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Pathology

DIAGNOSTIC UTILITY OF COAGULATION PARAMETERS (PROTHROMBIN TIME, ACTIVATED PARTIAL THROMBOPLASTIN TIME, AND D-DIMER) IN DIFFERENTIATING BENIGN AND MALIGNANT BREAST LESIONS: A PROSPECTIVE HISTOPATHOLOGICAL CORRELATION STUDY

KEY WORDS: Breast Carcinoma, D-dimer, Prothrombin Time, Hypercoagulability.

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ABSTRACT

This prospective cross-sectional study evaluates the diagnostic efficacy of a baseline preoperative coagulation profile—comprising Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), and quantitative plasma D-dimer levels—in differentiating benign breast lumps from malignant breast lesions, and correlates these indices with established tumor staging criteria. Sixty female patients presenting with palpable breast lumps who underwent Fine Needle Aspiration Cytology (FNAC) followed by definitive surgical excision were enrolled. Coagulation metrics were quantified using an automated Erba ECL 105 analyzer and correlated with post-surgical histopathological parameters. Preoperative mean values for all parameters were significantly higher in the malignant cohort compared to the benign arm:PT (18.63 +/- 4.3 vs. 13.69 +/- 1.1 s , p = 0.0071), aPTT (41.755 +/- 3.9 vs. 32.283 +/- s, p =0.00091), and plasma D-dimer (901.94 +/- 420.5 vs. 162.969 +/- 78.4 ng/mL , p = 0.0002).Within the malignant subgroup, plasma D-dimer scaled significantly with increasing tumor size (p = 0.0045), higher Nottingham histological grade (p = 0.0029), and advanced axillary regional nodal burden (p = 0.0017). Furthermore, a simultaneous elongation or rise across all three coagulation metrics was uniquely linked to the presence of structural lymphovascular invasion (p = 0.000179). Preoperative evaluation of baseline coagulation pathways, particularly plasma D-dimer, offers a rapid, highly reproducible, and cost-effective screening adjunct that optimizes risk stratification in primary breast carcinoma.

INTRODUCTION

Breast masses represent an exceptionallY common presentation in the female population globally [1]. While a substantial majority of palpable breast lumps are confirmed to be benign entities, breast carcinoma remains the leading primary malignancy among women and a significant contributor to cancer-related mortality [2]. Accurate early distinction and risk stratification are fundamental to tailoring multi-disciplinary treatment pathways and improving clinical outcomes [1, 2]. Diagnostic frameworks routinely deploy triple assessment; however, establishing reliable, non-invasive, peripheral blood-based biomarkers that match the biological impact of local neoplastic progression represents a relevant clinical objective [3].

A subclinical prothrombotic or hypercoagulable state is a recognized feature of solid organ malignancies [1, 2]. Tumors alter vascular hemostasis via direct mechanisms, including the constitutional expression of Tissue Factor (TF) and secretion of protease activators like Cancer Procoagulant, alongside indirect loops mediated by pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF- \alpha) and Interleukin-1 (IL-1) [2]. This ongoing low-grade systemic activation of the clotting cascade results in the continuous consumption of circulating clotting factors, which can alter standard clinical coagulation screens like Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) [2].

Simultaneously, the continuous degradation of cross-linked fibrin networks by host plasmin produces predictable systemic elevations of cross-linked fibrin split fragments, notably plasma D-dimer [2, 4]. This study evaluates the efficacy of baseline preoperative PT, aPTT, and quantitative D-dimer levels in differentiating benign from malignant masses, and analyzes their correlation with histopathological indicators of tumor aggression [4, 5].

MATERIALS AND METHODS

This prospective cross-sectional study was conducted in the Department of Pathology, Index Medical College Hospital & Research Centre, Indore, over a five-month period from July 2024 to November 2024. Sixty female patients presenting with clinically palpable breast massesreferred for Fine Needle Aspiration Cytology (FNAC) who subsequently underwent definitive operative resection were included via simple random sampling. The study protocol utilized standard diagnostic investigations on standard clinical volumes without modifying patient care protocols.

- **Inclusion Criteria**: Patients with palpable breast masses who underwent initial FNAC followed by definitive institutional surgery providing specimens for final histopathological confirmation.
- **Exclusion Criteria:** History of distant metastatic disease at baseline; oral or systemic anticoagulant therapy within the preceding 3 months; history of thromboembolic events [1, 2]; severe chronic liver disease; active pregnancy; or major trauma/surgery within the preceding 3 months.

Preoperatively and concurrent with diagnostic FNAC, peripheral venous blood was collected into 3.2% buffered sodium citrate tubes (9:1 ratio). Samples were centrifuged at 2500 \times g for 15 minutes to isolate platelet-poor plasma. PT, aPTT, and quantitative plasma D-dimer concentrations were evaluated on an automated Erba ECL 105 single-channel analyzer following standard manufacturer calibration guidelines.

Surgical tissue products were fixed in 10% neutral buffered formalin, grossed, sectioned, and processed for routine Hematoxylin and Eosin (H&E) staining. Malignant tumors were classified according to architectural subtype, modified Bloom-Richardson (Nottingham) histological grade, pathological tumor size (pT status), axillary lymph node status (pN status),

and the presence or absence of lymphovascular invasion (LVI).

Continuous data were expressed as mean values +/- standard deviations and analyzed using independent unpaired Student's *t*-tests and one-way Analysis of Variance (ANOVA) via SPSS version 26.0, where a p -value <0.05 was considered statistically significant.

RESULTS

The baseline age of the 60 female participants ranged from 16 to 74 years, with an overall mean age of 37.2 years. Confirmed histopathological evaluation split the study cohort equally into 30 benign lesions and 30 malignant invasive breast carcinomas. Fibroadenoma was the most common benign diagnosis (n=22 , 73.3%), while Invasive Ductal Carcinoma (IDC), Not Otherwise Specified (NOS), was the predominant malignant subtype (n=28 ,93.3%), as structured below in Table 1.

Table 1: Patient Age Distribution and Baseline Histopathological Subtypes (N=60)

Parameter / Pathological Metric	Cohort Category	Frequency (n)	Percentage (%)
Age Distribution (Years)	< 30	18	30.0%
	31–50	27	45.0%
	> 50	15	25.0%
Benign Histopathology (n=30)	Fibroadenoma	22	73.3%
	Fibrocystic Changes	8	26.7%
Malignant Histopathology (n=30)	Invasive Ductal Carcinoma (IDC)	28	93.3%
	Invasive Lobular Carcinoma (ILC)	2	6.7%

Preoperative mean values for all three parameters were significantly prolonged or elevated in the malignant group compared to the benign cohort: PT (18.63 +/- 4.3 vs. 13.69 +/- 1.1 s , p = 0.0071), aPTT (41.755 +/- 3.9 vs. 32.283 +/- 2.1 s , p = 0.00091), and plasma D-dimer (901.94+/- 420.5 vs. 162.969 +/- 78.4\text{ ng/mL} , p = 0.0002), as summarized below in Table 2.

Table 2: Comparative Analysis of Coagulation Parameters Between Benign and Malignant Cohorts

Coagulation Parameter	Benign Cohort (n=30)	Malignant Cohort (n=30)	t-value	p-value	Statistical Inference
Mean PT (seconds)	13.69 +/-1.1	18.63+/- 4.3	3.24	0.0071	Highly Significant (p< 0.01)
Mean a PTT (seconds)	32.283 +/-2.1	41.755+/ -3.9	4.12	0.00091	Highly Significant (p< 0.001)
Mean D-Dimer (ng/mL)	162.969+/- 78.4	901.94+/ -420.5	5.86	0.0002	Highly Significant (p< 0.001)

Within the malignant cohort (n=30), the coagulation metrics were cross-analyzed against key clinicopathological markers, including Nottingham histological grading, tumor size categories (pT status), and axillary nodal burden (pN stage). One-way ANOVA demonstrated that plasma D-dimer values scaled in direct correlation with advanced clinical variables, showing highly significant elevations with expanding tumor metrics. Conversely, variations in mean PT and aPTT did not reach statistical significance across tumor grade, size, or nodal stages, as compiled below in Table 3.

Table 3: Malignant Cohort Coagulation Metrics Stratified by Tumor Stage, Size, and Nodal Burden (n=30)

Clinicopathological Parameter	Sub-Category Tier	Freq (n)	Mean PT (s)	Mean aPTT (s)	MeanD-Dimer (ng/mL)	p-value (ANOVA)
Nottingham Tumor Grade	Grade1	11	18.16	37.32	719.03	PT: 0.0770
	Grade 2	14	17.24	36.29	947.07	aPTT: 0.7300
	Grade 3	5	23.37	50.10	2521.90	D-Dimer: **0.0029**
Pathological Tumor Size (T)	<2 cm (T1)	2	18.009	38.072	651.080	PT: 0.0614
	2 - 5 cm (T2)	19	18.215	36.415	816.8271	aPTT: 0.0731
	> 5 cm (T3)	6	17.230	34.954	855.661	D-Dimer: **0.0045**
	Advanced Stage (T4)	3	17.700	35.790	1427.006	
Axillary Lymph Nodes (N)	0 Nodes (N0)	8	17.186	35.560	789.390	PT: 0.0630
	1–3 Nodes (N1)	12	17.152	35.480	791.390	aPTT: 0.1730
	4–9 Nodes (N2)	9	17.377	35.803	802.080	D-Dimer: **0.0017**
	> 10	1	37.900	50.100	2521.900	

Regarding the evaluation of structural lymphovascular invasion (LVI), histopathological tracking identified distinct tumor emboli in 3 malignant cases (10.0%), while LVI was absent in 27 cases (90.0%). Unlike the other parameters, the presence of LVI correlated with substantial, statistically significant prolongations across all three parameters simultaneously: mean PT was significantly prolonged to 27.10 s compared to 16.70 s in LVI-absent cases (p = 0.000831); mean aPTT extended significantly to 51.10 s vs. 43.79 s (p = 0.00172); and mean plasma D-dimer values more than doubled to 1891.013 ng/mL compared to 786.225 ng/mL in the LVI-absent group (p = 0.000179).

DISCUSSION

The results of this study demonstrate that patients with malignant breast lesions exhibit significantly prolonged PT and aPTT values, alongside elevated plasma D-dimer levels, compared to patients with benign masses (Table 2). This structural shift reflects subclinical consumption of circulating clotting factors, which occurs as a consequence of tumor-induced cascade activation. Malignant epithelial cells expose procoagulant proteins to the microvasculature, extending both extrinsic and intrinsic screening times during *in vitro* analyses. These observations are consistent with baseline findings by Kirwan et al. (2008) [3] and Tas et al. (2013) [8], who documented factor consumption trends and prolonged baseline clotting times across solid tumors.

Quantitative plasma D-dimer demonstrated a pronounced capacity for diagnostic differentiation, with mean levels in malignant cases (901.94 ng/mL increasing more than five-fold relative to the benign group (162.969 ng/mL (Table 2). This distinct separation underscores the diagnostic utility of D-dimer for characterizing palpable masses, as localized benign processes do not stimulate systemic fibrin production or reactive fibrinolysis. Invasive breast carcinomas, however, rely on stromal fibrin deposition to support cell migration and angiogenesis, which drives secondary plasmin-mediated degradation and subsequent systemic release of cross-linked fragments, matching findings from Lu et al. (2019) [4].

Beyond baseline differentiation, plasma D-dimer levels expanded in direct proportion to the morphological determinants of tumor aggression. D-dimer concentrations showed a significant stepwise escalation with increasing Nottingham grade, advancing tumor size status, and regional axillary nodal burden (Table 3). This relationship reflects the

enhanced procoagulant factor expression, tissue necrosis, and architectural remodeling characteristic of high-grade, high-volume tumors. Nodal migration involves physical cell transit through lymphatic channels, which triggers localized mechanical endothelial damage and intensifiessecondary fibrin turnover, corroborating findings from Patel et al. (2018) [5] and Halugodu and Sharma (2021) [7].

A highly notable trend was identified regarding the presence of lymphovascular invasion. Unlike other clinical variables, the LVI-positive subgroup demonstrated a concurrent, statistically significant prolongation of PT (27.10 s), prolongation of aPTT (51.10 s), and escalation of plasma D-dimer (1891.013 ng/mL. Tumor emboli within vascular and lymphatic spaces disrupt endothelial continuity and expose subendothelial matrices, accelerating intravascular factor depletion and secondary degradation, supporting evaluations by Siddiqui et al. (2021) [6]. Given that automated measurements of PT, aPTT, and D-dimer are reproducible, inexpensive, and globally accessible, incorporating these metrics into preoperative assessments can improve diagnostic safety and risk stratification.

CONCLUSION

Systemic coagulation parameters are significantly deranged in patients with malignant breast lesions compared to those with benign configurations. Preoperative prolongation of PT and aPTT, combined with elevations in plasma D-dimer, reflects the chronic prothrombotic state induced by invasive breast carcinoma. Within the malignant cohort, quantitative D-dimer serves as a surrogate marker for overall tumor burden, scaling with tumor size, architectural grade, and regional nodal metastasis (Table 3), while concurrent derangement across all three parameters reliably signals high-risk histopathological features such as lymphovascular invasion. These automated assays are rapid and cost-effective, making them suitable as adjunctive diagnostic and risk-stratification metrics [5, 6].

LIMITATIONS AND FUTURE DIRECTIONS

This study was restricted to 60 patients evaluated over a 5-month duration within a single tertiary academic medical center (Index Medical College, Indore), which limits the generalizability of the findings across wider demographic settings. Additionally, long-term post-treatment surveillance was not conducted, preventing the evaluation of these biomarkers in relation to overall survival or recurrence rates. Future multi-centric investigations with larger sample cohorts and longitudinal follow-up are needed to establish precise diagnostic thresholds and validate the role of coagulation profiling in predicting survival outcomes [4, 5].

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