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Evaluation of Bone Resorption in Type I diabetes Mellitus Patients by Measuring Urinary Total Deoxypyridinoline (U. DPD) as a Biomarker of Bone Resorption

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Abstract:

Background: Diabetes mellitus is a common disease in most parts of the world, the metabolic abnormalities of diabetes potentially affect bone metabolism, structure, mineral density, and bone resorption, as part of physiological bone turnover.

Aim: This study aimed to estimate urinary total deoxypyridinoline as a biomarker of mature bone collagen degradation and bone resorption in type I diabetes mellitus patients and evaluate its relationship with fasting blood sugar, serum bone minerals profile and the duration of disease and also to investigate the degree of severity of bone resorption and the probability of fracture risk.

Patients and methods:165patients with type I diabetes mellitus from Diabetes mellitus and Endocrine Disease Centre at AL -Nasiriyah city in Thi-Qar province, Iraq were studied.100 (60.6%) were female and 65(39.4%) were male, age was between(25-65) year, mean44.27±11.1,their duration with type 1 DM disease ranged from 5 months to 10 years, they were classified into three groups: the first group included 45 (27.3 %) patient their duration with type 1 DM were less than one year, the second group included 65 (39.4%) their duration with type I DM were 1 to 5 year and the third group included 55 (33.3%) patient their duration with disease were from 6 to 10 year. Fasting blood venous and first-morning urine samples were collected for biochemical investigation according to standard methods and Urine DPD was measured by the quantitative competitive enzyme immunoassay. Also, this study included 165 normal people selected as a control group. The study duration was from May to December 2017.

Results: A significant increased ($P<0.05$) in fasting blood sugar (266.96±33.8 mg/dl), inorganic phosphorous (9.5±0.72mg/dl), and U.DPD (132.34±18.67ng/ml) were seen in type 1 diabetic patients as compared to control group (80 ±5.31mg/dl, 3.38 ± 0.22 mg/dl, and 3.48 ±1.01 ng/ml) respectively. Total calcium (6.10 ± 0.49 mg/dl) and magnesium(1.48 ±0.41 mg/dl) levels in type I diabetic patients groups were found to be significantly lower ($P < 0.05$) than control group (8.89 ± 0.39 mg/dl and 1.87 ± 0.21 mg/dl) respectively. U.DPD positively related to fasting blood sugar and phosphate, but adverse relation founded with both total calcium and magnesium. There are significantly increased ($P < 0.05$) in fasting blood sugar, U.DPD, and inorganic phosphorus, also significantly decreased ($P < 0.05$) in total calcium and magnesium with the increased prolonged of disease.

Conclusion: Diabetic patients with type I have an elevation in urinary total deoxypyridinoline as a marker of bone resorption and degradation product of bone matrix which is related to hyperglycemia and abnormal bone mineral profile like hypocalcemia, hyperphosphatemia, and hypomagnesemia, also this study see that poor glycemic control and type IDM duration seem to play a key role given the lower bone density and that is mean those patients group underlying bone alterations and increased the probability of low bone density and quality then increased bone fracture risk.

Key words: Urinary total deoxypyridinoline, bone minerals profile, bone resorption, type I diabetes mellitus.

Introduction:

Type 1 diabetes mellitus (T1DM) results from an absolute deficiency of insulin, which is most commonly due to the auto-immunological destruction of the insulin-producing pancreatic β cells but which can be caused by other etiologies. As the prevalence of diabetes is increasing worldwide, research on impaired bone health in diabetes mellitus is gaining a lot of attention (Vikram *et al*, 2017).

Bone is a specialized connective tissue consisting primarily of glycoproteins and proteoglycans, together with cartilage, makes up the skeletal system and three

major cell types: osteoclasts, osteoblasts, and osteocytes. The fibers of bone are mostly composed of type-I collagen impregnated with mineral. Bone minerals found in the form of hydroxyapatite [Ca10 (PO4)6 (OH)2] (Datta *et al*, 2008). Bone is constantly undergoing a metabolic process called remodeling, this includes a degradation process or bone resorption, mediated by the action of osteoclasts, and a building process, bone formation, mediated by the action of osteoblasts. Approximately 90% of the organic matrix of bone is type I collagen a triple helical protein, its cross-linked by specific molecules that provide rigidity and strength. Cross-links of mature type I collagen in bone are Pyridinoline (PYD) and deoxypyridinoline (DPD) (Hesley *et al*, 1998, Delmas. 1993).

PYD and DPD are formed during the extracellular maturation of fibrillar collagens, they bridge several collagen peptides and mechanically stabilize the collagen molecule as in figure 1(A) (Von der. 1999).

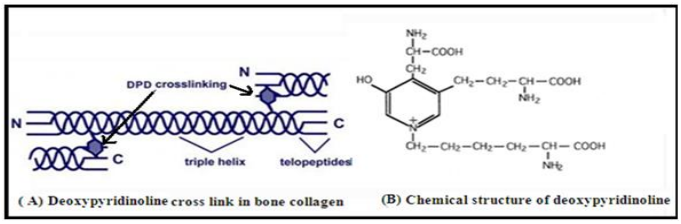

Figure1: Deoxypyridinoline. (A) Deoxypyridinoline cross linked in bone collagen (B) Chemical structure of deoxypyridinoline (Robins *et al*, 1994).

PYD is found in cartilage, bone, ligaments, and vessels, while DPD is almost exclusively found in bone and dentin. Since bone has a much higher turnover than cartilage, ligaments, vessels or tendons. DPD reflects degradation of mature collagen only specific to bone (Seibel,2005, Vasikaran *et al*, 2011), less invasive than blood and DPD isn't taken up from food also, DPD is independent of nutrition and therefore a specific marker of bone resorption (Delmas, 1993; Risteli and Risteli, 1993 and Hesley, 1998).

DPD is formed by the enzymatic action of lysyl oxidase on the amino acid lysine. In humans, the total pool of urinary DPD is approximately 45% free while the remaining fraction is bound to oligopeptides ranging from small linear peptides to very large cross-linked structures in excess of
10,000 Da. Free and binding cross-links appear in healthy individuals and those with metabolic bone diseases, thus providing the rationale for measuring the combined total forms of DPD. In contrast to formerly used parameters such as hydroxyproline. Deoxypyridinoline can be a more sensitive marker than hydroxyproline, with some advantages, such as its quantitation in a urine specimen and its high bone specificity (Delmas. 1993; Risteli .1993 and Rivero *et al*, 1997 and Hesley, 1998).

The relationship between diabetes and bone disease is complex (Isidro, 2010) in addition, osteoporosis is a silent disease without obvious symptom and evidence until the occurrence of fracture. The diagnosis of osteoporosis is based on the quantitative analysis of bone mineral density (BMD) by dual energy *x* - ray absorptiometry (DXA) (Lang *et al*, 2002).

During bone resorption, cross linked collagens are proteolytically broken down and the crosslink components are released into the circulation and the urine (Gunja-Smith *et al* 1981, Delmas,1991; Eastell *et al*, 1997). So , the measurement of specific degradation products of bone matrix provide analytical data of the rate of bone metabolism and bone resorption, because collagen type 1 represents more than 90% of bone matrix and deoxypyridinoline (DPD) crosslink stabilizers of collagen molecules, a major post-translational modification of collagen, plays important roles in the biological and biomechanical features of bone (Seibel. 2005; Saito *et al* 2014).

The present study aimed to estimate urinary total deoxypyridinoline as a biomarker of mature bone collagen degradation and bone resorption in type 1 diabetes mellitus patients compared to non-diabetics and evaluate if there is any possible relation with fasting blood sugar and serum bone mineral profile: total calcium, inorganic phosphorous, and magnesium, also the effect of disease duration to investigate severity degree of bone resorption and the probability of fracture risk.

Patients and methods:

165 patients with type 1 diabetes mellitus from Diabetes mellitus and Endocrine Disease Centre at AL - Nasiriyah city in Thi-Qar province, Iraq. Of those 165 patients there are 100 (60.6%) were female and 65 (39.4%) were male, age was (25-65) year, mean 44.27± 11.1, their duration with type 1 DM disease ranged between 5 months to 10 years, they were classified in to three groups according to the duration of type I DM: the first group included 45 (27.3 %) patient their duration with type 1 DM were less than one year, the second group included 65 (39.4 %) their duration with type 1 DM were 1 to 5 year and the third group included 55 (33.3%) patient their duration with disease were from 6 to 10 year. Also, this study included 165 healthy individuals selected as a control group, their age and

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gender matching with patients study group. The study duration was from May to December 2017.

For biochemical analysis of blood sugar, total calcium, inorganic phosphorous and magnesium fasting venous blood (2 ml) were collected without using tourniquet and serum samples separated as routine method and storage in -20 °C until analysis by using standard colorimetric methods using commercially available kits (Human, Germany) and spectrophotometer (APEL) (Mann, 1956, Trinder,1969; Daly and Ertingshausen, 1972 and Lorentz, 1982). Also, first morning urine samples were collected for measuring urinary deoxypyridinoline, urine sample separated by centrifuge for approximately 20 minutes at 1000 × g (or 3000 rpm) within 30 minutes after collection. The supernatants were collected carefully and stored frozen (-20°C) until testing. Urine Dpd was measured by the quantitative competitive enzyme immunoassay principle methods using commercially available kits (Elabscience, China) and ELISA instrumental is Biotek ELX50 Microplate strip washer and absorbance reader (Robins *et al*, 1994).

Statistic analysis:

Data analyzed by using statistical package for social sciences (SPSS) software version 19. Categorical variables expressed as frequency and percentages. Continuous variables expressed as a mean and standard deviation. the difference between quantitative variables in diabetic patients and control subjects done by using Independent Sample Student's t- test P-values < 0.05 was considered statistically significant. ANOVA was used for the comparative analysis of the three groups, followed by the multiple comparison, Post Hoc test (Tukey test). The correlation between variables was calculated by the Pearson test.

Results:

The biochemical investigation for 165 healthy individuals and 165 diabetes mellitus patients with type I shown in table 1, there are significant differences between patients with type I DM and healthy control group for all parameters (P < 0.05). The fasting blood sugar levels in type I diabetic patients (266.96 ± 33.8 mg/dl) were significantly (P < 0.05) higher than in control group(80 ± 5.31 mg/dl) as in figure 2. Serum Bone mineral profile (Figure 3) : Total calcium (6.10 ± 0.49 mg/dl) and magnesium (1.48 ± 0.41 mg/dl) levels in type I diabetic patients groups were found to be significantly (P < 0.05) lower than control group (8.89± 0.39 mg/dl and 1.87 ± 0 .21) for both TCa and Mg respectively. Significant elevated in serum phosphate (9.5 ± 0.72 mg/dl) was found compared to control group (3.38 ± 0.22 mg/dl).

Urinary total deoxypyridinoline significantly elevated in type I DM patients (132.34 ± 18.69 ng/ml) as compared to the control group (3.48 ± 1.01 ng/ml) as in figure 4.

Table 1: Some biochemical parameter of 165 patients with type I diabetes mellitus compared with control group

Parameters	Control (no 165) Mean ±SD	Type 1 DM patients (no 165) Mean ±SD
Fasting Blood sugar * (mg/dl)	80.19 ± 5.31	266.96 ± 33.8
Total blood calcium * (mg/dl)	8.89 ± 0.39	6.10 ± 0.49
Blood inorganic phosphorus* (mg/dl) (PHO)	3.38 ± 0.22	9.50 ± 0.72
Blood magnesium* (mg/dl)(Mg)	1.87 ± 0 .21	1.48 ± 0.41
Urinary total deoxypyridinoline* (ng/ml) (U.DPD)	3.48 ± 1.01	132.34 ± 18.69

*Significant difference at (P < 0.05).

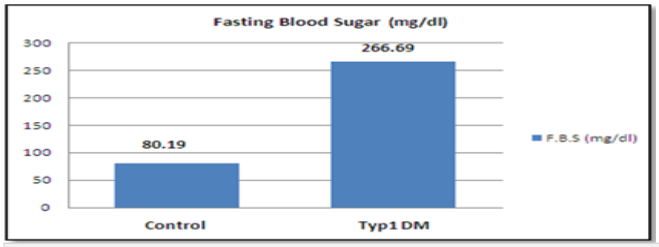

Figure 2: The concentration of fasting blood sugar (mg/dl) in 165 patient with type 1 diabetes mellitus and 165 healthy individual

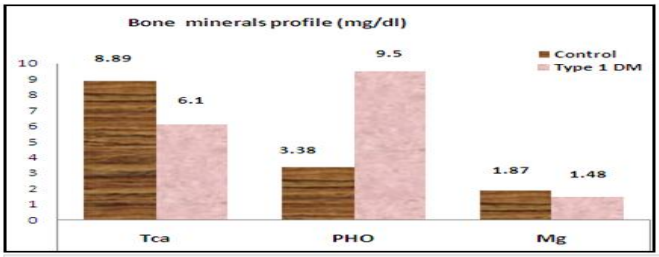

Figure 3: The concentration of serum bone mineral profile (mg/dl) in 165 patient with type 1 diabetes mellitus and 165 healthy individual

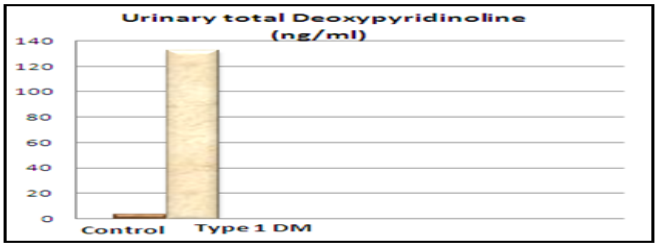

Figure 4: The concentration of urinary total deoxypyridinoline (ng/ml) in 165 patient with type 1 diabetes mellitus and 165 healthy individual

There is a positive relationship between increased in U.DPD and the increase in F.B.S as in figure 5. The relationship between U.DPD and serum bone minerals profile shown in figure 6, there is an adverse relation between U.DPD and both TCa and Mg as in figure A and C respectively, and a positive relation was found between U.DPD and PHO as in figure B

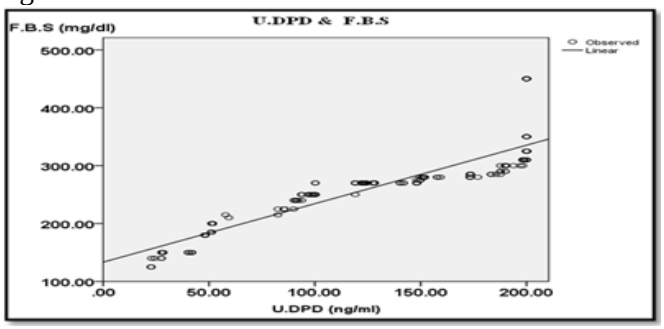

Figure 5: The relationship between urinary total deoxypyridinoline and fasting blood sugar level

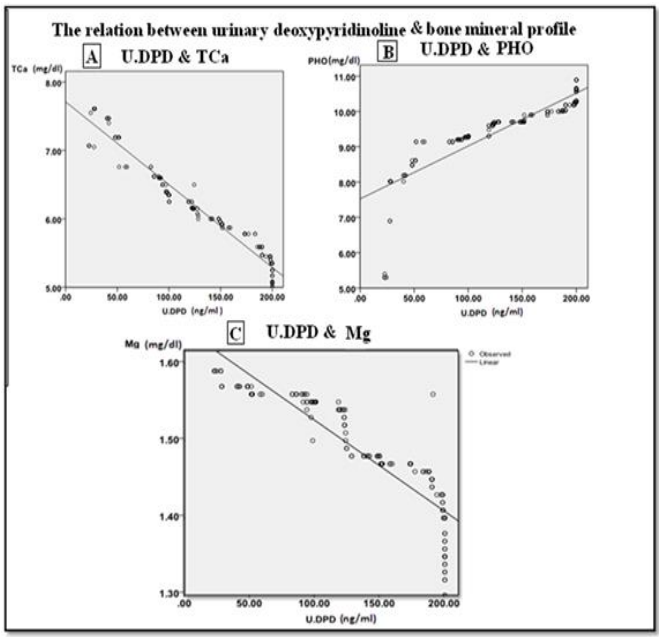

Figure 6: The relationship between urinary total deoxypyridinoline and bone minerals profile in all 165 patients with type 1 DM. A: Relationship between D.DPD and TCa; B: Relation between D.DPD and PHO; C: Relationship between D.DPD and Mg

duration on the chemical parameters: F.B.S, bone minerals profile and urinary total deoxypyridinoline. There are significantly increased ($P < 0.05$) in F.B.S, U.DPD, and PHO, also significantly decrease ($P < 0.05$) in TCa and Mg concentration with the increased prolonged duration of disease.

Table 2: Effect of diabetes mellitus duration on some biochemical parameters

Duration of type 1 DM	Patients		F.B.S * mg/dl	Serum bone minerals profile test *			U. DPD * ng/ml
	no	%		TCa mg/dl	PHO. mg/dl	Mg mg/dl	
< 1 year	45	27.3	201.19 ± 39.92	7.00± 0.38	8.46 ± 1.12	1.57 ± 0 .18	80.79 ± 25.54
1-5 year	65	39.4	264.69 ±10.79	6.01± 0.19	9.70± 0.21	1.48± 0 .18	121.44 ± 23.27
6-10 year	55	33.3	335.00 ± 50.71	5.30 ± 0.91	10.42± 0 .83	1.39 ± 0.05	194.79 ± 7.21
Total	165	100	266.96 ± 33.80	6.10 ± 0.49	9.50 ± 0.72	1.48 ± 0.41	132.34 ± 18.67

* Significant differences at ($P < 0.05$).

Discussion:

The mechanisms of diabetic effects on bone cells are very complex and several researchers have described different mechanisms that showed how DM induces osteoporosis and bone fractures through multiple pathways (Gurav, 2013). In this study patients with type I DM, have uncontrolled hyperglycemia, generally, it accepted that uncontrolled hyperglycemia occurs more commonly, because insulin therapy imposes regularity in food intake and particularly the intake of carbohydrates. A dietary pattern in these patients should include carbohydrate from fruits, vegetables, whole grains, legumes, and low-fat milk. Moreover, monitoring carbohydrate whether, by carbohydrate counting, exchanges or experienced-based estimation remains a key strategy in achieving glycemic control and maintain a stable body weight(Gin *et al*.1999).

The 165 patients with type 1 DM associated with severe hypocalcemia (6.1± 0.49 mg/dl), hyperphosphatemia (9.5 ± 0.72 mg/dl), and hypomagnesaemia (1.48 ± 0.41 mg/dl), theoretically abnormalities in bone minerals of these patients group , related to different factors such as volume depletion, its fact that, under conditions of renal failure, phosphorus cannot be excreted by the malfunctioning kidney, leading to hyperphosphatemia (Fry and farrington, 2006; Liamis *et al*, 2014). On another hand, hyperphosphatemic condition induces hypocalcemia by interfering with phosphorus excretion in the malfunctioning kidney (Blaine *et al*, 2015). In addition, phosphate binds ionized calcium and removes calcium from the bloodstream (Liamis *et al*, 2014). Like hyperphosphatemia, hypomagnesemia is another cause of hypocalcemia in diabetic patients (Pham *et al*. 2007). Mg 2+ depletion leads to hypocalcemia through impaired secretion of parathyroid hormone (PTH) or via bone and renal tubular resistance to the action of PTH and increased prevalence of hypoparathyroidism (Takiishi *et al*, 2010). Moreover, a small downward

shift in PTH secretion in patients with TIDM, as well as decreased parathyroid gland responsiveness to hypocalcemia in DM patients, have been reported (Heidbreder *et al*, 1986 and Schwarz *et al*, 1992). Magnesium deficiency is the most evident disturbance of metal metabolism in insulin-dependent diabetes mellitus. Mg deficiency decreases insulin sensitivity and insulin secretion. Moreover, Mg deficiency is inherently related to the pathogenesis and the development of not only diabetic microangiopathy but also lifestyle-related diseases, (Yokota. 2005). Many studies indicated positive correlations between decreased levels magnesium and poor glycemic control (Lin and Huang , 2015).

Urinary deoxypyridinoline (U. DPD) significantly elevated in typ1 diabetic patients and this study shows significant correlation with hyperglycemia, hypocalcemia, hyperphosphatemia, and hypomagnesemia. Severe elevation in urinary DPD concentration (132.34 ± 18.69 ng/ml) reflect severity degree of the degradation of mature bone collagen, data have accumulated that collagen cross-link formation affects not only the mineralization process but also microdamage formation (Vasikaran *et al*, 2011; Saito *et al* 2014). Consequently, collagen cross-linking is thought to affect the mechanical properties of bone. Furthermore, recent basic and clinical investigations of collagen cross-links seem to face a new era (Saito *et al*, 2014, Saito and Marumo, 2010).

Also, several mechanisms may be suggested to be responsible for low bone density, quality and suppress osteoblasts action (Saito *et al*. 2014). Theoretically, hyperglycemia, induced, oxidative stress and the production of advanced glycation end products (AGE) and free oxygen radical levels could induce tissue damage and abnormalities in antioxidant defenses in DM (Sheweita *et al*, 2007). Oxidative stress can impair the balance between proteolytic enzymes and their inhibitors that may cause losing or degradation of collagen crosslink results in increased bone inflammation effect on both osteoclasts and osteoblasts. (Schwartz *et al*, 2001; Nikolajczyk *et al*, 2011; Kuyumcu *et al*, 2012, and Sandukji and Starup-Linde, 2013). Potentially, this inflammation resulting in impaired bone formation (McCabe.2007, Botolin and McCabe 2006).

Chronic hyperglycaemia may also result in the non-enzymatic glycosylation of proteins (e.g. collagen) and other cell components (e.g. DNA), In diabetics, hyperglycemia may be associated with increased loss of calcium in the urine, This leads to a loss of calcium from the skeleton with a decrease in the bone mineral density (Raskin *et al*, 1978 and McNair *et al*, 1979). The plasma magnesium level has been shown to be inversely related to insulin sensitivity (Alikhani et al, 2007; Hamada *et al*, 2009 and Abd EL Dayem *et al*, 2011). This explanation accepted in this present study.

The statistic results for type 1 DM duration of three groups, there are significant differences between the three groups for all parameter, there are observant changing was seen, high changes in bone urinary DPD levels even after a long period of the disease. Bone minerals have been linked both to the acute metabolic and late chronic complication of diabetes (Elamin and Tuvemo, 1990).

A similar phenomenon is seen in some studies, the chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs (IDF Diabetes, 2013). lower bone density among those who have had diabetes for over five years (Lvers et al. 2001), other hand other studies found that long duration of diabetes more than 10 years are significantly increased all fracture risk (Sharma *et al*. 2007).

In conclusion , diabetic patients with type I have an elevation in urinary deoxypyridinoline (DPD) a marker of bone resorption and degradation product of bone matrix related to hyperglycemia and abnormal bone mineral profile like hypocalcemia, hyperphosphatemia, and hypomagnesemia, also ,poor glycemic control and diabetes duration are seem to play a key role given the lower bone density that meaning those patients group underlying bone alterations and increased the probability of low bone density and quality then increased bone fracture risk.

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