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Expression of Collagen type XI alpha 1 Gene as A Potential Biomarker of Breast Cancer

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Abstract

Background: Breast cancer is one of the most common types of cancer in the world. Collagen type XI alpha 1 (COL11A1) is an important component of collagen. It is involved in the regulation of multiple biological processes, including cell proliferation, differentiation and migration. COL11A1 is overexpressed in breast cancer and also predicts poor prognosis. In Bangladesh, this sector is still less explored and this study aims to find out the expression of the COL11A1 gene in female breast cancer patients. **Objective:** To determine the expression of the COL11A1 gene as a potential biomarker of breast cancer. **Methods:** A cross-sectional study was carried out in the Department of Biochemistry, Dhaka Medical College, Dhaka in collaboration with the Institution for Population and Precision Health, the University of Chicago, USA, from July 2022 to June 2023. In this study, 34 diagnosed female breast cancer patients were selected from the Department of General Surgery, Dhaka Medical College Hospital, Dhaka by purposive sampling. After informed written consent from each participant, 0.5 cm3 normal tissue and 0.5 cm3 breast tumour tissue was collected after modified radical mastectomy (MRM) in two separate Eppendorf, which contained DNA/RNA shields as a preservative. The tissue sample was sent in a biohazard bag temperature below (-80°c) to the University of Chicago, USA on dry ice for the subsequent laboratory procedures. Data was analyzed by Statistical Package for Social Science (SPSS) version 26. **Results:** In this study, median and IQR values of COL11A1 gene expression were significantly higher in tumor tissue compared to normal tissue of breast 3.91 (1.95–4.34) vs 4.19 (2.56–4.81), p=0.043. The expression of the COL11A1 gene in tumor breast tissue showed a significant positive correlation with the age onset of menarche (p=0.001) and a significant negative correlation with age (p=0.023). **Conclusion:** This finding implies that the COL11A1 gene is a biomarker of breast cancer. It might provide better information for the diagnosis, treatment plan and prognosis of breast cancer patients, and it has a significant positive correlation with the age of onset of menarche.

Keywords: Collagen type XI alpha 1 (COL11A1), Modified radical mastectomy (MRM)

Introduction

Breast cancer is a leading cause of cancer-related deaths among women worldwide.[1] Breast cancer affects an estimated 1.4 million people worldwide each year, with more than half of the 400,000 deaths happening in low- and middle-income countries.[2] Breast cancer has emerged as a major public health concern in both developed and developing countries.[3] Breast cancer remains the most common terrible cancer among women in Bangladesh. It has become a hidden burden, accounting for 69% of women's deaths. In Bangladesh, the incidence of breast cancer is predicted to be 22.5 per 100,000 females of all ages, with the most common age range being 15 to 44 years.[4] Breast cancer development is influenced by both hereditary and environmental factors.[5] Breast cancer has an insidious etiology, with few associated clinical symptoms and good diagnostic tools available early on. Statistics show that the majority of newly diagnosed breast cancers have progressed.[6] Currently, the comprehensive treatment options advocated around the world, which include primarily surgical treatment augmented by

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chemotherapy, endocrine therapy and targeted therapy have not demonstrated excellent therapeutic outcomes.[7] As a result, identifying new prognostic indicators and therapy targets for breast cancer is a critical issue that must be addressed during the diagnosis and treatment process.

COL11 A1 is the alpha 1 chain of type XI collagen, and its coding gene is found in band 1, region 2 of the short arm of chromosome 1. More and more research suggested that collagen, as an interstitial component of solid tumors, serves as a scaffold for tumor cell proliferation, invasion, and metastasis.[8] Given that COL11 A1 is strongly linked to breast cancer formation and progression, medication resistance, and a poor prognosis. There is also evidence that COL11 A1 is implicated in the development of breast cancer.[9] The level of COL11 A1 in breast cancer patients with positive lymph node metastases is more than 100 times higher than in primary breast cancer. The increased expression of the COL11 A1 gene in invasive ductal carcinoma suggested that it contributed to the progression from ductal carcinoma in situ to invasive ductal carcinoma. Additionally, COL11 A1 has been linked to malignant relapses of breast cancer.[10] Basic principles of microarray are base-pairing and hybridization. DNA microarray platforms for gene expression profiling were invented relatively recently, and breast cancer has been among the earliest and most intensely studied diseases using this technology. The new molecular profiling has enriched our understanding of breast cancer heterogeneity and yielded new prognostic and predictive information.[11] This analysis can be used for all aspects of the condition; including start, progression, invasion, and treatment resistance to elucidate the underlying molecular pathways.[12]

Materials and methods

Study design, study population, sample size and sampling

This descriptive cross-sectional study was conducted from July 2022 to June 2023 Department of Biochemistry, Dhaka Medical College, Dhaka in Collaboration with the Institution for Population and Precision Health, Department of Public Health Sciences, The University of Chicago, USA.

In this study, thirty-four (34) diagnosed female breast cancer patients were selected by purposive sampling technique. Evaluation of patients was done by history, clinical examination, mammography, FNAC, histopathology & immunohistochemistry reports from the Department of General Surgery (Breast Clinic), Dhaka Medical College & Hospital (DMCH) was selected as the study population. Patients receiving or receiving treatment for breast cancer and a history of any other cancer were excluded.

Data collection

The data was collected through face-to-face interviews with the patients. Before proceeding with the data collection, each respondent properly explained the details of the study, and written consent was taken from them. The collected data was checked and verified at the end of each day's work.

The questionnaire was developed by using the selected variables according to the specific objectives and was developed from a review of qualitative and quantitative literature for relevant items.

Tissue sample collection and preservation

After taking informed consent, collection of 0.5 cm^3 normal breast tissue and 0.5 cm^3 tumor breast tissue from the breast cancer patients after modified radical mastectomy (MRM) in two separate DNA/RNA shields containing Eppendorf that were preserved at room temperature (15-25°c) for further analysis. After getting national & international permission, tissue samples were sent by a biohazard bag at a temperature below -80°c to the University of Chicago on dry ice for the subsequent laboratory procedures like RNA extraction, library preparation, target enrichment, targeted RNA sequencing and gene expression.

Laboratory Procedure

RNA was extracted by using Quick-DNA/RNA™ Microprep Plus Kit. Quality control and concentration were measured by using Nanodrop 1000. Targeted RNA sequencing was done by using custom custom-designed Twist next-generation sequencing (NGS) Kit and measured at a concentration (ng/ul). Data was analyzed by microarray technique.

Data analysis

After completion of data collection, the data were checked and edited manually and verified before tabulation. Then data were coded, entered and analyzed by using a statistical package for the social sciences (SPSS) version 26 & the level of expression of the COL11A1 gene was observed by the Mann-Whitney U test and expressed as p-value (<0.05) level of significance. The correlation of the COL11 A1 gene with sociodemographic variables is assessed by the Spearman correlation test and expressed as a p-value (<0.05) level of significance.

Ethical considerations

For conducting the study, ethical clearance for the study was taken from the ethical review committee, Dhaka Medical College to review the scientific and ethical issues related to the research to obtain the required approval. The study did not seem to pose any potential risk. The nature, procedure, purpose, risk, and benefits of the study were explained in detail to the study subjects followed by taking informed written consent from them.

Results

Comparison of expression of COL11 A1 gene

Table 01 shows that a significant difference (p=0.043) is observed in the tumor breast tissue compared to the normal breast tissue of the study subjects.

Table 01: Comparison of expression of COL11 A1 gene between normal and tumor tissue of the breast of the study subjects (N=34)

COL11A1	Normal (n=34)	Tumor (n=34)	p-value*
Median IQR (25th to 75th percentile)	3.91 1.95–4.34	4.19 2.56–4.81	**0.043**

*p-value obtained by the Mann-Whitney U test, p<0.05 considered as a level of significance.

Comparison of the expression of the COL11 A1 gene between normal and tumor tissue of the breast shows that median and IQR values were significantly higher in tumor tissue compared to normal tissue of the breast **[Figure 01]**.

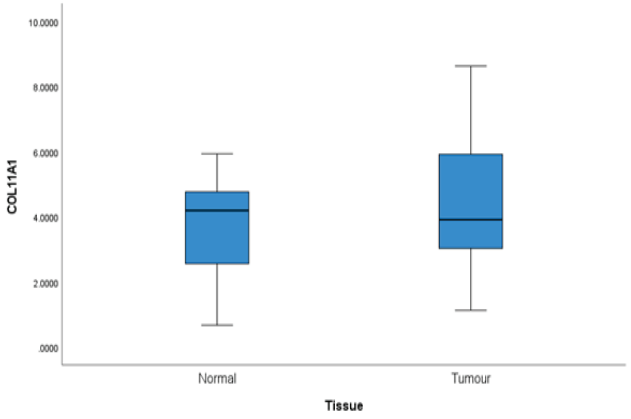

Figure 01: Whisker plot showing Comparison of expression of COL11 A1 gene between normal and tumor tissue of breast (N=34).

Correlation with sociodemographic variables

Table 02 shows the expression of the COL11A1 gene in tumor breast tissue showed a significant positive correlation with the age onset of menarche (p=0.001) and a significant negative correlation with age (p=0.023). On the other hand, this observation shows an insignificant positive correlation with BMI (p 0.622) and history of breastfeeding (p=0.220) and an insignificant negative correlation with a family history of breast cancer (p=0.322), contraceptive use (p= 0.514) and history of parity (p=0.160).

Table 02: Correlation of expression of COL11A1 with sociodemographic variables (N=34)

Variables	Spearman Correlation	
	rho-value	p-value
Age (years)	-0.276*	0.023
BMI	0.061	0.622
Family history of breast cancer	-0.122	0.322
Menarche (Years)	0.403**	0.001
Contraceptive user	-0.080	0.514
Parity	-0.172	0.160
Breastfeeding	0.151	0.220

Discussion

COL11 A1 gene was considered a biomarker for the diagnosis of early breast cancer lesions. In this study, the level of expression of the COL11 A1 gene was compared between normal breast tissue and tumor breast tissue of breast cancer patients and the level of expression was statistically significant (p 0.043). Which showed that the COL11 A1 gene was significantly higher in tumor breast tissue than in normal breast tissue.

Similar findings were observed that the expression of the COL11 A1 gene is elevated in breast cancer and is associated with poor survival, chemoresistance and recurrence.[13] COL11 A1 can promote cancer progression, metastasis and drug resistance and is used as a biomarker to identify patients with breast cancer.

The COL11 A1 gene was shown to be elevated in breast cancer, and it is also linked to resistance to treatment and cell invasion. Additionally, a high expression of COL11 A1 tends to predict distant metastases, a poor prognosis, and a short overall survival.[14] This closely resembles the findings of our investigation.

According to another study overexpression of the COL11 A1 gene has been demonstrated to increase chemoresistance; however, it is yet unclear how COL11 A1 functions in breast cancer stemness, tumor dormancy, inflammation, and recurrence; which contrasts with the current study.[15]

Conclusion

This study revealed that the COL11A1 gene is overexpressed in tumor tissue of breast cancer patients. So, it's a breast cancer biomarker. It may aid with the diagnosis, treatment strategy, and prognosis of breast cancer patients. It is significantly associated with breast cancer development, differentiation, progression, drug resistance, and a bad prognosis. Additionally, COL11A1 has been associated with malignant relapses in breast cancer. The COL11A1 gene correlates considerably positively with the onset of menarche and negatively with age.

Limitations

- The sample size was small and it does not reflect the picture of the entire country
- The sample was taken purposively, so there may be a chance of bias which can influence the result
- Time and resource constraints
- Highly expensive

Recommendations

- Further study with a large sample size should be carried out and appropriate designs are warranted
- For high-risk breast cancer populations, COL11A1 genetic testing may be performed

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