Original Article

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Pathology Section

Mucinous Carcinoma of Breast: A Histopathological and Immunohistochemical Study

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ABSTRACT

Introduction: Mucinous Carcinoma (MC) is a special type of breast cancer. It comprises 4% (1-7%) of all invasive breast cancer. MC is also called colloid breast cancer characterised by nests of cells floating in lakes of mucin. It is divided into two subtypes Pure Mucinous Carcinoma (PMC) and Mixed Mucinous Carcinoma (MMC). PMC classified into two main types according to its structural and cytological features type A (paucicellular) the classical variant with a large amount of extracellular mucin, and type B (hypercellular) a hypercellular variant with less mucin and often with neuroendocrine differentiation. Hormone receptors, Human Epidermal growth factor Receptor-2 (HER2) and MUC2 status play an important role in prognosis and management.

Aim: To study histopathological features of MC breast and Immunohistochemical (IHC) status.

Materials and Methods: A retrospective study of patients who presented with breast cancer were studied at Department of Pathology, Karnataka Institute of Medical Sciences, Hubballi, Karnataka, India. The study was analysed in June 2002 and authors included data from January 2021 to June 2022. The data included the age at diagnosis, Tumour (T), Lymph Nodes (N), and Metastases (M) (TNM) stage, presence and number of Lymph Node (LN) metastases, Oestrogen Receptor (ER), Progesterone Receptor (PR), HER2 and MUC2 status. The patients in whom the diagnosis of MC of breast was given on histopathology were only included in the study. Patients of breast cancer of other pathological types were excluded from the detailed study. Descriptive statistics like mean, tables, and charts were used with the help of Microsoft office 2007 to interpret the results.

Results: A total of 245 patients reported as invasive breast cancer during 18 months of study period, out of which 12 cases diagnosed as MC were taken in study. Amongst 12 cases, eight cases were PMC and four cases were MMC. The mean age at presentation was 63.37±16.38 years (eight PMC) and 60.0±19.30 years (four MMC). A total of 11 out of 12 cases were females and only one was male case (PMC). Majority of PMC 04 (50.0%) and MMC 03 (75.0%) were observed to be in TNM Stage 2, and four of 12 cases of MC had LN metastasis with no distant metastasis. MC showed higher expression of hormone receptors and lower expression of HER2/neu and MUC2 positivity, which corroborates with other studies and concluded that, MC with such an immunohistochemistry profile was prognostically better.

Conclusion: Mucinous Breast Carcinoma (MBC) is a rare type of breast cancer accounting for about 4% of all diagnosed breast cancers. These are associated with a better long-term prognosis than other breast cancers. Hormone receptors and HER2 status play an important role in prognosis and management. MUC2 also plays a major role in mediating the proliferation, apoptosis, metastases of breast cancer cells and determining the use of chemotherapy drugs.

Keywords: Human epidermal growth factor receptor-2, Mixed, Oestrogen receptor, Progesterone receptor, Pure

INTRODUCTION

The MC is a special type of breast cancer that presents with a large amount of extracellular mucin. It comprises 4% (1-7%) of all invasive breast cancer [1]. MC is also called colloid breast cancer characterised by nests of cells floating in lakes of mucin partitioned by delicate fibrous septae containing capillary blood vessels [2]. It is divided into two subtypes: PMC and MMC [3]. Hormone receptors and HER2 status play an important role in prognosis and management [4].

Mucins are high molecular-weight glycoproteins having oligosaccharides attached to serine or threonine residues of the mucin core protein backbone by O-glycosidic linkages. MUC2 expression is associated with the less aggressive biological properties of MC than invasive ductal carcinoma through the production of abundant extracellular mucin forming the characteristic configuration, since MUC2 is a gel-forming secretory mucin [5]. PMC classified into two main types according to its structural and cytological features: type A (paucicellular), the classical variant with a large amount of extracellular mucin, and type B (hypercellular), a hypercellular variant with less mucin and often with neuroendocrine differentiation [6]. MUC2 is not expressed in the normal breast, however expressed in 1/3rd of Ductal Carcinoma In-situ (DCIS) and Lobular Carcinoma In-situ (LCIS) lesions and in invasive breast cancer and reviewed in implicating MUC2 in cancer progression [7].

Study Objectives

- To study pathologic features of MC of breast.
- To determine features of PMC and MMC.
- To understand immunohistochemical role of MC of breast with literature correlation for prognosis.

MATERIALS AND METHODS

A retrospective study of patients, who presented with breast cancer was studied at Department of Pathology, Karnataka Institute of Medical Sciences, Hubballi, Karnataka, India. The study was analysed in June 2002 and authors included data from January 2021 to June 2022. All procedures performed in the current study were approved by Institutional Ethical Committee in accordance with the 1964 Helsinki declaration and later amendments (IEC Approval number 412/2020-2021, Date 22/01/2021).

Inclusion and Exclusion criteria: The patients in whom the diagnosis of MC of breast (both PMC and MMC) was given on histopathology were only included in the study. Patients of breast cancer of other pathological types were excluded from the detailed study.

The data included the age at presentation, gender of the subjects, TNM staging, LN metastases based on histopathological data, ER, PR, HER2 (HER2/neu), and MUC2 status. All the cases satisfying inclusion and exclusion criteria were included in the study.

Study Procedure

A brief history of illness was obtained from request forms. The specimens were than fixed in 10% neutral buffered formalin. Sections from representative areas were taken and paraffin blocks were made following standard protocol. Four micron sections were cut-stained with Haematoxylin and Eosin (H&E) according to standard procedures.

Diagnosis of MC was rendered based on histological features, i.e., nests and clusters of tumour cells floating in pools of extracellular mucin. IHC stains (supplied by PathnSitu Biotechnologies) were applied to evaluate ER, PR, HER2/neu, and MUC2 status. More than 1% nuclear expression of ER and PR in tumour cells was taken positive. For HER2/neu, strong and complete membranous expression in more than 10% tumour cells was labelled as positive. MUC2 positivity was indicated when more than 10% of tumour expressed. In addition, subcellular localisation of MUC2 expression was assigned as follows: luminal/apical, luminal/apical +cytoplasmic, and membranocytoplasmic expressions.

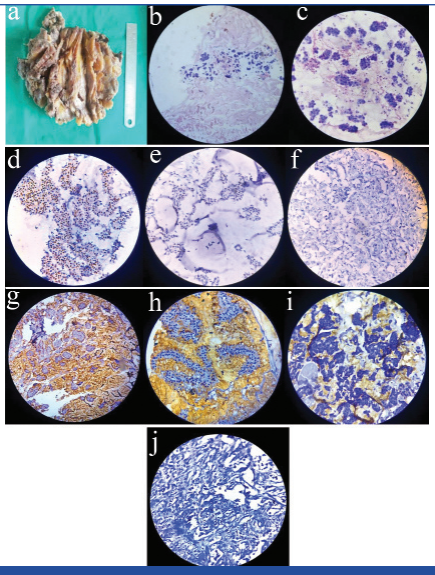
STATISTICAL ANALYSIS

Data was entered into Microsoft excel data sheet and was analysed using Statistical Package for the Social Sciences (SPSS) software, version 22.0 (IBM SPSS Statistics, Somers NY, USA). Normality of the continuous data, was tested by Kolmogorov-Smirnov test and the Shapiro-Wilk test. Continuous data was represented as mean and standard deviation. Categorical data was represented in the form of frequencies and proportions. Independent t-test was used as test of significance to identify the mean difference between two quantitative variables. Chi-square test was used as test of significance for qualitative data. The p-value (probability that the result is true) of <0.05 was considered as statistically significant, after assuming all the rules of statistical tests.

RESULTS

A total of 245 patients reported as invasive breast cancer during 18 months of study period, out of which 12 cases diagnosed as MC were taken in study. Then specimen was examined grossly and microscopically [Table/Fig-1].

Amongst 12 cases, 8 cases were PMC (66.66%) and 4 cases were MMC (33.33%). The mean age at presentation was 63.37±16.38 years among the cases with PMC, and 60.00±19.30 years among the cases with MMC. Eleven out of 12 cases (91.6%) were females and remaining 1 case (8.3%) was male, who got diagnosed with PMC. There exists no association of age at presentation and gender with respect to type of MC. TNM staging of PMC Grade one was one case with pT1c pN0 pM0. Grade two had four cases with pT2 pN0 pM0. Grade three had no cases. Grade four had three cases pT4 pNx pM0, pT4 pN0 pM0, pT4b pN1 pM0, respectively. MMC grade one and grade three no case was present. Grade two had three cases pT2 pN0, pT2a pN3a, pT2 pN1, respectively. Grade four had one case pT4b pN1a pM0. Majority of PMC (4 out of 8; 50.0%) and MMC (3 out of 4; 75.0%) were observed to be in TNM stage two. The study found no association between type of MC and TNM staging [Table/Fig-2].



[Table/Fig-1]: Mucinous Breast Carcinoma (MBC). (a) Gross image of breast showing well-circumscribed mass of 2.5x2.5x2 cm with gelatinous cut surface; (b) H&E-staining (100x) showing nests of tumour cells floating in pools of extracellular mucin; (c) H&E-stained section (400x) showing tumour cells with mild nuclear atypia; (d) ER-staining (400x, IHC) showing diffuse strong expression of tumour cells; (e) PR-staining (400x, IHC) depicting a strong nuclear expression in tumour cells; (f) HER2/neu (400x, IHC)-staining revealing lack of any membranous positivity. MUC2 staining patterns: (g) Luminal/Apical (400x); (h) Luminal/Apical+Cytoplasmic (400x); (i) Membranocytoplasmic(400x); (j) Negative (400x).

Parameters	Categories	Pure mucinous carcinoma (PMC) (n=08)	Mixed mucinous carcinoma (MMC) (n=04)	p-value
Age at presentation (in years)	Mean±SD	63.37±16.38	60.00±19.30	0.757[a]
Gender	Male	01 (12.5%)	0	0.460[b]
	Female	07 (87.5%)	04 (100%)	
TNM staging	1	01 (12.5%)	0	0.634[b]
	2	04 (50%)	03 (75%)	
	3	0	0	
	4	03 (37.5%)	01 (25%)	
LN Metastasis (Histopathological)	Positive	01 (12.5%)	03 (75%)	0.030*[b]
	Negative	07 (87.5%)	01 (25%)	
ER	Positive	08 (100%)	04 (100%)	-
	Negative	0	0	
PR	Positive	08 (100%)	04 (100%)	-
	Negative	0	0	
HER2/neu	Positive	0	0	-
	Negative	08 (100%)	04 (100%)	
MUC2	• Luminal/Apical	04 (50%)	0	0.029*[b]
	• Luminal/ Apical+Cytoplasmic	03 (37.5%)	0	
	• Membranocytoplasmic	01 (12.5%)	02 (50%)	
	Negative	0	02 (50%)	

[Table/Fig-2]: The comparison of characteristics in Pure Mucinous Carcinoma (PMC) and Mixed Mucinous Carcinoma (MMC).
a) Independent t-test; b) Chi-square test, *Statistically Significant

Based on histopathological data, 4 out of 12 cases (33.3%) had LN metastasis with no distant metastasis, where majority of PMC were LN negative for metastasis (7 out of 8; 87.5%), while majority of MMC were LN positive for metastasis (3 out of 4; 75.0%). The study also established association between type of MC and LN metastasis based on histopathological data [Table/Fig-2].

The ER and PR were positive and HER2/neu was negative in all the cases, irrespective of the type of MC. This suggested that all the MC cases presented with significant number of receptors for both oestrogen and progesterone, while negative for HER2 [Table/Fig-2].

On analysing the expression of MUC2, majority of PMC were luminal or apical (4 out of 8; 50.0%), while 3 out of 8 were luminal or apical+cytoplasmic (37.5%), and remaining 1 was membranocytoplasmic (12.5%). Among the cases with MMC, 2 out of 4 were membranocytoplasmic (50.0%), and remaining 2 were MUC2 negative (50.0%). Hence, the study showed statistically significant association between type of MC and MUC2 expression [Table/Fig-2].

DISCUSSION

The MC of the breast is rare in clinical practice and includes approximately 4% (1%-7%) of all invasive breast cancers [1]. Pure mucinous tumours have a good prognosis. Mixed mucinous cancers have a poor prognosis [3]. In the present study, we found that the proportion of MC is low, as only 12 cases of MC were identified compared to 245 cases of invasive breast cancer in the same study period. However, MC was noted to have better prognostic characteristics, such as lower tumour grade. Moreover, MC showed a prognostically better IHC profile, i.e., higher expression of hormone receptors and lower expression of HER2/neu and MUC2 positivity.

It is more common, especially in the peri-menopausal and postmenopausal age groups [2]. In the present study, the mean age at presentation was 63.37±16.38 years in PMC cases, which was comparatively higher than that in MMC cases with mean age of 60.00±19.30 years. This is similar to the studies such as Bagga PK et al., and Marrazzo E et al., where the mean age at presentation was 63.60 years and 64.40 years, respectively [2,8]. However, on the contrary, in the study by Skotnicki P et al., where the mean age in PMC was lower (64 versus 66 years) than in patients with MMC [9]. Also, in certain studies like Yang M et al., and Hashmi AA et al., the presentation of MC is observed in comparatively younger population, where the mean age was 55.28±15.73 years and 56.47±13.90 years, respectively [3,4].

MC of the breast is rare in clinical practice and includes approximately 4% (1-7%) of all invasive breast cancers. It is divided into pure and mixed subtypes. Even in our study, MC accounted for 4.8% (12/245) of all invasive breast cancers diagnosed during the 18 months study period, whilst PMC comprised of only 3.3% (8/245), and MMC of 1.6% (4/245). The study by Bagga PK et al., accounted for 1.5% (10/658) of all invasive breast cancers diagnosed during the 10 years study period, whilst PMC comprised of only 0.3% (2/658) [2]. Also, Hashmi AA et al., correlated with the present study, as 2.9% (38/1268) cases of MC were diagnosed in a span of eight years [4].

The PMC displays indolent behaviour, and mucin comprises the majority of the tumour volume. The authors found that the tumour size was T1 in one case, T2 in four and T4 in three cases of PMC, which is more in proportion with respect to MMC, where T2 and T4 were observed in three and one case, respectively. This can be substantiated by the findings from the study by Skotnicki P et al., which found T1-T2 TNM stage (91.4% vs 85.0%), and pT1 (44.3% vs 42.5%) of PMBC and MMBC, respectively [9]. The volume of mucin contributes to an overestimation of tumour size, and thus early detection could contribute to the very good prognosis.

Like IDC, MC metastasise to axillary LN, and therefore determining the nodal status is the most important factor in the management of MC. In our study, LN metastasis was noted in 33.3% cases of MC. It has also been reported in the literature [Table/Fig-3] that PMC is unlikely to metastasise to axillary LN and the presence of LN metastasis indicates that the tumour may be an MMC rather than PMC [8-10].

Studies	LN metastasis (histopathological)	
	PMC	MMC
• Ranade A et al., 2010 [10]	14.0%	39.0%
• Skotnicki P et al., 2016 [9]	10.0%	25.0%
• Marrazzo E et al., 2020 [8]	14.8%	43.4%
• Present study, 2022	12.5%	75.0%

[Table/Fig-3]: Comparison of Lymph Node (LN) metastasis (histopathological) with other studies [8-10].

According to the literature, ER and PR expression often found in a high percentage, and low rate of HER2/neu expression is observed in MC, which can be appreciated even in the present study, as shown in [Table/Fig-4] [1,3,4,7].

Studies	ER+	PR+	HER2/neu+
• Kim D et al., 2012 [7]	91.0%	74.4%	6.4%
• Yang M et al., 2013 [3]	96.4%	92.8%	0.0%
• Lei L et al., 2016 [1]	85.4%	72.9%	6.3%
• Hashmi AA et al., 2021 [4]	94.7%	78.9%	10.5%
• Present study, 2022	100.0%	100.0%	0.0%

[Table/Fig-4]: Comparison of hormonal (ER and PR) and HER2/neu status with other studies [1,3,4,7].

Secreted mucins like MUC2 can be intracellular, extracellular or both. In the present study, MUC2 expression was observed in 83.3% (10/12) cases (100.0% in PMC, and 50.0% in MMC). The study also established statistically significant association of MUC2 expression, with respect to type of MC. As there are less studies ours was is in correlation with the findings from the study by Kim D et al., [Table/Fig-5] [7]. Similarly, according to the study by Rakha EA et al., [5], MUC2 expression was noticed in around 81.3% of MCs, thereby signifying its role in determining the aggressiveness and prognosis of tumour. Also, the study by Astashchanka A et al., emphasises on the role of MUC2 in modulating the aggressiveness and prognosis of breast cancer [6].

MUC2 Expression		Present study, 2022	Kim D et al., 2012 [7]
Positive	Luminal/Apical	33.3%	34.6%
	Luminal/Apical+Cytoplasmic	25.0%	28.2%
	Membranocytoplasmic	25.0%	29.4%
Negative		16.7%	7.6%

[Table/Fig-5]: Comparison of MUC2 expression with previous literature.

Limitation(s)

Major limitations of the present study were, it was retrospective study, and number of MC cases was very low. Moreover, follow-up of the patients was not available to compare the difference in overall survival and disease-free survival between PMC and MMC cases. Further analysis of a larger number of patients is required to understand its prognostic significance.

CONCLUSION(S)

The MBC is a rare type of breast cancer accounting for about 4% of all diagnosed breast cancers. These are associated with a better long-term prognosis than other breast cancers. Hormone receptors and HER2 status play an important role in prognosis and management. MUC2 also plays a major role in mediating the

proliferation, apoptosis, metastases of breast cancer cells and determining the use of chemotherapy drugs like paclitaxel have shown to be effective in management.

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