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Bone Sialoprotein Level in Osteoporotic Diabetic Patients type II with Microvascular Complication

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Abstract:

Objective: The aim of this study is to assess the level of serum bone sialoprotein as a diagnostic biomarker in cases of diabetic bone diseases and its relation to diabetic microvascular complications**. Methods:** A total of 60 subjects; 30 diabetic patients type II with micro-vascular complications (retinopathy and/or nephropathy) as a case group and 30 healthy individuals serve as control group was recruited in this case–control study from diabetes and endocrinology clinic and internal medicine department in Beni Suef university hospital. The biochemical and metabolic parameters and bone turnover marker will be assessed in all patients. **Results:** Serum bone sialoprotein (BSP) was found to be significantly higher in diabetic patients with microvascular complication compared to normal control group. Moreover, bone sialoprotein (BSP) was positively correlated to osteoporosis of lumbar spine. **Conclusion:** Bone sialoprotein increase could be used as a biomarker of diabetic bone disease diagnosis and could be a predictor of spinal osteoporotic fractures in diabetic patients.

Keywords: bone sialoprotein, diabetes type II.

1. Introduction:

Osteoporosis is a prevalent public health disease; it affects millions of people all over the world. It is characterized by diminished bone mass and changes of bone microarchitecture [1], so it reduces bone strength which increase the predisposition to bone fracture. Bone density is defined as grams of mineral in area or volume. Also, it is determined by peak bone mass and amount of bone loss [2]

Diabetes Mellitus type II is a common age-related disease that considered a growing health and economic burden in older populations. Many studies suggest the strong relation between type 2 diabetes and the high risk of bone fragility [3].

Many studies have illustrated the high risk of bone fracture in diabetic patients even with normal or increased bone mineral density [BMD]. The risk of bone fracture in diabetic patients increases with longer disease duration, poor control, and presence of diabetic complications [4].

There are many studies reported the association between the complications of diabetes and reduced BMD. Decreased BMD proved to be associated in diabetic patients with neuropathy [4, 5], in patients with early f diabetic chronic renal disease [6] and in patients with retinopathy [7]. Some studies revealed that diabetes may decrease bone quality via regulation of bone cells [8].

Type II diabetes mellitus alters bone health in advanced stages of the disease when lack of insulin, glucose toxicity, advanced glycation end products [AGEs], fat metabolites including pro-inflammatory cytokines and adipokines pathway inhibition and, probably, microvascular disease of the bone all these factors sharing in impairment of the mechanotactic function of osteocytes, turnover of bone and collagen properties [9].

It was proved that bone turn over markers are more sensitive than BMD in assessment of risk of fracture in diabetic patients due to the relation between bone turn over markers and metabolism of glucose [10].

Bone sialoprotein, as a bone turn over marker, is a component of mineralized tissues such as bone, dentin, cement, and calcified cartilage. BSP is a significant component of the bone extracellular matrix and has been suggested to constitute approximately 8% of all non-collagenous proteins found in bone and cementum. [11].

Prior studies demonstrated that there was a higher level of bone sialoprotein in patients with high bone turn over as in postmenopausal female, patients with hyperparathyroidism, patients with Paget's disease, and breast cancer patients' that had metastasis to bone, with the highest levels in patients with untreated multiple myelomas, indicating a relationship with bone remodeling [12].

However, few studies address the association between sialoprotein bone turn over marker and diabetic microvascular complications. We hypothesize that circulating levels of sialoprotein can be helpful for earlier diagnosis of osteoporosis and prevention of bone fracture in type II diabetic patients.

2. Patients and Methods:

2.1Type, site and time of study:

This case–control study was conducted in diabetes and endocrinology clinic and internal medicine department in Beni Suef university hospital from January 2018 to June 2018.

The study included 2 groups:

- Diabetic type II includes 30 patients with diabetic micro-vascular complications [retinopathy and/or nephropathy].
- Control group included 30 healthy individuals.

2.2 Inclusion and exclusion criteria:

1 -Inclusion criteria

1. The diagnosis of type 2 diabetes carried out and/ or confirmed following the American Diabetes Association criteria [13].

2. The diagnosis of retinopathy carried out by Dilated indirect ophthalmoscopy coupled with biomicroscope and seven-standard field stereoscopic 30° fundus photography according to the American Academy of Ophthalmology recommendation [14].

3. Diabetic kidney disease is diagnosed according to diabetic nephrology reformulated classification released in 2014 [15].

4. Age between 30 -70

2 -Exclusion criteria

1. Cancer patients .

2. Chronic disorders. [renal failure, autoimmune diseases, and chronic immobilization]

3. Steroid treatment.

2.3 All patients were subjected to:

A. All patients were asked for demographic and clinical characteristics including:

 Sex, age, diabetes duration, age of onset of diabetes [years], cigarette smoking status, current use of medications, height and weight were measured using standard anthropometric techniques, body mass index was calculated as body weight [kg]/[height [m^2]

 Blood pressure was measured twice after a 10 min seated rest.

 FRAX score to assess fracture risk probability [lebanease version].

 Physical activity was assessed according to specific scale as follow:

Vigorous: [30 min, at least 5 days per week]

Moderate: [30 min, 3–4 days per week]

Low: [10–30 min, less than 3 days per week]

Very low: no daily activity [16].

B. Blood samples were collected after an overnight fasting. The sera were kept at −80 °C until analysis and the samples will be analyzed for the following - :

- Fasting glucose.
- Total cholesterol [TC], high-density lipoprotein [HDL], low-density lipoprotein [LDL], triglyceride [TG].
- Blood urea nitrogen [BUN], and creatinine.
- Urine microalbumin and creatinine levels .
- Glycated hemoglobin [HbA1c] .
- Serum bone sialoprotein as marker for bone turn over.
- ALT: alanine aminotransferase
- AST: aspartate transaminase
- Serum calcium [total]
- Estimated GFR calculated by CKD-EPI equation

The kit of assay of bone sialoprotein uses enzyme-linked immune sorbent assay [ELISA] based on the Biotin double antibody sandwich technology to assay the Human Bone sialoprotein. Add Bone sialoprotein to the wells, which are pre-coated with Bone sialoprotein monoclonal antibody and then incubate. After that, add anti BSP antibodies labeled with biotin to unite with streptavidin-HRP, which forms immune complex. Remove unbound enzymes

after incubation and washing. Add substrate A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of Human Bone sialoprotein [BSP] are positively correlated.

C. Radiological work up:

All patients were subjected to dual-energy x-ray absorptiometry or DEXA scan to assess the severity of osteoporosis. The assessment was done using lunar GE device in immunology unit in Beni-Suef university hospital. Interpretation of diagnosis of osteoporosis was done using bone density test results are reported using T-scores.

2.4 Statistical analysis:

Data analysis was performed using SPSS v. 25 (Statistical Package for Social science) for Windows. Description of quantitative variables was done in the form of mean, standard deviation (SD), description of qualitative variables was done in the form of numbers (No.) and %. Comparing between quantitative variables was carried out by independent t-test that was used to test the difference between the means of 2 groups of a scale variable. Comparing between categorical data was done using the Chi square test, to test the statistical difference between the 2 groups. Correlation was done to test the association between 2 scale variables. The significance of the results was assessed in the form of P-value that was differentiated into non-significant when P-value > 0.05 and significant when P-value ≤ 0.05.

2.5 Ethical Considerations and Review:

Study protocol was approved by Faculty of Medicine, Beni-Suef University, Research Ethics Committee.

3. Results :

The study included 30 diabetic patients with microvascular complications and 30 healthy controls with age 49.3±6.1 years and 49.9±5.9 years for both groups, respectively. There were 23.3% males in the diabetic group versus 33.3% in the control group while females represent 76.7% in diabetic group compared to 66.7% in the control group. Regarding the baseline characteristics, there is statistically significant low mean height with p-value <0.05 among diabetic patients with microvascular complications. On the other hand, there is no statistically significant difference with p-value >0.05 between study groups as regards age and blood pressure measures, anthropometric measures [weight, and BMI], smoking, and physical activity which indicated proper matching between study groups **[Table 1].**

Table [1]: Comparisons of demographic data and baseline characteristics in both study groups.

Variables [mean±SD]	Diabetic patients with microvascular complications group No=30 (%)	Healthy control group No=30 (%)	P-value
Age [years]	49.3±6.1	49.9±5.9	0.601
Sex no [%] Males Females	7[23.3%] 23[76.7%]	10[33.3%] 20[66.7%]	0.393
Disease duration [years]	**13.4**±4.3	-----	----
Blood pressure			
Systolic	133.3±29.9	125.6±7.4	0.300
Diastolic	80.3±16.5	78.7±6.2	0.703
Anthropometric measures			
Weight [kg]	75.3±11.1	80.8±10.8	0.070
Height [cm]	159.3±6.5	167.9±8.6	**<0.001***
BMI [kg/m^2]	30.2±5.6	28.8±4.7	0.104
Smoking no [%]			
No	29[96.7%]	24[80%]	0.105
Yes	1[3.3%]	6[20%]	
Physical activity no[%]			
5 days / week	0[0%]	1[3.3%]	0.108
2-3 days / week	1[3.3%]	4[13.3%]	
Daily	29[96.7%]	25[83.3%]	

P-value is significant

Regards the laboratory parameters, there is statistically significant low mean serum calcium, and e-GFR level also high mean of HBA1C%, triglyceride, AST, creatinine, and albumin/creatinine ratio among diabetic patients with microvascular complications compared to healthy controls with p-value <0.05. On the other hand, there is no statistical significance difference between study groups with p-value >0.05 as regards other investigations [cholesterol, and ALT level] **[Table 2].**

Table [2]: Comparisons between the studied groups regarding the laboratory investigations:

Variables [mean±]	Diabetic patients with microvascular complications group No=30	Healthy control group No=30	P-value
Serum Calcium mg/dL	**8.4**±0.46	8.8±0.59	**0.001***
HBA1C %	**9.3**±2	4.9±0.31	**<0.001***
Cholesterol mg/dL	191.4±23.5	179.4±15.1	0.070
Triglyceride mg/dL	**141.4**±35.8	101.2±14.3	**<0.001***
ALT U/L	29.2±15.5	24.9±3.5	0.074
AST U/L	**37.4**±14.8	25.5±3.3	**<0.001***
Creatinine mg/dL	**1.7**±0.4	0.8±0.1	**<0.001***
eGFR ml/min/1.73m2	**41.7**±16.5	84.6±3.1	**<0.001***
Albumin/ creatinine ratio mg/g	**965.9**±99.7	20.3±7.2	**<0.001***

P-value is significant

Concerning the sialoprotein level in both groups, there is statistically significant high mean of bone sialoprotein level among diabetic patients with microvascular complications compared to healthy controls with p-value <0.05.

The mean FRAX% score in diabetic patients with microvascular complications, T score AP, femur neck and radius was 2.5±1.8, 1.3±0.65, -1.4±0.83 and 2.1±1.6 for all parameters, respectively **[Table 3] .**

Table [3]: Comparisons of bone sialoprotein in the two studied groups.

Variable mean±SD	Diabetic patients with microvascular complications group No=30	Healthy control group No=30	P-value
Sialoprotein	**51.1±9.8**	**37.4±5.3**	0.001*

P-value is significant

There is no statistically significant linear correlation between bone sialoprotein and any of age, disease duration, and routine laboratory parameters [p-value >0.05] **[Table 4].**

Table [4]: Correlation between Bone sialoprotein with age, disease duration and different laboratory investigations in diabetic patients with microvascular complications.

Variables	Bone sialoprotein in diabetic patients with microvascular complications	
	r	p-value
Age [years]	-0.02	0.9
Disease duration[years]	0.08	0.7
Serum Calcium [mg/dL]	0.3	0.08
HBA1C %	0.2	0.3
Cholesterol mg/dL	0.1	0.6
Triglyceride mg/dL	0.2	0.2
ALT U/L	-0.09	0.6
AST U/L	-0.08	0.7
Creatinine mg/dL	-0.19	0.3
eGFR ml/min/1.73m2	0.09	0.6
Albumin/ creatinine ratio mg/g	-0.22	0.2

r: correlation coefficient

There is statistically significant moderate positive correlation with [r=0.46, p-value 0.01] between bone sialoprotein and T-score AP but there is no statistically significant correlation with p-value >0.05 with FRAX% and other T-Scores among patients with
microvascular complications, which indicated increase in bone sialoprotein will associated with increase in T-score AP.

4. Discussion:

The current study showed that there is statistically significant e high mean of bone sialoprotein level among diabetic patients with microvascular complications compared to healthy controls with p-value <0.05

This result is agreed with Mark Luedde et al.,2018 study who stated that different metabolic diseases as type 2 diabetes, hyperinsulinemia and obesity were associated with elevated serum levels of osteoblast related proteins including bone sialoprotein [17].

This result can be explained by the study conducted by Tsai and his colleagues, who illustrated that the osteogenic capability of diabetic patient's Human mesenchymal stem cells [hMSCs] was highly decreased in comparison to the same cells from non-diabetic subjects. Giving that the activities of MSCs are important to bone homeostasis, the downregulated osteogenic capability of hMSCs may result in decreased bone formation, contributing to development of osteoporosis [18].

Moreover, Chen in 2003 explained that as he found an evidence suggested that medial calcification in diabetic patients is an active, cell mediated process, like that observed in end-stage renal disease patients', in which vascular smooth muscle cells [VSMCs] expressed some bone matrix proteins that facilitate regulate the process of calcification. Many bone-associated proteins as bone sialoprotein, alkaline phosphatase, and type I collagen. These proteins have been identified in histologic sections of the vessels in patients with diabetes [19].

The current study showed that there is no statistically significant correlation with p-value >0.05 between bone sialoprotein and any of age, disease duration, laboratory parameters as serum calcium, HbA1c, ALT, cholesterol, triglycerides, creatinine, glomerular filtration rate and albumin creatinine ratio in diabetic patients with microvascular complications .

This result was supported by the result found by Wierzbicka,et al., (2018) who did not find any correlation between bone mineral density, bone structure or estimated bone strength parameters and proteins and duration of disease or degree of diabetes control in patients with microvascular complication [20].

Our results showed that there is statistically significant moderate linear positive correlation with p-value <0.05 between bone sialoprotein and T-score AP but there is no statistically significant correlation with p-value >0.05 with FRAX% and other T-Scores among patients with microvascular complications, which indicated increase in bone sialoprotein will associated with increase in T-score AP .

Also, this also match the results of Mosely, 2012 who found that type 2 diabetes patients have decrease bone mass that associated with decrease in bone formation and bony microangiopathy [21]. In addition to Ganeko et al.,2015 study, they explained that advanced glycation end products [AGEs] are considered risk factor for decreased bone formation because of sequential nonenzymatic addition of carbohydrate molecules to protein amino groups. AGEs are deposited in bone and decrease bone formation by increasing the inflammatory state [22].

5. Conclusion and Recommendations:

In conclusion the sialoprotein level is significantly increased in diabetic patients with

microvascular complications and significantly correlated with T-score AP. This study recommends strict control of microvascular complications among diabetic patients as it increase the bone turn over markers that affect the bone formation, density, function and make patient more susceptible to fracture.

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