Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2010; 35 (2) ; 145–150.

Research Article [Araştırma Makalesi]

Yayın tarihi 15 Haziran, 2010 © TurkJBiochem.com

[Published online 15 June, 2010]

Investigation of Serum Crosslinked N-telopeptides of Type I Collagen (NTx) Levels and Total Antioxidant Capacity in Patients with Type 2 Diabetes Mellitus

[Tip 2 Diabetes Mellitus Hastalarında Serum Tip I Kollajenin Çapraz Bağlı N-telopeptidleri (NTx) Düzeyleri ile Total Antioksidan Kapasitenin Araştırılması]

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Registered: 14 February 2009; Accepted: 15 March 2010

[Kayıt Tarihi : 14 Şubat 2009 ; Kabul Tarihi : 15 Mart 2010]

ABSTRACT

Purpose: Our aim was to investigate the serum cross-linked N-telopeptides of type I collagen levels and total antioxidant capacity in patients with type 2 diabetes mellitus.
Methods: 46 patients with type 2 diabetes mellitus (24M, 22F) aged 41 to 68 years (54.36 ± 7.92) and 34 healthy controls (17M, 17F) aged 40 to 69 years (52.91 ± 8.04) were enrolled in the study. Serum cross-linked N-telopeptides of type I collagen levels were measured by enzyme-linked immunosorbent assay method using a commertially available kit, total antioxidant capacity levels were measured by a colorimetric method based on the oxidation of 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) radical and hemoglobin A_{1c} levels were measured by high performance liquid chromatography technique.
Results and Conclusion: Serum cross-linked N-telopeptides of type I collagen levels of the patients were significantly higher ($p<0.01$) whereas total antioxidant capacity levels were significantly lower ($p<0.05$) than those of the controls. Also, there was a significant positive correlation between serum cross-linked N-telopeptides of type I collagen and hemoglobin A_{1c} levels ($r=0.301$, $p<0.05$) and a significant negative correlation between total antioxidant capacity and hemoglobin A_{1c} levels ($r=-0.382$, $p<0.01$) in the patients group.
In conclusion, our results show patients with type 2 diabetes mellitus have a significantly reduced total antioxidant capacity levels and there is a risk of bone resorption in these patients which can be estimated by measuring serum cross-linked N-telopeptides of type I collagen levels
Key Words: Type 2 diabetes mellitus, bone resorption, serum cross-linked N-telopeptides of type I collagen (NTx), hemoglobin A_{1c}, total antioxidant capacity (TAC)

ÖZET

Amaç: Amacımız tip 2 diabetes mellitus hastalarında serum tip 1 kollajen çapraz bağlı N-telopeptid düzeylerini ve total antioksidan kapasiteyi araştırmaktır.
Metotlar: Çalışmaya, yaşları 41-68 arasında değişen (54.36 ± 7.92) 46 tip 2 diabetes mellitus hastası (24E, 22K) ile yaşları 40-69 arasında değişen (52.91 ± 8.04) 34 sağlıklı kişi (17E, 17K) alındı. Serum tip 1 kollajen çapraz bağlı N-telopeptid seviyeleri ticari kit kullanılarak ELISA metodu ile, total antioksidan kapasite seviyeleri 2,2'-azino-bis (3-etilbenz-thiazoline-6-sulfonik asit) radikalinin oksidasyonuna dayanan kolorimetrik bir metot ile ve hemoglobin A_{1c} seviyeleri yüksek basınçlı sıvı kromatografi metodu ile ölçüldü.
Bulgular ve Sonuç: Hastaların serum tip 1 kollajen çapraz bağlı N-telopeptid seviyeleri kontrollerden anlamlı derecede yüksek ($p<0.01$), total antioksidan kapasite seviyeleri ise kontrollerden anlamlı derecede düşük ($p<0.05$) bulundu. Ayrıca, hasta grubunda serum tip 1 kollajen çapraz bağlı N-telopeptid ve hemoglobin A_{1c} seviyeleri arasında anlamlı bir pozitif korelasyon ($r=0.301$, $p<0.05$) ve total antioksidan kapasite ile hemoglobin A_{1c} seviyeleri arasında anlamlı bir negatif korelasyon ($r=-0.382$, $p<0.01$) bulundu. Sonuç olarak, bulgularımız tip 2 diabetes mellitus hastalarında total antioksidan kapasite düzeyinin önemli oranda azaldığını ve bu hastalarda serum tip 1 kollajen çapraz bağlı N-telopeptid seviyelerinin ölçümü ile belirlenebilen bir kemik rezorpsiyonu riski bulunduğunu göstermektedir.
Anahtar Kelimeler: Tip 2 diabetes mellitus, kemik rezorpsiyonu, serum tip 1 kollajen çapraz bağlı N-telopeptid (NTx), hemoglobin A_{1c}, total antioxidan kapasite (TAC)

Introduction

Osteoporosis is one of the most important metabolic bone diseases of diabetic patients. Although its vascular complications are well known, studies on bone involvement in patients with diabetes mellitus (DM) have generated conflicting results, largely because of the pathogenetic complexity of the condition (1,2).

Bone is continously remodeled through an ongoing cyclic process of osteoclast mediated bone resorption, followed by osteoblast mediated bone formation. These two events are normally coupled and interact with each other. Abnormalities in this process often results in changes in skeletal mass.

Bone loss is a chronic complication of DM associated with alterations in mineral and bone metabolism (3). Patients with recent onset of type 1 DM may have impaired bone formation because of the insulin deficiency, whereas in long-standing type 1 DM, vascular complications may account for low bone mass (2). Although several mechanisms have elucidated some pathophysiologic characteristics of abnormal bone metabolism in patients with type 2 DM, some contradictory effects have also been published. Obesity, prevalent in type 2 DM, is strongly associated with higher bone mineral density (BMD) probably through mechanical loading and hormonal factors including insulin, estrogen and leptin (4-6). However hyperglycemia may have several adverse effects on bone metabolism both in patients with poorly controlled type 1 DM and type 2 DM (2,7-9). Microvascular complications of diabetes and reduced blood flow to bone (10) and lower 25-hydroxyvitamin D_3 serum levels (11) may also contribute to bone loss and fragility.

Biochemical markers of bone turnover are used as tools for the estimation of bone turnover and future BMD. Approximately 90% of the organic matrix of bone tissue is type I collagen, the major component of bone. Collagen degradation during osteoclastic bone resorption forms the basis of new clinical bone metabolism assays (12,13); where measurements of specific degradation products of the bone matrix provide analytical data on bone metabolism rate. Among these assays, determination of urinary cross-linked N-telopeptides of type I collagen (NTx) concentrations have been reported to be a sensitive and specific marker of bone resorption in the context of metabolic bone disease (12-15).

Evaluation of experimental and clinical data promoted assays for serum NTx measurement to be a useful index of bone resorption (12,16-20); however, mostly for technical reasons, such as assay stability and variability, the serum assay has not become as widely used as the urinary NTx assay (12,16). However, since, serum markers of bone turnover have the advantage of not being influenced by the significant variability of, and errors associated with, creatinine measurement (21) or by the diuretic effect on the kidney caused by calcium supplementation (22), measurement of serum NTx level is still believed to be more advantageous than the measurement of urinary NTx level (16,17).

Oxidative stress leads to the induction of multiple cellular pathways ultimately leading both to the onset and subsequent complications of DM. Many biochemical pathways; glucose autoxidation, the non-enzymatic glycation of proteins, the accumulation of sorbitol via the aldose-reductase pathway influencing the generation of free radicals, which may lead ultimately to increased lipid peroxidation and depletion of antioxidants are a few examples promoted in chronic hyperglycemia state and thereby augment oxidative stress in subjects with type 2 DM (23,24). In addition, increased osteoclastic activity and decreased osteoblastic activity were reported to be associated with an imbalance between oxidant and antioxidant status in postmenopausal osteoporosis (25).

It has been reported that poor glycemic control was correlated with osteoporosis (26) and loss of bone mass was associated with increased oxidative stress (27). Therefore, the aim of this study was to investigate serum NTx and total antioxidant capacity (TAC) levels in patients with type 2 DM to evaluate potential risks associated with bone resorption and their contribution to oxidative stress.

Materials and Methods

Study subjects and samples

The subjects consisted of 46 patients with type 2 DM (24M, 22F) and 34 healthy controls (17M, 17F). The patients were aged 41 to 68 years (54.36 ± 7.92) and the controls were aged 40 to 69 years (52.91 ± 8.04). None of the patients and controls had formerly been diagnosed with any bone disease; no patients and controls were on menopause treatment. Current treatment of diabetes was evaluated.

Patients with type 2 DM recruited from the Department of Internal Medicine, Division of Endocrinology and Metabolism, Meram Faculty of Medicine, University of Selcuk, Konya, Turkey. The study protocol was approved by the Ethics Committee of Meram Faculty of Medicine, University of Selcuk, Konya, Turkey. All patients were informed of the details of the study and a written consent was received from each patient. Type 2 DM was diagnosed according to the "Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus" (28).

Fasting blood samples were obtained in EDTA tubes and hemoglobin A_{1c} (HbA_{1c}) levels were measured immediately. For NTx and TAC analysis, blood samples obtained in plain tubes were kept at room temperature for 30 min, then separated from the cells by centrifugation at 1469 g for 10 min. Serum samples were stored at −80 °C until biochemical analysis.

Measurement of the serum NTx levels

NTx levels were measured by a commercially available

kit based on enzyme-linked immunosorbent assay (ELISA) method (Osteomark NTx Serum, Wampole Laboraties, Inc., Dist. Princeton, NJ 08540, USA). The assay is a competitive-inhibition ELISA assay developed for the measurement of quantitive determination of NTx in human serum on a microtitre plate washer and ultra microplate reader (ELx50 and Elx800, BioTek Instruments, Inc., Vermont, USA, respectively). The NTx concentration values are reported in nanomoles of bone collagen equivalents per liter (nm BCE/L).

Measurement of the serum TAC levels

TAC levels of serum was determined using an automated measurement method which is based on the bleaching of the characteristic color of a more stable 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants (29) (Beckman Coulter, Fullerton, CA, USA). The ABTS radical cation is decolorized by antioxidants according to their concentrations and antioxidant capacities. The results are expressed in mmol Trolox equivalents/L.

Measurement of HbA_{1c} levels

HbA_{1c} levels were measured by the high performance liquid chromatography (HPLC) assay (30) (Variant II, Bio-Rad Laboratories, Hercules, CA, USA).

Serum glucose levels were measured by routine methods on autoanalyser (Beckman Coulter, Fullerton, CA, USA).

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc. Chicago, IL, Version 13.0). All data are expressed as mean ± standard deviation (SD). Statistical differences between the groups were evaluated using a χ^2 test and independent t-test. The correlations between variables were performed by Pearson's Correlation test. Differences were considered significant at a probability level of $p<0.05$.

Results

The physical characteristics and biochemical findings of the subjects are presented in Table 1. As seen from the table, serum NTx levels of the patients were significantly higher ($p<0.01$) whereas TAC levels were significantly lower ($p<0.05$) than those of the controls. Also, we have found a significant positive correlation between NTx and HbA_{1c} levels ($r=0.301$, $p<0.05$) and a significant negative correlation between TAC and HbA_1c levels ($r=-0.382$, $p<0.01$) in the patients group (Table 2, Fig. 1 and Fig. 2). There were significant positive correlations between glucose and HbA_{1c} levels in both groups ($r=0.474$, $p<0.01$ for the patients group and $r=0.400$, $p<0.05$ for the control group). Serum NTx and TAC levels did not differ between patients treated with insulin, patients treated with oral hypoglycemic agents (OHA) and patients treated with insulin plus OHA.

Discussion

Our study demonstrated that serum NTx level of patients with type 2 DM were significantly elevated whereas serum TAC levels were significantly decreased compared to those of the control subjects.

Since, NTx is a specific product of osteoclast proteolysis, our finding shows that serum levels of NTx may be used as a specific indicator of bone resorption in patients with type 2 DM. The significant correlation was found between serum NTx and HbA_{1c} levels in our study confirm this opinion. This is an important finding especially for routine purposes in the assessment and monitoring of bone turnover in diabetic patients. Indeed hyperglyce-

Table 1. The physical characteristics and biochemical parameters of the groups (mean ± SD).

	Patients	Control	P
Age (years)	54,36 ± 7,92 (ranges from 41 to 68)	52,91 ± 8,04 (ranges from 40 to 69)	NS
Gender (M/F)	24/22	17/17	NS
Menopause (-/+)	6/18	5/17	NS
BMI (kg/cm^2)	28.69 ± 5.20	26.55 ± 4.58	NS
Disease duration (years)	10.76 ± 5.61 (ranges from 3 to 22)	-	
Current treatment (insulin / OHA / insulin + OHA)	15/12/19	-	
HbA_{1c} (%)	8.98 ± 2.04	5.44 ± 0.30	<0.001
Ntx (Nm BCE/L)	24.63 ± 8.27	18.99 ± 7.78	<0.01
TAC (mmol Trol. equi./L)	1.09 ± 0.10	1.14 ± 0.10	<0.05
Glucose (mg/dl)	206.24 ± 80.31	101.38 ± 12.89	<0.001

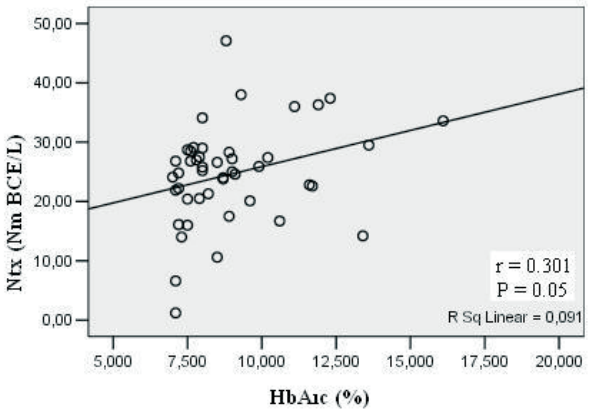

Figure 1. Correlation between patients NIx and HbA_{1c} levels

Table 2. The correlations between patients group parameters

Parameter	Glucose	Ntx	TAC
HbA_{1c}	0.474**	0.301*	-0.382**
TAC	-0.231	0.002	
Ntx	0.005		

*P<0.05

**P<0.01

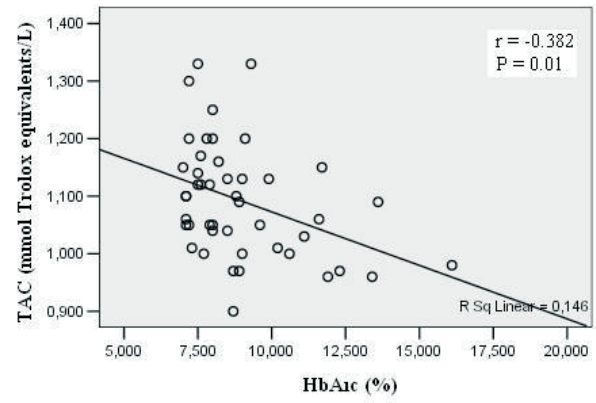

Figure 2. Correlation between patients TAC and HbA_{1c} levels

mia generates a higher concentration of advanced glycation end products (AGEs) in collagen which are reported to inhibit phenotypic osteoblast expression (7), increased osteoclast induced bone resorption (8), and sustained hyperglycemia further decreased osteoblastic cell function (4). Furthermore, the osteoclast related bone resorption is also promoted through sorbitol accumulation in the cell by the hyperglycemia state (4). Hypercalciuria associated with glycosuria may also induce bone loss (9). In addition, the expression of transcription factors regulating osteoblastic cell differentiation is restrained, and the apoptosis of those cells is promoted. As a result, osteoplasty is obstructed (4). Indeed, Suzuki et al. (31) have found that urinary excretion of C-telopeptide of type I collagen, deoxypyridinoline and NTx were significantly increased in male patients with type 2 DM when compared to controls.

In some previous studies, urinary NTx levels (14-16,18,32,33) and serum NTx levels (16-20,34,35) have been investigated for their potential use as a parameter and markers of bone resorption and BMD. For instance, Ebeling et al. (14) showed that urine N-telopeptide was an independent predictor of BMD in addition to age and serum bone alkaline phosphatase. Hamwi et al. (15) reported that in women with no hormone replacement therapy, only urinary C- and N-telopeptides correlated significantly with the lumbal T-score as an index for BMD. Garnero et al. (32) suggested urine levels of NTx might be a more relevant marker of bone turnover. Scariano et al. (17) found that total skeleton BMD was associated most strongly with serum NTx levels in elderly women. They also found serum NTx concentrations to significantly correlate with intact parathyroid hormone and thyroxin levels, thereby providing further evidence to the contention that NTx was a specific product of osteoclast proteolysis. Fall et al. (18) have reported that serum markers including NTx are useful measures of bone resorption in postmenopausal women and in men of similar age, in whom the use of such markers is likely to be helpful in the management of osteoporosis. Maeno et al. (19) have found that serum NTx may provide a clinically relevant serum assay to estimate bone turnover in hemodiyalysis patients. Our finding of increased serum NTx levels in type 2 diabetic patients shows a potential risk of bone resorption in these patients.

HbA_{1c} is also one of the early-stage glycation products used to evaluate metabolic control in diabetic patients and reflects the extent of exposure to glucose in the 4–8 weeks before testing. Some previous studies have investigated the correlation between HbA_{1c} and urinary NTx levels. For example, Hosoda et al. (36) have found no significant correlation between urinary NTx concentration and age, duration of diabetes and HbA_{1c} in postmenopausal woman with type 2 DM. Also, Fukui et al. (13) have found no significant correlation between urinary NTx concentration and age, duration of diabetes, HbA_{1c}, body mass index (BMI) in men with type 2 DM. On the other hand, Capoglu et al. (33) have investigated the effects of metabolic control on bone turnover markers in patients with type 2 DM over a period of 12 months, and reported a significant positive correlation between urinary NTx and HbA_{1c} levels. They have also found significant decreases in bone formation and resorption parameters including urinary NTx levels in parallel with HbA_{1c} levels and concluded that effective management of type 2 DM plays an important contribution to bone turnover improvement. This finding is in accordance with our finding. However, the underlying mechanism of the differences between our finding and those of Hosoda et al. (36) and Fukui et al. (13) is not clear at present. However, urinary markers of bone turnover may have been affected by the significant errors associated with, creatinine measurement (21) or by diuretic effect on the kidney

caused by calcium supplementation (22). Serum markers are not affected by such variations. Also, age, gender, disease duration and type of treatment they used in their patients may have interfered with their results. On the other hand, we have found a significant positive correlation between serum NTx and HbA_{1c} levels but there was no correlation between serum NTx and glucose levels. The underlying mechanism of this finding is not known and needs to be investigated.

It is widely postulated that the etiology of type 2 DM has a strong association with oxidative stress, originating from increased free radical production and decreased antioxidant potential (37). However, a number of studies on the role of oxidative stress in type 2 diabetes have shown inconsistent results (38), and this discrepancy may exist with respect to patient age, duration of diabetes, treatment methods, techniques used to measure oxidative stress etc. Even so, the most common results reported in patients with type 2 DM are increased oxidative stress and impaired antioxidative defense systems (24,37,38).

Our finding of significantly decreased TAC levels of patients with type 2 DM indicates increased oxidative stress in our patients. Since, increased oxidative stress is associated with loss of bone mass (25,27), it might be suggested that decreased TAC levels in type 2 DM might contribute to bone resorption in these patients. However, there was no correlation between serum TAC and NTx levels of the groups. Although, many studies have investigated serum TAC levels of diabetic patients (24,37), we have not found any study investigating the correlation between serum NTx and TAC levels in these patients.

In our study, we have not separated our subjects to male and female groups as in some other literature (33,34,36) and female subjects to pre- and post-menopausal groups. This is a limitation of our study. However, as seen from Table 1, there was no significant difference between the number of pre- and post-menopausal woman in the groups.

In conclusion, our results show that there is a potential risk of bone resorption in patients with type 2 DM which can be estimated and evaluated by measuring serum NTx levels. This finding is confirmed by a positive correlation between serum NTx and HbA_1c levels. Therefore, serum NTx level can be used as a marker of bone resorption in patients with type 2 DM. Also, our results confirmed a significantly reduced TAC level in these patients.

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