OSTEOPOROSIS IN DIABETICS AND ITS RELATION TO IGF-1

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ABSTRACT

Background: Osteoporosis is a global age-related health problem in both male and female elderly, affecting the microstructure of bone. Although osteoporosis is normally associated with old age and estrogen deficiency, diabetes mellitus (DM), also contributes to and/or aggravates bone loss in osteoporotic patients Diabetes can affect bone through multiple pathways including obesity, changes in insulin levels, higher concentrations of advanced glycation end products in collagen, microangiopathy, inflammation and lower insulin-like growth factor-I (IGF1).

Aim of the work: Studying the alterations in bone metabolism in diabetic patients and its relation to IGF1.

Subjects and methods: The study included 83 participants, 53 of them were diabetics, and 30 participants were age and sex matched healthy subjects. Patients with hepatic or renal diseases, post-menopausal females,males older than 50 years, steroid medication intake,smoking, alcohol intake and other endocrinal disease causing osteoporosis were excluded. Blood samples were obtained from all subjects to measure calcium,phosphorus, parathyroid hormone, HBA1c and IGF1. DEXA scan were done to all subjects to evaluate bone quality.

Results: IGF1 concentration did not show any significant difference between total diabetic patients and control but its concentration was lower in type 1 DM than type 2 but did not reach a significant value. Whole diabetic group showed significantly lower BMD when compared to controls. In addition , type 1 DM subgroup showed lower BMD than type 2 DM subgroup .Osteoporosis and/or osteopenia showed significantly higher incidence in whole diabetic group, type 1 DM subgroup when compared to controls and in type 1 DM subgroup when compared to type 2 DM subgroup,IGF1 was negatively correlated with HbA1C in type 1 DM. No other significant differences were found in laboratory data between different studied groups except for HbA1c which was significantly higher in whole diabetic groups in comparison to control group.

Conclusion: Diabetes mellitus either type 1 or type 2 can lead to bone defects but in type 1 DM more damage to bone occurred than in type 2, IGF1 concentration is lower in type 1DM than in type 2 DM and is negatively correlated with HA1C in type 1 DM.

INTRODUCTION

Osteoporosis is a global age-related health problem in both male and female elderly, affecting the microstructure of bone. Although osteoporosis is normally associated with old age and estrogen deficiency, diabetes mellitus (DM), also contributes to and/or aggravates bone loss in osteoporotic patients **(1).**

Diabetes can affect bone through multiple pathways including obesity, changes in insulin levels, higher concentrations of advanced glycation end products in collagen, increased urinary excretion coupled with lower intestinal absorption of calcium, inappropriate homeostatic response of parathyroid hormone secretion, complex alterations of vitamin D regulation, reduced renal function, lower insulin-like growth factor-I, microangiopathy, and inflammation. All these factors affect bone metabolism in diabetic patients but with different magnitudes according to the type of D.M **(2).**

Type 1 DM results from insulin insufficiency which leads to hyperglycemia in the young. Besides the usual neurovascular complications, both male and female patients with type 1 DM have low bone mass , which may eventually lead to an increased incidence of bone fractures **(3).**

On the other hand skeletal abnormalities in type 2 DM, or non-insulin-dependent DM, appear conflicting, and the exact explanation of this is still unknown. For example, some studies reported a higher bone meniral denisity (BMD) in elderly patients with type 2 DM when compared to age-matched non-DM volunteers **(4).** In contrast, several other investigators reported a negative effect of type 2 DM on BMD **(5).**

However evidence is accumulating that patients with type 2 diabetes who have complications,

are at increased risk of certain types of osteoporotic fractures irrespective of having a high, normal or low BMD **(6).**

Thus, type 1 and 2 DM induce skeletal complications of different magnitudes. This difference could be explained by some studies which reported that type 1 DM is characterized by low circulating insulin and IGF-1 levels usually occurs in young children prior to peak bone mass attainment, whereas type 2 DM is common in adults who have already attained peak bone mass **(7).**

AIM OF THE WORK

Studying the alterations in bone metabolism in diabetic patients and its relation to insulin like growth factor (IGF1).

SUBJECTS AND METHODS

The study included 83 participants, 53 of them were diabetic (cases) recruited from Mansoura General Hospital (diabetes outpatient clinics and inpatient wards) , and 30 participants were age and sex matched healthy subjects (control group). Ethical approval was obtained.

Inclusion criteria:

- Diabetic subjects either type 1 or type 2; (based on history, fasting plasma glucose concentration ≥126 mg/dl, 2 hour plasma glucose ≥ 200mg/dl, and HbA1c ≥ 6.5%).
- Females in child bearing period.
- Males age ≤ 50 years.

Exclusion criteria:

- Smoking
- Patients taking steroids
- Hepatic or renal impairment (serum creatinine > 1.5 mg/dl)
- Post menopausal female
- History of oopherectomy
- Males above 50 years

Study design:

The study is a case-control study included 83 participants, 53 of them were diabetic recruited from Mansoura General Hospital (diabetes outpatient clinics and inpatient wards) , 10 of them were type 1 and 43 were type 2. All type 1 DM patients received insulin, while 19 patients with type 2 received insulin and 24 received oral hypoglycemic drugs. Mean age of studied patients was 40.3 (SD=6.8) years. They comprised 12 males and 41 females. In addition, 30 healthy subjects of matched age and sex were included as control group.

Data collection:

All participants in the study were subjected to:

1- History taking

Including age, sex, history of chronic disease, history of hypertension, history of DM including diabetes duration, type of anti diabetes medications , presence or absence of complications, history of back pain , history of previous bone fractures, history of steroid , heparin or anticonvulsant drug intake or any other medications for chronic disease , history of previous surgical operations and history of malnutrition.

2- Clinical examination:

- Measurement of blood pressure.
- Measurement of body weight and height with calculation of body mass index (BMI).
- Head and neck examination to exclude any abnormalities as exopthalmous and goiter.
- Chest, cardiac and abdominal examination to exclude any organ failure.

3-Laboratory investigations included

Blood sampling was done for measuring, CBC, Sr. creatinine, liver function tests, HbA1c ,parathyroid hormone ,Ca, Ph, and IGF1.

HBA1C was measured by using Stanbio Glycohemoglobin Procedure No. 0350 for its quantitative colorimetric determination in whole blood. IGF1 was measured by using immunoenzymetic assay for its quantitative measurement in serum by DIASOURCE IGF1-EASIA kit catalogue KAP 1581.Parathormone was measured by using Calbiotech Intact-PTH ELISA Kit catalog No. PT019T, for its quantitative determinationin human serum.Calcium was measured by using ACCUCARE CALCIUM ARSENAZO IIIfor its quantitative determination in serum.Phosphorus was measured by Spectrum diagnostic phosphorus reagent for its in-vitro quantitative determination in human serum.

4-Radiological investigations:

Dual energy x ray absorptiometry (DEXA) on the spine was performed to all subjects and controls at Mansoura Children Hospital. The patient lies on a soft table. The scanner passes over the lower spine and hip.

STATISTICAL ANALYSIS

The statistical analysis of data was done by using excel program (Microsoft Office 2013) and SPSS (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 20.

Kolmogorov-Smirnov test was done to test the normality of data distribution. Qualitative data were presented as frequency and percentage. Chi square and Fisher's exact tests were used to compare groups. Quantitative data were presented as mean and standard deviation.

For comparison between two groups; student t-test, and Mann-whitney test (for non parametric data) were used. For comparison between more than two groups; ANOVA and Kruskal wallis (for non parametric data) were used.

Diagnostic performance was determined by constructing a ''receiver-operating characteristic'' (ROC) curve and calculating the area under the ROC (AUROC) curve. From these curves, sensitivities, specificities and the best cut-off values, were established, which were the values that maximized the sum of the sensitivity and specificity to identify patient status.

Spearman's rank correlation coefficient was used to examine the correlation between parameters.

Logistic regression was done for prediction of osteoporosis and/or osteopenia with DM patients, variables with $p<0.05$ in univariate analysis, were included into multiple regression analysis.

N.B: p is significant if ≤ 0.05 at confidence interval 95%.

RESULTS

1- Comparison of laboratory data of different studied groups (table 1)

HbA1c was significantly higher in total, type 1 DM and type 2 DM patients than controls. Otherwise no significant differences were found in laboratory data between different studied groups.

2- IGF1 concentration in the different studied groups (table 2)

No significant differences were found in IGF1 concentrations between different studied groups, but IGF1 concentration in type 1 DM subgroup was lower when compared to type 2 DM subgroup.

3-Comparison between radiological data of different studied groups (table 3)

Total, type 1 DM and type 2 patients showed significantly lower BMD when compared to controls. In addition , type 1 DM pateints showed lower BMD than , type 2 DM. T and Z scores were significantly lower in total DM patients and type 1 DM when compared to controls and in type 1 DM when compared to type 2 DM. Osteoporosis and/or osteopenia showed significantly higher incidence in total DM, type 1 DM when compared to controls and in type 1 DM when compared to type 2 DM.

4-Comparison between DM with and without osteoporosis and/or osteopenia regarding different studied parameters (table 4)

DM patients with osteoporosis or osteopenia showed significantly lower weight, lower BMI , longer disease duration, lower BMD, T and Z scores. Otherwise, no significant differences were found between those with and those without osteoporosis or osteopenia regarding other studied parameters.

5- Performance characteristics of calcium, phosphorus, PTH, HA1c and IGF1 levels in DM patients for potential prediction of osteoporosis (table 5)

ROC analysis was conducted to identify the optimal calcium, phosphorus, PTH, HA1c and IGF1 levels for potential prediction of osteoporosis and/or osteopenia within DM patents. All of these parameters poorly discriminated those with and those without OP in DM patients.

6- Logistic regression for prediction of OP within studiedDM patients (table 6)

Logistic regression for prediction of osteoporosis and/or osteopenia within studied DM patients was done, using age, sex, BMI, DM type, therapy, duration, cacium, phosphorus, PTH, HA1c and IGF1 as covariates. Age, BMI, type 1 DM and duration had significant association with osteoporosis and/or osteopenia in studied diabetic paints in univariate analysis. Variables with significant association with osteoporosis and/or osteopenia development in univariate analysis, were included into multiple regression analysis. Only type 1 DM was considered as an independent prognostic variable for osteoporosis prediction within DM patients in multiple regression analysis ($p=0.005$, OR=2.5, 95% CI=1.67-18.14).

Table (1) Comparison of laboratory data of different studied groups

	Control (n=30)			DM (n=53)									p^{TC} (Total DM versus Control)	P^{1C} (Type1 DM versus Control)	P^{2C} Type 2 DM versus Control)	$P^{1,2}$ (Type 2 DM versus Control)
				Total (n=53)			T1D (Type 1 DM) (n=10)			T2D (Type 2 DM) (n=43)						
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max				
Calcium (mg/dL)	9.2	6.5	11	9.5	5.4	10.8	8.1	6.5	10.8	9.5	5.4	10.5	0.515	0.234	0.231	0.075
Phosphorus (mg/dL)	3.8	2	5.5	4	2.0	5.7	3.9	2.9	5.7	4	2	5.7	0.274	0.129	0.174	0.648
PTH (pg/mL)	37.2	13.4	93.1	26.5	12.2	281.1	26.2	12.2	218.0	26.5	12.2	281.1	0.229	0.155	0.361	0.759
HA1c (%)	5.9	4.7	6.6	7.2	5.4	11.5	8	5.8	11.5	7.2	5.4	11.1	<0.001	<0.001	<0.001	0.187

PTC, comparison between total DM patients versus control; P1C, comparison between type 1DM patients versus control; P2C, comparison between type 2DMpatients versus control; P1,2, comparison between type 2DM versus type 1DM.

Table (2) IGF1 concentration in the different studied groups

	Control (n=30)			DM (n=53)									p^{TC} (Total DM versus Control)	P^{1C} (Type1 DM versus Control)	P^{2C} Type 2 DM versus Control)	$P^{1,2}$ (Type 2 DM versus Control)
				Total (n=53)			T1D (Type 1 DM) (n=10)			T2D (Type 2 DM) (n=43)						
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max				
IGF1 (ng/mL)	143.5	48	355	149	12.8	542.0	95.4	12.8	214	158	13.1	542	0.596	0.303	0.318	0.078

PTC, comparison between total DM patients versus control; P1C, comparison between type 1DM patients versus control; P2C, comparison between type 2DMpatients versus control; P1,2, comparison between type 2DM versus type 1DM.

Table (3) Comparison between radiological data of different studied groups

		Control (n=30)			DM (n=53) Total (n=53)			T1D (Type 1 DM) (n=10)			T2D (Type 2 DM) (n=43)			p^{TC} (Total DM versus Control)	P^{1C} (Type1 DM versus Control)	P^{2C} Type 2 DM versus Control)	$P^{1,2}$ (Type 2 DM versus Control)
		Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max				
BMD (g/cm^2)		1.2	1.1	1.5	1.18	0.9	1.5	1.02	0.9	1.4	1.18	0.9	1.5	0.005	0.002	0.027	0.011
T score		0.5	-0.9	2.7	-0.1	-3.1	2.7	-1.55	-3.1	1.0	0.1	-2.1	2.7	0.013	0.001	0.084	0.003
Z score (%)		-0.2	-2.4	1.9	-0.8	-2.6	1.9	-2.05	-2.6	0.1	-0.5	-2.6	1.9	0.027	0.004	0.112	0.015
		N	**%**		**N**	**%**		**N**	**%**		**N**	**%**					
DEXA	**Normal**	30	100		45	86.5		4	40		41	95.3					
	Osteopenia or osteoporosis	0	0		8	15.4		6	60		2	4.7		0.046	<0.001	0.509	<0.001

PTC, comparison between total DM patients versus control; P1C, comparison between type 1DM patients versus control; P2C, comparison between type 2DMpatients versus control; P1,2, comparison between type 2DM versus type 1DM.

Table (4) Comparison between DM with and without osteoporosis and/or osteopenia regarding different studied parameters

		DM with no OP (osteoporosis and/or osteopenia) (n=45)	DM with OP (osteoporosis and/or osteopenia) (n=8)	*P*
Age (years)	**mean±SD**	41.20±5.5	35.25±11	0.147
	Range	28-50	21-52	
Sex; N, %	**Male**	8 (17.8)	4 (50)	0.067
	Female	37 (82.2)	4 (50)	
Height (cm)	**mean±SD**	158.38±5.9	154.63±5.4	0.100
	Range	150-170	144-160	
Weight (kg)	**mean±SD**	95.53±17	75±7.4	0.002
	Range	58-120	65-93	
DM type; N (%)	**T1D**	4 (8.9)	6 (75)	<0.001
	T2D	41 (91.1)	2 (25)	
BMI (kg/m2)	**mean±SD**	38.1±6.7	31.3±3.4	0.007
	Range	22.7-53.3	28. 1-37.7	
Therapy; N (%)	**Insulin**	23 (51.1)	6 (75)	0.269
	OHG	22 (48.9)	2 (25)	
Duration (years); median (range)		5 (0.2-25)	10 (5-17)	0.037
Calcium (mg/dL); median (range)		9.5 (5.4-10.5)	9.05 (6.5-10.8)	0.610
Phosphorus (mg/dL); median (range)		4 (2-5.7)	3.9 (2.9-4.6)	0.190
PTH (pg/mL); median (range)		26.5 (12.2-281.1)	27.65 (15.3-218)	0.619
HA1c (%); median (range)		7.2 (5.4-11.1)	7.75 (6.3-11.5)	0.200
IGF1 (ng/mL); median (range)		149 (13.1-542)	141.5 (12.8-418)	0.576

Table (5) Performance characteristics of calcium, phosphorus, PTH, HA1c and IGF1 levels in DM patients for potential prediction of osteoporosis

	AUC (Area under the curve)	SE (Standard error)	*p*	95% CI (confedance interval)		Cut off	Sensitivity (%)	Specificity (%)
Calcium (mg/dL)	0.557	0.139	0.611	0.284	0.830	8.4	50	88.9
Phosphorus (mg/dL)	0.646	0.093	0.192	0.463	0.829	4.3	87.5	42.2
PTH (pg/mL)	0.556	0.104	0.619	0.351	0.760	20.2	87.5	35.6
HA1c (%)	0.643	0.107	0.201	0.434	0.852	6.3	100	31.1
IGF1 (ng/mL)	0.562	0.112	0.576	0.343	0.782	217	87.5	31.1

Table (6) Logistic regression for prediction of OP within studied DM patients

	Univariate				Multivariate			
	p	OR (Odd's ratio)	95% CI (confedance interval)		*p*	OR (Odd's ratio)	95% CI (confedance interval)	
Age (years)	.034	.879	.779	.991	.976	.999	.924	1.080
Sex (females versus males)	.058	.216	.044	1.052	-	-	-	-
BMI (kg/m^2)	.015	.831	.716	.965	.093	.915	.825	1.015
DM type (T1D versus T2D)	<0.001	3.910	2.497	19.121	.005	2.510	1.674	18.138
Therapy (Insulin versus OHG)	.225	2.870	.522	15.766	-	-	-	-
Duration (years)	.016	1.109	.975	1.261	.481	1.076	.878	1.318
Calcium (mg/dL)	.245	.708	.395	1.268	-	-	-	-
Phosphorus (mg/dL)	.196	.505	.179	1.422	-	-	-	-
PTH (pg/mL)	.598	1.003	.991	1.016	-	-	-	-
HA1c (%)	.136	1.436	.893	2.310	-	-	-	-
IGF1 (ng/mL)	.544	.998	.992	1.004	-	-	-	-

DISCUSSION

The aim of this work is to study the alterations in bone metabolism and IGF1 concentration under diabetic condition. We cannot neglect the great importance of aging as an important cause in determining osteoporosis,. This is explained by many ways; firstly calcium deficiency and secondary hyperparathyroidism **(8).**Secondly the rate of bone resorption is much more than bone formation with advances in age which could be attributed to IGF1 resistance that occur with aging **(9)** , Lastly

many studies suggest that the defect in bone in elderly patients is due to defective osteoblastogensis and hence IGF1 regulating system is responsible for maintenance of osteoblastic function, a decrease in IGF1 is suspected and was attributed to a decrease in growth hormone secretion, this was supported by another study that reported that there is a decrease of IGF1 concentration in trabecular and cortical bones of neck of femur of human to one third from the age of 29 up to 92 years. So in our study the mean age of our cases was 40.3 with standard deviation ± 6, to exclude aging as a cause of bone defect.

Estrogen deficiency is associated with osteoporosis. Post-menopausal females and those with history of oophrectomy were excluded also from the study as in elderly females, the rate of bone resorption increases and cannot match bone formation. And this can be explained by the increased sensitivity of the bone to resorping effect of PTH and the decrease in renal synthesis of 1,25 dihydroxycholcalceterol induced by estrogen deficiency **(9).**

A complex relationship exists between obesity and osteoporosis and since DM has a close relation with obesity, it is important to highlight this point and discuss the effect of obesity on bone. Some studies state that obesity exerts a protective effect on bone, while others denies this protective effect .These contradictory results come from the differences in the experimental design, sample structure and selection of covariates.

Both osteoblasts and adipocytes originate from the same origin; mesenchymal stem cells, so many factors can affect the differentiation of this stem cell to either type. As regard the studies that suggest the protective effect of obesity on bone, a logic explanation exists; the mechanical effect exerted by increased body weight on bone increases the bone mass to accommodate this load. When body mass index is increased , bone mass density is also increased **(10).**

This explanation alone may be unsatisfactory, it is well known that adipocytes is not an inert organ and it secretes different molecules that can affect bone metabolism. For example, estrogen , leptin, adiponectin and aromatase enzyme. Aromatase is an enzyme that converts testosterone to estrogen which stimulates bone formation and decrease bone resorption **(10).**Leptin act on the bone marrow mesenchymal cells encouraging its differentiation to osteoblasts rather than adipocytes **(11).** Adiponectin increses bone mass by stimulating osteoblastogensis and inhibits osteoclastogenesis.

On the other side, some studies deny the protective effect of obesity on bone. As regard the study that stated the positive mechanical effect of obesity on bone, after excluding this mechanical loading effect from the statistical analysis , there were negative correlation between bone and obesity **(12).** Also some environmental factors as increasing the physical activity and increasing milk ingestion, both lead to a decrease in bone loss and decrease in body weight **(10).**

In our study body mass index in the total diabetic patients was significantly higher than in controls ($p = 0.007$), type 2 diabetic patients showed a higher body mass index than type 1 ($p=0.01$). And in relation to bone mass density total diabetic patients showed significant low bone mass density in relation to controls ($p=0.005$). In addition type 1 diabetic patients showed a lower bone mass density, than type 2, ($p=0.011$).This supports the previous studies that deny the protective effect of obesity on bone and supports the negative effects of diabetes on bone.

After analysis of the statistical results, 1 patient with type 1 DM had osteoporosis and 5 had osteopenia from the total 10 patients and on the other hand, 2 patients with type 2 DM had osteopenia and the rest 41 patients had no bone abnormality. This suggests that type 1 DM has a more dangerous effect on bone than type 2 DM.

So it is evident that both type 1 and type 2 DM, can lead to bone complications of different magnitudes. Some studies explained this difference by stating that type 1 DM is

characterized by low circulating insulin and IGF-1 levels that occurs in young children prior to the peak bone mass is gained, while type 2 DM is common in adults who have already attained peak bone mass **(13).**

On the other hand, skeletal abnormalities in type 2 DM are confusing as some studies reported a higher bone mass denisity (BMD) in patients with type 2 DM when compared to age-matched non-DM subjects (Petit et al., 2010). In contrast, several other researcher reported a negative effect of type 2 DM on BMD **(5).**

In our study we compared diabetic males and females regarding the studied parameters, and one positive finding was noticed, males with type 2 DM suffered from osteopenia more frequent than females with type 2. This could be explained by the following, firstly males with type 2 DM were significantly older than females with type 2 DM ($p<0.005$). Secondly females with type 2 DM had BMI more than males with type 2 DM but did not reach the significant value ($p=0.06$).

IGF1 is one of the accused factors in determining the negative effect of DM on bone, IGF-I enhances osteoblastic differentiation, and helps to maintain the osteoblast phenotype, and also inhibits the collagenase activity. These IGF-dependent effects leads to an increase in bone matrix deposition and preserves the skeleton **(14).** We found that IGF1 concentration is lower in type1 DM than in type 2 but actually it did not reach a significant value ($p= 0.07$) , and this is may be due to the small number of type 1 DM patients (ten patients). This can also explain the lower BMD in type1 DM patients than that found in type 2 DM patients. Some studies reported that the level of IGF1 is related to age and the level decreases with aging, and we excluded elderly patients from our study.

Duration of diabetes plays an important role in determining bone mineral density (BMD) as it was found that patients who had type 1 diabetes for more than five (5) years were 12.25 times more likely to have fracture compared to subjects without diabetes **(15).** Our study supported these findings as patients with type 1 DM had a disease duration ranging from 2 to 25 years (median=10), those patents had a low BMD compared to control ($p=0.002$), and also had a bone defect osteopenia or osteoporosis when compared to controls ($p< 0.001$).

By comparing the laboratory data in our study among the different studied groups ,as regard calcium, phosphorous and PTH, no significant differences were detected among these groups indicating that there is no hypocalcemia and secondry hyperparathyroidism that usually occur with progression in age, making DM alone an indicator for the detected bone defects and thus excluding other causes of osteoporosis.

Hyperglycemia is usually associated with adverse health effects, affecting every part in the body of diabetic patients, type 2 diabetes show that bone turnover is suppressed in patients with poor glycemic control, and bone metabolism returns to normal with normalization of glycemia. It is not proved that if hyperglycemia per se is responsible or if the associated absolute or relative insulin deficiency might be involved in bone defects. It is believed that IGF-1 tend to be low in patients with poorly controlled diabetes mellitus and improve with improving glycemic control **(16).** This supports the results of our study as IGF1 was significantly negatively correlated with HA1C in type 1 DM only, so this may suggest that insulin deficiency is responsible for the bone defects and not hyperglycemia alone . Bone defects in type 1 DM was more than that detected in type 2 DM.

Limitations of the study and further recommendations

The number of type 1 DM subgroup is small about 10 subject which did not give a good statistical results , so it is recommended that in further studies measurement of the concentration of IGF1 should be done on a large number of type 1 DM patients and comparing it with normal subjects.

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