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CLINICOPATHOLOGICAL SIGNIFICANCE OF SERUM TUMOR MARKER CA 15-3 IN VARIOUS IMMUNOHISTOCHEMISTRY BASED MOLECULAR SUBTYPES OF BREAST CANCER

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ABSTRACT

Aim: The aim of the present study was to assess the correlation of CA 15-3 level with Clinico Pathological parameters and molecular subtypes of breast cancer.

Methods: This study was a prospective study conducted in the Department of Pathology, Government Medical College Patiala, Punjab and included 75 cases, with the approval of the Institutional Ethics Committee.

Results: In the present study, patients were divided into 7 groups according to age. The highest no of cases were recorded in the 51-60 age group i.e. 5th and 6th decade (33.3%). The mean age was 50.36 ± 12.04 years. The range of age was 25 - 80 years of age. In present study value of CA 15.3 above 30 IU/L was taken as significant, it was observed that out of 75 cases, number of cases with positive CA 15.3 value are 34 (45.3%). In the present study, out of total 75 cases studied, maximum cases were noted of subtype IDC NOS i.e. 70 (93.3%) followed by Lobular (5.3%) and Medullary (1.3%) carcinoma of breast. In the present study maximum number of cases were noted in T2 stage (2-5 cm) i.e. 47 (62.7%) followed by T1 (21.3%) and T3 (11%) and minimum cases were noted in T4 stage i.e. 1 (1.3%). Out of 75 cases, maximum cases were noted in N0 stage i.e. 56 (74.6%) followed by 8 cases each of N1 and N2 (21.3%) and minimum cases were noted in N3 stage i.e. 3 (4%). Out of 75 cases, 2 cases of distant metastasis were noted. Out of 75 cases, maximum no of cases observed were of Luminal A subtype i.e. 33 (44.0%) followed by Basal (24%) and HER 2 enriched (15%) subtype. A minimal no of cases

were observed in Luminal B (3%) subtype. As the tumour size increased the level of CA 15.3 also increased. p value was calculated and was found to be statistically significant between tumour size and Ca 15.3 level. (p value = <0.001). As the nodal involvement increased, the level of CA 15.3 also increased. p value was found to be statistically significant between nodal status and Ca 15.3 level. (p value = <0.001). There was a positive correlation between metastasis and CA 15.3 value which was found to be statistically significant with p value of <0.001.

Conclusion: The CA15-3 levels are associated with tumor burden indicators including tumor size and lymph node status and metastasis. The higher levels of CA 15–3 are more common in patients with larger tumor size, advanced axillary lymph nodal status and in metastatic breast cancer patients. No correlation was seen with histological grade.

Positive correlation was seen between serum CA 15.3 levels and luminal subtype of breast cancer. luminal A was the majority in molecular subtype and showed increased CA 15.3 levels.

Keywords: Serum tumor markers, CA 15-3, Breast cancer, Metastasis, Molecular Subtype.

INTRODUCTION

According to GLOBOCAN 2020 estimates, there are presently an estimated 2.3 million new cases of breast cancer worldwide, making it one of the most frequently diagnosed malignancies and the fifth leading cause of cancer-related deaths. Breast cancer is a general word that refers to several diseases with various histologic patterns, patterns of spread, therapeutic responses, patient outcomes, and imaging characteristics. As per the GLOBOCAN data 2020, in India, BC accounted for 13.5% of all cancer cases and 10.6% of all deaths with a cumulative risk of 2.81. Currently, the Breast Health Global Initiative (BHGI) is in charge of creating appropriate guidelines and strategies to offer the most effective breast cancer control globally1.

Numerous clinicopathological factors are significant in the prognosis and individualized care for each patient. Age, tumor size, tumor grade, lymph node status, molecular markers such as hormone receptor status and expression of human epidermal growth factor receptor 2, and serum tumor markers are crucial for screening, early identification of recurrence, and treatment.[2] Histologic grading is an easy and affordable way to evaluate tumor behaviour and patient prognosis, identifying individuals who may benefit from (neo) adjuvant therapy and are at risk for unfavorable outcomes. Modified Bloom-Richardson (MBR) score for histological grading includes three components: tubule development, nuclear pleomorphism, and mitosis.[3] Breast cancer is classified using Immunohistochemistry (IHC) into molecular subtypes, with triple negative breast cancer having the least favourable prognosis and luminal subtypes having the best prognosis.[4]

More precise patient stratification is required based on the relative risk of progression or recurrence. Due to these demands, various classification schemes have been developed that take IHC markers including Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (Her2) into account. The expression of biomarkers such as HER-2, PR, and ER is necessary for IHC characterization. Immunohistochemistry is used to determine ER and PR to identify patients who will benefit from endocrine treatment.[5] Estrogen

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receptor status is the most significant predictive factor in the management of breast cancer because the progesterone receptor is induced by estrogen and is a marker of an active estrogen receptor.[6] The HER2 protein is an oncoprotein and the second member of the family of tyrosine kinase receptors. Its overexpression is correlated with the grade of the tumor because it promotes unchecked cell growth, lower levels of apoptosis and angiogenesis, and a poorer prognosis.[7] There are some non-invasive biomarkers that are important for predicting the course of the disease, in addition to different clinicopathological factors. The two most often utilized markers are CA 15.3 and CEA. Data on serum marker levels are used as an additional diagnostic tool to gauge the severity of the condition and to track the effectiveness of treatment.[8] Serum markers are simple to measure and are crucial in a variety of malignant tumors, although their significance in breast cancer is still debatable. There is some link between tumor markers and tumor clinicopathology, and in some situations, these markers may provide helpful information regarding the phenotype of breast cancer at an early stage when the acquisition of tissue specimens is not possible.[9] While elevated CA 15-3 tumour marker levels are typically found in individuals with advanced breast cancer, various other kinds of advanced adenocarcinoma may also exhibit high concentrations of this marker. Aside from breast cancer, advanced carcinomas such as ovarian, pancreatic, lung, and intestinal cancer may cause increased CA 15-3 levels. Rarely, certain benign conditions such chronic hepatitis, liver cirrhosis, sarcoidosis, and megaloblastic anaemia may result in increased CA 15-3 levels.[10,11] Normal serum levels of the marker CA 15-3 for both men and women are less than 25 to 30 kIU/L, depending on the method of determination and the reagent and analyzer manufacturer.

The aim of the present study was to assess the correlation of CA 15-3 level with Clinico-pathological parameters and molecular subtypes of breast cancer.

MATERIALS AND METHODS

This study was a prospective study conducted in the Department of Pathology, Government Medical College Patiala, Punjab and included 75 cases, with the approval of the Institutional Ethics Committee.

Inclusion Criteria- Females with malignant breast tumours.

b. Exclusion Criteria- Inadequate tissue sample, male breast tumours, follow up cases.

All clinicopathological parameters like tumour size, lymph node status, tumour grade, sites of distant metastasis will be taken into consideration and biological parameters like hormone and growth factor receptors (IHC) for molecular subtyping. Preoperative CA 15.3 samples of known cases of breast cancer diagnosed cytologically and histologically were collected. Post-operative samples of breast which included predominantly mastectomies were received in department of pathology. Confirmatory histological diagnosis, grading as per MBR score and TNM staging was done. Paraffin blocks were used to make IHC slides of the sections required for IHC staining. After staining, ER, PR and HER 2 scoring and IHC based molecular subtyping was done and was correlated with CA 15.3. Several clinicopathological factors were also correlated with CA 15.3.

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Based on IHC interpretation, Molecular Subtyping was done. Luminal A subtype shows ER/PR positivity and negative HER2 staining. Luminal B subtype shows ER, PR and HER 2 positivity. Her 2 Enriched shows only HER 2 positivity and triple negative subtype as per its name is negative for all the antigens.

Statistical Tests:

The data was entered, cleaned and compiled.

The data was analyzed using Microsoft Excel Software 2016 and Epiinfo CDC Atlanta version 7.2.2.1.6.

1. Continuous variables was reported as Mean ± Standard Deviation
(SD) while categorical variables were expressed as absolute values and percentages.
2. Kolmogorov Smirnov and Shpiro-Wilk test will be used to check the normality of the data.
3. Unpaired t test was used if the data was normal otherwise Mann Whitney U test was applied to compare the means between the two groups.
4. ANOVA was used if the data was normal otherwise Kruskal Walis H Test was applied to compare the means between more than two groups.
6. p-value less than 0.05 was considered statistically significant.

RESULTS

Table 1: Patient details

Age (years)	**No. of cases (n=75)**	**Percentage of cases**
<20	0	0.0%
20-30	4	5.3%
31-40	12	16.0%
41-50	23	30.7%
51-60	25	33.3%
61-70	6	8.0%
>71	5	6.7%
Ca 15.3		
<30 (NEGATIVE)	41	54.6%
>30(POSITIVE)	34	45.3%
HISTOLOGICAL SUBTYPE		
IDC	70	93.3%
LOBULAR	4	5.3%

MEDULLARY	1	1.3%
TUMOUR SIZE		
T1 (<2 cm)	16	21.3%
T2 (2-5 cm)	47	62.7%
T3 (>5 cm)	11	14.7%
T4	1	1.3%
Nodal Status		
N0	56	74.6%
N1	8	10.6%
N2	8	10.6%
N3	3	4%
METASTASIS		
Yes	2	2.7%
No	73	97.3%
MOLECULAR SUBTYPE		
Luminal A	30	40.0%
Luminal B	4	5.4%
Her 2 enriched	16	21%
Basal	25	33%

In the present study, patients were divided into 7 groups according to age. The highest no of cases was recorded in the 51-60 age group i.e. 5th and 6th decade (33.3%). The mean age was 50.36 ± 12.04 years. The range of age was 25 - 80 years of age. In present study value of CA 15.3 above 30 IU/L was taken as significant, it was observed that out of 75 cases, number of cases with positive CA 15.3 value are 34 (45.3%). In the present study, out of total 75 cases studied, maximum cases were noted of subtype IDC NOS i.e. 70 (93.3%) followed by Lobular (5.3%) and Medullary (1.3%) carcinoma of breast. In the present study maximum number of cases were noted in T2 stage (2-5 cm) i.e. 47 (62.7%) followed by T1 (21.3%) and T3 (11%) and minimum cases were noted in T4 stage i.e. 1 (1.3%). Out of 75 cases, maximum cases were noted in N0 stage i.e. 56 (74.6%) followed by 8 cases each of N1 and N2 (21.3%) and minimum

cases were noted in N3 stage i.e. 3 (4%). Out of 75 cases, 2 cases of distant metastasis were noted. Out of 75 cases, maximum no of cases observed were of Luminal A subtype i.e. 33 (44.0%) followed by Basal (24%) and HER 2 enriched (15%) subtype. A minimal no of cases was observed in Luminal B (3%) subtype.

Table 2: Correlation of tumour size, nodal status, metastasis with CA 15.3 level

Tumour Size	Ca 15.3 (mean) ± S.D (IU/L)	p value	Significance
T1 (<2 cm)	17.22 ± 7.98	<0.001	HS
T2 (2-5 cm)	32.24 ± 20.08		
T3 (>5 cm)	59.07 ± 23.01		
T4	52.00 ± 0.00		
Nodal Status			
N0	23.18 ± 12.47	<0.001	HS
N1	31.29 ± 21.78		
N2	65.95 ± 17.34		
N3	43.80± 21.83		
Metastasis			
Yes	80.67 ± 1.88	<0.001	HS
No	31.93 ± 21.08		
Histological Grade			
Grade 1	18.61 ± 8.38	0.132	NS
Grade 2	30.97 ± 20.55		
Grade 3	37.94 ± 28.88		

As the tumour size increased the level of CA 15.3 also increased. p value was calculated and was found to be statistically significant between tumour size and Ca 15.3 level. (p value = <0.001). As the nodal involvement increased, the level of CA 15.3 also increased. p value was found to be statistically significant between nodal status and Ca 15.3 level. (p value = <0.001). There was a positive correlation between metastasis and CA 15.3 value which was found to be statistically

significant with p value of <0.001. As the histological grade increased, the mean value of CA 15.3 also increased but it was found to be statistically insignificant. (p value = 0.132).

Table 3: Correlation of receptor status with CA 15.3 level

		Ca 15.3 (mean) ± S.D (IU/L)	p value	Significance
ER	**Positive**	43.38 ± 26.09	0.005	HS
	Negative	24.82 ± 13.93		
PR	**Positive**	43.38 ± 26.09	0.005	HS
	Negative	24.82 ± 13.93		
HER2	**Positive**	25.57 ± 18.39	0.038	S
	Negative	36.21 ± 23.05		

The statistically significant correlation was found in the present study between positive ER, PR receptor status and CA 15.3 levels with p value of 0.005 in both the groups. Whereas HER 2 negative receptor status shows increase in CA 15.3 level and this was found to be statistically significant.

Table 4: Correlation of molecular subtype with CA 15.3 level

MOLECULAR SUBTYPE	Ca 15.3 (mean) ± S.D (IU/L)	p value
Luminal A	44.77 ± 26.24	0.025
Luminal B	33.00 ± 20.82	
Her 2 enriched	24.07 ± 18.58	
Basal	25.30 ± 10.35	

Table shows that correlation of molecular subtype with CA 15.3 was statistically significant in present study. p value was calculated to b 0.025. Luminal A subtype has the highest mean value of 43.36 ± 26.24.

DISCUSSION

Breast cancer is the most frequently occurring cancer in women and its incidence has been steadily increasing in India. Despite the rising incidence of breast carcinoma, the survival rate has been improved due to the awareness of the biological behaviour of breast carcinoma. Therefore, it is essential to identify reliable prognostic factors to guide decision-making during the treatment of breast cancer to improve prognosis. Along with the traditional pathological

factors such as tumor size, tumor grade, lymph node status and molecular markers including hormone receptor status, serum tumor markers have an important role in screening, early diagnosis of recurrence, and treatment of many malignancies. In recent years, the prognostic value of preoperative CA15-3 levels in breast cancer has gained much attention. Although its usefulness remains uncertain and American Society of Clinical Oncology guidelines do not recommend its use for follow-up. Measurement of circulating CA 15-3 levels is widely used for surveillance purposes in the clinical field. It is a fast, noninvasive, reproducible, and quantitative serum test.[12]

In the present study the majority of the study population lies in 51-60 years age group which might be due to it being the most common age group and a risk factor for the incidence of breast carcinoma due to hormonal effects. The age distribution in the present study was in concordance with the study done by Geng et al[13] and Shao et al.[2] MUC1 is overexpressed in breast cancer cells when compared to normal breast tissue and detected in peripheral blood by CA15-3 assay77

In the present study It was found that as the size of the tumor increased, the levels of CA 15.3 increased (T1 - mean CA 15.3 = 17.22, T2 - mean CA15.3= 32.24, T3 - mean CA 15.3 = 59.07). The statistical analyses were done to find the correlation between tumor size and CA 15.3. The study found that there was a statistically significant correlation between the two (p value <0.001). The findings was in accordance with the study done by other authors Park et al[14], Fang et al[15] and Uygur et al.[16]

The increase of MUC1 expression on the cell surface may precipitate invasion and metastasis of cancer cells as the CA15-3 has a role of cellular adhesion and cell to cell interaction.[17] In the present study as the nodal involvement increased from one node to more number of nodes in the cases of breast carcinoma, CA 15.3 level was found to be elevated. This was found to be statistically significant between the two (p value <0.001). This finding was in concordance with the study done by authors Park et al[14], Fang et al[15] and Shao et al.[2] In the present study, the breast carcinoma was graded histopathologically into Grade 1, Grade 2 and Grade 3. The statistical analysis was done between the histological grade and CA 15.3 levels. The study showed that as the grade of the tumor raised, the CA 15.3 levels also increased (Grade 1: mean CA15.3 =18.61, Grade 2 : mean CA 15.3 = 30.97 and grade 3 : mean CA 15.3 = 37.94). There was not found to be statistically significant correlation between the two variables (p value = 0.132). These results were in accordance with the studies conducted by Geng et al[13] and Park et al.[14]

CA15-3 is involved in cellular adhesion and cell-to-cell contact, an increase in MUC1 expression on cancer cells' cell surfaces could hasten their invasion and metastasis.[17] In the present study, metastasis of the tumor was statistically analyzed with CA 15.3 levels and which was found to be statistically significant (p value <0.001). This finding was in accordance with the study done by Geng et al[13] (p value = 0.017) and Uygur et al[16] (p value = 0.008). In the present study of 75 cases of breast cancer, Her 2 receptor status was compared with CA 15.3 which was found to be statistically significant (p value=0.038).The value of Ca 15.3 was raised in Her2 negative receptor status. This finding was in accordance with the study done by Uygur et al[16] (p value = 0.011). CA 15-3 is elevated more frequently in the luminal subtypes compared with the non-

luminal groups. A possible explanation for this relationship is that in less differentiated subtypes, such as the non-luminal subtypes or basal-like subtypes, tumor cells may arise from breast stem cells, which may not express certain antigens.[18]

In the present study, Breast carcinoma was categorized according to the molecular subtypes. The molecular subtypes were compared with CA 15.3 which was found to be statistically significant. Ca15.3 levels were significantly increased in the Luminal subtype of breast carcinoma in the present study. The least value was seen with the HER2 receptor. These findings were in accordance with the study done by authors Yerushalmi R et al[19] and Zobair et al.[20] The present study demonstrated that serum levels of CA 15-3 were associated with host tumor burden such as larger tumor size (>5 cm), more lymph node metastases (≥4), and advanced stage. Since CA 15-3 are directly associated with host tumor burden and the presence of serum tumor- associated antigens indicates vascularization of the tumor with the possibility of micrometastases, preoperative levels of serum tumor markers could be related to poor outcome. The elevation of CA15-3 levels was significantly greater in ER-positive breast patients. Simultaneously, the elevated rates of CA15-3 levels in different subtypes of breast cancer are also different, which may be explained in part by the different biological behaviors of different molecular subtypes. Luminal A accounted for the largest proportion in the present study.

CONCLUSION

The CA15-3 levels are associated with tumor burden indicators including tumor size and lymph node status and metastasis. The higher levels of CA 15–3 is more common in patients with larger tumor size, advanced axillary lymph nodal status and in metastatic breast cancer patients. No correlation was seen with histological grade. In luminal A subtype breast cancer, the incorporation of CA 15.3 would be helpful in clinical decisions concerning adjuvant therapy as Luminal A subtype is more sensitive to endocrine therapy and chemotherapy can be ceased in most of the Luminal A cases as per NCCN guidelines. There are following limitations of this study as the number of cases due to COVID were taken as 75, a comprehensive study with larger sample size is required. Molecular classification was done using only ER, PR and HER 2 and Ki67 was not included.

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