

ORIGINAL RESEARCH

## Comparative Study Of D-Dimer Levels In Patients With Carcinoma Breast Vs Age-Matched Controls: A Hospital Based Study

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### ABSTRACT

**Background:** To evaluate serum D-Dimer levels in carcinoma breast patients.

**Materials and Methods:** In this prospective comparative hospital-based study, 65 diagnosed cases of operable breast carcinoma not started on any treatment for cancer and 65 healthy women were included as cases and controls, respectively. Peripheral venous blood was analyzed for D-dimers detected by Quantitative immune-turbidimetric latex assay.

**Results:** It was observed that D-dimer levels significantly correlated with clinical stage ( $r=0.583$ ;  $p<0.05$ ), histological grade ( $r=0.655$ ;  $p<0.05$ ), ER/PR receptor ( $r=0.534$ ;  $p<0.05$ ), lymph node status ( $r=0.471$ ;  $p<0.05$ ) and presence of lymphovascular invasion ( $r=0.507$ ;  $p<0.05$ ).

**Conclusion:** D-dimer is a simple, non-invasive, quick laboratory investigation that may be considered a good indicator for determining the clinical stage, progression of disease and lymphovascular invasion.

**Keywords:** D-dimer, Carcinoma Breast, Fibrin Degradation Product.

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### INTRODUCTION

Breast carcinoma is the leading cause of carcinoma death in women, with more than 10,00,000 cases being reported worldwide annually.<sup>1</sup> Breast carcinoma has become the commonest malignancy in urban India, replacing cervical cancer.<sup>2</sup> In rural India, it is the second most common malignancy in females.

The hemostatic system plays a vital role in maintaining bleeding and clot formation balance. Interestingly, activation of coagulation and fibrinolysis is often present in cancer patients, especially in patients with metastatic disease.<sup>3-4</sup> The formation of the platelet-fibrin-tumor cells creates an extracellular microenvironment that can facilitate tumor cell growth and survival. Lymphovascular invasion (LVI) is the presence of tumor cells within a definite endothelial-lined space in the breast surrounding invasive carcinoma, a significant prognostic factor in invasive breast cancer, concerning local and distance recurrence<sup>5-6</sup> and poorer survival.<sup>7-9</sup> Lymphovascular invasion is also associated with other prognostic factors, including tumor size, grade and locoregional lymph node involvement.<sup>10</sup>

D-dimer, a plasmin-mediated degradation product of cross-linked fibrin. Recent articles reported that plasma D-dimer was elevated in patients with different malignancies before treatment, including lung cancer, prostate cancer, cervical cancer, breast cancer, and colorectal cancer.<sup>3</sup> However, the prognostic role of D-dimer in cancer is still controversial. There has been no systematic attempt to explore its prognostic value. There exists a gap in our knowledge regarding the relationship between quantitative D-dimer levels and the extent of disease involvement in breast cancer. In this context, this study was done to evaluate the correlation of D-Dimer levels with lymph nodes involvement and Lymphovascular invasion in carcinoma breast patients.

## MATERIALS & METHODS

This prospective age-matched comparative study was conducted in the department of surgery of a tertiary care institute in South India over two years (from Oct 2019 to Sept 2021). The test group comprised of 65 patients visiting the surgical oncology outpatient department and diagnosed with biopsy-proven invasive carcinoma breast. Patients on anticoagulants, anti-platelet drugs, history of smoking, or any other malignancies were excluded. The control group comprised 65 age-matched healthy women. The purpose of the study was thoroughly explained and informed written consent was taken to participate in the study. In addition, ethical clearance was taken from the institute's ethical committee.

### Laboratory Procedure

Citrated, platelet-poor plasma was prepared from venous blood collected by venipuncture. The blood was collected into 3.2% tri-sodium-citrate in a ratio of 9:1

D-dimer was detected by immunoassay using monoclonal antibodies specific for the cross-linked D-dimer domain in fibrinogen by using a quantitative immunoturbidimetric latex assay. Reference intervals: Normal plasma D-dimer values in healthy women were taken as < 0.25 mg/l. Precision was estimated based on two concentration levels of an analyte according to protocol EP-5 T (20 consecutive days, two runs per day, two repeats per run).

### Statistical Analysis

Quantitative data was depicted with the help of Mean and Standard deviation. Comparison among the study groups was made using an unpaired t-test. Qualitative data was presented with the use of a frequency and percentage table. Association among the study groups was assessed with the help of the Fisher test, student 't-test, and Chi-Square test. 'p-value less than 0.05 was taken as significant.

## RESULTS

A total of 65 cases of biopsy-proven breast cancer and 65 healthy controls were included. The majority of the patients (32.3%) were from 51-60 years. The mean age of the study participants in the case group was  $53.52 \pm 14.02$  years. [Table 1].

**Table 1: Distribution of patients(age wise)**

Age (years)	N	%
21-30 years	5	7.7%
31-40 years	6	9.2%
41-50 years	14	21.6%
51-60 years	20	32.3%
61-70 years	12	18.4%
71-80 years	7	10.8%

<b>Total</b>	65	100%
<b>Mean ± SD</b>	53.52 ±14.02	

9 out of 65 (13.8%) patients were at Stage I, while 14 (21.5%) and 20 (30.8%) patients were at Stage IIA and Stage IIB, respectively. 22 (33.9%) patients were at Stage IIIA. 10 (15.4%) patients were Grade I, while 21 (32.3%) and 34 (52.3%) patients were Grade II and Grade III, respectively. The total number of ER/PR positive cases was 78.5%. 39 (60%) patients presented lymph node-positive while 26 (40%) patients presented lymph node-negative. There was the presence of lymphovascular invasion in 46 (70.8%) patients. The mean D-Dimer level of patients was 3.04 ± 2.06 mg/l. [Table 2].

**Table 2: Distribution of patients as per D-Dimer Levels**

D-Dimer Level	N	%
<0.25 mg/l	7	10.8%
0.25 - 2 mg/l	15	23.1%
2 - 5 mg/l	30	46.1%
>5 mg/l	13	20%
<b>Total</b>	65	100%
<b>Mean ± SD</b>	3.04 ± 2.06	

There was a significant association of D-Dimer levels with clinical Stage, histological Grade, ER/PR status, lymph node status, and presence of lymphovascular invasion as per the ANOVA test ( $p < 0.05$ ). [Table 3-7].

**Table 3: Association of Clinical Staging and D-Dimer Levels of patients**

Clinical Staging	Mean	SD	p-value
Stage I	0.68	0.37	<b>&lt;0.05</b>
Stage IIA	1.42	0.78	
Stage IIB	3.56	1.66	
Stage IIIA	4.56	1.79	

**Table 4: Association of Histological Grade and D-Dimer Levels of patients**

Histological Grade	Mean	SD	p-value
Grade I	0.63	0.38	<b>&lt;0.05</b>
Grade II	1.98	1.22	
Grade III	4.40	1.71	

**Table 5: Association of ER/PR receptor and D-Dimer Levels of patients**

ER/PR receptor	Mean	SD	p-value
Yes	2.29	1.42	<b>&lt;0.05</b>
No	6.10	0.92	

**Table 6: Association of Lymph node status and D-Dimer Levels of patients**

Lymph node status	Mean	SD	p-value
Positive	4.04	1.63	<b>&lt;0.05</b>
Negative	1.53	1.69	

**Table 7: Association of presence of lympho-vascular invasion and D-Dimer Levels of patients**

Presence of lymphovascular invasion	Mean	SD	p-value
Yes	3.92	1.79	<b>&lt;0.05</b>
No	0.90	0.55	

It was observed that D-dimer levels significantly correlated with clinical stage ( $r=0.583$ ;  $p<0.05$ ), histological grade ( $r=0.655$ ;  $p<0.05$ ), ER/PR receptor ( $r=0.534$ ;  $p<0.05$ ), lymph node status ( $r=0.471$ ;  $p<0.05$ ) and presence of lympho-vascular invasion ( $r=0.507$ ;  $p<0.05$ ). [Table 8]

**Table 8: Correlation of D-Dimer with various parameters**

Parameters	D-Dimer	
	r	p
Clinical Stage	0.583	<b>&lt;0.05</b>
Histological Grade	0.655	<b>&lt;0.05</b>
ER/PR receptor	0.534	<b>&lt;0.05</b>
Lymph node status	0.471	<b>&lt;0.05</b>
Presence of lymphovascular invasion	0.507	<b>&lt;0.05</b>

## DISCUSSION

The fibrin d-dimer antigen is an unparalleled marker of the primary enzymatic dissolution product of cross-linked fibrin by plasmin dissolve the cross-linked fibrin to liberate fibrin degradation products and reveal the D dimer antigen. Plasma D dimer levels are elevated in many clinical conditions like smoking, infection, pregnancy, old age, trauma, tumors, and others<sup>12</sup>; a supplement to the diagnostic use of D dimer, it could be possible prognostic use in many conditions.

In a systematic review and meta-analysis, Li W et al. 11 in 2018 evaluated the prognostic value of elevated D-dimer levels in solid tumors. The authors reported different tumor sites, disease stages, cut-off values, and ethnicities. Meanwhile, patients with a high plasma D-dimer had a shorter PFS (HR = 1.46, 95% CI = 1.22–1.76;  $P < 0.001$ ), DFS (HR = 2.02, 95% CI = 1.56–2.62) and CSS (HR = 2.04, 95% CI = 1.58 – 2.64). The authors concluded that the pre-treatment plasma D-dimer analysis might provide useful information to predict prognosis in patients with solid tumors. Blackwell K et al. 13 study investigating the relationship between plasma D-dimer levels and extent of tumor involvement in operable breast cancer patients observed thirty-three patients (35%) had involved lymph nodes, with the mean number of positive lymph nodes being 3.28 (range, one to 16 positive lymph nodes). Five patients (3%) had ten or more involved lymph nodes. Five patients (3%) had five to nine active lymph nodes. Three patients with ten or more lymph nodes had preoperative palpable axillary lymph nodes documentation. None of the patients with five to nine involved lymph nodes were noted preoperatively to have palpable lymph nodes. In the present study, 26 (40%) patients had axillary lymph node dissection and ten lymph nodes removed at the time

of dissection. Determining lymph node status via standard lymph node dissection creates significant long-term side effects, including pain, numbness, and lymphedema. Many attempts have been made to predict lymph node status without undergoing a complete lymph node dissection.

The D-dimer level is a clinically significant marker for progression and points towards a relation between hemostasis and tumor progression. D-dimer, a fibrin degradation product, is a prognostic and predictive indicator in breast cancer patients.

D-dimer is a universally available, routinely measured, and simple, reproducible molecular marker. Plasma D-dimer is markedly elevated in patients with different malignancies before treatment, including breast cancer<sup>3</sup>. Ferroni P et al.<sup>3</sup> study reported that an elevated D-dimer level before treatment is associated with a more advanced tumor stage or a more advanced clinical progression; therefore, it may indicate a worse prognosis. It was noted in the present study that there was a significant increase in the D-Dimer level with the clinical Stage. This finding was similar to the studies of Harish S et al.<sup>14</sup>, Blackwell K et al.<sup>13</sup>, and Ghadhban BR<sup>15</sup>.

Harish S et al.<sup>14</sup> study showed a statistically significant difference in D-dimer levels based on clinical stage grouping ( $P = 0.002$ ). Study by Blackwell K et al.'s<sup>13</sup> reported results of correlation between D-dimer and clinical-stage showed no patients were Stage IIIB or stage IV. There was a statistically significant difference in D-dimer levels based on clinical stage grouping.

The current cancer prognostic evaluation system has its drawbacks in that patients with the same TNM stage often have different survival outcomes. D-dimer may serve as a complementary factor that can help us to improve prognosis estimation.

It was observed in our study that there was a significant increase in the D-Dimer level with the histological Grade. Ghadhban BR<sup>15</sup> prospective study revealed when there was a high grade of the disease, there was an elevation in the level of d-dimer and the difference was significant. Blackwell K et al.<sup>13</sup> study showed D-dimer levels correlated strongest with the number of positive lymph nodes in the pilot, prospective, and combined groups. In our study, the patients with lymph node-positive had significantly higher D-Dimer levels than those with lymph node-negative ( $4.04 \pm 1.63 \text{ mg/l}$  vs.  $1.53 \pm 1.69 \text{ mg/l}$ ). Similar observations were noted in Harish S et al.<sup>14</sup>, Ghadhban BR<sup>15</sup>, and Blackwell K et al.<sup>13</sup>. Blackwell K et al.<sup>13</sup> studies observed a significant difference between D-dimer levels in those patients with benign breast disease, ductal carcinoma-in-situ, lymph node-negative invasive carcinoma, and lymph node-positive invasive carcinoma. There was a significant difference between median D-dimer levels in patients having positive nodes compared with those patients with no nodal involvement. Elevated D-dimer levels correlated strongly with the presence of positive lymph nodes. It was observed in the present study that the D-Dimer level of patients with the presence of lympho-vascular invasion was significantly higher compared to patients without the presence of lympho-vascular invasion ( $3.92 \pm 1.79 \text{ mg/l}$  vs.  $0.90 \pm 0.55 \text{ mg/l}$ ). This is similar to Harish S et al.<sup>14</sup>, Dent R et al.<sup>16</sup>, Blackwell K et al.<sup>13</sup>, and Ghadhban BR<sup>15</sup>.

Harish S et al.'s<sup>14</sup> study observed D-dimer levels directly correlated with the extent of lymph node involvement and lympho-vascular invasion. D-dimer levels were associated strongest with the number of positive lymph nodes but not with tumor size or estrogen and progesterone receptor status. Study by Blackwell K et al.<sup>13</sup> found that lympho-vascular invasion and positive lymph nodes were significant in predicting D-dimer levels in univariate and multivariate linear regression models. A patient with a 2-cm tumor and an elevated level of D-dimer (200 ng/mL) was twice as likely (probability 5 0.42) to have involved axillary lymph nodes as those patients with low levels of D-dimer (5 ng/mL; probability 5 0.20).

It was observed in our study that D-dimer levels significantly correlated with clinical stage ( $r=0.583$ ;  $p<0.05$ ), histological grade ( $r=0.655$ ;  $p<0.05$ ), ER/PR receptor ( $r=0.534$ ;  $p<0.05$ ), lymph node status ( $r=0.471$ ;  $p<0.05$ ) and presence of lymphovascular invasion ( $r=0.507$ ;  $p<0.05$ ). This is comparable to Blackwell K et al.<sup>13</sup>, Ryu YJ et al.<sup>18</sup>, and Perisanidis C et al.<sup>17</sup>.

## CONCLUSION

Lymph node metastasis is the one of the most important prognostic factors in patients with carcinoma breast. Plasma d-dimer level is a good prognostic factor in breast carcinoma, especially in advanced breast carcinoma, and may also be considered a good indicator for determining clinical-stage progression of the disease, lymphovascular invasion.

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