



## ORIGINAL ARTICLE

# Changes in Inflammatory Markers Concentration in Diabetic and Non-diabetic Patients with Myocardial Infarction

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### ABSTRACT

Cytokines are important mediators of inflammatory and immune responses. Type 2 diabetes mellitus have a two- to four fold increased risk of myocardial infarction in patients. The aim of this study was to investigate the changes in cytokines concentration in elderly diabetic and non-diabetic patients with myocardial infarction. Human serum samples of normal older subjects ( $n = 31$ ), diabetic patients without myocardial infarction ( $n = 33$ ), older diabetic patients with myocardial infarction ( $n = 32$ ) and older non-diabetic with myocardial infarction ( $n = 30$ ) were investigated. The patients were selected on clinical grounds from National Institute of Cardiovascular Disease, Karachi and Jinnah Postgraduate Medical Centre, Karachi, Pakistan. Cytokine levels (interleukin-6, interleukin-8 and tumor necrosis factor- $\alpha$ ) were estimated by enzyme linked immunosorbent assay (ELISA).

Values of interleukin-6, interleukin-8 and tumor necrosis factor- $\alpha$  were significantly higher in diabetic and non-diabetic patients with and without myocardial infarction as compared with older control subjects. When diabetic patients with myocardial infarction compared with age matched non-diabetic patients with myocardial infarction showed significantly higher concentrations. A significant positive correlation was observed, between interleukin-6 and interleukin-8 and between interleukin-8 and tumor necrosis factor- $\alpha$  in diabetic patients with myocardial infarction. A significant positive correlation was observed, between interleukin-6 and interleukin-8 and between interleukin-6 and tumor necrosis factor- $\alpha$  in non-diabetic patients with myocardial infarction. Positive significant correlation was observed between fasting blood glucose and systolic blood pressure in diabetic patients with myocardial infarction.

Elevated levels of the inflammatory markers indicating that they play a role in the development of myocardial infarction. The results of the present study thus demonstrated that levels increased in both condition but are more severe in diabetic patients.

**Key words:** Myocardial infarction, inflammatory, cytokines, diabetes

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### INTRODUCTION

Myocardial infarction is the most important determinant of the excessive morbidity and mortality in type 2 diabetic patients [1]. Hyperglycemia can increase oxidative stress through several pathways, causing substantial damage to low-density lipoproteins (LDL), endothelial cells (ECs), and tissue cells, finally leading to endothelial dysfunction that is related to vascular low-grade inflammation [2]. This inflammatory process contributes to precipitate acute thrombotic complications [3]. Inflammatory markers are used to predict cardiovascular risk [4]. Several inflammatory markers have been identified in myocardial infarction; among them are cytokines, including interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [5,6]. Inflammatory cytokines (IL-6, IL-8 and TNF- $\alpha$ ) produced by lymphocytes and macrophages inhibit collagen synthesis by smooth muscle cells and enhance the expression of matrix metalloproteinases (MMPs), which lead to proteolytic destruction of connective tissue matrix of the vulnerable atheromatous plaque [7,8]. They also reduce expression of tissue inhibitors of MMPs and enhance collagen degradation in the atheromatous fibrous cap [9]. Many studies have revealed the central position of inflammation in the pathogenesis of atherosclerosis, which is the main cause of coronary artery diseases [10]. It is hypothesized that a prolonged activation of

inflammatory mediators leads to myocardial damage and dysfunction. IL-6 is a multifunctional cytokine that mediates inflammatory and stress induced responses. Its gene is located on chromosome 7 and a G/C polymorphism at position -174 of its promoter is associated with several inflammatory cardiovascular diseases [11,12,13]. TNF- $\alpha$  is a proinflammatory cytokine produced mainly by monocytes, macrophages, T and B lymphocytes. The TNF- $\alpha$  gene maps within the MHC class III region on chromosome 6 and a G/A polymorphism at position -308 of its promoter has functional implications as the A allele results in higher levels of transcription than the G allele [14]. Studies have shown that elevated TNF- $\alpha$  serum levels are associated with various inflammatory and autoimmune diseases. Many reports have been published on the association between inflammatory [mainly IL-6, IL-8 and TNF- $\alpha$ ] cytokines and myocardial infarction [15,16,17]. If cardiovascular disease is an inflammatory disease, it can be expected to be provoked by such an inflammatory status. The present study **aimed** to assess the changes in inflammatory markers concentration in diabetic and non-diabetic patients with myocardial infarction.

## RESEARCH DESIGN AND METHODS

### Subjects and Sample Collection

The study included one hundred fifty seven subjects. Out of them 31 were normal older subjects, 33 were older diabetic patients without myocardial infarction, 32 were older diabetic patients with myocardial infarction and 30 were older non-diabetic with myocardial infarction. The blood samples were collected from the subjects during the period of March 2004 to December 2007. The ethical committee of Ziauddin University approved the protocol and consent of the patients was obtained after the nature of the study was fully explained. The older subjects were selected who were over sixty years of age as control subjects. Sex, weight, duration of diabetes, duration of complication in diabetic and non-diabetic patients, type of diabetes and type of treatments received were also recorded. Physical examination including measurement of blood pressure was recorded. Individuals were classified as having diabetes mellitus if any of the following criteria were met [18]. Fasting serum glucose levels of 7.0 mmol/L or more, random glucose levels of more than 11.1mmol/L, current use of medications prescribed to treat diabetes (e.g. insulin or drugs). Older patients or those with more than one complication were excluded from the study. Diagnosed cases of myocardial infarction were included in the study on the basis of chest pain, ECG changes i.e. ST elevation and Q wave inversion and biochemical markers i.e. raised levels of troponin T, CKMB, AST and LDH. The patients were selected on clinical grounds from National Institute of Cardiovascular Disease, Karachi and Jinnah Postgraduate Medical Centre, Karachi, Pakistan.

Blood was collected in fasting state after a 10-h overnight fast. Samples were withdrawn by venous puncture and distributed equally into three tubes containing EDTA (for HbA<sub>1c</sub>), heparin (for glucose estimation) and tube with no anti-coagulant (for serum collection). The samples were then immediately stored on ice until processed. Clotted blood was centrifuged at 1,500 rpm for 30 min and the serum was separated and frozen at -70°C until analysis. Blood glucose was determined by glucose oxidase method, glycosylated hemoglobin (HbA<sub>1c</sub>) was determined calorimetrically using HbA<sub>1c</sub> kit (Bio Systems Reagents and Instruments, Spain). The IL-6, IL-8 and TNF- $\alpha$  concentrations were measured from stored frozen serum samples using a commercially available high-sensitivity ELISA test [Interleukin-6 ELISA kit (DRG-EIA 4640), Interleukin-8 ELISA kit (DRG-EIA 4700) and human necrosis factor- $\alpha$  ELISA kit (DRG-EIA 4641)] were obtained from DRG instruments GmbH, Germany.

### Statistical analysis

Data was analyzed using statistical program Prism. The results are presented as mean,  $\pm$  standard deviation,  $\pm$  standard error of mean. The statistical significance of the difference between two mean of various parameters between different groups was evaluated by one-way analysis of variance (ANOVA). The Bonferroni's post hoc test was used to determine which group mean differs. With this test software automatically adjusts the significant level for the multiple comparisons to avoid spurious significant differences being identified (any values below the level of 0.05 was considered as significant). The graphics were performed in the statistical program Prism and the Statistical significance (p) was set at 5%.

## RESULTS

Values of interleukin-6, interleukin-8 and tumor necrosis factor- $\alpha$  were significantly increased ( $P < 0.001$ ) in diabetic and non-diabetic patients with and without myocardial infarction as compared with older control subjects. When diabetic patients with myocardial infarction compared with age matched non-diabetic patients with myocardial infarction showed significantly higher ( $P < 0.001$ ) concentrations (fig.1). Fasting blood glucose and HbA<sub>1c</sub> were significantly higher in older diabetic patients with or without myocardial infarction as compared with older non-diabetic patients with myocardial infarction and older control subjects. The increase in the fasting blood glucose level in all older diabetic patients

with and without myocardial infarction correlates significantly with glycosylated hemoglobin concentrations. Also, the fasting blood glucose and glycosylated hemoglobin were not found to be different in older diabetic patients with and without myocardial infarction. When compared with age matched normal subjects, the older non-diabetic patients with myocardial infarction showed no significant difference in levels of fasting blood glucose and glycosylated hemoglobin. Body mass index of diabetic patients with myocardial infarction are significantly higher ( $P < 0.05$ ) as compared with non-diabetic patients with myocardial infarction. Blood pressure both systolic and diastolic of diabetic and non-diabetic patients with myocardial infarction was increased significantly as compared with control subjects (table 1).

A significant positive correlation was observed, between interleukin-6 and interleukin-8 ( $r= 0.89$ ) in diabetic patients with myocardial infarction (fig.2A), between interleukin-8 and tumor necrosis factor- $\alpha$  ( $r= 0.88$ ) in diabetic patients with myocardial infarction (fig.2B), between interleukin-6 and interleukin-8 ( $r= 0.93$ ) in non-diabetic patients with myocardial infarction (fig.2C), between interleukin-6 and tumor necrosis factor- $\alpha$  ( $r= 0.91$ ) in non-diabetic patients with myocardial infarction (fig.2D). Positive significant correlation was observed between fasting blood glucose and systolic blood pressure ( $r= 0.91$ ), in diabetic patients with myocardial infarction.

**Table 1:** Physical features of control subjects, diabetic patients without myocardial infarction and diabetic and non-diabetic patients with myocardial infarction

Parameters	Control subjects (31)	Diabetic patients without myocardial infarction (33)	Diabetic patients with myocardial infarction (32)	Non-diabetic patients with myocardial infarction (30)
Sex (F/M)	16/15	16/17	14/18	15/15
Age (years)	64.19 ±3.94 ± 0.70	64.18 ±3.31 ± 0.57	66.00 ±4.45 ± 0.78	65.73 ±4.68 ± 0.85
Weight (Kg)	63.61 ±6.82 ± 1.22	65.66 ±8.78 ±1.53	66.03 ±6.04 ± 1.07	63.33 ±6.90 ± 1.26
Height (m)	1.59 ±0.05 ±0.01	1.59 ±0.05 ±0.01	1.58 ±0.05 ±0.01	1.60 ±0.06 ±0.01
BMI (Kg/m <sup>2</sup> )	25.22 ±2.97 ±0.53	26.00 ±3.70 ±0.64	26.20 <sup>b</sup> ±2.91 ±0.51	24.78 ±3.12 ±0.57
Systolic BP (mmHg)	121.54 ±5.88 ±1.05	119.70 ±6.83 ±1.19	144.53 <sup>a</sup> ±24.27 ±4.29	139.16 <sup>a</sup> ±24.81 ±4.53
Diastolic BP (mmHg)	83.06 ±6.14 ±1.10	81.66 ±6.08 ±1.05	91.56 <sup>a</sup> ±9.45 ±1.67	87.33 <sup>a</sup> ±9.89 ±1.80
Fasting Blood Glucose (mmol/l)	5.09 ±0.58 ± 0.10	7.46 <sup>ab</sup> ±1.42 ± 0.24	8.97 <sup>ab</sup> ±1.68 ±0.29	5.07 ±0.67 ±0.12
Glycosylated Hemoglobin (HBA1c %)	4.97 ±0.46 ± 0.08	9.02 <sup>ab</sup> ±1.61 ± 0.28	9.26 <sup>ab</sup> ±1.52 ±0.26	5.09 ±0.58 ±0.10

The values are expressed as mean, ± standard deviation, ± standard error of mean. Units and numbers of cases are shown in parentheses.

**a - Significant as compared with control subjects**

**b - Significant as compared with non-diabetic patients with myocardial infarction**

## DISCUSSION

The role of cytokines in heart disease is subject of increasing interest. Many clinical investigations have focused on cytokines in heart failure or the pathogenesis of arteriosclerosis [19,20]. Cytokines, important effectors of a normal inflammatory response, have been implicated in the pathogenesis of wide range of cardiac diseases and an increased expression of proinflammatory cytokines has been demonstrated in myocardial infarction. The main aspect of our study was to assess the status of cytokines in elderly diabetic and non-diabetic patients with myocardial infarction. We found an elevation in the levels of proinflammatory cytokines in diabetic patients with myocardial infarction as compared with non-diabetic patients with myocardial infarction and control subjects. We also found an elevation in the levels of proinflammatory cytokines in non-diabetic patients with myocardial infarction as compared with control

subjects. These findings indicate levels of proinflammatory cytokines increased in both conditions but are more severe in diabetic patients with myocardial infarction compared with non-diabetic patients with myocardial infarction. These findings are echoed in earlier studies [6,21,22]. Inflammatory mediators have a potential role in this process, as in all stages of atherosclerosis. Coronary atherosclerotic plaque disruption or erosion with consequent thrombosis is the major cause of heart diseases [23]. As inflammation and thrombosis are intertwined pathologies in the natural history of atherosclerosis, measurements of inflammatory markers can help predict prognosis in patients with coronary artery disease.

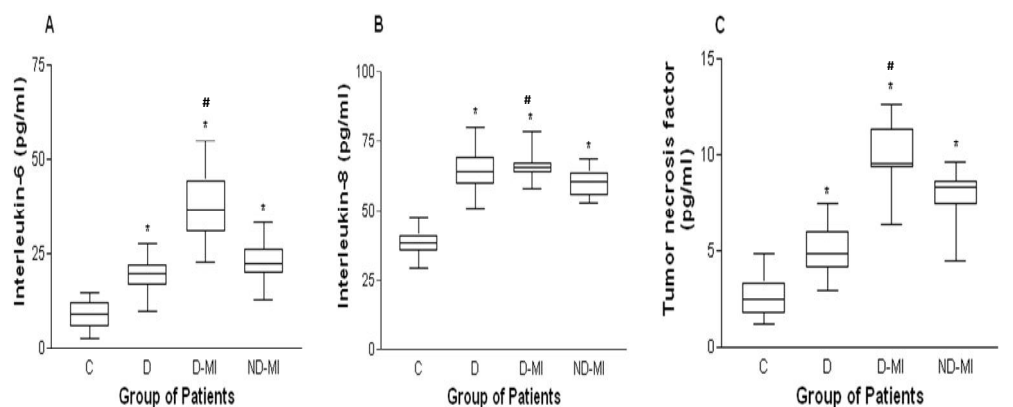


Fig. 1. levels of Interleukin-6 (A), interleukin-8 (B) and tumor necrosis factor-α (C) in diabetic and non-diabetic patients with and without myocardial infarction. Each column box in the figure represents mean±SEM for healthy control subjects (C; n=31), senile diabetics without myocardial infarction (D; n=33), senile diabetics with myocardial infarction (D-MI; n=32) and senile non-diabetics with myocardial infarction (ND-MI; n=30). \* P < 0.001 - statistically significant as compared with healthy control subjects. # P < 0.001 - statistically significant as compared with senile non-diabetics with myocardial infarction.

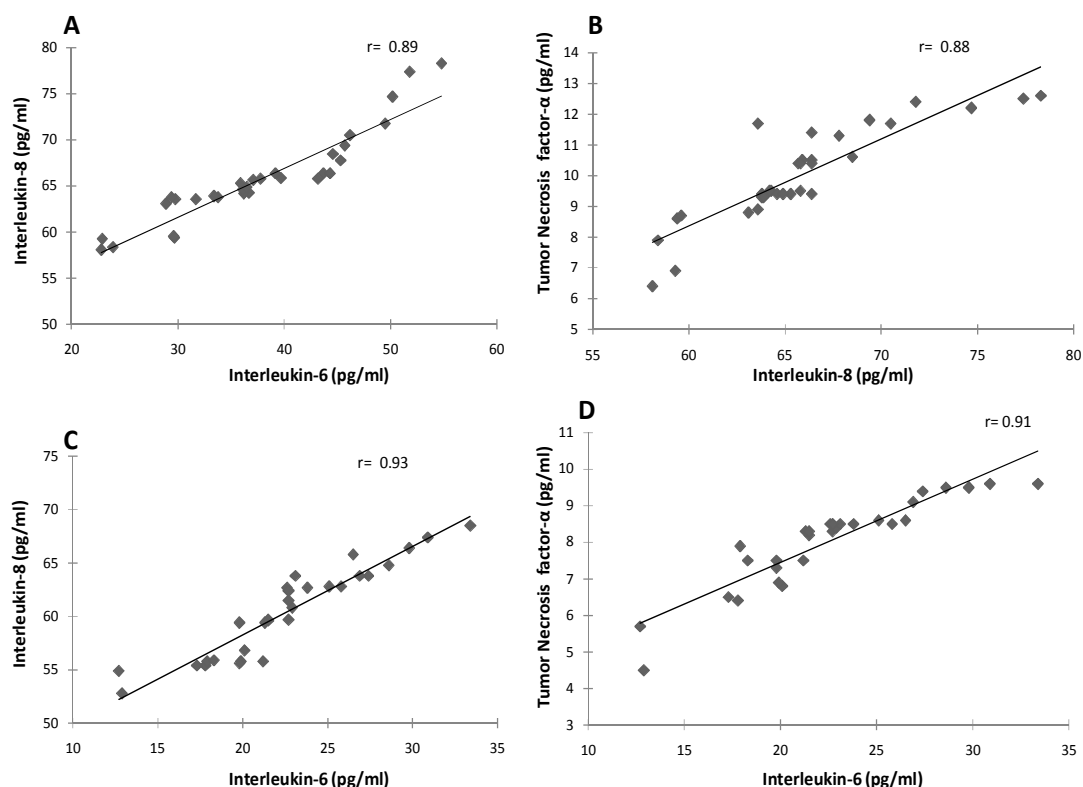


Fig. 2. Correlation of interleukin-6 vs. interleukin-8 in diabetic patients with myocardial infarction (2A), correlation of interleukin-8 vs. tumor necrosis factor-α in diabetic patients with myocardial infarction (2B), correlation of interleukin-6 vs. interleukin-8 in non-diabetic patients with myocardial infarction (2C), correlation of interleukin-6 vs. tumor necrosis factor-α in non-diabetic patients with myocardial infarction (2D).

Mizia-Stec et al [24] found that there was an increase in TNF-α levels in patients with acute myocardial infarction. Luo et al [25] reported elevated levels of serum IL-6 in patients with acute coronary syndrome. Koukkunen et al [26] showed a rise in IL-6 and TNF-α in patient with unstable angina. Wang et al [27]

also found higher levels of proinflammatory cytokines in patients with CAD than in controls. Interleukin-6 and IL-8 depress myocardial function by activation of inducible nitric oxide synthase (iNOS) and by stimulation of intercellular adhesion molecule-1 (ICAM-1) with accumulation of neutrophils and subsequent neutrophil dependent myocardial injury. They inhibit collagen synthesis and reduce the expression of tissue inhibitors of MMPs and enhance degradation of collagen in atheromatous fibrous tissue [28]. Tumor necrosis factor- $\alpha$  has been implicated in the pathogenesis as well as progression of atherosclerotic plaques in a number of ways. The possible mechanisms postulated include the enhanced surface expression of ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), E- and P-selectins on endothelial cells [28]. It also leads to increased chemokine and scavenger receptor expression. The parallel increase in anti-/proinflammatory cytokine levels in acute myocardial infarction may be an adaptive mechanism for the neutralization of action of myocardial injury caused by inflammatory mediators [6]. It has been suggested that inflammation may be a response to vascular injury caused by shear stress, oxidized lipids, smoking, and high levels of advanced glycation end products [29]. The pathogenesis of cardiovascular disease has long been described as an accumulation of lipids in a dysfunctional endothelial wall, driven by lifestyle-related factors such as smoking, dyslipidaemia, dysglycaemia, obesity, and hypertension. Since the end of the millennium, it has been commonly accepted that inflammatory processes play a prominent role in atherosclerosis, reclassifying cardiovascular disease as a chronic inflammatory disorder. Still, the exact balance between lifestyle and inflammation in its causality has remained unclear.

## CONCLUSION

Elevated levels of the proinflammatory cytokines including IL-6, IL-8 and TNF- $\alpha$  in myocardial infarction, indicating that cytokines play a role in the development of myocardial infarction. The results of the present study thus demonstrated that levels of proinflammatory cytokines increased in both condition but are more severe in diabetic patients with myocardial infarction compared with non-diabetic patients with myocardial infarction.

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