OXIDATIVE STRESS AND CORONARY STENTING

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Key words: Cardiovascular disease, Oxidative stress, Percutaneous intervention, Restenosis.

INTRODUCTION

Coronary artery bypass surgery and Percutaneous intervention (PCI) are the treatment modalities of coronary revascularization. PCI is a less expensive and less invasive procedure compared to Coronary artery bypass surgery and has become commonly used treatment for symptomatic coronary artery disease. Numerous studies have proved that long term death risk and reinfarction rates are similar between these two treatments. Stenting is a frequently performed procedure of PCI. Stenting can be done with two types of stents – bare metal and drug-eluting stent. In-stent restenosis remains an often seen complication [10 – 40%] following PCI procedure. However, various factors are shown to affect the outcome of stenting in a patient. Patient variables, stent and procedure characteristics interventional cardiologist

Outcomes of PCI have been impacted by various factors including angina, myocardial infarction or repeat revascularization during six months of follow up was measured.

PCI PROCEDURE

Under local anesthesia PCI procedure was carried out either through femoral or radial route. Depending on the patient characteristics interventional cardiologist

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ABSTRACT

Introduction: Percutaneous intervention compared to CABG has become commonly used treatment for symptomatic coronary artery disease. Various factors are shown to affect the outcome of stenting in a patient. This study was done to determine the changes that occur in oxidative stress parameters and its relation with coronary stent outcome during follow-up. Subjects and Methods: 57 patients were successfully followed up for six months for cardiac related adverse events. Blood samples were collected for serial measurements of Catalase, serum malondialdehyde, protein carbonylation and total antioxidant levels. Results: When compared with baseline [23.81±19.39 µmol/L] level, MDA showed a significant decrease after the procedure [11.91±9.78 µmol/L] which increased after 24 hours [18.06±16.54 µmol/L] and after 72 hours [20.81±18.11 µmol/L]. Catalase showed increase after 24 hours of procedure [33.62±16.00 U/L] when compared with baseline [7.91±5.66 U/L] which decreased at 72 hours [24.60±11.20 U/L] significantly, though it remained high compared to baseline. During follow up of six months four patients developed adverse cardiac events. Discussion: We conclude that PCI procedure is associated with injury to vessel wall which results in oxidative injury and this may contribute to restenosis and reinfarction.
decided the type of stent and use of glycoprotein IIb/IIIa receptor inhibitors. Both drug-eluting and bare metal stents were used. At the end of the procedure patency of the vessel was assessed by angiography and TIMI flow 3 achieved.

Clopidogrel 300 mg and aspirin 300 mg was given to all patients on the previous night and on the morning of the day of surgery. Nitroglycerine and Heparin 5000U were given during the PCI procedure. Another 2500U heparin was given to the patient if procedure extends beyond one hour. Patients were advised to take clopidogrel, aspirin and also atorvastatin for six months.

Sample collection
2 mL fasting venous blood sample was collected from the patients 24 hours prior to coronary stenting. 2 mL of arterial samples were collected immediately before the procedure and immediately after the procedure was completed. Another 2 mL of venous blood samples were collected from the patients after 24 hours of PCI procedure and after 72 hours. Samples were immediately analyzed for catalase levels and rest was separated and stored at −20°C for future analysis of serum malondialdehyde [MDA], protein carbonylation [PCO] and total antioxidant levels.

Assay methods

Estimation of lipid peroxides (MDA)
Serum malondialdehyde was measured by thiobarbituric acid reactivity by Satoh's method. Proteins were precipitated by trichloroacetic acid. Sulphuric acid hydrolyses the lipid peroxides from the protein to yield a MDA. This MDA reacts with thiobarbituric acid (TBA) to produce MDA-TBA adduct. On boiling in water bath, this gives a pink color, which is measured at absorbance of 540 nm using N-butanol as blank.

Assay of protein carbonylation (PCO)
PCO was measured based on modified Levine's method. Carbonylated protein present in serum reacts with 2,4-Dinitrophenyl hydrazine (DNPH) at room temperature under dark condition. Reacted proteins were precipitated by using 10% trichloroacetic acid. The precipitates were washed with ethanol: ethyl acetate mixture to remove excess 2, 4-DNPH. The washed pellets were redissolved in protein dissolving solution and measured at 366nm spectrophotometrically.

Estimation of catalase
RBC catalase activity was determined by the method of Aebi H. The method is based on monitoring of the rate of decomposition of H₂O₂.

Estimation of total antioxidant status
Total antioxidant level is measured by the method proposed by Benzie et al [1996]. At low pH reduction of ferric tripyridyltriazine (Fe³⁺TPTZ) complex to the ferrous form which has an intense blue colour can be monitored by measuring the absorbance. It is directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture.

Statistical analysis
Results were expressed as mean ± standard deviation. Repeated measures ANOVA followed by Bonferroni’s multiple comparison test was used. P value of < 0.05 was considered as significant for all statistical tests. All analyses were performed with SPSS statistical software (SPSS Inc., Chicago, Illinois).

RESULTS
57 patients who underwent successful coronary stent and followed up for six months participated in the study. Mean age of the participants was 53 years.

Table 1: Patients profile

<table>
<thead>
<tr>
<th>S No.</th>
<th>Characters</th>
<th>Total N = 57</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age [years]</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>Sex [F:M]</td>
<td>7:50</td>
</tr>
<tr>
<td>3</td>
<td>Smoking, n [%]</td>
<td>24 [42]</td>
</tr>
<tr>
<td>4</td>
<td>Obesity, n [%]</td>
<td>16 [28]</td>
</tr>
<tr>
<td>5</td>
<td>Hypertension, n [%]</td>
<td>19 [33]</td>
</tr>
<tr>
<td>6</td>
<td>Diabetes, n [%]</td>
<td>17 [30]</td>
</tr>
<tr>
<td>7</td>
<td>Dyslipidemia, n [%]</td>
<td>18 [32]</td>
</tr>
<tr>
<td>8</td>
<td>Type of stent</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>GP IIb/IIIa blocker used, n [%]</td>
<td>24 [44]</td>
</tr>
</tbody>
</table>

OXIDATIVE STRESS PARAMETERS
MDA, PCO, total antioxidant status and catalase were analyzed before 24 hours of PCI procedure, immediately before the procedure and immediately after the procedure and after 24 hours and 72 hours of procedure.

Table 2 shows the data of oxidative stress parameters in patients who underwent coronary stenting at various timed collections.

As shown in the figure 2, catalase showed increase after 24 hours of procedure [33.62±16.00 U/L] which decreased after 24 hours [18.06±16.54 µmol/L] and after 72 hours [20.81±18.11 µmol/L]. Changes in serial MDA measurement are depicted in the figure 1.

Comparison in oxidative stress parameters within and between bare metal and drug eluting stents
Alterations in oxidative stress parameters were compared within group of patients who were treated with bare metal stents and those who were treated with drug eluting stents. MDA, PCO and Catalase showed significant changes in patients treated with bare metal stent while MDA and Catalase showed significant changes in patients treated with drug eluting stent. Data are given in the table 3 and 4. But there were no significant changes in oxidative stress when compared between the patients treated with bare metal stent and drug eluting stent.

Follow up
During follow up of six months four patients developed adverse cardiac events. Two of the patients were diagnosed with angina and two with myocardial infarction. There was no significant correlation between alterations in oxidative stress parameters and development of adverse cardiac events.

DISCUSSION
When the balance between oxidant and antioxidant status in our body is disturbed it may lead to damage. During PCI procedure, placement of stent causes endothelial damage which may lead to recruitment of inflammatory cells and their activation and also activation...
of platelets. This may lead to production of reactive oxygen species.

Present study showed elevated MDA level after 24 hours and 72 hours of procedure. But the study by Apostolovic et al showed elevation after 6 and 12 hours after procedure and decrease at 24 and 72 hours. Elevation in oxidative stress is proposed to be due to plaque destabilization and release of free radicals from injured endothelial cells during procedure. In addition to injured endothelial cells neutrophils may also increase the production of reactive oxygen species which may play an important role in the development of complications following PCI.9

Catalase level was high after 24 hours of implantation which is in accordance with the study by Apostolovic et al who showed elevation after 24 hours of procedure. This increase is due to activation of antioxidant system after the procedure.9TAS showed no significant variations except that there was a significant decrease at 72 hours when compared to baseline level (0.86±0.23 vs 0.97±0.24 mmol/L, p =< 0.05). Baseline TAS level in this study was less (0.97±0.24 mmol/L) when compared to the study by Ciccek et al (1.27±0.043 mmol/L) who also showed an increase in TAS level after procedure.9

Restenosis after coronary stenting is thought to be mainly due to neointimal proliferation. The migration and proliferation of smooth muscle cells, induced by the production and release of growth factors, cytokines and extracellular matrix synthesis, result in neointimal formation and eventually represents the restenosis.10

In patients undergoing elective PCI, the use of drug-eluting stents has drastically reduced the incidence of restenosis compared with bare-metal stents in patients with comparable stent thrombosis.11 Conversely present study showed no significant difference in incidence of adverse event between patients treated with bare metal and drug eluting stents. There were no significant variations in oxidative stress parameters between patients who developed adverse events and those didn’t which is consistent with the study by Apostolovic et al.8

Hence we conclude that PCI procedure is associated with injury to vessel wall which results in oxidative injury and this may contribute to restenosis and reinfarction.

Figure 1: Changes in MDA levels during serial assessment

![Figure 1: Changes in MDA levels during serial assessment](image1)

Figure 2: Changes in Catalase levels during serial assessment

![Figure 2: Changes in Catalase levels during serial assessment](image2)

Table 2: Serial assessment of Oxidative Stress markers in patients who underwent coronary stenting

<table>
<thead>
<tr>
<th>Parameters</th>
<th>24 hours before PCI</th>
<th>Immediately before PCI</th>
<th>Immediately after PCI</th>
<th>24 hours after PCI</th>
<th>72 hours after PCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/L)</td>
<td>23.81±19.39</td>
<td>23.94±19.4</td>
<td>11.91±9.78</td>
<td>18.06±16.54</td>
<td>20.81±18.11</td>
</tr>
<tr>
<td>Catalase (U/L)</td>
<td>7.91±5.66</td>
<td>6.11±2.47</td>
<td>5.94±2.90</td>
<td>33.62±16.00</td>
<td>24.60±11.20</td>
</tr>
<tr>
<td>PCO (nmol/mg)</td>
<td>10.54±7.24</td>
<td>9.69±6.46</td>
<td>8.93±10.98</td>
<td>10.82±7.63</td>
<td>10.02±6.19</td>
</tr>
<tr>
<td>TAS (mmol/L)</td>
<td>0.97±0.24</td>
<td>0.88±0.18</td>
<td>0.90±0.21</td>
<td>0.92±0.22</td>
<td>0.86±0.23</td>
</tr>
</tbody>
</table>

* - Significant when compared to a, # - Significant when compared to b, * - Significant when compared to c, ! - Significant when compared to d

Table 3: Sequential assessment Oxidative stress markers of patients placed with bare metal stent.

<table>
<thead>
<tr>
<th>Variables</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/L)</td>
<td>25.2±21.04</td>
<td>25.41±20.95</td>
<td>11.98±9.41</td>
<td>19.19±17.78</td>
<td>22.36±19.55</td>
</tr>
<tr>
<td>Catalase (U/L)</td>
<td>7.51±5.30</td>
<td>5.97±2.49</td>
<td>5.39±1.91</td>
<td>30.95±14.45</td>
<td>22.87±10.35</td>
</tr>
<tr>
<td>PCO (nmol/mg)</td>
<td>11.65±8.01</td>
<td>10.02±5.84</td>
<td>6.78±4.6</td>
<td>10.62±7.47</td>
<td>9.55±16</td>
</tr>
</tbody>
</table>

MDA in µmol/L, Catalase in U/L, PCO in nmol/mg of protein

* - Significant when compared to a, # - Significant when compared to b, * - Significant when compared to c, ! - Significant when compared to d

Table 4: Sequential assessment of fibrinogen and Oxidative stress markers of patients placed with drug eluting stent.

<table>
<thead>
<tr>
<th>Variables</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/L)</td>
<td>21.22±16.06</td>
<td>21.22±16.28</td>
<td>11.77±10.69</td>
<td>15.97±14.07</td>
<td>17.93±15.16</td>
</tr>
<tr>
<td>Catalase (U/L)</td>
<td>8.64±6.37</td>
<td>6.39±2.48</td>
<td>6.95±4.02</td>
<td>38.57±17.86</td>
<td>27.81±12.26</td>
</tr>
</tbody>
</table>

MDA in µmol/L, Catalase in U/L

* - Significant when compared to a, # - Significant when compared to b, * - Significant when compared to c, ! - Significant when compared to d

REFERENCES

7. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996. 15;239(1):70-6