



Immunohistochemical Staining of HER-2, VEGF, ER and PR in Breast Tissues from Sudanese Patients

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ABSTRACT

Objective: This study investigated the expression of 4 receptors in breast cancer tissues in Sudanese patients. **Material and methods:** Total 81 cancerous breast tissues and 50 normal breast tissues were obtained from the Radiation and Isotope Center in Khartoum (RICK). The expression of human epidermal growth factor receptor-2 (HER-2), vascular endothelial growth factor (VEGF), estrogen receptor (ER) and progesterone receptor (PR) was investigated in the normal and cancerous breast tissues using the immunohistochemistry technique. The statistical correlation between the expression of the different proteins and the age and grade was also investigated. **Results:** The expression of ER and PR was positive in all the normal breast tissues while they were positive in 41(50.6%) and 28 (34.6%) of the cancerous tissues respectively. The staining of the HER-2 and VEGF was negative in all the normal breast tissues and they were positive in 57 (70.4%) and 32 (39.5%) of the cancerous tissues respectively. Most of the positive HER-2, VEGF, ER and PR samples are distributed between grade II and grade III with significant negative correlations. The majority of the positive cases for all the studied proteins were less than 64 years of age. There was an insignificant negative or positive correlation between the different studied proteins and the tumor grade. **Conclusion:** This study registered that HER-2 was positive in 70.4% of breast cancer tissues from Sudanese patients.

Keywords: Breast cancer, Immunohistochemistry, VEGF, HER-2, Hormonal status

INTRODUCTION

Breast cancer is classified into 4 classes depending on the expression of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2). The 4 classes are ER/PR+ and HER-2+, ER/PR+ and HER-2-, ER/PR- and HER-2+, ER/PR- and HER-2-. Each type of breast cancer has specific molecular, pathological and clinical features [1].

Vascular endothelial growth factor (VEGF) and its receptor are well known to be involved in the angiogenesis process of vascular endothelial cells. It is essential to the normal function of the cardiovascular and central nervous systems, hematopoietic stem cell and bone formation. VEGF and its receptor are registered to be up-regulated in many tumor cells [2].

The 4 antigen is essential in the field of breast cancer since, 3 breast cancer therapies target the ER and PR positive tissues, HER-2 positive and VEGF positive [3-5]. The ER-positive breast tumors are approximately 65% of all the breast cancer [6], the HER-2 positive breast tumors account for about 20-30% of the breast cancers and the triple negative breast tumors are approximately 170000 in every 1 million breast cancer cases (approximately 17%) [7,8].

Recently breast cancer is classified into 5 classes according to the expression of hormone receptors (ER and PR), HER and some genes like Ki-67 and BRCA1 [9]. The VEGF is highly expressed in more than 50% of the breast tumors, however, its expression is associated with different factors including lymph node metastasis and density of vascular structure [10,11]. This article investigated the expression of ER, PR, HER-2 and VEGF in cancerous and normal breast tissues from Sudanese subjects.

PATIENTS AND METHODS

Study Design and Population

This study can be classified as a qualitative and case-control study. Total 81 cancerous breast tissues and 50 normal breast tissues were studied. The samples were obtained from the archive of the Radiation and Isotope Centre in Khartoum (RICK). The cancerous and normal breast tissues were confirmed by the Hematoxylin and Eosin staining.

Ethical Clearance

This study was achieved after an ethical license was obtained from the authorities of the Sudan Academy of Sciences (SAS) and the RICK.

Staining of the tissues with the antibodies of the studied parameters

The immunohistochemical analysis of the estrogen, progesterone, HER-2, and VEGF was done according to the instructions of the producing companies. The producing companies of the estrogen, progesterone, HER-2 and VEGF were DAKO (Monoclonal rabbit anti-human ER alpha clone EP1, DAKO AS/AS+, Code number IS084), DAKO (Monoclonal mouse anti-human clone PGR 636, Code number IS053), THERMO (HER-2 monoclonal mouse anti-human clone MJD2, Code number 14-9757-82) and THERMO (Monoclonal mouse anti-human clone SP28, Code number MA5-14573) respectively.

Statistical Analysis

The Chi-square test of SPSS version 20 was used for the analysis of the results.

RESULTS

Generally, the age was clustered in 3 groups 19-44 years, 45-64 years and ≤ 65 years. The numbers of the subjects in the different age groups were 34, 31 and 16 respectively. The age groups of the normal subjects were containing 31, 14 and 5 persons, respectively.

The grades of the cancerous tissues were grade I (9), grade II (36) and grade III (36). The interesting result of this research was concerning the normal tissues; they were all positive for estrogen and progesterone while they were all negative for the HER-2 and VEGF (Figures 1-4).

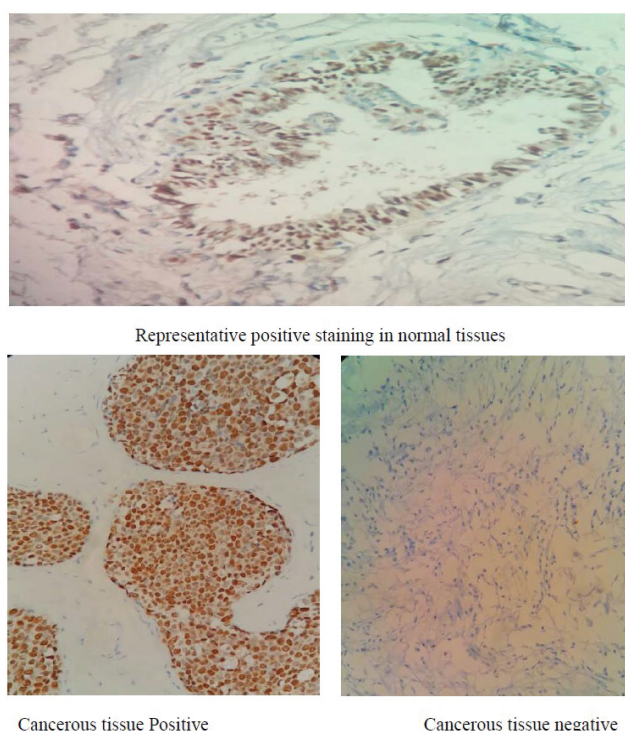
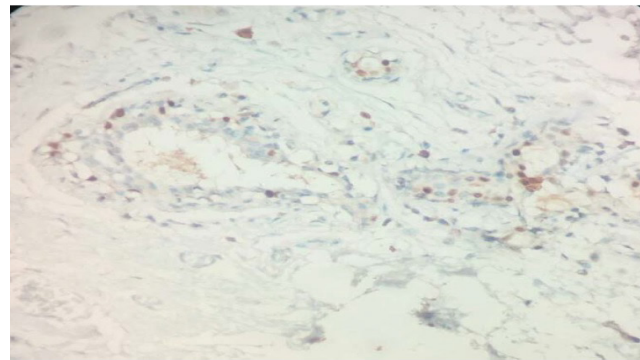
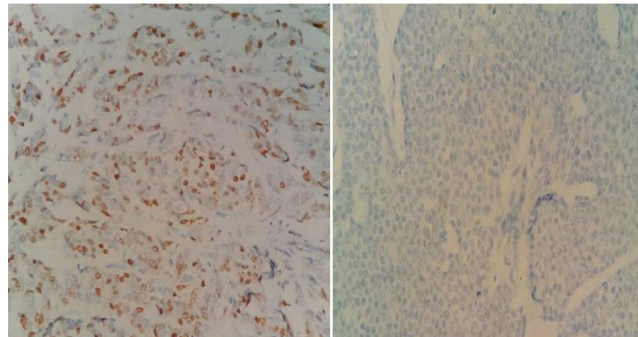


Figure 1 The staining results of estrogen receptor (ER) in the cancerous and normal breast tissues



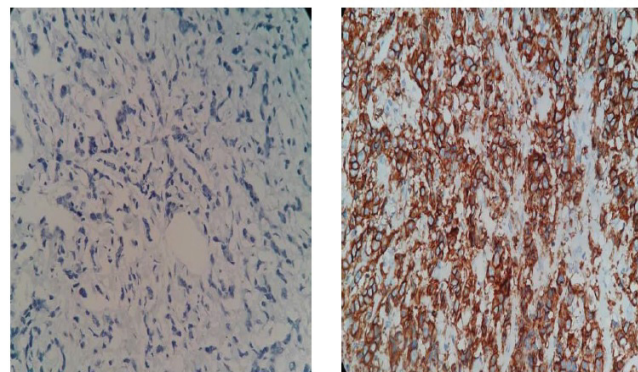
Representative positive staining in normal tissues



Cancerous tissues positive

cancerous tissues negative

Figure 2 The staining results of the progesterone receptor (PR) in the normal and cancerous tissues



cancerous tissue negative

cancerous tissue positive

Figure 3 The staining results of the HER2/neu in cancerous tissues cancerous

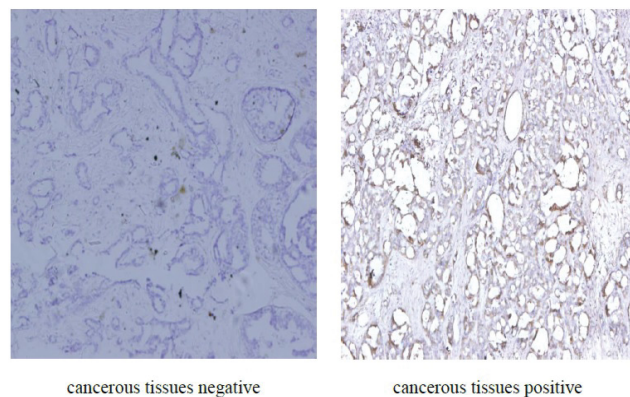


Figure 4 The Staining results of the VEGF in cancerous tissues

The cancerous tissues showed different expression patterns of the estrogen, progesterone, HER-2, and VEGF generally and at age group level (Table 1).

Parameter	Positive					Negative					Total
	19-44 Years	45-64 Years	≥65 Years	Total		19-44 Years	45-64 Years	≥65 Years	Total		
				No	%				No	%	
ER	15	17	9	41	50.6%	19	14	7	40	49.4%	81
PR	12	9	7	28	34.6%	22	22	9	53	65.4%	81
HER-2	26	24	7	57	70.4%	8	7	9	24	29.6%	81
VEGF	14	12	6	32	39.5%	20	19	10	49	60.5%	81

The percentages of the ER, PR, HER-2 and VEGF positive tissues were 50.6%, 34.6%, 70.4% and 39.5% respectively. However, the highest percentage was seen for the HER-2 compared to the international results (approximately 30%). Regarding the ER expression in the different age groups, the highest positive tissues were seen in the age groups (45-64) and (≥65). The PR and VEGF staining results showed the highest number of cases were negative for all the age groups. The highest number of positive tissues for HER-2 was seen in the age groups (19- 44) and (45- 64) (Table 1).

The staining of the parameters showed variable results in the three different grades (Table 2). However, grade II registered the highest number of positive tissues for ER while all the grades were with the highest numbers of positive tissues. The majority of the tissues were negative in all the grades for PR and VEGF (Table 2).

Table 2 The positive ER, PR, HER-2 and VEGF in the different grades of the cancerous breast tissues

Parameter	Positive					Negative					Total
	I	II	III	Total		I	II	III	Total		
				No	%				No	%	
ER	5	23	13	41	50.6%	4	13	23	40	49.4%	81
PR	4	15	9	28	34.6%	5	21	27	53	65.4%	81
HER-2	7	22	28	57	70.4%	2	14	8	24	29.6%	81
VEGF	5	15	12	32	39.5%	4	21	24	49	60.5%	81

The positively stained tissues for ER were higher in grade II while the negative cases for the PR registered the highest numbers in all the grades. The HER-2 staining results showed that the highest numbers of tissues were positive in all the grades. The majority of the tissues were negative for VEGF in grade II and III.

The breast cancer is diagnosed according to the staining of HER-2, ER and PR to different types. The types were 8 and their numbers of our study were; HER-2+, ER+ and PR+ (19, 23.5%); HER-2+, ER+ and PR- (11, 13.6%); HER-2+, ER- and PR- (26, 32.1%); HER-2+, ER- and PR+ (1, 1.2%); HER-2-, ER- and PR- (13, 16%); HER-2-, ER+ and PR- (3, 3.7%); HER-2-, ER+ and PR+ (8, 9.9%); HER-2-, ER+ and PR- (3, 3.7%); HER-2-, ER- and PR+ (0, 0%) (Table 3). The percentage of the triple negative tissues was 13/81 (16%).

Table 3 Types of breast cancer according to the expression of HER-2, ER, and PR

HER-2	ER	PR		Total
		Positive	Negative	
Positive	Positive	19	11	30
	Negative	1	26	27
Negative	Positive	8	3	11
	Negative	0	13	13

The triple negative cases were 13 (16%) and the triple positive cases were 19 (23.5%). However, the chi-square test showed that there was a significant correlation ($p \leq 0.000$). The Pearson's and Spearman correlation values for the cases of HER-2 positive and negative were 0.624 and 0.769 respectively. The results reflected a positive correlation between the expression of HER-2, ER, and PR.

This study tried to classify the breast cancer according to the staining of VEGF, ER and PR and classified the breast cancer into 8 types with different number positive and negative cases. The eight types were VEGF+, ER+ and PR+ (11, 13.6%); VEGF +, ER+ and PR- (5, 6.2%); VEGF +, ER- and PR- (16, 19.8%); VEGF +, ER- and PR+ (0, 0%); VEGF -, ER- and PR- (23, 28.4%); VEGF -, ER+ and PR- (9, 11.1%); VEGF -, ER+ and PR+ (16, 19.8%); VEGF -, ER+ and PR- (9, 11.1%); VEGF -, ER- and PR+ (1, 1.2%) (Table 4). The total VEGF positive tissues were 32 (39.5%) and the triple negative cases were 23/81 (28.4%).

Table 4 Types of breast cancer according to the expression of VEGF, ER, and PR

VEGF	ER	PR		Total
		Positive	Negative	
Positive	Positive	11	5	16
	Negative	0	16	16
Negative	Positive	16	9	25
	Negative	1	23	24

The triple negative cases were 23 (28.4%) compared to 11 (13.6%) triple positive. However, the chi-square test showed that there was a significant correlation ($p \leq 0.000$). The Pearson's and Spearman correlation values for the cases of HER-2 positive and negative were 0.724 and 0.628 respectively. The results reflected a positive correlation between the expression of HER-2, ER, and PR.

There were 4 types of breast cancer according to the staining of HER-2 and VEGF. The 4 types were HER-2+ and VEGF+ (22, 27.2%); HER-2+ and VEGF- (35, 43.2%); HER-2- and VEGF- (14, 17.3%); HER-2- and VEGF+ (10, 12.3%) (Table 5).

Table 5 The correlation between HER-2 and VEGF expression

HER-2	VEGF		Total
	Positive	Negative	
Positive	22	35	57
Negative	10	14	24

There was an insignificant correlation between the expression of HER-2 and VEGF. The Pearson's and Spearman correlation values were -0.029. There was a very weak negative correlation between the HER-2 and the VEGF.

There was a positive correlation between the ER and PR (Pearson correlation was 0.66) and the other correlations were weak (0.06, 0.02, 0.01). There were a weak inverse correlation between VEGF and ER, PR, and HER-2 (Pearson correlations were -0.01, -0.003 and -0.029 respectively). All the correlations were insignificant except for the correlation between ER and PR ($p \leq 0.000$).

The grade was weakly correlated to the expression of ER, PR, HER-2, and VEGF; the Pearson correlations were 0.21, 0.169, -0.08 and 0.139 respectively. All the correlations of the grade to the 4 parameters were insignificant, however, the grade correlation to the expression of the ER was closed to the significant value (p -value=0.06).

DISCUSSION

Our study showed that 50.6% of the breast cancer tissues were positive for ER and 34.6% were positive for PR. The total hormonal positive tissues were 85.2%. Our results of ER are comparable to the results of Huiyan who stated that the positive ER tissues for black women were 51.3% as compared to 62.5% in white women [12].

Ahmed, et al., in 2011, found that the expression of ER and PR in Yemeni breast cancer patients was 43.8% and 27% respectively [13].

Another study showed that the positive hormonal tissues (ER and PR positive) in 1387 cancerous breast tissues was 1073 (77.4%) compared to 85.2% for the ER and PR positive cases in our study population [14,15]. However, other authors stated that the ER+/PR+ percentages was 54% and 65% [6,16].

The percentage of HER-2 positive breast cancer in our study was 70.4%. This percentage is very high and it is not registered previously. The percentage of HER-2 positive breast cancer is ranging from 15% to 30% [13,17-19]. The highest percentage of the HER-2 positive in our study population reflects the aggressiveness of breast cancer in Sudan. However, this highest percentage does not reflect the real situation in Sudan because our study was not community-based.

Our study showed that the triple positive tissues (HER-2+, ER+, and PR+) constituted 23.5%. The triple positive tissues percentage was very high when compared to the previous studies. Previous studies registered 10%, 10.3% and less than 50% of the HER-2 positive tumors [15,17,20]. However, HER-2 positive tumors are associated with aggressive tumors and a reduced survival rate. The percentage of HER-2 positive breast cancer in our study was 70.4%.

The percentage of the triple negative breast cancer tissues in our study was 16%. Our result is similar to the previous studies findings since the triple negative breast cancer is ranging from 6% to 39%. However, different factors affect the triple negative percentage including the race (white, black or Hispanic), gender and geographical area [8,21].

We have obtained VEGF positive tissues with a percentage of 39.5% compared to the variable results registered by previous studies starting from 37% up to 87% [22-26]. It is well known that VEGF positive breast cancers are aggressive breast cancers.

CONCLUSION

The correlation statistics of our study showed that there was no strong correlation between the parameters except for the ER and PR and all the correlations were insignificant. There was an inverse correlation between the VEGF and all the parameters. All the parameters showed no correlation to the grade of the breast cancer. However previous studies stated that there was a positive and negative correlation between ER, PR, HER-2 and VEGF and the breast cancer grade [27-29].

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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