Role of Plasma D-Dimer Levels in Breast Cancer Patients and Its Correlation with Clinical and Histopathological Stage

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Abstract Breast cancer, the most common female malignancy, represents a heterogeneous group of tumors, which present both varied behaviors and response to therapy. This study was done with an attempt to evaluate the role of D-dimer in patients of carcinoma breast, in predicting lymph node metastasis in carcinoma patients and to look for relationship of these markers with histopathologic parameters. Clinical data was obtained from clinical examination of patients admitted in the Department of Surgery with history of breast lump and confirmed with fine needle aspiration cytology (FNAC). Clinical staging was done using TNM staging. D-dimer level was measured prior to commencement of treatment, i.e., modified radical mastectomy (MRM) or neoadjuvant chemotherapy and finally 6 months after surgery or completion of 6 cycles of chemotherapy. The characteristics of the study population Out of 60 study cases minimum age of the patient being 30 years and maximum being 74 years. Of all histopathologic variables examined, D-dimer levels directly correlated with extent of lymph node involvement and lymphovascular invasion, D-dimer levels correlated strongest with the number of positive lymph nodes, but not with tumor size, estrogen receptor status, and progesterone receptor status. This study clearly shows that plasma D-Dimer levels are elevated in carcinoma breast patients. Increased D-Dimer levels are an important marker of clinical stage, lymphovascular invasion, lymph node involvement, and tumor metastasis.

Keywords Breast cancer · D-Dimer · Bloom-Richardson grading · Metastasis · Early breast cancer

Introduction

Breast cancer, the most common female malignancy, represents a heterogeneous group of tumors, which present both varied behaviors and response to therapy. The development of distant metastases is the primary cause of death in breast cancer patients. Thrombosis in cancer is often migratory and may involve superficial veins and relatively unusual sites. The hypercoagulable state in malignancy is due to a complex interaction of tumor cells and their products with the host cells leading to various degrees of impairment of the normal defense mechanisms that ordinarily protect the host against thrombogenesis. Tumor cells can activate directly the blood clotting cascade and cause thrombosis or can induce procoagulant properties and inhibit the anticoagulant ones of the vascular endothelial cells, platelets, monocytes, and macrophage. The association of breast cancer and hemostasis activation has been previously reported and runs in-line with disease progression. Fibrin in the tumor may increase endothelial motility, worsen angiogenesis, and contribute to an increased risk of thrombosis in patients with breast cancer. 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, and XI levels, and a decrease in antithrombin III, are seen in cancer patients. Extracellular remodeling of fibrin is essential for angiogenesis in tumors, and activation of intravascular fibrin formation and degradation has been shown to occur in the plasma of breast cancer patients. This study was done with an attempt to evaluate the role of D-dimer in patients of carcinoma breast in predicting lymph node metastasis in carcinoma patients and

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Table 1 Patient characteristics

Characteristics		Total nos.	Percentage
Menopausal status	Premenopausal	32	53%
	Postmenopausal	28	46%
Histological grade	Grade I	12	20%
	Grade II	16	26%
	Grade III	32	53%
Histology	In situ	11	18.3%
	Invasive ductal carcinoma	49	81.6%
Lymph node	Positive	38	63.3%
	Negative	22	36.6%
ER/PR receptor	Positive	48	
	Negative	12	
Staging	I	10	
	II	30	
	III	20	

to look for relationship of these markers with histopathologic parameters which are known to have predictive and prognostic values in carcinoma breast.

Materials and Methods

A total of 60 cases of carcinoma breast admitted in the Department of Surgery, JSS Hospital Mysore, during the period from October 2014 to September 2016 were included in the study.

Inclusion Criteria All the cases of carcinoma breast as per F.N.A.C and H.P.E report irrespective of age that was undergoing surgical treatment were included in this study.

Exclusion Criteria (1) Cases of metastatic breast carcinoma and (2) Condition alter the coagulation factors such as warfarin therapy, thrombotic episodes, disorders of coagulation, liver disease, pregnancy, major trauma within 3 months, recent surgery within 3 months.

Clinical data was obtained from clinical examination of patients admitted in the Department of Surgery with history of breast lump and was confirmed with FNAC. Clinical staging was done using TNM staging.

Table 2 D-dimer level in patient population

D-Dimer (mg/l)	Nos. of patients	Percentage
< 0.25	6	10%
0.25–2	12	20%
2–5	28	46%
> 5	14	23%

Table 3 Plasma D-Dimer levels and histopathologic correlates

	Spearman Correlation	P
Lymph node status	0.51	0.0001
Tumor size	0.26	0.008
ER/PR status	−0.27	0.019
Histologic grade	0.50	0.002
Lymphovascular invasion	0.47	0001

D-dimer level was measured prior to commencement of treatment, i.e., modified radical mastectomy (MRM) or neo-adjuvant chemotherapy and finally 6 months after surgery or completion of 6 cycles of chemotherapy.

After MRM, the tissue specimens were examined for the following characteristics: tumor size, presence or absence of invasiveness, histological grade—according to Bloom Richardson's scoring, lymph vascular invasion, ER, PR status, axillary lymph node status.

Blood specimen: D-dimers are detected by immunoassays using monoclonal antibodies specific for the crosslinked D-dimer domain in fibrinogen. Specimen collection and preparation: Citrated, platelet-poor plasma is prepared from venous blood collected by venipuncture. The blood is collected into 3.2% tri-sodium-citrate at a ratio of 9:1. The citrate concentration must be adjusted in patients with a HCT > 55%. Plasma should be separated from the cells as soon as possible after the specimen is obtained. D-dimers are stable for 8 h in citrated plasma maintained at room temperature, for 7 days if stored at 2–8 OC, and up to 2 months at −20 0 C. Method used: Quantitative immunoturbidimetric latex assay. Reference intervals: Normal value of plasma D-dimer in healthy women is < 0.25 mg/l. Dynamic range and linearity: The D dimer procedure is linear from 0.25–7.5 µg FEU/ml. Precision: Estimated on two concentration levels of analyte according to protocol EP-5 T (20 consecutive days, 2 runs per day, 2 repeats per run).

Results

The characteristics of the study population out of 60 study cases are minimum age of the patient being

Fig. 1 D dimer levels with tumor volume: above distribution chart comparing the d dimer levels with the volume of the tumor. Levels of D-dimer elevated linearly with the tumor volume

Fig. 2 Relation between D Dimer levels with clinical palpable lymph nodes $p = 0.005$, relation between lymph node status clinically and d dimer levels. The mean d dimer level in patients with clinically palpable lymph nodes is 2.78 which is significant when compared with no palpable lymph nodes

30 years and maximum being 74 years. Rt. Sided breast lump was more common accounting for 56.67%. Most common location of breast lump was in the upper outer quadrant (60%).

Most of these lumps had nodular surface, irregular margins, were hard in consistency, and were not fixed to chest wall and overlying skin. According to the histological grade tumor differentiation, 20% of the patients were GI, 26% were GII, and 53% were GIII. Forty-six percent of the patients were at a postmenopausal status (Tables 1, 2 and 3).

The tumor's histologic characteristics identified 81.6% as IDC. Thirty-eight patients (63.1%) presented lymph node positive, whereas 22 (36.6%) patients presented lymph node negative. Of all histopathologic variables examined, D-dimer levels directly correlated with extent of lymph node involvement and lymphovascular invasion, D-dimer levels correlated strongest with the number of positive lymph nodes but not with tumor size, estrogen receptor status, and progesterone receptor status (Fig. 1).

Elevated D-dimer levels and tumor size were significant in predicting the presence of positive lymph nodes (Fig. 2).

D-Dimer and clinical stage: The results of correlation between D-dimer and clinical stage are listed in (Table 4). No patients were stage IIIB or stage IV. There was a statistically significant difference in D-dimer levels based on clinical stage grouping ($P = 0.002$).

Table 4 D- dimer level with clinical stage

Clinical stage	Mean	SD	Median
2A	1.31	1.78	.63
2B	1.74	1.41	1.25
3A	2.87	2.33	2.25

There is linear increase in the d dimer level with the clinical stage. Mean d dimer levels in patients with TNM stage 2A is 1.31, whereas in stage 3A it increased to 2.87. Which is statistically significant ($p < 0.05$)

Fig. 3 D-dimer levels with the grade of the tumor. D dimer levels are significantly elevated with higher grade

Discussion

Breast cancer is the most common cancer in females, especially in developing countries like India. With the development of medical knowledge and advent of newer therapies now, surgeons are aiming for the complete cure of the disease [1]. In this context, there is increased need for prognostic and predictive indicators to monitor disease process, response to the treatment, and recurrence so that we can intervene at appropriate time and with appropriate alternative therapy. Our study is an attempt to find out D- dimer, a product of fibrin degradation, as a prognostic and predictive indicator in the breast cancer patients. Available prognostic factors in the literature include lymph node status, tumor size, and estrogen/progesterone receptor (ER/PR) status [2, 3]. Additional factors include grade, presence of lymphovascular invasion, age, and ethnicity (Fig. 3). Certain biologic factors, including ER/PR and HER2/neu, plasminogen activator, and plasminogen activator inhibitor type 1 are both prognostic and predictive.

D-dimer level is a clinically important marker for progression and points towards a relation between hemostasis and tumor progression. This study is in partial agreement with this relation and these results strongly indicate interactions between angiogenesis and homeostasis in breast cancer. The process of metastasis involves multiple tumor-host interactions [4]. To survive, metastatic cancer cells must leave the primary tumor, migrate into the lymphovascular system, and establish a new blood supply at their metastatic site. Fibrin remodeling is almost certainly involved in all steps of metastasis and has been proven to play a crucial role in new vessel formation [4, 5]. This study confirms previous studies showing upregulated fibrinolytic activity (presence of plasma D-dimer) in malignant disease and increased levels of fibrinolytic activity (increased D-dimer levels) in metastatic disease. In addition, linear regression modeling showed a tight

Fig. 4 D-dimer levels with vascular invasion of tumor D dimer levels are significantly elevated when tumor involves the blood vessels

Fig. 5 Scatter plot between D-dimer levels with Bloom Richardson's scoring of the tumor on histopathological examination

relationship between the presence of lymphovascular invasion and elevated D-dimer levels (Fig. 4).

Clinically palpable lymph nodes, clinical stage are considered prognostically significant. In our study, the mean D-dimer level in patients with clinically palpable lymph nodes is 2.78 which is statistically significant ($p = 0.005$) when compared with no palpable lymph nodes.

When compared D dimer levels with the TNM staging, there is linear increase in the d dimer level with the clinical stage. Mean D-dimer levels in patients with TNM stage 2A is 1.31, whereas in stage 3A it increased to 2.87 which is statistically significant ($p < 0.05$).

We compared histopathological variables with D-dimer levels to grade of the tumor, lymphovascular invasion of the tumor, Bloom Richardson's scoring are considered predictive markers for prognosis of the disease (Fig. 5). In grade 2 tumors, the mean D-dimer levels are 0.44, whereas in grade 3 tumors, mean D-dimer levels are 2.67. ($p = 0.001$). Those tumors which invaded the vessels mean D-dimer levels are 1.97 which is higher than those not invaded ($p = 0.02$).

The mean D-dimer level is 0.39 when Bloom Richardson's scoring is 6, 1.24 when BRS is 7, and 2.67 when BRS is 8 ($p = 0.0001$) (Fig. 6). The correlation coefficient between these two variables is 0.719 which is statistically very significant. The mean D-dimer levels are more in hormone receptor negative patients compared to the ER/PR positive (Fig. 7).

A similar study done by L Y Dirix et al. in Belgium on 30 breast cancer patients showed that they were correlated D-dimer levels with tumor load and progression kinetics and

Fig. 6 D-dimer levels with bloom Richardson's scoring The mean dimer level is 0.39 when BRS is 6; it increased to 2.67 when it is 8

Fig. 7 D dimer levels with ER/PR status Estrogen and progesterone receptor negative status considered bad prognostic factor. In patients with negative receptor status having high median d dimer levels compared to the patients with positive receptor status

concluded that the level of D-dimers positively correlated with tumor load ($p = 0.001$) [6].

A.P.B Batschauer conducted similar study in Brazil on 31 breast cancer patients concluded that there is definite increase in levels of D- dimers in triple negative breast cancer patients but plasma D-dimers were not correlated with clinical and histopathological findings ($p > 0.213$) [7].

In another study done by Ketut sue ga in Indonesia on 79 breast cancer patients concluded that D-dimer levels positively correlated with clinical stage of solid cancers [8].

Recently in India, a similar study done by Bhavesh devkaran on 90 breast cancer patients concluded that increased D-dimer levels is an important marker of lymphovascular invasion, lymph node involvement, and tumor metastasis [9].

Our study results showed that the D-dimer levels were not statistically correlated with tumor volume ($p = 0.71$), ER/PR status (0.07), other clinical and histopathological variables like lymph node involvement ($p = 0.001$), clinical stage ($p = 0.05$), lymphovascular invasion (0.002), grade of the tumor ($p = 0.001$), and BRS ($p = 0.001$). There is considerable decrease in the levels of D-dimer in response to the treatment, i.e., MRM and chemotherapy ($p = 0.0006$) and correlation coefficient 0.906 (Fig. 8) [10, 11].

Fig. 8 Comparing preoperative D-dimer levels with postoperative level. Preoperative mean D dimer levels are 1.90; after treatment, that is MRM and chemotherapy, mean D-dimer levels are 0.66. ($p = 0.0006$) correlation 0.906

Conclusion

The diagnosis and treatment of carcinoma breast requires a multi-disciplinary approach involving the surgeon, radiologist, pathologist, and medical oncologist. Treatment planning and survival depend on the stage of disease at the time of diagnosis. Lymph node metastasis is the most important prognostic factor. This study clearly shows that plasma D-Dimer levels are elevated in carcinoma breast patients. Increased D-Dimer levels are an important marker of clinical stage, lymphovascular invasion, lymph node involvement, and tumor metastasis.

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