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The Relation between Exon Variations of KIT Gene and Clinical Pathological Factors of Breast Cancer

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ABSTRACT

Background & Objective: As the most common cancer type, breast cancer has been recognized as the second mortality cause among women. The *KIT* proto-oncogene is one of the important factors involved in tumor development. The previous findings have demonstrated an increased copy number and overexpression of this gene under the influence of breast cancer development.

Materials & Methods: This study aimed to investigate the relationship between the copy number variation (CNV) of all exons of *KIT* gene and estrogen receptor (ER), progesterone receptor (PR), HER2, P53, stage, tumor size, Ki67, Annexin V, histological type, age, molecular subtype, and node status by surveying breast cancer tissues collected from 64 patients. The CNV exons and clinicopathological variables were assessed by multiplex ligation-dependent probe amplification (MLPA), hematoxylin and eosin (H&E) staining, and immunohistochemistry techniques.

Results: Sixty percent of cases in exon 17, 60% in exon 18, and nearly 67% in exon 19 with increased CNVs had a tumor size of 2-5 cm; these results were significant. Also, patients with an increased exon 7 CNV were significantly in stage 3. Other exons did not exhibit significant relation to other clinicopathological variables (P>0.05).

Conclusion: Exons 7, 17, 18, and 19 are the key coding domains of tyrosine kinase, involving the activation of various upstream transcription factors that regulate apoptosis, cell differentiation, proliferation, and angiogenesis. Variation in exons can influence drug resistance. The results of this study can contribute to the diagnosis and treatment of breast cancer, although their confirmation requires further examinations.

Keywords: KIT gene, Clinical pathology factors, Breast cancer

Introduction

The *KIT* gene is a proto-oncogene located at 4q12, which spans 21 exons. This gene is one of the important factors in angiogenesis and tumor proliferation. Exons 1-9 of this gene code the extracellular domain, while exons 10 and 11 are involved in coding the transmembrane and juxtamembrane domains (JM), respectively. Exons 13-21 are responsible for the split tyrosine kinase domain **(1)**.

The *KIT* gene is activated by binding of its ligand, the stem cell factor. Phosphorylation cascade activation is followed by the activation of various transcription factors. Malignant tumors, such as breast cancer, could be suppressed by inhibiting the *KIT* gene **(2, 3)**.

Several studies have indicated the key role of *KIT* overexpression in the development of various cancers, such as gastrointestinal carcinoma, leukemia, and melanoma, in which insertion/deletion nucleotide and increased copy number variation (CNV) can result in the *KIT* overexpression **(4-9)**.

In contrast with other cancers, the increased copy number gene is highly frequent in breast cancer, while in other mutations are rarely observed **(10-16)**.

Our previous study showed a 55% increment in the level of *KIT* expression in breast cancer tissues **(17)**, while other studies demonstrated that nearly 28% of the samples had CNV of exons **(18)**. Variation of exons could lead to diverse protein structures and functions without its overexpression.

On the other hand, several *KIT* mutations are clinicopathologically relevant to responses toward inhibitor drugs.

In gastrointestinal stromal tumors (GISTs), for example, the majority of *KIT* mutations are seen in exon 11 (juxtamembrane domain) and exon 17 (tyrosine kinase domain), while mutations in *KIT* exons 2, 8, and 9 (extracellular domain) or exons 13 and 14 (tyrosine kinase domain) are less frequent. Interestingly, patients with variations in exon 17 are

resistant to imatinib drug **(19, 20)**. This issue is of crucial significance in the medicinal regime of patients with *KIT* gene mutations.

In this regard, the current study is the first research analyzing the relationship between deletions and duplications of *KIT* gene exons and clinicopathological variables, such as estrogen receptor (ER), progesterone receptor (PR), HER2, P53, stage, tumor size, Ki67, Annexin V, histological type, age, molecular subtype, and node status, among Iranian women suffering from breast cancer.

Material and Methods

Patients

Tissues were collected from 64 patients with breast cancer. The study included females with primary, sporadic breast cancer with no history of treatment regardless of their age or histopathological subtype who referred to Mehrad Hospital (Tehran, Iran).

DNA extraction was carried out on the tumor tissue samples obtained from tumor regions. The quality and quantity of DNAs were assessed by agarose gel electrophoresis and NanoDrop ND 2000 spectrophotometer.

Multiplex Ligation-Dependent Probe Amplification

KIT gene deletion and duplication were assayed using the P354-A2 kit, which investigated all CNVs of 21 exons in the *KIT* gene. Multiplex ligation-dependent probe amplification (MLPA) was conducted according to the protocol of the MRC Company, whose results were used for gel electrophoresis. Polymerase chain reaction (PCR) products were separated on an ABI3730XL capillary sequencer. The variation of the copy number of the *KIT* gene was determined using Coffalyser (ver. 140721.1958). According to the guideline of MRC, results below 0.7 or 1.3-2 were interpreted as deletion and low-level amplification of genes, respectively (Figure 1).

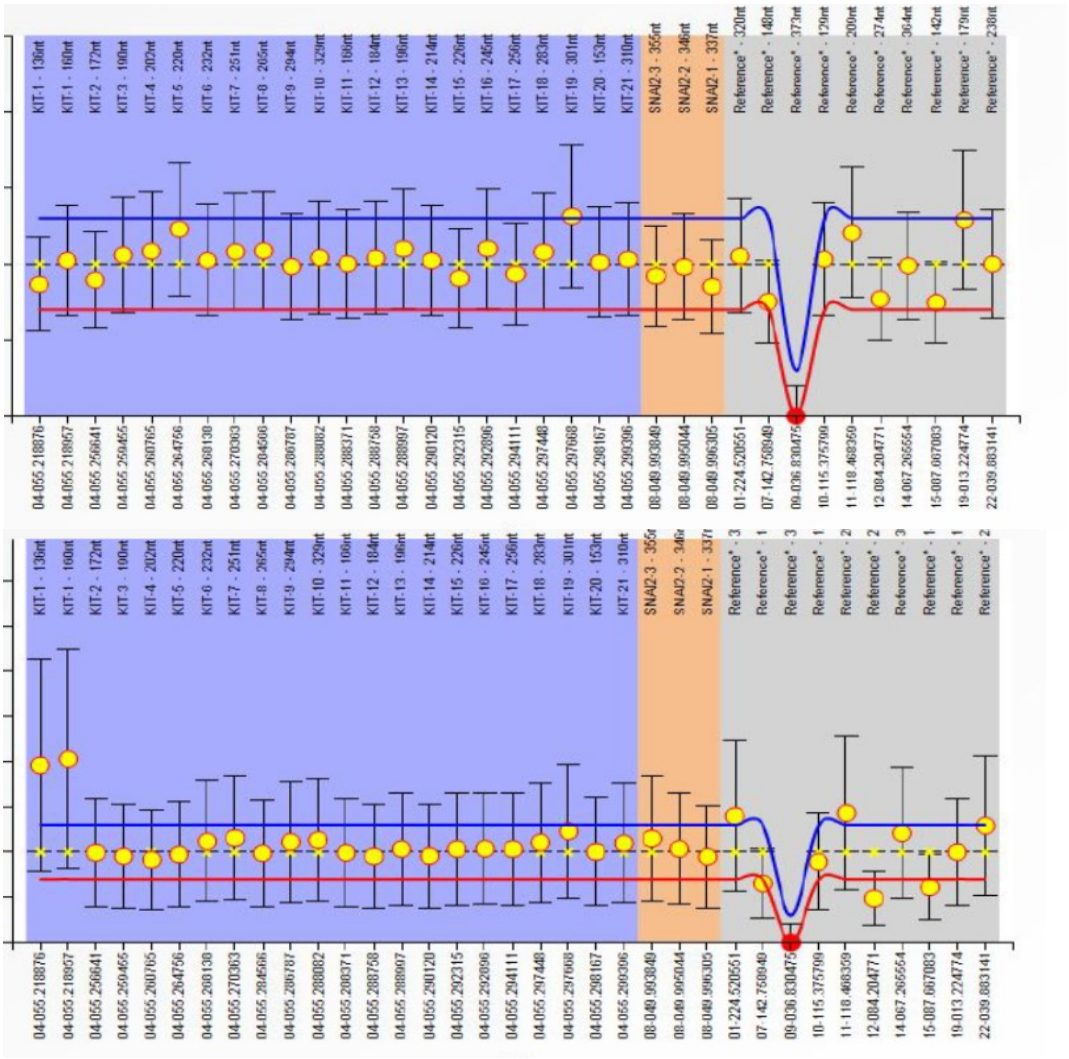

Figure 1. A: MLPA result of a sample on Coffalyser software without amplification of *KIT* B: MLPA result of a sample on Coffalyser software, which shows amplification of *KIT*

Histopathology

Tissue sections were prepared, stained by hematoxylin and eosin (H&E), and studied by a pathologist to confirm the tumor diagnosis and characterization. Immunohistochemical staining was carried out for ER, PR, and HER2/neu on paraffin blocks. The pathological stage of the disease was also determined. The association between the variation of exons and ER, PR, HER2, P53, stage, tumor size, Ki67, Annexin V, histological type, age, molecular subtype, and node status was statistically analyzed.

Statistical Analysis

Data analysis was achieved using SPSS 19.0 (SPSS Inc., Chicago, Ill., USA). Fisher's exact tests were used to assess the association between the variation in the copy number of *KIT* and clinicopathological variables. A P-value of less than 0.05 was considered statistically significant.

Results

The mean age of studied cases was 51 years (ranging from 30 to 76 years); their histological subtypes included 95% invasive ductal carcinoma and 4.9% invasive lobular carcinoma, while their tumor size was classified < 2 cm (8.5%) and 2-5 cm (91.5%).

Further, 16.1% of the patients were classified as grade 1, whereas 74.2% and 9.7% of the cases were grades 2 and 3, respectively.

These cases had an overall mean of 7.48 for P53, 8.97 for Ki67, and 8.66 for Annexin V. Further, 6.8% of the cases were in stage 1, while 79.7% and 13.6% were in stages 2 and 3, respectively.

Also, 46.8% of the samples were HER2-positive (hence, 53.2% were HER2-negative). Moreover, 69.4% and 61.3% of the subjects were ER-positive (30.6% ER-negative) and PR-positive (38.7% PR-negative), respectively.

Tables 1, 2, and 3 present the correlation between variations in *KIT* exons and clinicopathological variables.

Our results showed a significant correlation between increased copy number in exons 17-19 and the enhancement of tumor size (P<0.05). Also, a significant correlation was found between increased copy number in exon 7 and the cancer stage (P<0.05).

Other exons did not show a significant relationship with other clinicopathological variables (P>0.05).

Table 1. The relation between CNV in *KIT* gene exons, ER, PR, HER2, and tumor size

Tumor size			HER2 status			PR status			ER status			Exons/ Copy Number Variation	
P-value	2-5cm	<2cm	P-value	Negative	Positive	P-value	Negative	Positive	P-value	Negative	Positive		
0.06	44 95.7%	2 4.3%	0.71	24 50%	24 50 %	0.76	18 37.5%	30 62.5%	1.00	15 31.3%	33 68.8%	NO	1
	10 23.1%	3 76.9%		9 64.3%	5 35.7%		6 42.9%	8 57.1%		4 28.6%	10 71.4%	Yes	
0.10	49 94.2%	3 5.8%	0.27	28 50.9%	27 49.1%	1.00	21 38.2%	34 61.8%	0.08	19 34.5%	36 65.5%	NO	2
	5 71.4%	2 28.8%		5 71.4%	2 28.6%		3 42.9%	4 57.1%		0 0%	7 100%	Yes	
0.30	51 92.7%	4 7.3%	0.26	29 50%	29 50%	1.00	23 39.7%	35 60.3%	0.30	19 32.8%	39 67.2%	NO	3
	3 75%	1 25%		4 100%	0 0%		1 25%	3 75%		4 100%	0 0%	Yes	
0.30	51 92.7%	4 7.3%	0.26	29 50%	29 50%	1.00	23 39.7%	35 60.3%	0.30	19 32.8%	39 67.2%	NO	4
	3 75%	1 25%		4 100%	0 0%		1 25%	3 75%		4 100%	0 0%	Yes	
1.00	49 90.7%	5 9.3%	0.57	29 36.4%	27 16.4%	0.39	33 58.9%	23 41.1%	0.16	19 33.9%	37 66.1%	NO	5
	5 100%	0 0%		4 66.7%	2 33.3%		1 16.7%	5 83.3%		0 0%	6 100%	Yes	
0.30	51 92.7%	4 7.3%	0.60	30 51.7%	28 48.3%	0.63	22 37.9%	36 62.1%	1.00	18 31.0%	40 69.0%	NO	6

Tumor size			HER2 status			PR status			ER status				
P-value	2-5cm	<2cm	P-value	Negative	Positive	P-value	Negative	Positive	P-value	Negative	Positive	Exons/ Copy Number Variation	
	3 75%	1 25%		3 75%	1 25%		2 50%	2 50%		1 25%	3 75%	Yes	
0.30	51 92.7%	4 7.3%	0.19	29 50%	29 50%	0.63	22 37.9%	36 62.1%	1.00	18 31.0%	40 69.0%	NO	7
	3 75%	1 25%		4 100%	0 0%		2 50%	2 50%		1 35%	3 75%	Yes	
0.23	52 92.9%	4 7.1%	0.64	29 50%	29 50%	1.00	23 39.0%	36 61.0%	0.54	19 32.2%	40 67.8%	NO	8
	2 66.7%	1 33.3%		3 100%	0 0%		1 33.3%	2 66.7%		0 0%	3 100%	Yes	
0.36	50 92.6%	4 7.4%	0.56	29 50.8%	28 49.2%	1.00	22 38.6%	35 61.4%	0.31	19 33.3%	38 66.7%	NO	9
	4 80%	1 20%		4 80%	1 20%		2 40%	3 60%		0 0%	5 100%	Yes	
0.10	49 94.2%	3 5.8%	0.68	28 50.9%	27 49.1%	1.00	21 38.2%	34 61.8%	0.08	19 34.5%	36 65.5%	NO	10
	5 71.4%	2 28.8%		5 71.4%	2 28.6%		3 42.9%	4 57.1%		0 0%	7 100%	Yes	
0.10	49 94.2%	3 5.8%	0.68	28 50.9%	27 49.1%	1.00	21 38.2%	34 61.8%	0.08	19 34.5%	36 65.5%	NO	11
	5 71.4%	2 28.8%		5 71.4%	2 28.6%		3 42.9%	4 57.1%		0 0%	7 100%	Yes	
0.07	50 94.3%	3 5.7%	0.67	28 49.1%	29 50.9%	0.69	22 40%	33 60%	0.08	19 34.5%	36 65.5%	NO	12
	4 66.7%	2 33.3%		5 71.4%	2 28.6%		2 28.6%	5 71.4%		0 0%	7 100%	Yes	
1.00	49 90.7%	5 9.3%	0.61	29 50.9%	28 49.1%	0.64	23 40.4%	34 59.6%	0.31	19 33.3%	38 66.7%	NO	13
	5 100%	0 0%		4 80%	1 20%		1 20%	4 80%		0 0%	5 100%	Yes	
1.00	52 91.2%	5 8.8%	0.76	31 51.6%	28 48.4%	0.51	24 40%	36 60%	1.00	19 31.7%	41 68.3%	NO	14
	2 100%	0 0%		2 100%	0 0%		2 100%	0 0%		0 0%	2 100%	Yes	
1.00	49 90.7%	5 9.3%	0.13	28 49.1%	29 50.9%	0.14	24 42.1%	33 57.9%	0.31	19 33.3%	38 66.7%	NO	15
	5 100%	0 0%		5 100%	0 0%		5 100%	0 0%		0 0%	5 100%	Yes	
0.23	52 92.9%	4 7.1%	0.46	30 50.9%	29 49.1%	1.00	23 39.0%	36 61.0%	0.54	19 32.2%	40 67.8%	NO	16
	2 66.7%	1 33.3%		3 100%	0 0%		1 33.3%	2 66.7%		0 0%	3 100%	Yes	
0.05	51 94.4%	3 5.6%	0.56	29 50.9%	28 49.1%	1.00	22 38.6%	35 61.4%	0.31	19 33.3%	38 66.7%	NO	17
	3 60%	2 40%		4 80%	1 20%		2 40%	3 60%		0 0%	5 100%	Yes	
0.05	51 94.4%	3 5.6%	0.91	29 51.8%	27 48.2%	1.00	22 39.3%	34 60.7%	0.16	19 33.9%	37 66.1%	NO	18
	3 60%	2 40%		4 66.7%	2 33.3%		4 66.7%	2 33.3%		0 0%	6 100%	Yes	
0.02	48 96%	2 4%	0.44	27 51.9%	25 48.1 %	1.00	20 40%	32 60%	0.15	18 31.7%	34 68.3%	NO	19
	6 66.7%	3 33.3%		4 40%	6 60%		4 40%	6 60%		1 10%	9 90%	Yes	

Tumor size			HER2 status			PR status			ER status			Exons/ Copy Number Variation	
P-value	2-5cm	<2cm	P-value	Negative	Positive	P-value	Negative	Positive	P-value	Negative	Positive		
0.10	49 94.2%	3 5.8%	0.86	29 52.7%	26 47.3%	0.41	20 36.4%	35 63.6%	0.08	19 34.5%	36 65.5%	NO	20
	5 71.4%	2 28.6%		4 57.1%	3 42.9%		4 57.1%	3 42,9%		0 0%	7 100%	Yes	
0.16	47 94%	3 6%	0.47	28 52.8%	25 47.2%	0.72	20 37.7%	33 62.3%	0.25	18 34%	35 66%	NO	21
	7 77.8%	2 22.2%		5 100%	4 0%		4 44.4%	5 55.6%		1 11.1%	8 88.9%	Yes	

Table 2. **The relation between the CNV in *KIT* gene exons, stage, histological Type, Annexin V, and Ki67**

Ki67			Annexin V			Histological Type			Stage			Exons/ Copy Number Variation	
P-value	Negative	Positive	*P*-value	Negative	Positive	*P*-value	[b]ILCA	[a]IDCA	*P*-value	III	II	I		
0.43	39 81.3%	9 18.8%	0.71	38 79.2%	10 20.8%	1.00	4 8.5%	43 91.5%	0.06	4 8.7	39 84.8%	3 6.5%	NO	1
	13 92.9%	1 7.1%		10 71.4%	4 28.6%		0 0%	14 100%		4 30.8%	8 61.5%	1 7.7%	Yes	
0.58	45 81.8%	10 18.2%	0.65	43 78.2%	12 21.8%	1.00	4 7.4%	50 92.6%	0.56	7 13.5%	42 80.8%	3 5.8%	NO	2
	7 100%	0 0%		5 71.4%	2 28.6%		0 0%	7 100%		1 14.3%	5 71.4%	1 14.3%	Yes	
0.30	48 82.8%	10 17.2%	0.56	44 75.9%	14 24.1%	1.00	4 7%	53 93%	0.18	7 12.7%	45 81.8%	3 5.5%	NO	3
	4 100%	0 0%		4 100%	0 0%		0 0%	4 100%		1 25%	2 50%	1 25%	Yes	
1.00	48 82.8%	10 17.2%	0.56	44 75.9%	14 24.1%	1.00	4 7%	53 93%	0.18	7 12.7%	45 81.8%	3 5.5%	NO	4
	4 100%	0 0%		4 100%	0 0%		0 0%	4 100%		1 25%	2 50%	1 25%	Yes	
0.57	46 82.1%	10 17.9%	0.61	44 78.6%	12 21.4%	1.00	4 7.3%	51 92.7%	0.68	7 13%	45 79.6%	3 7.4%	NO	5
	6 100%	0 0%		4 66.7%	2 33.3%		0 0%	6 100%		1 20%	4 80%	0 0%	Yes	
0.51	49 84.5%	9 15.5%	1.00	45 77.6%	13 22.4%	1.00	4 7%	53 93%	0.18	7 12.7%	45 81.8%	3 5.5%	NO	6
	3 75%	1 25%		3 75%	1 25%		0 0%	4 100%		1 25%	2 50%	1 25%	Yes	
1.00	48 82.8%	10 17.2%	0.56	44 75.9%	14 24.1%	1.00	4 7%	53 93%	0.02	6 10.9%	46 83.6%	3 5.5%	NO	7
	4 100%	0 0%		4 100%	0 0%		0 0%	4 100%		2 50%	1 25%	1 25%	Yes	
1.00	49 83.1%	10 16.9%	1.00	45 76.3%	14 23.7%	1.00	4 6.9%	54 93.1%	0.10	7 12.5%	46 82.1%	3 5.4%	NO	8
	3 100%	0 0%		3 0%	0 100%		0 0%	3 100%		1 33.3%	1 33.3%	1 33.3%	Yes	
0.36	47 82.5%	10 17.5%	0.57	43 50.8%	14 49.2%	1.00	4 7.1%	52 92.9%	0.26	7 13.0%	44 81.5%	3 5.6%	NO	9
	5 100%	0 0%		5 100%	0 0%		0 0%	5 100%		1 20%	3 60%	1 20%	Yes	

Ki67			Annexin V			Histological Type			Stage			Exons/ Copy Number Variation	
P-value	Negative	Positive	*P*-value	Negative	Positive	*P*-value	[b]ILCA	[a]IDCA	*P*-value	III	II	I	
0.58	45 81.8%	10 18.2%	0.65	43 78.2%	12 21.8%	1.00	4 7.4%	50 92.6%	0.56	7 13.5%	42 80.8%	3 5.8%	NO	10
	7 100%	0 0%		5 71.4%	2 28.6%		0 0%	7 100%		1 14.3%	5 71.4%	1 14.3%	Yes	
0.58	45 81.8%	10 18.2%	1.00	42 76.4%	13 23.6%	1.00	4 7.4%	50 92.6%	0.56	7 13.5%	42 80.8%	3 5.8%	NO	11
	7 100%	0 0%		6 85.7%	1 14.3%		0 0%	7 100%		1 14.3%	5 71.4%	1 14.3%	Yes	
0.58	45 81.8%	10 18.2%	0.18	44 80%	11 20%	1.00	4 7.4%	50 92.6%	0.35	7 13.2%	43 81.1%	3 5.7%	NO	12
	7 100%	0 0%		4 57.1%	3 42.9%		0 0%	7 100%		1 16.7%	4 66.7%	1 16.7%	Yes	
0.58	47 82.5%	10 17.5%	0.57	43 50.8%	14 49.2%	1.00	4 7.1%	52 92.9%	0.69	7 13%	43 79.6%	4 7.4%	NO	13
	5 100%	0 0%		5 100%	0 0%		0 0%	5 100%		1 20%	4 80%	0 0%	Yes	
1.00	50 83.3%	10 16.7%	1.00	46 76.7%	14 23.3%	0.51	4 6.8%	55 93.2%	0.36	7 12.3%	46 80.7%	4 7%	NO	14
	2 100%	0 0%		2 100%	0 0%		0 0%	2 100%		1 50%	1 50%	0 0%	Yes	
0.58	47 82.5%	10 17.5%	1.00	44 77.2%	13 22.8%	1.00	4 7.1%	52 92.9%	0.69	7 13%	43 79.6%	4 7.4%	NO	15
	5 100%	0 0%		4 80%	1 20%		0 0%	5 100%		1 20%	4 80%	0 0%	Yes	
1.00	49 83.1%	10 16.9%	1.00	45 76.3%	14 23.7%	1.00	4 6.9%	54 93.1%	0.10	7 12.5%	46 82.1%	3 5.4%	NO	16
	3 100%	0 0%		3 0%	0 100%		0 0%	3 100%		1 33.3%	1 33.3%	1 33.3%	Yes	
0.58	47 82.5%	10 17.5%	1.00	44 77.2%	13 22.8%	1.00	4 7.1%	52 92.9%	0.27	7 13.0%	44 81.5%	3 5.6%	NO	17
	5 100%	0 0%		4 80%	1 20%		0 0%	5 100%		1 20%	3 60%	1 20%	Yes	
0.57	46 82.1%	10 17.9%	0.12	45 80.4%	11 19.6%	1.00	4 7.3%	51 92.7%	0.26	7 13.0%	44 81.5%	3 5.6%	NO	18
	6 100%	0 0%		3 50%	3 50%		0 0%	6 100%		1 20%	3 60%	1 20%	Yes	
0.19	42 80.8%	10 19.2%	0.44	41 78.8%	11 21.2 %	1.00	4 7.8%	47 92.2%	0.28	6 12%	41 82%	3 6%	NO	19
	10 100%	0 0%		7 70%	3 30%		0 0%	10 100%		2 22.2%	6 66.7%	1 11.1%	Yes	
0.58	45 81.8%	10 18.2%	1.00	42 76.4%	13 23.6%	1.00	4 7.4%	50 92.6%	0.56	7 13.5%	42 80.8%	3 5.8%	NO	20
	7 100%	0 0%		6 85.7%	1 14.3%		0 0%	7 100%		1 14.3%	5 71.4%	1 14.3%	Yes	
0.33	43 81.1%	10 19.9%	1.00	41 77.4%	12 22.6%	1.00	4 6.8%	48 93.2%	0.63	6 12%	40 80%	4 8%	NO	21
	9 100%	0 0%		7 77.8%	2 22.2%		0 0%	9 100%		2 22.2%	7 77.8%	0 0%	Yes	

aIDCA = Invasive Ductal Carcinoma
bILCA= Invasive Lobular Carcinoma
[c]*P*-value from Fisher's Exact Test.

Table 3. **The relation between CNV in *KIT* gene exons, subtype, age, and node status**

Node status			Age			Subtype				Exons/ Copy Number Variation	
P-value	Negative	Positive	P-value	≥40 years	< 40 years	P-value	ERBB2	Basal Like	luminal B	luminal A		
1.00	23 50%	23 50 %	0.37	41 89.1%	5 10.9%	0.47	10 20.8%	10 20.8%	27 56.3%	1 2.1%	NO	1
	7 53.8%	6 46.2%		11 21.4%	3 78.6%		5 35.7%	1 7.1%	8 57.1%	0 0%	Yes	
0.70	27 42.9%	25 57.1%%	1.00	46 86.8%	7 13.2%	0.55	13 23.6%	9 16.4%	30 54.5%	3 5.4%	NO	2
	3 71.4%	4 28.6%		6 85.7%	1 14.3%		0 0%	2 28.6%	5 71.4%	0 0%	Yes	
1.00	28 50.9%	27 49.1%	0.44	49 87.5%	7 12.5%	0.57	13 22.4%	11 19%	31 53.4%	3 5.1%	NO	3
	2 50%	2 50%		3 75%	1 25%		0 0%	0 0%	4 100%	0 0%	Yes	
1.00	28 50.9%	27 49.1%	0.44	49 87.5%	7 12.5%	0.56	13 22.4%	11 19%	31 53.4%	3 5.1%	NO	4
	2 50%	2 50%		3 75%	1 25%		0 0%	0 0%	4 100%	0 0%	Yes	
1.00	27 50%	27 50%	0.52	48 87.3%	7 12.7%	1.00	12 21.4%	10 17.9%	31 55.4%	3 5.4%	NO	5
	3 60%	2 40%		4 80%	1 20%		1 16.7%	1 16.7%	4 66.7%	0 0%	Yes	
0.35	29 37.9%	26 62.1%	0.44	49 87.5%	7 12.5%	1.00	13 22.4%	11 19%	32 55.2%	2 3.4%	NO	6
	1 25%	3 75%		3 75%	1 25%		0 0%	0 0%	3 75%	1 25%	Yes	
1.00	28 50.9%	27 49.1%	0.44	49 87.5%	7 12.5%	1.00	12 20/7%	11 19%	32 55.2%	3 5.1%	NO	7
	2 50%	2 50%		3 75%	1 25%		1 25%	0 0%	3 75%	0 0%	Yes	
0.61	29 50%	27 50%	0.35	50 39.0%	7 61.0%	0.79	13 22%	11 18.6%	32 54.2%	3 5.1%	NO	8
	1 33.3%	2 66.7%		2 66.7%	1 33.3%		0 0%	0 0%	3 100%	0 0%	Yes	
0.56	29 53.7%	25 46.3%	0.48	48 88.9%	6 11.1%	0.75	13 22.8%	10 17.5%	31 54.4%	3 5.3%	NO	9
	1 20%	4 80%		4 80%	1 20%		0 0%	1 20%	4 80%	0 0%	Yes	
1.00	26 50%	26 50%	1.00	46 86.8%	7 13.2%	0.81	12 21.8%	9 16.4%	31 56.4%	3 5.4%	NO	10
	4 57.1%	3 42.9%		6 85.7%	1 14.3%		1 14.3%	2 28.6%	4 57.1%	0 0%	Yes	
0.25	28 53.8%	24 46.2%	1.00	46 86.8%	7 13.2%	0.54	13 23.6%	9 16.4%	30 54.5%	3 5.4%	NO	11
	2 28.6%	5 71.4%		6 85.7%	1 14.3%		0 0%	2 28.6%	5 71.4%	0 0%	Yes	
1.00	27 50.1%	26 49.1%	0.69	47 87%	7 13%	0.54	13 23.6%	10 18.2%	29 52.7%	3 5.4%	NO	12
	3 50%	3 50%		5 83.3%	1 16.7%		0 0%	1 14.3%	6 85.7%	0 0%	Yes	
0.67	28 51.9%	26 48.1%	0.52	48 87.3%	7 12.7%	1.00	12 21.1%	10 17.5%	32 56.1%	3 5.3%	NO	13
	2 40%	3 60%		4 80%	1 20%		1 20%	1 20%	3 60%	0 0%	Yes	

Node status			Age			Subtype				Exons/ Copy Number Variation	
P-value	Negative	Positive	P-value	≥40 years	< 40 years	P-value	ERBB2	Basal Like	luminal B	luminal A		
0.23	30 52.6%	27 47.4%	0.25	51 40%	7 60%	1.00	13 21.7%	11 18.3%	31 55%	3 5%	NO	14
	0 0%	2 100%		1 50%	1 50%		0 0%	0 0%	2 100%	0 0%	Yes	
0.67	28 51.9%	26 48.1%	0.52	48 87.3%	7 12.7%	0.85	12 21.1%	11 19.3%	31 54.4%	3 5.4%	NO	15
	2 40%	3 60%		4 80%	1 20%		1 20%	0 0%	4 80%	0 0%	Yes	
0.61	29 50%	27 50%	0.35	50 39.0%	7 61.0%	0.79	13 22%	11 18.6%	32 54.2%	3 5.1%	NO	16
	1 33.3%	2 66.7%		2 66.7%	1 33.3%		0 0%	0 0%	3 100%	0 0%	Yes	
0.19	29 53.7%	25 46.3%	0.52	48 87.3%	7 12.7%	0.74	13 22.8%	10 17.5%	31 54.4%	3 5.3%	NO	17
	1 20%	4 80%		4 80%	1 20%		0 0%	1 20%	4 80%	0 0%	Yes	
0.67	28 51.9%	26 48.1%	0.52	48 87.3%	7 12.7%	0.16	13 23.2%	10 17.9%	30 53.6%	3 5.4%	NO	18
	2 40%	3 60%		4 80%	1 20%		0 0%	1 16.7%	5 83.3%	0 0%	Yes	
0.29	27 54%	23 46 %	0.61	44 88%	6 12%	0.15	12 23.1%	9 17.3%	28 53.8%	3 5.4%	NO	19
	3 33.3%	6 66.7%		8 80%	2 20%		1 10%	2 20%	7 70%	0 0%	Yes	
0.70	27 51.9%	25 48.1%	1.00	46 86.8%	7 13.2%	0.31	13 23.6%	8 14.5%	31 56.4%	3 5.4%	NO	20
	3 42.9%	4 57.1%		6 85.7%	1 14.3%		0 0%	3 42.9%	4 57.1%	0 0%	Yes	
0.73	26 52%	24 48%	1.00	44 86.3%	7 13.7%	0.62	11 20.8%	8 15.1%	31 58.5%	3 5.7%	NO	21
	4 44.4%	5 55.6%		8 88.9%	1 11.1%		2 22.2%	3 33.3%	4 44.4%	0 0%	Yes	

Discussion

Our results indicated that 60% of cases in exon 17, 60% in exon 18, and about 67% in exon 19 had a tumor size of 2-5 cm with an increase in CNVs; no significant relation was, however, found between other exons and tumor size.

These exons are key coding domains of tyrosine kinase, involving the activation of various upstream transcription factors that regulate apoptosis, cell differentiation, proliferation, and angiogenesis **(1, 20)**. Previous studies on phyllodes tumors have suggested an increase in exon 18 CNVs due to *KIT* overexpression **(15)**. Thus, an increase in the copy number of these exons may explain the rise in kit tyrosine kinase activity with an effective role in the tumor size enhancement. On the other hand, a study on phyllodes tumors demonstrated that 2 out of 13 *KIT* cases possess exon 17 alterations and protein overexpression **(21)**.

Our research confirmed other studies indicating that *KIT* can be a key factor in tumor development and breast cancer malignancy **(22)**. Exon 17 is one of the most important exons in other cancer types, such as GISTs, in terms of mutation frequency **(23, 24)**. On the other hand, variation in this exon can be due to resistance to imatinib **(25-27)**.

In this study, almost all the patients with a normal copy number of exons 7 were in stage 2, while those with increased CNV were in stage 3; these results were significant. Exon 7 codes the key domain of receptor tyrosine kinase, whose variation may influence stage development. These findings are in line with previous results highlighting the importance of *KIT* variation and overexpression for stage development **(28-30)**.

Most cases with no variation in exon 12 were HER2-positive, while those showing an increase of CNV exon

12 were HER2-negative (P>0.05). This exon encoded JM and can affect protein activity. The small sample size may be the main reason for insignificant results.

This study also demonstrated that most of the cases with increased exon 5 CNV were PR-positive, whereas those showing increased exons 15, 18, and 20 were PR-negative. One study revealed a correlation between enhanced copy numbers in exons 15 and 18 and their overexpression in phyllodes tumors **(15)**. All the cases with the increased exons 3 and 4 were ER-negative, although it was not statistically significant (P>0.05).

Two studies showed *KIT* expression in 42% and 30% of triple-negative breast cancers **(16, 31)**. Most studies have generally found that kit positivity may be more common in ductal carcinomas, HER2+, ER/PR2, and prognostically unfavorable tumors **(32, 33)**.

Furthermore, the correlation between variation in *KIT* exons and node status was also addressed. The cases with a normal copy number of exons 2, 6, 8, 9, 11, and 13-21 were node-negative, while those with increased copy numbers were mostly node-positive; our results were not significant. Exons 11, 13, 17, 2, and 8 play a key role in most cancer types, such as GISTs, as their mutation rate can dramatically influence malignancy **(27)**.

Conclusion

This study was carried out following our previous studies on the role of *KIT* gene CNVs and their expression in breast cancer. A significant relationship was found between the major exons involving the coding of key domains and tumor size and stage in some cancer types. These factors are indicative of the rate of tumor development. On the other hand, they are influential in diagnosis and follow-up processes. Further studies with larger populations are necessary to confirm our results.

kit-repressing drugs, such as imatinib used in the treatment of GIST, can also be employed for treating breast cancer. The variation in exon 17, i.e., in GIST, can be due to resistance to imatinib drug. Thus, further studies are needed to investigate this issue for the prevention of drug resistance. Moreover, previous studies have shown that the response to imatinib has a closer relationship with *KIT* mutational activation but not the expression of kit protein **(27-34)**. Some of the mutations of this study are different from the activating mutations reported in GISTs, reflecting genetic instability in malignant tumors. In breast cancer, however, an increase was observed in the copy number of the *KIT* gene, unlike other cancers **(4, 5, 12, 15, 35, 36)**.

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Conflict of Interest

All authors declare that they have no conflict of interests.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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