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ORIGINAL ARTICLE

Nestin as a Prognostic Marker in Ductal Carcinoma of The Breast

Mona George Shafeek, Nehal Selim El Sayed, Abeer Mohamed Abd El Bary, Mennat Allah Hesham Mohamed Fikry*

Pathology Department, Faculty of Medicine, Zagazig University, Egypt.

***Corresponding author:**
Mennat Allah Hesham Mohamed Fikry
Pathology Department,
Faculty of Medicine, Zagazig University, *Egypt*.
E-mail:
Fikrymenna45@gmail.com

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ABSTRACT

Background: Breast cancer is a heterogeneous disease that not only varies greatly between different patients but also even within each individual tumor. Nestin, a type VI intermediate filament expressed mainly in neural progenitor cells. In addition to being expressed in myoepithelial cells of normal mammary glands, Nestin has higher expression rates in triple negative breast cancers than other cancers .

We aimed to clarify its pathological role in breast carcinoma and implications of this role.

Methods: a cross-sectional study includes a comprehensive sample of 40 paraffin embedded tissue blocks from patients with breast cancer lesions. Samples are stained by routine (Hematoxyline and Eosin stain) then stained by a mouse anti-human polyclonal Nestin antibody.

Results: Immuohistochemical examination showed that Nestin was located in cytoplasm of tumor cells. Positive Nestin expression was found in 35.5 % of studied cases and distributed as 30.8% in ductal carcinoma in situ cases and 69.2% of infiltrating duct carcinoma .Statistically significant relation (P=0.008) was found between Nestin expression and tumor grades with highest expression was observed in GIII . Comparing Triple negative breast cancer and non TNBC cases as regarding Nestin expression there was a significant relation (P=0.04)

Conclusion: Nestin may have a role in breast cancer development as it was expressed in DCIS as well as IDC. Nestin expression is related to tumor grading suggesting that Nestin may be involved in breast carcinoma progression. Relation between Nestin expression and triple negative status indicating that Nestin is expressed in breast cancers with highly aggressive potency.

Keywords: Nestin, DCIS; ductal carcinoma in situ, IDC; invasive ductal carcinoma, TNBC; triple negative breast cancer .

INTRODUCTION

It is expected that in 2020, breast cancer will be detected in more than 1.97 million women worldwide, and 622,000 women will die of this illness [1]. Invasive breast cancer influences 1 of every 8 ladies in the United States (12.4%) during their lifetime [2]. Discrete breast cancer molecular subtypes that vary in gene expression forms affecting the prognosis as well as the therapeutic aims present in the cancer cells [3]. Unconventional gene expression profiling methods clarify that the list of intrinsic genes that distinguish these subtypes is now made up of several clusters of genes relating to estrogen receptor (ER) expression (the luminal cluster), human epidermal growth factor 2 (HER2) expression, proliferation, and a exclusive cluster of genes called the basal cluster [4]. Basal –like (Triple negative) the negativity for hormone receptors (estrogen receptor and progesterone receptor) and HER2 status [5]. TNBC accounts for 10% to 20% of all breast cancers [6].TNBC is described as larger tumors, poor differentiation, elevated mitotic index and tumor necrosis. Frequently metastases in visceral organs, lungs and CNS are noticed. This form has the poorest prognosis, and very often the disease relapses in the first three years, and p53 mutations are very regularly detected [7].

Nestin is individual of the class VI family of intermediate filaments (IFs) proteins, originally known as an indicator of neural stem cells and later confirmed to be expressed in BC and other cancer types. Nestin is a huge protein (> 1600 amino acids) containing a short N end and an unusually long C end which cooperates with other IFs including vimentin, desmin, or internexin, later shaping heterodimers and mixed polymers [8]. Assembly and disassembly of IFs are basic and dynamic process that impact and react to intra-cellular

signaling cascades controlling variation of vital processes, containing proliferation, migration, and survival [9]. In normal breast tissue, Nestin is expressed in the basal/myoepithelial cells of the mammary gland. [10]. Nestin has not been very much described in breast cancer but there is proof propose that this protein may assume a role in the regulation of mitosis. Nestin was found profoundly in basal breast cancer subtype (ERα−/PR−/Her2−) yet not in the Her2 subtype (ERα−/PR−/Her2+) or luminal epithelial phenotype (ERα+/PR+) [11].

Further studies are needed to evaluate clinicopathological correlation of Nestin with this rare and highly malignant subtype. To the best of our knowledge, this study is the first to be done in Zagazig university hospitals.

METHODS

Patients and clinical data

Cross-sectional study carried out on 40 cases of breast carcinoma achieved from the Archives of the Pathology Lab. of Zagazig University, In the period from January 2017 to January 2019. comprehensive sample diagnosed histopathologically as 8 cases ductal carcinoma in situ 32 cases invasive breast carcinoma (5 cases grade I, 13 cases grade II 14 cases grade III).

All cases were obtained by radical mastectomy and excisional biopsy. All tissue samples were formalin- fixed and paraffin-embedded. We collected the clinical, pathological, and immunohistochemical (estrogen receptor [ER], progesterone receptor [PR] and human epidermal growth factor 2 [Her2] information from the medical records of the patients. Histological typing and grading have followed the World Health Organization classification and modified Bloom-Richardson grading. [12]. The study was carried out with full local ethical measurements. All collected blocks were cut at 4 microns and stained with hematoxylin and eosin (H&E) stain to verify the diagnosis.

Written informed consent was obtained from all participants .

The study protocol was approved by the Ethics Committee of Faculty of Medicine, Zagazig University and the study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Immunohistochemical staining:

After cutting our blocks into 4 µm, the sections were deparaffinised with xylene, rehydrated in different alcohol grades, and located in 0.5% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase action. Antigen retrieval was done by incubation in 0.01 M citrate buffer (pH 6.0) for 5 min in a pressure cooker. The sections were exposed to the primary antibody for 60 min at room temperature. We used Streptavidin-biotin- complex using anti nestin antibody , for Nestin (mouse polyclonal anti nestin (1:50) - clone 10c2) (Code No sc-23927, Multilink. Detection kit, HRP, Santa Cruz biotechnology, INC) was used in this study diaminobenzidine (DAB) was used as the chromogen. Sections of myoepithelial and basal cells of breast are used as positive control. Negative control was used buffer instead of primary antibodies.

Evaluation of immunohistochemical staining

Regarding Nestin expression in tumor cells was classified semi-quantitatively according to the following criteria: 0 if <1% of neoplastic cells discretely expressed Nestin in their cytoplasm; 1+ if ≥1 and <10% of morphologically unequivocal neoplastic cells discretely expressed Nestin in their cytoplasm; and 2+ if ≥10% of morphologically unequivocal neoplastic cells discretely expressed Nestin in their cytoplasm. Samples scored as 1+ or 2+ were considered positive [13].

STATISTICAL ANALYSIS

The results from the analysis of the continuous variable are expressed as a mean ± standard deviation (SD). Analysis of categorical data was performed using the x^2 or Fisher's exact test, All statistical analyses were done using SPSS Statistical Package for the Social Sciences software (version 20; SPSS, Chicago, IL). The $P \leq 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Clinico-pathological results are shown in table 1

This study was conducted on 40 patients with breast lesions, their mean age of 45.23±13.08 years , 35% of age group ≤50 and 65% of age group >50 years, 32.5% are premenopausal females, 62.5% post-menopausal and 5% with no menopausal data.

Regarding tumor grading of the cases,20 % of cases are DCIS , 12.5% of cases are of grade I, 32.5% are of grade II and 35% grade III . The cases have lymph nodes involvement is positive in 40% (24 cases) and negative in 60% (16 cases).

-Relation between Nestin expression and clinico pathological parameters of all cases are shown in table 2

In the present study, Nestin immue-histochemical expression in the tumor cells of all studied cases showing (27 cases) 32.5% are Nestin+ve and 67.5% (13 cases) are Nestin-ve. Classified according to grading system of breast cancer as following ductal carcinoma in situ 8 cases ; 4 cases are Nestin positive and 4 cases are Nestin negative . Grade I 5 cases; all are Nestin negative . All grade II 12 cases are Nestin negative and one is Nestin positive. Grade III 14 cases ; 6 cases are Nestin negative and 8 cases are Nestin

positive . There is significant relation between Nestin expression and Tumor grade (P = 0.008). As regard patient's age and menopausal status, no significant relation with Nestin expression was found (P= 0.750 and 0.697 respectively)

Relation between Nestin expression and clinicopathological parameters of infiltrating duct carcinoma cases (32 cases) are shown in table 3

Regarding immunophenotypic profile of studied cases, 65.7% were positive for estrogen receptors, 59.4% were positive for progesterone receptors and 46.9% positive for HER2. Only 4 cases (12.5%) were triple negative**.** Among the studied IDC cases, there was a highly significant inverse relation between Nestin expression and both ER and PR status (P <0.001), but no significant relation between Nestin expression and HER2 status was found (P=0.1).Comparing TNBC (4 cases) and non-TNBC (28cases) as regard Nestin expression, there was a significant relation (P=0.04). Nestin expression was found in 33.3% of cases with positive lymph nodes with no significant relationship (P=0.99).

Table 1: Clinico-pathological results of studied cases

Variable		
Age (Years): (40 cases)		
Mean ± SD	45.23±13.08	Range 18-65
	No.	**%**
age groups:		
≤50	14	35.0
>50	26	65.0
Menopausal distribution (40 cases)		
Pre-	13	32.5
post	25	62.5
No data	2	5.0
Tumor grade : (40 cases)		
DCIS	8	20
I	5	12.5
II	13	32.5
III	14	35
Lymph nodes : (32 cases)		
Negative	20	60
Positive	12	40

Table 2 : Relation between Nestin expression and clinicopathological parameters of all cases

Variable	**Nestin-ve N=27**		**Nestin+ve N=13**		χ^2	**P value**
	No.	**%**	**No.**	**%**		
Age group:						
≤50	**9**	**33.3**	**5**	**38.5**	**0.1**	**0.750**
>50	**18**	**66.7**	**8**	**61.5**		
Menopausal distribution:						
Pre-	**8**	**29.6**	**5**	**38.5**		
post	**18**	**66.7**	**7**	**53.8**	**0.72**	**0.697**
no data	**1**	**3.6**	**1**	**7.7**		
Tumor grade:						
DCIS	**4**	**14.8**	**4**	**30.7**	**11.61**	**0.008**
I	**5**	**17.4**	**0**	**0.0**		**(S)**
II	**12**	**56.5**	**1**	**11.1**		
III	**6**	**26.1**	**8**	**88.9**		

Table 3: Relation between Nestin expression and clinic pathological parameters of infiltrating duct carcinoma cases (32 cases)

Variable	Nestin-ve N=23		Nestin+ve N=9		χ^2	P value
	No.	%	No.	%		
ER:						
negative	2	8.7	9	100.0	fisher test	<0.001
positive	21	91.3	0	0.0		HS
PR:						
negative	4	17.4	9	100.0	fisher test	<0.001
positive	19	82.6	0	0.0		HS
HER 2:						
negative	12	51.8	5	53.8	fisher test	1.0
positive	11	48.2	4	46.2		
Triple negative status :						
Yes	1	4.3	3	33.3	fisher test	0.04
No	22	95.7	6	66.7		(S)
Lymph node :						
Negative	14	60.9	6	66.7		0.99
Positive	9	39.1	3	33.3	0.018	

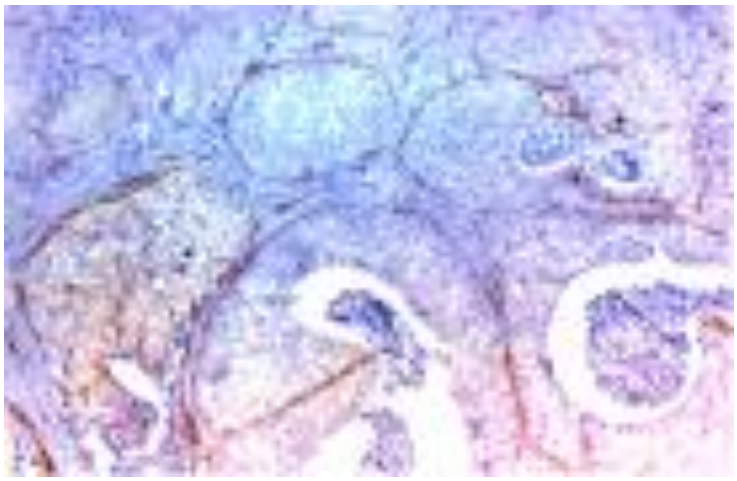

Figure 1. Ductal carcinoma in situ, comedo type showing Nestin expression in myoepithelial cells with focal expression in malignant cells (X100)

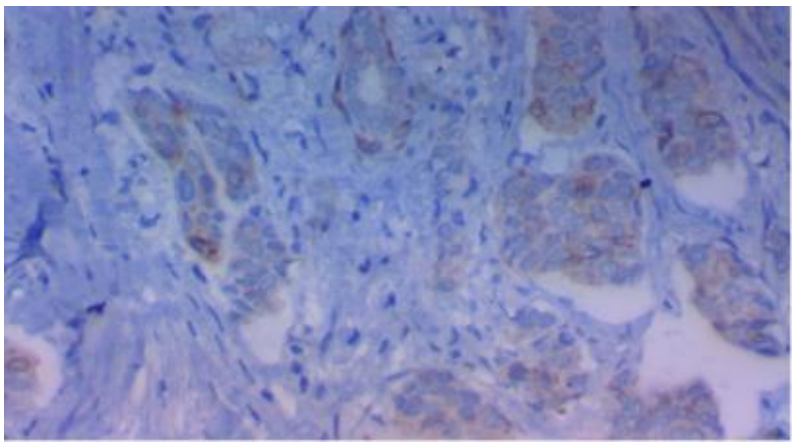

Figure 2. Invasive ductal carcinoma grade II showing Nestin expression in malignant cells (X400

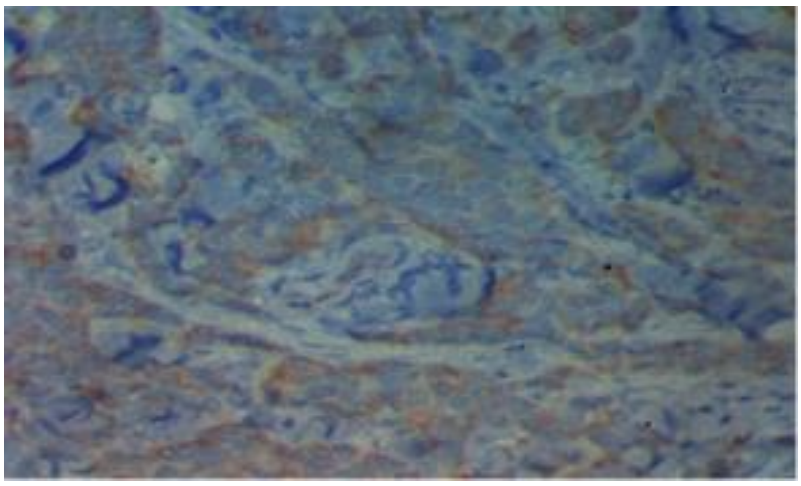

Figure 3. Invasive ductal carcinoma grade III showing Nestin expression in malignant cells (X400)

Figure4. A case of triple negative breast cancer grade III showing Nestin expression in malignant cells (X400)

DISCUSSION

Breast cancer is the most common form of cancer and one of the most important causes of death among women worldwide with a higher mortality rate in less developed countries [14].

The most important clinicopathologic parameters affecting the biological behavior and treatment of breast cancer include patient's age, tumor grade, lymph node involvement, estrogen-progesterone receptor status and human epidermal growth factor (HER2) overexpression. Prognostic factors can be useful to identify poor clinical outcomes and select patients who will receive adjuvant therapy [8].

This study included 40 cases of breast carcinoma distributed as 8 cases (20%) of ductal carcinoma in situ (DCIS) and 32 cases (80%) of invasive ductal carcinoma (IDC). Nestin expression in tumor cells was detected in 32.5% (13/40) of cases. This result was parallel to that obtained by Gao et al., who observed Nestin expression in 37.6% (41/109) of their cases [15]. In the present study, Nestin expression was distributed as 30.8% of DCIS and 69.2% of IDC cases .This result was consistent with the results of Liu et al., who studied 150 cases of breast cancers and found lower expression rate of Nestin in DCIS than IDC cases [13].

In the current study, Nestin expression was detected in myoepithelial cells at the periphery of normal breast ducts and DCIS. This pattern was also observed by Li et al., who concluded that Nestin is expressed in the basal/myoepithelial layer of the mammary gland and is a selective marker of basal epithelial breast tumors [11].

The mean age of cases of the present study was 50.23 ±13.08 years. 65 % of cases were >50 years indicating that breast cancer is a disease of any age but show specific increasing incidence with age. Premenopausal females were 32.5%, while 62.5% were post-menopausal and 5% with no menopausal data. No significant relation was found between Nestin expression and both age (P= 0.750) and menopausal state (P = 0.697). Although the cases in the present study were randomly collected, these findings were in agreement with the study performed by Gao et al. They reported no significant difference between the age of >40 and ≤40 years groups as regards Nestin expression (39.8% vs 18.2%; P=0.161) [15].

As regarding immunophenotypic features of the studied IDC cases, 65.7% of cases were positive for estrogen receptors (ER), 59.4% were positive for progesterone receptors (PR) and 46.9% were positive for HER2. Triple negative (TNBC) cases were represented by 12.5%. These percentages coincided with those obtained by Nowak et al., who studied 124 IDC specimens. They found 72.6% of cases positive for ER, 63.7 % positive for PR, 44.4% positive for HER2 and 15.3% of cases were triple negative [16].

In the present study, Nestin expression was detected in all (100%) ER and PR negative tumors and absent in ER and PR positive cases with a highly significant relation (P < 0.001). On the other hand, no significant relationship between Nestin expression and HER2 status was found (P =0.1). This was in agreement with the study of Asleh et al., who found significant relation (P=0.001) between Nestin expression and ER status and insignificant relation with HER2 status (P=0.6) [17] .Also, Gao et al., reported that expression rate of Nestin in ER- and PR- negative tumors was significantly higher than ER- and PR-positive cases (P= 0.011 and P = 0.036, respectively) in contrary to HER2 expression (P= 0.120) [15] . Moreover, Li et al., failed to detect Nestin expression in all tumors (0/16) with HER2 subtype and (0/16) tumors with a luminal epithelial phenotype (ER+/PR+) [11]

Findings of the present study showed a significant difference between triple negative and non-triple negative cases as regards Nestin expression (P = 0.04).This result coincided with Nowak et al., who found a significantly higher Nestin expression in triple negative cases than other cancers (P<0.001) [16]. Similar finding (P=0.002) was reported by Piras et al. They concluded that Nestin expression may characterize tumors with an aggressive clinical behavior, suggesting that the presence of Nestin in tumor cells can be considered as an important factor lead to a poor prognosis [18].

Regarding histological grade, there was a statistically significant relation with Nestin expression (P=0.008) as G III cases showed higher expression (88.9%) than GI and GII (11.1% and 0%, respectively). This finding was in agreement with the results of Liu et al., who found Nestin expression in 21/36 (58.3%) of GIII cases with a statistically significant value (P= 0.001) [13].Also**,** a significantly higher expression of Nestin in low differentiated tumors (P=0.021) was observed by Tampaki et al., who studied 141 cases of cancer breast [19].

Considering lymph node involvement in the current study, 12/32 IDC cases (37.5%) showed lymph node metastasis. Nestin expression was found in 33.3% of cases with positive lymph node compared to 66.7% without nodal metastasis with non-significant relationship (P = 0.99). Similarly, Gao et al., reported that the rate of Nestin expression between those with and without lymph node metastasis was not significantly different (P=0.769) [15]. In the contrary to these results, Asleh et al., studied 3641 cases and illustrated Nestin expression in 63% of cases with no lymph node involvement versus 33 % with positive lymph nodes with a significant relationship (P= 0.005) .This discrepancy in results can be attributed to a larger number of cases in their study [17].

CONCLUSION

Nestin may have a role in breast cancer development as it was expressed in DCIS as well as IDC. Significant relation between Nestin expression and tumor grading suggesting that Nestin may be involved in breast carcinoma progression. Significant relation between Nestin expression and triple negative status indicating that Nestin is expressed in breast cancers with highly aggressive potency . It established that Nestin might be novel potential marker for breast cancer but larger studies are needed to inspect the prognostic effect of Nestin expression in breast cancers.

RECOMMENDATIONS

An extended study with a large patient population is needed to clarify the efficacy of Nestin as a prognostic marker.

conflict of interest: No

financial disclosure : No

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