Clinical significance of Tissue factor-positive platelets and reticulated platelets in coronary artery disease patients

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ABSTRACT
Background: Acute coronary syndrome (ACS) is a set of signs and symptoms due to thrombotic events in coronary artery; it ranges from unstable angina to acute myocardial infarction (AMI). Platelets play a major role in coagulation process by their surface expressed protein and also by their cytoplasmic granules. In this work we try to evaluate the tissue factor (TF) positive platelets and reticulated platelets (RP) in patients with ACS. Methods and Results: One hundred and twenty patients with coronary artery disease were enrolled in this study, 60 patients with AMI, and 60 angina patients plus 50 apparently healthy subjects as a control group. Flow-cytometric analysis of TF positive platelets (+ve CD142) and reticulated platelets (RP) (+ve TO) in patients and control groups showed that the percent of TF +ve and RP platelets were significantly high in angina group (3.56±1.11) (2.34±0.58), and higher in patients with AMI (6.45±1.47) (6.54±2.12) when compared to control group (0.88±0.24) (0.96±0.28) respectively. Moreover, patients with a worse outcome of AMI had a significantly higher TF +ve and RP platelets (7.16±0.94) (7.95±1.55) than AMI patients with good outcome (5.9±1.44) (6.54±2.1) respectively. Conclusions: The higher percent of RP and TF +ve platelets in coronary artery disease patients contribute to the risk of coronary artery thrombotic occlusion, AMI development and predict a worse outcome of AMI.

KEYWORDS: Acute myocardial infarction, tissue factor-positive platelets, reticulated platelets

INTRODUCTION

Acute coronary syndrome (ACS) refers to any group of clinical symptoms compatible with acute myocardial ischemia and includes unstable angina (UA), non-ST-segment elevation myocardial infarction (MI) (NSTEMI), and ST-segment elevation myocardial infarction (STEMI) [1].

It results from rupture of atherosclerotic plaque which causes approximately 75% of fatal myocardial infarction, whereas superficial endothelial erosion accounts for the remaining 25% [2]. Atherosclerosis is a chronic inflammatory process in which thrombus formation on a ruptured atherosclerotic plaque is the pathological basis of an acute arterial thrombotic event such as myocardial infarction [3].

Platelets play an important role in cardiovascular disease both in the pathogenesis of atherosclerosis [4] and in the development of acute thrombotic events. Their importance in coronary disease and in acute coronary syndromes is indirectly confirmed by the benefit of anti-platelet agents in these disorders [5].
Tissue Factor (TF) is the main cellular initiator of blood coagulation, and it is also currently considered the protein that links pro-inflammatory and pro-thrombotic mechanisms in the progression of atherosclerosis [6]. TF protein has been found to be increased in coronary plaques, thus playing a crucial role in human coronary syndromes [7].

Platelets not only express TF protein [8] but they also contain its specific mRNA which has been shown to be translated into protein [9].

The platelet-derived TF may contribute to fibrin formation and to the propagation and stabilization of a thrombus but can also participate, as recently shown, in several cellular processes that stimulate atherogenesis such as angiogenesis and cell migration, both of which are associated with plaque growth and under certain circumstances, plaque weakening leading to destabilization of the lesion [10].

Reticulated platelets (RPs) are the youngest forms of circulating platelets that contain residual messenger RNA (mRNA). Those cells are larger and possibly more active than non-RPs. They indirectly indicate the state of marrow production [11].

Whereas platelets persist in the circulation for 7–10 days, RPs have a much shorter lifespan (<1 day) [12]. Accelerated platelet turnover has attracted increasing attention owing to high level of immature platelet which are not inhibited by aspirin and have an increased thrombotic potential [13]. They contain megakaryocyte-derived mRNA and thus have the translational capacity necessary for protein synthesis. Moreover, immature platelets are characterized by a higher number of dense granules and an increased platelet volume than older platelets. Finally, larger platelets have been shown to be enzymatically and metabolically more active and to have a higher thrombotic potential than smaller platelet [14].

We aim to study the expression of tissue factor, the important extrinsic pro-coagulant, on the surface of platelets as well as reticulated platelets in ACS patients.

MATERIALS AND METHODS

Patient Population:

Sixty patients with acute myocardial infarction and sixty angina patients were enrolled in this study. They were (85) males and (35) females, their ages ranged from 44 – 72 years old. Patients with acute myocardial infarction were selected from intensive care unit in Menoufia University hospital, while patients with angina were selected from outpatient clinic. They were diagnosed according to The Joint European Society of Cardiology/American College of Cardiology (ESC/ ACC) Committee [15].

Patients using drugs interfering with platelet functions except low dose aspirin were excluded.

The selected patients were compared to 50 normal non-smoker control persons not taking any drugs known to interfere with platelet functions. They were (36) males and (14) females ranged between 45 – 69 years old.

All participants (relatives) gave written informed before enrolment in the study and the study protocol was approved by the Ethical Committee of our scientific committee.

All participants in this study were subjected to:

I – Full medical history taking with stress on:

- Family history, past history; includes history of diabetes, hypertension, smoking and recurrent chest pain, drug history.

II – Clinical assessment and follow up:

- Acute myocardial patients were classified according to the Killip classification [16], received the appropriate treatment and followed up for 30 days. AMI patients with a worse outcome were considered as patients with a high Killip class (III and IV), worse ischemic complications (extended infarction, re-infarction and recurrent infarction) or sudden death [17].

III – Laboratory investigations:

Sample collection:

- Eight milliliters (8 ml) of venous blood were collected from all studied subjects under complete aseptic precautions. 2 ml were collected in EDTA vacutainer tubes, for complete blood count measurement. 2.7 ml whole blood (WB) were collected into evacuated citrated tube containing (1/10 volume of 0.129 mol/L sodium citrate) (Vacutainer, Becton Dickinson) for preparation of platelets rich plasma and subsequently used for assessment of reticulated platelets and tissue factor expression on the platelets.

- The remaining part was collected in another plain vacutainer tube, the blood was left to clot then centrifuged at 2000 g for 10 minutes then the clear supernatant sera was separated from the sedimented RBCs for lipid profile, troponin I and highly sensitive CRP.
1 – **Complete blood count (CBC)**:
CBC was analyzed by fully automated ADVIA-2120 hematological analyzer (Bayer Diagnostics, Newbury, United Kingdom) with examination of Leishman stained smears for morphological assessment.

2 – **Quantitative measurement of troponin I**:
The assay is performed by PATHFAST®, a fully automatic immunoassay analyzer, which combines the progressive chemiluminescence technology with the patented magtration technology.

3 – Lipid profile and high sensitive C-reactive protein (hs-CRP) were analyzed by Beckman Coulter AU480 fully automated auto analyzer.

4 – Flow cytometry analysis of reticulated platelets and TF expression on platelets by BD FACSCalibur (BD Immunocytometry Systems, San Jose, California, USA).

**Monoclonal antibodies and TO:**
1 – FITC (Fluorescein iso-thio-cyanate) conjugated mouse monoclonal anti-human CD61 code 61F-100T; clone C17 for flowcytometry, Immunostep, Spain.
2 – PE (phycoerythrin) conjugated mouse monoclonal anti-human CD61, code 61F-100T, clone C17 for flowcytometry, Immunostep, Spain.
3 – PE (phycoerythrin) conjugated mouse monoclonal anti-Human CD142 PE, code 12-1429, Clone HTF-1 for flowcytometry, eBioscience, Inc.
4 – Thiazole orange (TO): Retic-COUNT (Becton Dickinson BD RETIC-COUNT) catalog No. 349204 for flowcytometry, Becton Dickinson, USA.

**Principle of the test:**

**A – Preparation of Platelet-rich plasma (PRP) for assessment of reticulated platelets and TF expression on platelets:**
The citrated blood tube was centrifuged at 200 g for 15 minutes. To avoid leukocyte contamination, the top third of the PRP was aspirated. Then it was placed in another tube and washed twice with 3 ml PBS by centrifugation for 5 min at 1200 g. The supernatant was decanted and the sediment was suspended in 500 μl of PBS and used as a source of platelets for the experiments. Platelet and leucocytes counts were determined by automated ADVIA-2120 hematological analyzer. Leukocyte contamination was less than 1 leukocyte/10⁶ platelets.

**B – Sample staining:**

**Tissue factor expression on platelets:**
A volume of 10 μl of PE-labeled anti-CD142 Ab and FITC-labeled anti-CD61 Ab were added to 20 μl of PRP, mixed well and incubated at room temperature for 15 min in the dark, the platelets were then washed three times by 3 ml PBS and finally reconstituted in 200 μl 2% ultra-pure paraformaldehyde for flow cytometric analysis.

**Reticulated platelets:**
The principle depends on enumeration of CD61 and thiazole orange positive cells. Detection of CD61 was performed using PE-labeled anti CD61 monoclonal antibodies. Staining of RNA remnants of immature platelet (reticulated platelet) was performed by thiazole orange reagent. [18,19].

A volume of 10 μl of PE-labeled anti CD61 Abs was added to 20 μl of PRP, mixed well and incubated at room temperature for 15 min in the dark. Then one ml of thiazole orange was added to the previous tube, capped and mixed gently then incubated in the dark at room temperature for 15 minutes. The platelets were washed three times by 2 ml PBS and finally reconstituted in 200 μl 2% ultra-pure paraformaldehyde for flow cytometric analysis.

Auto control and FITC, PE conjugated mouse IgG antibodies were used as isotype controls for the quantification of background fluorescence.

**C – Flow cytometric analysis:**
Data were acquired on a FACS caliber flow cytometer (BD immune cytometry systems, San Jose, CA). The instrument set up was checked weekly using QC windows beads (flow cytometry standard, San Juan, Puerto Rico). Forward scatter, side scatter and fluorescence measurements were made with logarithmic amplifiers and flow cytometric two parameters dot plots and quadrant statistics were generated by cell quest software (Becton Dickinson immune-cytometry systems). Gating was done on platelet on the basis of the forward and side scatter as well as the characteristic CD61 positive staining. Ten thousand events were acquired and analyzed. Two colors and light scattering properties were applied to determine the percentage of tissue factor positive platelet
Statistical analysis:
The data were processed on an IBM-PC compatible computer using SPSS version 18 (SPSS Inc., Chicago, Illinois, USA). Continuous parametric variables were presented as means ± SD, whereas for categorical variables numbers (%) were used. Chi-square test was used for qualitative variables. The difference between two independent groups was performed by student’s t test and by Mann Whitney (U) test for parametric continuous variables and nonparametric variables, respectively. For more than two groups, one-way analysis of variance test was used for parametric data and Tukey HSD Post-hoc Test was further used to find means that are significantly different from each other. Binary logistic regression analysis and Odd's ratio were used to analyze multi-risk factors to determine whether there is effect modification, or to assess the relationships of several exposure or risk factors on an outcome simultaneously. A p. value less than 0.05 was considered statistically significant.

Results:
The characteristics of the study groups are show in table (1). No statistically significant difference exists between studied groups as regard to age or gender. Also no statistically significant difference exists between AMI group and angina patients as regard to smoking and diabetes. The healthy control group did not include smokers or diabetics.

hs- CRP levels show statistical difference between the studied groups (p. value < 0.001). AMI patients had higher levels than healthy control (p. value < 0.001) and angina patients (p. value < 0.001). The angina patients had higher levels of hs- CRP when compared to control (p. value < 0.01).

The lipid profile shows statistically significant difference between the included groups. The AMI patients had statistically higher levels of cholesterol, triglycerides and LDL-c, with lower level of HDL-c when compared with healthy control (p. value < 0.001).

Similarly, AMI patients had statistically higher levels of cholesterol and LDL-c when compared to angina patients (p. value < 0.001, < 0.01 respectively) but no statistically significant difference was noted as regard to the level of triglycerides and HDL-c between AMI patients and angina patients.

The angina patients had statistically higher levels of cholesterol, triglycerides and LDL-c and lower levels of HDL-c when compared with healthy control (p. value < 0.01, < 0.01 and <0.001, < 0.001 respectively).

As regard to the platelet parameters, no statistically significant difference exists between the studied groups as regard to the platelet count.

There was statistically significant difference between the studied groups as regard to TF +ve platelets and reticulated platelet (p. value < 0.001, < 0.001). AMI patients had higher percentage of TF +ve platelets and reticulated platelets than healthy control (p. value < 0.001, < 0.001) and angina patients (p. value < 0.001, < 0.001).

The angina patients had higher percentage of TF+ ve platelets and reticulated platelet when compared to control (p. value < 0.001, < 0.001).

The contributing factors of high TF +ve platelets and reticulated platelets are show in table (2). Smokers had higher percentages of TF +ve platelets and reticulated platelets than non- smokers (p. value < 0.001 and 0.001 respectively).

Diabetics had higher percentages of TF +ve platelets and reticulated platelets than non- diabetics (p. value < 0.01 and < 0.01). Similarly hypertensives had higher percentages of TF +ve platelets and reticulated platelets than normotensives (p. value 0.04 and < 0.01 respectively).

As shown in table (3), the TF +ve platelets and reticulated platelets were an independent risk factors for AMI with Odd's ratio of (2.3), (1.9) and p. value of (0.02), (0.045) respectively.

In table 4, AMI patients with a worse outcome had significantly higher percentage of TF +ve platelets and reticulated platelets when compared to AMI patients with a good outcome (p. value < 0.001, < 0.01).
### Table 1: Comparison of clinical and laboratory parameters between the studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=50)</th>
<th>AMI (n=60)</th>
<th>Angina (n=60)</th>
<th>Test of significance</th>
<th>p-value</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.8 ± 7.3</td>
<td>58.2 ± 8.4</td>
<td>59.6 ± 10.3</td>
<td>F: 2.54</td>
<td>0.08</td>
<td>0.33</td>
<td>0.06</td>
<td>0.66</td>
</tr>
<tr>
<td>Sex, male, N (%)</td>
<td>36 (72)</td>
<td>47 (78.3)</td>
<td>38 (63.3)</td>
<td>X²: 3.3</td>
<td>0.19</td>
<td>X²: 0.59</td>
<td>p: 0.44</td>
<td>X²: 0.93</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>48 (50)</td>
<td>12</td>
<td>X²: 96.5</td>
<td>&lt; 0.001</td>
<td>X²: 70.9</td>
<td>p: &lt;0.001</td>
<td>X²: 76.3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>50</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Yes</td>
<td>51</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>50</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>2.59 ± 0.66</td>
<td>3.2 ± 0.9</td>
<td>F: 147.6</td>
<td>&lt;0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>156.1 ± 38.7</td>
<td>234.4 ± 51.1</td>
<td>193.5 ± 68.5</td>
<td>F: 27.8</td>
<td>&lt;0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>112.2 ± 30.1</td>
<td>166.6 ± 68.8</td>
<td>145.9 ± 51.3</td>
<td>F: 14.1</td>
<td>&lt;0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.08</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>95.7 ± 31.5</td>
<td>44.9 ± 44.9</td>
<td>128.6 ± 19.2</td>
<td>F: 32.6</td>
<td>&lt;0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>44.9 ± 8.1</td>
<td>35.6 ± 7.9</td>
<td>38.6 ± 7.1</td>
<td>F: 20.4</td>
<td>&lt;0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.08</td>
</tr>
<tr>
<td>Platelet (10⁹/µl)</td>
<td>5.5 ± 1.67</td>
<td>3.98 ± 1.11</td>
<td>4.0 ± 0.9</td>
<td></td>
<td>&lt; 0.001</td>
<td>2.11</td>
<td>0.42</td>
<td>458</td>
</tr>
<tr>
<td>TF +ve platelets (%)</td>
<td>0.88 ± 0.24</td>
<td>6.45 ± 1.47</td>
<td>3.56 ± 1.11</td>
<td>F: 350</td>
<td>&lt;0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RP (%)</td>
<td>0.96 ± 0.28</td>
<td>6.54 ± 2.12</td>
<td>2.34 ± 0.58</td>
<td>F: 276</td>
<td>&lt;0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.005</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**AMI**: Acute myocardial infarction  **TF**: Tissue factor  **RP**: Reticulated platelets  
*p* for comparison between the 3 groups  **P1** for comparison between control group and AMI group  
**P2** for comparison between control group and angina group  **P3** for comparison between AMI group and angina group

### Table 2: Relationship between TF +ve platelets, reticulated platelets % and other risk factors in acute coronary syndrome patients

<table>
<thead>
<tr>
<th>Data</th>
<th>TF +ve platelets</th>
<th>Reticulated platelets %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X ± SD</td>
<td>t. test</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker (N.98)</td>
<td>5.5 ± 1.67</td>
<td>4.0</td>
</tr>
<tr>
<td>Non-smoker (N.22)</td>
<td>3.98 ± 1.11</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (N.99)</td>
<td>5.34 ± 1.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Negative (N.21)</td>
<td>4.11 ± 0.93</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (N.103)</td>
<td>5.22 ± 1.65</td>
<td>2.00</td>
</tr>
<tr>
<td>Negative (N.17)</td>
<td>4.38 ± 1.14</td>
<td></td>
</tr>
</tbody>
</table>

**X**: mean, **SD**: Standard deviation, **t**: Student t test, **U**: Mann Whitney U

### Table 3: Binary multivariate logistic regression analysis for risk factors of acute cardiovascular diseases

<table>
<thead>
<tr>
<th></th>
<th>SE</th>
<th>Wald χ²</th>
<th>p. value</th>
<th>Odds ratio</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>4.7</td>
<td>0.09</td>
<td>0.5</td>
<td>0.45</td>
<td>0.11</td>
<td>12.9</td>
</tr>
<tr>
<td>Smoking</td>
<td>8.6</td>
<td>0.8</td>
<td>0.3</td>
<td>0.26</td>
<td>0.0</td>
<td>27.4</td>
</tr>
<tr>
<td>DM</td>
<td>5.8</td>
<td>0.004</td>
<td>0.9</td>
<td>1.01</td>
<td>0.0</td>
<td>65.7</td>
</tr>
<tr>
<td>High Cholesterol</td>
<td>7.4</td>
<td>0.02</td>
<td>0.08</td>
<td>1.98</td>
<td>0.01</td>
<td>41.2</td>
</tr>
<tr>
<td>High TG</td>
<td>1.8</td>
<td>0.01</td>
<td>0.9</td>
<td>0.37</td>
<td>0.0</td>
<td>36.7</td>
</tr>
<tr>
<td>High LDL</td>
<td>8.5</td>
<td>0.1</td>
<td>0.09</td>
<td>1.43</td>
<td>0.1</td>
<td>39.3</td>
</tr>
<tr>
<td>Low HDL</td>
<td>5.1</td>
<td>0.003</td>
<td>0.9</td>
<td>2.01</td>
<td>0.0</td>
<td>25.6</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.48</td>
<td>0.23</td>
<td>0.63</td>
<td>1.12</td>
<td>0.11</td>
<td>37.3</td>
</tr>
<tr>
<td>Hypertension</td>
<td>35.52</td>
<td>0.02</td>
<td>0.90</td>
<td>1.07</td>
<td>0.0</td>
<td>41.5</td>
</tr>
<tr>
<td>Previous cardiovascular intervention</td>
<td>37.81</td>
<td>0.0</td>
<td>0.99</td>
<td>1.3</td>
<td>0.0</td>
<td>48.52</td>
</tr>
<tr>
<td>TF +ve platelets %</td>
<td>4.5</td>
<td>5.8</td>
<td>0.02</td>
<td>2.3</td>
<td>2.9</td>
<td>16.9</td>
</tr>
<tr>
<td>RP %</td>
<td>0.59</td>
<td>2.98</td>
<td>0.045</td>
<td>1.9</td>
<td>1.01</td>
<td>19.87</td>
</tr>
<tr>
<td>Constant</td>
<td>2.4</td>
<td>3.5</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SE**: standard error, **CI**: Confidence interval
Table 4: TF +ve platelets and reticulated platelets in AMI patients with a good and worse outcome

<table>
<thead>
<tr>
<th></th>
<th>Worse outcome (Killip III, IV, extensive, re-infarction, recurrent infarction and sudden death)</th>
<th>Good outcome (N. 38)</th>
<th>t. test</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF +ve platelets</td>
<td>7.16 ± 0.94</td>
<td>5.9 ± 1.44</td>
<td>3.66</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Reticulated platelets</td>
<td>7.95 ± 1.51</td>
<td>6.54 ± 2.1</td>
<td>2.75</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

X = mean, SD = Standard deviation, t = Student t test, U = Mann Whitney U

Fig. 1: Flow cytometry Gating Strategy for analyze the tissue factor expression on platelets in healthy and patient’s groups

Flow cytometry gating strategy to analyze the tissue factor (TF) expression on platelets in the studied groups. (A) Forward against side scatters histogram for platelets rich plasma with gating on platelets. (B) Analysis of platelets as regard to TF (dual histogram CD61 FITC/CD142 PE) in healthy control (0.94 % TF-positive platelets). (C) Analysis of platelets as regard to TF (dual histogram CD61 FITC/CD142 PE) in angina patient (3.67 % TF-positive platelets). (D) Analysis of platelets as regard to TF (dual histogram CD61 FITC/CD142 PE) in AMI patient (6.89 % TF-positive platelets). (A): FSC/SSC histogram. Gating around platelets (B): CD61/CD142 histogram. Gate on TF +ve platelets in control subject (C): CD61/CD142 histogram. Gate on TF +ve platelets in patient with angina (B): CD61/CD142 histogram. Gate on TF +ve in patient with AMI
Platelet-dependent thrombus formation is a key event in the pathogenesis of ACS [20]. Hyper-reactivity of platelets is a tool that can prospectively identify subjects at risk for these conditions. Here we study reticulated platelets and TF +ve platelets as two possible mechanisms by which platelet can pathologically hyper-react leading to progression to AMI.

In our study, AMI patients had statistically significant higher levels of reticulated platelets and TF +ve platelets when compared to angina patients and controls. Also angina patients had higher levels of these parameters in relation to the healthy control subjects.

We can't judge whether these findings preceded and precipitated the acute coronary event or they actually were a subsequent to the disease or a combination of both. On one hand, as a subsequent to atherosclerotic plaque rupture, reticulated platelets and TF +ve platelets which have a more thrombogenic potential can lead to a fixed and persistent thrombotic occlusion [20], micro-embolization [21], vasoconstriction [22], release of pro-inflammatory mediators [23], cardiac myo-necrosis and progression of the ischemic condition to AMI. On the other hand, the acute event is able to elicit an inflammatory response with the release of a variety of pro-inflammatory cytokines, that may increase expression of TF by platelets as a link between links pro-inflammatory and pro-thrombotic mechanisms in the progression of atherosclerosis [24] and influence platelet turnover with the subsequent mobilization and release of newly formed large, reticulated, reactive platelets from the bone marrow [25] with an increased hemostatic potential that may contribute to increased potential of coronary thrombus formation [26].

According to our study, these highly thrombogenic platelets were found at higher level in AMI in comparison to angina patients and control subjects and are accused for AMI development. This is based on their high thrombogenic activity [12,27] and reduced response of reticulated platelets to antiplatelet drugs [13].

Previous studies showed higher levels of reticulated platelets [26, 27] and TF +ve platelets [28,29] in AMI patients than controls.

No difference was noted as regard to age, sex and platelet counts among the studied groups. No differences were present between AMI and angina patients as regard to smoking, DM and hypertension. AMI patients had higher serum total cholesterol, LDL-cholesterol and hs-CRP compared to angina patients and control. As regard to triglycerides level, no difference was present between AMI and angina patients but both AMI and angina patients had higher levels than control group.

In our study, high levels of reticulated and TF +ve platelets were related to known risk factors of coronary artery disease like smoking, diabetes mellitus and hypertension. These findings were supported by several previous studies [30:33]. Atherosclerosis associated with these diseases is the reason for increased reticulated and TF +ve platelets through increasing platelet turn over and producing an inflammatory status. These diseases also can trigger an up regulation of the megakaryocyte–platelet system, either through a direct effect on megakaryocyte, an effect on humoral effectors of endomitosis, or by damaging endothelium and thereby increasing platelet turnover [34].
Martin et al., 2012 suggested a theory that cardiac risk factors could cause low grade increase in platelet consumption over a long or short time. These pathological conditions have an effect that mimics hemorrhage and would be sensed by a putative hemorrhagic response sensor leading to increased production of immature reticulated platelets [35]. Alternatively, this risk factor may urge the megakaryocytes to autonomously overproduce platelets without increased platelet consumption [36]. These large, dense, hyperactive platelets, which are lifesaving in the setting of true hemorrhage, might cause thrombosis of the coronary artery, because of their hyperactivity; in the presence of endothelial dysfunction in that artery or exposed sub-endothelium at the site of plaque rupture.

In our study, the reticulated platelets and TF +ve platelets were independent risk factors for development of atherosclerosis. Cardiac patients with high TF +ve platelets had 2.3 fold increased chance to develop AMI in comparison with those with normal TF +ve platelets. In case of reticulated platelets, cardiac patients with high reticulated platelets had 1.9 fold increased chance to develop AMI in comparison with those with normal reticulated platelets.

Moreover, the high levels of reticulated and TF +ve platelets didn't only identify high risk of development of AMI, but also these high levels can predict a tendency for a worse outcome of AMI including worse Killip classes, sudden death and increased ischemic complications. This reflects the more thrombogenic activity of these platelets that can lead to more extensive coronary thrombosis, more myocardial damage and less response to anti-platelet.

**Conclusion:**

This study suggested that reticulated and TF +ve platelets as possible means by which platelets can hyper react in coronary artery disease patients leading to progression to AMI and can even lead to a worse outcome of AMI. We recommend measuring of these parameters may be necessary for careful dealing with such high risk patients.

REFERENCES