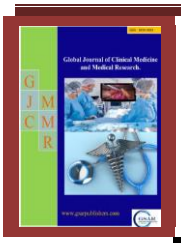

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A STUDY OF COL2A1 EXPRESSION IN TRIPLE-NEGATIVE BREST CANCER PATIENTS

BY

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Abstract

Collagen, type II, alpha 1 also known as COL2A1, is a human gene that provides instructions for the production of the pro-alpha1(II) chain of type II collagen. Mutations in COL2A1 can cause type II collagenopathies, which is important for genetic counseling, reduce the risk of recurrent cases in families, and improved clinical follow-up of patients. The present study aim to study clinical significance of COL2A1 protein expression in Triple Negative Breast cancer patients. Paraffin embedded breast tumour tissues from 50 Female TNBC patients were enrolled in the study. COL2A1 expression was studied by immunohistochemistry method and correlated with clinicopathological parameters and as well as disease status. As a result, COL2A1positivity was seen in 42% of tumor cells of TNBC patients. A trend of higher incidence of COL2A1 expression was noted in patients with lymph node negative status and patients with low BR score tumors, A significant higher incidence of COL2A1 expression was noted in patients with remission as compared to patients with relapse, A significant higher incidence COL2A1 expression was observed with respect to diseases free survival analysis in univariate survival analysis. Overall present study indicated that, Loss of COL2A1 expression was associated with positive lymph node status, advancement of tumor grade, disease metastasis and worse prognosticator predicting reduced associated with disease free survival of TNBC. This indicates association of loss of collagen II protein with disease aggressiveness in TNBC.

Keywords: *ER, PR, Her-2-neu, COLA21, TNBC*

INTRODUCTION

Cancer is the second leading cause of death, limiting life expectancy. The most common malignancy in women is breast cancer. Female breast cancer is the most common cancer worldwide as for 2020, with 2.3 million new cases and 6,84,996 deaths reported [1].

Triple-negative breast cancer is ER-negative, PR-negative, and HER2-negative. They constitute about 20% of all breast cancers. The basal-like (BL1 and BL2), claudin-low, mesenchymal (MES), luminal androgen receptor (LAR), and immunomodulatory (IM) subgroups are further divided into the TNBC subtype, with the first two accounting for 50–70% and 20–30% of cases, respectively. The clinical outcomes, phenotypes, and pharmacological sensitivities of each of these are additionally unique. Genetics, race, age, overweight and obesity, breastfeeding practices, and parity all influence the risk of developing TNBC. TNBC is distinguished by its aggressiveness, propensity for early relapse, and increased propensity to present at an advanced stage. In addition to enhanced genomic instability, it has a high rate of proliferation. It is a heterogeneous tumour with poor differentiation, high proliferative rate, and subgroups with varying prognosis from a histological perspective [2].

Location of the COL2A1 gene on the long (q) arm of chromosome 12 between positions 13.1 and 13.2[3]. The pro-alpha1(II) chain of type II collagen is produced by a human gene called collagen, type II, alpha 1, also known as COL2A1. Type II collagenopathies are a group of abnormalities that have been linked to mutations in the collagen type II alpha-1 gene (COL2A1) [4]. which is essential for genetic counseling, can decrease the possibility of recurrent cases in families, and can enhance patient clinical follow-up [5]. The alpha-1 chain of type II collagen is a fibrillar collagen found in cartilage and vitreous humour of the eye. Osteoarthritis, skeletal dysplasia, knee osteoarthritis, congenital toxoplasmosis and stickler syndrome, and many other conditions are associated with mutations in the COL2A1 gene [6].

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The current study has focused on assessing the role of COL2A1 protein expression in Triple Negative Breast cancer patients, learn immunohistochemistry technique, and correlated with clinical and pathological parameters as well as diseases status.

MATERIALS AND METHOD:

Patients Characteristics

This retrospective study was approved by Institutional Scientific Review Board and Ethics Committee, included 100 triple-negative breast cancer patients treated at Gujarat Cancer and Research Institute during the time period of 2015 to 2018. Detailed clinical history of patients like age, menopausal status, disease stage, histopathological findings, treatment offered, and disease status was recorded from the case files maintained at the Medical Record Department of the Institute. Disease staging was done according to AJCC classification. Patients subjected to Neo-adjuvant treatment and HIV/HCV/HBsAg positive patients were excluded from this study.

Immunohistochemical localization

Localization of markers COL2A1 expression was analysed by immunohistochemistry, which was performed on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Ventana, USA). Primary antibody COL2A1 was procured commercially from Assay Genie. The primary and secondary antibodies were incubated as follows: COL2A1 for 32 minutes at 37ºC with dilution 1:200, and HRP multimer for 8 minutes.

Scoring

Two individual observers scored the sections under microscope. Nuclear staining pattern was noted for COL2A1. The percentage of positive tumor cells corresponded to the following scores: i)0, staining in <1% of tumor cells; ii) +1, staining in 1-30%; iii) +2, staining in 31-60%; and iv) +3, staining in >60% of tumor cells. For statistical analysis COL2A1 0 and +1 were clubbed as negative and +2 and +3 were clubbed as positive.

Statistical analysis

Statistical analysis was carried out using SPSS statistical software version 27 (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). P values ≤ 0.05 were considered to be statistically significant.

RESULT

Patient characteristics with outcome

This retrospective study included 100 patients, 66% had age ≤ 50 years, whereas 34% patients had age >50, and 68% patients had post-menopausal status. In relationship to pathological characteristics, more than 50% were of T2 tumor size, positive lymph node status, early stage, IDC subtype, diseases grade III, and high BR score (Table 1). The primary treatment offered to the patients was surgery followed by adjuvant chemotherapy and radiotherapy. The maximum follow-up period was 108 months with a median follow-up of 34 months.

Table 1: Correlation of COL2A1 expression with clinicopathological parameters and diseasestatus

parameters		COL2A1 expression		
	N (%)	Negative	Positive	P
		N (%)	N (%)	
Age (years)	**100 (100)**	**58 (58)**	**42 (42)**	
≤ 50	66 (66)	36 (54)	30 (45)	0.49
> 50	34 (34)	22 (65)	12 (35)	
Menopausal status	**100 (100)**	**58 (58)**	**42 (42)**	
Pre-menopausal	32 (32)	20 (62)	12 (37)	0.65
Post-menopausal	68 (68)	38 (56)	30 (44)	
Tumor size	**50 (100)**	**58 (58)**	**42 (42)**	
T1 (≤ 2 cm)	08 (08)	08 (100)	00 (00)	0.17
T2 (≥ 2 cm to ≤ 5 cm)	72 (72)	38 (53)	34 (47)	
T3 (≥ 5 cm)	16 (16)	08 (50)	08 (50)	
T4 (≥ 10cm to ≤ 20cm)	04 (04)	04 (100)	00 (00)	
Lymph node status	**100 (100)**	**58 (58)**	**42 (42)**	

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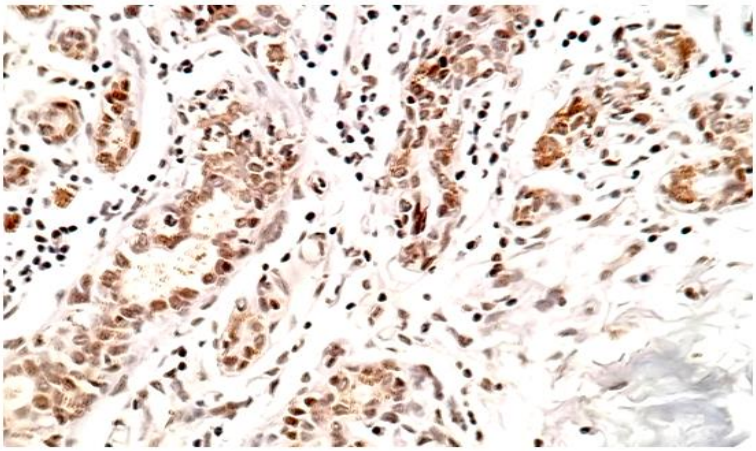
Negative	34 (34)	14 (41)	20 (59)	**0.08**
Positive	66 (66)	44 (67)	22 (33)	
Stage	**100 (100)**	**58 (58)**	**42 (42)**	
Early (stage IIA+ IIB)	58 (58)	32 (55)	26 (45)	0.63
Advanced (stage IIIA+ IIIC)	42 (42)	26 (62)	16 (38)	
Histopathology	**100 (100)**	**58 (58)**	**42 (42)**	
IDC	96 (96)	56 (58)	40 (42)	0.34
IDC + DCIS	02 (02)	00 (0)	02 (100)	
ILC	02 (02)	02 (100)	00 (0)	
Histological grade	**100 (100)**	**58 (58)**	**42 (42)**	
Grade I	04 (04)	00 (00)	4 (100)	0.14
Grade II	42 (42)	22 (52)	20 (48)	
Grade III	54 (54)	36 (67)	18 (33)	
BR score	**90 (100)**	**52 (58)**	**38 (42)**	
Low (5)	04 (04)	00 (00)	04 (100)	**0.08**
Intermediate (6-7)	34 (34)	16 (47)	18 (53)	
High (8-9)	52 (52)	36 (69)	16 (31)	
Disease status	**100 (100)**	**58 (58)**	**42 (42)**	
Remission	50 (50)	20 (40)	30 (60)	**0.01**
Relapse	50 (50)	38 (76)	12 (24)	
Disease status	**100 (100)**	**58 (58)**	**42 (42)**	
Alive	90 (90)	50 (56)	40 (44)	0.29
Death	10 (10)	08 (80)	02 (20)	

COL2A1 expression

Nuclear expression of COL2A1 was noted in 42% of the cases of TNBC patients (Figure 1). No significant correlation of COL2A1 expression was observed with age and menopausal status. A trend of higher incidence of COL2A1 expression was noted with lymph node-negative patients and low BR score of tumors (p=0.08) which indicated loss of COL2A1 associated with LN metastasis and advancement of tumor grade (Table 1). Significant higher incidence of COL2A1 expression was noted in patients with disease remission (p=0.01), suggested loss of COL2A1 associated with disease metastasis (Table 1).

Figure 1: Positive nuclear staining of COL2A1 in TNBC patients (40X)



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COL2A1 expression in relation to survival

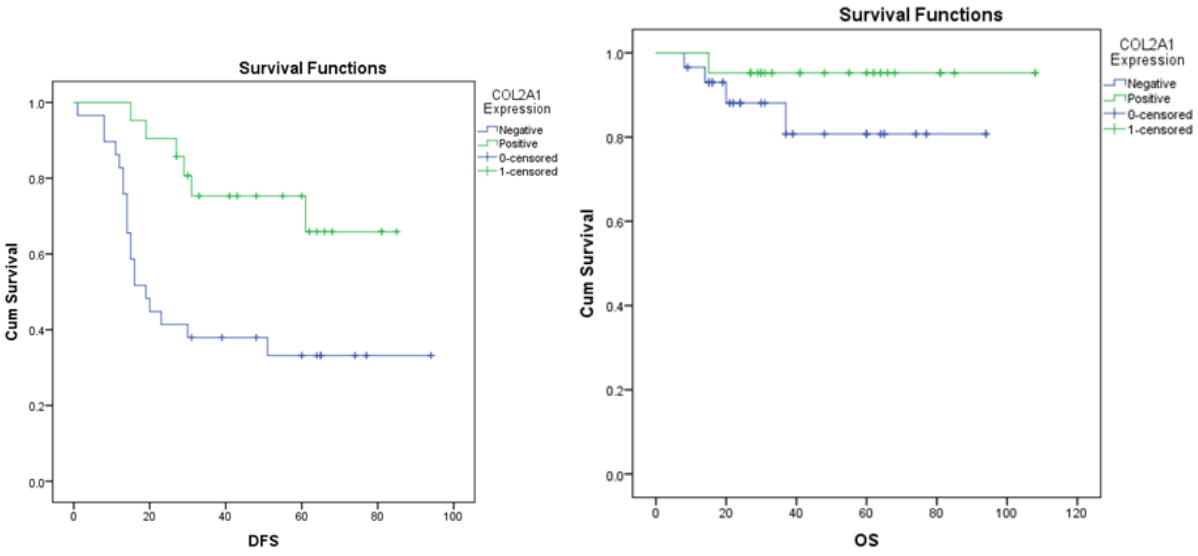
According to Kaplan and Meier univariate survival analysis, with respect to DFS, a significant higher incidence of disease relapse was noted in COL2A1 negative patients (66%, 38/58) than in COL2A1 positive patients (29%, 12/42). While with respect to OS, a trend of similar incidence of death was noted in COL2A1 negative patients (14%, 08/58) and COL2A1 positive patients (05%,02/42) (Table 2, Figure 2B)

Table 2: Univariate analysis of COL2A1 expression

COL2A1 expression	**N**	**DFS in months Mean ± SE**	**Remission N (%)**	**Relapse N (%)**
Negative	58	42.65 ± 4.98	20 (34)	38 (66)
Positive	42	67.77 ± 4.20	30 (71)	12 (29)
			Log rank = 16.59, df = 1, p = 0.000	
COL2A1 expression	**N**	**OS in months Mean ± SE**	**Alive N (%)**	**Dead N (%)**
Negative	58	80.36 ± 4.45	50 (86)	08 (14)
Positive	42	103.57 ± 3.05	40 (95)	02 (05)
			Log rank = 1.446, df = 1, p = 0.229	

Figure 2:(A) Kalpan – Meier survival analysis for disease-free survival (DFS)

(B) Kalpan – Meier survival analysis for overall survival (OS)



(A) **(B)**

DISCUSSION:

In the present retrospective study, COL2A1 exhibited nuclear staining and 42% (42/100) of COL2A1 was accounted in TNBC patients. While during the study of gastric cancer Yan Z et al., (2013) have been observed a higher incidence of COL2A1 expression with positive patients (75.9%, 44/58) [6]. When COL2A1 expression was correlated with clinical parameters like age and menopausal status, there are no significance association was observed with this study. Further, COL2A1 was correlated with pathological parameters, with tumor size, a higher incidence of COL2A1 expression was noted in T2 tumor size and T3 tumor size as compared to the T1 tumor size and T4 tumor size. In relation with the lymph node status, this study showed a trend of higher incidence of COL2A1 expression was noted in patients with lymph node-negative status than lymph node-positive status. Further, in relation with stage, higher incidence of COL2A1 expression was noted with IDC, ILC, and IDC+ DCIS subtype. This study represents similar incidence of COL2A1 expression was noted in patients with early stage of disease as compared to patients with advanced early stage of disease. Correlation with histological grade showed a higher incidence of COL2A1 expression in well-differentiated tumours as compared to

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moderately differentiated and poorly differentiated tumours**.** In relationship to BR score, this study showed trend of higher incidence of COL2A1 expression was noted in patients with low BR score tumours as compared to intermediate BR score tumours and high BR score tumours. In the present study, a significance higher incidence of COL2A1 expression was noted in patients with remission than patients with relapse. Similarly, COL2A1 expression was higher in alive patients than in dead patients. The disease-free survival and overall survival analysis was carried out by kalpan and meier univariate survival analysis with respect to DFS, a significant higher incidence of diseases relapse was noted in COL2A1 negative patients. While respect to OS, during this study similar incidence of death was noted in COL2A1-negative patients. This study suggested COL2A1 expression was associated with favourable parameters suggesting good prognostic indicator in TNBC. During the study of ovarian cancer, Ganapathi MK et al., (2016) have been observed higher incidence of COL2A1 expression was independently associated with DFS [7].

In conclusion, COL2A1, a type II Collagen Alpha 1 Chain which adds structure and strength to connective tissue. Loss of COL2A1 expression was associated with positive lymph node status, advancement of tumor grade, and disease metastasis. Further, loss of COL2A1 protein expression was associated with worse prognosticator predicting reduced associated with disease-free survival of TNBC. This indicates association of loss of collagen II protein with disease aggressiveness in TNBC.

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