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Expression of Calretinin in Carcinoma Breast in Correlation with Various Histological and Molecular Subtypes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Breast carcinoma is the second most common cancer following lung cancer. The incidence of cancer accounts for 24.2% and cancer death is about 15% among females. There are various prognostic and predictive factors for assessing the patient encountering carcinoma breast. Among the various prognostic factors, histological subtype and molecular subtype play a key role. The calretinin expression in carcinoma breast was seen in high-grade breast carcinomas and basal like subtype of carcinoma breast. Calretinin expression was predominantly noted in high grade II and III carcinomas as in other studies and other histological subtypes like metastatic carcinomas which are considered high grade tumours. The present study aimed to correlate the various clinicopathological parameters with the calretinin expression. The expression of Calretinin was seen mainly in grade II and grade III IDC- NST and metaplastic carcinomas. Among the molecular subtypes, the Basal like subtype and the Luminal B subtype showed calretinin expression. The calretinin expression was associated with tumours with a high proliferative index with Ki67 index >14%. This implies the importance of prognostic significance of calretinin expression, as cases with a high proliferative index are likely to have poor prognosis.

Keywords: Carcinoma breast; calretinin; epidermal growth factor receptor; lymph node.

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1. INTRODUCTION

Carcinoma of the breast is one of the leading causes of death worldwide. It is the second most common cancer following lung cancers. According to the statistics provided by GLOBECON 2018 carcinoma breast accounts for an estimated 2,088,849 (11.6%) new cases per year irrespective of the geographical distribution and the gender. The breast cancer death rate is estimated to be 626,679 (6.6%). Among females the incidence of breast cancer accounts for 24.2% and the cancer death is about 15% [1].

In general, the high rates of breast cancer in developed countries are the consequence of a higher prevalence of known risk factors related to menstruation (early age at menarche, later age at menopause), reproduction (nulliparity, late age at first birth, and fewer children), exogenous hormone intake (oral contraceptive use and hormone replacement therapy), nutrition (alcohol intake), and anthropometry (greater weight, weight gain during adulthood, and body fat distribution); whereas breastfeeding and physical activity are known protective factors2. These trends likely reflect a combination of demographic factors allied to social and economic development, including the postponement of childbearing and having fewer children, greater levels of obesity and physical inactivity, and an increase in breast cancer screening and awareness [1,2].

The breast cancer in male is less predominant than female accounting for about approximately 1% of all cases while causes being hormonal imbalance, radiation exposure and genetic predisposition [3-6]. The prognosis of carcinoma breast is mainly dependent upon the tumor size, histological subtype, histological grading, lympho-vascular invasion, the lymph node status and molecular sub-typing. The high grade ductal carcinoma-No Special Type (NST), solid lobular carcinoma and micropapillary carcinoma are classified with poor prognosis. The basal like subtype has shown poor prognosis [7-10].

The identification of the basal like subtype is done mainly using the immunohistochemical expression of estrogen receptors (ER), progesterone (PR) receptors, human epidermal growth factor receptor 2 (HER2) and Ki67. Various other markers like cytokeratins- CK5/6, CK14 and 17; epidermal growth factor receptor (EGFR) and Vimentin can also be used. Calretinin, an intracellular calcium binding protein that is mainly expressed in non-neoplastic and neoplastic tissue. Various studies have expressed the significance of expression of calretinin in high-grade carcinomas and basal type carcinomas and also showed association with poor survival rates [10-13].

2. MATERIALS AND METHODS

2.1 Inclusion Criteria

Lumpectomy, all mastectomies received and diagnosed as carcinoma breast by histo-pathological examination of both sexes and all age group.

2.2 Exclusion Criteria

The tru-cut and incision biopsies from the breast lump reported as carcinoma; benign breast diseases. Patients who have undergone surgery, post-chemotherapy or radiation therapy as this might modify the morphology and antigen expression.

2.3 Sample Size

32 cases of carcinoma breast which has satisfied the inclusion criteria are included in the study.

3. RESULTS

The present study included a sample of 32 patients diagnosed as carcinoma of breast fulfilling the inclusion criteria. All of them had undergone lumpectomy, mastectomy or modified radical mastectomy as first line of treatment. The data was analyzed for the following parameters namely sex distribution, age, laterality, histopathological subtypes, positive lymph nodes, histopathological grade, and molecular subtypes statistically and represented in pie charts, bar and diagrams.

The study showed that the carcinomas incidence was higher in female patients with 96.87% (31 cases) and male patients with 3.12% (1 case) incidence.

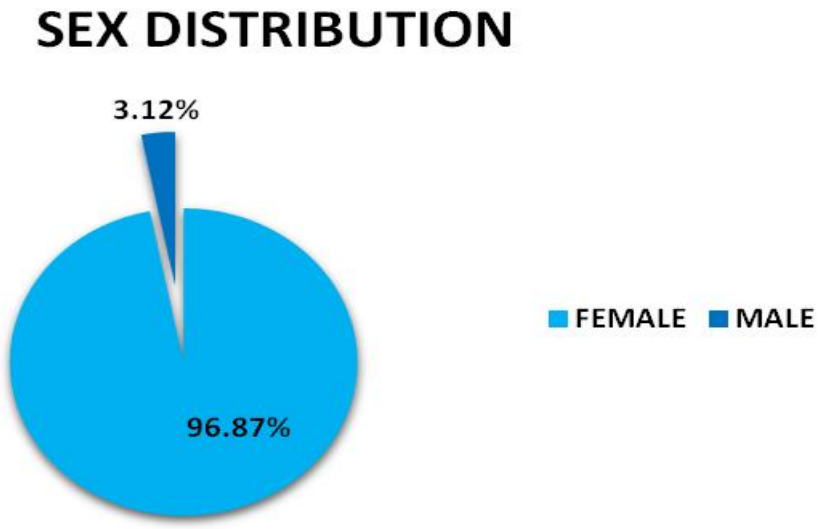
3.1 Laterality

Out of the 32 patients, 18 of them had left breast involvement, out of which 12 were showing negative, 4 were weak positivity, 1 moderate positivity and 1 strong positivity for Calretinin expression. 14 patients had right breast involvement out of which 8 of them were

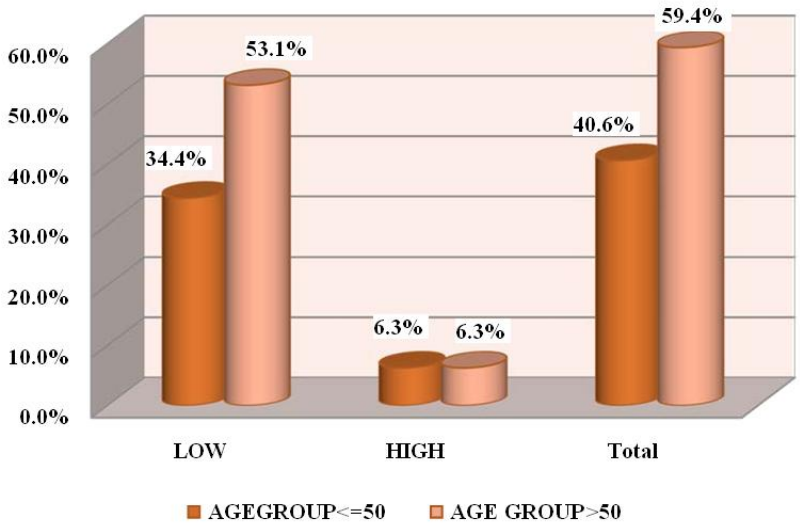
negative, 4weak positivity, 1 moderate positivity and 1 strong positivity for Calretinin expression. Statistical analysis found out the association was not significant with P value (0.788).

3.2 Histopathological Subtypes of Carcinoma Breast

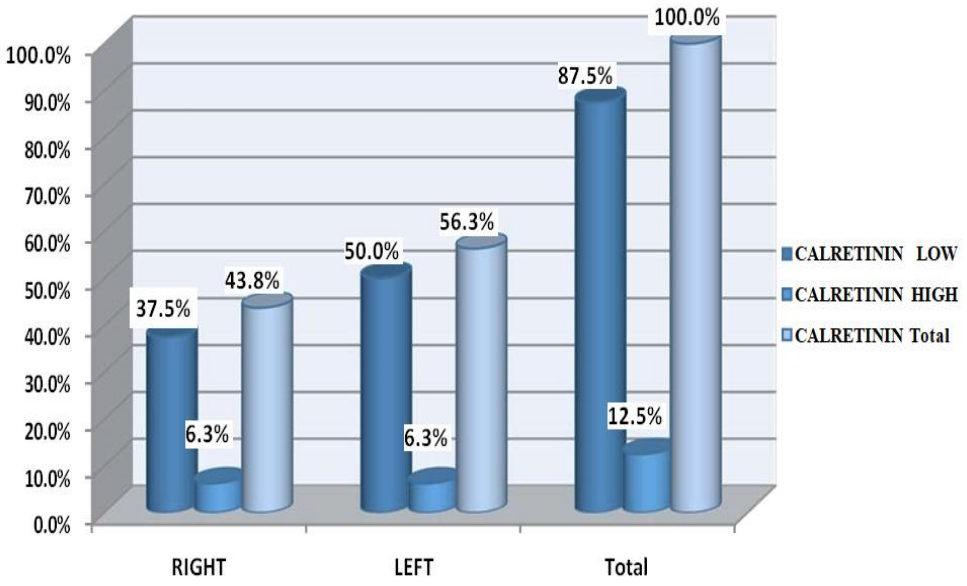
The carcinoma breast showed a spectrum of diagnosis histopathologically. They were mainly classified as non-invasive and invasive carcinoma. The non-invasive mainly included DCIS (1-Solid variant, 1-Comedo type)which contributed 3 cases among the 32 cases diagnosed constituting 9.4% among which 2 cases showed low positivity and 1 case showed negative Calretinin expression. The invasive carcinoma includes Invasive ductal carcinoma-NST constituting 22 cases -69% followed by metaplastic carcinoma constituting 4 cases – 12.5%, one case each of Mucinous carcinoma, cystic papillary carcinoma and Mixed ductal and lobular carcinoma constituting 3.1% each.



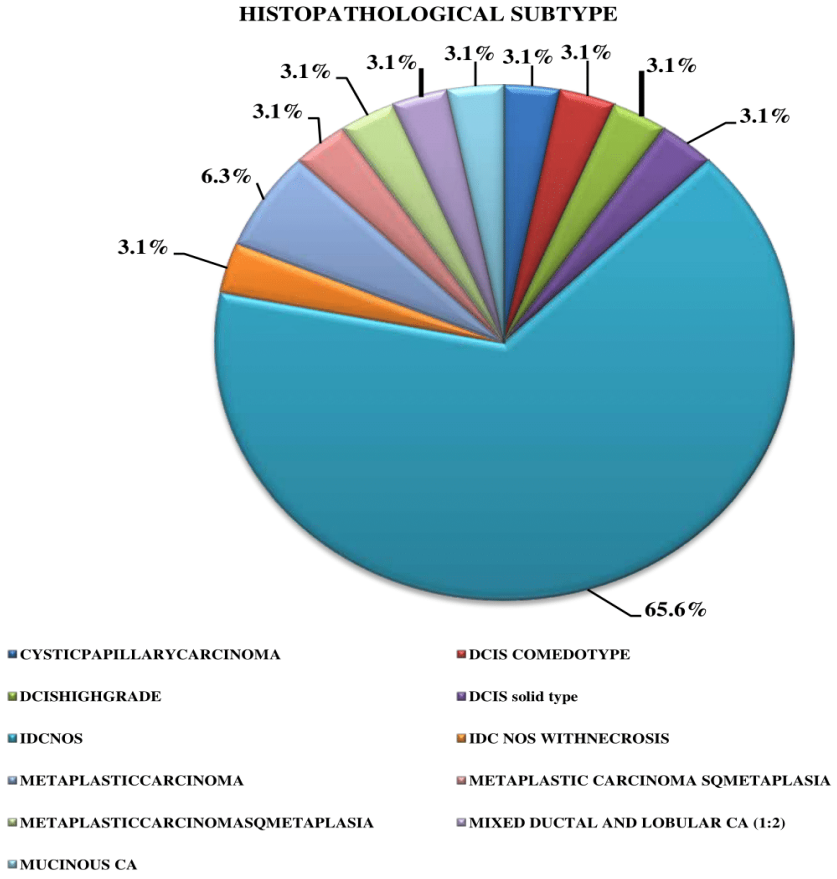
Graph 1. Sex distribution among carcinoma breast



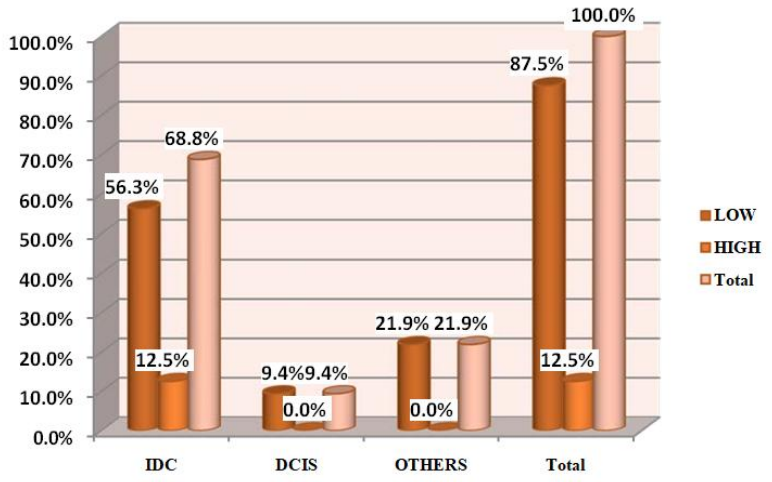
Graph 2. Correlation of calretinin expression with age



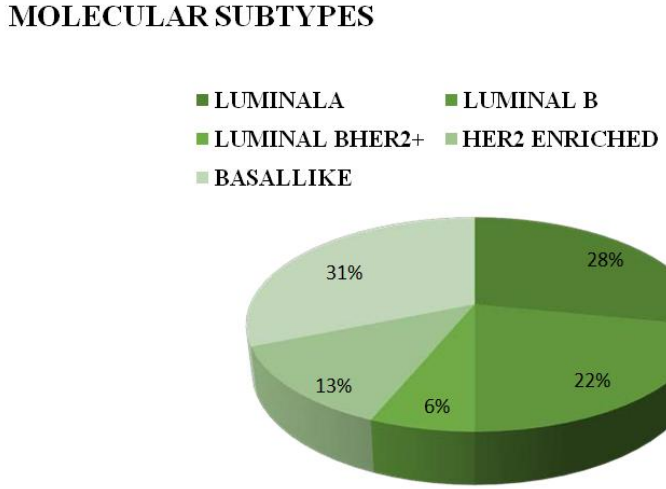
Graph 3. Correlation of calretinin expression andlaterality



Graph 4. Histopathological spectrum of carcinoma breast



Graph 5. Correlation of calretinin expression with histopathological subtypes of carcinomabreast



Graph 6. Spectrum of molecular subtypes of carcinoma breast

Among the 22 cases of IDC 14 cases showed negative calretinin expression, 4 cases showed weak positivity, 1 moderate positivity and 3 cases showed high positivity which includes one case of IDC with necrosis. Among the 4 metaplastic carcinoma 2 cases showed low calretinin expression. The cystic papillary carcinoma, mucinous carcinoma and the mixed ductal and lobular carcinoma showed negative calretinin expression. Statistical analysis found out the association was not significant with P value (0.354).

The histological grading is done based upon the three parameters- tubule formation, nuclear pleomorphism and the mitotic rate as discussed before. Accordingly, the invasive ductal carcinomas are gaded I, II and III. Among grade I tumours 3 cases showed calretinin negativity. Among grade II tumours 9 cases showed calretinin negativity, 3 cases showed weak calretinin positivity, 2 cases showed moderate positivity and 2 cases showed high positivity. Among grade III tumours 2 cases showed negativity, 1 case showed high positivity. Statistical analysis found out that the correlation was not significant with P- value 0.400.

3.3 Hormone Receptor

All the 32 cases were stained and tested for estrogen receptor and progesterone receptor

positivity. 16 cases showed estrogen positivity and 12 cases showed progestrone positivity. Two cases showed ER negative and PR positive which according to few studies is considered technical artifacts57,58,59 and thus the case diagnosed as DCIS high grade showed high PR positive (5+3) and ER negative was considered a technical artifact and thus the case was concluded ER positive and classified under LUMINAL-A. The other case diagnosed as IDC grade III has ER negative and PR was low positive (2+1) and thus the PR staining was considered negative and the case classified under Basal like.

Among 18 cases with estrogen positivity, 8 cases showed calretinin expression and among the 12 cases with progesterone positivity, 5 cases showed calretinin positive.

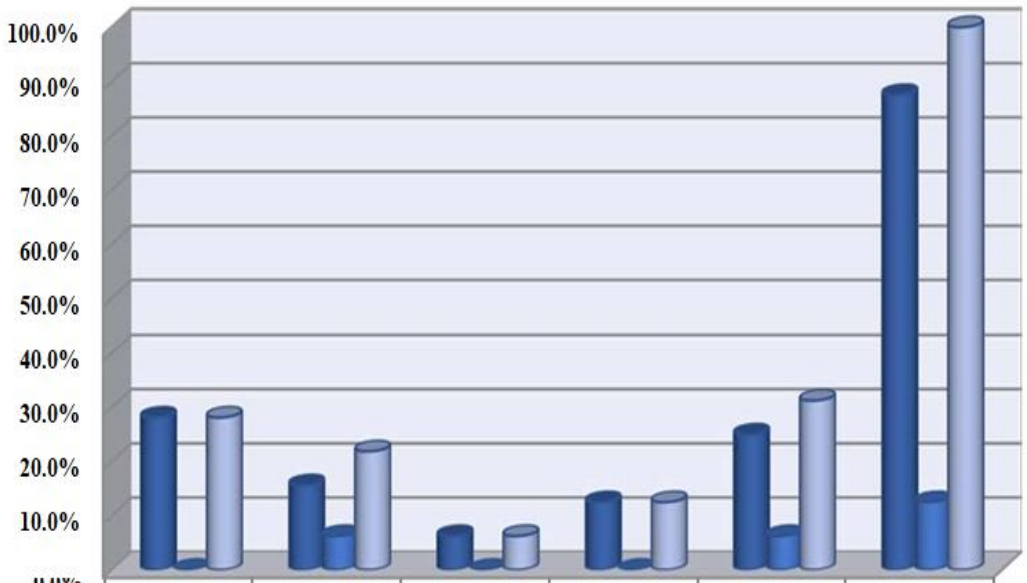
3.4 HER2 Receptor

HER2 showed positivity in 6 cases. Among these 4 cases were HER2 enriched and 2 cases were Luminal BHER2 positive and among 6 HER2 positive cases 1 case showed calretinin positivity.

3.5 Proliferative Index

The proliferative index was done using Ki67 immunohistochemistry. The cases with a nuclear positivity of >14% were considered high and <14% was considered low. 19 out of 32 cases showed high value and 13 cases showed low value. Out of the 19 cases with high proliferative rate 9 cases show calretinin expression. Out of the 13 with low proliferative index, 3 cases showed calretinin expression.

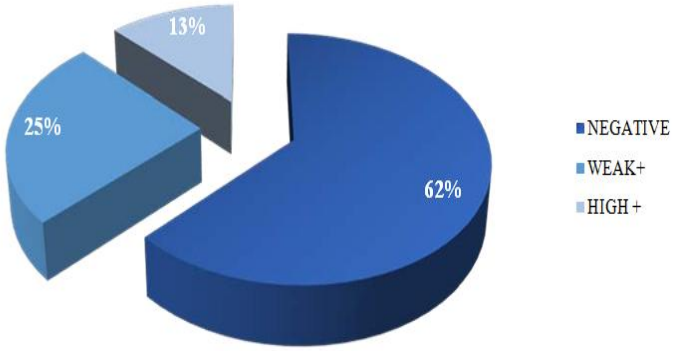
Calretinin expression is interpreted and noted and was compared with various other parameters like age, laterality, lymph node positivity, histopathological subtypes, and molecular subtypes. The calretinin shows negativity in 20 cases, low positive in 8 cases, moderate positive in 2 cases and strong positive in 2 cases. A total of 28 cases are classified under low positive and 4 cases under high positive.



	LUMINAL A	LUMINAL B	LUMINAL B HER2 +	HER2 ENRICHED	BASAL LIKE	TOTAL
LOW	28.1%	15.6%	6.3%	12.5%	25.0%	87.5%
HIGH	0.0%	6.3%	0.0%	0.0%	6.3%	12.5%
TOTAL	28.1%	21.9%	6.3%	12.5%	31.3%	100.0%

Graph 7. Correlation of calretinin expression and molecular subtypes of carcinoma breast

CALRETININ:



Graph 8. Frequency of calretinin expression

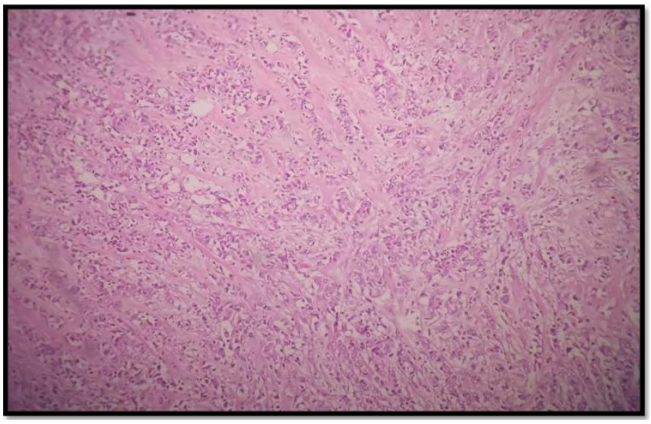


Fig. 1. Invasive ductal carcinoma-NST- grade III 100x

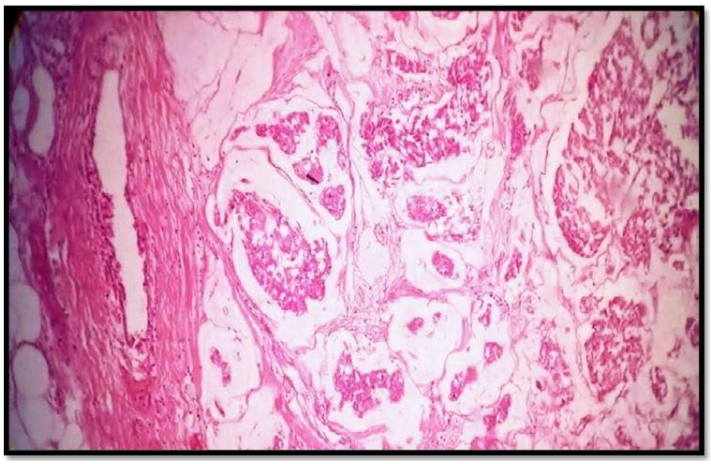


Fig. 2. Mucinous carcinoma- low grade- 100x

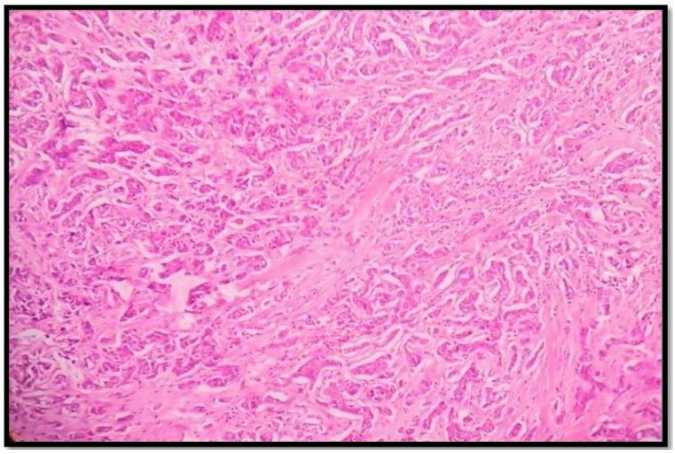


Fig. 3. Metaplasticcarcinoma-100x

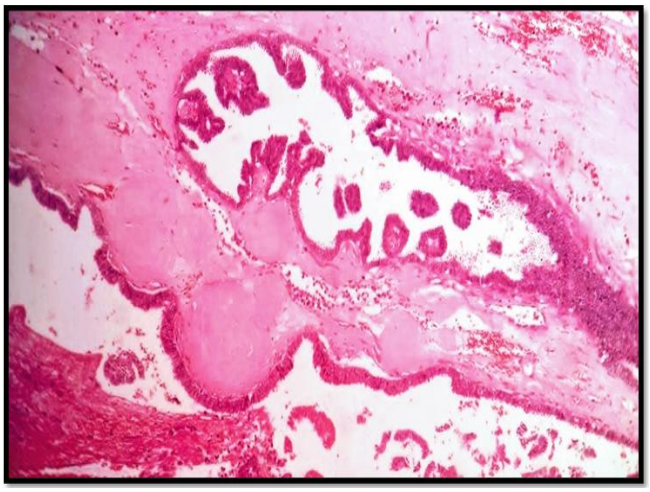


Fig. 4. Cystic papillary carcinoma-100x

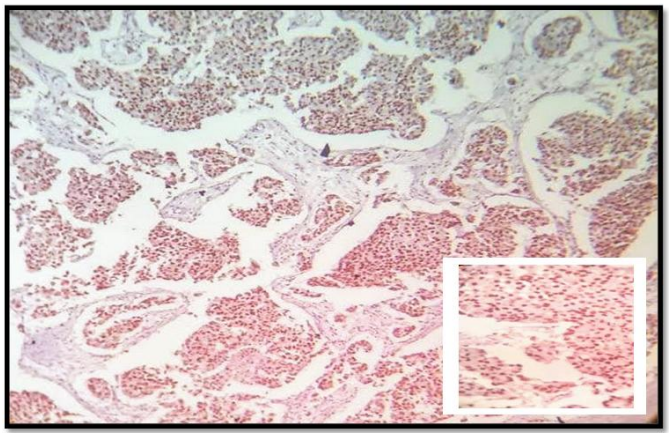


Fig. 5. 100x-estrogn receptorimmunohistochemical nuclear positivity (ALLRED 5+3). Inset-high power 400x

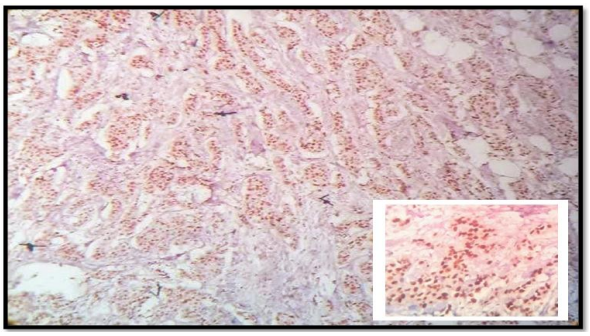


Fig. 6. 100x-progestrone receptorimmunohistochemical nuclear positivity (ALLRED 5+3). Inset-high power 400x

4. DISCUSSION

In our present study the age of the patient and the expression of calretinin was correlated. Low expression of calretinin was seen more in patients >50 years of age. The high expression of calretinin was seen equally in patients in 50 and <50 age groups. The was no significant correlation (P=0.542) and thus both are considered independent parameters. A study conducted by Paz et al. [14] has shown the mean of 50 years at the time of diagnosis of carcinoma breast among the 100 cases studied. A study conducted by Cherry Bansal et al. [15] has shown the mean of 55 years among the 45 cases studied.

Another study conducted by Han sunkang et al. [16] has showed the mean age of 46.8 years at the time of diagnosis of carcinoma breast. Our study results were consistent with other studies.

The study by Aboobacker et al. [17] also showed no significant correlation between age and Calretinin expression (p>0.05). Another study by Faraag et al. [14] showed that calretinin expression is independent of age of the patient.

The various other studies showed that the age of the patient is independent of calretinin expression which is also observed in our present study.

In our present study, the maximum numbers of patients were diagnosed as IDC –NST, 22 out of 32 cases. 3 cases of DCIS were noted and the other types include Mucinous carcinoma, metaplastic carcinoma, cystic carcinoma and mixed ductal and lobular carcinoma. The expression of calretinin was seen maximum in IDC cases with 18 cases showing low positivity and 4 cases showing high positivity. All the 4 metaplastic carcinoma showed low positivity. A significant correlation could not be established (P= 0.354) probably due to sparse distribution of other subtypes.

The study by Aboobacker et al. [17] had a total study population of 30 cases with 28 cases of IDC and 2 cases belonging to other subtypes. The study show15 cases of IDC showing high positivity and 13 cases showing low positivity and no significant correlation has been shown (P=0.92). Another study by Micello et al. [18] has shown that the maximum incidence of calretinin expression was noted in Medullary carcinomas (p<0.05) and IDC (<0.001).

The study discussed by Lugli et al. [11] has showed Strong calretinin expression was found in 44.4% of medullary carcinomas, 25% of apocrine carcinomas, 14.3% of papillary carcinomas, 1.9% of invasive ductal carcinoma and 4.4% of ductal carcinoma in-situ (DCIS).Farrag et al. [19] has showed that majority of his cases of IDC showed calretinin positivity, with 65% of weak positivity and 35% with high expression and among the other cases the maximum cases showed high expression.

Our study has shown results similar to the study by Aboobacker et al. [17] as both the studies has been conducted in a low study population whereas the other studies compared shows a higher study population which can explain the significant correlation.The study by Farrag et al. [19] has showed maximum number of cases under grade I and II and among these cases maximum showed low expression and the grade III tumours, maximum showed high calretinin expression (p<0.05).

A study by Powell et al. [20] has showed that 80% of calretinin positive cases were grade III. Taliano et al. [13] have showed that among

grade III tumours 31% has exhibited high calretinin positivity and 69% shows low calretinin expression. In our study the distribution of cases among the different grades was not similar to the studies compared. A total of 3 cases of grade III could only be asses due to the low study population.

The modified radical mastectomy cases were assessed for the lymph node status. Among the 26 cases 10 cases did not have any nodal spread and among those cases 3 cases showed high calretinin expression and 7 cases showed low expression. The 3 cases with more than 10 lymph node spread showed only 1 case with high expression of calretinin. Thus there was no significant correlation found among these parameters (P=0.495).

Aboobucker et al. [17] has showed that the high expression of calretinin was seen maximally in cases with lymph node spread (p>0.05). Farrag et al. [19] have showed that the low expression of calretinin was higher in cases without lymph node spread (p>0.05).

The molecular sub typing was done based on the expression of the hormonal receptors and proliferative inde-x calculation by immune histochemistry. Our present study showed 10 cases of basal like type, 9 Luminal A, 7 Luminal B, 2 Luminal B with HER2 positivity and 4 HER2 enriched cases. Among the basal like type 2 cases showed high expression and 8 cases showed low expression and among Luminal B 2 cases showed high expression and 5cases showed low expression. Luminal B with HER2 positivity showed low expression. All the Luminal A cases showed low expression of calretinin.

Aboobucker et al. [17] has showed maximum calretinin expression of the luminal subtypes followed by the basal subtype and the least in the HER2 enriched type (p>0.05). Taliano et al. [13] has showed the maximum expression of the calretinin in the basal subtype followed by Luminal B subtype, Luminal A subtype and least in the HER2 enriched subtype and unclassified cases (p>0.05).The study by Faraag et al. [19] has showed the high expression of calretinin more in the basal subtype and the low expression was maximum in the Luminal A subtype and least in the HER2 enriched subtype.

The molecular subtypes and their prognostic significance have been well recognized through the enormous literatures reviewed. The calretinin expression is established in basal like subtype and the Luminal B subtype and low in HER2 enriched cases. Our study results are similar to other studies thus emphasizing the expression of calretinin in molecular subtypes with poor prognosis.

5. CONCLUSION

Our study certainly signifies the expression of calretinin in cases with unfavorable prognosis as defined by the histological subtype, histological grading and the molecular sub-typing which implies the prognostic significance of Calretinin expression in carcinoma breast. However there are several potential limitations to the study that could affect the interpretation of the results which is mainly the low study population which has influenced the statistical analysis of the study. Further studies with more study population can be carried out for a statistically significant analysis and a significant correlation.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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