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Research Article

A hospital Based Observational Study to Identify Risk of Breast Cancer Associated Pten and Its Pseudogene (ptenp1) Levels in Patients Underwent Surgery

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ABSTRACT
Background: The tumour suppressor gene, PTEN, has previously been demonstrated to be involved in breast tumorigenesis and tumor progression. PTEN Gene expression is regulated by its processed pseudogene PTENP1 (Phosphatase and Tensin Homolog Pseudogene 1), PTEN Gene expression is also regulated by PTEN targeting small noncoding RNAs such as microRNAs (miRNAs), and by sense and antisense transcripts of the PTENP1 long noncoding RNA. To activate or repress PTEN gene transcription, several transcription factors bind directly to the PTEN gene promoter. Methods: This hospital based observational study was conducted on 24 breast cancer patients underwent surgery at tertiary care centre of North India. The eligible recruited study participants according to AOCG inclusion criteria were enrolled for the present study. Blood samples were extracted and later serum was isolated at 5000xg for 30 min. Cancerous and non-cancerous tissue specimens were also taken out from the same participant. Serum and tissue levels of PTEN and its pseudogene PTENP1 were measured using the ELISA method. A detailed questionnaire was used to fill the information. Risk factors for determining their poor health outcomes were analyzed using bivariate and multivariate Cox-proportional hazard regression models. Results: The level of tumour suppressor gene, PTEN in tissue, was abnormally expressed in the breast carcinoma tissues and serum compared to non-carcinoma tissues was found to be statistically significant (177.4+23.52 v/s 289.5+31.33; $p<0.001$) and 68.26+8.42 v/s 179.65+42.17) respectively. The risk factors that were contributed to poor health outcome and this transition includes educational status (AHR 1.58, 95%CI: 1.12, 2.29), low income (AHR 1.73, 95%CI: 1.19, 2.57) and age (AHR 1.39, 95%CI: 1.06, 1.88). Conclusion: The results of the present study concluded that due to non-awareness of risk factors associated with poor health outcome in patients with breast cancer includes age, education and income. These results indicate that PTEN and its pseudogene PTENP1 are important for the tumorigenesis, development and prognosis of breast cancer. Government driven awareness program should be integrated with robust training of households for eradicating breast cancer.

Keywords: PTEN Gene, PTENP1 Pseudogene, sncRNAs, miRNAs, lncRNAS, Sense and Antisense transcript, Ubiquitination, Epigenetic regulation, Chromatin Remodelling Complexes, Histone Modifying Enzymes.

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INTRODUCTION

Breast cancer is among the most common malignant tumors, with its prognosis influenced by various internal and external factors (1-3). Some contributing factors include an unhealthy lifestyle, socio-psychological, and environmental influences. Previous studies indicate that 5% to 10% of breast cancer cases are linked to genetic mutations and family history. However, 20% to 30% are due to modifiable factors within breast cells (4). Cancerous tumors consist of cells that have the capacity to spread and invade surrounding tissue. Breast cells rarely undergo disruptions that impair their normal function and homeostasis. In contrast, non-cancerous breast conditions may involve atypical hyperplasia and cysts, which are associated with changes that lead to benign tumors, such as intraductal papilloma's (4). According to recent data from the World Health Organization (WHO), 2.3 million women worldwide were diagnosed with breast cancer by 2022, contributing to an estimated 670,000 deaths (5). Extensive research has been conducted to investigate the pathogenesis and genetic interactions involved in the regulation and metastasis of breast cancer. In addition to other factors, genetic interactions play a major role in breast cancer development and metastasis. Among these, the phosphatase and tensin homolog deleted on chromosome 10 (PTEN), also known as mutated in multiple advanced cancers 1 (MMAC1), and its pseudogene (PTENP1) play a critical role in breast cancer cell proliferation, migration, and survival (7-8). Previous studies have shown that PTEN dephosphorylates PIP3 to PIP2, negatively regulating cellular homeostasis through the pro-proliferative and anti-apoptotic PI3K/Akt pathway (8-9). Conversely, reduced PTEN levels contribute to malignancy, with its expression, function, and cellular half-life being regulated at transcriptional, post-transcriptional, and post-translational levels (10-11).

MATERIAL AND METHODS

Study Design

This hospital based observational study was conducted on 24 breast cancer patients underwent surgery at tertiary care center of Kanpur Nagar district of North India. According to the census of Kanpur held in the year 2011, out of the total Kanpur Nagar population for 2011 census, 65.83 percent lives in urban regions of district. In total 3,015,645 people lives in urban areas of which males are 1,622,546 and females are 1,393,099.

Inclusions Criteria

1. AOCG breast cancer screening guideline.
2. Patients giving consent

Exclusions Criteria

1. Minor less than 18 years of age
2. The disagreement of written consent by patients.

Sampling Procedure and Data Management

The study subjects were selected from tertiary care centre of North India. Surgeons along with the trained personnel collected the data from breast cancer patients who underwent surgery. The outcome of this study is to identify the risk factors of breast cancer associated PTEN and its Pseudogene. Local language was implemented in all interviews. Anthropometric measurements including height, weight and BMI were recorded. Data was collected on the paper-based structured questionnaire and later entered into Microsoft Excel format for statistical analysis. Written consent was obtained from all participants prior to enrollment, and the study design was explained to them in their local language. On the day of surgery, both non-cancerous and cancerous breast tissues were collected from each patient in sterile tubes containing Trizol reagent. Whole blood samples were also taken from the same patients for serum isolation. Immediately after collection in the operating room, all samples were stored at -80°C until further processing.

Outcome Variables

Outcome variables studied include risk factors of breast cancer associated PTEN and its pseudogene levels in patient underwent surgery.

Independent Variables

Independent variables were categorized into household elements such as socioeconomic status (high income/middle income/low income and slum), type of family (nuclear or joint), monthly household income, residence (urban and rural) and geographical location, and clinical history, including the patient's age, gender, tumour location, sample size, final diagnosis and tumour size, tumour grade, Nottingham score, lymph node involvement, and vascular or perineural invasion in the case of tumoral tissues.

Statistical Analysis

Statistical analysis was performed with Origin Pro software. Mean $\pm$ standard deviation (SD) and percentage were used for continuous and categorical data respectively. Chi-square test along with analysis of variance (ANOVA) was used to determine the differences between categorical variables and continuous variables respectively. Odds Ratio (OR) was calculated to determine risk factors. P values <0.05 were considered to be statistically significant.

ELISA quantification of PTEN and PTENP1

The collected blood samples were processed for serum isolation by centrifuging the blood for 30 minutes at 1500 g. Serum levels of PTEN and its pseudogene PTENP1 were measured using commercially available ELISA kits, following the manufacturer's instructions (Biosource International, CA, USA). This assay employed a quantitative sandwich enzyme immunoassay technique utilizing antibodies. The optical density was recorded at 450 nm, and the levels of PTEN and PTENP1 were calculated accordingly.

RESULTS

The median age of the study participants was 41 years and out of 24 participants, 51% of the breast cancerous tissue showed lymph node positive metastasis while 49% presented node negative distribution. The religious distribution showed that 98% of the study participants were Hindu and two percent Muslims with 82% of the participants with lower income group and 10% among middle income group and 2% among high income group. All women participants of the present study were married with around 84% have children. The results of the present study showed that 35% of the participants ever heard the term breast cancer and it was found that 94% of the women of the lower income group were not aware of the breast cancer and its associated risk factors. Nearly 78% of the women had education above high school. Awareness related to breast cancer and its risk factors were found to be more in women aged between 25-34 years compared to younger age (Table 1).

Table.1 Adjusted odds ratio of socio-demographic characteristics.

Characteristics	**Percentage**	**Adjusted Odds Ratio- With 95% C.I.**
18–24	43.4	1
25–34	53.3	1.68 (0.79–3.86)
35–44	46.7	1.59 (0.64–3.92)
45–55	50.2	2.33 (0.92–5.61)
Illiterate	33.7	1
Literate	66.3	3.89 (2.15–6.14)
Hindu	48.7	1
Non-Hindu	51.6	1.22 (0.58–2.54)
SC/ST/OBC	51.2	1
Others	47.8	1.26 (0.84–1.89)
Nuclear	50.4	1
Joint/extended	41.5	0.65 (0.42–1.11)
Not working	47.5	1
Working	57.3	1.52 (0.91–2.72)
Unmarried	45.7	1
Married	49.5	1.64 (0.76–3.52)

It was observed that literacy was found to be strong predictor of breast cancer in study population (Table 1) and it's positively correlated with breast cancer (AOR: 3.89; CI: 2.15–6.14, P<0.001). In this study, risk factors associated with the PTEN and its pseudogene was evaluated among the study participants and found that progressive age is among the major risk factor after education for breast cancer progression and expression of PTEN and its pseudogene (Table 2).

Table.2 Risk Factors awareness among breast cancer patients

Characteristics		Started menstruating at an early age (< 12 yrs)	Woman having late age at menopause	Nulliparous woman	First baby after age 30 years	Woman used oral contraceptives/ pills	Woman who is obese	Woman consumes high fat	Woman undergone hormone replacement therapy	Not given breastfeeding to the child	Family history of BC	Woman having past history of BC
Age Group (Years)	**18–24**	16.2	12.4	28.2	20.1	28.2	32.5	32.3	28.6	32.3	32.2	44.3
	25–34	5.3	13.7	25.8	22.5	20.6	22.5	41.6	12.2	50.2	32.5	44.5
	35–44	2.7	8.8	20.2	10.2	20.4	11.3	30.5	14.4	36.3	29.3	34.9
	45–55	4.8	8.5	24.5	13.5	19.6	19.4	31.4	8.6	35.5	30.3	37.5
Education	**Illiterate**	2.6	8.4	18.3	9.8	22.3	15.5	36.2	9.8	37.6	20.7	40.4
	Literate	6.4	11.2	26.4	17.5	20.5	21.2	32.8	14.9	39.4	35.3	38.5
Religion	**Hindu**	5.5	11.3	23.6	15.4	20.7	18.9	33.3	13.2	38.3	31.2	39.4
	Non-Hindu	5.3	0	27.7	16.6	22.3	27.8	33.5	16.8	44.5	27.8	33.9
Caste	**SC/ST/OBC**	5.4	16.8	22.3	11.8	22.2	16.4	36.5	11.8	40.2	22.3	39.5
	Others	5.6	7.2	24.9	17.3	20.5	20.9	32.4	14.5	38.7	35.4	39.3
Family type	**Nuclear**	5.2	9.4	22.3	16.2	20.8	18.5	30.7	13.8	34.3	32.3	36.7
	Joint/extended	7.7	15.3	32.2	12.4	22.4	22.7	50.2	10.1	62.7	25.1	52.8
Employment	**Not working3**	4.3	9.4	22.5	12.3	20.6	15.4	31.7	11.2	36.3	28.5	36.9
	Working	11.8	13.5	29.6	27.5	22.8	36.3	43.3	20.8	50.2	40.5	50.2
Marital status	**Unmarried**	14.4	11.3	31.5	25.8	22.7	25.8	34.4	27.4	34.8	40.2	45.8
	Married	4.2	10.2	22.7	13.7	20.5	18.2	33.8	11.3	39.4	29.2	38.2

The level of tumour suppressor gene, PTEN in tissue, was abnormally expressed in the breast carcinoma tissues and serum compared to non-carcinoma tissues was found to be statistically significant (177.4±23.52 v/s 289.5±31.33; $p<0.001$) and (68.26±8.42 v/s 179.65±42.17; $p<0.001$) respectively. Moreover, the levels of PTENP1 in cancerous tissue and serum were significant.

DISCUSSION

This study focused on the risk factors associated with PTEN and its pseudogene in breast cancer patients of North India. It was unfortunate that only less than two third of the study participants were aware of such risk factors of breast cancer. Previously conducted studies also showed similar pattern of awareness of risk factors of breast cancer in India and Ethopia (12-14). However, another study of same country but different region showed greater awareness related to these risk factors of breast cancer in women aged between 18-70 years of the age (15). Our observations showed that education and age played vital role in breast cancer progression and its positively correlated with the PTEN and its pseudogene and considered to be most important risk factors in breast cancer in our study population. Multivariate analysis showed education and age as positive predictors in breast cancer, similar observation was made by previously published study (12). In our study participants it was observed that awareness of risk factors was found to be poor and same pattern was observed by several published study from same country (12, 16-18). Based on substantial evidence, the progression of breast cancer is associated with the downregulation of the PTEN and PTENP1 genes, as reflected by the correlation between their expression levels and the clinical status of breast cancer patients. In this study involving 24 breast cancer patients, we found a significant reduction in PTEN and PTENP1 expression in both cancerous tissues and serum compared to normal tissues. Similar findings have been previously reported, showing markedly decreased expression of these genes in gliomas, breast cancer, and both benign and malignant epithelial thyroid tumors (19-21). This pronounced downregulation may result from methylation at the gene's transcriptional regulatory sites, as suggested by earlier studies (22-23). It was observed that very women were found to be aware of these risk factors in the society associated with breast cancer due to impairment in their biological clock and among which age is also the major predictors among them. This reduced awareness may be contributed due to low awareness related to self-breast assessment.

CONCLUSION

In conclusion, we assessed PTEN and its pseudogene at both the protein and gene levels, finding significant downregulation in cancerous tissues and serum of breast cancer patients compared to non-cancerous tissues from the same individuals. These findings suggest that limited awareness of risk factors, including age, education, and income, contributes to poor health outcomes in breast cancer patients. This highlights the critical role of PTEN and its pseudogene PTENP1 in breast cancer tumorigenesis, progression, and prognosis. To effectively combat breast cancer, government-led awareness programs should be complemented by comprehensive household training initiatives.

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Conflict of Interest Disclosures

The authors declare that they have no conflict of interest.

Ethical Statement

In all stages of this study, the principles of the Helsinki Declaration (Ethical Principles for Medical Research, including Humanities) and the Ethics Committee of the GSVM Medical College, Kanpur has been observed. This study was confirmed approved by the Ethics Committee GSVM Medical College, Kanpur.

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