Original Article

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# No Relationship between Osteocalcin and the Microvascular Complications of Type 2 Diabetes

## Tip 2 Diyabetin Mikrovasküler Komplikasyonları ile Osteokalsin Arasında İlişki var mı?

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### Abstract

**Purpose:** Osteocalcin, one of the osteoblast-specific proteins is produced and secreted by osteoblasts. Although osteocalcin has been thought to play a role only in bone formation, recent studies have shown that it also enhances insulin sensitivity and insulin secretion from pancreatic beta cells. Several studies have reported lower serum osteocalcin levels in patients with type 2 diabetes mellitus (T2DM) than in non-diabetic individuals; and a negative correlation between microvascular complications and osteocalcin levels in type 1 diabetes mellitus (T1DM) patients. We investigated the relationship between microvascular complications and serum undercarboxylated osteocalcin (uOC) levels in T2DM patients.
**Material and Method:** One hundred seventy-nine patients with T2DM aged between 30 and 60 years were randomly included in the study; 101 patients with microvascular complications formed the patient group, and the other 78 without a microvascular complication formed the control group. The patients were evaluated for diabetic nephropathy, retinopathy and neuropathy. The ELISA method was used to measure uOC levels.
**Results:** The two groups were statistically similar with regard to age, gender and body mass index (BMI). Duration of diabetes was shorter in control group than in patient group ($p<0.05$). HbA1c level in patient group was significantly higher than in controls ($p<0.05$). There was no statistically significant difference in uOC levels between the groups ($p>0.05$).
**Discussion:** There was no relationship between serum osteocalcin levels and the microvascular complications of T2DM. *Turk Jem 2014; 18: 126-131*
**Key words:** Type 2 Diabetes Mellitus, undercarboxylated osteocalcin, microvascular complications
**Conflict of interest:** The authors reported no conflict of interest related to this article.

### Özet

**Amaç:** Osteokalsin, osteoblastlar tarafından salınan osteoblasta özgü proteinlerden birisidir. Yakın bir zamana kadar sadece kemik yapımında rol oynadığı düşünülen osteokalsinin, son zamanlarda insülin duyarlılığını ve pankreas beta hücrelerinden insülin salınımını arttırdığına dair bilgiler yayınlanmıştır. Tip 2 Diyabetes Mellitus (DM) hastalarında serum osteokalsin düzeyi, tip 2 DM'si olmayanlara göre daha düşük seviyelerde tespit edilmiştir. Tip 1 DM'de mikrovasküler komplikasyonlar ile osteokalsin düzeyi arasında negatif ilişki olduğu gösterilmiştir. Bu çalışmada, tip 2 DM hastalarındaki mikrovasküler komplikasyonlar ile serum andekarboksile osteokalsin düzeyleri arasındaki ilişkiyi araştırmayı amaçladık.
**Gereç ve Yöntem:** Tip 2 DM tanısı ile takip edilen, 30-60 yaş arası hastalar rastlantısal olarak çalışmaya alınmıştır. Çalışmanın hasta grubu diyabetin mikrovasküler komplikasyonu olan hastalardan (101 kişi), kontrol grubu ise (78 kişi) mikrovasküler komplikasyonu olmayan tip 2 DM hastalarından oluşturuldu. Hastalar diyabetik retinopati, nefropati ve nöropati açısından araştırıldı. Andekarboksile osteokalsin ölçümü ELISA yöntemiyle yapıldı.
**Bulgular:** İki grup arasında yaş, cinsiyet ve vücut kütle indeksi açısından istatistiksel olarak fark yoktu. Komplikasyon gelişmemiş grubun diyabet süresi komplikasyon gelişen gruba göre daha kısaydı ($p<0,05$). Komplikasyon gelişen grubun HbA1c değeri istatistiksel olarak daha yüksek olarak bulundu ($p<0,05$). Her iki grubun andekarboksile osteokalsin düzeyi açısından anlamlı fark saptanmadı ($p>0,05$).
**Tartışma:** Serum andekarboksile osteokalsin düzeyi ile tip 2 DM'nin mikrovasküler komplikasyonları arasında ilişki saptanmadı. *Turk Jem 2014; 18: 126-131*
**Anahtar kelimeler:** Tip 2 Diabetes Mellitus, andekarboksile osteokalsin, mikrovasküler komplikasyonlar
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## Introduction

Although osteocalcin is one of the osteoblast-specific proteins secreted by osteoblasts, recent studies showed that it is secreted by megakaryocytes and adipocytes (1,2,3,4). Osteocalcin was thought to have an active role in bone formation only. Recently, it has been reported that insulin sensitivity and insulin secretion from pancreatic beta cells are affected by the lack of osteocalcin in osteocalcin-deficient mice (5). On the other hand, insulin signalling in the osteoblast activate osteoclasts, thus, osteocalcin accumulation in the extracellular matrix increases. Finally, these findings support that insulin affects the osteoblasts that play an important role in glucose metabolism (6).

In individuals with type 2 diabetes mellitus (T2DM), serum osteocalcin levels have been observed to be lower than in those who do not have T2DM (7,8,9,10,11,12,13). Especially, the impacts of the undercarboxylated form of osteocalcin on glucose balance and energy metabolism have been reported (5).

Ferron et al. found that osteocalcin inversely correlated with glucose and HbA1c in patients with T2DM before and after glycemic control. Glycemic control is improved the osteoblast function, increased IGF-1 levels and bone formation in diabetic patients. Animal studies showed that daily osteocalcin injection improved glucose metabolism and prevented the development of T2DM (14).

The chronic complications of diabetes occur with contributions of factors, such as hyperglycemia, dyslipidemia, and atherosclerosis (15). Studies on the relationships of osteocalcin levels with glucose metabolism, lipid metabolism and atherosclerosis are suggestive of a possible relationship between osteocalcin levels and development of diabetic microvascular complications. Serum osteocalcin levels were significantly lower in type 1 diabetic patients with retinopathy and/or proteinuria than in type 1 diabetic patients without microangiopathic compications (16). In a study, patients with T2DM with nephropaty had higher osteocalcin levels than type 2 diabetic patients without nepropathy and healthy controls (17). A few studies have found conflicting results about the relationship between T2DM and microangiopathic complications (16,18). The aim of this study was to investigate the relationship between the microvascular complications of T2DM and serum undercarboxylated osteocalcin (uOC) levels.

## Materials and Methods

Patients diagnosed with T2DM who were between the ages of 30 and 60 years and were followed up between August 2011 and December 2011 were randomly selected for the study. Informed consent forms in accordance with the Declaration of Helsinki were obtained from all subjects and the protocol was approved by the local ethics committee. The patient group of the study consisted of patients with microvascular complications (n=101) and the control group consisted of T2DM patients without microvascular complications (n=78). Patients with conditions such as multiple myeloma, bone tumors, bone metastases, hypercalcemia associated with malignancy, hyperthyroidism, bone fractures, chronic liver disease, stage 4-5 chronic renal failure, osteomalacia, hyperparathyroidism, hypoparathyroidism, and Paget's disease and those on medications (vitamin D, antiepileptic drugs, steroids, heparin, coumadin and glitazone) that could affect the osteocalcin levels were excluded from the study.

The patients were examined for retinopathy, nephropathy, and neuropathy, which are the microvascular complications of diabetes. Patients who have not been screened for diabetic retinopathy within the past year were evaluated for retinopathy in consultation with the eye diseases clinic. With diabetic nephropathy screening purposes, albuminuria in spot urine (microalbumin/creatinine ratio in urine samples) were evaluated: those with under 30 mg/g were classified as normal; 30-299 mg/g as microalbuminuria, and 300 mg/g or over were classified as macroalbuminuria. Serum creatinine levels were measured. The patients were surveyed for diabetic neuropathy symptoms and were examined for distal symmetric polyneuropathy. The participants were grouped according to the presence of microvascular complications.

Each patient's body mass index (BMI) was calculated. HbA1c, calcium, phosphorus, 25-hydroxyvitamin D [25(OH)D], parathormone (PTH), triglycerides (TG), total cholesterol and HDL cholesterol values were measured. Serum LDL cholesterol levels were calculated using the Friedewald formula [LDL=TK–(TG/5+HDL)].

The blood samples drawn to investigate the serum uOC levels of patients were centrifuged for 5 to 10 minutes at 4000 RPM; and serum samples were obtained. Until investigated concurrently, they were stored at -80 °C. The uOC levels in all samples were measured simultaneously via ELISA kit (Cusabio(R), Wuhan, P.R. of China).

### Statistical Analysis

Data analysis was conducted with SPSS (Statistical Package for the Social Sciences) version 15.00 for Windows. Descriptive statistics with a normal distribution are presented as mean ± standard deviation; those with a non-non-normal distribution are presented as median (min-max); and nominal variables are presented as number of cases and percentage (%).

The significance of the difference between the two groups was evaluated based on student's t-test for means and the Mann-Whitney U test for medians. In case of more-than-two group comparisons significance of mean differences between the groups were evaluated with ANOVA test and of medians using the Kruskall-Wallis test. Nominal variables were evaluated using Pearson's chi-square or Fisher's exact test.

Spearman's correlation coefficient was used to investigate the association between two continuous variables. A p value of less than 0.05 was considered statistically significant.

## Results

Of the 179 T2DM patients between 30 to 60 years of age who were included in this study, 101 were in the group with microvascular complications (patient group) and 78 were in the group without microvascular complications (control group). There were 26 male and 52 female participants in the control group; and 41 males and 60 females in the patient group. The two groups were statistically

similar in terms of age and gender distribution. While the average duration of diabetes was 69 months in the control group, it was 84 months in the group with multivascular complications (the duration of diabetes was taken as 1 year for those with diabetes for less than 1 year). The duration of diabetes in microvascular complication group was significantly longer than that in the control group ($p<0.05$). There was no statistically significant difference between the two groups in terms of BMI ($p>0.05$).

The average HbA1c value in the control group was 6.79% (min:4.03-max:12.9) while it was 8.55% (min:4.99-max:16.26) in the microvascular complication group: HbA1c levels in the patient group were significantly higher than in the control group ($p<0.05$).

The uOC level comparison of the two groups did not yield a significant difference between the groups with an average uOC level of 1.78 ng/mL (min:0.29-max:13.5) in the control group and 1.9 ng/mL (min:0.26-max:44.62) in the patient group ($p>0.05$).

The participants in the patient group were grouped based on the presence of retinopathy, nephropathy, and neuropathy. uOC levels in 40 patients with retinopathy were compared with that in the control group. Average uOC levels in retinopathy patients (n=40) and the control group were 1.37 ng/mL (min:0.39-max:19.6) and 1.78 ng/mL (min:0.29-max:13.5), respectively; no significant difference was found in uOC levels between the two groups ($p=0.637$).

A possible association between uOC levels in controls and the 54 patients with nephropathy was investigated. Average uOC levels in participants with nephropathy and those in the control group were 1.93 ng/mL (min:0.32-max:46.62) and 1.78 ng/mL (min:0.29-max:13.5), respectively: No significant difference was observed in uOC levels between the two groups ($p=0.244$).

We evaluated uOC levels in patients with neuropathy the average uOC levels were 1.95 ng/mL (min:0.26-max:46.62) and 1.78 ng/mL (min:0.29-max:13.5) in the neuropathy patients group and the control group respectively: No significant difference between the two groups was observed in terms of uOC levels ($p=0.970$).

Figure 1 displays a comparison of the uOC levels in control group and the microvascular patients with retinopathy, nephropathy, and neuropathy.

No statistically significant difference was found in the comparison of the number of microvascular complications and uOC levels (n=101) ($p> 0.05$).

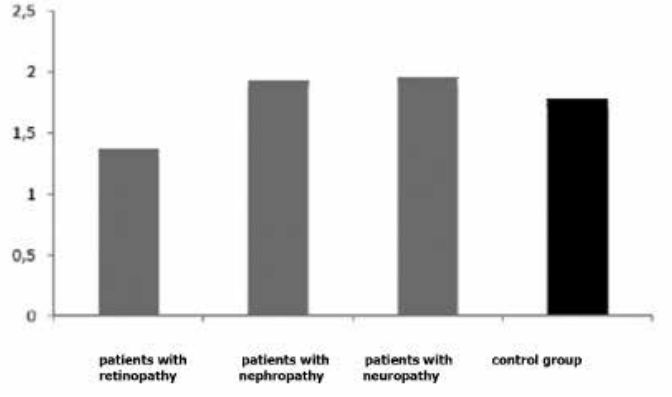


**Figure 1.** Comparison of the undercarboxylated osteocalcin levels between microvascular complications groups and the control group

The associations between the uOC levels and the other parameters included in this study were investigated. Associations between patients' age and uOC levels were investigated and no significant association was found (n=179) ($p>0.05$). A statistically significant association was not found (n=178) between BMI and uOC levels either ($p>0.05$). There was no significant relationship between the HbA1c levels and uOC levels, and no significant relationship of uOC levels with triglyceride, total cholesterol, LDL and HDL levels (n=179) was detected ($p>0.05$). While no significant relationship of uOC levels with calcium, phosphorus, and 25(OH)D levels was observed, a statistically significant positive correlation was found between PTH and uOC levels (n=179) ($p<0.05$) ($r=0.012$). When the groups were evaluated separately, there was no statistically significant correlation of osteocalcine with HbA1c, LDL, HDL, TG, PTH, P, Ca, Cre in patients without complications (n=78), however, in group with complications (n=101), osteocalcine statistically positively correlated with Cre ($p=0.01$, $r=0.23$) and PTH ($p=0.02$, $r=0.23$).

## Discussion

There is evidence that osteocalcin regulates insulin secretion, insulin resistance, and energy expenditure in addition to its role in bone metabolism (19). In recent studies, bone has been defined as an endocrine organ (20,21). The aim of this study was to identify the relationship between microvascular complications and serum uOC levels in T2DM patients.

T2DM patients with and without microvascular complications were compared in this study. Duration of diabetes and HbA1c levels in the patient group with complications were higher than those in patients without complications, as expected. These results indirectly suggest that duration of diabetes and poor glucose control facilitate the development of microvascular complications.

In numerous studies, osteocalcin levels in healthy individuals have been proven to increase by age (22,23,24). The participants in patient and controls groups were selected from the 30 to 60 years old age group, when osteoporosis is not frequently observed, and all participants were T2DM patients. Based on the data collected from the participants included in this study, no significant association was observed between patients' age and uOC levels ($p>0.05$).

In 2007, in an experiment on mice, Lee et al. concluded that undercarboxylated form of osteocalcin has an active role in glucose balance and energy metabolism (5). Hence, it has been experimentally proven that the skeleton via osteocalcin can also be responsible for the endocrine regulation of energy metabolism in the body (5).

Undercarboxylated osteocalcin increases insulin secretion and glucose tolerance (25). Total osteocalcin level has been measured in many studies that investigated the role of osteocalcin in energy metabolism (Total osteocalcin consists of both carboxylated and undercarboxylated osteocalcin). However, there are fewer studies conducted on uOC. Proceeding from our knowledge on the active role of undercarboxylated form of osteocalcin on glucose balance and energy metabolism (5), we planned to study uOC.

While an inverse relationship between osteocalcin and HbA1c levels has been reported in various studies, (8,20,26,27,28,29,30) a statistically significant relationship between uOC and HbA1c

levels has not been determined in this study. In addition, though Kanazawa et al. have detected an inverse relationship of osteocalcin with HbA1c, fat percentage, and body fat percentage in postmenopausal women, multiple regression analyses have not yielded significant results (30). In another study, P. Pietschmann et al. has failed to detect a significant association between osteocalcin and HbA1c levels (16). HbA1c indicates blood glucose levels over the past 3 months, while osteocalcin indicates the momentary osteocalcin. Moreover, some studies have even demonstrated that osteocalcin levels can vary at different times of the day or seasonally across the year (31,32,33). We thougt that uOC level is unstabil in a day therefore cannot use a stabil parameter like HbA1c. It would be a more effective way to measure blood glucose levels after injecting uOC exogenously in order to obtain concrete evidence on whether uOC actually regulates blood glucose levels or not. We were able to find two experimental studies on this topic in the literature. The first one was a study conducted by Yoshikawa et al. They have demonstrated that ablation of osteoblasts in adult mice affected glucose metabolism. As in case of osteocalcin deficiency, a partial ablation in this cell population have resulted in hypoinsulinemia, hyperglycemia, glucose intolerance, and reduced insulin sensitivity; and with osteocalcin administration, glucose intolerance in those mice improved and blood glucose and insulin levels have returned to normal (34). The second study by Ferron et al. investigated the therapeutic potential of intermittent osteocalcin application in mice and concluded arguing that daily osteocalcin injections can regulate glucose metabolism and therefore prevent the development of T2DM (15).

Several studies in the literature investigated the relationship between osteocalcin and obesity lipid profile. In an experimental study where mice were put on a high fat diet, the group that received osteocalcin along with the diet had smaller fat pads and within the normal range triglyceride levels compared to the group that did not receive osteocalcin. This particular study reported that osteocalcin prevents obesity and T2DM (35). Kanazawa et al. have obtained results evident of an inverse relationship of osteocalcin levels with body fat percentage and visceral/subcutaneous fat ratio among male T2DM patients, independent of age, duration of diabetes, height, and renal functioning (30). A study by Sayınalp et al. on this topic, however, failed to detect an association between BMI and osteocalcin (27). An association between BMI and osteocalcin levels was investigated in this study as well, but no such relationship of statistically significance was observed.

Kanazawa et al., in another study, determined that the baseline osteocalcin levels in T2DM patients measured prior to glycemic control negatively correlated with the changes in triglyceride levels and positively correlated with the changes in the HDL cholesterol levels; and that the changes in osteocalcin levels negatively correlated with baseline triglyceride levels. These findings support the argument that osteocalcin has a role in glucose and lipid metabolisms (29). However, while some studies have detected an inverse association between osteocalcin and lipid values; there are studies that failed to demonstrate an association. Daniela Gradinaru et al., in their study named Evaluation of Osteocalcin Levels in Elderly Patients with Type-2 Diabetes Mellitus, did not report a significant relationship between osteocalcin and lipid values (total cholesterol, triglycerides, LDL, cholesterol, HDL cholesterol) (7). No significant relationship was found between lipid values and uOClevels in this study. However, further studies are needed on this topic as there are studies reporting inconsistent results.

Pietschman et al. (16) and Pasaoglu et al. (36) in their study on patients with type 1 DM with retinopathy and/or proteinuria determined that serum osteocalcin levels were significantly lower in complicated diabetic patients than in those without complication. Osteocalcin levels might have decreased due to development of diabetic microangiopathy causing impairments in bone vascularization that impacts bone formation. Pietschman et al. reported that bone formation and therefore the osteocalcin levels were affected by microvascular complications in type 1 DM patients (16). In our study, the two groups that were classified based on the presence of microvascular complication were compared and found not to be significantly different in terms of uOC levels ($p>0.05$). Pietschman et al., on the other hand, compared T2DM patients with and without microangiopathy and detected that the osteocalcin levels in patients with microangiopathy were lower, but failed to demonstrate a statistically significant difference between the osteocalcin levels in the two groups. In an another study, 27 diabetic patients with microvascular complications were evaluated in terms of osteocalcin levels which were statistically significantly lower than in without complications group (18). Inversely, Inukai et al. reported that osteocalcin levels were higher in diabetic patients with microalbuminuria, retinopathy and macroalbumunuria (37,38) Chen et al. showed that osteocalcin levels were higher in patients with diabetic nepropathy than in those without (17). Probably, the reason for the different reports about osteocalcine are related with using different osteocalcine formations (osteocalcin, carboxylated osteocalcin and undercarboxylated osteocalcin) and problem of standardization. The participants in the patient group in our study who had retinopathy, nephropathy, and neuropathy were grouped separately. Although the difference was not statistically significant, the mean uOC level in patients with retinopathy was lower than that in controls. The mean uOC levels in patients with nephropathy and neuropathy were higher than that in the control group, however, no significant difference was detected in uOC levels between the two groups. In this study, although not statistically significant, there was an increase in osteocalcine levels but, in another similar study, Chen et al. reported statistically significantly increased osteocalcin levels (17).

There is no statisticaly significant relationship between uOC levels and diabetic microvascular complications.

While the glucose control was detected to be poorer in the microvascular complication group than that in the group with no microvascular complications, the lack of a significant change in their uOC levels suggests that uOC is not the only active pathway in glucose control. Whether or not having developed a complication, all participants in this study were T2DM patients. Guided by the

studies investigating the role of osteocalcin in energy metabolism up to the present the uOC levels of both groups were expected to be lower, compared to the normal population. However, we did not have the opportunity to verify this due to recruiting only diabetic participants. As no statistically significant difference was detected in uOC levels between the T2DM patients with and without microvascular complications in this study, we believe that the uOC level is not the only factor in the development of microvascular complication in patients with T2DM.

Vitamin D increases the osteocalcin synthesis (39) and plays a role in the realization of the gamma-carboxylation of osteocalcin (40). It has been reported that the uOC level is inversely associated with the 25(OH)D level (40). However, we did not find a statistically significant association between 25(OH)D, uOC, and HbA1c levels. The mean 25(OH)D levels in both the patient and the control groups was determined to be low in this study (12.11 and 12.15 ng/mL, respectively). While the uOC levels were determined not to have a significant association with calcium, phosporus, and vitamin D, a statistically significantly positive correlation was identified between the uOC and PTH levels, as expected (n=179) ($r=0.012$; $p<0.05$). When the groups evaluated separately, osteocalcine levels in group with complications (n=101) statistically significantly positively correlated with with Cre ($p=0.01$, $r= 0.23$) and PTH ($p=0.02$, $r=0.23$). Increased creatinine and PTH levels increase the osteocalcin level by accelerating the bone turnover.

## Conclusion

Osteocalcin is part of the complex communication system between the bone and the organs responsible for energy metabolism such as pancreas and fat tissue (19). Osteocalcin has been shown to have a role in pancreatic insulin secretion, increasing the insulin sensitivity in peripheral tissue, and regulation of glucose and lipid metabolism (21). uOC acts as a regulatory hormone for glucose metabolism and fat mass (30). In various studies, serum osteocalcin levels in T2DM patients have been shown to be lower than in those without diabetes (8,20,26,27,28,29,30). We investigated the association between T2DM-related microvascular complications and uOC levels and did not find a significant association. We did not determine the uOC levels to have any association with HbA1c, TG, LDL, HDL, and total cholesterol levels.

We believe that reliable future studies can resolve the contradictions in the literature regarding the impact of osteocalcin on metabolic regulation. If these effects of osteocalcin are proven in humans as well, it will provide the medical community with more effective methods and resources in the prevention and treatment of diabetes and osteoporosis.

**Conflicts of Interest**

There are no conflicts of interest.

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