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A STUDY OF OCT3/4 EXPRESSION IN HER2/NEU POSITIVE BREAST CANCER PATIENTS

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ABSTRACT

Oct3/4 is found in germ cells is crucial to control pluripotency during normal development. There is mounting evidence for an association between Oct3/4 and carcinogenesis. Thus Oct3/4 immunohistochemistry has been found in studies to be a useful diagnostic technique for pluripotent germ-cell cancers, providing fresh insights into the cancer's histological heterogeneity. The aim is to study role of Oct3/4 expression in HER2/neu positive breast cancer patients. Paraffin embedded breast tumour tissues from 50 HER2/neu positive female breast cancer patients were enrolled in the study. Oct3/4 expression was studied by immunohistochemistry method and correlated with clinical and pathological parameters as well as disease status. Oct3/4 positivity was seen in 16% of HER2/neu positive breast cancer patients. No significant correlation of Oct3/4 expression with age, menopausal status, tumour size, histopathology, grade and BR score was observed. A trend of higher incidence of Oct3/4 expression was noted with lymph node positive (30%) than lymph node negative patients (07%). A significant higher incidence of Oct3/4 expression was noted in patients with advanced stage of disease (32%) as compared to patients with early stage of disease (04%) and in patients with relapse (44%) than patients in remission (10%). A significant higher incidence Oct3/4 expression was observed with respect to disease free survival in univariate survival analysis. In multivariate survival analysis, for disease free survival metastasis entered at step 1 and grade entered at step 2. For overall survival, metastasis is entered at step 1 as significant factor. The Oct3/4 gene expression may be implicated in self-renewal and tumorigenesis via activating its downstream target genes. It might serve as an important biomarker for contemplating the carcinogenesis, progression, metastasis, or invasive potential and prognosis of breast carcinoma. A larger prospective follow-up studies are recommended to further explore Oct3/4 significance.

Keywords: *Breast cancer, Immunohistochemistry, Oct3/4, HER2/neu positive*

INTRODUCTION

Cancer is a biological illness characterized by a complex interplay of genetic and environmental variables that orchestrate carcinogenesis, i.e., the process by which normal cells are transformed into cancer cells [1]. Breast cancer has surpassed lung cancer as the most commonly diagnosed malignancy, with an estimated 2.3 million new cases (11.7%). It is by far the most common cancer in the world as of 2020, with 7.8 million women surviving who had been diagnosed in the previous five years. HER2/neu positive subtype of breast cancer is defined by a high level of HER2 expression. Amplification of HER2 gene and increased expression of the HER2 protein have been linked to tumours with a higher histological grade, a higher proliferative index, and a higher propensity to metastasize, all of which led to shorter disease-free survival and a worse prognosis. However, medications that block HER2 activity, such as humanized monoclonal antibodies (Trastuzumab) and molecular receptor tyrosine kinase inhibitors (Lapatinib), may be effective in treating these malignancies [2]. Cancer stem cells are distinguished by their ability to self-renew and differentiate into a variety of cell types, some of which are tumorigenic. CSC are engaged in the development and growth of the primary tumour, as well as the development of metastasis and recurrence, at every stage of cancer development [3]. The implication of stem cells in breast cancer's origin and development has been debated for many years. Breast CSCs have

also been linked to prognostic significance and the ability to impact therapy outcomes in a number of studies. Breast cancer stem cell targeting holds a great promise for preventing metastasis, reducing the risk of drug resistance and decreasing recurrence [4]. Oct3/4 is found in embryonic stem and germ cells and is crucial in the control of pluripotency during normal development. In the mammalian embryo, Oct3/4 is required for the identification of the pluripotent founding cell population [5]. There is mounting evidence for an association between Oct3/4 and carcinogenesis. Increased Oct3/4 expression (up to 150% of normal expression) in ES cells promotes the potential of these cells to form tumours in syngeneic hosts, from 4% to >80%, whereas inactivation causes regression of a malignant phenotype. Up-regulation of Oct3/4 expression, on the other hand, has been linked to tumour heterogeneity, metastasis, and resistance to conventional cancer therapy [6]. Oct3/4 immunohistochemistry has been found to be a useful diagnostic technique for pluripotent germ-cell cancers, providing fresh insights into the cancer's histological heterogeneity [7]. Studies have shown that expression of Oct3/4 was variable ranging from 35% to 65% of the cases [8, 9, 10]. The current study has focused on assessing the role of OCT3/4 in HER2/neu positive breast cancer patients. Furthermore, to correlate their expression with various clinicopathological parameters.

MATERIAL AND METHODS

Patient characteristics

This retrospective study was approved by institutional scientific and ethics committees, included 50 HER2/neu positive breast cancer patients treated at The Gujarat Cancer & Research Institute. Detailed clinicopathological history such as age, menopausal status, tumour size, lymph node status, histopathological type, stage, histopathology grade, BR score, metastasis and treatment offered were recorded from the case files maintained at the Medical Record Department of the Institute. Disease staging was done according to AJCC classification. Disease status was assessed by clinical examination, radiological investigations and biochemical investigations.

Immunohistochemical Localization

Localization of markers Oct3/4 expression was analyzed by immunohistochemistry, which was performed on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Ventana, USA). Primary antibody Oct3/4 was procured commercially from Epredia. The primary and secondary antibodies were incubated as follows: Oct3/4 for 32 minutes at 37°C with dilution 1:50, and HRP multimer for 8 minutes.

Scoring

Two individual observers scored the sections under microscope. Cytoplasmic staining pattern was noted for Oct3/4. For Oct3/4, scoring was done using the ASCO and CAP guidelines 2007 where immunoreactivity scored as 0 for negative (no cytoplasmic staining), 1+ (faint or incomplete cytoplasmic staining), 2+ (10-30% with strong cytoplasmic staining) and 3+ (<30% tumour cells with complete cytoplasmic staining). For statistical analysis Oct3/4 negative and 1+ were clubbed as negative.

Statistical analysis

Statistical analysis was carried out using SPSS statistical software version 26 (SPSS Inc. USA). Mean, Standard error (SE) of mean and median were calculated and Pearson's Chi-square test with Pearson's correlation coefficient (r) was used to assess correlation and significance between two parameters. Univariate survival analysis was carried out by Kaplan Meier and Log Rank statistics was used to assess the prognostic significance of disease-free survival (DFS) and overall survival (OS), Multivariate survival analysis was performed using Cox regression model with forward stepwise (likelihood ratio) method. P values ≤ 0.05 were considered to be statistically significant.

RESULT

Patient characteristics with outcome

This retrospective study included 50 patients, 64% had age ≤ 50 years, whereas 36% patients had >50 years and 60% patients had postmenopausal status. In relation to pathological characteristics, more than 50% were of T2 tumour size, negative lymph node status, IDC

subtype, disease stage II, histological grade II and no metastasis (Table 1). The primary treatment offered to the patients was surgery followed by adjuvant chemotherapy, radiotherapy, hormonal o targeted therapy. The maximum follow-up period was 77 months with a median follow-up of 50 months.

Table 1 Correlation of Oct3/4 expression with clinicopathological parameters and disease status

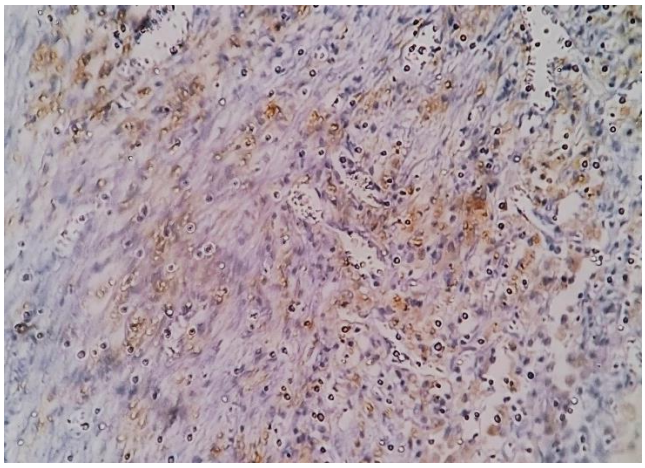
Parameters		**Incidence of Oct3/4 expression**		
	N (%)	**Negative**	**Positive**	**P**
		N (%)	**N (%)**	
Age (years)	**50(100)**	**42(84)**	**08(16)**	
≤ 50 years	32(64)	26(81)	06(19)	0.76
> 50 years	18(36)	16(89)	02(11)	
Menopausal status	**50**	**42(84)**	**08(16)**	
Pre-menopausal	20(40)	17(85)	03(15)	1
Post-menopausal	30(60)	25(83)	05(17)	
Tumor Size	**50(100)**	**42(84)**	**8(16)**	
T1 (≤ 2 cm)	08(16)	06(75)	02(25)	0.62
T2 (≥2cm to ≤5cm)	38(76)	33(87)	05(13)	
T3 (≥5cm)	04(08)	03(75)	01(25)	
Lymph node Status	**50(100)**	**42(84)**	**08(16)**	
Negative	30(60)	28(93)	02(07)	0.07
Positive	20(40)	14(70)	06(30)	
Histopathology	**50(100)**	**42(84)**	**08(16)**	
IDC	29(58)	25(86)	04(14)	0.91
IDC+DCIS	21(42)	17(81)	04(19)	
Stage	**50(100)**	**42(84)**	**08(16)**	
Early (stage IA + IB + IIA)	28(56)	27(96)	01(04)	0.02
Advanced (stage IIB + IIIA + IIIB + IIIC)	22(44)	16(68)	07(32)	
Grade	**50(100)**	**42(84)**	**08(16)**	
Grade I	03(06)	02(67)	01(33)	0.22
Grade II	26(52)	24(92)	02(08)	
Grade III	21(42)	16(76)	05(24)	
BR score	**50(100)**	**42(84)**	**08(16)**	
5	03(06)	02(67)	01(33)	0.37
6	07(14)	07(100)	00(00)	
7	19(38)	17(90)	02(10)	
8	17(34)	13(77)	04(23)	
9	04(08)	03(75)	01(25)	

Disease status	**50(100)**	**42(84)**	**08(16)**	
Remission	41(82)	37(90)	04(10)	0.04
Relapse	09(18)	05(56)	04(44)	
Metastasis	**50(100)**	**42(84)**	**08(16)**	
No metastasis	41(82)	37(90)	04(10)	0.0001
Recurrent (breast)	03(06)	00(00)	03(100)	
Lung	04(08)	04(100)	00(00)	
Ovary	01(02)	00(00)	01(100)	
Bone	01(02)	01(100)	00(00)	
Disease status	**50(100)**	**42(84)**	**08(16)**	
Alive	46(92)	38(83)	08(17)	0.84
Dead	04(08)	04(100)	00(00)	

Oct3/4 expression

Cytoplasmic expression of Oct3/4 was noted in 16% of the cases of HER2/neu positive patients (Figure 1). No significant correlation of Oct3/4 expression was observed with age and menopausal status. A trend of higher incidence of Oct3/4 expression was noted with lymph node positive patients (p = 0.07) (Table 1). Oct3/4 expression was significantly higher in patients with advanced stage of disease (p = 0.02) (Table 1). Similarly, significant higher incidence of Oct3/4 expression was noted in patients with in patients having relapse (p = 0.04) (Table 1). Among the metastasis site significant correlation of Oct3/4 expression was observed with recurrent patients as compared to other metastasis site (p = 0.001) (Table 1).

Figure 1: Positive cytoplasmic Oct3/4 staining of breast tumour cells of cancer patients (40X)



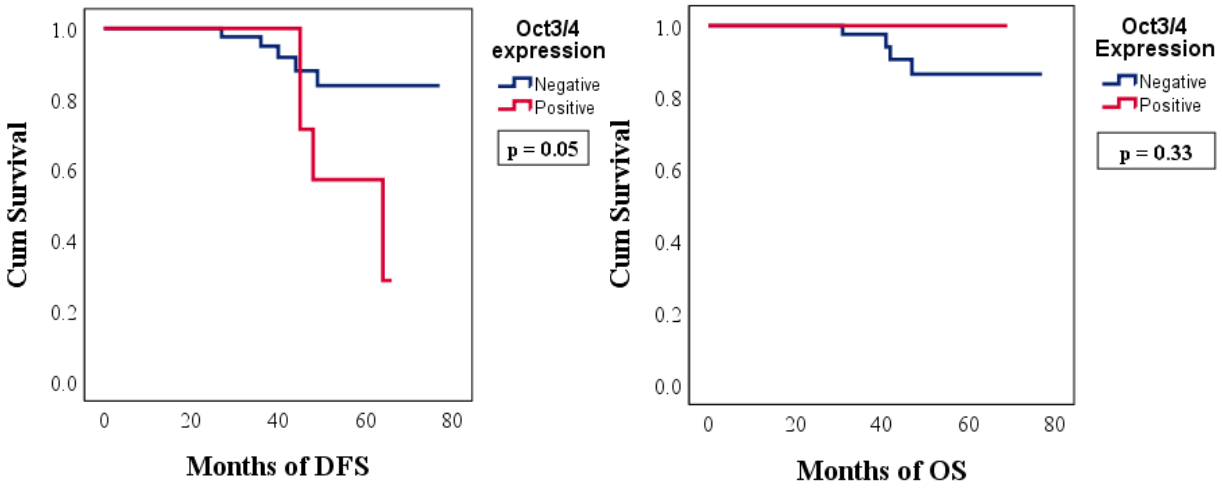
Oct3/4 expression in relation to survival

According to Kaplan and Meier univariate survival analysis, with respect to DFS, a significant higher incidence of disease relapse was noted in Oct3/4 positive patients (50%, 04/08) than in Oct3/4 negative patients (12%, 05/42). While with respect to OS, similar incidence of death was noted in Oct3/4 negative patients (09%, 04/42) and Oct3/4 positive patients (00%, 00/08) (Table 2, Figure 2A). No mean OS months were noted as no patients with positive Oct3/4 expression died (Table 2, Figure 2B). Oct3/4 expression when correlated with treatment offered to the patients, with respect to disease free survival. No significant correlation of Oct3/4 expression with treatment offered to the patients, with respect to disease free survival and overall survival was obtained.

Table 2 Univariate analysis of Oct3/4 expression

Oct3/4 expression	**N**	**DFS in months Mean ± SE**	**Remission N (%)**	**Relapse N (%)**
Negative	42(84)	71.12 ± 2.44	37(88)	05(12)
Positive	08(16)	46.86 ± 3.59	04(50)	04(50)
			Log rank = 0.57, df = 1, p = 0.05	
Oct3/4 expression	**N**	**OS in months Mean ± SE**	**Alive N (%)**	**Dead N (%)**
Negative	42(84)	-	38(91)	04(09)
Positive	08(16)	-	08(100)	00(00)
			Log rank = 0.95, df = 1, p = 0.33	

Figure 2: (A) Kaplan - Meier survival analysis for disease free survival (DFS) (B) Kaplan - Meier survival analysis for overall survival (OS)



(A) **(B)**

Multivariate survival analysis using Cox regression model with forward stepwise (likelihood ratio) method was carried out to evaluate the prognostic significance of clinical and pathological parameters such as age, menopausal status, tumour size, lymph node status, disease stage, histopathology, histological grade, BR score, metastasis site, treatment and Oct3/4. Metastasis entered at step 1 as significant factor (Wald statistic = 21.87, df = 1, Exp (B) = 5.59, p = 0.001) and Grade entered at step 2 (Wald statistic = 4.59, df = 1, Exp (B) = 4.63, p = 0.03). With respect to overall survival metastasis entered at step 1 as significant factor (Wald statistic = 8.70, df = 1, Exp (B) = 3.80, p = 0.03) (Table 3)

Table 3 Multivariate analysis of Oct3/4 expression

							95% CI for Exp (B)	
Patients	**Step**	**Variables**	**Wald statistics**	**df**	**P**	**Exp(B)**	**Lower**	**Upper**
DFS	1	Metastasis	21.87	1	0.001	5.59	2.70	11.50
	2	Grade	4.59	1	0.03	4.63	1.13	18.85
OS	1	Metastasis	8.70	1	0.03	3.80	1.56	9.24

DISCUSSION

In the present study, Oct3/4 expression was noted in 16% of HER/2neu positive breast cancer patients. While other studies found that expression of Oct3/4 was variable ranging from 35% to 65% of the cases [8, 9, 10]. A trend of higher incidence of Oct3/4 expression was noted in lymph node positive patients but other studies obtained a significant association with LN [8].

Present study indicate that significantly higher incidence of Oct3/4 expression was seen in patients with advance stage of the disease and with metastasis site, similar results were obtained by Zhang JM et al., 2018 and Chen SF et al., 2013, respectively. The possible reasons were that up regulation of Oct3/4 could regulate multiple genes and pathways to induce tumor cells proliferation/invasion, and repress cells apoptosis/differentiation, resulting in the acceleration of tumor growth and metastasis [11, 12].

According to Kaplan Meier univariate survival analysis with respect to DFS significantly higher incidence of disease relapse was noted in Oct3/4 positive patients. Similar results were obtained by previous studies [11, 13, 14]. Oct3/4 overexpression could directly reflect stem-cell properties, which has been correlated with strong resistance of therapies, including conventional systemic chemotherapy and radio-therapy, thereby contributing to the resistance to therapies and increasing relapse as suggested by the study of Zhang JM et al. (2018) [11].

In multivariate survival analysis for DFS, metastasis entered at step 1 and grade entered at step 2. For OS, metastasis is entered at step 1 as significant factor. Studies of Abou Gabal et al. (2016) and Gwak et al. (2017) gave similar results [8, 13]. Study of Zhang JM et al. (2018) suggested that Oct3/4 could target multiple pathways including JAK/STAT pathways, Wnt/β-catenin pathway and AKT pathway, which are the potentially actionable signaling pathways, accelerating cells proliferation and invasion and resulting in tumor growth and poor differentiation, thereby increasing recurrent rate [11].

Oct3/4 expression was not statistically significant with conventional clinicopathological parameters due to inclusion of small number of patients in this study. Oct3/4 expression was significantly correlated with TNM stage and DFS which could be served as an independent biomarker to predict worse prognosis.

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