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CLINICAL STUDY OF OSTEOCALCIN AND ITS RELATION TO DIABETES MELLITUS

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ABSTRACT

The research included estimate the concentration of osteocalcin in control and diabetic patients (Type I and Type II). The results demonstrated that the normal mean of osteocalcin in serum was (28.07 ± 4.79 ng/mL) in control group for both sexes, their ages range between (less or equal than 15-more or equal than 65) year. also, the results demonstrated a significant decrease in the concentration of osteocalcin in serum of type I and type II diabetic patients compared with control and between type I diabetic patients compared with type II and it was affected by age and sex, in control and diabetic patients, also showed a significant decrease according to BMI in control and diabetic patients (Type I and II), aswellas showed significant decrease of osteocalcin in serum of fasting and smoking of type I and type II diabetic patients compared with control, while found a significant increase according to menopausal of females and year's seasonals in control and diabetic patients (Type I and II), whereas showed a significant graduated decrease in the concentration of osteocalcin when glucose concentration increase in diabetic patients, while found a significant increase in the concentration of osteocalcin in the patients who treating with insulin, whereas showed a significant decrease in the concentration of osteocalcin when the disease's period increase in diabetic patients as well as the patients affected by other diseases compared with diabetes mellitus only.

KEYWORDS: Osteocalcin, Diabetes, Insulin, Year's seasonals, Disease's period.

INTRODUCTION

Diabetes mellitus is a systemic, chronic metabolic disease characterized by hyperglycemia, dyslipidemia, glucosuria and various concomitant clinical and clinical symptoms, as well as a high risk of early death. There are three major types of diabetes: type1, type2 and gestational

diabetes.[1] Type II diabetes is characterized by a progressive worsening in secretion functions of the produced insulin and the development of peripheral resistance to insulin, rather than a deficit in insulin production.[2]

Studies showed that may be there was relation between diabetes mellitus and discovered hormone (osteocalcin).

The name osteocalcin (*osteo* (Greek): for bone; *calc* (Latin): for lime salts; *in*; for protein) derives from Ca^{2+} affinity of this protein and from the abundance of this protein in bone tissue.[3] Osteocalcin, (OC), also called bone Gla protein or BGP or BGLAP is the most abundant noncollagenous protein of bone matrix, was the first member of the calcium binding and vitamin K-dependent protein family not associated with blood coagulation[4] osteocalcin was discover originally in chicken bone by Hauschka et al[5] and bovine bone by Price et al[6] as proteins that contain calcium-binding gamma-carboxyglutamate. Also in cattle bone by Price et al.[7] After discover osteocalcin in the bone, it was also detected in blood.[8] Also osteocalcin has been isolated from human.[9] Osteocalcin is expressed in osteoblasts, chondrocytes, odontoblasts, cementoblasts, cementocytes, besides it was reported that most non-osseous tissues express osteocalcin such as in ovary, kidney, pancreas, spleen, thymus, prostate and testis, osteocalcin immunoreactivity has been detected also in neurons.[10,11] It was reported that adipose tissues releases, osteocalcin, suggesting a possible link between adipose tissue and osteocalcin in the regulation of metabolism.[12] Osteocalcin is synthesized as a prepromolecule (molecular weight ~11000) consisting of a 23-residue leader sequence with a signal peptide, a 28-residue propeptide and a 49 residue mature protein, the hydrophobic leader sequence targets the protein for secretion and is cleaved by a signal peptidase co-translationally, after cleavage the pro-osteocalcin is γ-carboxylated because the pro-region contains a γ- carboxylation recognition site homologous to vitamin K-dependent blood coagulation factors, subsequently, the propeptide is removed intracellularly and the mature protein with a molecular weight of approximately 5900 is secreted from the cell.[11,13]

Bones are typically thought of as calcified, inert structures, but researchers at Columbia University Medical Center have now identified a surprising and critically important novel function of the skeleton, they've shown for the first time that the skeleton is an endocrine organ that regulates systemic glucose and energy metabolism, which makes it a major determinant of the development of type 2 diabetes.[11,14] The researchers found that osteocalcin, a protein made by bone- forming cells (osteoblasts) under the control of insulin,

was not a mere structural protein, but rather a hormone with totally unanticipated and crucial functions, osteocalcin fulfills the endocrine functions via its binding to the G-protein coupled receptor, GPRC6A, osteocalcin directs the pancreas'beta cells, which produce the body's supply of insulin, to produce more insulin, at the same time, osteocalcin directs fat cells to release a hormone called adiponectin, which improves insulin sensitivity.[15] This discovery showed for the first time that one hormone has a synergistic function in regulating insulin secretion and insulin sensitivity, and that this coordinating signal comes from the skeleton, additionally, osteocalcin enhances the production of insulin-producing beta cells, which is considered one of the best, but currently unattainable, strategies to treat diabetes.[16] Studies have shown that there is an association between osteocalcin and both adiposity and glucose metabolism.[17]

Aims of the research

Because there is few previous studies in Iraq about osteocalcin so, there was suggestion to study it in control and diabetic patients (Type I and II).

MATERIALS AND METHODS

This study included (70) healthy subjects (35 female, 35 male), with age matching to the patients group as control. Also, (75) patients (37 female, 38 male) with diabetes mellitus from al-waffa center for diabetic patients in Mosul city, were divided into two groups:

Group I: (22) patients from both sexes, their ages range between (less or equal than 15) years old and (16-35) years old with type I diabetes mellitus.

Group II: (53) patients from both sexes, their ages rang (36-49) years old, (50- 64)years old and (over or equal than 65) years old with type II diabetes mellitus.

Fasting & non fasting blood samples were taken and serum were separated and used to estimate the following clinical parameters:

-Osteocalcin: was measured by enzyme linked immunosorbent assay (ELISA) technique[18] using Epitope Diagnostics, Inc kit (USA). This analysis was performed in the immunity laboratory in Al-Salam hospital in Mosul city by using(BIO-TEK INSTRUMENTS, INC), USA.

-Blood glucose: was determined by the enzymatic colorimetric method[19], using Randox kit (United Kindom).

-Body mass index (BMI): was calculated as weight in kilogram divided by the squared height in meters.[20]

Data Analysis: The data obtained in the current study was analyzed using Statistical Package for Social Sciences (SPSS).

1. Standard statistical methods were used to determine the mean and standard error.
2. T-test to compare between two parameters.
3. On way Anova (Duncan-test) to compare between more than two parameters.
4. P-Value≤ 0.05 was considered to be statistically significant.[21]

RESULTS AND DISCUSSION

The results in (table 1) showed that the normal mean of osteocalcin (28.07 ± 4.79 ng/mL) in control and this was agreement with those obtained by other investigators (22)(27.5 ± 11.2 ng/mL) and approximate to the results obtained by other investigators (23,24,25,26) in control (4.6-39.5 ng/mL), (6.52-29.74 ng/mL), (14.0-42.0ng/mL) and (18.8-28.6ng/mL) respectively.

Table (1) The concentration of osteocalcin in control and diabetic patients according to age and sex.

Age (year)	Concentration of osteocalcin (ng/mL) mean ± S.E					
	Control group		TypeI Diabetes		Type II Diabetes	
	Male	Female	Male	Female	Male	Female
(≤ 15)	35.19 ± 4.33	24.01 ± 6.14	*21.72 ± 2.31	*12.35 ± 2.12		
(16-35)	31.37 ± 3.65	18.51 ± 4.89	*20.01 ± 2.59	*9.66 ± 4.09		
(36-49)	29.22 ± 3.48	15.04 ± 5.96			*18.95 ± 3.12	*8.48 ± 3.50
(50-64)	27.81 ± 4.04	35.78 ± 4.34			*18.28 ± 3.07	*26.59 ± 3.74
(≥ 65)	26.66 ± 3.11	37.13 ± 4.46			*17.67 ± 3.01	*27.43 ± 6.27
Total mean ±S.E	28.07 ± 4.79		*15.93 ± 3.15		*19.56 ± 3.26	

*Significant difference at p≤0.05.

1. Osteocalcin concentration in diabetic patients compared with control

Table (1) demonstrates that diabetic patient (type I and II) have a significantly lower osteocalcin as compared with control. The table also demonstrates that there was a significant lower osteocalcin in type I diabetic patients compared with type II diabetic patients. Type I diabetic patients represents the lowest mean and control represents the highest mean. These results were in agreement with those done by[11,17], the cause of the decreasing of osteocalcin in type I and type II compared with control may be due to osteoblast activity decreasing[27], or

it may be due to insulin secretion decrease which activate the secretion of the hormone active form of osteocalcin.[28]

2. Osteocalcin Concentration in diabetic patients compared with control group according to the age and sex

The results in table(1) showed a significant decrease in osteocalcin concentration with age increase for all age groups of males and for age groups (≤ 15) years old, (16-35) years old, and (36-49) years old of females, this indicates that osteocalcin was affected by age. The cause of osteocalcin reduction might be due to decrease osteoblast activity with age increase[29], while found a significant increase in osteocalcin concentration in the age groups (50-64) years old and (≥ 65) years old for postmenopausal females compared with premenopausal females, The cause of osteocalcin increase might be due to estrogen concentration decrease at menopause lead to imbalance between bone formation and bone resorption which this lead to make bone resorption more than bone formation, and then imbalance incidence in osteocalcin concentration in all from blood and bone so the osteocalcin concentration in blood could be more than bone in the postmenopausal females, therefore, osteocalcin concentration could be a marker for assessing the risk of osteoporosis in postmenopausal females.[30] And when we compare between males and females with all age group, the results found a significant increase in osteocalcin concentration for age groups (≤ 15) years old, (16-35) years old, and (36-49) years old for males comparable with females, the cause of increase might be due to that males bones could be longer and widen comparable with females bones(31), while showed a significant increase in osteocalcin concentration for age groups(50-64) years old and (≥ 65) years old for postmenopausal females comparable with males, this increase increase might be due to estrogen concentration decrease at menopause and its effect on osteocalcin concentration.[32]

3. Osteocalcin concentration in diabetic patients compared with control group according to BMI

Table (2) demonstrates that there was significant decrease of osteocalcin in control and type I and type II diabetic patients by increasing BMI, the cause may be due to that role of osteocalcin in lipids catabolism when it concentration increase in blood and this indicates that obesity disease correlate with diabetes disease and with bone formation decrease[33] and because of insulin resistant generation and leptin release from adipose tissue and it concentration increase in blood in obese lead to inhibition the hormone active form of

osteocalcin through role of leptin on brain through its role in sympathetic nervous system increasing.[34]

Table (2) The concentration of osteocalcin in control and diabetic patients according to BMI.

BMI kg/m^2	Concentration of osteocalcin (ng/mL) mean±S.E		
	Control group	Type I Diabetes	Type II Diabetes
25≥ normal weight	43.44 ± 8.68	*23.96 ± 2.88	*27.01 ± 8.62
26-29 over weight	29.61 ± 2.58	*13.87 ± 3.66	*18.26 ± 2.30
30≤ obese	12.15 ± 1.78	*8.59 ± 2.05	*10.57 ± 0.46

*Significant difference at p≤0.05.

4. The concentration of osteocalcin in control and diabetic patients according to menopausal of females

Table (3) demonstrates that there was significant increase of osteocalcin in control and type I and type II diabetic patients in postmenopausal females compared with premenopausal females, The cause of osteocalcin increase might be due to estrogen concentration decrease at menopause lead to imbalance between bone formation and bone resorption which this lead to make bone resorption more than bone formation, and then imbalance incidence in osteocalcin concentration in all from blood and bone so the osteocalcin concentration in blood could be more than bone in the postmenopausal females, therefore, osteocalcin concentration could be a marker for assessing the risk of osteoporosis in postmenopausal females.[30,35]

Table (3) The concentration of osteocalcin in control and diabetic patients according to menopausal of females.

menopausal of females	Concentration of osteocalcin (ng/mL) mean±S.E	
	Control group	Diabetic group
premenopausal	16.77 ± 4.74	*9.18 ± 3.27
postmenopausal	36.35 ± 3.94	*26.83±3.53

*Significant difference at p≤0.05.

5. The concentration of osteocalcin in control and diabetic patients according to fasting

Table (4) demonstrates that there was significant decrease of osteocalcin in type I and type II diabetic patients compared with control in serum of fasting individuals compared with non fasting individuals, these results were in agreement with those found by[36], The cause of reduction might be due to insulin and insulin like-growth factor-1 concentration decrease in fasting individuals which it have anabolic effects on bone and it have role in activation 25(OH)D-1α-hydroxylase in kidney.[34]

Table (4) The concentration of osteocalcin in control and diabetic patients according to fasting.

	Concentration of osteocalcin (ng/mL) mean±S.E		
	Control group	Type I Diabetes	Type II Diabetes
Non fasting	34.92 ± 8.33	*19.05 ± 7.21	*26.46 ± 8.02
fasting	21.65 ± 4.66	*11.11 ± 4.69	*13.29 ± 3.98

*Significant difference at p≤0.05.

6. Osteocalcin concentration in diabetic patients according to the smoking

Table (5) demonstrates that there was significant decrease of osteocalcin in type I and type II diabetic patients compared with control in serum of smoking individuals compared with non smoking individuals, these results were in agreement with those found by[37], The cause of reduction might be due to affect of nicotine & nonnicotine tobacco smoke which lead to osteoblast activity decrease either directly or through hormonal changes.[38]

Table (5) The concentration of osteocalcin in control and diabetic patients according to smoking.

	Concentration of osteocalcin (ng/mL) mean±S.E	
	Control group	Diabetic group
smoking	22.71 ± 4.61	*10.71 ± 2.21
Non smoking	30.76 ± 6.31	*21.95 ± 4.39

*Significant difference at p≤0.05.

7. Osteocalcin concentration in diabetic patients according to the year's seasonals

Table (6) demonstrates that there was significant graduated increase of osteocalcin in control and type I and type II diabetic patients in summer seasonal compared with spring and winter seasonals, the cause of increase might be due to vitamin D absorption through the skin from UVB radiation via sunlight, which it lead to osteocalcin synthesis increase.[39,40]

Table (6) The concentration of osteocalcin in control and diabetic patients according to year's seasonals.

year's seasonals	Concentration of osteocalcin (ng/mL) mean±S.E	
	Control group	Diabetic group
winter	19.67 ± 4.60	*10.62 ± 2.93
spring	26.73 ± 3.98	*16.87 ± 5.81
summer	39.08 ± 9.22	*25.94 ± 8.12

*Significant difference at p≤0.05.

8. Osteocalcin concentration in diabetic patients according to the blood glucose concentration

To found the relation between the osteocalcin concentration and glucose concentration, the groups of diabetic patient was divided according to the concentration of glucose into three concentrations as it is shown in table (7).

Table (7) The concentration of osteocalcin in diabetic patients according to glucose concentration.

Glucose concentration mmol/L	Concentration of osteocalcin (ng/mL) mean±S.E	
	Type I Diabetes	Type II Diabetes
7.5-10	*22.91 ± 3.58	*27.53 ± 4.72
10.1-14	*14.13 ± 3.68	*16.66± 4.02
14.1-20	*10.66 ± 4.66	*11.79 ± 3.59

*Significant difference at $p \leq 0.05$.

Table (7) demonstrates that there was significant graduated decrease of osteocalcin in type I and II diabetic patients when concentration of glucose increase.

The cause may be due to that osteocalcin has role in glucose concentration decrease when glucose concentration rises in blood, through its role in enhance insulin secretion directly vis its binding to GPRC6A receptor on pancreas β cell or indirectly via its role in its in enhance glucagon-like peptid-1,GLP-1) secretion from endocrine intestinal cell, aswell as osteocalcin enhanced glucose uptake via its binding to GPRC6A receptor on muscle, liver and adipose tissues.[41]

9. Osteocalcin concentration in diabetic patients according to the insulin

The results showed a significant increase in osteocalcin concentration in individuals treating with insulin(24.22 ± 5.53 ng/mL) compoared to individuals non treating with insulin(11.58 ± 2.65 ng/mL). These results were in agreement with those found by[41], this increase might be due to role of insulin in enhanced of secretion and releasing the hormone active form (uncarboxylated osteocalcin) to blood via positive feedforward loop which connect bone tissue and pancreas β cell.[43]

10. Osteocalcin concentration in diabetic patients according to disease's period

Table (8) demonstrates that there was significant graduated decrease of osteocalcin in type I and II diabetic patients when disease's period increase. The cause may be due to osteoblast activity decrease with disease's period increase, therefore osteocalcin secretion decrease and

osteocalcin concentration decrease in the blood, as well as disease's period increase lead to diabetic patients confronting increasing to bone diseases.[44]

Table (8) The concentration of osteocalcin hormone in diabetic patients according to disease's period.

disease's period	Concentration of osteocalcin (ng/mL) mean±S.E
(<1 year)	*26.95 ± 7.92
(1-5 year)	*15.80 ± 4.22
(>5 year)	*8.41 ± 1.55

*Significant difference at p≤0.05.

11. Osteocalcin concentration in diabetic patients according to some other disease

Table (9) demonstrates that there was significant graduated decrease of osteocalcin in diabetic patients affected by other diseases compared with diabetes mellitus only. The cause may be due to osteocalcin role in the carbohydrates and lipid metabolism, so imbalance incidence in glucose and lipid concentrations and it concentration increase in blood lead to osteocalcin concentration decrease, also the results showed osteocalcin decrease in diabetic patients affected by hypertension and hyperlipidemia compared with diabetic patients affected by bone diseases, this reduction might be due to osteoblast activity decrease in diabetes. This indicates that ostecalcin could be help in detect risk diabetic osteopenia development.[30]

Table (9) The concentration of osteocalcin in diabetic patients according to some other diseases.

		Concentration of osteocalcin (ng/mL) mean±S.E
Diabetic group		19.19 ± 9.75
Diabetic group + other diseases	1. bone diseases	*15.10 ± 2.87
	2.hypertension	*10.18 ± 1.00
	3.hyperlipidemia	8.10 ± 1.22
	4. hypertension and hyperlipidemia	*5.25 ± 0.59

*Significant difference at p≤0.05.

CONCLUSIONS

From this research we concluded that osteocalcin hormone has a major role in diabetes mellitus (type I and II), because its relation with BMI, Insulin and glucose, which lead to consider it as a marker for diabetes and obesity. This discovery potentially opens the door for novel therapeutic avenues for the prevention and treatment of type 2 diabetes.

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