Evaluation of Serum Troponin and Cytokines as Predictors of Ischaemic Heart Disease in Diabetic Patients

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ABSTRACT

The present study aims to evaluate the importance of the determination of serum levels of troponin I, vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF - β) and tumor necrosis factor alpha (TNF - α) as predictors of ischemic heart diseases in high risk diabetic patient group susceptible to myocardial vascular occlusion. Subjects and Methods: The study was performed on 40 subjects classified into three groups: two groups of type 2 diabetes mellitus patients (the first with recent myocardial infarction and the other with ischemic heart disease), in addition to healthy controls. All members of the study were subjected to thorough clinical and ECG examinations; in addition to the estimation of levels of plasma glucose and serum creatine kinase (CK), CK-MB, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), troponin I, VEGF, TGF - β and TNF - α. Results: A significant increase was present in enzymatic activity of CK, CK-MB, AST, and LDH and in troponin I levels in the infarction group compared to both control and ischemic groups. In the latter two groups, troponin I was detectable in 0 and 54% of the subjects respectively. The results of the study showed significant elevation in serum VEGF, TGF - β and TNF - α in cases of myocardiac ischemia compared to control group. The elevations of these parameters were not significant in acute myocardial infarction group compared to controls as the samples were taken directly after admission to the hospital. Conclusion: Serum troponin I seems to be an extremely sensitive marker of myocardial infarction. On the contrary, the change in serum VEGF, TGF - β and TNF - α seem to be insignificant in the very early stage of myocardial infarction which indicate relatively slow induction of these growth factors in response to ischemia and hypoxia. VEGF could also be responsible for the compensatory stage of ischemic patients most probably due to its angiogenic effect and collateral formation which play a role in the protection from vascular occlusion. On the other hand the elevated levels of TNF - α and TGF - β could play a role in the pathogenesis of the ischemic changes.

RIASSUNTO

Valutazione della troponina e delle citochine del siero come predittori di cardiopatia ischemica in pazienti diabetici

Lo scopo del presente studio è di valutare l'importanza della determinazione della troponina I, del fattore di crescita vascolare endoteliale (VEGF), del fattore di trasformazione della crescita beta (TGF-β) e del fattore di necrosi tumorale alfa (TNF-α), come predittori di cardiopatia ischemica in un gruppo di pazienti diabetici ad alto rischio, suscettibili di sviluppare occlusioni vascolari miocardiche. Sono stati inclusi nello studio 40 soggetti, suddivisi in tre gruppi: 2 gruppi di pazienti affetti da diabete mellito di tipo 2 (il primo con infarto miocardico recente, l'altro con cardiopatia ischemica) e un gruppo di controlli sani. Tutti i soggetti dello studio erano sottoposti ad approfonditi esami clinici ed; in aggiunta venivano misurati nel plasma la concentrazione di glucosio, e nel siero la creatina chinasi (CK), l'isoenzima CK-MB, la aspartato amminotransferasi (AST), la lattato deidrogenasi, la troponina I, VEGF, TGF-β e TNF-α. Si osservava un aumento significativo delle attività di CK, CK-MB, AST, e LDH e della concentrazione di troponin I nel gruppo con infarto in confronto sia con i controlli sia con il gruppo degli ischemici. Negli ultimi due gruppi troponina I era misurabile nel 0 e 54% dei soggetti, rispettivamente. Si dimostrava inoltre un aumento significativo di VEGF, TGF-β e TNF-α nei casi di ischemia del miocardio in confronto ai controlli. L'aumento di questi parametri non era significativo rispetto ai controlli nel gruppo dell'infarto acuto del miocardio, per i quali i campioni erano ottenuti direttamente dopo il ricovero ospedaliero.In conclusione, la troponina I del siero sembra essere un marcatore estremamente sensibile di infarto del miocardio. Al contrario, le variazioni del concentrazioni nel siero di VEGF, TGF-β and TNF-α non sembrano essere significative nei primissimi stadi dell'infarto del miocardio, ad indicare una induzione relativamente lenta di questi fattori di crescita in risposta alla ischemia ed alla ipossia. VEGF potrebbe anche essere responsabile per lo stadio compensatorio dei pazienti ischemici, molto probabilmente a causa dei suoi effetti angiogenici e relativi effetti collaterali, che giocano un ruolo nella protezione dalla occlusione vascolare. D'altro canto, gli elevati livelli di TNF-α e di TGF-β potrebbero giocare un ruolo nella patogenesi delle modificazioni ischemiche.

INTRODUCTION

Diabetes mellitus is a chronic illness frequently associated with cardiovascular complications. Patients with diabetes have a two to five times greater risk of cardiovascular disease than nondiabetic subjects. Much of the morbidity and mortality associated with diabetes predominantly reflects its deleterious effects on micro- and macrocirculation (1,2).

Impaired myocardial performance independent of the vascular disease has also been documented in the hearts of chronic diabetics. This impairment could be attributed to abnormalities in the contractile and regulatory proteins. Troponin I (TpI) is one of the sarcomeric proteins involved in regulation of myocardial muscle contraction. Phosphorylation of cardiac (TpI) is associated with altered enzymatic activity and depressed myocardial contractility. This phosphorylation which is cAMP dependent is enhanced in diabetic patients and greatly affects myocardial performance (3).

It is becoming evident that diabetes results in increased expression of angiogenic growth factors in numerous tissues as a response to both hyperglycemia and tissue ischemia. The manifestations of diabetic cardiovascular complications also implicate the actions of these growth factors in their development (4).

Vascular endothelial growth factor (VEGF), also known as vasopermeability factor and vasculotropin, is a 45-kDa homodimeric glycoprotein with potent vascular permeability and angiogenic effects (5) . VEGF is primarily mitogenic for endothelial cells and its expression was initially found to be markedly increased in rapidly growing, highly vascularized tumours (6). VEGF is crucially involved in the pathogenesis of a number of different angiogenic diseases, including psoriasis, rheumatoid arthritis and diabetic vasculopathy (7,8). In patients with diabetes and coronary or peripheral vascular disease VEGF may induce development of cardiac and limb vascular collateralization, respectively (9).

Plasma levels of transforming growth factor beta (TGF - β) are elevated in type 2 diabetes and may contribute to the occurrence of diabetic micro- and macrovascular complications (10). TGF - β is a potent inducer of the extracellular matrix expansion and cardiac fibrosis (11,12). Moreover, TGF - β is thought to play a role in atherosclerotic heart disease as well as in development of idiopathic cardiomyopathy (13,14).

Tumor necrosis factor alpha (TNF - α) is a proinflammatory cytokine that seems to play a crucial role in the pathogenesis of atherosclerosis and coronary heart disease (15). High glucose concentrations can increase the stimulated TNF - α production capacity, with possible important consequences for patients with diabetes mellitus (16). TNF - α also, through its interaction with the insulin signaling cascade, stimulation of lipolysis, and negative regulation of peroxisome proliferator - activated receptor gamma (PPAR gamma), an important insulin - sensitizing nuclear receptor, plays a role in the pathogenesis of obesity - linked insulin resistance and type 2 diabetes (17,18,19).

The present study aims to evaluate the interplay between factors suggested to be associated with ischemic myocardial injury and the angiogenic effect of VEGF in diabetic patients with history of ischemic heart diseases and myocardial infarction to evaluate their possible usefulness as predictors of vascular myocardial occlusion.

SUBJECTS AND METHODS

Forty subjects (age: 40 - 60 years) were included in the study and were divided into 3 groups: 2 groups of type 2 diabetes with 10 - 15 years history of the disease (group II and III) and a group of healthy controls (group I).

Group I: included 12 (5 males and 7 females) normoglycemic non - diabetic subjects without any manifestations of cardiac disorders.

Group II: included 13 diabetic patients (6 males and 7 females) attending the Outpatient Diabetic Clinic. All these subjects showed history of ischemic heart disease, exercise - induced angina and/or ischemic electro-cardiographic (ECG) changes.

Group III: included 15 diabetic patient (6 males and 9 females) admitted to the intensive care unit for suffering from acute myocardial infarction within the first 24 hours of the condition.

All members of the study were subjected to thorough clinical and ECG examinations and to the following biochemical evaluations:

- Colorimetric determination of plasma glucose (20) and serum enzyme activity of CK(21), CK - MB (22), AST (23) and LDH (24).
- Serum level of Troponin I using the chemiluminescent immunoassay system (Immulite) using kits supplied by DPC (Diagnostic Products Corporation), Los Angelos, USA (25).
- Serum level of VEGF (26), TGF - β (27) and TNF - α (28) using quantitative sandwich immunoassay technique using kits supplied by R and D systems Inc, USA.

Serum Collection: Using a serum separator tube, serum was allowed to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000 x g. Serum was removed and stored at - 20° C.

STATISTICAL ANALYSIS

Results are expressed as means • standard deviations (SD). Qualitative data were compared by Fisher's exact test. Comparison of means of groups was performed by one - way analysis of variance (ANOVA) with post - hoc testing. Pearson's correlation coefficient (r) was performed to assess the degree of association between the different variables(29).

RESULTS

Results of the study are shown in tables 1 and 2 and figures (1 - 3)

Table 1
Fasting plasma glucose and serum levels of CK, CK-MB, AST, LDH and Troponin I in the different groups

	Group I (Controls)	Group II (Ischemia)	Group III (Infarction)
F. glucose (mg/dL)	81.9 ± 5.1	185.2 ± 19.9 a	242.0 ± 112.1 ab
CK (U / L)	73.3 ± 5.6	84.3 ± 8.8	1120 ± 490 ab
CK - MB (U / L)	2.9 ± 1.1	3.3 ± 1.3	44.2 ± 37.1 ab
AST (U / L)	22.2 ± 2.5	27.7 ± 2.3	174.6 ± 97.7 ab
LDH (U / L)	132.2 ± 15.1	139.7 ± 12.8	425.3 ± 117.0 ab
Troponin I(ng/mL)	undetectable	0.16 ± 0.2	59.3 ± 49.7 ab

Values are expressed as mean ± S.D.
(a) denotes that the difference between the mean of the group and the corresponding mean of the control group is statistically significant ($p < 0.05$)
(b) denotes that the difference between the corresponding means of the two diseased groups is statistically significant ($p < 0.05$)

Table 2
erum levels of VEGF , TGF - β and TNF - α in the different groups

	Group I (Controls)	Group II (Ischemia)	Group III (Infarction)
VEGF (pg/mL)	255.7 ± 94.4	465.7 ± 162.2 ab	355.2 ± 430.1
TGF β (pg/mL)	987.5 ± 355.9	1474.6 ± 473.3 ab	1306.6 ± 585.7
TNF α (pg/mL)	12.6 ± 3.5	28.9 ± 12.1 ab	17.0 ± 6.6

Values are expressed as mean ± S.D.
(a) denotes that the difference between the mean of the group and the corresponding mean of the control group is statistically significant ($p < 0.05$)
(b) denotes that the difference between the corresponding means of the two diseased groups is statistically significant ($p < 0.05$)

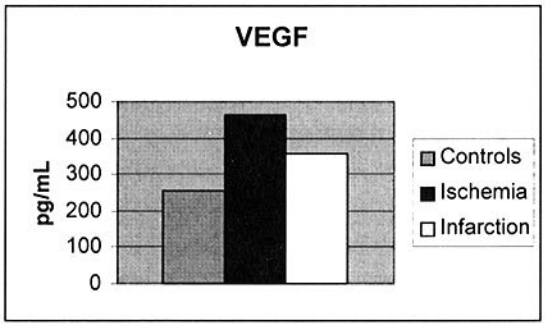

Figure 1
Serum VEGF (pg / mL) in controls, ischemia and infarction groups

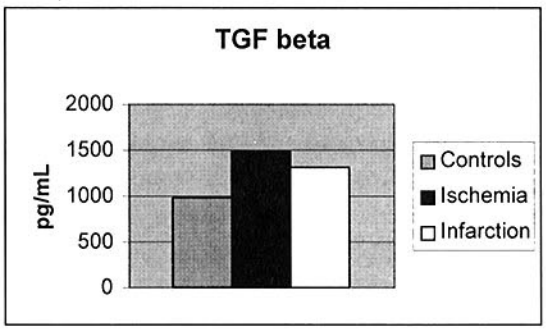

Figure 2
Serum TGF - β (pg / mL) in controls, ischemia and infarction groups

Table 1 shows a significant increase in enzymatic activity of CK, CK-MB, AST, and LDH in the infarction group compared to both control and ischemic groups; there was no significant difference between the latter two groups regarding the same parameters. Troponin I was elevated in all patients with infarction (100 %) and detectable in 7 patients with ischemia (54 %) and undetectble in all controls. A significant positive correlation was present between troponin I and each of CK, CK - MB, AST and LDH ($r = 0.78, 0.90, 0.83$ and 0.69 respectively.)

In table 2 and figures 1 - 3 show a significant elevation in serum VEGF, TGF - β and TNF - α between the ischemia group and each of the infarction group and the control group. Levels of the previous parameters were higher in the infarction group compared to the control; however the

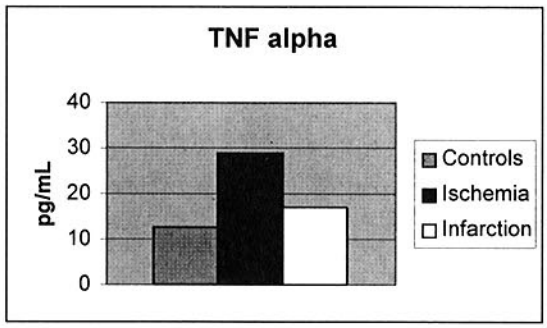

Figure 3
Serum TNF - α (pg / mL) in controls, ischemia and infarction groups

difference was insignificant.

DISCUSSION

Over the past several years, dramatic progress has been made in our understanding of the mechanisms and molecules involved in the pathogenesis of the diabetic cardiovascular disease.

In the present study, serum TpI was of detectable value in all patients with myocardial infarction and in 54 % of the patients with ischemic heart most probably due to its release from the injured myocardium. This finding denotes that, TpI is an extremely sensitive marker that could be of value in diagnosis of ischemic heart even without the presence of any changes in cardiac enzymes routinely used in diagnosis of infarction. These results are in accordance with La Vecchia et al.,(30) who proved that cardiac TpI represents a highly sensitive marker for myocardial cell death. It was detected in the blood of 25 - 33 % of the studied patients with severe heart failure and its presence may help to identify high risk subgroup who faces very poor short term prognosis and was the most powerful predictor of mortality at 3 months duration. Lucia et al., (31) reported that cardiac TpI serum level is not only a strong index of myocardial necrosis but also of ongoing myocyte injury and hemodynamic impairment predictive of poor outcome. They reported that cardiac TpI serum level is increased within the first 6 hours, remaining above normal until the seventh day. They also reported that TpI level is higher in unstable than in stable anginous patients and normal subjects but not in stable angina with respect to healthy controls. Lee et al., (32) reported that serum cardiac troponins were found to increase to some extent in one third of stable angina patients who have acute ischemia and thus could be used as a predictor for vascular occlusion in ischemic patients. Del-Carlo and O'Connor (33) also found detectable levels of cardiac troponins in patients with advanced congestive heart failure; levels which correlated with the severity and prognosis of the condition. They reported that possible mechanisms that release cardiac troponins in congestive heart failure include: ventricular remodeling, presence of coronary disease, abnormalities of coronary microcirculation, and reduced coronary reserve.

In the present study VEGF is elevated in patients with myocardial ischemia and infarction compared to controls, however the difference is significant only with the ischemia group.

VEGF is induced by hypoxia as well as by growth factors such as angiotensin II and platelet - derived growth factor in vascular smooth muscle cells (34). Besides its angiogenic properties, VEGF is also a potent vascular permeability factor and stimulates monocyte migration through endothelial cells(35). These events are key steps in the initiation and progression of atherosclerosis mediated by the concerted action of multiple growth factor cytokines and lipids(4).

When the myocardium is deprived of blood, a process of ischemia, infarction and myocardial remodeling is initiated. The formation of Coronary collateral vessels reduces the degree of myocardial ischemia and functional deficit following acute coronary occlusive episodes and a correlation exists between collateral coronary blood flow and myocardial viability in patients with recent myocardial infarction (36,37).

Several observations suggest that VEGF may play a significant role in this adaptive process. In vivo oxidative stress caused by systemic inhalational hypoxemic hypoxia increases cardiac VEGF protein expression and may trigger myocardial angiogenesis (38). VEGF mRNA also may be overexpressed in peripheral blood mononuclear cells in response to cardiac muscle damage in acute myocardial infarction and in cultured rat cardiac cells in response to hypoxia (39,40).

Serum VEGF levels reflect myocardial ischemia; the levels were elevated in patients with myocardial infarction and raised gradually to peak between 7 - 14 days (41,42,43). The latter 2 authors reported that serum VEGF levels were higher in patients with acute myocardial infarction than in patients with stable angina. Soeki et al.,(44) reported that VEGF levels in patients with preinfarction angina were higher than in patiens with no preinfarction angina. However, in accordance with our results Tamura et al., (45) reported that VEGF serum levels were similar to the baseline levels on day 0 (day of admission to the hospital) in acute myocardial infarction patients, and showed a remarkable increase by days 7 and 14 according to its role in the reconstructing process of infarcted myocardial tissue.

In the present study TGF - β was elevated in the two patients group but the elevation was significant in the group with myocardial ischemia. The elevated TGF - β in ischemic patients plays a role in induction of myocardial fibrosis by increasing matrix protein expression.

In accordance with the present results, Blann et al., (46) reported increased serum levels of TGF - β receptors in serum of patients with atherosclerosis. Wang et al., (47) reported an increase in active TGF - β with both the occurrence and severity of coronary heart disease which is independent of standard risk factors. Mazzone et al., (48) found elevated TGF - β in patients with silent ischemia. On the contrary Grainger et al., (49) reported that patients with advanced atherosclerosis have less active TGF - β in their sera than subjects with normal coronary arteries. Tashiro et al., (50) reported that patients with ischemic heart disease had significantly lower TGF - β than controls; and concluded that the process of atherosclerosis is inhibited by TGF - β. Erren et al., (51) reported that patients with coronary heart disease showed lower levels of active TGF - β than in controls. A negative correlation was found between TGF - β and the extent of coronary atherosclerosis. Sun et al., (52) reported increased TGF - β 1 and TGF - β 1 mRNA at the site of myocardial infarction. Extensive myocardial remodeling occurs after transmural myocardial infarction. The infarcted myocardium is being replaced by scar tissue after gradual resor-

ption of the necrotic tissue. The scar tissue is an ongoing dynamic process in which TGF - β seems to play an active role in the complex remodeling (53). Also, substantial evidence implicates TGF - β in the pathogenesis of restenosis. Adenovirus mediated antagonism of TGF - β at the site of coronary angioplasty proved to reduce luminal loss and stimulates the formation of dense collagenous adventitia which prevents constrictive remodeling by acting as an external scaffold (54).

TNF - α has been implicated in the pathogenesis of heart failure and ischemia - reperfusion injury. The cytokine influences the transendothelial migration of monocytes by altering the barrier function of endothelial cells. Furthermore, it elicits the expression of proteolytic enzymes by macrophages and smooth muscle cells within the atherosclerotic plaque (55).

TNF - α mean values are elevated in the two diseased groups of the present study, but this elevation is significant only in ischemic group compared to normal controls.

Pudil et al., (56) reported that the plasma TNF - a level was elevated throughout the time of observation (96 hours) in myocardial infarction without any significant peak. Halawa et al., (57) showed increased plasma levels of TNF - α in patients with acute myocardial infarction with maximum in the third day of infarction. There is a correlation between infarct size and concentrations of TNF - α The myocardial necrosis induces complement activation and free radical generation, triggering a cytokine cascade initiated by TNF - α release (58). Mizia-Stec et al., (59) reported that serum concentrations of TNF - a were increased in patients with stable and unstable angina. These increased concentrations do not reflect the clinical state of patients. Ridker et al.. (60) reported that levels of TNF - α increase with acute ischemia. Plasma concentrations of TNF - α are persistently elevated among post - myocardial infarction patients at increased risk for recurrent coronary events. These data support the hypothesis that a persistent inflammatory instability is present among stable patients at increased vascular risk. Balbay et al., (61) reported that TNF - α levels were comparable in acute infarction and stable angina but higher than the levels of healthy controls. Stamm et al., (62) reported that TNF - α is expressed in myocardium in experimental animals during compensated pressure overload hypertrophy and contributes to post ischemic myocardial dysfunction. Furthermore, the authors proved that inhibition of TNF - α signaling significantly improves post ischemic contractile function, myocardial energetics and intracellular calcium handling. Shames et al.,(63) reported that over-production of TNF - α following myocardial ischemia - reperfusion contributes to cardiac dysfunction, and anti-TNF-alpha has therapeutic potential for myocardial protection in cardiac surgery with obligatory ischemia. They concluded that ischemia alone induces TNF - α gene expression and peptide synthesis in the myocardium that are associated with NF-kappaB activation. Non-myocytes constitute the main source of myocardial TNF - α following ischemia.

Thus, it could be concluded that serum troponin I seems to be an extremely sensitive marker of myocardial infarction. On the contrary to this elevation in serum Troponin I level, the change in serum VEGF, TGF - β and TNF - α seem to be insignificant in the very early stage of myocardial infarction which indicate relatively slow induction of the growth factors in response to ischemia and hypoxia.VEGF could also be responsible for the compensatory stage of ischemic patients most probably due to its angiogenic effect and collateral formation which play a role in the protection from vascular occlusion. On the other hand the elevated levels of TNF - α and TGF - β could play a role in the pathogenesis of the ischemic changes.

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