth and results in a worse prognosis (Minuti et al., 2012; Raghav et al., 2012). C-KIT, a member of the oncogene family, is also a transmembrane receptor tyrosine kinase. If you have a KIT mutation, people will have uncontrolled cell proliferation (Heinrich et al., 2003; Soria et al., 2011). We aimed to discover the clinicopathological characteristics and identify the most prevalent oncogenes that connected with the development of this specific subtype of breast cancer in Saudi women since there are few publications regarding HER2-positive breast cancer patients in Saudi Arabia.

2. Materials and methods

2.1. Participants in this study

The current research was carried out in Saudi Arabia's western region. 126 Saudi women with stage I-IV breast cancer were involved in the study. The ethical committee at Umm Al-Qura University approved the IRB. A histopathologist analyzed all tumor tissues after staining them with hematoxylin and eosin (H&E) (Goud et al., 2020). All clinical information and pathological factors were gathered from the patient's medical records and pathology reports. We excluded any patient who ever had any type of therapy such as chemotherapy, radiotherapy, or hormonal therapy before collecting the tumor tissue for the analysis.

Tumor tissue were collected from 126 patients. A 10-nm-thick chunk of each sample was taken and preserved in Eppendorf safe-lock tube designed for DNA extraction. The genomic DNA from the 126 patients' tissue was isolated using the Qiagen kit with DNA FFPE according to the methodology specified (Goud et al., 2020). After the extraction process was completed, the concentration of the extracted DNA was measured by two methods. The NanoDrop and Qubit techniques were used to quantify the concentration of genomic DNA, and the remaining 126 genomic DNA was preserved in the freezer until further usage. After optimizing all genomic DNA to 10 ng/μl, molecular analysis for the NGS approach was performed.

2.2. Immunohistochemistry and scoring &

Four μm thickness section were cut from paraffin blocks and stained with an anti-HER2 clone 4B5 for HER2.). HER2 scoring was graded 0–3 as follow: score 0: (denotes negative &staining or membrane staining in greater than 10% of tumor cells, score&1: weak incomplete membranous&staining in < 10% of tumor cells& (1 +). Score 2+: weak to moderate complete membranous&staining in < 10% of tumor cells. The score 2 + is deemed as equivocal. With a staining percentage of over&ten percentage of tumor cells exhibiting strong complete membranous&staining, this is classified as score 3 + . For the purpose of this study, only HER2 3 + score cases were include in the study (Elston & Ellis, 2002).

2.3. DNA extraction

Following the completion of the inclusion and exclusion criteria, we chose 126 tumors identified in breast cancer women. The clinician was provided tumor tissue, and DNA was extracted using the Qiagen kit. To double-check the DNA quantity, both the NanoDrop and the Qubit were used to assess DNA concentration, and we finally adjusted on 10 ng for each DNA sample. The optimized samples were kept in the freezer until the NGS technology was used.

2.4. Identifying the genetic variations using Next-Generation Sequencing (NGS)

Illumina second generation sequencing equipment was used for NGS analysis (Illumina, San Diego, CA, USA). NGS analysis was carried out in 126 cases with 20 ng of genomic DNA that was optimized with 10 ng of genomic DNA for sequencing. The performance of fifteen genes was assessed using TruSight Tumor 15. The quality of the pooled libraries was assessed using Qubit, a dsDNA extremely sensitive assay. Paired-end reads were used for sequencing on the MiSeq Platform. The MiSeq reporter software was used to further analyze the DNA sequence data after evaluating and comparing all of the reads to the hg19/GRCh37 reference sequence. The tissue sample variations were accurately identified using the BaseSpcae Variant Interpreter. Because the modifications were recognized as somatic malignant breast tumors (SNOMEDCT) version 4.0.7.6, the problem was found to be extremely rare. We increased the threshold values of genotyping quality, read depth, and Indel repeat length in order to limit the number of false positives in our sample. Prior study was conducted at our institute in conjunction with the NGS analysis.

2.5. Statistical analysis

We applied Chi-Square statistical test to study the correlation between HER2 expression, clinicopathological variables and mutation in these genes: *c-MET, c-KIT* and *PIK3CA*. Using the 22nd version of SPSS software, statistical analysis was performed with an obtained data and positive association was considered when p value was defined as < 0.05.

3. Results

3.1. Clinical and pathological features

The current study was conducted on 126 cases. The clinicopathological*characteristics of the women were represented

Table 1
Clinical and Pathological characteristics for the participants.

Clinicopathological Characteristics		N	%
Age	Less than 50	52	41.3%
	More than 50	74	58.7%
Tumor Size	Less than 2	36	28.6%
	2–5	80	63.5%
	More than 5	10	7.9%
Necrosis	Absent	73	57.9%
	Present	53	42.1%
Lymphovascular invasion	Absent	74	58.7%
	Present	52	41.3%
Tumor Grade	Grade 1	25	19.8%
	Grade 2	48	38.1%
	Grade 3	53	42.1%
Lymph Node Metastasis	Negative	32	25.4%
	Positive > 3	47	37.3%
	Positive < 3	47	37.3%
Stage	Stage I	16	12.7%
	Stage II	47	37.3%
	Stage III	38	30.2%
	Stage IV	25	19.8%
HER2 Status	Negative	86	68.3%
	Positive	40	31.7%

in Table 1. In brief, the mean age of patients was 54.7 ± 12.9 years (29–87 years). HER2-positive cases were 40 (31.7%) while the remaining 68 cases (68.3%) were HER2-negative.

3.2. Correlation between HER2 positive status and the clinicopathological variables

Young age, large tumor size, lymph node metastases, tumor grade, and stage were all found to be statistically correlated with HER2 positivity (p = 0.01) (See Table 2).

3.3. Frequency of gene mutations

In this cohort, *c-MET* mutations were found in 52 of 126 patients (41.3%), *PIK3CA* mutations were found in 40 of 126 patients (31.7%), and *c-KIT* mutations were found in 26 of 126 patients (20.6%). Furthermore, as compared to HER2-negative patients, HER2-positive cases were statistically correlated with these mutant genes: *c-MET* (p = 0.001), PIK3CA (p = 0.0001), and *c-KIT* (p = 0.001). Other genes that have been shown to contain mutations include EGFR (55.5 %, 70/126), PDGR (39.7%, 50/125), KRAS (23.8 %, 30/126), and BRAF (3 %, 4/126). The latter genes, on the other hand, did not appear to be linked to HER2-positive status (See Table 3).

4. Discussion

An aggressive subtype of breast cancer which has a very bad prognosis and low overall survival, is designated as HER-2 positive breast cancer. The estimated number of HER2-positive breast cancer patients is about 15–20% of the total (Alqahtani et al., 2020). During this investigation, however, we found that HER2-positive subtype accounted for 31.1% of the total breast cancer cases.

This result is consistent with many studies in Arab populations which reported higher rates of HER2-positive subtype (Alnegheimish et al., 2016; Al-Tamimi et al. 2009). Zekri et al. explained the possible causes for the higher HER2 overexpression rate in Saudi Arabia are due to the high of variability in the genetic background beside early development of breast cancer at younger age (Zekri et al., 2021). Similar to previous reports, HER2-positive patients in our cohort, were commonly >50 years old. Furthermore, there were significant correlation between HER2-positivity and tumor size, tumor grade, lymph node metastasis and metastasis (Zekri et al., 2021).

Recent advances in neoadjuvant therapy have markedly changed treatment strategies for breast cancer patients, especially those with HER2-positivestatus. Trastuzumab, a specific HER2-targeting antibody, has enormously improved the survival of HER2-positive patients and it is considered the standard first line therapy. However, this efficacy, resistance to trastuzumab limits its potential. It is reported that about two-thirds of HER2-positive patients cannot benefit from HER2-targeted therapy (Zhao et al., 2018). Trastuzumab is known to target specifically the extracellular domain of the HER2 receptor and subsequently inhibit the downstream of *PIK3*/Akt pathway. As a consequence, mutation of the *PIK3CA* gene is an important cause for trastuzumab resistance (Minuti et al., 2012).

Our study describes the genetic background of HER2-positive cases and in order to highlight the mutational pattern of genes in this specific subtype, we examined the genetic mutation of our breast cancer cases using NGS. Based on our data, mutation of *c-*

Table 2
Clinical and Pathological features of HER2-positive patients.

Clinicopathological Characteristics		HER 2				P-value
		Negative		Positive		
		N	%	N	%	
Age	Less than 50	29	55.8%	23	44.2%	0.012
	More than 50	57	77.0%	17	23.0%	
Tumor Size	Less than 2	16	44.4%	20	55.6%	<0.001
	2–5	65	81.3%	15	18.8%	
	More than 5	5	50.0%	5	50.0%	
Necrosis	Absent	53	72.6%	20	27.4%	0.218
	Present	33	62.3%	20	37.7%	
Lymphovascular invasion	Absent	49	66.2%	25	33.8%	0.558
	Present	37	71.2%	15	28.8%	
Tumor Grade	1	25	100.0%	0	0.0%	<0.001
	2	28	58.3%	20	41.7%	
	3	33	62.3%	20	37.7%	
Lymph Node Metastasis	Negative	32	100.0%	0	0.0%	<0.001
	Positive > 3	32	68.1%	15	31.9%	
	Positive < 3	22	46.8%	25	53.2%	
Stage	I	16	100 %	0	0.0%	<0.001
	II	42	89.4%	5	10.6%	
	III	23	60.5%	15	39.5%	
	IV	5	20.0%	20	80.0%	

Table 3
The frequencies of the significant genes' mutations in our cohort study.

Significant genes		HER2 Status				P-value
		Negative		Positive		
		N	%	N	%	
c-MET	No mutation	64	86.5%	10	13.5%	<0.001
	Mutation	22	42.3%	30	57.7%	
c-KIT	No mutation	75	75.0%	25	25.0%	0.001
	Mutation	11	42.3%	15	57.7%	
PIK3CA	No mutation	81	94.2%	5	5.8%	<0.0001
	Mutation	5	12.5%	35	87.5%	

MET gene was the most frequent mutation in HER2-positive cases, followed by *PIK3CA* and *c-KIT*. Mutations in other genes were also detected such as *EGFR*, *PDGR*, *KRAS* and *BRAF*, however, these mutations were not significantly associated with HER 2-positive status and further investigation are suggested. Consistent with our results, many studies showed that mutation in *PIK3CA* gene is frequent in HER-2-positivesubtypes; however, these studies could not detect any significant association between mutation of *PIK3CA* gene and resistance to trastuzumab (Berns et al., 2007; Thorpe et al., 2015). However, and to the best our knowledge, no available literatures have studied the correlation between *c-MET* and/or *c-KIT* mutation and Her-2-positive breast cancer in Saudi patients.

5. Conclusion

The current study showed that HER2-positive breast cancer is associated with aggressive behavior such as lymph node metastasis, high grade tumor and tumor stage. Furthermore, our study is unrivaled as it is the first in Saudi Arabia to study gene expression signatures of HER2-positive breast cancer. Our study demonstrated that HER2-positive tumors exhibited high incidence of *c-MET*, *PIK3CA*, and *c-KIT* mutations than HER2-negative subtype. These findings suggest the presence of high mutational burden in HER2-positivepatients in Saudi population which could be implicated in the resistance to trastuzumab. Therefore, targeting these mutations would be a promising therapeutic strategy in the treatment of HER2-positive patients. Nevertheless, the significance of other gene mutations detected in this study justifies further and deep analysis of the mutational profile of HER2-positive breast cancers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the Institute of Scientific Research and Revival of Islamic Heritage and Deanship of Scientific Research at Umm Al-Qura University (Project No. 43509003) for the financial support.

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