INTERRELATIONSHIP BETWEEN BLOOD GLUCOSE LEVEL AND INCIDENCE OF BONE DISEASE IN DIABETES

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SUMMARY: Patients, with high blood glucose (diabetes) suffer from a number of disorders including bone disease.

The present investigation was undertaken to study the probable mechanism by which bone disturbances occur in patients with varying levels of serum glucose.

In females with serum glucose within the range of 110-270 mg/dL serum ionized calcium (Ca^{2+}) level decreased from 4.42±0.50 to 3.46±0.48 mg/dL ($p<0.05$), whereas serum total calcium (Ca) level did not change significantly. Serum phosphorous concentration increased from 4.21±0.56 to 5.19±1.21 mg/dL ($p<0.05$) and serum alkaline phosphatase (ALP) activity elevated from 69.84±9.70 to 103.53±30.73 IU/L respectively ($p<0.05$).

In male with the same serum glucose levels (110-270 mg/dL) serum total calcium (Ca) level decreased from 9.77±0.50 to 7.7±2.12 mg/dL ($p<0.05$) and serum ionized calcium (Ca^{2+}) decreased from 4.18±0.65 to 3.45±0.43 mg/dL ($p<0.05$). Serum phosphorous concentration elevated from 3.90±0.60 to 5.5 ± 1.58 mg/dL ($p<0.05$), and serum ALP was elevated from 69.85±9.71 to 106±34.5 IU/L. Elevated serum ALP activity was related to bone fraction isoenzymes as examined by heat stability test (56°C in 10 min). Serum protein level in both male and female patients was decreased in comparison to healthy controls.

The probable relationship between hyperglycemia and occurrence of bone disease in diabetic patients has been discussed in this manuscript.

Key Words: Serum alkaline phosphatase, diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a recognized metabolic disorder which affected many population of the world. Only approximately 10 millions American population suffer from this disease and it is the third leading cause of death in the United States (1). A large number of syndromes and diseases might be associated with an inappropriate elevation of high fasting plasma glucose.

Renal dysfunction might occur following hyperglycemia with subsequent vitamin D deficiency which is very important in calcium homeostasis and in turn bone metabolism (2).

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Table 1: Determination of serum parameters related to bone metabolism in 66 diabetic female patients and healthy female controls.

Serum parameters	Control	Serum glucose (mg/dL)		
	73-81	110-160	160-200	200-270
Total Ca (mg/100)	9.70±1.16	9.31±2.21	9.21±1.70	9.16±1.92
Ionized Ca (mg/100)	4.42±0.51	3.46*±0.43 p<0.05	3.43*±0.50 p<0.05	3.51*±0.51 p<0.05
Phosphorous Ca (mg/100)	4.21±0.56	4.84±1.33	5.04±1.10	5.70*±1.20 p<0.05
ALP (IU/L)	69.84±9.70	104.18*±30.06 p<0.05	104.31*±38.83 p<0.05	102.10*±23.30 p<0.05
Total protein (g/100)	6.73±0.68	5.72*±1.8 p<0.05	5.72±1.66	5.36*±1.29 p<0.05

Blood was withdrawn from both controls and diabetes and sera were prepared. The serum levels of total calcium, ionized calcium, phosphorous, alkaline phosphatase and protein were determined according to the methods. Each figure is the mean±SE. The number of controls was 10 post-graduate female students.
* indicates statistical significant.

It has been postulated that diabetic bone disease is characterized to low bone turnover resulting from either impaired secretion of parathyroid hormone (PTH) or refractoriness of osteoblasts to PTH. In this regard, observations of Inaba *et al.* (3) show that there is impaired PTH secretion which might be due to the low bone turnover in those patients maintained on regular hemodialysis. Paula *et al.* (4) has also postulated the relationship between diabetes bone disease and secretion of parathyroid hormone. These authors concluded from their investigations that PTH secretion is impaired in patients with poor controlled diabetes.

Diabetic osteoporosis has been also reported by a number of other researchers throughout the world (5-7). Osteomyelitis secondary to diabetic foot infections can lead to proximal amputation if not diagnosed in time and treated accurate manner. Observations of Kaleta *et al.* (8) show that in combination with clinical suspicion in diabetic foot infection the erythrocyte sedimentation rate is highly predictive of osteomyelitis, and that the value of 70 mm/h is the optimal cutoff to predict accurately the presence or absence of bone infection in diabetes.

Up to our present knowledge no comprehensive study has been reported in the literature in concern with the level of serum glucose and the appearance of bone disturbances in male and female diabetic patients. Therefore, the present investigation has been established to study the relationship between changes in serum bone related parameters and occurrence of bone disease in diabetic male and female patients.

MATERIALS AND METHODS

Sixty six female and thirty four male diabetic patients all referred from Diabetic Research Center of Isfahan University of Medical Science and also from Isfahan Institute of diabetes. Male and female healthy post-graduate students took part in this project as control groups.

Blood samples from patients and healthy controls were withdrawn. Sera were separated from blood cells using centrifugation technique and immediately used for glucose and other biochemical determinations.

Serum glucose was determined by the method of Trinder (9). Serum calcium and phosphorous concentrations were determined using methods of Grindler (10) and Ghalambor (11).

Table 2: Determination of serum parameters related to bone metabolism in 34 diabetic male patients and healthy male controls.

Serum parameters	Control	Serum glucose (mg/dL)		
	82-92	110-160	160-200	200-270
Total Ca (mg/100)	9.77±0.50	8.30±1.96	7.80*±2.01 p<0.05	7.00±2.41
Ionized Ca (mg/100)	4.18±0.65	3.70*±0.45 p<0.05	3.46*±0.5 p<0.05	3.21*±0.35 p<0.05
Phosphorous Ca (mg/100)	3.90±0.60	5.10±1.95	5.48*±1.60 p<0.05	5.94*±1.19 p<0.05
ALP (IU/L)	69.85±9.71	105.98*±30.06 p<0.05	106.31*±38.83 p<0.05	107.40*±23.35 p<0.05
Total protein (g/100)	7.72±0.27	6.12*±0.80 p<0.05	6.17*±1.2 p<0.05	6.45*±1.22 p<0.05

Blood was withdrawn from both controls and diabetes and sera were prepared. The serum levels of total calcium, ionized calcium, phosphorous, alkaline phosphatase and protein were determined according to the methods. Each figure is the mean±SE. The number of controls was 10 post-graduate male students.
* indicates statistical significant.

Alkaline phosphatase activity was determined by the method of Bowers and McComb (12) using para-nitro phenyl phosphate as substrate. Heat stability test was used to determined bone isoenzyme alkaline phosphatase as reported by Moss and Whitby (13). Students t-test was used for statistical analysis ($p<0.05$).

All chemicals used in this project were of analytical grade and purchased from Sigma Chemical Company.

RESULTS

Initial blood analysis was carried out to determine fasting blood glucose of sixty six female and thirty four male patients. All patients were then grouped according to the level of their fasting blood glucose.

Serum levels of total and ionized calcium, phosphorous, alkaline phosphatase and protein were then determined and compared with healthy controls. Data obtained are presented in Tables 1 and 2.

Female patients were grouped to three classes according to the level of their serum glucose as (110-160, 160-200 and 200-270 mg/dL). Serum total calcium did not change significantly ($p<0.05$) in diabetic patients. Whereas serum ionized calcium was reduced by approximately 22% in comparison to healthy female controls. The average serum ionized calcium in all three groups were (3.46±0.43, 3.43±0.50 and 3.50±0.51 mg/dL) and of controls it was 4.42±0.5 mg/dL. Serum phosphorous level was elevated in patients by 23% in comparison to the controls.

The average serum phosphorous in all three group female diabetic patients were (4.84±1.33, 5.04±1.11 and 5.7±1.20 mg/dL) in comparison to the level of controls (4.21±0.56 mg/dL). Serum alkaline phosphatase level was then determined. It was found that there was approximately 48% elevation in ALP activity of diabetics in comparison to that of the control group.

The ALP activities were (104.18±30.06, 104.31±38.83 and 102.10±23.30 IU/L) in the diabetics in comparison to 69.84±9.70 IU/L for healthy controls.

Same serum biochemical parameters were then determined in 34 diabetic male patients. Data obtained are presented in Table 2. Unlike, serum total calcium in female patients, there was a 21% elevation in comparison to serum total calcium in healthy controls.

Accordingly, their serum total calcium was (8.30±1.96, 7.80±2.01 and 7.0±2.4 mg/dL) in comparison

Table 3: The effect of heat (56°C) on sera alkaline phosphatase activity in diabetic patients.

Patients no	Enzyme unit pre-incubation	Enzyme unit post-incubation	% Remaining activity	Patients no	Enzyme unit pre-incubation	Enzyme unit post-incubation	% Remaining activity
1	144.1	10.7	7.4	15	95.3	9.7	10.1
2	164.1	20.5	12.5	16	141.2	4.9	3.5
3	141.4	11.2	7.9	17	125.2	11.5	9.2
4	146.1	14.3	9.8	18	135.9	4.2	3.1
5	144.3	12.4	8.6	19	151.5	17.8	11.8
6	140.4	12.1	8.6	20	125.6	19.7	1.3
7	148.4	15.3	10.2	21	140.3	11.9	8.5
8	142.6	15.5	10.9	22	158.9	18.3	11.5
9	132.8	10.0	7.5	23	145.4	15.1	10.4
10	110.6	9.1	8.2	24	147.8	23.9	16.2
11	118.2	13.4	11.3	25	145.9	11.9	7.6
12	99.3	3.8	3.8	26	143.1	14.2	9.9
13	132.9	9.7	2.7	27	144.1	11.7	8.1
14	100.4	10.0	10.2	28	125.1	12.3	9.9

Sera were prepared and total alkaline phosphatase activity were determined in patients before and after heating at 56°C. Data obtained are presented as pre- and post-incubated sera alkaline phosphatase. The percent of remaining alkaline phosphatase was also presented.

to 9.77± 0.50 mg/dL for the controls. Serum ionized calcium were reduced approximately by 18% (3.7±0.45, 3.46±0.5 and 5.94±1.19 mg/dL) in comparison to the serum ionized calcium for healthy controls (4.18±0.65 mg/dL).

When serum phosphorous concentration was determined in male diabetic patients it was found that approximately 40% elevation in serum phosphorous was the same when compared with its controls.

Serum phosphorous level in healthy control was 3.90 ±0.60 mg/dL and in male diabetic patients were 5.10±1.95, 5.48±1.60 and 5.94±1.19 mg/dL respectively.

Approximately the same elevation in ALP activity was seen in comparison to the level seen in female diabetic patients (Table 2).

The serum protein concentration was decreased in both male and female with varying serum glucose levels. The serum elevated alkaline phosphatase was then tested for bone alkaline phosphatase isoenzymes fraction by heating the elevated serum at 56°C for 10 min.

Data presented in Table 3 show that majority of elevated alkaline phosphatase was related to bone fraction since less than 10 per cent of activity remained following incubation.

DISCUSSION

The present investigation was conducted on 66 female and 34 male diabetic patients with fasting blood glucose levels within the range of 110 to 270 mg/dL. In this project attempts have been made to explain the probable relationship between hyperglycemia (elevation of serum glucose) and the appearance of bone disease in female and male patients. In female patients serum total calcium did not alter significantly, whereas ionized calcium concentration decreased by approximately 22% (Table 1). In male patients both serum total and ionized calcium decreased by approximately 21% in comparison with healthy controls (Table 2). This discrepancy in total calcium level might be due to the menstrual cycle in female patients which by itself could influence on bone metabolism (3). Observations of

Paula *et al.* postulated that PTH secretion is impared in patients with poorly controlled diabetes. They suggested that hypocalcemia in these patients caused an elevation of PTH with subsequent bone disturbances (4).

Inaba *et al.* (3) reported that serum PTH levels were significantly lower in hemodialyzed patients with diabetes than those without diabetes. They have found that serum osteocalcin and deoxypyridinoline levels were significantly lower in patients with diabetes. Although serum bone specific alkaline phosphatase and pyridinoline levels did not differ significantly between the two groups of patients.

Our heat stability test showed that the elevated serum alkaline phosphatase was bone fraction isoenzyme in both male and female (Table 3).

Our observations show that changes in the serum phosphorous and alkaline phosphatase concentrations of both sexes were comparable and increased in parallel to the gradual elevation of serum glucose. Our findings are consistent with the former studies by Stephen *et al.* and also by Maxwell *et al.* who indicated, the elevation of bone alkaline phosphatase by visual interpretation of electrophoresis patterns (14, 15).

Insulin has been reported of influence the phosphorus excretion by renal tubules. The higher the level of plasma glucose, the higher the level of phosphorus as indicated in Tables 1 and 2.

It was also shown that insulin and insulin like growth factors (IGF-1, IFD-2) have an influence on bone metabolism itself and other growth factors, cytokines and hormones determine changes in diabetic bone metabolism (16). On the other hand, amylin is a 37 amino acid peptide co-secreted with insulin by pancreatic beta-cells. Its absence in diabetic is frequently associated with osteopenia. Amylin binds to calcitonin receptors, lowers plasma calcium concentration, inhibits osteoclast activity, and stimulates osteoblasts. Observations of Horcajada-Molteni (17) showed that addition of amylin to induced diabetic rat by streptozotocin, improved bone indices, apparently by inhibiting resorption and stimulation bone formation.

We may now reach to this conclusion that the lower the level of insulin in diabetes the higher the blood glucose concentration and the higher plasma phosphorus concentration which in turn might be lead to the reduction of serum calcium and the elevation of plasma alkaline phosphatase following bone resorption.

More investigation should be done to elucidate the exact mechanism by which bone disease appeared in patients with high level of blood glucose.

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