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**Original Research Article**

# Correlation of coagulation markers with axillary lymph node metastasis in carcinoma breast

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## ABSTRACT

**Background:** Lymph node metastasis has been shown to be the most important prognostic marker for carcinoma breast. Lymph node involvement is not only a prognosis marker, but also an important guiding factor in deciding surgical plan as well as postoperative therapy. The aim of the study was to assess role of coagulation markers (D-dimer and factor VIII) value as predictive markers of lymph node metastasis in breast cancer
**Methods:** A prospective study was conducted in the Department of Surgery in with help of Departments of Biochemistry, Pathology and Radiology, KAPV&MGMGH, Trichy. 50 female patients who were diagnosed and treated as carcinoma breast in the department of surgery, MGMGH Trichy. The following variables were studied: 1. Age of patient 2. TNM stage of patients. 3. D-dimer levels 4. Factor VIII levels.
**Results:** There was statistically significant relationship between mean values of D-dimer and factor VIII levels and advancing stage of disease, number of pathological axillary lymph node involved, increase in histological grade of tumor, Lymphovascular invasion.
**Conclusions:** D-dimer and factor VIII may be used as yardstick for systemic adjuvant therapy in patients with node negative, <1 cm carcinoma breast. D-dimer may prove to be a safe, convenient and easily available biomarker which can be combined with conventional sentinel node biopsy in clinically node negative breast cancer to assess metastatic-disease in axilla and reduce false negative results.

**Keywords:** Antithrombin, Carcinoma, Thrombosis

## INTRODUCTION

Carcinoma Breast is the second most common carcinoma in females, and its incidence is mounting every year.[1] Considering the magnitude of the problem, it becomes of paramount importance to consider about various characteristics of a tumour and patient while planning out the treatment protocol for the patient. Lymph node metastasis has been considered to be one of the most important prognostic markers for carcinoma breast.[2,3] Lymph node involvement is not only a prognostic marker but also an important guiding factor in deciding the surgical plan. Presently, sentinel lymph node biopsy is considered the gold standard to detect the involvement of axillary lymph nodes in carcinoma breast with clinically negative axilla. Search for a marker to predict the metastasis of lymph nodes in clinically negative axilla has been a matter of research for long. There is evidence that the various factors involved in the coagulation and fibrinolytic system play a vital role in tumor pathology and angiogenesis.[3] The important initial step in tumor metastasis is fibrin deposition and remodeling in extracellular matrix of the tumor. Development of sustained angiogenesis is important not only for tumor

growth but also for their metastasis. The important steps in metastatic cascade are the invasion of extracellular matrix and vascular dissemination and homing of tumor cells. In the extracellular matrix, the cross-linked fibrin forms the essential framework for the migration of endothelial cell during angiogenesis and also for the migration of cancer cells. Remodeling of fibrin in extracellular matrix is important for neovascularization in tumors. The formation and degradation of intravascular fibrin have been noted in the plasma of breast cancer patients. The emerging paradigm is that tumor angiogenesis, and hence the metastasis is controlled by the balance between angiogenic factors and anti-angiogenic factors.[4,5]

The molecular basis of the angiogenic switch involves an increase in angiogenic factors and a decrease in angiogenic inhibitors. The following changes were noted in cancer patients, including increase in platelet count, fibrinogen, and fibrin degradation products like D-dimer, and increase in factors V, VII, VIII, IX and X or XI levels and a decrease in antithrombin III.[6] This current study is an attempt to evaluate role of these coagulation markers with special reference to D-dimer and Factor VIII in patients with carcinoma breast, in predicting lymph node metastasis in carcinoma breast patients and their relation with other acknowledged risk factors of nodal involvement and to look for relationship of these markers with histopathologic parameters which are known to have predictive and prognostic value in carcinoma breast.

D-dimer and factor VIII may be used as the yardstick for systemic adjuvant therapy in patients with node-negative, <1 cm carcinoma breast. D-dimer may prove to be a safe, convenient and easily available biomarker which can be combined with conventional sentinel node biopsy in clinically node-negative breast cancer to assess metastatic-disease in the axilla and reduce false negative results. The significant postoperative decrease in D-Dimer and Factor VIII may provide objective criteria to assess completion of surgery. These findings need to be validated with large multicentric prospective studies with long-term follow up.

***Aim***

To assess role of coagulation markers (D-dimer and Factor VIII) value as predictive markers of lymph node metastasis in breast cancer.

## METHODS

A prospective study was conducted in the Department of Surgery in with help of Departments of Biochemistry, Pathology and Radiology. All diagnosed patients of carcinoma breast were included in the study. Exclusion Criteria: Severe blood coagulation disorders, conditions known to increase coagulation marker levels like disseminated intravascular coagulation (DIC), myocardial infarction (MI), sickle cell disease with vasoocclusive crisis, thromboembolic events, and mechanical valve repair, carcinoma breast in males. Clinical staging using TNM classification was performed in all enrolled patients and based on all information available before first treatment. The included documenting tumour size, node status and metastatic workup including USG abdomen and chest x-ray. American Joint Committee on Cancer (AJCC), TNM staging system was used for staging of the patients. The NycoCard D-Dimer single test was used which is an in vitro test for measuring the D-dimer (fibrin degradation product in plasma. The test is based on an immuno metric flow through principle. D-dimer molecules are trapped on a membrane carrying D-dimer specific mono clonal antibodies when the sample was soaked into the device. Then the conjugate solution is added containing monoclonal antibodies conjugated with ultra-small gold particles. The D-dimer on the membrane will bind the gold antibody conjugate in a sandwich type reaction. This reaction imparts reddish appearance to the membrane with color intensity proportional to the D-dimer concentration. The color intensity is evaluated using NycoCard READER II.

## RESULTS

In the present study, 50 patients of breast cancer were included into the study. All these patients had histologically proven disease. The purpose of the study was to explore the significance of D-Dimer (product of fibrin degradation) and factor VIII as a prognostic marker in breast cancer and its relationship with other variables such as histological characteristics, lymph node status.

**Table 1: TNM stage and baseline levels of D-dimer and factor VIII.**

| TNM stage | Number of (% age) | D-dimer (Mean) | Factor VIII (Mean) |
|---|---|---|---|
| | | Baseline (ng/ml) | Baseline (%) |
| Stage I | 1(2) | 200 | |
| Stage II A | 6 (12) | 267± 163.29 | 250.7± 214.46 |
| Stage IIB | 13(28) | 415±328.75 | 233.8±125.22 |
| Stage IIIA | 9(16) | 700±663.32 | 332.7±249.94 |
| Stage IIIB | 19(38) | 605±599.51 | 332.3±151.44 |
| Stage IIIC | 1(2) | 2300 | 670.9 |
| Stage IV | 1(2) | 1100 | 558.9 |
| P Value | | 0.029 | 0.004 |

We also attempted to find variables which can be used to predict lymph node metastasis in node negative breast cancer (clinically N0). 40% of patients were of early

Breast cancer with only 2% patient in stage I.60% patients were of locally advanced and advanced breast cancer out-of these 4% were advanced breast cancer patients.

**Table 2: Tumor size and baseline levels of D-dimer and Factor VIII.**

| TNM size | Number of (%) | D-dimer (Mean) | Factor VIII (Mean) |
|---|---|---|---|
| | | Baseline (ng/ml) | Baseline (%) |
| T1 | 1(2) | 200 | 125.4 |
| T2 | 8 (16) | 275±148.80 | 198.4±181.72 |
| T3 | 20 (40) | 460±518.57 | 255.1±197.16 |
| T4 | 21 (42) | 652±684.03 | 359.2±167.86 |
| P Value | | .076 | .076 |

The increase in mean values of D-dimer and Factor VIII with advancing stage of disease was found to be significant (p=0.029 for D-dimer and 0.004 for Factor VIII) using Kruskal Wallis test suggesting relation of these markers with tumor load of patient.

**Table 3: Lymph node status and baseline levels of D-dimer and Factor VIII.**

| Axillary Lymph nodes (Pathological) | No. of (% age) | D-dimer (Mean) | Factor VIII (Mean) |
|---|---|---|---|
| | | Baseline (ng/ml) | Baseline (%) |
| N0 | 18(36) | 344±268.37 | 237.1±113.99 |
| N1 (1-3) | 13 (26) | 561±522.07 | 284.7±209.94 |
| N2 (4-9) | 11(22) | 554±454.67 | 327.3.±183.03 |
| N3 (10 or more) | 8 (16) | 712±936.55 | 348.6±209.82 |
| P Value | | 0.029 | 0.032 |

The increase in mean values of D-dimer and Factor VIII with increasing Tumor size was not significant (p=0.076 and p=0.076) Spearman's coefficient of rank correlation.

**Table 4: Baseline and post surgery levels of D-dimer and Factor VIII.**

| Coagulation markers | Baseline | Post chemotherapy | P value |
|---|---|---|---|
| D-DIMER (ng/ml) | 568.00± 569.08 | 156.00±70.45 | <0.0001 |
| Factor VIII (%) | 304.18± 184.39 | 177.39±79.63 | <0.0001 |

The increase in mean values of D -dimer and Factor VIII with increasing lymph node involvement was found to be significant (p=0.029 for D-dimer and 0.032 for Factor VIII) Spearman's coefficient of rank correlation.

**Table 5: Histological grade and baseline levels of D-dimer and factor VIII.**

| Histologic grade | Number of (% age) | D-dimer (Mean) | Factor VIII (Mean) |
|---|---|---|---|
| | | Baseline (ng/ml) | Baseline (%) |
| I | 5(10) | 460±328.63 | 195.2±59.70 |
| II | 28 (56) | 492±506.20 | 299±186.40 |
| III | 17 (34) | 723±702.25 | 344.6±197.74 |
| P Value | | 0.500 | 0.351 |

**Table 6: Lymphovascular invasion and baseline levels of D-dimer and Factor VIII.**

| Lymphovascular invasion | No. of (%) | D-dimer (Mean) | Factor VIII (Mean) |
|---|---|---|---|
| | | Baseline (ng/ml) | Baseline (%) |
| Present | 28 (56) | 750±685.02 | 366.5±208.01 |
| Absent | 22 (44) | 336±227.92 | 224.8±109.15 |
| P Value | | 0.018 | 0.021 |

**Table 7: Mitotic index and baseline levels of D-dimer and Factor VIII.**

| Mitotic | No. of (% age) | D-dimer (Mean) | Factor VIII (Mean) |
|---|---|---|---|
| | | Baseline (ng/ml) | Baseline (%) |
| 1 Point | 32 (64) | 478±467.48 | 367.9± 211.86 |
| 2 Points | 18 (36) | 727±701.93 | 231.4± 129.41 |
| 3 Points | 0 (0) | 0 | 0 |
| P Value | | 0.068 | 0.007 |

**Table 8: Univariate analyses for pridictive values of clinicopatholog1cal parameters for lymph node metastasis.**

| Parameter studied | P value |
|---|---|
| Size | 0.840 |
| Mitotic index | 0.034 |
| Histologic grade | 0.123 |
| Lymphovascular invasion | <0.0001 |
| D-dimer | 0.042 |
| Factor VIII | 0.135 |

The reduction in D-dimer and Factor VIII values after Surgery were significant for both D-dimer (p=0.000) and Factor VIII (p=0.000) Wilcoxon signed rank test.

The difference between mean values of D-dimer and Factor VIII according to Histologic grade was not significant (p=0.500 for D-dimer and 0.351 for Factor VIII) Kruskal Wallis test.

The difference between mean values of D-dimer and Factor VIII according to lymphovascular invasion was significant (p=0.018 for 0-dimer and 0.021 for Factor VIII) Mann Whitney U test.

The difference between mean values of Factor VIII according to Mitotic index was significant (p value.007) the difference between mean values of D-dimer according to Mitotic index was not significant (p=0.068) Kruskal Wallis test.

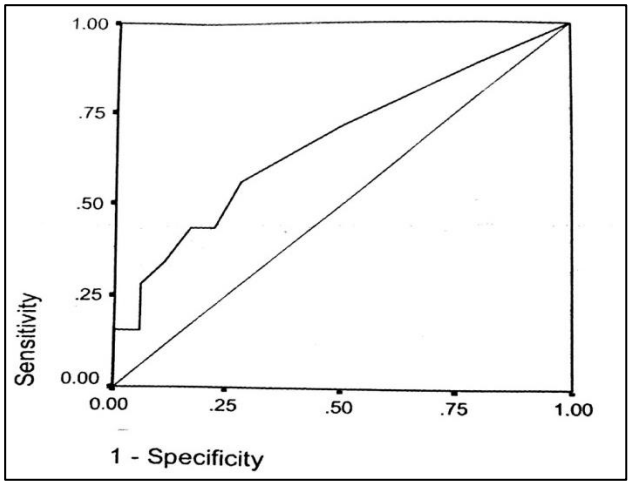


**Figure 1: D-dimer as predictor of lymph node metastasis.**

Mitotic index, Lymphovascular invasion and baseline D-dimer value were found to be statistically important predictive factors for lymph node metastasis after univariate analysis.

A receiver operating characteristic (ROC) curve analysis with D-dimer as variable and lymph node metastasis as state 250 ng/ml as cut off value for D-dimer to predict lymph node metastasis. (Area under curve=0.672, p=0.045 under non parametric assumption with sensitivity and specificity of 71.9% and 50% respectively.

## DISCUSSION

Our study showed that there is progressive increase in D-dimer and Factor VIII levels as the stage of disease progresses. This increase is found to be statistically significant.[7-10] There was no statistically significant difference found between either D-dimer or Factor VIII levels according to increase in tumor size.[8] In contrast to our result, cited study showed a significant correlation between tumor size and D-dimer levels.[9] This may be due to the small number of patients in our study group and the large variance possible in mean tumor size. We did not find any significant relationship between D-dimer and Factor VIII levels and histologic grade of the tumor. Similar results were reported by other studies when D-dimer and Factor VIII levels were compared with histologic grade of tumor. Lymphovascular invasion has been proved to have prognostic significance for risk of recurrence. At 20 years of follow-up study noted a correlation between Lymphovascular invasion and the risk of recurrence and death. For the tumour to metastasize and to survive at the metastatic site it must invade lymphovascular system and establish its own blood supply. Extracellular remodeling of fibrins involved in all steps of metastasis and plays a critical role in neovascularization. In a study linear regression modeling exhibited a significant relation between raised D-dimer levels and the Lymphovascular invasion.[9] This relationship suggests a possible, yet unproven, biologic mechanism for the entrance of D-dimer fragments into the circulation. In present study we found statistically significant relationship of D-dimer and Factor VIII levels with Lymphovascular invasion by the tumor cells, this relationship suggests a possible role of these markers as prognostic factors in carcinoma Breast. We found statistically significant relationship between Factor VIII levels and mitotic Index of tumor but relationship between D-dimer levels and mitotic index of tumor was not found to be significant.[9] We found significant relationship between D-dimer and Factor VIII levels and number of lymph nodes involved pathologically.[8,9] Another study showed this relationship to be significant only when number of lymph nodes is more than 10.[8] As axillary lymph node involvement is most important prognostic factor, this relationship inspires confidence in suggesting D-dimer and Factor VIII levels as prognostic factors further. These factors may be used in those patients who are node negative for an individualized assessment and use of systemic therapy. In our study Univariate analysis for predictive value of various clinicopathological parameters for lymph node metastasis showed that Mitotic index, Lymphovascular invasion and baseline D-dimer value were statistically important predictive factors for lymph node metastasis. In another study, it showed elevated D-dimer and tumor size as significant markers for predicting lymph node metastasis in breast cancer.[9] The most important prognostic indicator for patients with early-stage carcinoma breast is the axillary lymph nodal status and there is a direct relationship between the number of involved axillary nodes and the risk for distant recurrence.[11] The five year survival rate for patients with negative nodal disease is 82.8% versus 28.4% for >13 positive nodes.[12,13]

## CONCLUSION

D-dimer and factor VIII may be used as yardstick for systemic adjuvant therapy in patients with node negative, <1cm carcinoma breast. D-dimer may prove to be a safe, convenient and easily available biomarker which can be combined with conventional sentinel node biopsy in clinically node negative breast cancer to assess metastatic-disease in axilla and reduce false negative results. Moreover D-dimer alone or in combination with other biomarkers may also provide an alternative to conventional sentinel node biopsy to assess metastatic

disease in axilla. Significant postoperative decrease in D-Dimer and Factor VIII may provide objective criteria to assess completion of surgery. These findings need to be validated with large multicentric prospective studies with long term follow up.

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