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Assessment of Inflammatory and Cardiovascular Markers in Type 2 Diabetes without Clinical Evidence of Cardiac Events

Kardiyovasküler Hastalık Belirtileri Olmayan Tip 2 Diyabetik Hastalarda İnflamatuvar ve Kardiyovasküler Belirteçlerin Değerlendirilmesi

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Abstract

Purpose: There is no doubt that several inflammatory markers were detected in type 2 diabetes (T2D). Cardiovascular events were also associated with T2D or complicated T2D. Co-existence of cardiovascular and inflammatory biomarkers in T2D patients in the absence of cardiovascular morbidity have been mentioned in a few articles. This study aimed to assess the association of C-reactive protein as inflammatory marker with serum NT-proBNP (a diagnostic marker of heart failure) with nitric oxide (a marker of vascular endothelial function) in T2D patients without clinical evidence of heart failure.

Material and Method: A total of 75 T2D patients recruited from the Center of Diabetes and 25 healthy subjects served as controls were enrolled in the study. Patients without clinical evidence of heart failure or recent infection were included in the study. Serum C- reactive protein, NT-proBNP, and nitric oxide were determined.

Results: High serum NT-proBNP levels (≥600 pg/ml) indicating the presence of moderate to severe heart failure was detected in 10.7% of subjects. Serum nitric oxide levels were significantly lower (60.98±30.75 µmol) compared with those in healthy subjects (120.3±12.5 µmol). Serum nitric oxide significantly and inversely correlated with serum NT-proBNP level ($r=-0.228$, $p<0.05$). Seven out of 14 patients with positive C-reactive protein had significantly high serum NT-proBNP level (≥600 pg/ml). Patients with positive C-reactive protein significantly have low serum nitric oxide level compared with those expressed negative C-reactive protein reaction.

Discussion: Biomarkers of cardiovascular events in T2D patients without clinical evidence of heart failure are detected in the presence of inflammatory process. *Turk Jem 2014; 18: 75-78*

Key words: Type 2 diabetes, NT-pro-brain natriuretic peptide, c-reactive protein, nitric oxide
Conflict of interest: The authors reported no conflict of interest related to this article.

Özet

Amaç: Tip 2 diyabetik hastalarda birçok inflamatuvar belirteç yüksek saptanmaktadır. Kardiyovasküler olaylar da tip 2 diyabet veya komplike tip 2 diyabet ile ilişkilendirilmektedir. Kardiyovasküler morbiditesi olmayan tip 2 diyabetiklerde kardiyovasküler ve inflamatuvar belirteçler az sayıda çalışmada değerlendirilmiştir. Bu çalışmada kalp yetmezliği olmayan tip 2 diyabetik hastalarda C-reaktif protein (CRP) (inflamatuvar belirteç olarak), NT-proBNP (kalp yetmezliği göstergesi olarak) ve nitrik oksit (NO) (vasküler endotel fonksiyou göstergesi olarak) değerlendirilmiştir.

Gereç ve Yöntem: Diyabet merkezinde 75 hasta ve 25 sağlıklı kontrol çalışmaya dahil edildi. Enfeksiyon ve kalp yetmezliği tablosu olmayan hastalarda CRP, NT-proBNP, nitrik oksit değerlendirildi.

Bulgular: Katılıcıların %10,7 sinde orta veya ağır kalp yetmezliği göstergesi olan yüksek NT-proBNP (≥600 pg/ml) saptandı. Serum NO düzeyleri sağlıklı kontrollere göre anlamlı olarak düşük saptandı (120,3±12,5 µmol vs. 60,98±30,75 µmol). Serum NO düzeyleri ile NT-proBNP düzeyleri arasında anlamlı ilişki saptandı ($r=-0,228$, $p<0,05$). CRP yüksekliği olan 14 hastanın 7 sinde NT-proBNP anlamlı yüksek saptandı (≥600 pg/ml). Pozitif CRP izlenen hastalarda NO anlamlı olarak düşük saptandı.

Tartışma: Klinik olarak kalp yetmezliği olmayan hastalarda inflamatuvar durum ile birlikte kardiyovasküler belirteçlerde bozulma izlenmiştir. *Turk Jem 2014; 18: 75-78*

Anahtar kelimeler: Tip 2 Diyabet, NT-pro-brain natriuretik peptid, c-reaktif protein, nitrik oksit
Çıkar çatışması: Yazarlar bu makale ile ilgili olarak herhangi bir çıkar çatışması bildirmemişlerdir.

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Introduction

Endothelial dysfunction is one of the precursor key steps in the development of atherosclerosis in diabetic subjects. Decreased nitric oxide (NO) production, increased oxidative stress and impaired function of endothelial progenitor cells are the main mechanisms involved in the accelerated atherosclerotic process observed in patients with type 2 diabetes (T2D). Diabetes mellitus and heart failure are associated with diminished release of NO into the arterial wall because of impaired synthesis or excessive oxidative degradation (1).

Diabetic patients present more frequently with acute pulmonary edema than non-diabetics, have more often acute coronary syndrome as precipitating factors of acute heart failure, and multiple co-morbidities such as renal dysfunction, arterial hypertension, anemia and peripheral vascular disease (2).

High incidence of diastolic dysfunction in asymptomatic diabetics is correlated with the duration of diabetes, HbA1c levels, obesity indices and diabetic microangiopathies (3). Moreover, patients with diabetes compared with those without diabetes were significantly older and more often had other cardiovascular risk factors, signs of heart failure and high the Global Registry of Acute Coronary Events (GRACE) score at admission (4). More intensive glycemic control in patients with T2D did not reduce the occurrence of heart failure events (5). Current evidence indicates the presence of several biochemical and molecular changes associated with diabetes that lead to diastolic dysfunction or American Heart Association stage B heart failure (6). In experimental animal model the increased C-reactive protein expression exacerbated left ventricular dysfunction after myocardial infarction (7).

Elevated circulating N-terminal pro brain natriuretic peptide (NT-proBNP) in T2D is a strong predictor of the excess overall and cardiovascular mortality independent of conventional cardiovascular risk factors (8). NT-proBNP is of prognostic value that discriminates acute coronary syndrome patients at higher risk (9).

The aim of this study was to demonstrate the interrelation between serum NT-proBNP (a biomarker of heart failure), NO (a biomarker of endothelial dysfunction) and C-reactive protein (an inflammatory biomarker) in T2D without clinical evidence of heart failure.

Materials and Methods

This study was done in the Department of Pharmacology, College of Medicine, Al-Mustansiriya University in cooperation with the Layla Qasim Diabetic Center in Erbil- Kurdistan, and the Laboratories of Specialized Center for Cardiac Surgery, Ibn Al-Bitar Hospital in Baghdad. Patients with T2D referred to the Layla Qasim Diabetic Center in Erbil, Kurdistan-Iraq were enrolled in this study. The study approved by the scientific committee at Hawler Medical University, College of Pharmacy. The study approved by the scientific committee at Hawler Medical University, College of Pharmacy". The study was conducted according to the guidelines from the Declaration of Helsinki with approval from a local ethical review board. Written informed consent was obtained from each participant to donate a sample of venous blood for diagnostic biochemical tests. The criteria of inclusion was the presence of T2D. The criteria of exclusion included clinical evidences of heart failure and/or ischemic heart, and the presence of other diabetic complications.

A total of 75 (25 males and 50 females) participants fulfilled the inclusion criteria. Non diabetic subjects (12 males and 13 females) were also included in this study. The anthropometric measurements, including weigh, height and the calculated body mass index (weight/height2), fasting and HbA1c% were determined. Fasting venous blood samples were obtained from the participants and the sera were separated for determination of C-reactive protein, NT-proBNP and NO.

Serum proBNP levels were determined in the Laboratories of Specialized Center for Cardiac Surgery, İbn Al-Bitar Hospital using the technique of Enzyme Linked Fluorescent Assay (VIDAS NT-proBNP automated test for use on the VIDAS instrument). The principle of this assay is a one step immunoassay sandwich method with a final fluorescent detection (ELFA) and the range of measurement is 20-25000 pg/ml). Levels below 100 pg/ml indicate no heart failure, 100-300 pg/ml suggest the presence of heart failure, >300 indicate mild heart failure, >600 ml indicate moderate heart failure and >900 pg/ml indicate severe heart failure.

Serum C-reactive protein (LINEAR chemical SL Company, Barcelona, Spain) determined qualitatively using the cut-off level of ≥6 mg/L as a positive test indicating the presence of inflammation.

Serum NO was determined as described below (10): - briefly, 500 µl of serum was added to 50 µl HCl (6.5 M) and 50 µl sulfanilic acid (37.5 mM). After incubation for 10 minutes, 50 µl naphthylethylenediamine dihydrochloride (12.5 mM) was added and was incubated for a further 30 minutes and then centrifuged for 10 minutes at 1000 g. The absorbance at 540 nm was immediately recorded. The concentration of NO as nitrate/nitrite was calculated from the standard curve of lithium nitrate.

Statistical Analysis

The results are expressed as absolute number, percent, mean ± SD. The data analyzed using the student's t-test (one paired, two tailed), simple correlation test and odd ratio taking the $p \leq 0.05$ as lowest limit of significance.

Results

Table 1 shows the characteristics of the study. The mean age of the patients was 41 years and the majority of the patients had family history of diabetes. The median duration of diabetes was 8 years and all patients were poorly controlled by the evidence of HbA1c% which was more than 7% (Table 1). The majority of patients were overweighed and only 10.7% (8 out of 75) were obese class I (Table 1). Serum NT-proBNP levels were ranged from 10 to 8368 pg/ml and 8 (10.7%) patients had high serum level of NT-proBNP (≥600 pg/ml) indicating the presence of moderate to severe heart failure (Table 2). Figure 1 shows significantly low serum NO (60.98±30.75 µmol, $p<0.001$) levels in patients compared with healthy subjects (120.3±12.5 µmol). Serum NO significantly and inversely correlated with serum NT-proBNP levels in patients with T2D mellitus (Figure 2). Further analysis revealed that 14 out of 75 (18.7%) patients

had positive C-reactive protein, seven of them (50%) showed significant high serum level of NT-proBNP (≥600 pg/ml). Patients with positive C-reactive protein had significantly lower serum NO level compared with those expressed negative C-reactive protein reaction (Table 3). The other determinants are duration of disease, HbA1c%, fasting serum glucose did not show significant differences regarding the positive C-reactive test.

Discussion

This study shows that insidious heart failure is detected in T2D by the measurement of serum NT-proBNP levels. This biomarker inter-relates with C-reactive protein and nitrogen species indicating that these markers are involved in heart failure associated with T2D. In diabetes, coronary artery disease and cardiomyopathy are the most frequent and insidious complications (11). Diabetic cardiomyopathy is characterized by left ventricular diastolic dysfunction that can be detected in 52%-60% of well-controlled subjects. Hyperglycaemia induce myocardial inflammation and is associated with decreased NO levels as well as worsening in the endothelial function (12). The results of this study are in agreement with those of another study that showed increased serum NT-proBNP and C-reactive protein levels in idiopathic and dilated cardiomyopathy (13). Moreover, increased C-reactive protein and nitrogen species that demonstrated in congestive heart failure are due to dilated cardiomyopathy in dogs (14). Metabolic dysfunction occurring in diabetes explained the increased NT-proBNp level which indicated heart failure that resulted from diabetic cardiomyopathy. In diabetic heart, inhibition of glycolytic enzymes disturb the flux of substrate through the glycolytic pathway and leads to the diversion of glycolytic intermediate substrate through another pathway, which in turn mediates the onset of diabetic cardiomyopathy (15). Endothelial dysfunction represented by low NO and high peroxynitrite levels is demonstrated in this study. A recent study focused on the role of hyperglycemia in inducing oxidative stress and leading to changes in cellular signaling manifested by production of several

Table 1. Characteristics of the study

Number	75
Gender	
Male: Female	25:50
Age (Year)	41.0±4.23
Family history of diabetes	
1st degree relative	32
2nd degree relative	29
Body mass index (kg/m^2)	27.78±1.62
Under weight	0
Normal	2
Overweight	65
Obese class I	8
Obese class II	0
Obese class III	0
Duration of diabetes (year)	8.21±1.42
Fasting serum glucose (mg/dl)	211.2±26.04
HbAc1 (%)	8.65±0.72

Table 2. Distribution of cases according to their serum NT-proBNP level

Serum level of NT-proBNP (pg/ml)	Diabetic patients (n=75)	Healthy subjects (n=25)
<100 (no heart failure)	61 (81.33%)	19 (76%)
100-300 (suspected heart failure)	3 (4%)	4 (16%)
300-600 (mild heart failure)	3 (4%)	2 (8%)
600-900 (moderate heart failure)	1 (1.33%)	0
>900 (severe heart failure)	7 (9.33%)	0

Table 3. Interaction between vascular, cardiac and inflammatory markers in T2D patients

	Positive C-reactive protein (≥6 mg/L) (n=14)	Negative C-reactive protein (<6 mg/L) (n=61)
Duration of disease (year)	8.0±1.357	8.26±1.44
Fasting serum glucose (mg/dl)	208.1±22.07	221.9±27
HBA1c (%)	8.261±0.782	8.65±0.72
Serum nitric oxide (µmol)	44.11±19.2*	64.85±31.7
Serum NT-proBNP (pg/ml)		
<100	5	55
100-300	1	3
300-600	1	2
600-900	0	1
>900	7**	0

The results are expressed as mean ± SD. *p=0.003, **p=0.001 compared with negative C-reactive protein

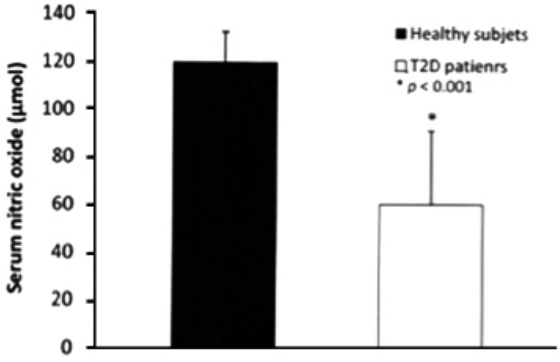

Figure 1. Significant changes in serum nitric oxide in T2D patients compared with healthy subjects

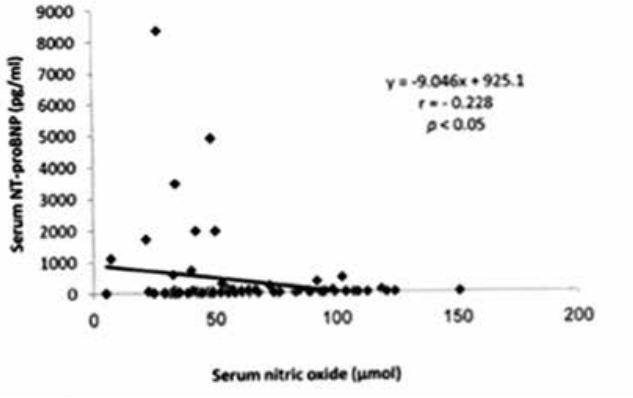

Figure 2. Relationship between serum nitric oxide and NT-proBNP in T2D patients

vasoactive and cardioactive factors. Therefore, dysfunction of both vascular endothelial cells and cardiomyocytes play a role in the pathogenesis of diabetic cardiomyopathy (16). Accordingly, the endothelial dysfunction occurring in T2D is associated with changes in vas-regulation (demonstrated by low serum NO), inflammatory activation (demonstrated by positive C-reactive protein test), and altered cardiac cell function (demonstrated by high serum NT-proBNP level) (17). It is concluded that increased serum NT-proBNP level in the absence of clinically diagnosed heart failure is observed in T2D and it may serve as a diagnostic biomarker of insidious heart failure. The inflammatory marker inter-relates with biomarkers of heart failure and vascular endothelial dysfunction.

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Conflicts of Interest

There are no conflicts of interest.

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