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Assessment of Role of Platelet Aggregation in Metastatic Breast Cancer Patients

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Abstract

Background: To assess role of platelet aggregation in metastatic breast cancer patients. **Methods:** 40 cases (Group I) of metastatic breast cancer patients and equal number of healthy control (Group II) subjects were included. Platelet aggregation studies in vitro using ADP and Thrombin were performed using an optical aggregometer. Detection of platelet aggregation was done by Chrono log series 490 dual and four channel optical aggregometer systems. **Results:** There were 4 subjects in group I and 12 in group II having ADP <60, 26 subjects in group I and 28 in group II with ADP 61-72 and 10 subjects in group I with ADP >72. Low thrombin <58 was seen in 8 in group II, normal thrombin between 61-72 was seen among 11 in group I and 32 in group II and high thrombin >82 among 29 in group I respectively. Amongst patients with normal platelet count, 14 patients had platelet aggregation with ADP in the normal range and 4 patients had platelet aggregation with ADP in the lower range. In patients with high platelet count, 12 showed aggregation in the normal range, and 10 patients showed aggregation in the higher range which was statistically significant (P< 0.05) (Table III, Graph II). **Conclusion:** Platelet aggregation has an important part to play in the tumor metastasis of breast cancer patients.

Keywords:- Breast cancer, Platelet aggregation, Tumor metastasis.

INTRODUCTION

Breast cancer is the most common cancer among females in the world. It has now become the most common female cancer in urban India with an annual incidence of approximately 144,000 new cases being reported.[1,2] Although early detection, precise resection using wide margins and systematic adjuvant therapy has improved survival, distant metastasis remains the leading cause of breast related mortality.[3]

Blood platelets play an important role in hemostasis and arterial thrombosis through their activation triggered by several different agonists.[4] Blood platelets from patients with cancer exhibit a variety of qualitative abnormalities.[5] These include reduced, increased, or spontaneous platelet aggregation and their impaired adhesion, and hypersensitivity to various platelet agonists.[6] Besides crucial role of platelets in coagulation and maintaining hemostasis following mechanical injury of the vasculature, platelets contain a plethora of bioactive molecules in

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their granules and express different receptors on their surfaces that also contribute to inflammation, cancer progression, and metastasis.[7] In the initial minutes, when tumor cells detach from the primary tumor and access the blood, platelets are the first host cells they encounter. Circulating tumor cells (CTCs) have been defined as tumor cells circulating in the peripheral blood of patients which shed from either the primary tumor or its metastases.[8] They cause activation of host platelets that leads to shape change, protein release and membrane based changes and further help in promote metastasis.[9,10] Considering this, we selected present study to assess role of platelet aggregation in metastatic breast cancer patients.

MATERIAL AND METHODS

The present observational study conducted among 40 cases (Group I) of metastatic breast cancer patients of either genders. Enrolment of patients were done after they agreed to participate in the study. Ethical clearance was obtained before starting the study. We selected equal number of healthy control (Group II) subjects also. Inclusion criteria was clinically and histopathologically confirmed metastatic breast cancer patients. Exclusion criteria was patients on chemotherapy and radiotherapy.

Data pertaining to subjects was recorded. Routine clinical details and investigations were performed. Platelet aggregation studies in vitro using ADP and Thrombin were performed using an optical aggregometer. Other parameters like platelet count, histological grade and surrogate molecular classification was also correlated with platelet aggregation. Detection of platelet aggregation was done by Chrono log series 490 dual and four channel optical aggregometer systems. Results of the study was tabulated and assessed using Mann Whitney U test. P value less than 0.05 was considered significant.

RESULTS

There were 4 subjects in group I and 12 in group II having ADP <60, 26 subjects in group I and 28 in group II with ADP 61-72 and 10 subjects in group I with ADP >72. A significant difference was observed (P< 0.05) [Table 1].

Low thrombin <58 was seen in 8 in group II, normal thrombin between 61-72 was seen among 11 in group I and 32 in group II and high thrombin >82 among 29 in group I respectively. A significant difference was observed (P< 0.05) [Table 1, Figure 1].

Amongst patients with normal platelet count, 14 patients had platelet aggregation with ADP in the normal range and 4 patients had platelet aggregation with ADP in the lower range. In patients with high platelet count, 12 showed aggregation in the normal range, and 10 patients showed aggregation in the higher range which was statistically significant (P< 0.05) [Table 3, Figure 2].

Table 1: Comparison of platelet aggregation with ADP in both groups

Range	Group I	Group II	P value
ADP <60	4	12	<0.05
ADP 61-72	26	28	>0.05

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ADP >72	10	0	<0.05

Table 2: Platelet aggregation with thrombin in both groups

Range	Group I	Group II	P value
Low thrombin <58	0	8	<0.05
Normal thrombin 61-72	11	32	<0.05
High thrombin >82	29	0	<0.05

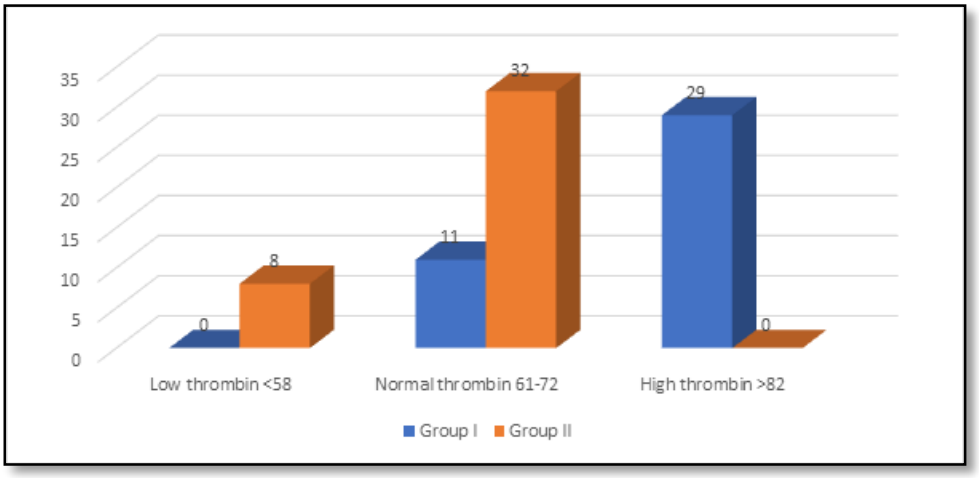

Figure 1: Platelet aggregation with thrombin in both groups

Table 3: Correlation of ADP with platelet count in cases

Range	Platelet count 1.5- 4 lac	Platelet count >4 lac	Total
ADP <60	4	0	4
ADP 61-72	14	12	26
ADP >72	0	10	10

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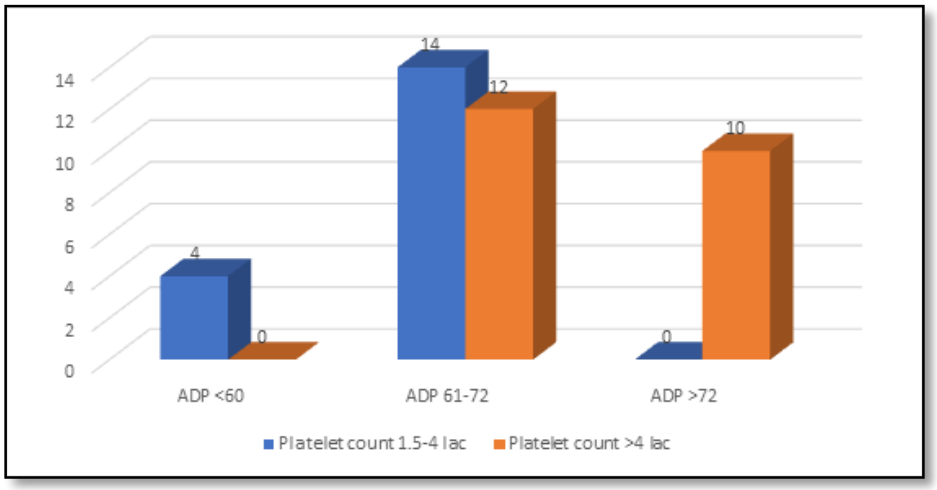

Figure 2: Correlation of ADP with platelet count in cases

DISCUSSION

In this study role of platelet aggregation in metastatic breast cancer patients were assessed.[11,12] Tumor cells that enter the blood circulation have to cope with high shear rates and the immune surveillance, e.g., the assault of natural killer cells.[13] Only a very small percentage of tumor cells in the circulation ends up in a metastatic foci, making this process very inefficient.[14,15] Platelets protect circulating tumor cells (CTCs) by encasing tumor cells in a thrombus, protecting them from cytolysis by natural killer cells.[16] For a stable adhesion between platelets and tumor cells, tumor cells activate platelets by distinct mechanisms, which are also the reason for hypercoagulation and increased risks of thrombosis in cancer patients. Tumor cells release soluble mediators like ADP, thromboxane A2 (TXA2), or high-mobility group box 1 (HMGB1), which ligates with toll-like receptor 4 (TLR4) to instigate a local platelet activation.[17]

There were 40 breast cancer female patients. Equal number of healthy subjects were taken. There were 4 subjects in group I and 12 in group II having ADP <60, 26 subjects in group I and 28 in group II with ADP 61-72 and 10 subjects in group I with ADP >72. Singla et al,[18] studied the role of platelet aggregation in metastatic breast cancer patients using ADP and thrombin among 30 cases (n = 30) of metastatic breast cancer and 30 controls (n = 30) of non metastatic breast cancer which were clinically diagnosed and histopathologically confirmed. In this study, increased aggregation was seen with ADP and thrombin in the metastatic cases and none showed increased aggregation in the non metastatic breast cancer patients. Also, high platelet count and higher histological grade correlated with increased aggregation. However, no correlation was seen between platelet aggregation and the surrogate molecular classification.

We observed that low thrombin <58 was seen in 8 in group II, normal thrombin between 61-72 was seen among 11 in group I and 32 in group II and high thrombin >82 among 29 in group I respectively. Stravodimou et al,[19] showed that patients with thrombocytosis were more likely to have metastases at diagnosis with breast cancer while the normal platelet group was

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more likely to have developed metastatic disease at a time later in the course of the disease. There is a complex interplay between platelets and tumor cells. Thrombocytosis induces formation of large platelet hetero aggregates. These hetero aggregates of platelets and tumor cells can embolize in the microcirculation and aid in the process of extravasation of tumor cells in metastatic sites. Platelets in their granules carry numerous bioactive molecules and growth factors like PDGFα that promotes EMT which gives a mesenchymal phenotype to the epithelial cells that promotes metastasis.

Ward et al,[20] revealed that cancer cell-expressed adhesion GPCR CD97 induced platelet activation which leads to lysophosphatidic acid (LPA) release from platelets. LPA in turn enhances tumor cell invasiveness and vascular permeability to promote transendothelial migration. Some cancer cells express tissue factor (TF) on their cell membranes, which activates the plasmatic coagulation cascade and finally generates thrombin which in turn induces platelet activation. Besides the activation of the coagulation cascade and platelets, thrombin is of key importance for almost every step of the metastatic cascade. Thrombin favors tumor cell proliferation and tumor growth, e.g., by activation of PAR-1 and fibrinogen. In tumor microenvironment, thrombin-stimulated fibroblasts and macrophages secrete monocyte chemotactic protein which fosters protumorigenic myeloid cell invasion.

CONCLUSIONS

It was observed that platelet aggregation has an important part to play in the tumor metastasis of breast cancer patients.

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