Research article

Relationship between cathepsin K and total oxidative state in diabetes mellitus female patients with osteoporosis in Iraq

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ABSTRACT

Introduction and Aim: Diabetes mellitus patients almost always struggle with a metabolic condition known as chronic hyperglycemia. According to the World Health Organization, osteoporosis is a progressive systemic skeletal disorder that is characterized by decreasing bone mass and microstructural breakdown of bone tissue that increases susceptibility to fracture and increased risk of breaking a bone. Here, we aimed to compare the levels of CatK and total oxidative state in patients with diabetes and osteoporosis among the female Iraqi population and study the possible relationship between them.

Materials and Methods: This study included 40 females with diabetes (Group G1), 40 with diabetes and osteoporosis (Group G2) and 40 normal healthy females (Group G3) as controls. All participants were checked for their height, weight and BMI. Blood drawn from everyone was analyzed for fasting blood sugar, HbA1c, Cathepsin K, TOS, TAC and MDA. Data obtained was subjected to statistical analysis.

Results: According to the findings, the levels of cathepsin K increased significantly (P<0.001) from Group 1 to Groups 2 and 3, as measured by their mean and standard deviation values. The results of total oxidant status, expressed as means and standard deviations (Mean ± SD), demonstrated a high significant (P< 0.001) decline from group G1 to groups G2 and G3.

Conclusion: Cathepsin K was observed to be linked to both type 2 diabetes and bone loss. In postmenopausal Iraqi women with type 2 diabetes, Cathepsin K may serve as a biomarker for both diabetes and osteoporosis.

Keywords: Cathepsin K; TOS; T2DM; OP.

INTRODUCTION

High blood glucose levels are a hallmark of the metabolic condition known as diabetes mellitus. The hormone insulin is secreted by pancreatic beta cells, which aid in the uptake of glucose into cells for use as fuel and in other metabolic processes. Insufficient insulin production by the pancreas leads to high blood glucose levels and diabetes mellitus (1). Overproduction of insulin and sustained elevations in blood sugar, known as chronic hyperglycemia, have been linked to a variety of micro- and macrovascular complications in people with diabetes mellitus (2). Chronic hyperglycemia is connected with a wide range of medical complications, including ketoacidosis, nephropathy, retinopathy, neuropathy, and hypertension.

Chronic hyperglycemia has been linked to increased rates of osteoporosis in the diabetic community, especially among women (3), according to recent scientific research. Osteoporosis is a disorder of the skeleton characterized by low bone mass, increased bone fragility, and an increased risk of fracture (4). Fragility fractures most typically occur in the hip, spine, and wrist in diabetic older women (5, 6).

Cathepsins are protease enzymes that are vital for normal physiological functions including digestion, coagulation, immune response, adipogenesis, hormone liberation, bone resorption etc., (7). Based on the serine,

cysteine, or aspartic acid residues the Cathepsins are further divided into 11 classes (B, H, L, S, C, K, O, F, V, X, and W) in humans (8). The last ten years have seen a significant increase in interest in Cathepsin K due to its role as the predominant cysteine Cathepsin in osteoclasts and its involvement in the breakdown of extracellular matrix during bone remodeling. Pharmaceutical companies are interested in Cathepsin K because it plays a role in the release of thyroid hormones from thyroglobulin at neutral pH in thyroid epithelial cells (6). Although it is also found in lesser concentrations at several other tissues, Cathepsin K is robustly expressed in osteoclasts (7). Recent studies have also demonstrated serum CatS and CatD to be significantly increased in patients with type 2 diabetes (9, 10). In the present study we aimed to compare the levels of CatK and total oxidative state in patients with diabetes and osteoporosis among the female Iraqi population and study the possible relationship between them.

MATERIALS AND METHODS

The current study was carried out at the Baghdad Ministry of Health's Endocrinology and Diabetes Center. The study's 100 participants were randomly assigned to one of three groups (G1, G2, or G3). Forty healthy adults, aged 40 to 55, made up the G1 control group. Forty people who had type 2 diabetes but not osteoporosis made up the G2 group. The G3 group

included 40 people with both type 2 diabetes and osteoporosis. Regular procedures for measuring height and weight were followed. The BMI was computed into dividing the weight at the square of the height (kg/m^2).

Each participant gave 5 ml of blood, 3 of which was placed in a sterile tube and allowed to clot at room temperature for 30 minutes, and 2 in an EDTA-containing tube. The serum extracted by centrifuging the tube for 10 minutes at 3500 g was used in the analysis of biomarkers such Fasting Blood Sugar (FBS), HbA1c, Cathepsin K, TOS, TAC and MDA. FBS concentrations were determined using a spectrophotometer (company, country). HbA1c and Cathepsin K concentrations were determined using an ELISA kit (RayBiotech Inc, USA), Human high sensitivity C-reactive protein (TOS and TAC) concentrations were determined using ELISA kits (My BioSource company, USA), and MDA concentrations were determined using an ELISA kit manufactured by Bioassay Technology Laboratory (BT Lab), China.

The t-test, mean, and standard division were used to compute the data. The t-test was used to see if there were any differences between the groups studied. The significance was calculated at P≤0.001value.

RESULTS

A total of 120 women participated in the trial; 40 served as healthy comparisons (Group G1), 40 had type 2 diabetes (Group G2), and 40 had both osteoporosis and type 2 diabetes (Group G3). The mean level of parameters studied for individuals in the three groups is presented in Table 1. Result for BMI was significantly higher (P<0.001) in the patient groups (G2 and G3) compared to healthy individuals (G1). Plasma levels of glucose (FBS) were observed to be significantly higher in type 2 diabetic patients (G2) as well as patients with diabetes and osteoporosis (G3) compared to healthy female controls (G1). A comparison of FBS levels between G2 and G3 individuals revealed that, while significant, FBS levels in the G3 group decreased marginally (Table 1). However, results for HbA1c showed that although a significant increase was seen in patient groups (G2 and G3) compared to controls, the values between patients in G2 and G3 remained almost the same and non-significant. TOS, TAC and MDA values were significantly increased in type 2 diabetic patients (G2 group) and in patients with diabetes and osteoporosis (G3) compared to healthy controls (G1). However, a comparison of the TOS, TAC, and MDA values between the patient groups revealed that patients with diabetes and osteoporosis (G3) had significantly higher values than patients with only type 2 diabetes (Table 1). In contrast, Cathepsin K level was observed to be significantly lowered in both the patient groups compared to healthy controls. Between the G2 and the G3 group the Cathepsin K values were further significantly lowered in patients with diabetes and osteoporosis compared to patients with only diabetes (Table 1).

Table 1: The mean ± SD levels of parameters of healthy controls and patient groups

Parameter	G1 (control)	G2 (T2DM)	G3 (T2DM and osteoporosis)	G1 Vs G2	G1 Vs G3	G2 Vs G3
Age (year)	43.55±2.440	43.91±2.41	43.92±2.251	NS	NS	NS
BMI (kg/m^2)	25.74±3.352	30.26±2.890	28.50±3.392	HS	HS	HS
FBS	82.65±5.771	204.75±65.720	159.3±28.86	HS	HS	HS
HbA1C	4.74±0.430	8.64±1.420	8.64±1.391	HS	HS	NS
Cathepsin K	1.860± 0.30	1.50±0.20	1.17±0.34	HS	HS	HS
TOS	9.65 ± 1.86	14.22± 2.94	22.62±4.73	HS	HS	HS
TAC	37.85±4.84	75.6±5.19	95.47 ±5.70	HS	HS	HS
MDA	23.36 ±3.37	37.07±17.62	79.72±5.20	HS	HS	HS

NS=Not significant; HS = Highly significant.

Table 2: Correlation between Cathepsin K levels with BMI, FBS, HbA$_1$C, TOS, TAC, MDA

Parameter	G2 T2DM		G3 Type 2DM with osteoporosis	
	r	p-value	r	p-value
BMI	-0.002	HS	-0.06	HS
FBS	0.14	HS	0.13	HS
HbA1C	0.22	HS	0.09	HS
TOS	0.03	HS	-0.100	HS
TAC	-0.24	HS	-0.093	HS
MDA	0.07	HS	-0.076	HS

HS = Highly significant.

Spearman's correlation was used to examine the relationship between plasma Cathepsin K and the measured values of the parameters of interest, BMI, FBS, HbA1c, TOS, TAC, and MDA among patients with type 2 diabetes as well as in patients with type 2 diabetes and osteoporosis. As seen from Table 2, a significant negative association was seen between Cathepsin K to BMI (r= -0.002) and TAC (r= -0.24) in patients with type 2 diabetes mellitus. Similarly, in patients with type 2 diabetes, Cathepsin K was seen to

be exhibit significant negative correlation with BMI (r= 0.06), TOS (r=0.100), TAC (r=0.093) and MDA (r= -0.076) (Table 2).

DISCUSSION

According to a recent study, the prevalence of T2DM increased the BMI in postmenopausal women (11) Lower BMI categories are associated with older ages, greater rates of current smoking, and low rates of type 2 diabetes mellitus and hypertension in both men and women. FBS is associated with T2DM and postmenopausal osteoporosis. In osteoporosis, FBS is used to diagnose improper glucose metabolism. FBS levels may help explain how AGM affects osteoporosis risk. Fasting glucose and osteoporosis are not well understood. Fasting glucose and two hours after a glucose load are used to diagnose diabetes, impaired glucose tolerance, and impaired fasting glucose. Impaired fasting glucose may affect osteoporosis; however, the evidence is weak. We found that IFG strengthens bones and reduces osteoporosis (12). Fang et al., discovered that HbA1C didn't differ between type 2 and osteoporosis patients. Higher blood sugar or HbA1c levels cause muscle mass loss in several ways. Insulin resistance and AGEs are the biggest concerns. IL-6, TNF-alpha, and C-reactive protein are linked to insulin resistance in type 2 diabetes mellitus. Muscle protein metabolism involves protein production and degradation. Calpain activity, macrophage autophagy, and apoptosis, which need ATP, are controlled by inflammatory signals (13). In current study, Cathepsin K may regulate obesity, glucose intolerance, and diabetes mellitus. Cathepsin K, the strongest mammalian cysteine protease, is elastase and collagenase. Cathepsin K helps cells recycle protein, degrade collagen, and repair the extracellular matrix. The study indicated that Cathepsin K deletion reduces energy metabolism-related hyperglycemia, cathepsin K inhibition lowers obesity-induced blood glucose and insulin increases and improves systemic glucose utilization (14). The study found that the serum Cathepsin K levels of diabetic postmenopausal women were significantly higher than those of healthy postmenopausal women. This shows that both managed and uncontrolled type 2 diabetes in postmenopausal women are associated with increased bone resorption and a higher risk of fracture (15). This result agrees with results reported by Adolf et al., (16) where serum Cathepsin K levels were reported to decrease with advancing age. Osteoclasts have high levels of cathepsin K expression; however, this protease is also produced and may have functions in tissues apart from bone. That means it's not just osteoclasts affected. Ovarian, chordoma, rheumatoid arthritis synovial fibroblast, thyroid, and bronchial cells have all been found to produce cathepsin K (16). The oxidative stress index suppresses bone synthesis and promotes bone resorption, causing postmenopausal osteoporosis. Osteoporotic women had lower antioxidant enzyme ROS defense. Postmenopausal women without estrogen lose bone faster and have changed blood levels of cytokines (interleukin-1, tumor necrosis factor alpha, granulocyte-macrophage colony stimulating factor, interleukin-6; 17).

Korkmaz and coworkers (18) found that osteoporotic women had a much larger mean serum TOS rise compared to postmenopausal control women. Numerous diseases, such as cancer, atherosclerosis, rheumatoid arthritis, osteoarthritis, fibromyalgia, diabetes, and osteoporosis, have been linked to oxidative stress. When the human body's oxidative and antioxidative defenses are out of whack, it experiences oxidative stress. Increased osteoclastic activity and decreased osteoblastic activity are hallmarks of postmenopausal osteoporosis, which is associated with much greater levels of oxidative stress in patients than in controls. Therefore, combining therapy with an antioxidant-rich diet may open up novel prospects for enhancing current osteoporosis treatment choices (17). Total oxidative stress is much higher in diabetics than in healthy controls, as shown by a 2017 study by Rani and Mythili in the blood, the increased malondialdehyde release may be attributable to oxidative stress resulting from diabetes-related lipid peroxidation (19). Type 2 diabetic postmenopausal women had higher serum TAC levels. This study examines type 2 diabetic postmenopausal women's antioxidant capacity. Diabetes and lipid peroxidation are connected, Low antioxidant defenses Lipid peroxidation produces various byproducts. Type 2 diabetics have greater plasma levels. Plasma and bodily fluid antioxidant activity is often measured by total anti-capacity. Total anti-capacity and Milano di aldehyde suggest oxidative stress (20). Malondialdehyde (MDA has the ability to create protein adducts by precisely altering the lysyl residues of proteins, which explains why it is associated with both osteoporosis in women and type 2 diabetes (21). These findings showed no statistically significant difference between osteoporotic and non-osteoporotic females. Blood levels of MDA were found to be significantly greater in patients with bone disorders. This study's findings corroborated those of previous research showing no association between MDA levels with osteoporosis, the severity of postmenopausal osteoporosis may not be accurately assessed using MDA (22). However, both postmenopausal women with and without osteoporosis had elevated levels of MDA in their serum. Lipoproteins and cellular membrane lipids are the primary substrates for peroxidation. As a measure of lipid damage due to free radicals, MDA levels are considered a sign of oxidative stress. Lipid peroxidation leads to elevations in Milano di aldehyde (23). Hyperglycemia and oxidative stress were associated with T2DM without osteoporosis in postmenopausal women's MDA levels. OS in DM is caused by non-enzymatic, enzymatic, mitochondrial, and hyperglycemia-induced MDA-lipid interactions.

Hyperglycemia in T2DM raises plasma MDA and lipid peroxidation, increasing coronary lipid risk factors (24).

CONCLUSION

Our study demonstrates that Cathepsin K to be associated with type 2 diabetes as well as osteoporosis. Hence, Cathepsin K could be considered as a biomarker in the diagnosis of diabetes and also in predicting developing osteoporosis in type 2 DM postmenopausal women in Iraq.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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