ORIGINAL RESEARCH PAPER

General Surgery

SIGNIFICANCE OF TUMOUR MARKERS(CEA AND CA15-3) IN CARCINOMA BREAST

KEY WORDS: CEA, CA15-3, TNM staging, Tamoxifen.

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ABSTRACT

INTRODUCTION: Breast cancer is a major public health problem for Women throughout the world. In the United States, breast cancer remains the most frequent cancer in women and the second most frequent cause of cancer death.
Objective:To study the significance of Tumour Markers (CEA and CA15-3) in carcinoma breast.
MATERIAL AND METHODS: This study was conducted in KRISHNA HOSPITAL and MEDICAL RESEARCH CENTRE, KARAD during the period from June 2017 to June 2019.The study comprises of a total of 50 cases that were admitted in this hospital. On admission case history was noted and the patients were examined clinically. In this study the TNM staging system was followed and blood samples were collected in plain bulbs. The study was approved by Institutional Ethical Committee. Informed consent was obtained from each patient prior to sample collection.
RESULTS AND CONCLUSIONS: In 13/24 cases with abnormal CA15-3 value in postoperative follow up period, there are 54% patients with metastatic disease.In 4/24 cases with preoperative abnormal CA15-3, metastasis occurred in 75% and loco-regional recurrence occurred in 50%.In 8/50 cases with preoperative abnormal CA15-3, 75% patients presented with locally advanced disease.In 9/24 cases with abnormal CEA value in postoperative follow up period, there are 67% patients with metastatic disease.In 15/24 cases with preoperative abnormal CEA, metastasis occurred in 40% and in 16/24 cases with preoperative abnormal CEA, loco-regional recurrence occurred in 25%.In 32/50 cases with preoperative abnormal CEA, 63% patients are presented with locally advance disease.

INTRODUCTION:

Breast cancer is a major public health problem for Women throughout the world. In the United States, breast cancer remains the most frequent cancer in women and the second most frequent cause of cancer death. Since 1990, the death rate from breast cancer has decreased in the United States by 24% and similar reductions have been observed in other countries. Mathematical models suggest that both the adoption of screening mammography and the availability of adjuvant chemotherapy and tamoxifen have contributed approximately equally to this improvement.

Although breast cancer has traditionally been less common in non industrialized nations, its incidence in these areas is increasing. Roughly half of these newly diagnosed patients are node-negative, however 30% of these cases progress to metastatic disease. There are number of tumour markers that can help clinicians to identify and diagnose which breast cancer patients will have aggressive disease and which will have an indolent course. These markers include estrogen and progesterone receptors, DNA ploidy and percent-S phase profile, epidermal growth factor receptor, HER-2/neu oncogene, p53 tumour suppressor gene, cathepsin D, proliferation markers and CA15-3. CA15-3 is most useful for monitoring patients post-operatively for recurrence, particularly metastatic diseases. 96% of patients with local and systemic recurrence have elevated CA15-3,which can be used to predict recurrence earlier than radiological and clinical criteria. A 25% increase in the serum CA15-3 is associated with progression of carcinoma. A 50% decrease in serum CA15-3 is associated with response to treatment. CA15-3 is more sensitive than CEA in early detection of breast cancer recurrence. CA15-3 levels are also increased in colon, lung and hepatic tumours.

CarcinoembryonicAntigen(CEA) and Cancer Antigen 15-3(CA15-3) are the most thoroughly investigated tumour markers in breast cancer. Circulating levels of CEA and CA15-3 have become established diagnostic tools as fast, noninvasive, reproducible, quantitative parameters in follow up care and monitoring therapy of breast cancer patients. CEA is a member of immunoglobulin superfamily. The human CEA gene family is clustered on chromosome 19q and comprises 29 genes. Of these, 18 are expressed, with 7 belonging to the CEA subgroup and 11 to the pregnancy specific glycoprotein subgroup. When isolated from liver metastasis, CEA is a glycoprotein consisting of approx 60% carbohydrate and a molecular mass of approx 180-200 kDa. Most of the carbohydrate is comprosed of mannose, galactose, N-acetylglucosamine, fucoe and sialicacid. CA15-3 assay measures that protein product of the MUC-1 gene located on chromosome 1q. MUC1 protein is a large transmembrane glycoprotein containing a large extracellular domain, a membrane spanning sequence and a cytoplasmic domain.

The CA15-3 is a glycoprotein, helps in cell adhesion, immunity and metastasis. The antigen is defined by reacting with two monoclonal antibodies DF3 and 115D8. The DF3 antibody was raised against a membrane enriched fraction of a human breast carcinoma. The 115D8 antibody was prepared against human milk fat globulin membrane and may be directed to a carbohydrate epitope. Serum CEA and CA15-3 are established prognostic markers in breast cancer patients. This study is aimed to correlate the serum marker levels with the clinical stage of the disease, size of the tumour, nodal status and for detecting metastasis, loco-regional recurrence in female breast cancer patients. If a correlation is found, it could provide clinicians an indication of prognosis, thus enabling appropriate adjuvant therapy.

AIMS AND OBJECTIVES:

1. To study the significance of Tumour Markers (CEA and CA15-3) in carcinoma breast.

MATERIAL AND METHODS:

Ethical Statement:

The Study made the standards outlining the declaration of Helsinki and Good Epidemiological practices. This study did not change or modify the laboratory of clinical practices of each centre and differences of practices were kept as they are.The data collection was anonymous and identifiable patient information was not submitted.

Individual researchers were responsible for complying with local ethical standards and hospital registration of study.

This study was conducted in KRISHNA HOSPITAL and MEDICAL RESEARCH CENTRE, KARAD during the period from June 2017 to June 2019. The study comprises of a total of 50 cases that were admitted in this hospital. On admission case history was noted and the patients were examined clinically. In this study the TNM staging system was followed and blood samples were collected in plain bulbs. The study was approved by Institutional Ethical Committee. Informed consent was obtained from each patient prior to sample collection.

Tumourmarkers(CEA and CA15-3) were analysed by sandwich ELISA method using C.L.I.A Technology. (Fully automated Bidirectionally interfaced ChemiluminescentImmuno assay)

CEA : 50 microlitre of the patient's samples and 100 microlitre anti-HRP (Horse Radish Peroxidase) conjugate were added to the microwells coated with monoclonal antibody(Mab). The antibody-enzyme conjugate solution contained goat anti CEA antibody conjugated to HRP. CEA in the patient's serum bound to anti CEA MAb on the well and the anti-CEA-HRP second antibody then bound to CEA. Unbound protein and HRP conjugate was washed off by wash buffer(phosphate buffer saline). Upon the addition of the TMB (3 3' 5 5' tetra methylbenzidine) substrate, the intensity of colour is proportional to the concentration of CEA in the samples. The colour development was stopped with addition of stop solution(1N Hcl).

CA15-3 ELISA test: this test is based on the principle of a solid phase enzyme linked immunosorbent assay. The assay system utilizes a polyclonal anti-CA15-3 antibody directed against intact CA15-3 for solid phase immobilization on the microtitre wells. A monoclonal anti-CA15-3 antibody conjugated to a horse radish peroxidase(HRP) is in the antibody-emzyme conjugate solution. The test sample is allowed to react first with the immobilized polyclonal antibody. The wells are washed to remove any unbound antigen. The monoclonal-HRP conjugate is then reacted with the captured antigen, resulting in the CA15-3 molecules being sandwiched between the solid phase and enzyme-linked antibodies. The wells are washed with water to remove unbound labeled antibodies. A solution of TMB reagent is added, resulting in the development of a blue colour. The colour development is stopped with the addition of Stop solution, which changes the colour to yellow, and the absorbance is measured using a spectrophotometer at 450nm. The concentration of CA15-3 is directly proportional to the colour intensity of the test sample.

OBSERVATIONS AND RESULTS:

Table no 1:Age Distribution

Age in years	*number of patients*	*Percentage(%)*
26-35	4	8%
36-45	10	20%
46-55	15	30%
56-65	17	34%
Above 65	4	8%

Table no 2:Clinical stage of the disease

Clinical Stage	*Number of Patients*	*Percentage(%)*
I	2	4%
II	20	40%
III	26	52%
IV	2	4%

Table No 3: Relation of preoperative abnormal CEA with clinical stage

Clinical Stage	*Number of patients with increased value*	*Percentage(%)*
I	1	2%
II	11	22%
III	18	36%
IV	2	4%

Table no 4: Relation of preoperative abnormal CEA with tumour size in 50 patients

Tumour Size	*Patients with increased value*	*Patients with normal value*	*Total number of patients*
Less than or equal to 5 cm	14(54%)	12(46%)	26
More than 5 cm	18(75%)	6(25%)	24
Total	32	18	50

Table no 5: Relation of preoperative abnormal CEA with axillary lymph node in 50 patients

Axillary lymph node	*Patients with increased value*	*Patients with normal value*	*Total number of patients*
PRESENT	15(68%)	7(32%)	22
ABSENT	17(60%)	11(40%)	28
Total	32	18	50

Table no 6: Relation of preoperative abnormal CA15-3 with clinical stage

Clinical stage	*Number of patients with increased value*	*Percentage(%)*
I	1	2%
II	1	2%
III	4	8%
IV	2	4%

Table no 7: Relation of preoperative abnormal CA15-3 with tumour size in 50 patients

Tumour Size	*Patients with increased value*	*Patients with normal value*	*Total number of patients*
Less than or equal to 5 cm	2(8%)	24(92%)	26
More than 5 cm	6(25%)	18(75%)	24
Total	8	32	50

Table no 8: Relation of preoperative abnormal CA15-3 with axillary lymph node in 50 patients

Axillary lymph node	*Patients with increased value*	*Patients with normal value*	*Total number of patients*
PRESENT	4(18%)	18(82%)	22
ABSENT	4(15%)	24(85%)	28
Total	8	42	50

Table no 9: Relation of preoperative abnormal CEA with locally advanced disease in 50 patients

CEA	*Developed*	*Not Developed*	*Total number of patients*
Patients with normal value	8(44%)	10(55%)	18
Patients with increased value	20(62%)	12(38%)	32
Total	28	22	50

Table no 10:Relation of preoperative abnormal CA15-3 with locally advanced disease in 50 patients

CA15-3	*Developed*	*Not Developed*	*Total number of patients*
Patients with normal value	22(52%)	20(48%)	42
Patients with increased value	6(75%)	2(25%)	8
Total	28	22	50

RESULTS:

1) In 13/24 cases with abnormal CA15-3 value in

postoperative follow up period, there are 54% patients with metastatic disease.
2) In 4/24 cases with preoperative abnormal CA15-3, metastasis occurred in 75% and loco-regional recurrence occurred in 50%.
3) In 8/50 cases with preoperative abnormal CA15-3, 75% patients presented with locally advanced disease.
4) In 9/24 cases with abnormal CEA value in postoperative follow up period, there are 67% patients with metastatic disease.
5) In 15/24 cases with preoperative abnormal CEA, metastasis occurred in 40% and in 16/24 cases with preoperative abnormal CEA, loco-regional recurrence occurred in 25%.
6) In 32/50 cases with preoperative abnormal CEA, 63% patients are presented with locally advance disease.

DISCUSSION AND SUMMARY:

Fifty cases of carcinoma of breast in which tumour markers were studied during the period from June 2017 to June 2019. All the cases were admitted in this institution, investigated and treated accordingly but tumour marker were sent to outside standard laboratory, Mumbai. The cases were followed up, for a minimum period of 24 months.

1) Elevated serum levels of CA15-3 are found in breast cancer patients with distant metastasis.
2) The main clinical application of CA15-3 is for monitoring patients with diagnosed breast carcinoma and preclinically detecting recurrences
3) Elevated CA15-3 levels are more common in metastatic breast cancer patients than CEA.
4) Patients with abnormal CEA and CA15-3 had no significant association with locally advanced disease.
5) Measurement of preoperative abnormal serum CA15-3 showed significant correlation with tumour size, but no significant association with clinically palpable axillary lymph node.
6) Measurement of preoperative abnormal serum CEA did not show any significant correlation with tumour size and clinically palpable lymph node.
7) Patients with preoperative abnormal CA15-3 had significant association with loco-regional recurrence and metastasis.
8) Patients with postoperative abnormal CA15-3 and CEA during follow up, had significant association with metastasis.
9) Patients with preoperative abnormal CEA had no significant association with loco-regional recurrence and metastasis.
10) We recommended that levels of CA15-3 be controlled in all patients with a normal and abnormal level of CA15-3 after primary diagnosis for early detection of recurrent and metastatic disease.
11) Patients with high postoperative concentration of CA15-3 have a worse outcome than those with low concentration.

CONCLUSIONS:

- There is significance of Tumour markers in Carcinoma of breast.
- The use of CA15-3 for early detection of metastasis seem to be promising.
- It appears that CA15-3 is valuable prognostic indicator in following up breast cancer patients as it had significant association with both metastasis and loco-regional disease.

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