Imbalances of Pro-inflammatory cytokines in myocardial infarction patients

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Abstract: To assess levels of Interleukin-1β and CRP, in Diabetic and Non-diabetic Myocardial Infarction patients, prior to and post angioplasty. 200 patients were recruited in the study. MI patients between the age of 40 and 60 years. Patients came to NICVD with complaints of chest pain, positive Troponin T test and ECG was the confirmatory test for MI. They were divided into 2 groups 100 patients each. First group comprised of MI patients without DMT-II and second group comprised of MI patients with DMT-II. Serum triglycerides, cholesterol, LDL and HDL, FBS, by enzymatic kits, Insulin by RIA. HbA1C, Interleukin-1 β and CRP by ELIZA. Interleukin1β and CRP were significantly higher (P<0.001) in patients at the time of the infarction, prior to angioplasty as compared post angioplasty levels in both groups, which indicate their importance in development of ischemia and MI. FBS and Insulin were significantly higher (P<0.001), while HDL and HbA1C were significantly lower (P<0.001) in MI without DMT-II when compared to MI with DMT-II. BMI, SBP pressure were significantly higher (P<0.001) in MI patients with DMT-II when compared with MI patients without DMT-II. Interleukin1β and CRP were found to be significantly higher prior to angioplasty as compared to post angioplasty levels in both groups which confirms their role in development of ischemia and MI.

Keywords: Interleukin, myocardial infarction, diabetes mellitus, coronary angioplasty, C reactive protein.

INTRODUCTION

Interleukin-1β

The family of Interleukin 1(IL-1 β) comprises of four proteins which share sequence homology: IL-1α, IL-1β, IL-1 receptor antagonist, and IL-18 (Dinarello et al., 1997). Immune activity of IL-1β has been found in monocytes, macrophage, EC, and VSMC in human as well as in experimental atherosclerotic plaque; it is produced by EC, VSMC, macrophage and monocytes.

IL-1β is strongly pro-inflammatory for multiple cell types as it initiates expression of other inflammatory cytokines; it is induced by pro-inflammatory stimuli, like TNFα (Chamberlain et al., 2006; Tedgui et al., 2006). IL-1β facilitates extravagation in early lesion formation by increasing leukocyte/EC interactions (Bevilacqua et al., 2011). It also induces cytokine expression in every cell present in the lesion autocrine and paracrine. It not only initiates but maintains local inflammatory response.

IL-1α and IL-1β correlate with progression of atherosclerotic plaques in humans; the expression in healthy coronary arteries is minimal, increased in atherosclerotic plaques, and high in complicated plaques (Dewberry et al., 2000). Inhibition is seen with the treatment of IL-1 blockers in progression of atherosclerosis (Bhaskar et al., 2011). Direct effects of IL-1β are seen in insulin resistance (Donath et al., 2011), thrombosis (Bevilacqua et al., 1984) and metabolic derangements in obesity (Chamberlain et al., 2009). As IL-1β direct plasma level determination is difficult, only few studies have shown increased levels of IL-1β in patients with atherosclerotic events (Ikonomidis et al., 1999; Saitoh et al., 2000). Prognosis after acute coronary syndromes IL-1β levels were not favorable (Correia et al., 2010, Orn et al., 2010).

Hwang et al suggested that IL-1beta neutralizes the acute phase of MI which is caused by cardiac rupture and thus have a protective role. (Hwang et al 2001). Reduced fatty streak area was seen in ApoE mice that were given a decoy infusion of IL-1 receptor (Ellhage et al., 1998) Increased macrophage infiltrate with marked increased lesion area was seen in ApoE double knockout mice (Merhi-Soussi et al., 2005). A 30% decrease in atherosclerosis was seen in IL-1β/ApoE double knockout mice (Kirii et al., 2003). A therapy given to rheumatoid arthritis patients who included IL-1 neutralization showed beneficial symptoms like delaying the progression of the disease and joint destruction. In post-infarction cardiac remodeling and heart failure the emerging role of IL-1 family has been shown by the recent evidences (Nam et al., 2010). Pilot studies, preclinical models and observation data have suggested, a beneficial role of IL-1 blockade in a variety of pathologic processes including...
C-reactive protein
C-reactive protein (CRP) is an acute phase protein that is produced by stimulation of pro-inflammatory cytokines in the liver (Packard et al., 2000). It is strongly associated with cardiovascular risk (Boekholdt et al., 2006). In heart diseases it is a valuable tool to see the status of inflammation in patients because ease of measurement and the availability of the method. Numerous studies have shown both low- and high-sensitivity CRP to be associated with heart failure. Studies have shown high CRP levels in the elderly who prone to develop heart failure (Berton et al., 2003; Yin et al., 2004; Chirinos et al., 2005; Cesari et al., 2003).

A study that involved 4691 subjects from the general population, it was found that the relative risk of hospital admission for HF was twofold in those whose CRP levels were above 3 mg/L (Engstrom et al., 2009). It is seen in a study that there is an association between CRP and the stage of HF and it claims that CRP levels predict the probability of readmission to hospital due to deterioration in the functional stage of HF (Yin W-Het al., 2004; Alonso-Martinez et al., 2002). Some studies have found a stronger correlation between CRP and left ventricular ejection fraction and others have found no statistically significance between them (Berton et al., 2003; Yin et al., 2004; Alonso-Martinez et al., 2002). C-reactive proteins concentrations are of prognostic significance in unstable angina have been showed in a recent study (Liuzzo et al., 1994).

Objective of the study
To determine the role of pro-inflammatory cytokines Interleukin-1 β and CRP in Myocardial Infarction patients with and without Diabetes Mellitus Type II.

MATERIALS AND METHODS

Study design: Observational study
200 patients were recruited in the study they were MI patients between the age of 40 and 60 years. Patients came to NICVD with complaints of chest pain positive Troponin T test and ECG was the confirmatory test for MI (Inclusion criteria). Legal requirement was fulfilled by taking informed consent either from patient or his relatives before inducting them in the study. Ethical approval was taken from NICVD and Sindh University Jamshoro (Ref. No: ERC-11/2017). BMI was calculated after measuring Height and weight. Blood pressure and pulse was recorded. Exclusion criteria included person’s suffering from hyperglycemic or hypoglycemia, diabetic ketoacidosis, inherited disorders of lipid and lipoprotein metabolism, deranged liver functions, Cerebrovascular accidents, and acute infections.

10 ml blood was taken after 10-12 hours fasting, after centrifugation Serum was collected and stored at –80°C for different parameters which included Serum Triglycerides, Serum Cholesterol, Serum HDL-Cholesterol, Fasting blood glucose which were assessed using automated enzymatic kits cobas integra provided by Merck & Roche. LDL-Cholesterol was calculated using Friedwald formula. Serum Insulin levels were done by radioimmunoassay (RIA) from Merck. HbA1C, Serum IL-1 β and CRP were measured by ELIZA with commercial kits from Gesendet: Donnerstag (DRG Instruments GmbH) Germany.

TOSHIBA Infinix 2000 was used by consultant cardiologist to perform Angiography on MI patients after confirmation on the basis of history, signs and symptoms, ECG findings and positive Troponin T test.

STATISTICAL ANALYSIS

Data analysis was performed using the statistical package for the Social Sciences (SPSS ver.23). P value was determined by Students t test. P<0.05 was considered statistically significant. Gender and smoking were assessed by applying Chi Square test. Data is shown as mean and standard deviation. Sample size was determined by simple random sampling.

RESULTS

100 MI patient’s diabetes and 100 MI patients with diabetes mellitus type II (DMT-II) were included in the study. Table 1 shows the baseline and physical parameter of MI patients with and without diabetes mellitus type II. Age, duration of diabetes and dystolic blood pressure was found to be non-significant between the two groups. BMI, systolic blood pressure were significantly higher (P<0.001) in MI patients with DMT-II when compared with MI patients without DMT-II. Gender and smoking were found to be non-significant among the two groups when Chi Square test was applied to them.

Table 2 Fasting blood sugar and insulin levels was significantly higher (P<0.001) in patients of MI with DMT-II when compared with MI without DMT-II. Triglycerides, Cholesterol and Low density lipoprotein were found non-significant in the two groups, while High density lipoprotein and Glycosylated hemoglobin were significantly lower (P<0.001) in MI without DMT-II when compared to MI with DMT-II.
Table 1: Baseline Characteristics of Patients n=200

<table>
<thead>
<tr>
<th></th>
<th>Myocardial Infarction N (100)</th>
<th>Myocardial Infarction with Diabetes Mellitus Type II N(100)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Male/Female)</td>
<td>68 / 32</td>
<td>63 / 37</td>
<td>0.457</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>55 ± 4</td>
<td>56 ± 3</td>
<td>0.079</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.55 ± 0.01</td>
<td>1.54 ± 0.01</td>
<td>0.201</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>68.22 ± 1.29</td>
<td>70.47 ± 2.49</td>
<td>0.001</td>
</tr>
<tr>
<td>Body Mass Index (BMI)</td>
<td>27.57 ± 2.2</td>
<td>29.47 ± 4.71</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of DM (Years)</td>
<td>-</td>
<td>12 ± 3</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>20</td>
<td>25</td>
<td>0.404</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>130 ± 5</td>
<td>129 ± 8</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80 ± 4</td>
<td>81 ± 6</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard Deviation (SD). Student’s t test is applied to obtain significance P-<0.001

Table 2: Biochemical Parameter of patients in relation to Blood Glucose and Blood lipid Levels n=200

<table>
<thead>
<tr>
<th></th>
<th>Myocardial Infarction</th>
<th>Myocardial Infarction with Diabetes Mellitus Type II</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Blood Glucose (mg/dl)</td>
<td>80 ± 4</td>
<td>132 ± 16</td>
<td>0.001</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>5 ± 0.70</td>
<td>7 ± 0.81</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting Insulin (µIU/mL)</td>
<td>11 ± 2</td>
<td>19 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum Triglycerides (mg/dl)</td>
<td>190 ± 25</td>
<td>185 ± 26</td>
<td>0.172</td>
</tr>
<tr>
<td>Serum Total Cholesterol (mg/dl)</td>
<td>204 ± 22</td>
<td>196 ± 24</td>
<td>0.013</td>
</tr>
<tr>
<td>Serum LDL Cholesterol (mg/dl)</td>
<td>147 ± 30</td>
<td>144 ± 37</td>
<td>0.557</td>
</tr>
<tr>
<td>Serum HDL Cholesterol (mg/dl)</td>
<td>24 ± 5</td>
<td>27 ± 7</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 3: Levels of Inflammatory Cytokines in Myocardial Infarction patient’s pre and post angioplasty n=100

<table>
<thead>
<tr>
<th></th>
<th>Pre angioplasty</th>
<th>Post angioplasty</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-1β (pg/ml)</td>
<td>5.81 ± 0.58</td>
<td>3.06 ± 0.53</td>
<td>0.001</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>32.66 ± 7.83</td>
<td>1.85 ± 0.28</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard Deviation (SD). Student’s t test is applied to obtain significance P-<0.001

Table 4: Levels of Inflammatory Cytokines in Myocardial Infarction patients with Diabetes Mellitus Type II, pre and post angioplasty n=100

<table>
<thead>
<tr>
<th></th>
<th>Pre angioplasty</th>
<th>Post angioplasty</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-1β (pg/ml)</td>
<td>6.46 ± 0.98</td>
<td>3.60 ± 0.57</td>
<td>0.001</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>38.25 ± 7.92</td>
<td>2.57 ± 1.08</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard Deviation (SD). Student’s t test is applied to obtain significance P-<0.001

DISCUSSION

Serum interleukin 1-beta
It is found that Serum Interleukin 1-beta (IL-1β) concentrations in diabetic and non-diabetic Myocardial Infarction (MI) patients were raised at the time of Ischemia/MI as compared to the levels done after the inflammation has subsided that is post angioplasty. Matsumori et al stated that in healthy controls and the patients who completely recovered after MI the IL-1β concentrations were undetectable (Matsumori et al., 1994). In early course of MI, IL-1β plays as important mediator in inflammatory process (Gruzdeva et al., 2017). In early phase acute MI, IL-1 receptor antagonist have
been detected (Latini et al., 1994). In our study the raised levels of IL-1β in patients prior to and post angioplasty are related to pro-inflammatory response at the time of ischemia and reperfusion. IL-1β and coronary artery disease leading to atherosclerosis is linked by considerable evidence, is due to the inflammation in vascular wall (Ross et al., 1986). The raised active inflammatory response in vascular wall during and after ischemia. Endothelial dysfunction in atherosclerosis is attributed to impaired production/secreton of vasodilators such as endothelial derived relaxing factor Nitric Oxide (NO) (Ishizaka et al., 1991). It is of great interest that the enzyme responsible for NO production the NO-Synthase, its metabolism is regulated by IL-1β (Tsujino et al., 1994; Cunha et al., 1994, Szabo et al., 1993). The atherogenesis which leads to high morbidity and mortality rates can be controlled or somewhat inhibited by using IL-1β receptor antagonist (Dinarello et al., 1993, Alexander et al., 1992) which in turns reduces the vascular inflammation in atherogenesis and thus plays as a protective role in MI (Hamsten et al., 1995, Brown et al., 1990, Maulik et al., 1993).

More in-depth studies which also involve histopathological evidences are needed to link IL-1β concentrations and ischemia/MI. It can give an insight to the role of IL-1β and Ischemic Heart Disease (IHD) (Van Tassell et al., 2017). In sub-acute phase of AMI IL-1β loss of viable myocardium which promotes cardiac dilation and dysfunction and suppresses cardiac contractility and β-adrenergic receptor responsiveness. When IL-1β Inhibitors given during AMI they tend to have the potential to prevent adverse cardiac remodeling and/or heart failure (Toldo et al., 2017). In future serum IL-1β levels can/will be used for the diagnosis of IHD patients.

**C-reactive protein**

An increase level in markers of inflammation acute phase proteins and cytokines play important role in inflammatory processes by determining plaque stability, destabilization and rupture of atherosclerotic plaques, leading to acute cardiovascular events (Nikolaos et al., 2015). For the prediction of CHD and as a causative factor C-reactive protein (CRP), a prototype marker of the inflammatory process, is the most studied (Shrivastava et al., 2015). CRP can be measured inexpensively with available high-sensitivity assays it has no diurnal variation, levels are stable over long periods, have shown variation, levels are stable over long periods, have shown significant rise in the levels at the time of ischemia and decrease after the angioplasty was done and the inflammation got subsided shows that these cytokines play an important role in the cascade of ischemia/Myocardial Infarction and rupture of plaque.

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