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SERUM OSTEOCALCIN LEVELS IN TYPE I DIABETES MELLITUS*

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Serum osteocalcin levels are a marker of bone formation. In this study, bone and mineral metabolism in type I diabetes mellitus (DM) were investigated, and the changes related to diabetic microvascular complications were examined. Serum calcium (Ca), inorganic phosphate (P), osteocalcin (OC) and parathyroid hormone (PTH) levels were measured in 42 type I diabetic subjects. Diabetics were subdivided into those with or without complications. Age and sex-matched control subjects were used for comparisons with the diabetic groups. Serum P and PTH levels were not different from those of controls. Serum Ca levels were significantly increased ($p < 0.001$) although the values were within the normal range. OC levels were significantly lower in the complicated (retinopathy and/or protenuria) diabetic group ($p < 0.005$). In Type I diabetes mellitus, the serum OC level is influenced by the presence of microvascular complications. *Key words: osteocalcin, Gla protein, diabetes mellitus.*

Altered bone and mineral metabolism has previously been reported in both experimental and clinical diabetes mellitus (DM)[1-3]. Decreased bone mass as well as disturbed calcium (Ca) and vitamin D homeostasis have been described in DM. However, the effects of diabetes on bone and mineral metabolism in humans is still far from clear, since there are conflicting reports where decreased, normal or increased bone mass have been reported[4-6].

Osteocalcin (OC) or bone g-carboxyglutamic acid (Gla) protein is a small, noncollagenous protein of the bone matrix with three residues of vitamin K-dependent Gla[7,8]. Circulating levels of OC, a specific product of osteoblasts, reflects the rate of bone turnover with high sensitivity and has clear advantages over other parameters of bone metabolism[8,9].

In recent years, serum OC levels in DM and in several metabolic bone diseases have been studied, and the results in diabetic patients have been contraversial[8,10,11]. Also, the effects of type microvascular complications and treatments on bone metabolism in diabetic patients are not yet clear (Fig. 1).

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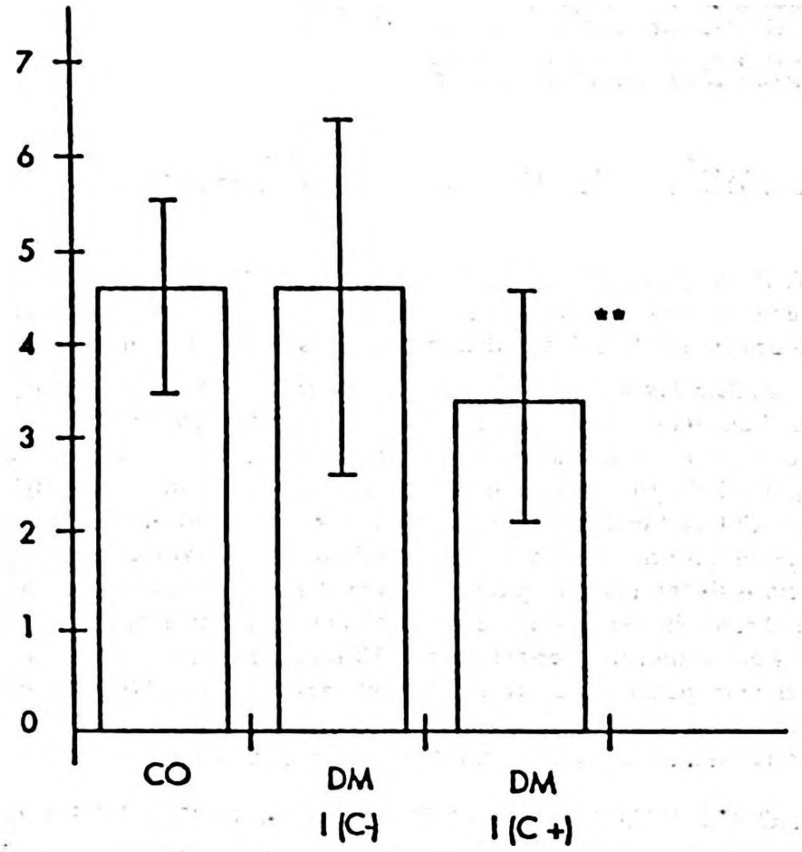

Fig. 1: Mean serum osteocalcin (OC) levels in diabetic patients and in control subjects.
CO : Control subjects.
I (C-) : Type I diabetics without complications.
I (C+): Type I diabetics with complications.
Vertical lines (I) inticate ± SD values **: $p < 0.005$.

In our yprevious study[12], although the serum PTH levels were not significantly changed, 1.25 $(OH)_2$ vitamin D_3 levels were found to be decreased in newly diagnosed type I diabetic patients who had no previous insulin treatment. The present study was undertaken to determine serum OC, Ca and inorganic phosphate (P) levels in type I DM and to investigate whether changes in these values were related to diabetic microvascular cmoplications (retinopathy, nephropathy and neuropathy).

Material and Methods

Subjects

Forty-two patients with type I DM, routinely attending the Diabetic Clinics of the Departments of Pediatrics and Endocrinology at the Faculty Hospital of Erciyes University, were included in this study. The patients were free from diseases other than DM, and no subject was taking any medication known to interfere with bone metabolism. Type I diabetics were then subdivided into those with or without microvascular complications (e.g. retinopathy, neuropathy and

nephropathy). Retinopathy was diagnosed after fundoscopic examination; in doubtful cases fluorescein fundus angiography was performed by ophthalmologists. While diabetic neuropathy was diagnosed following the neurological examination including electromyography, nephropathy was diagnosed with the help of laboratory tests such as blood urea nitrogen (BUN), creatinine, microalbumin and creatinine clearence.

All diabetic subjects had been on insulin replacement therapy since diagnosis. Twenty-six age- and sex-matched control subjects with no clinical or biochemical evidence of bone or metabolic disease were also included in the study. Details of the diabetic and control subjects are given in Table I.

Table I: Details of Diabetic and Control Subjects

	n	Sex (M/F)	Age (yr) mean ± SD	Duration of Diabetes (yrs)	HbA_{1C} (%)	Fasting Glucose (mg/dl)	Serum Creatinine (mg/dl)
Controls	26	12/14	14 ± 3	–	< 7	80 ± 5	0.8 ± 0.04
Diabetics	42	20/22	14 ± 3	1-10	9.9 ± 0.7	169 ± 53	0.9 ± 0.05

Patients' results were compared with those of the control group. Student's test was used for statistical analysis.

Laboratory Methods

As a circadian rhythym of OC in human serum has been reported[13], blood samples were drawn between 8:00 and 9:00 a.m. in the overnight fasting state, before insulin injections. Blood sampling in the patients and controls was equally distributed throughout the year.

Serum was separated from the samples without delay and kept at -20 °C until the time of OC assay.

OC levels were measured by radioimmunoassay using the Incstar^{125}I. RIA Kit. Parathyroid hormone (PTH) levels were measured by DPC (RIA) kit. Serum fasting glucose, total Ca, P and creatinine levels were measured on the same day by Technican RAXT autoanalyser, using routine Biotrol Kits. Also, Hemoglobin (HbA_{1c}) levels in erythrocytes were measured by Biotrol Kit on the same day.

Results

The results obtained in diabetic patients as compared to control subjects are outlined in Tables I and II. The fasting serum glucose and HbA_{1C} levels of diabetics were greater than those of control subjects (Table I).

In type I diabetics, serum Ca levels were significantly increased ($p < 0.05$), although the values were within the normal ranges for age- and sex-matched human serum. Serum inorganic P and PTH levels were not different from those of controls ($p > 0.05$) (Table II).

Table II: Serum OC, Ca, P and PTH levels in Diabetic Patients and Control Subjects

	n	Osteocalcin (ng/ml)	Ca (mg/dl)	P (mg/dl)	PTH (ng/ml)
Control	26	4.64 ± 0.92	9.3 ± 1.1	4.0 ± 0.8	0.46 ± 0.2
Type I (Comp -)	29	4.66 ± 1.96	10.4 ± 0.4 ≠**	3.8 ± 0.9	0.44 ± 0.1
Type I (Comp +)	13	3.37 ± 1.17 Ø***	9.9 ± 0.7 ≠*	4.2 ± 1.0	0.44 ± 0.2

Mean ± SD values are given
(Comp -) = Diabetic patients without complications.
(Comp +) = Diabetic patients with complications.
Ø: Less than control *: $p < 0.05$.
≠: Greater than control **: $p < 0.01$ ***: $p < 0.005$.

Serum levels of OC were significantly lower in diabetic patients with microvascular complications than in controls. Also, serum OC levels of the complicated group decreased more than those of the noncomplicated group ($p < 0.02$).

Discussion

Diabetic children and adults have decreased bone mass as shown by photon absorptiometry and radiometry[1, 3]. This osteopenia is evident not only in the peripheral skeleton but also in the spine of diabetic women[5], and diabetic osteopenia differs from typical osteoporosis in character[6]. The pathogenesis of diabetic osteopenia in humans remains unclear.

Despite normal serum Ca levels, disturbed Ca metabolism has been seen in diabetic patients and is thought to be related to a skeletal defect[14]. Studies on experimental and human diabetes have showed normal[14-16], low[5] and high[10, 17] serum Ca levels, Glachen et al.[11] found that the levels of ionized Ca were not changed in diabetic rats. In the present study, ionized Ca levels could not be measured.

In our study, when we examined the Ca and P levels, serum Ca levels were higher in patients with type I DM than in controls ($p < 0.01$), although these high values were within normal limits for human serum. On the other hand, PTH was not significantly different. Serum P levels are not changed in diabetics. Auwerx et al.[5] found decreased serum Ca (9.3 ± 0.3 mg/dl) and P (3.0 ± 0.6 mg/dl) levels in type I diabetic patients (Age 41.5 ± 13.5 years, disease duration 18.3 ± 7.8 years). Pedrazzoni et al[10]. reported increased Ca (9.6 ± 0.3 mg/dl) and normal P (2.8 ± 0.7 mg/dl) levels in type I diabetics (Age 49.1 ± 1.55 years, disease duration 8.9 ± 5.9 years). Hough et al.[17], who also found high levels of serum Ca in diabetic rats, suggested that this may be due to increased intestinal Ca absorption. These different findings may have resulted from differences in the study groups and their durations of disease.

Serum concentrations of OC have been shown to reflect the synthesis of OC by osteoblast and thus are a parameter of bone formation[7, 18]. The source of OC in

plasma is new osteoblastic synthesis and not a release from resorbed bone; it is cleared mainly by glomerular filtration in the kidneys[19]. Vitamin K1 is essential for OC biosynthesis, and synthesis is stimulated by 1.25 $(OH)_2$ Vitamin D_3[20]. OC may be a valuable marker for decreased bone remodeling in insulinopenic diabetes[11].

In the present study, OC levels were lower in type I diabetics who have complications than in controls. Also, serum OC levels were diminished in complicated diabetics as compared to noncomplicated diabetics. However, in type I diabetics without complications, OC levels were not different from those of controls. This may be partly related to the regular treatment of our patients with insulin. The increasing effect of insulin replacement therapy on serum OC levels has been suggested in a previous report[10]. A direct influence of insulin on in vitro OC synthesis has also been reported[21]. Guarneri et al.[22] showed that serum OC values were lower in newly diagnosed diabetic children than in controls. After 15 days of insulin therapy they found no difference between diabetics and controls. These results support the effect of insulin on OC. Pedrazzoni et al.[10] suggested that the low levels of OC they found in insulin-dependent and non-insulin-dependent diabetics might be due to a reduction in the levels of 1,25-dihydroxyvitamin D_3. They said that the reduction of OC in their diabetic population was related to the type of treatment, with diet-controlled subjects showing greater differences from the expected normal values than those treated with oral hypoglycemic agents or insulin. Our previous study[23] showed serum 1.25 $(OH)_2$ vitamin D_3 levels to be decreased in newly diagnosed type I diabetics who did not have insulin therapy. This result may indicate that diminished serum OC levels were due to decreased 1.25 $(OH)_2$ vitamin D_3.

Verhaeghe et al.[24] showed that plasma OC concentrations were markedly decreased in spontaneously diabetic rats after 3 to 4 weeks of diabetes. The half life of [^{125}I] OC was similar in diabetic and non-diabetic rats, indicating that decreased plasma OC was due to decreased synthesis. They suggested that the number and/or function of osteoblasts are severely suppressed in diabetes, resulting in decreased plasma OC levels. In the literature, only Pietschmann et al.[25] have examined the effects of microvascular complications on serum OC levels in diabetic patients. They found that OC levels were significantly lower in type I diabetics with retinopathy and/or protenuria than in type I diabetics without microangiopathy. In this respect, the results of the present study support their findings. Therefore, bone formation as reflected by serum OC levels seems to be influenced by the presence or absence of microvascular complications in type I DM.

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