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IMMUNOHISTOCHEMICAL EXPRESSION OF STATHMIN 1 IN PATIENTS WITH INVASIVE BREAST CARCINOMA

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ABSTRACT

Background: Breast cancer is the most frequently diagnosed cancer and it is the leading cause of cancer death among females worldwide, with an estimated 2,261,419 new cases and 684,996deaths in 2020. Several recent studies have demonstrated that Stathmin1 expression may be closely associated with prognosis in patients with breast cancer. Stathmin1 (STMN1), also known as oncoprotein 18, is a cytosolic phosphoprotein and a key regulator of cell division due to its microtubule depolymerization in a phosphorylation-dependent manner. **Aim of the study:** evaluate the immunohistochemical expression of stathmin1 in patients with invasive breast carcinoma and its association with several prognostic factors. **Patients and Methods:** In this study, we used formalin-fixed paraffin-embedded tissue blocks from 50 patients of invasive breast carcinoma including different grades and types. Histological sections were taken for hematoxyline and eosin staining to assess histopathological features. Monoclonal antibody for STMN1 was used for immunohistochemical staining of tissue sections and STMN1 expression was semi quantitatively scored. The Association between clinicopathological parameters and stathmin1 expression was done. **Results:** Fifty cases of invasive breast carcinoma were studied, 32 cases (64%) show high expression and 18 cases (36%) show low expression. Significant association with tumor grade, ER hormonal negativity, lympho-vascular invasion and triple negative breast carcinoma (TNBC), and no significant association was found with tumor histological type, IHC based subtype, PR hormonal status and HER-2 status. **Conclusions:** STMN1 according to this study prove to be a powerful marker in predicting the prognosis of breast carcinoma. With further work up and more extensive studies it may be of routine use in the near future.

KEYWORDS: Stathmin1, Breast carcinoma, Immunohistochemical expression, hormonal receptors.

INTRODUCTION

Breast carcinoma is the most common malignant tumor and the second most common cause of carcinoma death in women, with more than 2.3 million cases occurring worldwide annually.[1] About 55% of the global burden is currently experienced in developed countries, but incidence rates are rapidly rising in developing countries.[2] The large majority of breast cancers are detected during the postmenopausal years. However, breast cancer can develop at any age, from childhood to old age.[3]

As a heterogeneous disease, comprehensive gene expression profiling has distinguished four major molecular subtypes of breast cancer with different clinical outcomes: luminal A, luminal B, HER2/neu and triple negative.[4] The luminal A and B subtypes are collectively referred to as the luminal type, which accounts for 65–70% of breast cancers. Compared with other breast cancers, patients with luminal subtypes benefit from endocrine therapies and have a better prognosis. However, long-term recurrence remains a major clinical problem.[5]

Traditional prognostic factors in patients with invasive breast cancer include lymph node status (the single most important prognostic factor), tumor size, histologic type, and histologic grade. Lymphatic vessel invasion is also considered by many to be an important prognostic factor. Numerous other biologic markers have been proposed as aids to assess the prognosis in mammary carcinoma. These include the following: markers of proliferation (e.g., S-phase fraction, thymidine labeling index, and

immunostaining with antibodies to Ki-67); oncoproteins (HER2/neu or c-*erb*-B2); tumor suppressor genes (e.g., p53); angiogenesis, as indicated by stromal micro vessel density; and bone marrow micro metastases.[6]

Recently, numerous studies have demonstrated that Stathmin1 is overexpressed in a variety of cancers, including urinary bladder, oral, ovarian, lung, nasopharyngeal, liver, esophageal, colorectal, and breast cancer; it has a positive correlation with nodal involvement, distant metastasis, clinical stage, and a poor prognosis.[7,8]

Stathmin is a cytosolic phosphoprotein proposed to act as a relay integrating diverse cell signaling pathways, notably during the control of cell growth and differentiation. Its name derived from the term ‘stathmos', the Greek word for ‘relay'. It is also called by different names (e.g., p17, p18, p19, 19 K, metablastin, oncoprotein 18, LAP18, and stathmin1, Op18/stathmin). Stathmin represents a critical intermediate during signal transduction in modulation and control of microtubule polymerization, and may also be considered as one of the key regulators of cell division for its ability to destabilize microtubules in a phosphorylation-dependent manner.[9]

In this study we aimed to evaluate the immunohistochemical expression of stathmin 1 in different types of invasive breast carcinoma & its association with several prognostic factors like hormonal receptors, tumor subtype and grade.

Method: This retrospective cross-sectional study was carried out in Babylon training center for Pathology during the period from April 2019 to December 2020.

The study group composed of formalin-fixed paraffin-embedded tissue blocks from randomly selected 50 cases of breast carcinoma. Re-evaluation of all the slides was done by two expert pathologists to confirm the diagnosis.

Biopsy type was tru-cut biopsy n=13, excisional biopsy n=25 and mastectomy specimen n=12.

These cases, their associated clinical data and their results for ER, PR, and HER-2 status were collected from laboratory of histopathology in Al-Hilla Teaching Hospital in Babylon and from some private laboratories in this governorate.

From each tissue block, 2 sections of 5 micrometer thickness were obtained for hematoxylin \ eosin (H & E) staining method ,and **Primary antibody (Stathmin1)**: Stathmin1 Protein concentrated, 0.1 ml, dilution 1: 50 - 1 :200, Bio SB, Clone EP247.

The criterion for positive immunohistochemical reaction is dark brown precipitate in the cytoplasm for Stathmin-1. The score was assessed by calculation of modified Allred score[10], which is a semiquantitative system for each slide according to the following: the proportion of the stained tissue (0, none; 1, 0-1%; 2, 1-10%; 3, 10-33%; 4, 33-66%; and 5, 66-100%) and staining intensity (0, none; 1, weak; 2, intermediate; and 3, strong) both incorporated to produce total scores of 0 or 2 through 8. A score of 0-3 was defined as low STMN1 expression and a score of 4-8 was defined as high STMN1 expression.

RESULTS

Fifty cases of invasive breast carcinoma were studied. Patients age range (29-85) years with mean age 54.42 years, all patients were females. According to the Nottingham modification of the Bloom–Richardson system of breast carcinoma , grade 1 breast carcinoma was reported in 1 (2%) case , grade 2 in 33 (66%) cases and grade 3 in 16 (32%) cases .The types of breast carcinoma included in this study were invasive ductal carcinoma no special type which comprise 39 (78%) of cases ,invasive lobular carcinoma in 5 (10%) of cases and other types which include (invasive ductal carcinoma with medullary feature 2cases ,invasive ductal carcinoma with invasive lobular carcinoma 2 cases ,invasive papillary carcinoma 1case and solid papillary carcinoma 1case) , all comprise 6 (12%) of cases. According to IHC based subtype, (38) cases where in the luminal A&B group, (7) cases in HER-2 enriched group and (5) cases in the TNBC group. Hormonal profile and HER-2 status were as following: 38 (76%) of cases were ER +ve, 21 (42%) PR +ve and 16 (32 %) HER-2 +ve. Different types of biopsies were obtained which include: tru-cut, excisional biopsy and mastectomy specimens. (table1)

In this study 32 (64%) of cases express STMN-1 highly and 18 (36%) of cases show low STMN-1 expression.

Table 1: Distribution of breast carcinoma cases according to different clinicopathologic parameters (age, gender, type, tumor grade, type of biopsy hormonal profile and HER-2 status).

Clinicopathological parameter		Breast carcinoma cases	
		Number	Percentage
Age	21-40	6	12%
	41-60	34	68%
	61-80	9	18%
	81-100	1	2%
Gender	Female	48	96%
	Male	2	4%
Type	IDC NST	39	78%
	ILC	5	10%
	Others	6	12%
Grade	G1	1	2%
	G2	33	66%
	G3	16	32%
Type of biopsy	Tru-cut biopsy	13	26%
	Excisional biopsy	25	50%
	Mastectomy	12	24%
Hormonal profile	ER +VE	38	76%
	ER –VE	12	24%
	PR +VE	21	42%
	PR –VE	29	58%
IHC based subtype	Luminal A&B	38	76%
	HER-2 enriched	7	14%
	TNBC	5	10%
HER-2 status	HER-2 +VE	16	32%
	HER-2 –VE	34	68%

Table 2: Association of STMN-1 expression and breast carcinoma pathological parameters.

pathological parameter		Number, percentage (%)		Total	
		High expression	Low expression		
Histological type	IDC NST	27 (69.23)	12 (30.77)	39 (78)	0.32
	ILC	2 (40)	3 (60)	5 (10)	
	Others	3 (50)	3 (50)	6 (12)	
Tumor grade	G2	18 (54.54)	15 (45.45)	33 (67.4)	(0.02)
	G3	14 (87.5)	2 (12.5)	16 (32.6)	
Hormone profile	ER+VE	21 (55.26)	17(44.74)	38(76)	(0.02)
	ER-VE	11 (91.6)	1(8.4)	12(24)	
	PR+VE	12(57.14)	9(42.86)	21(42)	(0.38)
	PR-VE	20(68.96)	9(31.04)	29(58)	
HER-2 Status	HER-2 +VE	13(81.25)	3(18.75)	16(32)	(0.08)
	HER-2 –VE	19(55.88)	15(44.12)	34(68)	
IHC based subtype	Luminal A&B	21(55.26)	17(44.74)	38(76)	(0.05)
	HER-2 enriched	7(100)	0(0)	7(14)	
	TNBC	4(80)	1(20)	5(10)	
LVI* Status	LVI	20(74)	7(26)	27(73)	(0.02)
	No LVI	2(20)	8(80)	10(27)	

***LVI: lympho- vascular invasion**

No significant association between STMN-1 expression and histological type, PR status, HER-2 status and IHC based subtype of breast cancer was found.

There is significant association between STMN-1 expression and grade, ER profile and lympho-vascular invasion of breast carcinoma.

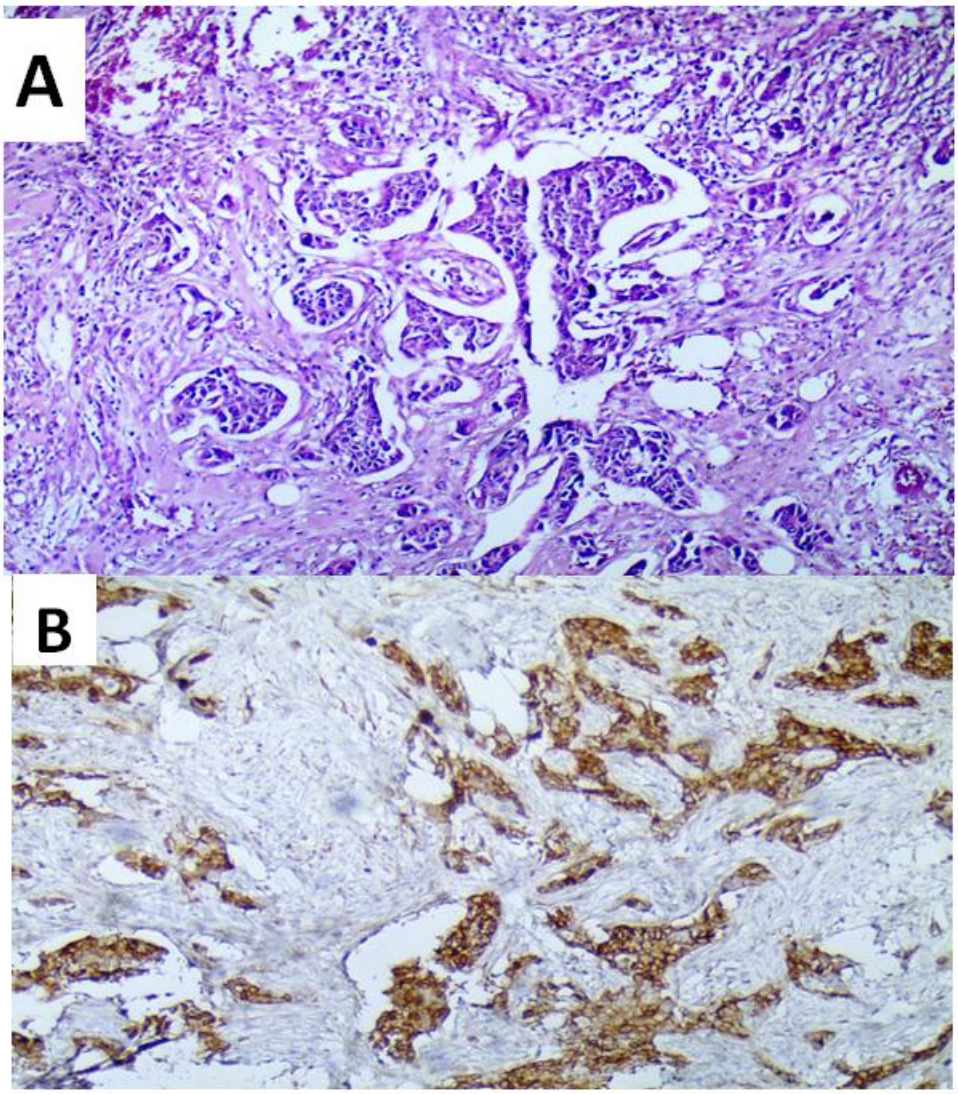

Figure 1: a. invasive ductal carcinoma Grade III(H&E, 200X), b. this tumor revealed high STMN1 expression (score 7) (STMN1, 200X).

DISCUSSION

The complicating issue of breast carcinoma lies in the disease heterogeneity associated with diverse morphologies, molecular characteristics, clinical behavior, and response to therapeutics are well documented. Reaching a prognosis in breast cancer has depend on numerous clinico-pathological parameters such as age, tumor size, grade, and individual molecular markers such as hormone receptor, human epidermal growth factor status (HER2) and Ki67.[11]

It is certain that axillary lymph nodes metastasis is the single most important prognostic factor to predict the overall survival and guide the management options in breast cancer cases. However, this factor alone or in combination with previously mentioned clinico-pathological parameters failed to predict the precise prognosis in number of cases.[12]

In an attempt to achieve the perfection, many new factors which are either biological or molecular in nature have been studied. With the aim that these factors can not only predict the prognosis but are also able to identify 'high risk' group of patients. One of the most important among newly studied markers was Stathmin1.

Stathmin1 is a cytosolic phosphoprotein with a dynamic relation with microtubules necessary for mitosis in cell cycle. It is the signature marker for PI3K pathway activated in neoplasia of solid tumors including breast carcinoma.

Stathmin, as a microtubule destabilizing cytosolic phosphoprotein which has profound influence on cell proliferation, differentiation and cellular motility is an accurate signature IHC marker of PI3K pathway.[13]

This study presents a review of 50 cases of invasive breast carcinoma classified according to WHO classification 2019 with the aim to investigate the

frequencies of various clinico-pathological and histopathological features and their association to STMN-1 expression in predicting the prognosis of invasive breast cancer.

In reviewing previous studies **Xia-Ying Kuang et al.**[14] **Cecilie Askeland et al,**[15] used a staining index (SI) was calculated according to the intensity and percentage of positive cells. A semiquantitative grading system incorporating staining intensity (0, no staining; 1, weak; 2, moderate; 3, strong) and the percentage of cells stained (0, no staining; 1, < 10%; 2, 10–50%; and 3, > 50% of tumor cells) was applied. The SI was calculated by multiplying the results of these two variables and ranged from 0 to 9. A cutoff representing the upper quartile (SI > 4) was used to define high levels of staining, whereas others were defined as low levels of staining.

Depending on our search results, we found no association between STMN-1 expression and histopathological types of breast carcinoma (P-value = 0.32), the reason for that may be that the sample size (*n* = 50) was too small to attempt reasonable statistical association. Despite that and in keeping with our study, **Rastko Golouh et al,**[16] study had also notice the same result with (P-value = 0.34).

In agreement with the finding in patient material in current study **Cecilie Askeland et al**[15], **Rastko Golouh et al**[16] and **Xia-Ying Kuang et al,**[14] found that tumor nuclear grade was significantly higher in the STMN1overexpression group with (P-value =0.002), (P-value =0.01) and (P-value=0.02) respectively, that were near our results (P-value=0.02) , and in the study by **G Brattsand**[17], **PA Curmi et al,**[9] at mRNA level of STMN1 using western blot and RT PCR technique respectively, overexpression was also associated with higher tumor grades , giving further enforcement to our search results.

Furthermore, several other factors are also known to associate with ER loss, some of which are: proliferation[18], overexpression of cycline E.[19] and mutations in the *p53* gene.[20] Parenthetically overexpression of stathmin1 thus amazingly points out to highly proliferative primary breast carcinomas showing signs of aggressiveness.

To evaluate the expression level of STMN1 with hormonal profile of breast carcinoma patient's information was gathered from patients' reports and were studied in association with data obtained from our study, we have noticed that loss of estrogen receptors was found in high STMN1 expression group of patients with (P-value=0.02), like most reviewed studies of **Sayaka Obayashi et al,**[10] **Cecilie Askeland et al,**[15] and **S C Drury et al.**[21] And at molecular level in the study of **PA Curmi et al,**[9] while **Xia-Ying Kuang et al,**[14] study show no significant association. The difference may be excused due to different primary antibody source, larger study group, and the nature of tissue blocks.

It is believed that PR expression provides good prediction with regard to which patient is more likely to respond to endocrine therapy.[6]

Analysis of the patients' data for association between STMN1 overexpression to PR hormonal status show no significant association (P-value=0.38), agreeing with **Rastko Golouh et al**[16], **Xia-Ying Kuang et al**[14] and in contrast to the results of **Cecilie Askeland et al**[15]**, Koyel Das et al**[13]**, Sayaka Obayashi et al**[10]. This difference may be attributed to the type of the scoring system used and size of the sample.

HER2 is a biomarker that is not only a marker of poor prognosis in breast carcinoma but also a predictive marker of treatment response.[22]

Koyel Das et al,[13] **Cecilie Askeland et al,**[15] and **Sayaka Obayashi et al,**[10] even with studying relatively larger groups obtain similar finding to our result in seeking STMN-1 expression in relation to HER-2 status which revealed that there is no significant association were found between STMN1 expression and HER-2 status of breast carcinoma , despite the fact that HER-2 expression being ominous prognostic factor in breast carcinoma patients and associated with a higher mortality and a relapse rate without targeted treatment.[23]

On the other hand, only **Xia-Ying Kuang et al,**[14] study had found opposite results, probably due to the larger number of patients included in their study, depending only on mastectomies as type of biopsy which will provide more amount of tissue for proper sampling and better assessment of HER-2 status of these tumors.

At the ninth **St Gallen,**[24] meeting in 2005, LVI was added to the prognostics for node-negative patients. Compared to patients with no LVI, a 60% higher mortality rate was observed for node-negative breast carcinoma patients having positive LVI,[25]

Sayaka Obayashi et al,[10] was the only study that investigate the relation between lympho-vascular invasion and STMN-1 expression in breast cancer with group study of 237 and had found no significant association between them in contrast to our finding in which we had found significant association with high STMN-1 expression and lympho-vascular invasion, p-value= (0.002).This could be explained by the fact that all cases of invasive breast carcinoma with lympho-vascular invasion were of high grade. This finding is of significant prognostic relationship between high stathmin-1 expression and worse prognosis in patients with breast cancer.

According to IHC-based subtypes, the STMN-1 expression level was significantly higher in the HER-2

enriched and TNBC with (100%), (80%) of cases, respectively. This indicates an association between IHC-based subtype and STMN-1 expression, a result supported by **Sayaka Obayashi et al.**[10]

Since that breast carcinoma with triple negative phenotype has no endocrine therapy options and do not benefit from HER2 inhibitors, so molecular-targeted therapies against TNBCs are crucially needed.

Saal LH et al,[26] have found that TNBCs have loss of PTEN more frequently, and the PI3K pathway is strongly activated in these tumors. PTEN loss significantly associated with STMN1 expression in the **Sayaka Obayashi et al,**[10] study and STMN1 expression becomes a good marker of the PI3K pathway activation.[27]

In this study, STMN1 expression was significantly higher in the TNBCs, there by measurement of STMN1 expression may be a clinically beneficial test for the stratification of patients for anti-PI3K pathway therapy and for monitoring therapeutic efficacy.

CONCLUSIONS

Our results provide substantial evidence that STMN-1 expression defined by IHC on paraffin sections of invasive breast carcinoma point to strong associations between high expression and several poor prognostic factors in breast cancer (higher tumoral grade, negativity of ER hormone receptor, lympho-vascular invasion and TNBC) than other clinico-pathological and histopathological features. Together these results favor a role for STMN1 in predicting aggressiveness and disease progression and that this phosphoprotein could be in the near future a powerful prognostic factor in the field of breast carcinoma prognostic and predictive markers.

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