Deep Vein Thrombosis - Role of Platelet Derived Micro particles in Coagulation

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ABSTRACT

Introduction: Several parameters may help in identification of DVT. Though the most important diagnosis of DVT is by compression Ultrasound but there are several other parameters as D Dimer, P-selectin which may help in identification of DVT. Platelets are one of the important factors which may be altered in patients with DVT. We wanted to look into the status of Platelet Derived Micro particles (PDMP).

Methods: A total of 10 patients and 10 controls were included in the study. We included all acute DVT patients as confirmed by duplex scan and excluded those patients who were on antiplatelet or anticoagulants. Isolation of platelet derived microparticles was done after blood sampling with 2 ml of blood collected in blue top sodium citrate vials from each case and control were sent to Biochemistry laboratory and studied within 1-2 hrs. Agonist induced conformational change in integrin αβ results in high affinity binding of fibrinogen to platelet surface. PAC-1 antibody specifically recognizes open conformation of αβ. Therefore, PAC-1 antibody was used to study the effect of agonist on integrin activation.

Results: Among all 10 patients (6 males and 4 females), 8 patients were in age group 20-39 years. Platelet derived microparticles are 3-4 times raised as compared to healthy individual. Median number of platelet derived microparticles of all cases was 4461.5/10⁵ platelet cells and median number of platelet derived microparticles of all controls was 1868.0/10⁵ platelet cells.

Conclusion: Mean number of PDMPs in each case with control which showed that mean number of microparticles were significantly raised in cases (4.09±1.2 vs 3.06±0.5; p=0.029).

Key Words: DVT, Platelets, Micro particles, Micro vesicles, Platelet Derived Micro particles (PDMP)

INTRODUCTION

Deep vein thrombosis (DVT) is a potentially deadly condition caused by a blood clot that forms in a vein – most commonly deep veins in leg. Thrombus can spontaneously form in the larger veins of the lower limb, obstructing blood flow from the leg back to the heart.

The estimated incidence of deep vein thrombosis is between 60 and 160 cases per 100,000 people, with approximately 260,000 cases occurring annually in the United States¹,². There is no significant difference in incidence between men and women³. The incidence increases with age and sharply rises after age of 45 years⁴. Untreated deep vein thrombosis can however result in fatal pulmonary embolism, which occurs in an estimated 50,000 people per year in the US². Without prophylaxis, venous thrombosis occurs after approximately 20% of all major surgical procedures with associated embolism after 1-2% of such procedures⁵.

Activated platelets can release microparticles, which are small membrane vesicles. Platelet microparticles are highly
procoagulant, because they contain the exposed, anionic phospholipid, phosphatidylserine, which facilitates the assembly of components of the coagulation cascade. The coagulation cascade is required for thrombin generation and conversion of fibrinogen to fibrin, which in turn strengthens the platelet thrombus.

**MATERIALS AND METHODS**

A total number of 10 patients and 10 controls were included in the study. We included all acute DVT patients as confirmed by duplex scan and excluded those patients who were on antiplatelet or anticoagulants. We also excluded patients with associated co-morbidities like Tuberculosis, Diabetes, inflammatory Diseases or other chronic illnesses.

**a) Isolation of platelet derived microparticles**

Blood sampling- 2 ml of blood collected in blue top sodium citrate vials from each case and control were sent to Biochemistry laboratory and studied within 1-2 hrs.

Procedure-

- Blood sample was centrifuged at 180g for 10min. at 22°C
  - Aspirin (1M) added 1μl/ml of PRP(platelet rich plasma) and incubated at 37°C for 15min.
  - Centrifuge at 800g for 10min.
  - Centrifuge at 1200g for 2min.
  - Supernatant(100μl) taken and PAC-1 antibody(5μl) added

**b) Measurement of PAC-1 binding by Flow Cytometry**

Principle: Agonist induced conformational change in integrin αβ results in high affinity binding of fibrinogen to platelet surface. PAC-1 antibody specifically recognizes open conformation of αβ. Therefore, PAC-1 antibody was used to study the effect of agonist on integrin activation.

Procedure-

- Supernatant + PAC-1 antibody mixed and incubated in dark at room temperature for 30min.
  - 200 μl of FACS Sheath added
  - Analysed by Flow Cytometry (Using CellQuest Pro software)

**RESULTS**

Among all 10 cases with distribution to sex, 6 patients were male and 4 were female. Maximum number of cases 8 was in age group 20-39 years.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total number of platelet derived microparticles/lakh of cells</th>
<th>Mean number of microparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>6434</td>
<td>4.05</td>
</tr>
<tr>
<td>Patient 2</td>
<td>728</td>
<td>2.94</td>
</tr>
<tr>
<td>Patient 3</td>
<td>1122</td>
<td>2.76</td>
</tr>
<tr>
<td>Patient 4</td>
<td>12901</td>
<td>5</td>
</tr>
<tr>
<td>Patient 5</td>
<td>14910</td>
<td>6.68</td>
</tr>
<tr>
<td>Patient 6</td>
<td>2489</td>
<td>4.26</td>
</tr>
<tr>
<td>Patient 7</td>
<td>8783</td>
<td>5.13</td>
</tr>
<tr>
<td>Patient 8</td>
<td>659</td>
<td>2.62</td>
</tr>
<tr>
<td>Patient 9</td>
<td>8682</td>
<td>4.14</td>
</tr>
<tr>
<td>Patient 10</td>
<td>2329</td>
<td>3.33</td>
</tr>
</tbody>
</table>
Table 1 showing total number of platelet derived microparticles per one lakh of platelet cells and mean number of microparticles in all cases. Almost in all cases platelet derived microparticles were more than 1000/lakh of cells with maximum upto 8000 microparticles. Mean number of microparticles was more than 2.6 in all cases with maximum upto 5.1.

Table 2: Comparison of total number of Platelet Derived Microparticles between case and control

<table>
<thead>
<tr>
<th>No. of microparticles/1,00,000 cells</th>
<th>Case</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>4461.50</td>
<td>1868.00</td>
<td>0.049</td>
</tr>
<tr>
<td>(1710.75-9812.50)</td>
<td>(840.50-2557.00)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 showing comparison of number of platelet derived microparticles between cases and controls, done by Mann Whitney U- test. Median number of platelet derived microparticles of all cases was 4461.5/10^5 platelet cells and median number of platelet derived microparticles of all controls was 1868.0/10^5 platelet cells. It was found that number of platelet derived microparticles was raised in cases when compared with controls which was statistically significant (p-value=0.049).

Table 3: Comparison of mean number of Microparticles between case and control

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean±SD</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>4.0910±1.2684</td>
<td>2.371</td>
<td>0.029</td>
</tr>
<tr>
<td>Control</td>
<td>3.0630±0.5204</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 showing comparison of mean number of microparticles between case and control, done by student t-test. Mean of mean number of microparticles of all cases was 4.09 and mean of mean number of microparticles of all controls was 3.06. When compared the mean number of microparticles of cases was found to be raised which was statistically significant with p-value=0.029.

**DISCUSSION**

Deep venous thrombosis is a serious disease not only because of the risk of developing pulmonary embolism, but also of its risk for long term sequelae. Venous thromboembolism comprises DVT and/or pulmonary embolism and either of them can be asymptomatic. Despite adequate therapy, 1% to 8% of patients in whom pulmonary embolism develops will die, whereas others will experience long-term complications such as postphlebitic syndrome (40%) and chronic thromboembolic pulmonary hypertension (4%). Diagnosis of DVT is based on direct visualization of the thrombus and lack of compressibility on gray scale and identification of either a persisting filling defect or thrombus in the color column of the vessel lumen or absence of flow on Doppler Ultrasonography (US).

The small vesicles liberated by cells into the extracellular milieu are collectively known as extracellular vesicles. Extracellular vesicles stored in multivesicular bodies (or a-granules in platelets) and released by exocytosis have dimensions ranging between 50 and 150nm and are called exosomes. Extracellular vesicles produced by plasma membrane budding and shedding appear larger (100–1000nm) and are called microvesicles. Extracellular vesicles participate in coagulation, affect vascular function, can play roles in cellular proliferation and differentiation, are involved in inflammation and mediate cell–cell communication. Microvesicles originating from platelets, erythrocytes, endothelial cells, and leukocytes are present in the blood circulation, with those from platelets being the most abundant followed by extracellular vesicles derived from erythrocytes. Upon activation, platelets are extremely potent at producing microvesicles, which are historically known as microparticles.

Platelet derived microparticles (PDMPs) are produced by platelet activation or by physical stimulation under various conditions. Loss of membrane phospholipid asymmetry coincides with microparticle formation. In unactivated platelets, phosphatidylserine is absent from the outer leaflet and is almost uniquely comprised in
the inner leaflet of the plasma membrane. Upon activation, a rise in cytosolic calcium via nonselective ion channels and the mitochondrial permeability transition pore, and enzymes such as floppases and scramblase, are involved in the promotion of phosphatidylserine exposure.

PDMPs contain inner granules and membranous microvesicles released from activated platelets and membranous fragments produced by mechanical destruction and exhibit coagulative activity. The main model used to date to study the involvement of MPs in venous thrombosis is the inferior vena cava (IVC) ligation model. However, this model is most likely not the best for examining the participation of MPs given that MP delivery at the site of injury is obviously impeded by the ligation. Ramacciotti et al. demonstrated that in this model, the injection of MPs positively impacts the weight of the thrombus formed.

N. Inami et al, (2003) studied the role of P-selectin and platelet derived microparticles in patients of pulmonary embolism. This study included 6 cases of pulmonary embolism (PE), 11 post-operative cases (PO) and 10 controls. Platelet derived microparticles were raised in cases when compared with controls (PDMP/10^4 platelets: PE: 529±96, PO: 459±61, Control: 388±54). In our study, we included 10 cases of diagnosed DVT without having comorbidities like diabetes, inflammatory diseases and 10 healthy controls. Because there were several reports that microparticles may be increased in other conditions also. Median number of PDMPs/10^5 platelet cells in all cases were significantly raised as compared to controls (4461.50 vs 1868.00; p=0.049). We also compared mean number of PDMPs in each case with control which showed that mean number of microparticles were significantly raised in cases (4.09±1.2 vs 3.06±0.5; p=0.029).

**CONCLUSION**
Platelet derived microparticles are 3-4 times raised as compared to healthy individual. This study may help in pathogenesis of DVT.

**REFERENCES**


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