Original Article

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Pathology Section

Immunoexpression of Nanog and Nestin in Egyptian Women Predicts Outcome in Breast Carcinoma

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ABSTRACT

Introduction: Breast cancer is the commonest malignant tumour in females. Drug resistance and disease relapse are common problems occurring during treatment. Cancer Stem Cells (CSC) are implicated in tumourigenesis and resistance to therapy. Nanog is a transcription factor important for regulation of Embryonic Stem Cell (ESC) maintenance and survival. Whereas, Nestin, is a class VI intermediate filament protein that was primarily found during development in neural stem cells. They have been observed in CSC in several neoplasms.

Aim: To evaluate the expression of Nanog and Nestin in breast carcinoma in Egyptian women and its relation to clinicopathologic parameters and prognosis.

Materials and Methods: The current study was a prospective cohort study conducted in Zagazig University hospitals in Egypt in General surgery Department, Pathology Department and Clinical Oncology and Nuclear medicine Department during the period between September 2015 and September 2019. The study evaluated the immunohistochemical expression of Nanog and Nestin in 74 breast carcinoma cases.

Results: The immunoexpression of Nanog and Nestin were related to high tumour grade, advanced TNM stage (TNM Classification of Malignant Tumors), nodal infiltration, lympho-vascular invasion, ER negative status, PR negative status and high Ki67 expression. Nestin expression was significantly associated with 4-year Disease Free Survival (DFS), and 4-year Overall Survival (OS) ($p<0.001$).

Conclusion: Nanog and Nestin were poor prognostic markers of breast carcinoma patients and Nestin is superior to Nanog in predicting patients' outcome.

Keywords: Breast carcinoma, cancer stem cells, Disease free survival, Overall survival, Pluripotency, Prognosis

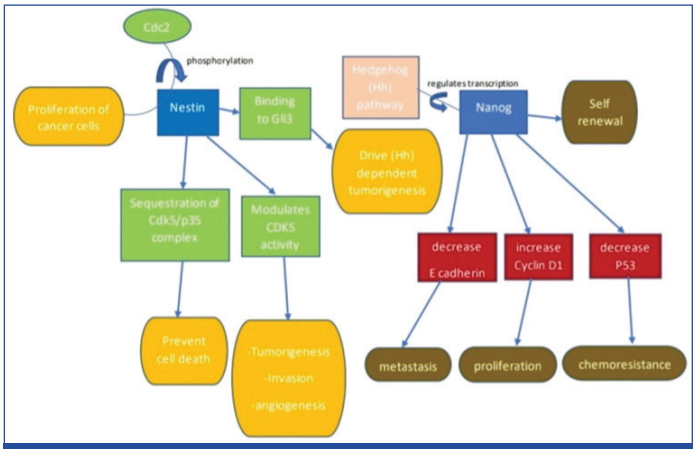
INTRODUCTION

Breast cancer is the most common cancer in females and is considered the second commonly occurring cancer throughout the world. Over two million new cases were reported in 2018 [1]. In Egypt, breast cancer is the commonest cancer in women representing 38.8% of female cancer [2]. Although diagnosis and treatment of breast cancer have been developed, drug resistance and disease relapse are common problems [3]. Tumour recurrence in cancer breast is caused by intratumour heterogeneity and the presence of a subpopulation of cells defined as CSCs which can overcome conventional treatment and initiate malignancy again [4].

Nanog is a regulatory gene located on chromosome 12; it is a key transcription factor responsible for maintaining pluripotency and self-renewal of ESC [5]. It is expressed also in CSCs therefore it is closely related to tumour initiation, relapse, metastasis, chemo and radio-resistance [6]. Nanog over-expression has been found to accentuate spontaneous changes in expression of EMT-related genes in CSC [7]. An enhanced Nanog expression was observed in many cancers, such as cancer prostate, brain tumours, and colorectal carcinoma [8,9].

Nestin is a type VI intermediate filament protein that shares in the organisation of cytoskeleton and is encoded by the NES gene. It is expressed in embryonic progenitor cells and some adult stem cells [10]. Nestin can participate in activation of EMT process through regulation of the Wnt/β-catenin pathway [11].

Normal breast ducts showed Nestin expression in basal myoepithelial layer, in neoplasia [12], Nestin expression is observed in CSC and newly-formed tumour blood vessels. The interaction between CSC and endothelial cells lining of blood vessels in the tumour stroma promotes Nestin mediated cytoskeletal changes leading to neovascularisation [Table/Fig-1] [10,13].



[Table/Fig-1]: Nanog and Nestin pathway.

Overexpression of Nestin has been associated with poor prognosis in several neoplasms including melanoma [14], tumours of central nervous system [15], pancreatic carcinoma [16] and prostate cancer [17].

The current study was conducted with the objectives of investigating the immunohistochemical expression of Nanog and Nestin in breast carcinoma in Egyptian women and analysing the relation of these markers to clinical and pathologic variables and prognostic parameters in an attempt to provide data for the effectiveness of using these markers as predictors for patients' outcome and disease progression as well as to make recommendations for improving treatment program.

MATERIALS AND METHODS

Patient history and tissue preparation: This prospective cohort study was conducted in Zagazig University hospitals in Egypt in General Surgery Department, Pathology Department and Clinical Oncology and Nuclear medicine Department during the period between September 2015 and September 2019. The

immunohistochemistry was done on paraffin-embedded breast carcinoma samples. Seventy-four breast carcinoma cases were obtained from Egyptian females operated in General Surgery Department, Faculty of Medicine, Zagazig University. The samples were transferred to Pathology Department, Faculty of Medicine, Zagazig University where they were diagnosed histopathologically and routine immunohistochemical staining for ER, PR, KI67 and HER2 status was done and registered for breast carcinoma subtyping. Breast carcinoma patients were treated according to American Joint Committee on Cancer (AJCC) staging and indications [18]. In addition, they underwent follow-up in Clinical Oncology and Nuclear Medicine Department. Metastatic cases and patients who received neoadjuvant treatments were excluded from this study. Breast subtypes were classified according to immunohistochemical expression of Oestrogen Receptor (ER), Progesterone Receptor (PR), (Ki67) expression and Human Epidermal Growth Factor Receptor 2 (HER2). Luminal A was characterised by ER and/or PR positive expression and HER2 negativity with low Ki67 expression. Luminal B was characterised by expression of ER and/or PR. high Ki67 expression or by triple positive expression of ER, PR and HER2. The HER2 subtype was ER and PR negative and HER2 positive. The triple-negative subtype was ER, PR and HER2 negative [19].

Ethical consideration: This study was performed with approval of ethics committee of our institution, (ZU-IRB: 5674/15-8-2015) and in accordance with the Helsinki Declaration of 1975 as revised in 2000 for studies involving humans [20]. Informed consent was obtained from all participants included in the study about the use of their data in the research.

Immunohistochemical Staining

Immunohistochemical staining steps were applied on 4 micrometer thickness representative tissue sections prepared on positively-charged slides. For removal of paraffin-xylene solution absolute ethyl alcohol was used. Then washing slides in running water, drying and adding 1% hydrogen peroxide mixture. After 10 minutes methyl alcohol was put to the solution to reach the boiling temperature, the slides were autoclaved in 100°C for 15 minutes after that they were cooled to room temperature. The sections were washed with buffer wash. After that, tissue sections were incubated for an hour with Nanog monoclonal antibody (ab109250; Abcam, Boston, MA, USA) at a dilution of 1:150 and Nestin monoclonal antibody (10c2 sc-23927, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) using 1:50 dilution. Next, sections were washed twice with buffer wash. After adding DAB solution, the slides were washed again in buffer wash. After that, tissue sections were stained with Mayer's haematoxylin.

Evaluation of immunohistochemical stain: The intensity of Nanog nuclear staining was categorised as: 0 (no staining); 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The extent of expression was measured as percent of stained cells in total tumour area as follows: 0 (none of tumour cells), 1 (1-50% positive tumour cells), and 2 (50-100% positive tumour cells). The final score was the sum of the percentage score and the intensity score. Tumours with final score of less than 2 were considered low and those with final score of 3-5 were considered high [21].

The expression of Nestin was categorised semi quantitatively as: 0 if less than 1% of tumour cells separately expressed Nestin in their cytoplasm; 1+ if more than 1 and less than 10% of morphologically unmistakable tumour cells separately expressed Nestin in their cytoplasm; and 2+ if >10% of morphologically unmistakable tumour cells separately expressed Nestin in their cytoplasm. A score of 1+ or 2+ was considered positive expression [22].

Positive and negative control: Positive control for Nanog was Seminoma tissue while positive endothelial cells in breast carcinoma tissues served as internal positive control for Nestin. 19Negative control slides were prepared by substitution of the primary antibodies by Phosphate-Buffered Saline (PBS).

STATISTICAL ANALYSIS

Continuous variables were expressed as the mean±SD. Categorical variables were compared using Pearson's chi-square test or Fisher's-exact test. Spearman correlation test used for correlation analysis. Kaplan and Meier method used to estimate overall and disease free survival and log rank test compared survival curves. OS was calculated as the time from diagnosis to death or the most recent follow-up contact (censored). DFS was calculated as the time elapsed from date of starting treatment to date of progression or the most recent follow-up contact that patient was known as relapse free. The p-value <0.05 was regarded as indicator for significant differences. The collected data were statistically analysed using (SPSS 22.0 for Windows; SPSS Inc. Chicago, Illinois, USA).

RESULTS

Patients Characteristics

A total number of 74 female breast carcinoma patients with mean age (46.95±12.7 years), and and median of 48 (ranging between 20 and 64 years), Thirty-five (47.3%) patients were luminal A, 16 (21.6%) were luminal B, 8 (10.8%) were HER2 enriched and 15 (20.3%) were triple negative. Grading of breast carcinoma was according to the criteria of Nottingham modification of the Bloom-Richardson system [23]. All clinicopathologic data and hormone status of the studied cases are outlined in [Table/Fig-2,3].

Relation between Nanog Expression and Clinicopathological Parameters in Breast Carcinoma Patients [Table/Fig-2,3,4a-f]

A statistically significant association was found between Nanog expression and T stage (p=0.0445), lymph node metastasis (p=0.0192), advanced TNM stage (p=0.0418), high grade (p=0.0278), lympho-vascular invasion (p=0.0075), ER negative status (P=0.013), PR negative status (p=0.0016) and high Ki67 expression (p=0.013).

No significant relation was found between Nanog expression and age, histologic type or HER2 status (p>0.05).

Relation between Nestin Expression and Clinicopathological Parameters in Breast Carcinoma Patients [Table/Fig-2,3,4g-l]

Nestin expression showed a statistically significant association with tumour stage (p=0.015), nodal metastasis (p=0.0017), advanced TNM stage (p=0.001), high histologic grade (p=0.0342), lympho-vascular invasion (p=0.0036), ER negative status (p=0.0056), PR negative status (p=0.0017) and high Ki67 expression (p=0.025).

No significant relation was recorded between Nestin expression and age, histologic type or HER2 status (p>0.05).

Association between Breast Cancer Subtypes and Nanog Expression [Table/Fig-5a]

Positive Nanog expression was found in fifteen cases of luminal A (42.9%), 9 cases of luminal B (56.2%), 6 cases of HER2 (75%) and 13 cases of triple-negative (86.7%) breast cancer.

There was a significant association between high Nanog expression and triple-negative subtype compared with non-triple negative breast cancer subtypes together (p=0.012).

Association between Breast Cancer Subtypes and Nestin Expression [Table/Fig-5b]

Positive Nestin expression was found in five cases of luminal A (14.3%), 4 cases of luminal B (25%), 2 cases of HER2 (25%) and 9 cases of triple-negative (60%) breast cancer.

		Nanog			Nestin		
		Low	High		Negative	Positive	
Parameters	**Total 74**	**31 (41.9%)**	**43 (58.1%)**	**p***	**54 (73%)**	**20 (27%)**	**p***
Age				**0.61**			**0.087**
<50	36 (48.6)	14 (45.2)	22 (51.2)		23 (42.6)	13 (65)	
>50	38 (51.4)	17 (54.8)	21 (48.8)		31 (57.4)	7 (35)	
Histological type				**0.596**			**0.752**
Ductal carcinoma	59 (79.7)	23 (74.2)	36 (83.7)		44 (81.5)	15 (75)	
Lobular carcinoma	11 (14.9)	6 (19.4)	5 (11.6)		7 (13)	4 (20)	
Others	4 (5.4)	2 (6.4)	2 (4.7)		3 (5.5)	1 (5)	
T stage				**0.0445**			**0.015**
T1	14 (18.9)	10 (32.2)	4 (9.3)		13 (24.1)	1 (5)	
T2	37 (50)	14 (45.2)	25 (58.1)		29 (53.7)	8 (40)	
T3	23 (31.1)	7 (22.6)	14 (32.6)		12 (22.2)	11 (55)	
N stage				**0.0192**			**0.0017**
N0	29 (39.2)	17 (54.8)	12 (27.9)		27 (50)	2 (10)	
N1-3	45 (60.8)	14 (45.2)	31 (72.1)		27 (50)	18 (90)	
TNM stage				**0.0418**			**<0.001**
I	11 (14.9)	8 (25.8)	3 (7)		10 (18.5)	1 (5)	
II	35 (47.3)	15 (48.4)	20 (46.5)		31 (57.4)	4 (20)	
III	28 (37.8)	8 (25.8)	20 (46.5)		13 (24.1)	15 (75)	
Grade				**0.0278**			**0.0342**
I	22 (29.7)	14 (45.2)	8 (18.6)		19 (35.2)	3 (15)	
II	28 (37.8)	11 (35.5)	17 (39.5)		22 (40.7)	6 (30)	
III	24 (32.5)	6 (19.3)	18 (41.9)		13 (24.1)	11 (55)	
Lympho-vascular invasion				**0.0075**			**0.0036**
Negative	55 (74.3)	28 (90.3)	27 (62.8)		45 (83.3)	10 (50)	
Positive	19 (25.7)	3 (9.7)	16 (37.2)		9 (16.7)	10 (50)	

[Table/Fig-2]: Nanog and Nestin expression in breast cancer tissue in relation to clinicopathologic parameters.
*Chi-square test; TNM stage: TNM classification of malignant tumors (TNM); T: size or direct extent of the primary tumor; N: degree of spread to regional lymph nodes; M: presence of distant metastasis

		Nanog			Nestin		
		Low	High		Negative	Positive	
Parameters	**Total 74**	**31 (41.9%)**	**43 (58.1%)**	**p***	**54 (73%)**	**20 (27%)**	**p***
ER				**0.013**			**0.0056**
Negative	29 (39.2)	7 (22.6)	22 (51.2)		16 (29.6)	13 (65)	
Positive	45 (60.8)	24 (77.4)	21 (48.8)		38 (70.4)	7 (35)	
PR				**0.0016**			**0.0017**
Negative	30 (40.5)	6 (19.4)	24 (55.8)		16 (29.6)	14 (70)	
Positive	44 (59.5)	25 (80.6)	19 (44.2)		38 (70.4)	6 (30)	
HER2				**0.732**			**0.724**
Negative	61 (82.4)	25 (80.6)	36 (83.7)		44 (81.5)	17 (85)	
Positive	13 (17.6)	6 (19.4)	7 (16.3)		10 (18.5)	3 (15)	
Ki67				**0.013**			**0.025**
Low	45 (60.8)	24 (77.4)	21 (48.8)		37 (68.5)	8 (40)	
High	29 (39.2)	7 (22.6)	22 (51.2)		17 (31.5)	12 (60)	
Breast subtypes				**0.012**			**0.0013**
Triple negative	15 (20.3)	2 (6.5)	13 (30.2)		6 (11.1)	9 (45)	
Non triple negative	59 (79.7)	29 (93.5)	30 (69.8)		48 (88.9)	11 (55)	

[Table/Fig-3]: Nanog and Nestin expression in breast cancer tissue in relation to hormone status.
*Chi-square test; ER: Oestrogen receptor; PR: Progesterone receptor (PR); HER2: Human epidermal growth factor receptor 2

There was a highly significant relation between positive Nestin expression and triple-negative subtype compared with non-triple negative breast cancer subtypes together (p<0.001).

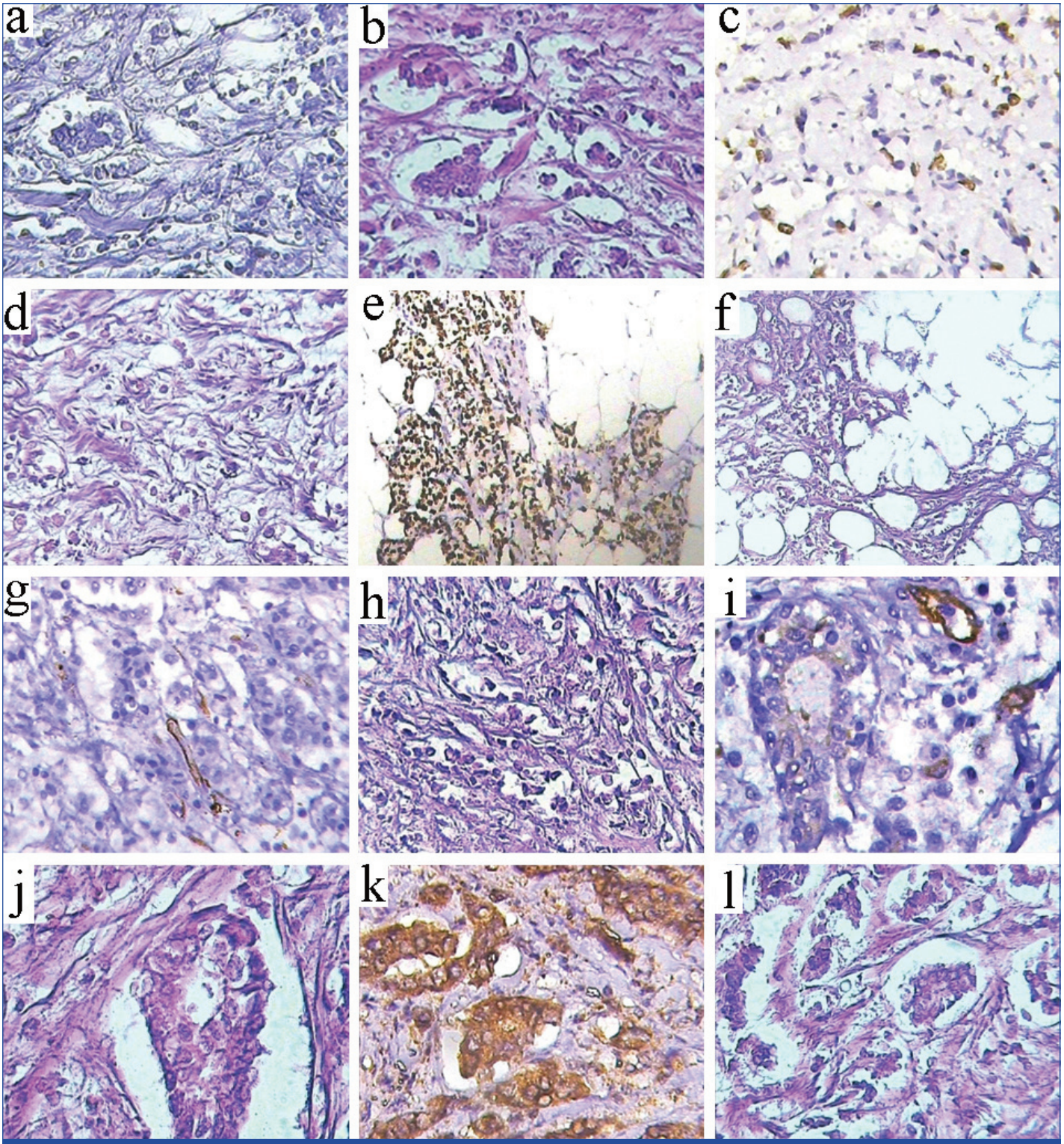
Correlation between Nanog and Nestin Expression in Breast Carcinoma Tissue [Table/Fig-6]

There was a weak positive correlation between the expression of Nanog and Nestin in breast carcinoma tissue (R=0.1467), however, the correlation was statistically non-significant (p=0.2).

Clinical Outcome of Patients in Relation to Markers Expression [Table/Fig-7,8,9a,b,c] and [Table/Fig-10a,b,c]

There was a significant correlation between positive expression of Nestin and death, distant metastasis, and local recurrence, 4 years OS and 4 years DFS (p<0.001).

There was no significant association between Nanog expression and the clinical outcome.

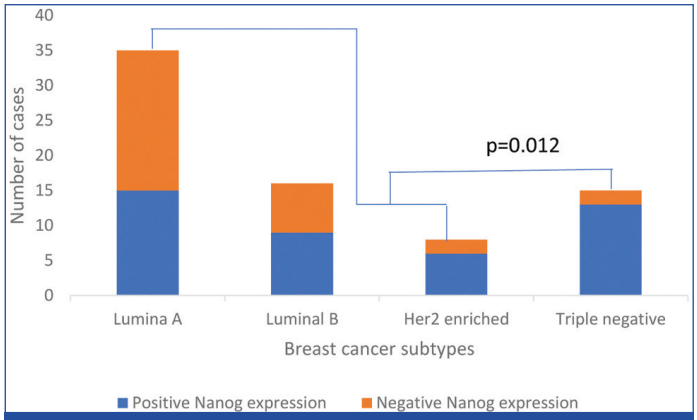


[Table/Fig-4]: Immunohistochemical staining for Nanog and Nestin in breast carcinoma tissue; (a) Negative Nanog expression in tumour cells; (b) H&E staining of the same case; (ABC, DAB chromogen X400); (c) Low nuclear expression of Nanog in tumour cells; (d) H&E staining of the same case; (ABC, DAB chromogen X400); (e) High nuclear expression of Nanog in tumour cells; (f) H&E staining of the same case; (ABC, DAB chromogen X100). (g) Negative Nestin expression in tumour cells with intense staining of endothelial cells; (h) H&E staining of the same case;(ABC, DAB chromogen X400); (i) Weak cytoplasmic expression of Nestin in tumour cells (score 1); (j) H&E staining of the same case; (ABC, DAB chromogen X400); (k) strong cytoplasmic expression of Nestin in tumour cells (score 2); (l) H&E staining of the same case; Magnification (ABC, DAB chromogen X400).

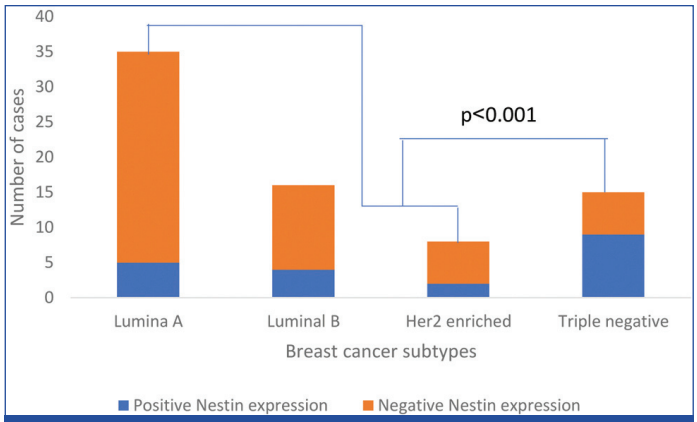
DISCUSSION

Nanog is a transcription factor documented as an important regulator in maintaining ESC survival [5], in a number of studies, overexpression of Nanog has been associated with poor prognosis and short survival [8,9,21], considering Nanog as a therapeutic target could decrease tumour recurrence as it has been related to chemoresistance mechanisms [7,8]. Whereas, Nestin, is a class VI intermediate filament protein that was primarily found during development in neural stem cells [10,16]. High expression of nestin has been linked to poor prognosis in several studies on different tumours [14-17]. Some studies have reported that Nestin inhibition reduced tumour proliferation and invasion of various neoplasms, hence Nestin-targeted therapy may be effective with minimal side effects [10,16]. Cancer stem cell population and their role in cancer pathology have been widely studied, and currently CSCs are claimed to be involved in cancer initiation and progression.

Expression of these stem cell markers in breast carcinoma tissue, and their absence in normal breast tissue proposes either amplification of local stem cells or returning of somatic breast cells to a stem cell-like condition [12,24]. Moreover, radiation induced reprogramming of non-breast cancer stem cells to breast cancer stem cells is accompanied by increase in expression of these markers [6]. Therefore, it will be possible to develop certain drugs precisely affect CSC population and settle novel strategies for treatment.



[Table/Fig-5a]: Nanog expression in breast cancer subtypes.



[Table/Fig-5b]: Nestin expression in breast cancer subtypes.

Nestin expression	Nanog expression	
	High (n=43)	Low (n=31)
Positive (n=20)	14 (70%)	6 (30%)
Negative (n=54)	29 (53.7%)	25 (46.3%)
(R)=0.1467 p-value=0.2*		

[Table/Fig-6]: Correlation between Nanog and Nestin immunoexpression in breast carcinoma.
*Pearson correlation test

and tumour grade, nodal involvement and tumour stage [25]. Also, Han J, et al., reported similar relation with T stage (p=0.003) and clinical stage (p=0.037) [28]. However, Wang D et al., and Nagata T et al., did not notice any connection between Nanog expression and TNM stage [7,21].

The current study did not find significant association between Nanog expression and age or tumour size, some previous studies showed significant association with tumour size but not with age [7,27].

In this study, we reported significant relationship between high Nanog expression and negative ER status, PR status and high Ki67 expression but not with HER2 status, on the other hand, Jin C et al., found significant association between Nanog expression and high Ki67 and HER2 expressions while no correlation was found with ER and PR status [27]. These differences may be due to different number of cases, variable scoring and cut off points or different method sensitivities.

Regarding breast subtypes, the present study found significant association between Nanog expression and triple negative breast subtype (p=0.0013) in relation to other subtypes, this agrees with previous studies [24,29] and disagrees with Gawk JM et al., who observed more frequent Nanog expression in luminal A and luminal B subtypes [30].

In this work, after a median follow-up period of 48 months, 9 patients (12.2%) died and 6 (8.1%) patients had each of distant metastasis and local recurrence, it was found that there was no significant association between Nanog expression and the clinical outcome however positive Nanog was associated with triple negative breast cancer subtype which is commonly linked to poor survival. This was contradictory to Zhao L et al., meta-analysis on some human solid tumours which stated that positive Nanog expression was significantly associated with poor DFS and OS and recommended the performance of extra studies on Nanog to validate these results [31].

On the other hand Arif KH et al., indicated that Nanog is not only responsible for tumourigenesis, but also has a role in tamoxifen

Clinical outcome		Total N=74		Nanog				p*	Nestin				p*
				Negative N=31		Positive N=43			Negative N=54		Positive N=20		
		N	%	N	%	N	%		N	%	N	%	
Death	No	65	87.8%	28	90.3%	37	86.0%	0.579	53	98.1%	12	60.0%	**<0.001**
	Yes	9	12.2%	3	9.7%	6	14.0%		1	1.9%	8	40.0%	
Metastasis	No	68	91.9%	30	96.8%	38	88.4%	0.191	53	98.1%	15	75.0%	**0.001**
	Yes	6	8.1%	1	3.2%	5	11.6%		1	1.9%	5	25.0%	
Local recurrence	No	68	91.9%	29	93.5%	39	90.6%	0.658	52	96.2%	16	80.0%	**0.023**
	Yes	6	8.1%	2	6.4%	4	9.3%		2	3.7%	4	20.0%	

[Table/Fig-7]: Clinical outcome of patients in relation to markers' expression.
*Chi-square test

The present study demonstrated that Nanog expression was predominately observed in nuclei of 43 breast carcinoma cases (58.1%), this is close to Saravi OE, et al., [25] that reported nuclear Nanog expression in (55.8%) of breast carcinomas, also Finicelli M, et al., [26] detected Nanog in 44.5% of studied breast cancer cases.

While Wang D et al., and Jin C et al., reported Nanog expression in 36.51% and 29.17% of studied cases respectively, this variation may be attributed to ethnic differences [7,27].

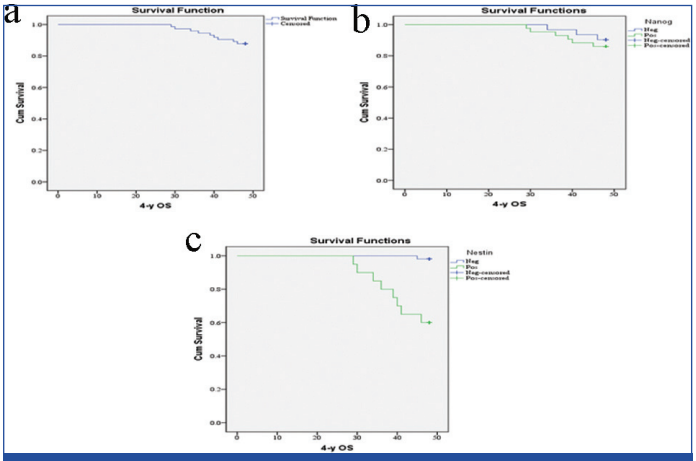
In this work, we found significant association between Nanog expression and clinicopathologic parameters such as, T stage, nodal metastasis, TNM stage, grade of differentiation, and lympho-vascular invasion. These findings were near to Saravi OE et al., who found similar association between Nanog expression resistance and it is negatively related to apoptosis pathway [32].

In the current study, Nestin expression was observed in the cytoplasm of 20 breast carcinoma cases (27%); this result is near to Nowak A et al., who reported Nestin expression in 31.5% of studied cases [10]. Other studies reported lower level of expression [11,22,33-35]. This may be due to deficient number of cases or different scoring methods.

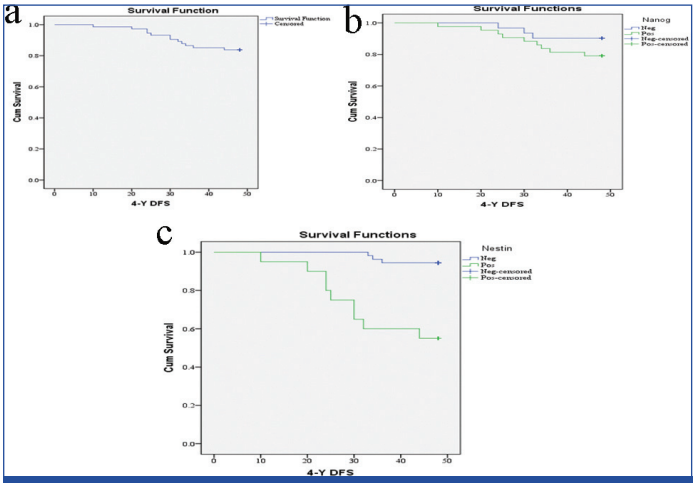
In this work, a significant association was recorded between Nestin expression and T stage, nodal metastasis, TNM stage, histologic grade, and lympho-vascular invasion. This is consistent with Shaban MI and El- Goday SF who found significant connection with grade and vascular invasion [36]. Other previous studies showed

Markers		Survival rate %	Survival time (Months) Mean±SE	(95% CI)	p*
4-year overall survival					
Overall		87.8%	46.8±0.5	(45.87-47.64)	
Nanog	Negative	90.3%	47.3±0.5	(46.3-48.22)	0.556
	Positive	86.0%	46.4±0.7	(45.05-47.74)	
Nestin	Negative	98.1%	47.9±0.1	(47.84-48.05)	<0.001
	Positive	60.0%	43.6±1.4	(40.73-46.37)	
4-year disease-free survival					
Overall		83.8%	44.8±0.9	(43.03-46.64)	
Nanog	Negative	90.3%	46.1±1	(44.08-48.18)	0.204
	Positive	79.1%	43.9±1.4	(41.2-46.61)	
Nestin	Negative	94.4%	47.2±0.4	(46.4-48.08)	<0.001
	Positive	55.0%	38.4±2.7	(33.01-43.69)	

[Table/Fig-8]: Mean survival time and survival rates in relation to each marker.
After a median follow-up period of 48 months with range (29-48) months
*log rank test



[Table/Fig-9]: Kaplan meier curve of 4-year overall survival: (a) for the studied group; (b) in relation to Nanog expression; (c) in relation to Nestin expression.



[Table/Fig-10]: Kaplan meier curve of 4-year disease-free survival: (a) for the studied group; (b) in relation to Nanog expression; (c) in relation to Nestin expression.

significant association with high histologic grade [10,22,33,35] and nodal infiltration [22,33].

The present study did not found relation between Nestin expression and patients' age or tumour size, in accordance with a study made by Lui C et al., [34]. While other studies found significant association between Nestin expression and young age [11,33,34].

In this work, there was significant relation between Nestin expression and ER, PR negativity, and high Ki67 expression, in agreement with previous studies [10,22,33,35,37]. Regarding Nestin expression in breast cancer subtypes, the current study found significant association with triple negative subtype. In agreement with this study, several studies found significant relationship between triple negative breast cancer and Nestin positivity [10,11,14,22,33,34,38]. In this work, there was a significant correlation between positive expression of Nestin and death, distant metastasis, and local recurrence, 4-y OS and 4-y DFS (p<0.001). This is consistent with Zhao Z et al., who found significant association between Nestin expression and poor survival in Chinese patients with triple-negative breast cancer [39]. They concluded also that, Nestin High but not Nestin Low breast CSC, can produce mammospheres in vitro, and provoke solid tumours in vivo. Furthermore, knockdown of Nestin expression improved the spontaneous apoptosis and inhibited EMT process and the activation of Wnt/β-catenin pathway in breast CSC. Their data therefore suggested that Nestin may be considered as therapeutic target for triple-negative breast cancer and delivered new understandings into the regulating role of Nestin in the cellular processes in breast cancer stem cells.

Also, the results of this study are consistent with Nowak A et al., study which showed that positive Nestin expression was associated with a shorter patient overall survival (P=0.02) [10]. Also, the data obtained from this work are matching with Neradil J and Veselska R study which showed that Nestin expression is associated with reduced survival in breast cancer patients [13]. In this work, there was a weak positive correlation between Nanog expression and Nestin expression in breast carcinoma tissue (R=0.1467), however, the correlation was statistically non-significant (P=0.2). In line with this study, co-expression of both markers was reported in prostate cancer cell lines [40], gall bladder carcinomas [41] and oral squamous cell carcinoma [42].

Limitation(s)

There were some limitations of this work such as small number of studied cases, difficulties in follow-up of patients during survival studies and financial problems, so further studies on larger population are recommended.

CONCLUSION(S)

The current study demonstrated that Nanog and Nestin are related to poor prognostic clinicopathologic parameters of breast carcinoma, whereas Nestin is a superior predicting marker of patients' outcome to Nanog. They might be implicated in breast cancer progression and metastasis, and being predominantly expressed in triple negative breast cancer subtype. This might give the chance of therapy to those patients with this type of breast cancer who could not be treated with hormonal therapy or Herceptin, and are treated by chemo or radiotherapy with probability of drug resistance or relapse. Therefore inclusion of these markers in the current immunohistochemical panel used for planning therapy, and targeting these stem cell markers may be beneficial to guide in new therapeutic strategies substituting the traditional ones. Further studies on larger population are necessary to explore possible underlying mechanisms and to validate ability to improve therapy.

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