Cytokeratin expression is unrelated to hormone receptor expression in breast carcinoma

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Abstract

Introduction: Invasive breast cancer (IBC) is a heterogeneous entity, showing distinct molecular features and biologic behaviour. Immunohistochemistry (IHC) based molecular classification has been recommended for clinical decision making. Variable expression of cytokeratins (CK) is now a major therapeutic determinant in addition to the hormone receptor status, clinical stage and grade of the tumour.
Aims and Objectives: This study was conducted to assess the expression of CK 8/18, CK 19, CK5/6 and CK 20 in patients of invasive breast carcinoma and its correlation with hormone receptor status and clinicopathological profile.
Materials and Methods: IHC staining for CK 8/18, CK 19, CK5/6 and CK 20 and ER, PR, Her2neu was applied on sections of 43 cases of carcinoma breast using avid in biotin peroxidase technique. The distribution and intensity of staining was recorded and statistical analysis was done using chi square and fischers test. P value of <0.05 was considered significant.
Observations: CK 8/18, CK 19, CK 20 and CK 5/6 was observed in 72%, 83.7%, 13.9% and 18.6% of cases respectively. There was no significant difference of various Cytokeratin expression with ER, PR and Her2neu expression. One fourth of CK5/6 positive cases also expressed ER and PR. CK8/18 correlated positively with CK 19 expression as both are luminal markers.
Conclusion: Breast cancer cases could be subdivided into different cellular phenotypes based on the expression of luminal and basal CK. There was no correlation of CK expression with hormone receptor expression. This study has further characterized the heterogeneous nature of breast carcinoma using IHC which may facilitate the physician in making patient diagnosis, prognostication and outcome.

Keywords: Breast cancer, Cytokeratin, Hormone receptor.

Introduction

Breast cancer is the most common female cancer worldwide representing nearly a quarter of all cancers.[1] Breast cancer is the leading cause of cancer among Indian females with age adjusted rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women. An increasing trend in the incidence rates of carcinoma breast has been reported from the various registries of national cancer registry project. Average age of occurrence of the breast cancer in India reveals that it occurs a decade earlier than in the Western population.[2]

Carcinoma breast is a heterogeneous disease which can be characterised into various groups based on their clinical, morphological, immunohistochemical and molecular characteristics.[3] Attention to various groups as separate entities is important due to their different clinical behaviour and progression.

In addition to ER, PR and her2neu status, variable cytokeratin expression in breast cancer is a novel prognostic marker.

The normal breast is composed of two cell layers, an inner luminal cell population and a distinct outer cell layer juxtaposed to the basement membrane, termed the basal layer.[4] The luminal and basal layers have different immunoprofile. Cytokeratins are intermediate filament forming proteins which are expressed in different combinations in these distinct epithelial cell types. Basal cells typically express CK5/6 and CK 17, while luminal cells typically express Cytokeratins 8 and 18.[5]

It has been suggested that stratified cytokeratin expressing carcinomas differ from simple epithelial keratin expressing carcinomas with respect to their morphology, hormone receptor expression, biological behaviour with respect to progression, prognosis and treatment response.[6,7] This emphasizes that in addition to the known clinicopathological parameters like histological type, grade, LN status and stage, there is a need for immunoexpression profiling of IBC to be able to define the tumour subsets which are likely to behave aggressively.

Aims and Objectives

1. To study the expression of Cytokeratins 8/18, 19, 5/6 and 20 and Estrogen receptor (ER), Progesterone receptor (PR) and HER2/Neu in patients of invasive breast carcinoma.
2. To evaluate the correlation between expression of Cytokeratins and hormone receptors with clinicopathological parameters.

Materials and Methods

The study was conducted in the Departments of Pathology and Surgery, Maulana Azad Medical College and associated Lok Nayak Hospital, over a period of 1 year. Total of 43 untreated cases of breast carcinoma were included in the study. Patients who received preoperative chemotherapy were excluded from the study.

The previous medical history was obtained which included history of menarche and menopause, parity, age at child of first birth, family history, intake of oral

contraceptive, hormone replacement therapy, and history of radiation, alcohol and tobacco. All relevant history of the lump with respect to the duration, ulceration, eczematous changes and nipple discharge was also noted.

General physical examination and a detailed local examination of the breast was done. The clinical staging was done according to AJCC.[8] The tumors were classified morphologically and histological grading was done as per modified bloom Richardson grading.

Immunohistochemistry was done by the biotin avidin technique. The various immunohistochemical markers used were: ER (rabbit monoclonal ERα antibody, clone SP1, Dako), PR (rabbit monoclonal antibody, clone PgR636, Dako), HER2/neu (mouse monoclonal antibody, clone CB11, Biogenex), CK 8/18 (mouse monoclonal antibody, clone 5D3, Diagnostic BioSystems), CK19 (mouse monoclonal antibody, clone RCK108, Dako), CK20 (mouse monoclonal antibody, clone Ks20.8, Dako) and CK5/6 (mouse monoclonal antibody, clone D5/16 B4, Dako).

Expression of ER and PR was interpreted as positive when more than 10% of tumor cells showed positive nuclear staining. Expression of HER2/neu was from scored[9] from 0 to 3. It was considered positive with a score of 2 or 3. Cytokeratin expression was considered when at least 10% of cells showed strong cytoplasmic and/or membranous staining.

The morphology and immunohistochemistry of all the cases was statistically analysed using the chi square test, Fischer's test wherever applicable and spearman rank correlation. p value of <0.05 was considered significant.

Results and Discussion

A total of 43 cases diagnosed as breast carcinoma were included in the present study. Of these cases, invasive breast carcinoma no special type (NST) was the largest category comprising 95.4% of all the cases with one case each of metaplastic carcinoma and colloid carcinoma (2.3%) (Fig. 1A, 1B). The patient's age ranged from 25 to 70 years. Mean age of the patients was 49.7 years with a standard deviation of 12.29 years and 27.9% patients were younger than 40 years of age (Graph 1). Similar finding was observed by Kakarala[10] et al where 16.2% of Asian Indian breast cancer patients were younger than 40 as compared to 6.23% of Caucasian women. The reason for the lower age in India could be underdiagnosis and underreporting in elderly population[11] therefore screening mammography should be performed at an early age.

Majority of tumors (55.8%) were in the size range of 2 to 5 cm with a mean size of 3.5 cm. Lymph node metastasis was observed in 44.2% of the cases in agreement with Mijung[12] et al with 51.9% nodal metastasis. Staging was done according to the AJCC guidelines[8]. Majority of the cases presented with stage 2 (69.8%) followed by stage 1(20.9%). Most of the cases were of T2 N0 M0 (46.5%). The findings reveal that breast cancer frequently presents with higher stage in Indian population. Kakarala[10] et al also documented that Asian women present with higher stage than Caucasians. The

reasons could be due to lack of awareness, fear of disease and psychological reasons. In developed countries, the use of routine screening mammography has led to the detection of early lesions. Whereas in India, due to lack of screening mammography and no nationwide breast cancer screening program, longer duration of the undiagnosed tumor leads to much higher stage at presentation[11].

The clinical and pathological staging was done. The cases in stage I increased from 4.6% to 20.9% with a decline in stage II cases from 83.7% in clinical stage to 69.8% in pathological staging. This is explained by the fact that not all clinically palpable lymph nodes show metastasis microscopically. Out of all cases with clinically palpable lymph node, 7 cases revealed no metastasis microscopically. These cases on microscopy showed reactive hyperplasia of the lymph node and caseating granulomas were noted in a case suggestive of concurrent tuberculosis.

On microscopy, majority of the tumors belonged to grade 2 (63.4%) followed by grade 3 (31.7%) (Fig. 2A, Graph 2). This is in agreement with Dutta[18] et al who documented 76% grade 2 tumors. Infiltrative margin was observed in 74.4% of cases. Peritumoral fibrosis was noted in 69.8% of cases while necrosis in 53.5%, calcification in 25.6% and inflammatory infiltrate in 93% of cases. The intensity of pattern of inflammatory infiltrate was graded as absent (0), mild (1), moderate (2) marked (3) and with germinal centre (4).[8] Most of the tumors (37.2%) showed grade 1 inflammatory infiltrate followed by grade 2 (30.2%) and grade 3 (23.3%) (Fig. 1C). No significant difference was obtained of lymphoplasmacytic infiltrate with tumor stage and necrosis.

The prognostic impact of adipose tissue invasion (ATI) by the tumour cells was studied and graded (Fig. 1D). It was evident in 76.7% of cases. Adipocytic infiltrate was observed in the larger tumor (p value=0.02) and in the higher stage (p value=0.034) as compared to the small size and lower stage respectively. While adipocytic infiltrate was observed in 33.3% of tumors with size <5 cm, it was observed in 83.8% of those with >5cm (Graph 3). This is attributed to the fact that the larger the tumor size, the more probability of it being invasive into adjacent adipose tissue. Similar findings have been documented by Yamaguchi[14] et al who found close association of ATI with nodal metastasis, tumor size, and patient's age. However, we could not obtain any statistical difference in tumor grade and hormone receptor status with ATI.
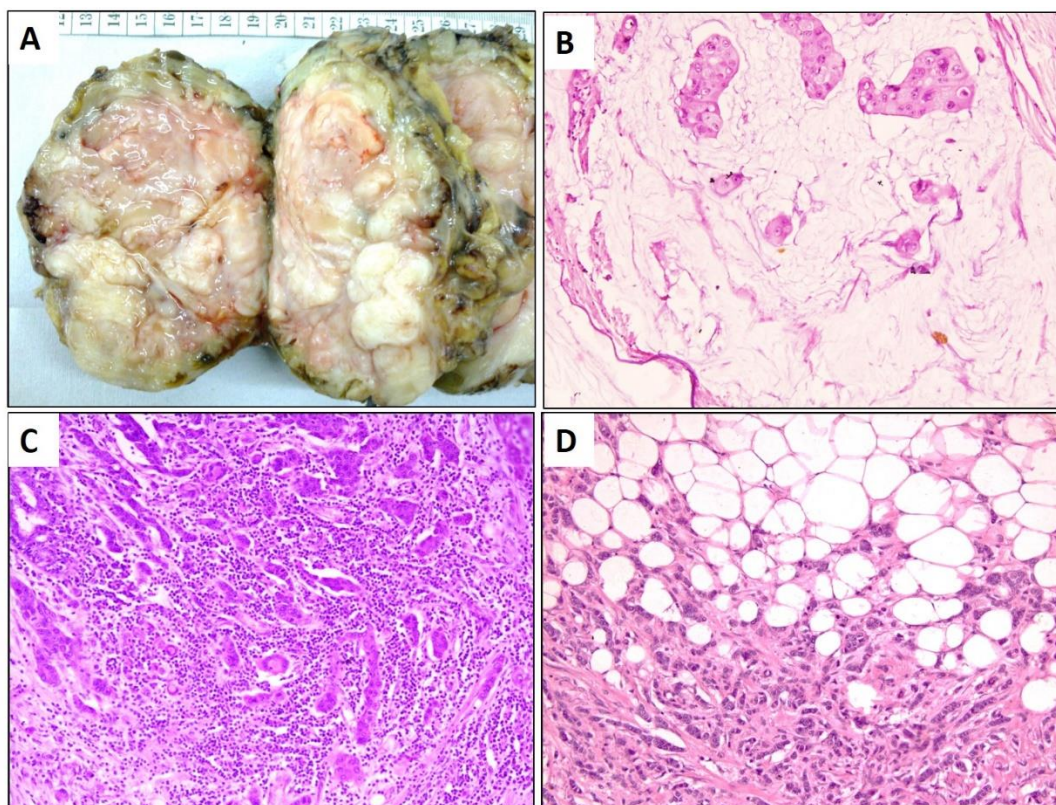
Fig. 1A: Cut section of carcinoma with lobulated grey white appearance, 1B: Colloid carcinoma showing islands of tumor cells in pool of mucin (H & E, 200X), 1C: IDC with grade 3 lymphocytic infiltrate (H & E,200X), 1D: IDC with prominent adipose tissue invasion (H & E, 100X)

Tumor Markers Expression with Clinical and Morphological Parameters

ER and PR were expressed in 30.2% and 25.6% of cases respectively (Fig. 2B, 2C). This receptor expression is similar to that described in most Indian studies. Kakarala[10] et al compared breast cancer developing in Indians/Pakistanis in the US with that of Caucasians and found a higher incidence of hormone negative tumors among Indians (30.6% Vs 21.8% in Caucasians. p value-0.0095%.).

There was an inverse association between inflammatory infiltrate and ER expression. 34% of ER negative cases revealed grade 3 or 4 lymphocytic infiltrate as compared to only 7.7% of ER positive cases (p=0.040 by Fischer's). This is supported by Suvarschala[15] et al and Kreike[16] et al who found that the infiltration of stromal lymphocytes into the tumor is reported to be predominantly present in ER negative breast carcinomas. Also, clinical stage 3 cancers had fewer inflammatory cells than stage 1 and 2 neoplasms.

A positive correlation was seen between ER and PR expression (r=0.314, p=0.04). Majority of the cases (58%) were negative for both ER and PR followed by ER positive and PR negative cases (16%). Shet[17] et al documented a hormone receptor expression of 53.5% as compared to 75-80% reported in the Western literature. This high incidence of hormone receptor negativity has been hypothesized due to younger age at presentation among Indian patients and higher histological grade[17] although other factor could be a reduced exposure to exogenous estrogens such as hormone replacement therapy and oral contraceptive pills, which leads to higher occurrence of ER negative tumors.

14% of cases expressed both ER and PR and ER&/or PR expression was observed in 42% cases. The two groups showed significant difference in respect to CK8/18 expression (p=0.037) indicating a higher expression of ER and PR in CK8/18 positive tumors. However no significant difference was obtained between these two groups, in terms of age, tumor grade, stage and nodal metastasis.

Her2 neu expression was observed in 18 cases (41.9%) which is similar to the frequency reported by Dutta[13] et al (57.2%) and Munjal[18] et al (40.2%) (Fig. 2D). Her2neu expression is higher among Indian patients in comparison to 25-30% frequency in the western literature.[19,20] This may be due to inherent higher Her2 immunoreactivity in Indian women.[18]

CK8/18 is identified as a luminal marker and was positive in 72% of cases (Fig 3A). This is in accordance with Dalia[21] et al who demonstrated 88.7% positivity and 80% expression was noted by Lerma[22] et al.

CK8/18 correlated positively with CK 19 expression (r= 0.45, p value=0.002) as both CK8/18 and CK19 are expressed in luminal epithelial cells (Graph 4). There was no significant difference of CK 8/18 expression with CK5/6, CK 20 expression and hormone receptors positivity.

We observed that one fourth of CK5/6 positive tumors were positive for both ER and PR and 35.5% of CK8/18 positive tumors expressed ER and 25% of these expressed PR (Graph 5). Similar findings were reported by Dalia[21] et al who

demonstrated higher percentage of tumours expressing CK8/18 and CK19 coexpressed ER. While only 29.8% of CK5/6 positive tumors were ER positive.

CK 19 was expressed in 83.7% of cases similar to the frequency observed by Parikh[23] (79.5%) and Dalia[21] et al (92. 8%) (Fig. 3B). No statistical significance was observed between CK 19 expression and other IHC markers.

On comparing CK8/18 and/or CK19+ cases with both CK18 and 19 negative tumors, there was no statistical difference in relation to tumor margins (p=0.6), necrosis (p=0.65), inflammatory infiltrate (p=1) and ATI (p=0.3) in the two groups.

CK5/6 is identified as basal marker and was noted in 18.6% of cases (Fig. 3C). This is in concordance with the study by Dalia[21] et al who observed CK5/6 expression in 17.6% of the cases. They also documented an inverse correlation between the luminal (CK8/18, CK19) and the basal CK5/6. However, we failed to find any significant association between luminal and basal type cytokeratins.

Multivariate analysis suggests that overall CK5/6 positive tumors are associated with poor prognosis, higher relapse and shorter disease-free interval.[24] We found that majority of CK 5/6 positive cases belonged to tumor grade 2 (62%) and pathological stage I/II (87%). In addition, there was significantly more lymphoplasmacytic infiltrate (r=0.32, p=0.035) possibly indicating a better immune response. Out of tumors that expressed CK 5/6 and Smooth muscle actin (SMA), pushing border was observed in 66.7% of cases and necrosis was observed in 66.7% of cases, although it was not statistically significant.

An inverse relation has been documented between CK5/6 positivity and ER, PR expression.[12,25] On the contrary, it has been seen in various studies that 14-45% of basal CK positive tumors still expressed at least one of ER, PR or Her2neu[26,27] but the present study did not reveal any difference of ER, PR and Her2neu expression with CK5/6 positivity. In fact, one fourth of these cases also expressed ER/PR positivity signifying that this small group of CK5/6 positive breast carcinoma is prognostically a better group. This contradictory finding reflects the difference in Indian genetic profile vis-à-vis the western counterpart.

CK 20 was expressed in 13.95% of the cases (Fig. 3D). Moll[28] et al also documented that most of the large series of breast carcinoma were negative for CK20. This infrequent staining of CK20 in sparse cells of carcinomas derived from CK20 negative tissues like breast may indicate the loss of regulatory control mechanisms in individual cells and its diagnostic tumor characterisation in such rare CK positive cells has little significance.

Interestingly, a positive correlation was observed between CK 20 and CK 5/6 expression (r=0.353, p=0.02). The reason for such an observation is not entirely clear and the prognostic significance of CK20 in breast carcinoma is still unexplored.

Tumours that expressed one or more of the luminal marker (CK8/18 and/or CK19) together with CK5/6 comprised 13.9% of all cases. These cases were referred to as basiluminal carcinoma. The occurrence of such a group points to the stem cell hypothesis of breast carcinogenesis, which can subgroup breast cancer into various phenotypes: a stem cell phenotype (CK5/6+), an intermediate glandular phenotype (CK5/6+, CK8/18+) and a differentiated glandular phenotype (CK8/18+).[21]
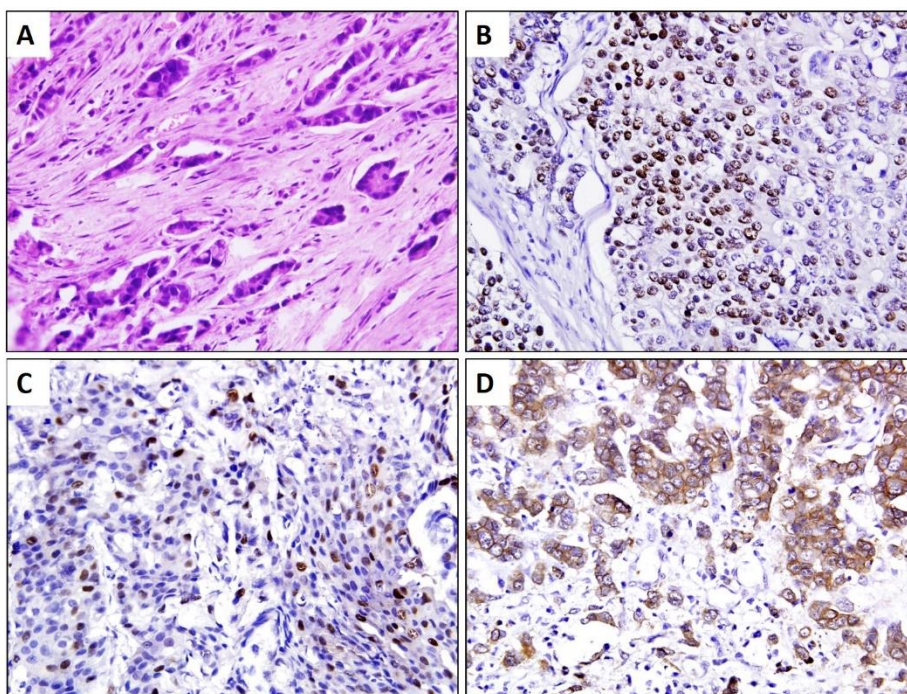

Fig. 2A: Grade 2 IDC (H & E, 400X), 2B: ER nuclear expression (streptavidin biotin, DAB, 400X, 2C: PR expression (streptavidin biotin, DAB 400X), 2D: Her2Neu expression (streptavidin biotin, DAB 400X)
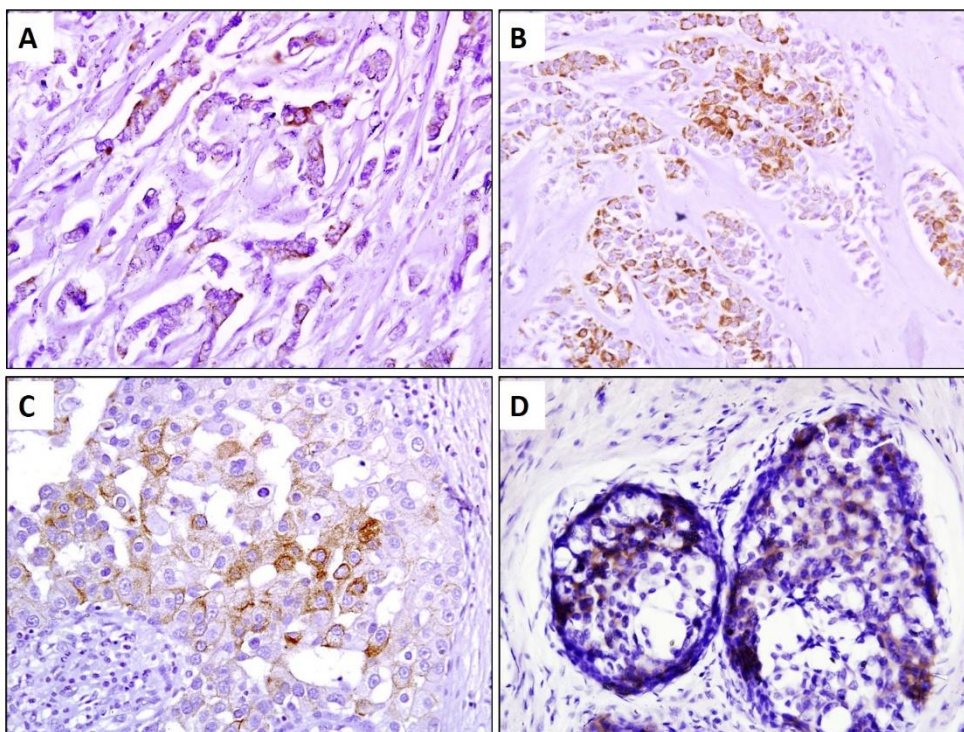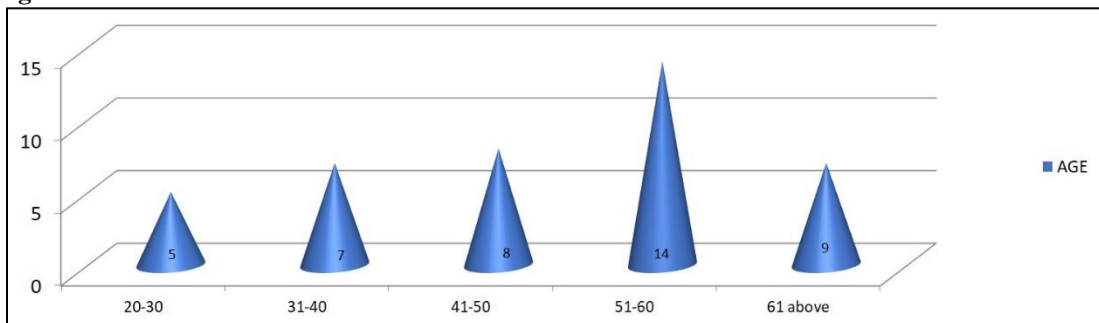
Fig. 3A: CK 8/18 expression in tumor cells (streptavidin biotin, DAB 400X), 3B: CK 19 strong membranous expression (streptavidin biotin, DAB 100X), 3C: Strong membranous positivity of CK5/6 (streptavidin biotin, DAB, 400X), 3D: CK20 expression in cribriform DCIS (streptavidin biotin, DAB, 200X)
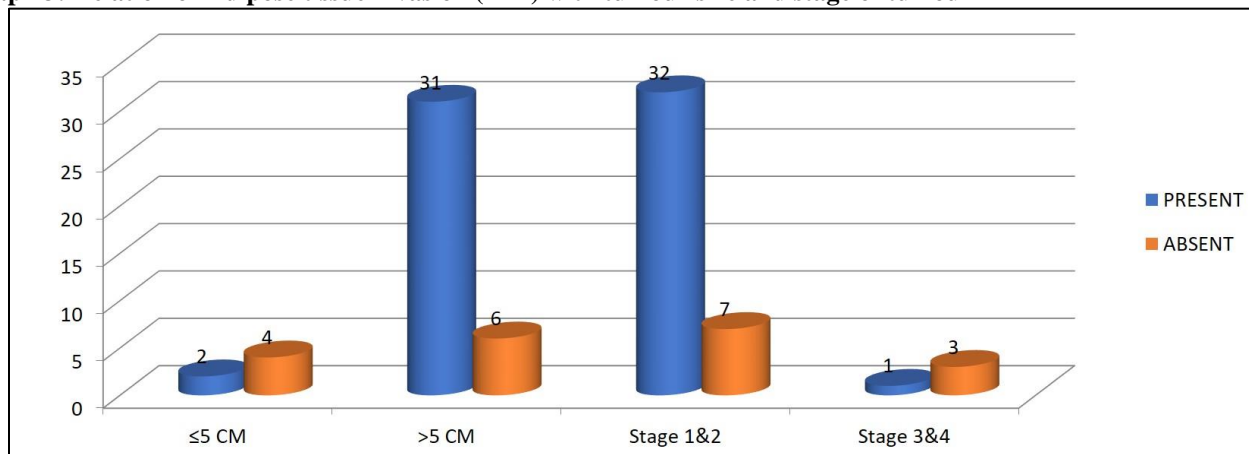
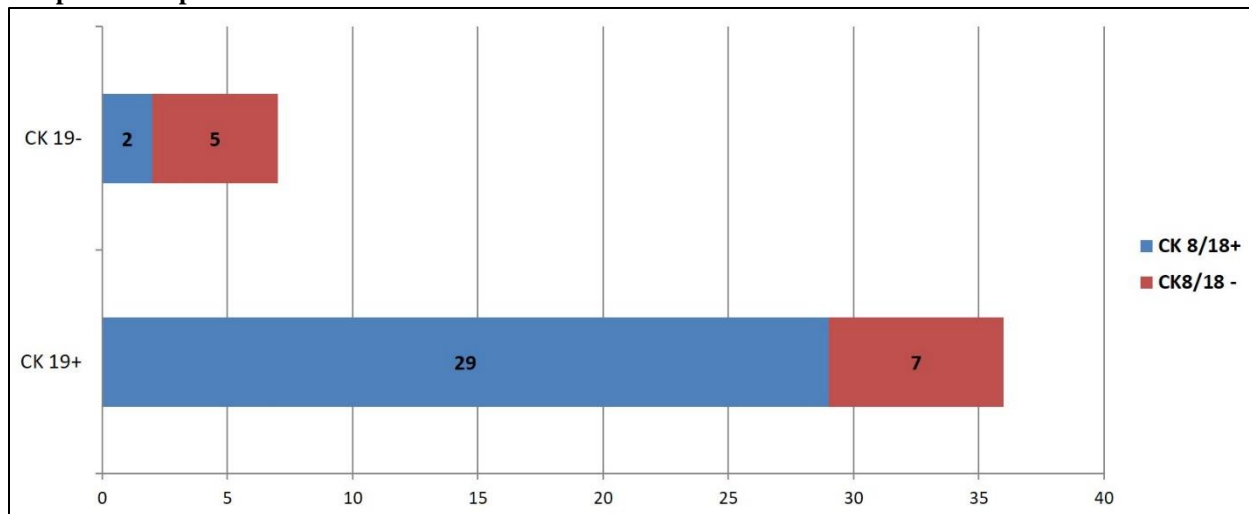**Graph 1: Age distribution of cases of IBC**

Graph 2: Distribution of IDC NST cases according to the histological grade
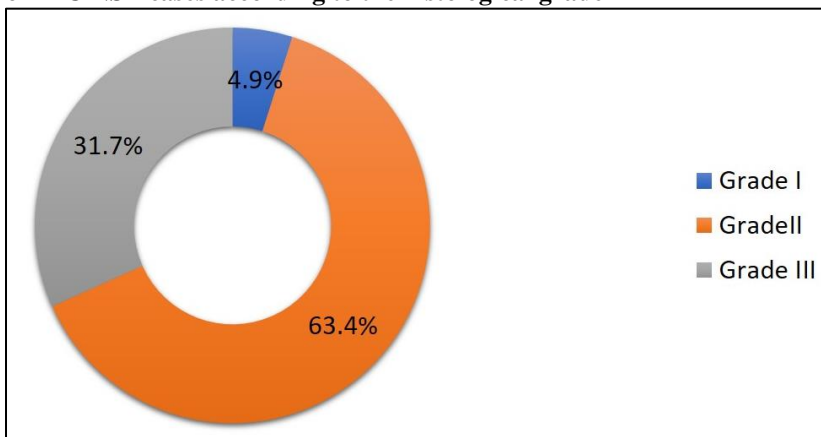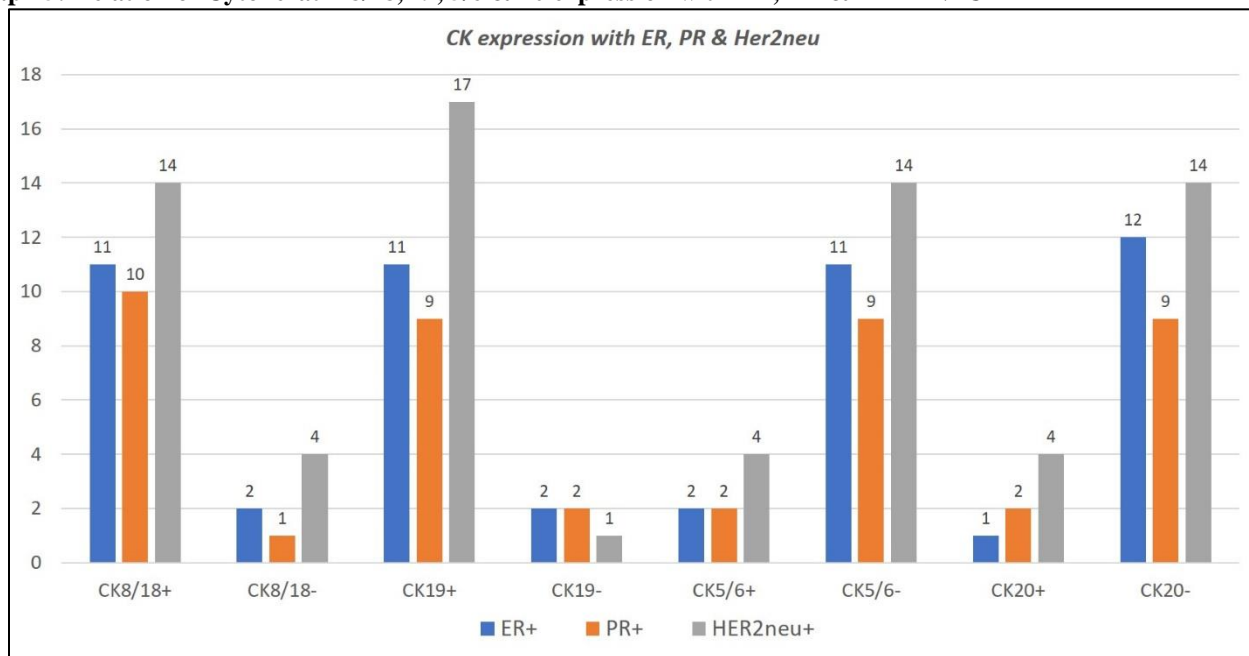
Graph 3: Relation of Adipose tissue invasion (ATI) with tumour size and stage of tumour

Graph 4: Coexpression of CK8/18 & CK 19

Graph 5: Relation of Cytokeratin 8/18, 19, 5/6 & 20 expression with ER, PR & HER2NEU

Three of the cases did not express either basal or luminal cytokeratins, however SMA expression was observed in one of the case. Thus, two cases were regarded as null phenotype. It is possible that this phenotype could reflect non-epithelial derivation or a dedifferentiation to a more primitive subclass.

On combining the results of the luminal markers together with the basal cytokeratin expression, the cases could be subdivided into four different cellular phenotypes:

1. Luminal phenotype (74.4%): which expressed one or more of luminal cytokeratin (CK8/18 or CK19)
2. Combined luminal and basal phenotype (13.9%): which were positive for one or more of luminal marker along with CK5/6.
3. Basal phenotype (7.0%): which expressed only basal cytokeratin (CK5/6) or SMA.
4. Null phenotype (4.6%): that was negative for both luminal as well as basal markers.

The cases which expressed at least one of the basal CK or SMA were identified (20.9%) separately, as this group is shown to behave differently in terms of poor survival, less disease-free interval, metastasis to brain, absence of ER and PR and overexpression of Her2.

We observed that 34.9% cases were negative for ER, PR and Her2neu and were regarded as triple negative tumors. Out of these cases, CK5/6 was expressed in 26.7% of cases and CK8/18 and CK 19 was positive in 66.7% and 73.3% respectively. This is in contrast to Rakha[29] et al who demonstrated CK5/6 and/or CK17 expression in 55.7% of cases. This may be due to less number of cases included in our study and it is possible that high expression of CK5/6 in these tumors may be responsible for poor clinical outcome. This also suggests that although majority of basal like tumors are triple negative, not all triple negative tumors express basal cytokeratin.

It is important to identify this subtype as these tumors do not respond to ER and Her2 targeted therapies and so EGFR targeted therapy is an option for some of these tumours which express EGFR.[16]

Recent cDNA gene expression analysis and Tissue microarray (TMA) IHC studies have proposed two distinguishable groups with luminal and basal phenotypes that have different cytogenetic alterations and protein expression patterns.[24] We identified four distinct profiles: luminal, combined luminal and basal, basal and null. These findings indicate different cellular profiles in breast cancer and each of these may reflect alternative pathways of epithelial differentiation during carcinogenesis.

Conclusion

Cytokeratins are used as differentiation markers in breast cancer, since their expression is thought to remain stable in carcinogenesis.[30] Breast cancer may be luminal or basal with regard to CK phenotype, with some tumors expressing both basal and luminal CK. This is supported by microarray expression profiling that classifies IBC into prognostically and clinically relevant distinct molecular subtypes. More work is required to further comprehend if the expression of these cytokeratins independently impacts the biologic behaviour and if therapeutic strategies focussing on this new classification would aid in better response to therapy.

Conflict of Interest: None.

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