RESEARCH ARTICLE

CORRELATION OF FOUR RECEPTORS EXPRESSION AND LYMPH NODE METASTASIS IN SUDANESE BREAST CANCER PATIENTS

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ARTICLE INFO

ABSTRACT

Objectives: The aim of this study was to investigate the correlation between the imunohistochemical staining of four receptors and the metastasis of breast cancer to the lymph nodes and the age. The investigated receptors were estrogen and progesterone receptor (ER and PR), human epidermal growth factor-2 (HER-2) and vascular endothelial growth factor receptor (VEGF).

Material and Methods: This study was retrospective study. One hundred and twenty one breast cancer tissues were retrieved from the radiation and isotope centre in Khartoum (RICK). The expression of the receptors was investigated by immunohistochemistry.

Results: Forty eight samples (39.7%) were with negative lymph nodes and 73 (60.3%) had positive lymph node metastasis. Regarding the immunohistochemical staining of the ER was positive in 23 (47.9%) out of the 48 lymph node negative tissues and it was positive in 33 (45.2%) tissues out of the 73 lymph node positive tissues. The PR positive staining results in the negative and positive lymph nodes tissues were 17 (35.4%) and 22 (30.1%) respectively. Thirty breast tissues (62.5%) were positive for HER-2 out of the 48 negative lymph node tissues compared to 57 (78.1%) HER-2 positive tissues out of the 73 tissues with lymph node involvement. Concerning the VEGF, it was positive in 17 (35.4%) of the lymph node negative tissues and in 25 (34.2%) of the tissues with positive lymph node metastasis. The spearman correlation showed that there was a weak, negative and insignificant correlation between ER, PR and VEGF and the lymph node involvement while there was positive and insignificant correlation between HER and the lymph node metastasis. There was significant positive correlation between the collective results and the lymph node metastasis.

Conclusion: Collective staining results of ER, PR, HER and VEGF were useful as predictors for lymph node metastasis.

INTRODUCTION

It is known that some tumor markers are associated with the invasion of tumors in their surrounding tissues and metastasis to the lymph nodes and distant tissues like the CA15-3, hepatocyte growth factor and integrins (Ceng *et al*, 2015; Martin *et al*, 2013). There are two types of estrogen receptors; ERα and ERβ and they are nuclear receptors. The amino acid sequence of the two receptors is almost the same except in the amino terminal. The estrogen receptors bind estrogens like the 17β etsradiol. Together the estrogens and their receptors play a central role in the normal reproductive and sexual function of the males and females.

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Also they have many biological effects on different systems in males and females including the muscloskeletal, nervous, immune and cardiovascular (Heldring *et al*, 2007). Progesterone receptors are nuclear receptors and they are of three types; A, C and B. The progesterone receptor B is the full length receptor while the progesterone receptor A and C are amino terminal truncated forms. The progesterone hormone binds the progesterone receptors and acts as a proliferative factor in milk ducts of the breast and anti proliferative in the ovaries and reproductive tract (Daniel *et al*, 2011). Human Epidermal growth factor Receptor -2 belongs to the four member human epidermal growth factor receptors; HER-1, HER-2, HER-3 and HER-4. It is a trans membrane protein with 1255 amino acid. It activates cell proliferation, survival, differentiation and angiogenesis in many tissues (Nida Iqbal and Naveed Iqbal, 2014)

There are four types of vascular endothelial growth factors; A, B,C, D. However, the gene of the VEGF contains five members; the four genes of the VEGF and the gene of the phosphatidylinositol- glycan biosynthesis F protein (PIGF). The VEGF is essential for the angiogenesis, lymphogenesis and vasculogenesis in the developing embryos and post-natal children (Quoc T. Ho and Calvin J. Kuo, 2007). The four receptors or growth factors are well known to be over expressed in breast cancer and they are used as targets for breast cancer therapies (National Cancer Institute, 2018). We did not obtain any research correlating between the expression of ER, PR, HER and VEGF and the lymph node metastasis in black or Sudanese breast cancer patients, most of the previous studies were done in cancer patients of Asian origin [Su *et al.*, 2016; Li Hao *et al.*, 2007; Hussain Gadelkarim Ahmed *et al.*, 2011] (Su *et al*, 2016; Li Hao *et al*, 2007; Ahmed *et al*, 2011). The objective of this article was to investigate the correlation between the immunohistochemical staining of ER, PR, HER-2 and VEGF and the metastasis of breast cancer to the lymph nodes in Sudanese patients.

MATERIAL AND METHODS

Study design, population and ethical clearance

This retrospective study was done in Sudan. The one hundred and twenty one breast tissue samples were collected from the archive of the Radiation and Isotope Centre in Khartoum (RICK). This study was conducted after an ethical and academic license was obtained from the Sudan Academy of Sciences (SAS) and the authorities of RICK. Staining of the tissues with the antibodies of the studied parameters. The cancerous breast tissues were confirmed by the Hematoxylin and Eosin staining. The immunehistochemical staining of the estrogen, progesterone, HER-2 and VEGEF was carried out depending on the producing companies instruction sheets. The estrogen, progesterone receptors were investigated using DAKO kits (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). The HER-2 and VEGF were analyzed using THERMO kits (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).

Statistical analysis

The cross tab with correlation test of SPSS version 20 was used for the statistical analysis of the results.

RESULTS AND DISCUSSION

RESULTS

The study subjects were divided to three groups according to the age of their patients; 20- 44, 45- 64 and 65 or above. The number of tissue samples with metastasis to lymph nodes were 73 compared to 48 with free lymph nodes (Table 1). The ER staining results showed a percentage of 47.9% positive tissues from the cancerous tissues without lymph node involvement. The ER was positive in 45.2% of the cancerous breast tissues which metastasized to the lymph nodes (Table 2). However, the overall positive tissues for ER were (56\121, 46.3%).

The number of the positive tissues for the progesterone receptor was 39 with a percentage of 32.2%. The positive PR staining in the tissues with free lymph node and in the tissues with involved lymph nodes were 35.4% and 30.1% respectively (Table 3). The HER-2 positive tissues were 87 (71.9%), however, the HER-2 was the highest marker with positive cases in the lymph node free and involved cancerous breast tissues. The HER-2 positive numbers in the lymph node free and involved tissues were 62.5% and 78.1% respectively (Table 4). The staining results of the VEGF was comparable to the results of the PR since the positive tissues without lymph node involvement was 17 (35.4%) and the positive tissues with lymph node involvement was 25 (34.2%) (Table 5). The overall positive tissues for VEGF irrespective of the lymph node status were 42 (34.7%). Collective staining results of the four markers showed the presence of fourteen types of breast cancer as shown in Table 6. The type with the highest incidence was the HER+ VEGF- ER- PR- and it was the type with the highest lymph node metastasis (Table 6). The correlations showed that the only significant correlation was the correlation between the collective staining results and the lymph node involvement. However, all the correlation were negative except for the HER and the collective staining results (Table 7). The total number of the tissue samples was 121, 73 of them were with metastasis to lymph nodes and 48 were without metastasis to lymph nodes. The highest number of samples were within the age group 45- 64 while the highest lymph node involvement percentage was seen in the age group 20- 44.

Table 1. Description of the study subjects

Age group	Lymph node status		Total
	Free	Involved	
20- 44	15	30	45
45- 64	22	32	54
65≤	11	11	22
Total	48	73	121

Table 2. The results of the Estrogen Receptor (ER) staining

Lymph node status	ER	Age group			Total
		20- 44	45- 64	65≤	
Free	Negative	9	12	4	25
	Positive	6	10	7	23
	Total	15	22	11	48
Involved	Negative	18	18	4	40
	Positive	12	14	7	33
	Total	30	32	11	73
Total lymph nodes (free and involved)	Negative	27	30	8	65
	Positive	18	24	14	56
	Total	45	54	22	121

Table 3. The results of the Progesterone Receptor (PR) staining

Lymph node status	PR	Age group			Total
		20- 44	45- 64	65≤	
Free	Negative	11	14	6	31
	Positive	4	8	5	17
	Total	15	22	11	48
Involved	Negative	22	23	6	51
	Positive	8	9	5	22
	Total	30	32	11	73
Total lymph nodes (free and involved)	Negative	33	37	12	82
	Positive	12	17	10	39
	Total	45	54	22	121

The ER was positive in 23 (47.9%) out of the 48 free lymph node tissues and it was positive in 33 (45.2%) tissues out of the 73 tissues with lymph node involvement.

The age group ≥ 65 was the highest age group with positive ER tissues 14/22 (63.6%)

The results of the Vascular Endothelial Growth Factor (VEGF) staining

The VEGF was positive in 17 (35.4%) out of the 48 free lymph node tissues and it was positive in 25 (34.2%) tissues out of the 73 tissues with lymph node involvement. Irrespective of the lymph node involvement 42 (34.7%) tissues were positive for VEGF the PR was positive in 17 (35.4%) out of the 48 free lymph node tissues and it was positive in 22 (30.1%) tissues out of the 73 tissues with lymph node involvement.

Table 4. The results of the Human Epidermal growth factor Receptor-2 (HER-2) staining

Lymph node status	HER-2	Age group			Total
		20- 44	45- 64	65≤	
Free	Negative	5	6	7	18
	Positive	10	16	4	30
Total		15	22	11	48
Involved	Negative	7	5	4	16
	Positive	23	27	7	57
Total		30	32	11	73
Total lymph nodes (free and involved)	Negative	12	11	11	34
	Positive	33	43	11	87
Total		45	54	22	121

The HER-2 was positive in 30 (62.5%) out of the 48 free lymph node tissues and it was positive in 57 (78.1%) tissues out of the 73 tissues with lymph node involvement. The overall positive tissues for HER-2 was 87 (71.9%).

Table 5. The results of the Vascular Endothelial Growth Factor (VEGF) staining

Lymph node status	VEGF	Age group			Total
		20- 44	45- 64	65≤	
Free	Negative	8	16	7	31
	Positive	7	6	4	17
Total		15	22	11	48
Involved	Negative	19	21	8	48
	Positive	11	11	3	25
Total		30	32	11	73
Total lymph nodes (free and involved)	Negative	27	37	15	79
	Positive	18	17	7	42
Total		45	54	22	121

Table 6. Collective positive staining results

SN	Staining Results	Lymph node		Total
		Free	Involved	
1	All positive	4	7	11
2	All negative	9	4	13
3	VEGF+ HER+ ER+	1	2	3
4	VEGF+ ER+ PR+	1	1	2
5	HER+ ER+ PR+	9	8	17
6	VEGF+ HER+	6	10	16
7	VEGF+ ER+	2	0	2
8	HER+ ER+	3	10	13
9	HER+ PR+	1	0	1
10	ER+ PR+	2	5	7
11	VEGF+	3	5	8
12	HER+	6	20	26
13	ER+	1	0	1
14	PR+	0	1	1
	Total	48	73	121

The highest number of tissues were HER+, VEGF-, ER- and PR- (26, 21.5%). From the 26 HER+ samples 20 were with

lymph node metastasis. The second type was HER+ ER+ PR+ (17, 14.05%). In the third, fourth and fifth orders were VEGF+ HER+, HER+ ER+ and all negative respectively.

Table 7. Correlations and significance results

SN	Staining Results	Lymph node	
		Pearson correlation	Sig (2tailed)
1	ER	-0.02	0.77
2	PR	-0.05	0.55
3	HER	0.17	0.06
4	VEGF	-0.01	0.9
5	Collective	0.19	0.04

The correlations between the staining of the parameters and the lymph node involvement was weak. However, there was insignificant negative correlations between the lymph node involvement and the staining of ER, PR and VEGF. The HER was positively correlated to the lymph node involvement while the collective staining of the parameters was significantly positively correlated to the lymph node involvement.

DISCUSSION

Our findings can be summarized as follows;

- The percentage of the positive tissues for ER, PR, HER and VEGF were 46.3%, 32.2%, 71.9% and 34.7%.
- There was insignificant negative correlation between the expression of ER, PR and VEGF and the lymph node involvement.
- There was insignificant positive correlation between the staining of HER-2 and the involvement of lymph nodes.
- There was a significant positive correlation between the collective staining results of the studied parameters and the lymph node involvement.

The incidence of ER- positive in our study population was 46.3% compared to 47.4% in USA in the period between 1980-2008 (278759\588720). However, the incidence of ER-positive are variable depending on different factors (William F Anderson *et al*, 2011). Regarding the correlation between the ER staining and the lymph node involvement in Sudanese breast cancer patients, we have registered an insignificant negative or inverse relationship. Similar to our finding studies in Slovak republic and Netherland found that the ER was not a predictive factor for non sentinel lymph nodes (Dyttert *et al*, 2016; Ingridvan den Hoven *et al*, 2016). Another study stated that negative estrogen receptor was associated with metastasis to lymph nodes which means positive ER staining was not correlated to metastasis of breast cancer to lymph nodes (Sang Uk Woo *et al*, 2007). Unlike our findings, an Italian and Egyptian studies found a significant positive correlation between ER- positive and lymph node metastasis (D'Andrea *et al*, 2007; Elsayed M Ali *et al*, 2014). The incidence of PR-positive breast cancer in USA in the period between 1992-1998 increased from 65% to 67.7% (Christopher I. Li *et al*, 2003) compared to 32.2% in our study. We have found a negative correlation between PR expression and lymph node involvement. However, Elsayed M Ali and his colleagues found a significant positive correlation between PR expression and lymph node involvement (Elsayed M Ali *et al*, 2014). One research studied the association of luminal A, luminal B breast cancers and the lymph node involvement in Brazilian patients and it concluded that luminal A and luminal B breast cancers were associated with lymph node metastasis. Luminal A

involves ER+ and\or PR+ and HER- while luminal B includes ER+ and\or PR+ and HER+ (Helio Rubens de Oliveira Filho *et al*, 2015). HER+ constituted 79.1% of our study population, it a higher percentage compared to majority of the literature (American Cancer Society, 2017; M. Parton *et al*, 2004; Masataka Sawak *et al* 2006). Concerning the association of the lymph node involvement and the immunohistochemical expression of HER, we have registered an insignificant positive correlation. M.R. Araújo and his research team in 2016 stated that HER-2 was highly expressed in breast tumors metastasized to lymph node compared to primary breast cancers (Araújo *et al*., 2016) (Araújo *et al*, 2016). Similarly, a Chinese study concluded that both luminal A and B showed a significant probability to invade lymph nodes (Chengshuai Si *et al*, 2014). The VEGF was expressed in 34.7% of our study population, this percentage is low compared to the study done USA by Ying Liu and his colleagues who registered a percentage of 72.5% out of 1788 invasive breast cancers (Ying Liu *et al*, 2011). With regard to the correlation between the lymph node involvement and the expression of VEGF, we have found an insignificant negative correlation. However, Fei Su concluded that highly expressed VEGF was associated with lymph node metastasis in Asian breast cancer patients (Su *et al*, 2016). Unlike the finding of Fei Su, Monireh Halimi and her research group found that there was no significant correlation between VEGF expression and axillary lymph node metastasis in invasive ductal carcinoma patients (Monireh Halimi *et al*, 2012).

Conclusion

The ER, PR and VEGF expression in Sudanese breast cancer patients were in significantly and negatively correlated to lymph node metastasis while the HER-2 expression was insignificantly (p= 0.06) positively correlated. The collective staining results were predictive for lymph node metastasis since there was significant (p= 0.04) correlation.

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