Original article:
Comparison of levels of Myeloperoxidase, C-reactive protein and Uric acid in patients of Stable and Unstable angina

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Abstract:

Background: Coronary artery disease (CAD) is the leading cause of morbidity & mortality worldwide and comprises mainly stable angina, unstable angina & acute myocardial infarction. In the present study, we investigated plasma myeloperoxidase (MPO) level along with uric acid and C-reactive protein (CRP) in patients with stable and unstable angina.

Methods: A total of 150 subjects were recruited and categorized into control group (n=50), stable angina (SA) group (n=50), unstable angina group (n=50). Plasma myeloperoxidase level was measured by a modified sandwich ELISA assay, uric acid was measured by enzymatic colorimetric reagents, CRP was measured by latex turbidity method assay.

Results: Plasma Myeloperoxidase (MPO) levels were significantly increased in unstable angina (340.94 ± 45.28) and stable angina (283.26 ± 30.68) as compared to control mean value (112.12 ± 29.07ng/ml). CRP was significantly raised in stable angina [3.73 ± 0.72], and in unstable angina [18.22 ± 5.75] as compared to control mean value [1.39 ± .643mg/l]. While serum uric acid levels were also significantly increased in unstable angina [5.58 ± 0.76], and stable angina [3.55 ± 0.62], when compared with control mean value of uric acid [2.52 ± 0.51mg/dl]. The values of MPO, CRP & Uric acid were also significantly higher in unstable angina as compared to stable angina.

Conclusion: Myeloperoxidase level is increased in stable and unstable angina patients and this increase is statistically significant when compared to the control group. This increase is in accordance with increase in other inflammatory marker like serum CRP and serum uric acid. Similarly these values were also higher in unstable than stable angina. Hence myeloperoxidase serves as a marker of the inflammation.

Keywords : Uric acid , Coronary artery diseases, Stable angina, C-reactive protein

Introduction:
Coronary artery disease (CAD) continues to be a significant cause of mortality and morbidity. Stable angina is the most common presentation of coronary artery disease. The pain usually goes away in a few minutes after rest or after taking angina medicine. Stable angina is a fore-runner of myocardial infarction.
Unstable angina is a serious condition that requires emergency treatment. It is a sign that acute myocardial infarction could occur soon. Unlike stable angina, it does not follow a pattern. It can occur without physical exertion and is not relieved by rest or medicine. Unlike early treatment of myocardial Ischaemia to prevent necrosis with treatments such as fibrinolysis, coronary artery bypass grafting and percutaneous coronary intervention have improved outcome. Over time it has become clear that in order for such treatments to be of maximal benefit, timely diagnosis
is important. Here biomarkers become important, to help us improve our diagnostic accuracy of the disease, as treatments are not without risk. Furthermore, biomarkers also provide prognostic information about the disease, which then aids clinicians in deciding how aggressively they need to treat the disease.

The newly identified myeloperoxidase (MPO, EC 1.11.1.7) is a lysosomal dimeric hemeoprotein, composed of two 59 kDa and two 13.5 kDa subunits. It is a unique peroxidase that catalyzes the conversion of hydrogen peroxide (H$_2$O$_2$) and chloride to hypochlorous acid.$^{1,2}$ Myeloperoxidase is an enzyme secreted by a variety of inflammatory cells, including neutrophils, certain tissue macrophages, and monocytes, such as those found in atherosclerotic plaque. The enzyme is not released until degranulation and leukocyte activation. It is the strong oxidant with powerful antimicrobial activity and has broad-spectrum reactivity with biomolecules.$^3$ Increased plasma myeloperoxidase (MPO) is thought to be associated with increased mortality rate of hospitalized patients with coronary artery disease (CAD).$^{2,5,6,7}$ Despite the fact that elevated MPO occurs in CAD patients, the underlying mechanisms remain unknown. Prior studies showed that MPO concentration is markedly elevated in acute-phase response induced by inflammation and expression during acute-phase response.$^{8,9}$ Inflammatory processes play important role in determining plaque stability.$^9$

Our work has focused on whether markers of inflammation like MPO; CRP & Uric acid helps to improve risk stratification between stable angina & unstable angina. While single biomarker measurement may stratify the risk, combination of measurements of a marker of inflammation may improve upon risk stratification. The aim of this study was to analyze the MPO values and to compare MPO with CRP and uric acid in patients with stable & unstable angina.

**Method:**

**Study design and subjects**

We included 100 consecutive patients admitted to the cardiac care unit for ischemic chest pain during the 24 hours before admission. They were categorized based on symptomatology & ECG findings into stable angina & unstable angina. We excluded patients with concomitant inflammatory diseases, and patients on treatment with immunosuppressive or anti-inflammatory agents. The study was approved by the ethics committee of the institute attached to hospital, and all patients gave written informed consent to participate.

**Laboratory methods**

Complete clinical data and blood samples for laboratory measurements were collected at admission. ECG was obtained immediately on admission or in the OPD on arrival of the patient. A 2D echocardiogram was performed within the initial 24 hours. Serum CRP, uric acid and plasma MPO levels were measured on admission.

The plasma MPO concentration was quantified by a sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies with some modifications.$^{10,11}$ We measured serum CRP levels with a latex turbidity method assay. Uric acid was measured with enzymatic colorimetric reagents.

**Statistical analysis**

Statistical calculations were performed with SPSS 11.0 statistical software package. All values were expressed as mean ± standard deviation (SD), unless
otherwise indicated. When two groups were compared, the significance was evaluated by unpaired Student’s t test; when multiple groups were compared, the significance was evaluated by one-way analysis of variance (ANOVA). A P value <0.05 was considered statistically significant.

**Results:**

**Clinical characteristics of study subjects**

The age of patients with stable angina was ranging from 45.76 ± 9 years while for unstable angina it was 44.96 ±7.76 years. Age of the pt was not significant statistically for either of the group.

As shown in table 1, there were no significant differences in gender, age, and diastolic blood pressure, Hb level & Serum creatinine between the three groups. Compared with the control group, all patients with stable & unstable angina had a significantly higher systolic blood pressure. Serum CPK levels were significantly high in stable(127.67±16.20 )& & unstable angina( 256.92 ±20.02) , twice the control values in stable angina while two & half times the control values in unstable angina . Serum CPK-MB levels were also significantly high in stable (23.38 ±3.71) & unstable angina (36.43 ± 6.17), again twice the control values in stable angina while thrice the control values in unstable angina

**Table 1:-** Clinical and biochemical data of controls and patients with stable and unstable angina patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control group (n=50)</th>
<th>SA group (n=50)</th>
<th>Unstable angina (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>37/13</td>
<td>39/11</td>
<td>39/11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.24 ±9.58</td>
<td>45.76 ±9.41</td>
<td>44.96±7.76</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>123.20 ±6.61</td>
<td>128.76 ±7.60</td>
<td>126.20 ±10.38</td>
</tr>
<tr>
<td>Diastolic</td>
<td>68.54±4.68</td>
<td>74.90 ±5.21</td>
<td>76.42 ±6.46</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>13.11 ±.93</td>
<td>11.94 ±1.57</td>
<td>13.02 ±1.24</td>
</tr>
<tr>
<td>WBC (x10⁹/µL )</td>
<td>6.85 ±1.05</td>
<td>7.54 ±1.35</td>
<td>9.42 ±1.56</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.57 ±.13</td>
<td>0.83 ±.15</td>
<td>0.91 ±.17</td>
</tr>
<tr>
<td>Serum total CPK (IU/L)</td>
<td>101.71 ±37.92</td>
<td>127.67 ±16.20</td>
<td>256.92 ±20.02</td>
</tr>
<tr>
<td>Serum CK-MB (IU/L)</td>
<td>10.52 ±5.58</td>
<td>23.38 ±3.71</td>
<td>36.43 ±6.17</td>
</tr>
</tbody>
</table>
Table 2: The mean ± SD values of myeloperoxidase, C-reactive protein, and uric acid, in controls and patients with stable angina and unstable angina

<table>
<thead>
<tr>
<th>Data</th>
<th>MPO ng/ml</th>
<th>CRP mg/L</th>
<th>Uric acid mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=50)</td>
<td>112.12 ±29.07</td>
<td>1.39 ±0.64</td>
<td>2.52 ±0.51</td>
</tr>
<tr>
<td>Stable angina (n=50)</td>
<td>283.26 ±30.68</td>
<td>3.73 ±0.72</td>
<td>3.55 ±0.62</td>
</tr>
<tr>
<td>Unstable angina (50)</td>
<td>340.94 ±45.28</td>
<td>18.22 ±5.75</td>
<td>5.58 ±0.76</td>
</tr>
</tbody>
</table>

As shown in table 2, in the present study, serum MPO levels were significantly raised in stable angina [283.26 ±30.68] & unstable angina [340.94 ±45.28], as compared to the controls [112.12 ±29.07]. MPO levels were found to be two & half times the control values in stable angina while three times the control values in unstable angina. In our study the patients showed a high statistically significant increase (P<0.0001) in myeloperoxidase level, as compared to normal controls.

In the present study, serum CRP levels were significantly raised in stable angina [3.73 ± 0.72], unstable angina [18.22 ± 5.75], as compared to the controls [1.39 ± 0.64]. CRP levels were found to be two & half times the control values in stable angina while fifteen times the control values in unstable angina. The study revealed a statistically significant increase of serum uric acid in stable and unstable angina patients as compared to healthy controls (p<0.0001).

Discussion:
Coronary artery disease continues to be a major cause of morbidity and mortality worldwide. It remains a leading cause of death in India and represents an enormous cost to health care system. Myocardial ischemia results from reduction of coronary flow to such an extent that supply of oxygen to the myocardium does not meet the oxygen demand of myocardial tissue.

In this study, we investigated the levels of plasma MPO, CRP and Uric acid in patients with SA and unstable angina. Our results showed that the patients with SA and unstable angina had increased plasma MPO concentration, increased serum CRP, & serum uric acid levels as compare to controls. In our study MPO levels were found to be two & half times the control values in stable angina while three times the control values in unstable angina. CRP levels were found to be two & half times the control values in stable angina while fifteen times the control values in unstable angina.
values in unstable angina, Serum Uric acid levels were found to be one & half times the control values in stable angina & twice the control values in unstable angina. Interestingly we also observed higher values of all three markers like MPO (one & half times the values in stable angina) , CRP (six times the values in stable angina) & Uric acid (one & half times the values in stable angina) in patients with unstable angina implying greater degree of inflammation & plaque vulnerability in patients with unstable angina than stable angina.

Even though we could not compare these biomarkers with coronary angiographic findings, our results may suggest degree of inflammation & plaque vulnerability. However this needs further clarification with extensive study sample & correlating it with coronary angiographic findings. These results suggest that MPO may be a positive modulator for CRP, and uric acid metabolism in vivo. To our knowledge, this may be the first study comparing levels of plasma MPO with CRP, uric acid in patients with stable and unstable angina in India. Several prior studies have reported elevated CRP values in patients with unstable coronary artery disease. However the data so far available in India are relatively very few and hence more studies are requested to precisely define the role of MPO.

**Conclusion:**

In summary, our study showed that plasma MPO concentration is significantly increased, like that of CRP and uric acid in patients with unstable angina as well as stable angina. However these values are significantly more in patients with unstable angina, it implies us about greater degree of inflammation & plaque vulnerability in patients with unstable angina than stable angina. The use of MPO and other novel inflammatory markers may significantly add to our ability to identify patients presenting with CAD who are at high risk for future cardiovascular events.

**Limitations of our study:**

MPO assessment have used different methods, thus standardization of method is needed.

Increased MPO is not specific for cardiac diseases, as activation of neutrophils and macrophages can occur in any infectious, inflammatory process, therefore, more studies are necessary to clarify these points.

Comparison is not done between these biomarkers & Coronary angiographic findings

Sample size is small.

Coronary angiography was not performed for diagnosis of coronary artery disease

**References:**


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