

PRO-INFLAMMATORY CYTOKINES INTERLEUKIN 6 AND TUMOR NECROSIS FACTOR- α IN DIABETIC AND NON-DIABETIC MYOCARDIAL INFARCTION PATIENTS TREATED BY ANGIOPLASTY

Farhan Jaleel¹, Allah Bux Ghanghro², Saima Aqil³,
Muhammad Sameer Qureshi⁴, Tariq Ashraf⁵

¹Department of Biochemistry, Liaquat College of Medicine and Dentistry, Karachi-Pakistan.

²Department of Biochemistry Sindh University, Jamshoro-Pakistan

³Department of Biochemistry Altamash Institute of Dental Medicine, Karachi-Pakistan

⁴Department of Biochemistry Dow University of Health Sciences Karachi-Pakistan

⁵National Institute of Cardiovascular Diseases Karachi-Pakistan

Address for Correspondence:

Farhan Jaleel

Department of Biochemistry, Liaquat College of Medicine and Dentistry, Karachi-Pakistan

Emails: fjaleel@yahoo.com

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Contribution

FJ conceived, designed and did statistical analysis & manuscript writing. ABG, SA, and MSQ, did data collection and manuscript writing. TA did review and final approval of manuscript

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ABSTRACT

Objective: To evaluate the levels of pro-inflammatory cytokines Interleukin-6, Tumor necrosis factor- α before and after angioplasty in patients of myocardial infarction with and without diabetes mellitus type II.

Methodology: This cross sectional study included patients between the age of 40 and 60 years presented to National Institute of Cardiovascular diseases, Karachi. Patients were divided into 2 groups. First group comprised of myocardial infarction patients, second group included patients with myocardial infarction and diabetes mellitus type 2. Serum Interleukin-6, Tumor Necrosis Factor- α , serum triglycerides, cholesterol, LDL cholesterol HDL cholesterol, FBS, HbA1C and Insulin concentrations were determined. P value was determined by Students t test. Chi Square test was applied to gender and smoking.

Results: We included 200 patients. The patients were divided into 2 groups and each group comprised of 100 subjects. First group comprised of Myocardial Infarction patients, second group Myocardial Infarction with Diabetes Mellitus type 2 patients. Values of fasting blood glucose, glycosylated hemoglobin and insulin were significantly increased in myocardial infarction patients with diabetes as compared with myocardial infarction patients without diabetes ($p < 0.001$). Serum HDL cholesterol concentrations were significantly decreased in myocardial infarction patients without diabetes ($p < 0.001$). When values of cholesterol, triglycerides, LDL cholesterol, were compared, it was observed that they were non-significant. Interleukin-6 Tumor necrosis factor- α were found to be significantly higher in both groups prior to angioplasty as compared with post angioplasty levels ($p < 0.001$).

Conclusions: The study indicates that high levels of pro-inflammatory cytokines are found at the time of ischemia which play an important role in the cascade of MI. Levels were higher in patients regardless of diabetes mellitus status.

Key Words: Interleukin, Myocardial Infarction, Diabetes Mellitus, Inflammation, Coronary Angioplasty.

INTRODUCTION

Cardiovascular disease (CVD) is the leading global cause of death and disability, and approximately half of these cases are attributed to coronary heart disease (CHD).¹ Effective predictive and diagnostic methods are important for reducing the global burden of CHD on public health and its associated cost. Insulin resistance in diabetes mellitus type 2 (DMT2) is the pathogenesis which is found in general as well as in diabetic populations and is related to cardiovascular risk factors which includes hyperglycemia, arterial hypertension, obesity, thrombosis and smoking and dyslipidemia.^{2,3} Many clinical trials have shown that insulin resistance is a predictor of cardiovascular events like ischemic heart disease (IHD) and myocardial infarction (MI).^{4,5} Inflammation and pro-inflammatory cytokines have been important pathogenic factors in development of insulin resistance and diabetes mellitus.^{6,7} Pro-inflammatory cytokines are only secreted by immune cells was an old thought. After studying myocardium it is seen that every nucleated cell in it, secretes pro-inflammatory cytokine either due to stress or injury. In MI the myocytes have activated cytokine cascade which is established in numerous studies.⁸⁻¹⁰ The release and induction of pro-inflammatory cytokines like IL-6, TNF- α and Interleukin 1-beta are steadily described in experimental models of MI.¹¹⁻¹³ These cytokines play an important role in IHD and gives a link between inflammation, insulin resistance and DMT2. The release of interleukin-6 causes insulin resistance and free fatty acid mobilization which in turn increases the risk of ischemic heart diseases.^{14,15} In DMT2 which leads to cardiovascular events like IHD and CAD IL-6 is an independent predictor.^{16,17}

IL-6 has intense effects on cardiac myocytes both in the protection and apoptosis and promotion of cardiac hypertrophy.¹³ In atherosclerosis IL-6 may have direct pro- and anti-atherogenic effects on its development. The Pro-atherogenic effects are stimulation of vascular smooth muscle proliferation endothelial cell activation and platelet activation while athero-protective effects involve lowering of plasma low density lipoprotein (LDL) via upregulated LDL receptor gene expression^{19-22,24}

Interleukin -6 is a pro-inflammatory especially expressed in human atherosclerosis plaque and increased in serum in patients with coronary artery disease (CAD).^{26,27}

In injury and repair of cardiac tissue TNF- α exerts diverse effects on all cells. It improves cardiomyocyte apoptosis, suppresses cardiac contractility and stimulates expression of chemokines, adhesion molecules and leukocytes.^{28,29} It regulates extracellular metabolism in cardiac fibroblast by enhancing matrix metalloproteinases activity by decreasing collagen synthesis.³⁰⁻³³ TNF- α antagonize insulin stimulated receptors signaling and is elevated in people having insulin

resistance. TNF- α in ischemic myocardium, if present in lower dose it may induce angiogenesis and if in higher dose it may induce development of connective tissue leading to scar formation. High levels of TNF- α has been found in patients with coronary artery disease especially in ruptured plaque.^{28,29} Stress to myocardium releases pro-inflammatory cytokines TNF- α , which produce abnormal contractile function in myocyte. Receptors that bind to TNF- α may be able to prevent and even reverse the damage. Pre-treatment with TNF- α antibody may diminish the myocardial infarct size, resembling ischemic pre-conditioning.³⁴

Objective of the study was to determine the levels of pro-inflammatory cytokines Interleukin-6 and Tumor necrosis Factor- α in Myocardial Infarction patients with and without Diabetes Mellitus Type II at the time of MI, prior going for Angioplasty and compare it to post angioplasty levels.

METHODOLOGY

This cross sectional study included patients between the age of 40 and 60 years presented to National Institute of Cardiovascular Diseases with complaints of chest pain. Myocardial Infarction was confirmed by ECG and Troponin T test. Informed consents were taken from all the subjects either personally or via relatives as legally required prior to participation in the study following approval of the study by the Ethical Committees of NICVD and Sindh University Jamshoro. Convenient sampling was done to recruit the subjects. Height and weight were measured and BMI was calculated. Systolic and diastolic blood pressure and pulse was recorded. Patients having acute metabolic complications like hypoglycemia, diabetic ketoacidosis, hyperglycemic states, cerebrovascular accidents, acute infections, inherited disorders of lipid and lipoprotein metabolism and/or family history and deranged liver functions were excluded from the study.

The patient's blood samples were drawn after an overnight fast for around 10-12 hours; Serum was collected after centrifugation and stored at -80°C for subsequent assay. The different biochemical parameters which includes:

Fasting blood glucose was determined by glucose oxidase kit method from Merck. HbA1C was determined by automated kit on cobas integra provided by Roche. Serum Insulin levels were done by radio immunoassay (RIA) from Merck. Serum Triglycerides, was done by enzymatic kit method obtained from Merck. Serum-Cholesterol, Serum HDL-Cholesterol which were done by enzymatic kit method obtained from Merck and LDL-Cholesterol was calculated using Friedwald formula.

Serum levels of IL-6 and TNF-alpha were measured by enzyme-linked immunosorbent assays with commercial kits from Gesendet: Donnerstag (DRG Instruments GmbH) Germany. Angiography was performed on TOSHIBA Infinix

2000 by a consultant cardiologist on the basis of history, signs and symptoms, Troponin I levels and ECG findings.

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. Chi Square test was applied to gender and smoking.

RESULTS

The study included 200 patients of Myocardial Infarction (MI). Out of 200 patients 100 were those with MI without diabetes and 100 were MI patients having diabetes mellitus type II (DMT2).

Baseline and physical parameter of patients of MI with and without diabetes mellitus type II are shown in table 1. Chi Square test was applied to gender and smoking and they were found to be non-significant among the two groups. Age, duration of diabetes and diastolic blood pressure was also non-significant between the two groups. BMI, systolic blood pressure were significantly higher ($p < 0.001$) in MI patients with DMT2 when compared with MI patients without diabetes. The pro-inflammatory cytokines Interleukin-6 and Tumor necrosis factor alpha were significantly higher

($p < 0.001$) in MI patients at the time of the infarction, prior to angioplasty as compared to levels which were done after the angioplasty has been done (Table 2).

The levels of pro-inflammatory cytokines Interleukin-6 and Tumor necrosis factor alpha of MI patients with diabetes mellitus type II which are shown in Table 3, the levels of cytokines were found to be significantly higher ($p < 0.001$) prior to angioplasty when compared with levels of post angioplasty.

Lipid levels between the two groups are shown in figure 1. Triglycerides, Cholesterol and Low density lipoprotein were found non-significant when compared between the two groups, while High density lipoprotein was significantly lower ($p < 0.001$) in MI without diabetes when compared to MI with diabetes mellitus type II.

The diabetic indices Fasting blood glucose, Glycosylated hemoglobin and Fasting insulin levels are shown in Figure 2, FBS was significantly higher ($p < 0.001$) in patients of MI with DMT2 when compared with MI patient without diabetes. Glycosylated hemoglobin was significantly lower in ($p < 0.001$) MI patients when compared with MI with DMT2. Fasting insulin level was significantly higher among MI with DMT2 when compared with MI without diabetes.

Table 1: Baseline Characteristics of Patients (n=200)

| Variables | Myocardial Infarction without Diabetes Mellitus Type II n(100) | Myocardial Infarction with Diabetes Mellitus Type II n(100) | p-Value |
|--|--|---|---------|
| Gender (Male /Female) | 68 / 32 | 63 / 37 | 0.457 |
| Age (Years) | 55 \pm 4 | 56 \pm 3 | 0.079 |
| Height (m) | 1.55 \pm 0.01 | 1.54 \pm 0.01 | 0.201 |
| Weight (Kg) | 68.22 \pm 1.29 | 70.47 \pm 2.49 | 0.001 |
| Body Mass Index (BMI) (kg/m ²) | 27.57 \pm 2.2 | 29.47 \pm 4.71 | 0.001 |
| Duration of Diabetes Mellitus (Years) | - | 12 \pm 3 | - |
| Smoking | 20 | 25 | 0.404 |
| Systolic Blood Pressure (mmHg) | 130 \pm 5 | 129 \pm 8 | 0.001 |
| Diastolic Blood Pressure (mmHg) | 80 \pm 4 | 81 \pm 6 | 0.012 |

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

Table 2: Levels of Inflammatory Cytokines in Myocardial Infarction Patient's pre and Post Angioplasty (n=100)

| | PRE ANGIOPLASTY | POST ANGIOPLASTY | p -Value |
|---|------------------|------------------|----------|
| Interleukin -6 (pg/ml) | 20.41 \pm 2.53 | 5.73 \pm 1.13 | 0.001 |
| Tumor Necrosis Factor- α (pg/ml) | 27.08 \pm 5.20 | 0.96 \pm 0.13 | 0.001 |

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

Table 3: Levels of Inflammatory Cytokines in Myocardial Infarction Patients with Diabetes Mellitus Type II, pre and Post Angioplasty (n=100)

| | PRE ANGIOPLASTY | POST ANGIOPLASTY | p-Value |
|---|------------------|------------------|---------|
| Interleukin -6 (pg/ml) | 20.96 \pm 3.30 | 6.58 \pm 1.19 | 0.001 |
| Tumor Necrosis Factor- α (pg/ml) | 29.71 \pm 5.78 | 1.85 \pm 0.32 | 0.001 |

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

Figure 1:Lipid Profile in Myocardial Infarction Patients with and without Diabetes Mellitus Type II (n=100)

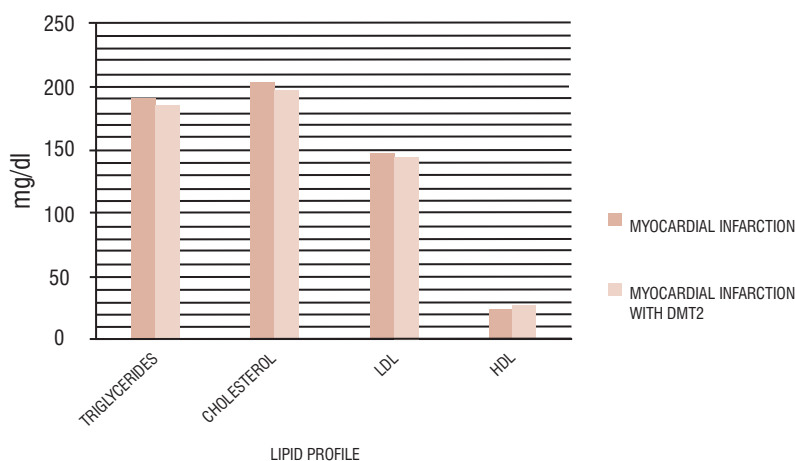
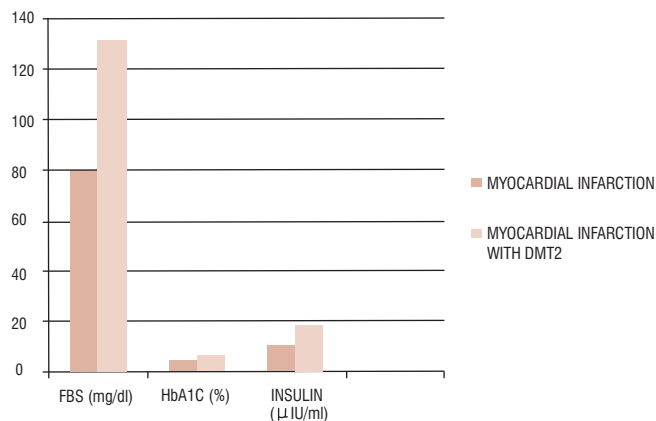


Figure 2:Diabetic Indices in Myocardial Infarction patients with and without Diabetes Mellitus Type II (n=100)



DISCUSSION

Our study focused on the levels of pro-inflammatory cytokines especially Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF- α) in Myocardial Infarction (MI) patients with and without Diabetes Mellitus Type II (DMT2). The subjects selected were patients who had an episode of MI which was confirmed by ECG and Troponin I. The patients were treated with angioplasty. There were two groups one with MI without DMT2 and the other MI with DMT2. In both the groups' blood samples were taken twice once at the time of MI and other after two months of the treatment with angioplasty so that the inflammation has subsided and the patients is back to normal condition. When the lipid levels were compared between the two groups' it was found that triglycerides, cholesterol and low density lipoprotein were non-significant among them.

High density lipoprotein (HDL) was found to be significantly lower ($p < 0.001$) in patients of MI without DMT2 when compared with patients of MI with DMT2.

It is well known that low levels of HDL increases the chances of deposition of cholesterol in vessels which in turn enhances the chances of formation of atherosclerosis in arteries especially of heart which leads to coronary artery diseases.

The diabetic indices fasting blood glucose, Glycosylated hemoglobin and insulin levels were compared in both the groups' and the levels were significantly increased ($p < 0.001$) in MI with DMT2 as compared to MI without DMT2.

IL-6 and TNF- α levels were assessed in both the groups' prior to angioplasty at the time of MI and compared with the levels of post-angioplasty, it was observed that the levels were significantly increased ($p < 0.001$) in samples taken prior to angioplasty as with samples taken after the treatment with angioplasty.

In our study we have found that when the event of MI takes place the levels of (IL-6) and (TNF- α) rises which further enhances the cascade of ischemia.

Increased levels of IL-6 in circulation causes; insulin resistance and mobilization of free fatty acids which in turn enhances the risk of ischemic heart disease.¹⁴ IL-6 is also found to be a Proinflammatory cytokine especially in human atherosclerosis plaque and coronary artery disease.^{26,27}

IL-6 plays an important role both as in protection and damage in myocardium.³⁹ When the levels of IL-6 are low in circulation they are cardio-protective, as the levels rises it contributes to worsen the heart.⁴⁰ High levels of circulating IL-6 were found in patients with end stage heart failure and dilated cardiomyopathy (DCM).⁴¹ In a study in which Rheumatoid arthritis (RA) patients were given an anti-IL-6

mAb therapy, it was found that the anti-IL-6 resulted in improvement of clinical symptoms and laboratory findings in RA patients.⁴²

TNF- α is also found to be increased in ruptured plaque. In low levels it stimulates angiogenesis but as the level rises it induces the development of connective tissue leading to scar formation.^{28,29}

The increased levels of cytokine prior to angioplasty at the time of MI indicate that they play an important role in the cascade of ischemia leading to MI, raising the possibility that these cytokine can be used as a noninvasive biomarker for atherosclerosis development. The treatment with anti-IL-6 and Anti TNF- α therapy might be helpful in preventing atherosclerosis and MI.

CONCLUSION

The patients of MI with and without diabetes mellitus type II both have increased levels of IL-6 and TNF- α prior to angioplasty at the time of the ischemia as compared to post angioplasty levels. This indicates that high levels of pro-inflammatory cytokines are found at the time of ischemia which plays an important role in the cascade of MI.

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