

## CORRELATION OF COAGULATION MARKERS WITH AXILLARY LYMPH NODE METASTASIS IN CARCINOMA BREAST

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### ABSTRACT

#### BACKGROUND

The relationship between malignancy and thrombosis is known for over 100 years. Thrombocytosis, an increase in fibrinogen and fibrin degradation products like D-dimer arise in factors V, VII, VIII, IX and XI levels and a decrease in antithrombin III are seen in cancer patients. Extracellular remodeling of fibrin is essential for angiogenesis in tumours and activation of intravascular fibrin formation and degradation has been shown to occur in the plasma of breast cancer patients.

The objectives of this study are-

1. To correlate plasma level of coagulation markers (D-dimer) with clinico-pathological stage of carcinoma breast.
2. To assess role of coagulation markers (D-dimer) value as predictive markers of lymph node metastasis in breast cancer.
3. To assess changes in D-dimer values according to increasing (pathological) lymph node involvement.

#### MATERIALS AND METHODS

This study was conducted in the Department of Surgery in Dr. S. N. Medical College, Jodhpur, Rajasthan, India and included 25 patients admitted in surgery ward from January 2016 to June 2017 (Prospective study). HPE of breast specimen was done and pathological examination report were analysed with Plasma D-Dimer value.

#### RESULTS

There was statistically significant correlation between mean values of plasma D-Dimer and advancing stage of disease, tumour size, histological grade and lymphovascular invasion.

#### CONCLUSION

Plasma D-Dimer level is elevated in breast carcinoma, especially in those with advanced stage. Increased D-Dimer level is an important marker of clinical stage, lymphovascular invasion, lymph node involvement and tumour metastasis. So preoperative plasma D-Dimer may prove to be a safe, convenient and easily available predictive marker of advanced breast carcinoma.

#### KEYWORDS

Carcinoma; Thrombosis; Antithrombin.

**HOW TO CITE THIS ARTICLE:** Choudhary GS, Bairwa MS, Choudhary A, et al. Correlation of coagulation markers with axillary lymph node metastasis in carcinoma breast. J. Evolution Med. Dent. Sci. 2017;6(91):6449-6453, DOI: 10.14260/jemds/2017/1402

#### BACKGROUND

Breast cancer is the most common cancer in women and accounts for 29% of all cancers diagnosed each year in women all over the world.<sup>1</sup> Considering the magnitude of problem, it becomes of paramount importance to take various patients and tumour characteristics into consideration while chalking out the treatment plan for the patient. Lymph node metastasis has been shown to be the most important prognostic marker for carcinoma breast.<sup>2,3</sup> Lymph node involvement is not only a prognosis marker, but also an important guiding factor in deciding surgical plan as well as postoperative therapy. Presently, sentinel lymph node biopsy is considered gold standard to assess lymph node metastasis in carcinoma breast with clinically negative axilla.<sup>4</sup>

*'Financial or Other Competing Interest': None.*

*Submission 04-10-2017, Peer Review 10-11-2017,*

*Acceptance 18-11-2017, Published 27-11-2017.*

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*DOI: 10.14260/jemds/2017/1402*



Search for a marker, which can predict lymph node metastasis in clinically negative axilla has been a matter of research for long.<sup>5</sup> There is ample evidence that the components of the coagulation/ fibrinolytic system play an important role in cancer biology and angiogenesis. Fibrin deposition and remodeling in extracellular matrix of the tumour is an important initial step in tumour metastasis. For a tumour to successfully metastasise from its primary location, it must undergo several obligate steps including the invasion into either the lymphatic or vascular lumen, transportation through the circulation and establishment of viability in target tissue. Cross linked fibrin in the extracellular matrix serves as a stable framework for endothelial cell migration during angiogenesis and tumour cell migration during invasion. Extracellular remodeling of fibrin is essential for angiogenesis in tumours and activation of intravascular fibrin formation and degradation has been shown to occur in the plasma of breast cancer patients. It is reported that various abnormalities including thrombocytosis, an increase in fibrinogen and fibrin degradation products like D-dimer, a rise in factors V, VII, VIII, IX levels and a decrease in antithrombin III are seen in cancer patients. The present study is an attempt to evaluate role of these coagulation markers with special reference to D-dimer in

patients of carcinoma breast, in predicting lymph node metastasis in carcinoma patients and their relation with other known risk factors of lymph node metastasis and to look for relationship of these markers with histopathologic parameters, which are known to have predictive and prognostic value in carcinoma breast.<sup>6</sup>

**MATERIALS AND METHODS**

This study is done on 25 patients admitted in Surgical Department, Dr. S. N. Medical College, Associated Group of Hospitals, Jodhpur, Rajasthan, India. Design of the study is a prospective study. All diagnosed patients of carcinoma breast encountered during the period of study were taken up in the study.

**Inclusion Criteria**

All diagnosed patients of Carcinoma breast.

**Exclusion Criteria**

- Blood coagulation disorders.
- Conditions known to increase coagulation marker levels like DIC, MI, vaso-occlusive crisis in sickle cell disease, thromboembolic events, CLL, mechanical valve repair.

**Methodology/ TNM Staging**

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.

**D-Dimer**

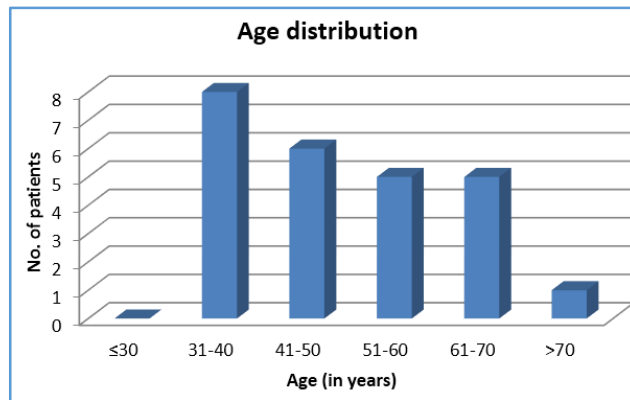
D-dimer levels were measured at the time of commencement of treatment.

The D-dimer levels were measured using single test, which is an in-vitro test for rapid determination of the fibrin degradation product D-dimer in plasma. The principle of test is based on an immunometric flow through principle. The plasma sample is applied to the test well of the device. When the sample has soaked into the device, D-dimer molecules are trapped on a membrane carrying D-dimer specific monoclonal antibodies. Then the conjugate solution is added containing monoclonal antibodies conjugated with ultrasmall 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 a colour intensity proportional to the D-dimer concentration. The colour intensity is evaluated using NycoCard Reader.

**RESULTS**

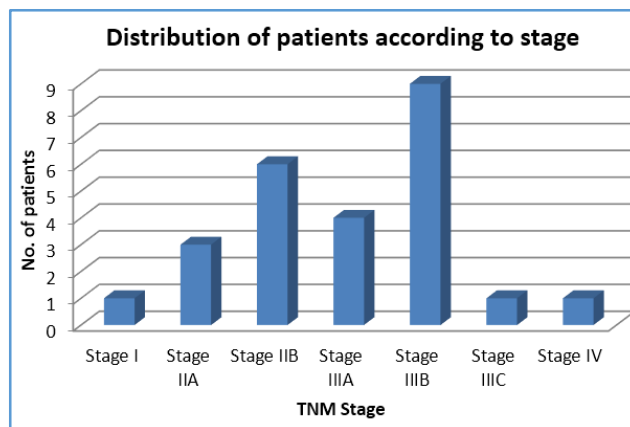
Age	No. of Patients	Percentage
≤30	0	0
31-40	8	32
41-50	6	24
51-60	5	20
61-70	5	20
>70	1	4
<b>Total</b>	<b>25</b>	<b>100</b>

*Table 1. Shows Age Distribution of Patients. Most of the Patients were in the Age Group of 31 - 60 Years and only 1 Patient was above 70 Years*



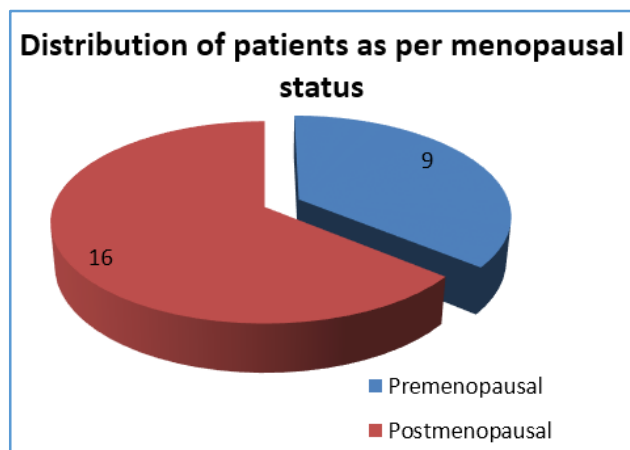
TNM Stage	Number of Patients	Percentage
Stage I	1	4
Stage IIA	3	12
Stage IIB	6	24
Stage IIIA	4	16
Stage IIIB	9	36
Stage IIIC	1	4
Stage IV	1	4
<b>Total</b>	<b>25</b>	<b>100</b>

*Table 2. Distribution of Patients according to TNM Stage of Diseases*



Menopausal Status	Number of Patients	Percentage
Pre-menopausal	9	36
Post-menopausal	16	64
<b>Total</b>	<b>25</b>	<b>100</b>

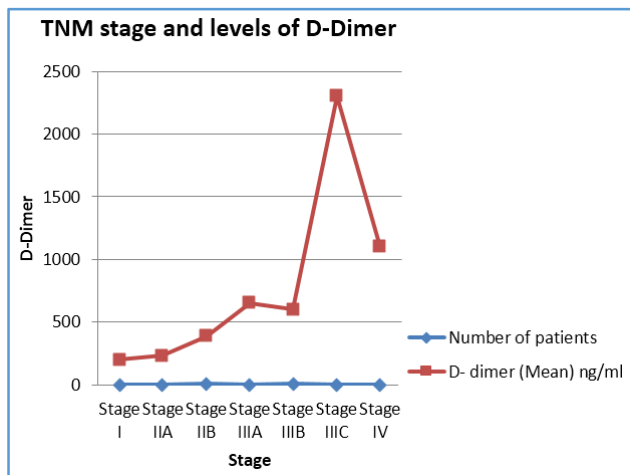
*Table 3. Distribution of Patients as per Menopausal Status*



TNM Stage	No. of Patients	D-Dimer (Mean) ng/mL
Stage I	1	200
Stage IIA	3	233.33
Stage IIB	6	383.33
Stage IIIA	4	650
Stage IIIB	9	605
Stage IIIC	1	2300
Stage IV	1	1100

**Table 4. Changes in D-Dimer values according to Advancing Stage of Disease**

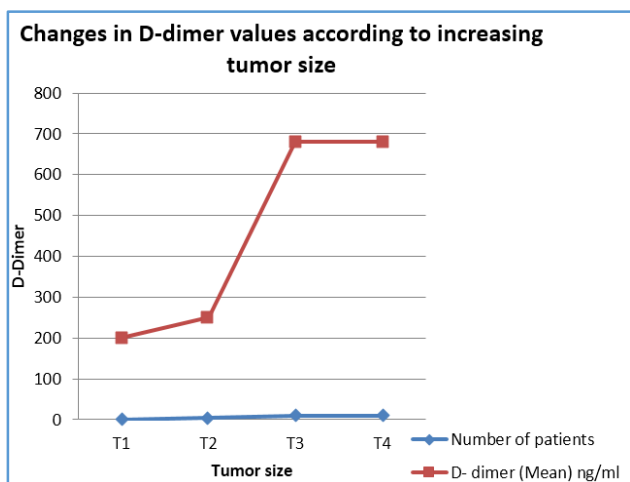
p value 0.016



Tumour Size	Number of Patients	D-Dimer (Mean) ng/mL
T1	1	200
T2	4	250
T3	10	680
T4	10	680

**Table 5. Changes in D-Dimer values according to Increasing Tumour Size**

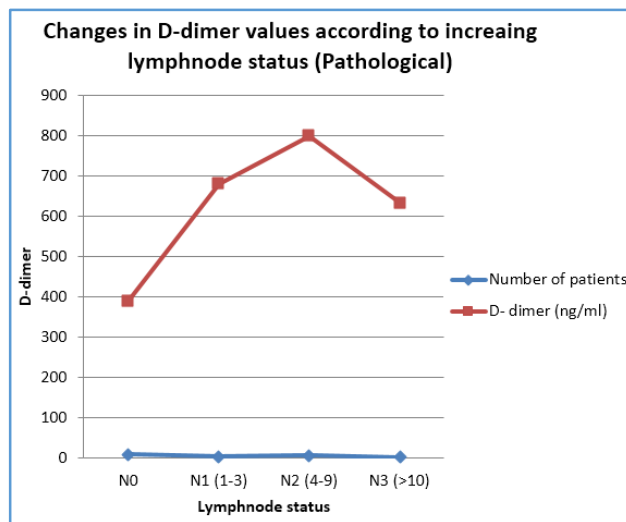
p value 0.014



Axillary Lymph Nodes (Pathological)	No. of Patients	D-Dimer (ng/mL)
N0	10	390
N1 (1-3)	5	680
N2 (4-9)	7	800
N3 (>10)	3	633.33

**Table 6. Changes in D-Dimer values according to Increasing Lymph Node Status (Pathological)**

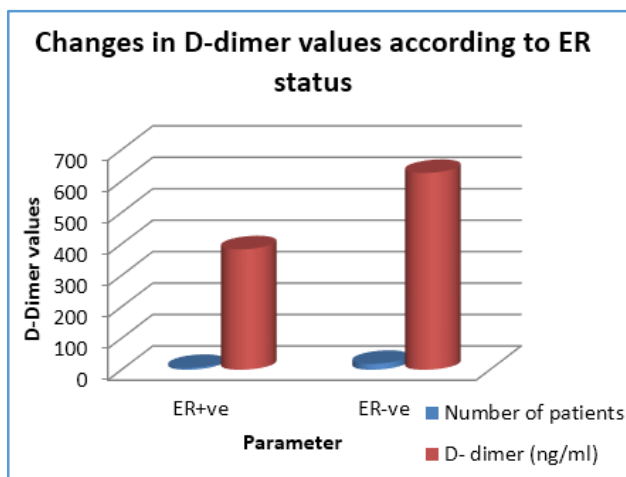
p value 0.0004



Parameter	No. of Patients	D-Dimer (ng/mL)
ER +ve	6	383.33
ER -ve	19	626.3

**Table 7. Changes in D-Dimer values according to ER Status**

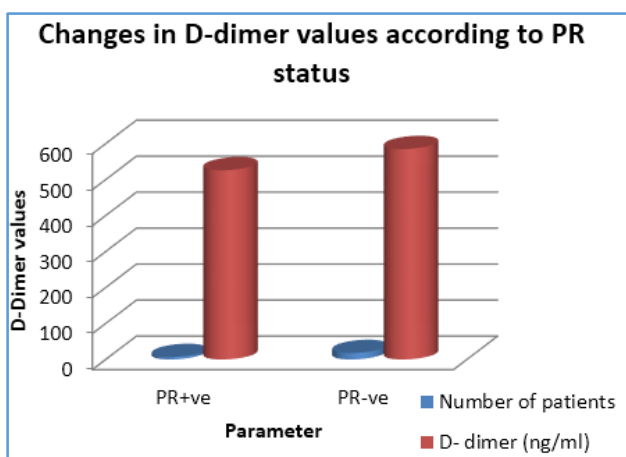
p value 0.56



Parameter	Number of Patients	D-Dimer (ng/mL)
PR +ve	7	526
PR -ve	18	585

**Table 8. Changes in D-Dimer values according to PR Status**

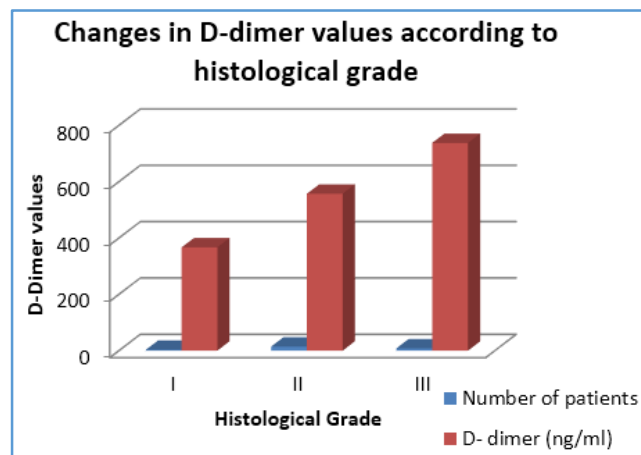
p value 0.644



Histological Grade	Number of Patients	D-Dimer (ng/mL)
I	3	366.66
II	14	557.14
III	8	737.5

**Table 9. Changes in D-Dimer values according to Histopathology**

p value 0.007



## DISCUSSION

It is accepted that coagulation and fibrinolytic system activation are associated with angiogenesis, tumoural growth, invasion and metastatic development. Levels of factor VIII and D-dimer are reported to be considerably high in cancer patients compared to healthy control subjects.<sup>7</sup> Procoagulant activity has been shown to be associated with stage of the disease.<sup>8</sup> Possible causes of increased procoagulants seem to be endothelial cell damage, endothelial synthesis (accelerated with the induction of tumour during angiogenesis), endothelial proliferation or an acute phase reaction due to vascular abnormalities, in addition to activation of the coagulation system as a result of increased tissue factor.

In the present study, 25 patients of breast cancer were included into the study. All these patients had histologically proven disease. Our purpose was to investigate the significance of D-dimer (product of fibrin degradation) as a prognostic marker in breast cancer and its relationship with other variables such as histological characteristics, lymph node status and markers (ER, PP and HER-2/neu). We also attempted to find variable, which can be used to predict lymph node metastasis in node negative breast cancer (clinically no).

Our study showed that there is progressive increase in D-dimer levels, as the stage of disease progresses. This increase is found to be statistically significant.<sup>9-12</sup>

We found a significant relationship between D-dimer and histologic grade of the tumour.<sup>9-10</sup>

We found significant relationship between D-dimer levels and number of lymph nodes involved pathologically.<sup>10</sup> As axillary lymph node involvement is the most important prognostic factor; this relationship inspires confidence in suggesting D-dimer levels as prognostic factors. Further, these factors may be used in those patients who are node negative for an individualised assessment and use of systemic therapy.

We did not find any significance between D-dimer levels with ER status of the patients. This may be due to a small sample size in our study.

## D-Dimer as Predictor of Lymph Node Metastasis

On receiver operating characteristic (ROC) curve analysis with D-dimer as variable and lymph node metastasis as state and 250 ng/mL as cut-off value for D-dimer to predict lymph node metastasis, we found it to be significant predictor with sensitivity and specificity of 71.9% and 50% respectively.

Presence or absence of axillary lymph node involvement and there is a direct relationship between the number of involved axillary node and the risk for recurrence. The 5 years survival for patients with node-negative disease is 82.8% compared with 73% for 1 - 3 positive nodes, 45.7% for 4 - 12 positive nodes and 28.4% for  $\geq 13$  positive nodes. These data demonstrate that the risk of recurrence is significant enough with lymph node positive disease to warrant adjuvant systemic therapy. Since, generally a future risk of distant recurrence of 20% or greater is regarded significant enough to consider the risks of therapy. Traditionally, the status of the axilla has been assessed by a standard axillary dissection in which level I and level II lymph nodes were removed. Recently, the use of sentinel node (SN) biopsy has become more common. Recent studies using a combination of blue dye and radiolabelled colloid have achieved detection rates of greater than 95%. Although, the ability of an experienced surgeon to accurately stage the axilla with sentinel node biopsy is accepted, 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. Adjuvant treatment is warranted in early breast cancer if nodes are positive or tumour size is  $> 1$  cm. Prognostic factors become important in a subset of patients where tumour is  $> 1$  cm and nodes are negative. This study highlights that D-dimer seems to be a promising biomarker in this group of patients. But, it definitely requires further prospective studies with long-term followup.

## CONCLUSION

This study of 25 patients of histologically diagnosed breast cancer patients was designed to evaluate the relationship of D-dimer level with various clinicopathological parameters and assess their usefulness as markers of lymph node metastasis in breast cancer. The following conclusions were drawn.

### There was Statistically Significant Correlation between Mean Value of D-dimer and Advancing Stage of Disease (p value 0.016)-

1. The mean values of D-dimer levels were found to be increasing tumour size and this was statistically significant (p value 0.014).
2. The mean values of D-dimer levels and their correlation with number of pathological axillary lymph node status was found to be statistically significant (p value 0.004).
3. The mean values of D-dimer were found to be lower in ER + tumour than ER - tumour. This difference was statistically not significant (p value 0.56).

4. The mean value of D-dimer was higher for PR negative tumours than PR positive tumours, but this was not statistically significant (p value 0.644).
5. The mean values of D-dimer were found to be elevated with increase in histological grade of tumour. This was statistically significant (p value 0.007).
6. D-dimer was found to be an independent predictive factor for lymph node metastasis thus providing an easy, simple, convenient and easily reproducible objective test which can be used as an alternative to conventional sentinel node biopsy method to find out the lymph node metastasis in axilla in cases of breast cancer.

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