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Investigation of Serum Interleukin-1beta and Nitric Oxide Levels in Diabetes Mellitus

Diabetes Mellitusta Serum İnterlökin-1beta ve Nitrik Oksid Düzeylerinin İncelenmesi

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Abstract

The free radical nitric oxide (NO), generated by the inducible form of nitric oxide synthase (iNOS), is a potential mediator of cytokine-induced beta-cell dysfunction. Macrophage derived interleukin-1beta (IL-1β) is important in eliciting beta-cell dysfunction and initiating beta-cell damage in response to microenviromental changes within islets. Destruction of pancreatic islet cells seems to be responsible for increased production of NO from activated macrophages. In human islet cells IL-1β, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) are required for iNOS expression. We presently investigated the relationship between NO and diabetes mellitus by the determination of the serum levels of stable end-products of NO, in various clinical patterns of diabetes mellitus and compared them with serum IL-1β in 85 diabetic patients and 23 healthy controls. In diabetes, serum NO concentration was statistically significantly higher as compared with healthy controls ($p< 0.05$). Between the serum levels of NO in types of diabetes and healthy controls, it was statistically significantly increased ($p< 0.01$ for IDDM; and $p< 0.05$ for NIDDM). In diabetic patients with nephropathy and retinopathy and without complications, the NO levels were found to be significantly higher when compared with healthy controls ($p< 0.05$, $p< 0.01$, $p<0.01$), respectively. We couldn't find any differences in serum levels of serum NO between the types of diabetes and in diabetic patients with complications. Serum IL-1β concentration was not detectable in 92 % of diabetic patients and in all of the healthy controls. Taken together, although NO and IL-1β work synergitically, it seems that it is hard to evaluate their role in diabetic patients by determining serum levels only. But, also in diabetic patients serum NO concentrations may be indicative of the pathogenesis of diabetes mellitus, which is not the case for IL-1β, as it is inefficiently detectable.

Key words: Cytokine, interleukins, diabetes mellitus

Özet

Bir serbest radikal olan nitrik oksid (NO), nitrik oksid sentaz enziminin indükleyici formu (iNOS) yoluyla üretilmekte olup, sitokin ile indüklenen, beta hücrelerinin fonksiyon bozukluğunda potansiyel aracı bir moleküldür. Aktive olmuş makrofajlardan türeyen interlökin-1beta (IL-1β), beta hücrelerinin fonksiyon bozukluğunun ortaya çıkmasında ve adacıkta, mikroçevrenin değişmesine yanıt olarak beta hücrelerinde hasarın başlamasında önemlidir. Pankreasta, adacık hücrelerinin yıkılımında aktive olmuş makrofajlardan türeyen NO sorumlu gözükmektedir. İnsan adacık hücrelerinde, IL-1β, tümör nekroz faktörü-alfa (TNF-α) ve interferon-gama (IFN-γ), iNOS ekspresyonu için gereklidir. Biz bu çalışmada, NO'nun stabil son ürünleri yoluyla serum düzeylerini diabetes mellitusun değişik klinik tablolarında saptayarak, serum IL-1β ile ilişkisini 85 diabetik hastada ve 23 sağlıklı kontrolde inceledik. Diabette, serum NO konsantrasyonları sağlıklı gruba göre istatistiksel düzeyde anlamlı olarak yüksek bulundu ($p<0.05$). Diabetin tipleri ile sağlıklı kontrol grubundaki serum NO düzeyleri karşılaştırıldığında, IDDM ve NIDDM'ta serum NO düzeyleri sağlıklı kontrollere göre daha yüksek ve istatistiksel olarak anlamlı bulunmuştur (sırasıyla; $p<0.01$ ve NIDDM için $p<0.05$). Nefropati, retinopati ve herhangi bir komplikasyonu olmayan diabetik hastalarda serum NO konsantrasyonları kontrollere göre önemli ölçüde yüksek ve istatistiksel olarak anlamlı bu-

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lundu (sırasıyla; $p< 0.05$, $p< 0.01$, $p< 0.01$). Diabetin tipleri ve komplikasyonlu hastaların serum NO düzeyleri arasında anlamlı bir fark gözlenmemiştir. Serum IL-1β konsantrasyonu diabetli hastaların % 92'sinde ve sağlıklı kontrol grubunda saptanamamıştır. Birlikte ele alındığında, NO ve IL-1β sinerjik olarak etki gösterse de, diabetik hastalarda sadece serum düzeylerini saptayarak rollerini değerlendirmek zor gibi görünmektedir. Fakat, aynı zamanda, diabetik hastalarda serum NO konsantrasyonları, yeterli olarak ölçülemeyen IL-1β'dan farklı olarak diabetin patogenezinde bir gösterge olarak yararlı olabilir.

Anahtar kelimeler: Sitokin, interlökinler, diabetes mellitus.

INTRODUCTION

Type 1, non-insulin dependent diabetes mellitus (IDDM) is a disease resulting from selective destruction of insulin-producing beta cells. Their secretion of different proinflammatory cytokines such as interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ), effects beta-cell fuction; and especially, interleukin-1beta (IL-1β) could play an important role immune-induced beta-cell damage (1-13). Type 1 cytokines initiate a cascade of immune/inflammatory processes in the islets (insulitis), culminating in beta-cell destruction. Type 1 cytokines activate cytotoxic T cells that interact specifically with beta-cells and destroy them. After that, macrophages produce pro-inflammatory cytokines (IL-1β, TNF-α, and IFN-γ) and oxygen and nitrogen free radicals that are highly toxic to islet beta-cells. Furthermore, the cytokines IL-1β, TNF-α, and IFN-γ, are cytotoxic for beta-cells, in large part by inducing the formation of oxygen free radicals, NO, and peroxynitrite in the beta-cells themselves (10).

Several of the deleterious effects of cytokines on rodent islets are mediated by the NO produced via the inducible form of nitric oxide synthase (iNOS). On the other hand, in vivo studies have given conflicting results; some studies suggested that NO synthase inhibitors do not supress streptozotocin-induced diabetes in mice, while others revelead that NO synthase inhibitors can reduce the incidence of insulin-dependent diabetes in rats. It was found that in vivo nitroprusside, a nitric oxide donor, also showed significant inhibitory effect on the severity of diabetes induced by alloxan. Alloxan treatment reduced NO generation, whereas L-arginine and sodium nitroprusside, when given along with alloxan, enhanced NO production in controls (1,14).

Correlation studies between cytokines expressed in islets and autoimmune diabetic, non-obese diabetic (NOD) mice and rats have demonstrated that beta-cell destructive insulitis is associated with increased expression of proinflammatory cytokines (IL-1β, TNF-α, and IFN-γ) and these may be directly cytotoxic to beta-cells by inducing NO and oxygen free radicals in the beta-cells. It was suggested that systemic administrations of a wide variety of cytokines can prevent IDDM development in NOD mice; however, a given cytokine may retard or accelerate IDDM development, depending on the dose and frequency of administration (10).

It was reported that culture of rat pancreatic islets with IL-1β results in the up-regulation of the iNOS and overproduction of NO. This is associated with reversible inhibition of both glucose-induced insulin secretion and islet glucose oxidation, and these effects are prevented by the iNOS inhibitor NG-monomethylarginine. IL-1β induced supression of islet glucose utilization is associated with a decline in islet glucokinase mRNA content, and in glucokinase protein synthesis, and all of these effects are prevented by NG-monomethylarginine. These findings suggest that IL-1β can downregulate islet glucokinase, which is the glucose-sensor apparatus, by an NO-dependent mechanism. Because reduction in islet glucokinase levels are known to cause a form of Type 2 diabetes mellitus, these observations raise the possibility that factors which increase islet NO levels might contribute to the development of glucose intolerance (12). In this study, we planned to measure serum NO and IL-1β levels in diabetic patients to show if there is any correlation in serum between the two parameters.

PATIENTS AND METHODS

Our series consisted of total 85 patients (mean age 55.4 ± 10.8 years); 54 female (mean age 55.4 ± 10.5 years) and 31 male (mean age 55.5 ± 11.2 years). The mean disease duration was 9 ± 7 years. These patients were subdivided into: a) 11 patients suffering from Type 1 (IDDM); b) 74 patients having Type 2 (NIDDM) diabetes mellitus. In addition, they werecategorized according to diabetic symptoms: a) 26 patients with nephropathy; b) 31 patients with retinopathy; and c) 28 of them without these complications. All patients were examined in good glycometabolic control reached by oral hypoglycemiant

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(71 cases) or insulin (14 cases) treatment. Thirtynine showed hypertension (arterial blood pressure over 160/95 mmHg, disease duration 7.8 ± 6.1 years). Twentythree individuals (16 female, 7 male subjects; mean age 31.1 ± 9.5) without internistic diseases were formed the normal control group.

After withdrawing the blood samples as soon as possible, they were centrifuged at 4000 rpm for 10 minutes at 4°C. Then serum samples were divided into aliquots and stored at -85 °C for IL-1β and insulin measurements. IL-1β and insulin measurements were performed each week on the collected samples. For NO measurements, after separation the serum samples were deproteinized as soon as possible using 3 % ZnSO4 (1:1). After deproteinization, the samples were centrifuged at 15000 rpm for half an hour. Then the supernatants were stored at -85 °C for determining NO concentrations. NO concentrations in all the samples were determined in two weeks.

Cytokine and insulin concentrations were done using the commercial BIODPC (Products Corporation, Los Angeles, CA, USA) kit (cat. no: LKL11 for IL-1β and cat no: LKIN1 for insulin) IMMULATE hormone autoanalyzer via the chemiluminescent enzyme immunometric method. The reference range for IL-1β was <5.0 pg/ml for healthy controls. So, IL-1β were not detectable in healthy groups in the given procedure. The reference range for insulin was 6-27 μIU/ml in the procedure. Serum glucose levels were determined using IL-900 autoanalyzer, via spectrophotometric colorimetric method. The normal range was given as 70-110 mg/dl in the procedure.

Serum NO was determined using the commercial kit of Boehringer Mannheim & Roche (Germany) (cat. no: 1 756 281), in which NO is detected via nitrite. The nitrate present in the sample is reduced to nitrite by reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of the enzyme nitrate reductase (NR). The nitrite formed reacts with sulfanilamide and N-(1-napthyl)-ethylenediamine dihydrochloride to give a red-violet diazo dye. The diazo dye is measured on the basis of its absorbance in the visible range at 550 nm on a microtiter plate. The absorbance was read using a microplate reader. It was also noted that serum samples must be deproteinized when using Griess reagent because the method interferes with serum proteins. So, even if the commercial kit was used, wed eproteinized the samples before NO determination. Serum NO concentrations were given as μM/L.

Nonparametric tests were used to analyze serum variables. Comparison of the data between different groups were performed using Students'-t test and Mann-Whitney U test. The correlations between the variables in serum were determined via Spearman rank correlation coefficient test. SPSS program was used for statistical analysis.

RESULTS

In diabetic and healthy controls, serum NO levels were found to be (15.28 ± 5.83 μM/L) and (7.64 ± 1.53 μM/L), respectively. In diabetes, serum NO level was statistically significantly higher than healthy controls ($p=0.01$; $p<0.05$). In IDDM and NIDDM, serum NO levels were found as (15.2 ± 3.0 μM/L) and (15.37 ± 11.25 μM/L), respectively. No differences were found within the types of diabetes. In Type 1 and Type 2 diabetes, serum NO levels were statistically significantly higher than healthy controls. (for IDDM $p=0.0022$; $p<0.01$ and for NIDDM, $p=0.01$; $p<0.05$) (Figure 1).

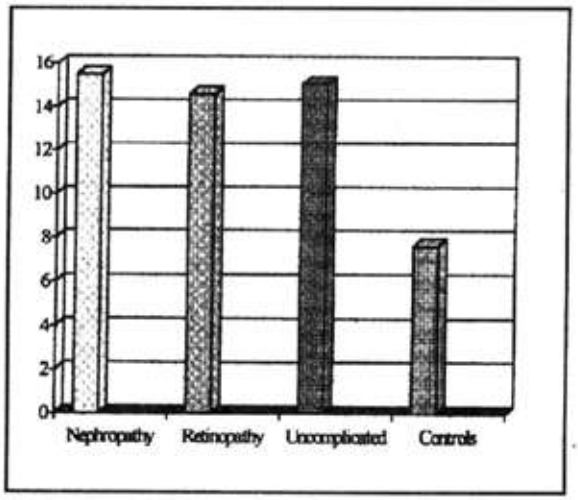

Figure 1. Comparison of serum NO (μM/L) levels in different types of diabetes mellitus

In the diabetic patients with nephropathy and retinopathy and in the diabetic patients without complications, serum NO levels were 15.49 ± 16.64 μM/L, 14.62 ± 7.25 μM/L, and 15.08 ± 10.12 μM/L, respectively. The differences comparison of the three groups with healthy controls, showed statistically significantly higher differences ($p=0.041$; $p< 0.05$ for nephropathy, $p=0.001$; $p< 0.01$ for retinopathy, and $p=0.0066$; $p<0.01$ for without complications) (Figure 2). There was no difference in the serum NO concentrations between the groups.

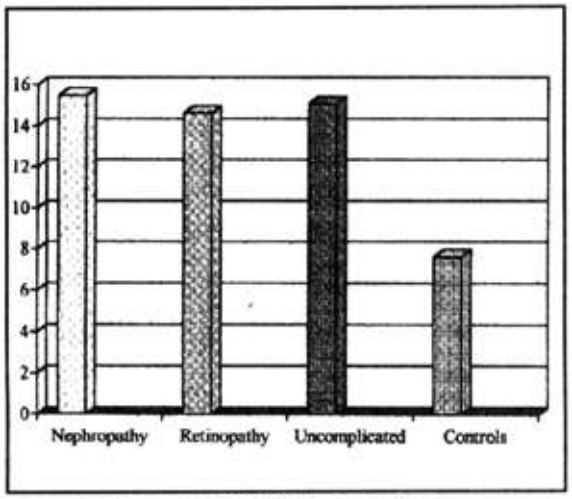

Figure 2. Serum NO (μM/L) levels in different types of diabetic complications.

Meanwhile, we determined serum IL-1β concentrations in diabetic patients. Serum IL-1β was not detectable in 92 % of patients and all of the healthy controls. We determined IL-1β in only seven of diabetic patients; 3 had retinopathy, 2 had nephropathy, and 2 of them had no complications. The mean value of IL-1β was (17.02 ± 5.06 pg/ml).

Table 1. The correlations of between serum NO and glucose and insulin levels in diabetic patients.

Parameters	Mean±SD	r	p
NO (μM/L)	14.69±10.53		
Glucose (mg/dl)	166.37±8.94	0.0466	0.745
Insulin (μIU/ml)	14.43±11.94	0.0271	0.860

In diabetic groups, the mean value for fasting glucose was 166.37 ± 8.94 mg/dl. There was no correlation between serum GLU and NO levels (r=0.0466; p=0.745). Also, insulin level was found to be 14.43 ± 11.94 μIU/ml in diabetic patients and there was no correlation between serum NO levels and insulin concentrations (r=-0.0271; p=0.860) (Table 1).

DISCUSSION

There has been various studies related with NO levels in diabetic mice or rats. However only a few articles touch upon the serum or plasma levels in diabetic individuals. Also, there hasn't been much research done in diabetic patients on the possible correlation of NO with cytokine (such as IL-1β, IL-6, TNF-α) levels in serum. Recently, Tessier et al (13), found that monocytes from Type 2 diabetic subjects produced larger amounts of NO in vitro. After oral 100g-glucose loading, NO concentrations increased according to baseline level. Zumsteng et al (2), reported that a combination of cytokines (IL-1β, TNF-α, and IFN-γ) inhibits insulin release, stimulates iNOS and the lack of NO production partially protected islets from cytokine-induced apoptosis. In addition to this, Ferlito et al (15), determined plasma NO in levels IDDM and NIDDM patients. They found similar NO concentrations between the IDDM and NIDDM, and healthy controls. There is considerable controversy on; a) whether human and rat islets respond differently to cytokines, b) the extent to which cytokine damage is mediated by induction of NO formation, and c) whether the effects of NO on islets can be distinguished from those reactive oxygen species or peroxynitrite.

Recently, Mohan et al (14), found that in vivo sodium nitroprusside, a NO donor, also showed significant inhibitory effect on the severity of diabetes induced by alloxan. Alloxan treatment reduced NO generation, whereas L-arginine and sodium nitroprusside, when given along with alloxan, enhanced NO production to control values in male Wistar rats. Induction of diabetes by alloxan in the experimental animals was associated with a marked elevation in plasma lactate, ketone body, and lipid peroxide levels with a simultaneous fall in plasma insulin and NO levels. Upon these results, they suggested that it was apparent that L-arginine and NO can prevent alloxan-induced beta-cell damage, and the development of diabetes, and restore the antioxidant status to near normal levels. Stevensen et al (16), reported that insulin can down-regulate the iNOS pathway in vivo. The iNOS pathway is up-regulated in diabetes prone rats and mice and is associated with an autoimmune process. However, the results demonstrated that macrophage NO production and iNOS mRNA expression are also elevated in rats or mice made diabetic by streptozotocin injection in which there is no primary autoimmune component. Insulin administration reduces NO production in auto-immune prone and streptozotocin-induced diabetic rodents and in-

sulin decreases macrophage NO production in normal host. Upon this evidence, they suggested that the autoimmune paradigm is inadequate to explain increased NO in diabetes.

Comparing our findings with the above reports, we can say that; a) In most of the experimental studies done on pancreatic cells or tissues or in blood, analytes have interactions with the systemic enviroment, and therefore it is hard to compare the data obtained in the experimental and blood research, b) our findings were closely related to those of Tessier and his co-workers. Like us, they worked in vitro and found that monocytes from Type 2 diabetic subjects produced larger amounts of NO. In addition, after the oral glucose loading, found increased NO for the diabetic subjects, c) the measurement of serum IL-1β gives less information, because it was detectable in 8 % of diabetic patients and not detectable at all in healthy groups. It is reported that, IL-1β reached all the investigated organs in the rats, was accumulated in kidneys and excreted in the urine. Possibly, it is also accumulated in the islets of Langerhans and responsible cytokine mediated beta-cell dysfunction, and d) the lack of correlation between glycemia and NO seems to indicate that the chronic exposure to high glucose levels is more relevant than the acute effects of hyperglycemia. In addition, it must be underlined that high glycaemic levels might exert a direct action on NO produced by cells.

In conclusion, it can be stated that serum NO levels are higher in diabetic patients with nephropathy or retinopathy and without any complications, as compared with controls. It can be inferred that NO plays an important role in the pathogenesis of diabetes mellitus in humans. To determine the mechanisms of NO action in diabetes mellitus, more intensive research is needed.

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