OBJECTIVE

To determine the changes in activity of plasma N-acetyl-β-D-glucosaminidase, a marker for inflammation as well as renal, pulmonary and cardiac damage and proinflammatory cytokines in patients undergoing coronary artery bypass grafting and find out the relationship between their plasma levels with clinical outcome of patients.

STUDY DESIGN

Cross-sectional study.

PLACE AND DURATION OF STUDY

The Aga Khan University, Karachi, from January to June 2003.

PATIENTS AND METHODS

N-Acetyl-β-D-glucosaminidase (NAG) activity and concentrations of tumor necrosis factor-alpha of (TNFα), interleukin 6 (IL-6), interleukin 8 (IL-8) and granulocyte-macrophage colony stimulating factor (GM-CSF) were monitored in plasma samples of 12 angina patients undergoing coronary artery bypass grafting (CABG), before, immediately after and 5 days post-surgical procedure. Serum glucose concentrations were also monitored in those patients. Patient’s clinical condition was monitored during this time period.

RESULTS

No significant increase was observed in plasma NAG activity (a marker of inflammation) or in plasma levels of TNFα, IL-6, IL-8 and GM-CSF immediately after surgery, indicating that cardiopulmonary bypass itself does not produce any significant amount of inflammation immediately after CABG. However, 5 days post surgery, there was a significant increase in plasma NAG activity (p=0.001), TNFα (p=0.047) and GM-CSF (p=0.045). There was no relationship between plasma NAG activity and clinical outcome because various parameters of renal, cardiac and pulmonary functions, though slightly affected, remained within the normal limits.

CONCLUSION

Increased levels of NAG and TNFα did not affect clinical outcome. However, data suggest that NAG can be a potential marker for inflammation and end organ damage following CABG. An increase in GM-CSF on day 5 following CABG indicates enhanced body’s defense mechanism against infection.

N-acetyl-β-d-glucosaminidase and inflammatory response after cardiopulmonary bypass

patients had either acute coronary syndrome or a non-ST elevation myocardial infarction before surgery. None had a recent major transmural myocardial infarction preoperatively. The study had been approved by the Research Ethics Committee of the institution. Informed consent was obtained from all the patients.

Three samples of blood were collected from each patient at different time intervals. The first set of blood samples was collected from a central venous catheter placed at the time of surgery and collected before systemic heparinization. Postoperatively, blood was collected from the same central catheter after administration of protamine. The samples were transported to the laboratory immediately or following a brief storage at 4°C. The day 5 samples were collected from a peripheral vein prior to patient discharge. Clinical status of patients was monitored throughout their stay in the hospital.

NAG activity was determined in plasma samples by the method of Whiting et al. described in a previous publication. One unit of NAG activity was defined as that which released 1 µmole of 4-methylumbelliferone per minute under assay conditions. TNFα, IL-6, IL-8 and GM-CSF in plasma samples were analyzed using the kits based on enzyme immunoassay by CHEMICON International Inc (Temecula, CA, USA).

Mean values have been presented as means±standard deviation (SD). Comparison of the two mean values at two different time intervals was carried out by paired sample t-test. One-way ANOVA followed by Tukey’s HSD test was used for comparison of the distribution of mean values of more than two groups. A p <0.05 was considered significant.

RESULTS

Demographic and clinical characteristics of patients have been listed in Table I. Mean values of NAG before, immediately after and 5 days postprocedure were found to be 5.7±1.6 U/l, 4.7±1.4 U/l and 7.3±1.4 U/l, respectively. NAG activity before and immediately after surgery were not significantly different indicating that there was little change in NAG activity during surgical procedure. However, NAG activity 5 days post-CABG was significantly increased (p=0.001).

Mean baseline NAG activity in diabetics was higher compared to non-diabetics (Table II). However, among the non-diabetics, there was a significant increase in plasma NAG activity (p=0.002) at day 5 post-CABG. Among diabetics, there was an increase in mean NAG activity on day 5 compared to baseline levels (7.95 ± 2.1 U/l vs. 6.92 ± 2 U/l), however, the increase was not statistically significant (p=0.158).

Plasma levels of cytokines, TNFα, IL-6, IL-8 and GM-CSF before CABG, immediately after CABG and 5 days post-CABG have been shown in Table III. There was a slight decrease immediately after CABG in the concentration of three proinflammatory cytokines (TNFα, IL-6 and IL-8). However, 5 days post-CABG, the levels of IL-6 and IL-8 return to baseline levels, while there is a significant increase in levels of TNF-α (p=0.047). Mean plasma levels of GM-CSF were found to be significantly increased in samples obtained 5 days post-CABG indicating boosted defense against infections in these patients at this point in time. There was no relationship between NAG and the clinical outcome. Postoperative serum creatinine levels, mean ventilatory time and heart function remained within normal limits in all the patients. Radiologically, there was no evidence of pneumonia in any of the study subjects. Moreover, none of them had any clinical infection during the postoperative period.

DISCUSSION

Cardiopulmonary bypass has been known to be one of the major causes of systemic inflammatory response after CABG and it may lead to multiple organ dysfunctions. In a study by Hirai et al., plasma levels of proinflammatory cytokines IL-6 and IL-8 significantly correlated with hepatic dysfunction and respiratory dysfunction, respectively, in patients following CABG. Wei et al. have demonstrated an association of increased systemic proinflammatory cytokine levels with postoperative myocardial dysfunction.

In pulmonary and renal damage, due to extracorporeal perfusion, plasma NAG activity has been shown to be significantly increased. The present results showed no significant increase either in the plasma NAG activity or in the plasma levels of TNFα, IL-6, IL-8 or GM-CSF immediately after the surgical procedure. However, there was a significant increase in plasma NAG activity...
at day 5 post-CABG. Though speculative at this moment, these findings suggest that cardiopulmonary bypass causes a systemic inflammatory response that might be responsible for organ damage within 5 days following surgical procedure. TNF-\(\alpha\) appears to be the major inflammatory cytokine produced by day 5 following surgery and its level was found to be increased along with that of NAG. It is clinically important to attenuate the inflammation that takes place during cardiopulmonary bypass and the inflammation of myocardium after ischemia and following re-perfusion, since these affect the clinical status of patients after cardiopulmonary bypass as well as myocardial functions. Different agents such as diltiazem,\(^{17}\) cimetidine\(^{18}\) and corticosteroids\(^{3}\) have been used to inhibit systemic inflammatory response to surgery. Off-pump coronary re-vascularization is another approach adopted to minimize physiological insult associated with CABG.\(^{19}\)

Though NAG and TNF-\(\alpha\) were found to be significantly increased on day 5 in patients undergoing CABG in this study, they did not affect the clinical outcome and, renal, pulmonary and cardiac function parameters remained within normal limits suggesting that anti-inflammatory processes neutralized any deleterious effects of the inflammatory cytokines.

GM-CSF is often considered to be responsible for host's defense against infections as it has been shown to increase neutrophil count as well as enhance the cytotoxic activity of macrophages and natural killer cells.\(^{20}\) Therefore, an increase on day 5 following CABG (Table III) shows enhanced body’s defense mechanism against infection. These results conform well to those reported by Hirai et al.\(^{16}\) who have also shown increased plasma levels of macrophage colony stimulating factor 48 hours post-cardiopulmonary bypass. This result (elevated levels of GM-CSF on day 5 post-CABG) is significant as most of the work done to-date has been on other cytokines associated with systemic inflammatory response following cardiopulmonary bypass. Elevation of the profile of GM-CSF as observed in this study is important as it will help us understand how body balances the proinflammatory processes and the defense against infection following cardiopulmonary bypass.

The mean plasma NAG activity values in acute myocardial infarction (AMI) patients and normal healthy subjects are 10.92 ± 7.5 U/l and 6.8 ± 2.2 U/l, respectively.\(^{8}\) In the present study, the mean baseline plasma NAG activity in angina patients undergoing CABG has been found to be 5.73 ± 1.6 U/l, which is not significantly different from the NAG activity in normal healthy subjects. This lends support to the initial hypothesis that AMI is an acute inflammatory condition and NAG activity in plasma would be expected to be elevated in it. However, angina patients would only exhibit elevated NAG activity following induction of pro-inflammatory cytokines, especially TNF-\(\alpha\). Similarly, enhanced baseline levels of NAG in diabetics compared to non-diabetics (Table II) is in line with the hypothesis that diabetes mellitus is an inflammatory disease,\(^{21}\) therefore, a marker of inflammation, such as NAG would be expected to be increased in this disease. The enhanced inflammatory response on day 5 might be responsible for some organ damage resulting in a further increase in plasma NAG activity. However, a simultaneous increase in plasma GM-CSF level at the same time post-CABG appears to suggest increased defense mechanism against infection.

A relatively small sample size of patients (n=12) may be considered as a limitation of this study. However, it is not uncommon to find in the international literature pilot level studies pertaining to changes in cytokines in cardiopulmonary bypass using a small number of patients. For example, Gormley et al. have monitored changes in cytokine levels in 8 patients undergoing cardiac surgery,\(^{10}\) while Hirai et al. studied such changes in 11 patients.\(^{16}\)

This data suggest that NAG can be a potential marker for inflammation and possibly for organ damage.

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**Table II:** Serum glucose levels and plasma NAG activity in diabetic and non-diabetic angina patients before, immediately after and 5 days post-coronary artery bypass grafting (CABG). (means ± SD)

<table>
<thead>
<tr>
<th>Patients</th>
<th>No.</th>
<th>Glucose concentration (mg/dl)</th>
<th>*P</th>
<th>NAG activity (U/l)</th>
<th>**P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Before CABG</td>
<td>Immediately after CABG</td>
<td>5 days Post CABG</td>
<td>Before CABG</td>
</tr>
<tr>
<td>Diabetic</td>
<td>4</td>
<td>218 ± 66</td>
<td>210 ± 37</td>
<td>214 ± 25</td>
<td>0.012</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>8</td>
<td>82 ± 21</td>
<td>153 ± 59</td>
<td>118 ± 35</td>
<td>5.14±0.99</td>
</tr>
</tbody>
</table>

*P compares mean glucose concentration before CABG with mean glucose concentration immediately after CABG by paired samples t-test.

**P compares all 3 mean values of NAG activity (before, immediately after and 5 days postprocedure) in the row using one-way ANOVA.

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**Table III:** Plasma levels of cytokines in angina patients before, immediately after and 5 days post coronary artery grafting (CABG) (means ± SD).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Plasma concentration (ng/ml)</th>
<th>*P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before CABG</td>
<td>Immediately after CABG</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>1.43 ± 1</td>
<td>1.06 ± 0.62</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.22 ± 1.66</td>
<td>1.2 ± 1.28</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.48 ± 0.44</td>
<td>0.41 ± 0.27</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>0.01 ± 0.011</td>
<td>0.011 ± 0.006</td>
</tr>
</tbody>
</table>

*P compares the 3 mean values of each cytokine by one-way ANOVA followed by Tukey’s HSD test.
following CABG using cardiopulmonary bypass. However, further studies using a large number of patients would be required to ascertain its role as a predictor of end-organ damage. Strategies to attenuate this proinflammatory response, such as, anti-TNF therapy, off-pump CABG, and increasing plasma GM-CSF level to boost body’s defenses, could play a role in reducing mortality and morbidity associated with cardiac surgery.

CONCLUSION

Increased levels of NAG and TNFα did not affect clinical outcome in patients undergoing cardiac surgery. However, the data suggest that NAG can be a potential marker for inflammation and end organ damage following CABG. An increase in GM-CSF on day 5 following CABG indicates enhanced body’s defense mechanism against infection. Strategies to boost the body’s defenses by increasing plasma GM-CSF level could be important in reducing mortality and morbidity associated with CABG.

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REFERENCES


