Determination of Osteocalcin, Bone Alkaline Phosphatase, and Lysyl Oxidase in Iraqi Acromegalic Patients

Eiman A. Abass
Dept. of Chemistry/ College of Education for Pure Science (Ibn Al-Haitham) / University of Baghdad
Baydaa Ahmed Abed
National Diabetes Center/AL-Mustansiriya University
Sohaib N. Mohsin
Dept. of Chemistry/ College of Education for Pure Science (Ibn Al-Haitham) / University of Baghdad
suhaibnayef@gmail.com
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Abstract

This study was aimed to determine bone formation markers (OST and BALP) and lysyl oxidase in diabetes and non-diabetes Iraqi acromegaly patients in addition to find the relationship among these parameters. The present study conducted 60 acromegalic patients (30 diabetes & 30 non diabetes) attending National Diabetes Center / AL-Mustansiriya University/Baghdad, and 30 healthy individuals as a control group aged (35-60) years. All patients were administrated Sandostatin drug, and they were diagnosed by physician in the hospital.FBG, GH, IGF-1, OST, BALP, and LOX were determined in all groups. The results showed a highly significant rise in all parameters (GH, IGF-1, FBG, OST, BALP, and LOX values in serum of all patients when compared with normal individuals. There were some positive highly significant correlations for lysyl oxidase with osteocalcin and a positive significant with BALP in acromegalic patients. We concluded that lysyl oxidase may be a novel biomarkers in endocrine diseases like acromegaly.

Key words: Acromegaly, bone formation markers, osteocalcin, BALP, lysyl oxidase.

Introduction

Acromegaly is a rare, chronic, advanced disease diagnosed by increased secretion of growth hormone (GH) with high concentrations of circulating insulin-like growth factor 1 (IGF-1). It is caused by a pituitary adenoma in the wide majority of cases. The clinical diagnosis, based on symptoms associated with GH raise, is often late due to the insidious nature of the disease [1]. The GH and (IGF-I) play a main role in the monitoring of skeletal health and early maturity. GH is important for acquisition of bone mass and the achievement of top bone mass, which is the main forecaster of osteoporotic fracture danger. Furthermore, during adult age, GH is significant in the maintenance of skeletal architecture through the organization of bone turnover [2].

GH reverses impacts of insulin on glucose and lipid digestion, resulting in metabolic complications in acromegalic patients. More of these is altered glucose digestion [3].Studies exploring the pathogenesis of changed glucose metabolism in acromegaly suggest GH excess induces insulin resistance by impairing the ability of insulin to suppress gluconeogenesis, decreasing peripheral glucose utilization , and decreasing insulin receptor numbers and restricting liking [4].GH and IGF-I are critical controllers of bone remodeling.GH invigorates the multiplication of cells of the osteoblastic heredity and influences the destiny of mesenchymal forerunners preferring osteoblastogenesis, chondrogenesis and contradicting adipogenesis [5].Notwithstanding its impacts on the separation of osteoblasts, GH animates, either forthrightly or by oblique through IGF-I, the separated capacity of develop osteoblast. GH additionally animates the carboxylation of osteocalcin, which is an indicator of osteoblastic work, and empowers generation of osteoprotegerin and its aggregation in bone matrix [6].Histomorphometric and clinical examinations exhibited that acromegaly patients have expanded bone turnover in strong association with action of sickness [7].Bone turnover markers are peptides released by osteoblasts, osteoclasts, or by parts of bone grid, secreted in the blood circulation in bone resorption and building [8, 9].Osteocalcin is a bone-specific protein created fundamentally by osteoblasts through bone development. In spite of the fact that the larger part of osteocalcin released by osteoblasts is saved in the extracellular of bone tissue, a little quantity of it enters the blood where it can be recognized. Flowing osteocalcin has been utilized as a part of clinical examinations as an indicator of bone metabolism while the protein expression has been useful as indicator of osteoblastic phenotype and bone development in vitro[10,11]. Bone-specific alkaline phosphatase (BALP) is synthesized by osteoblasts and is supposed to be required in the calcification of bone matrix, however its exact part in the development procedure is obscure. BALP is one of the various isoenzymes of alkaline phosphatase: bone, liver, kidney, digestive system, and placenta. In the serum of most normal people, bone and liver isoenzymes of the tissue non-specific AP gene prevail in around approach ratios. The distinction in glycosylation of the bone and liver isoenzymes (results of the same gene) have been used to produce particular antibodies against BALP. BALP is believed to be an exceptional particular marker of the bone-shaping action of osteoblasts [12, 13].

Lysyl oxidase (LOX, EC1.4.3.13) or (protein-lysine 6-oxidase, LOX) is a copper dependent enzyme that starts crosslinking of collagen and elastin by stimulating oxidative deamination of alpha-amino groups of lysine and hydroxylysine residues [14].The LOX family can be classified into two subsections comprising of LOX and LOXL1, and LOXL2 – LOXL4, respectively. LOX and LOXL1 each have exceptional propeptide districts with restricted like to each other, while LOXL2 – LOXL4 each have four preserved scavenger receptor cysteine-rich (SRCR) areas in their more extended propeptide locales. LOX biosynthesis incorporates excretion of the idle 50 kDa glycoprotein precursor thought to consist of copper and quinone

cofactors [15].The end enzyme reaction mechanism wanted for collagen cross-link creation is stimulated by the lysyl oxidase family [16].
This study aimed to determine bone formation markers (OST and BALP) and lysyl oxidase in diabetes and non-diabetes Iraqi acromegaly patients in addition to find the relationship among these parameters.

Materials & Methods

Subjects

Blood was obtained from 60 acromegalic (AC) patients (30 diabetes & 30 non-diabetes) in addition to 30 healthy individuals as a control group which enrolled in this study, aged (35-60) years. All patients were undergoing treatment by Sandostatin drug, and they were diagnosed by physician. In this study, specimens were collected during the period from October 2016 to May 2017 in the National Diabetes Center / AL-Mustansiriya University/Baghdad .Serum was frozen until used for analysis FBG, GH, IGF-1, OST, BALP, and LOX.

Methods

Determination of serum GH, and IGF-1 levels:

Human GH and IGF-1 were measured by a solid – phase ELIZA kit supplied by DRG Company, which intended for quantitative determination of their concentrations in serum [17, 18].

Estimation of fasting blood glucose (FBG) Level:

Fasting blood glucose was determined by enzymatic colorimetric method using glucose kit from Randox[19].

Determination of human osteocalcin (OST) level (ng/ml) and human bone alkaline phosphatase (BALP) level (IU/L):

Serum osteocalcin and bone alkaline phosphatase levels were determined by ELIZA kit from Cusabio Company**,** based on biotin double antibody sandwich technology to assay human OT and BALP levels.

Determination of serum lysyl oxidase (LOX) level:

Serum lysyl oxidase level was measured by ELIZA kit from Cusabio Company, based on biotin double antibody sandwich technology to assay serum LOX level.

Statistical Analysis:
Data were expressed as Mean ± SEM. The multiple variations between patients and normal groups were tested by using ANOVA test, Pearson's correlation coefficient. P-value of < 0.001 and < 0.05 that were considered highly significant and significant respectively.

Results & Discussion

The levels of diagnostic parameters in patients and control groups are abstracted in table (1).

ANOVA test showed a highly significant elevation in GH and IGF-1 levels in serum of acromegalic patients with diabetes (G1) and non-diabetes(G2) when comparing with control group ($p< 0.001$), also there was a difference in GH and IGF-1 levels between G1 and G2.

Acromegaly is an endocrine maladies described by excessive revolving growth hormone (GH) and insulin – like growth factor (IGF-1) levels, that usually come as consequence for pituitary adenoma [20].Growth hormone leads to synthesis of IGF-1. Both intemperate GH and IGF-1 can lead to metabolic changes and smooth tissue, bone, and organ enlargement. GH, or somatotropin, is in charge of the growth of whole cells and tissues [21].Most cases of acromegaly as a result of a GH secreting pituitary adenoma that leads to a rise levels of GH and insulin- like growth factor (IGF)-I [22,23].

Results, also showed a highly significant increase in FBG levels in patients groups when comparing with control group ($p< 0.001$), and FBG level in G1 was higher than that in G2.

GH and IGF-I are organized intermediary metabolism by either decreasing or strengthening insulin action [24].GH over abundance leads to insulin resistance and disability in pancreatic B cell work, making a great number of patients with acromegaly vulnerable to evolve diabetes mellitus [25].Diabetes mellitus might be an early complexities of acromegaly, frequently being available at diagnosis of the malady [26], that may be interfering with the explanation and understanding of diagnostic tests for acromegaly, and once in a while causing instability in picking the suitable rapeutic approach for the malady. Medications used to treat acromegaly might rise impact glucose homeostasis regardless of biochemical control of GH and IGF-I overabundance [27].

Table (2) illustrated serum osteocalcin (OST), bone alkaline phosphatase (BALP) levels as bone formation markers, and lysyl oxidase (LOX) level in control, G1, and G2, respectively. ANOVA test showed a highly significant elevation in OT, BALP, and LOX levels in patients groups when comparing with control ($p < 0.001$). Results in present study, showed that serum OST, BALP, and LOX levels in G2 were higher than their levels in G1. The concentration of osteocalcin in the blood cycle is an indicator for bone formation. In addtion to serum Ocn level in people is conversely connected with measures of glucose metabolism. Most human investigations don't concomitantly gauge other bone turnover markers to detach the role of osteocalcin as an index of bone building from its impact on glucose metabolism [28].

Osteocalcin is a bone matrix protein that has been related with same hormonal activities on energy and glucose metabolism. Animal and trial models have demonstrated that osteocalcin is liberated into the circulation system and works biological impacts on pancreatic beta cells and fat tissue [29].Osteocalcin is emitted exclusively by osteoblasts and thought to assume a job in the body's metabolic direction and is expert osteoblastic, or bone-forming naturally. It is likewise embroiled in bone mineralization and calcium ion homeostasis. Osteocalcin goes about as a hormone in the body, making beta cells in the pancreas produce additional insulin, and in the meantime guiding fat cells to produce adiponectin hormone, which elevates allergy to insulin [30]. Our finding was contrasted the results in Abdella N. et al. [31] study who found a high significant increase in BALP level in diabetic patients. The level of bone isoenzyme of ALP represent one of the most sensitive and specific indicators of bone disease.

Although deficiency of bone mineral is not generally considered as a major complication of diabetic patients have lower bone mass than natural individualss [32].Bone isoenzyme of ALP action was very elevated in 84% of the patients. Also, none of the 15 patients with idle acromegaly had increased bone isoenzyme action. The serum BALP gives a quantitative belief roundabout and not ultimate measure of the degree of bone development [33].

Markers of bone formation are immediate or roundabout results of dynamic osteoblasts communicated during different periods of their advancement and reflect diverse sides of osteoblast work. Through bone development, osteoblasts excreate in serum the forerunner of type 1 collagen, i.e. procollagen type 1 N-propeptide, which is considered as a dependable marker of osteoblast work [34].The study by Stephen T. Ward and his group [35] was the first that reports that the direct measurement of serum LOX concentration and showed an association between LOX level and diabetes, also serum LOX was increased with diabetes.It has long been known that there were widespread changes in collagen structure due to increased cross-linking of collagen fibers in patients with diabetes.

Collagen cross-links play an essential function in the expression of bone power and the suitable biological action of bone. The cross-links of collagen can be generally partitioned into two sorts: lysyl oxidase interposed cross- links and developed glycation final products [36].Lysyl oxidase stimulates the end known enzymetic step needed to collagen and elastin cross-linking. A cross-linked collagenous extracellular matrix is required for bone formation. Thus, due to its critical function in collagen accumulation, lysyl oxidase is likely important in bone development and pathology [37],and acromegaly is a rare hormonal condition that results from an excess amount of growth hormonal in the body, this extra amount of GH causes growth in the bone and soft tissues of the body. Acromegaly mostly affects the arms, legs, and face [38].

In this study, figures (1) and (2) showed a positive highly significant correlation ($p<0.001$) for lysyl oxidase with osteocalcin ($r= 0.52$) and a positive significant correlation ($p < 0.05$) for lysyl oxidase with bone alkaline phosphatase ($r = 0.32$) in Iraqi acromegalic patients .

In this study, it was determined that lysyl oxidase level in acromegalic patients in addition to relationship for lysyl oxidase with osteocalcin and bone alkaline phosphatase as bone formation markers for the first time in Iraqi acromegalic patients.

Table (1): (Mean ±SEM) of diagnostic parameters levels in control, diabetes and non-diabetes acromegaly patients groups.

Groups / Parameters	Control No.(30)	G1 patients No. (30)	G2 patients No.(30)	*P*-Value
GH (ng/ml)	2.13 ± 0.12	11.70 ± 3.12	6.18±1.22	$P < 0.001$
IGF-1 (ng/ml)	256.90 ± 14.87	669.70 ± 58.36	795.53±79.4	$P < 0.001$
FBG (mg/dl)	86.70 ± 1.34	167.56 ± 10.30	98.57±4.57	$P < 0.001$

Table (2): Levels (Mean±SEM) of bone formation markers (osteocalcin and bone specific alkaline phosphatase) and lysyl oxidase in control, diabetes and non-diabetes acromegaly patients.

Groups / Parameters	Control No.(30)	G1 Patients No.(30)	G2 patients No.(30)	*P*-Value
OST(ng/ml)	5.97 ± 0.3	20.47 ± 1.72	28.81±4.63	$P< 0.001$
BALP(IU/L)	9.30 ± 0.32	25.01 ± 1.10	27.50±1.04	$P < 0.001$
LOX (ng/ml)	3.40 ± 0.21	5.51 ± 0.44	8.73± 0.74	$P < 0.05$

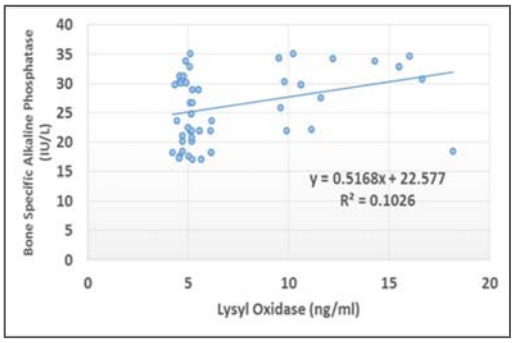

Figure (1): Correlation between LOX and BALP in acromegaly patients

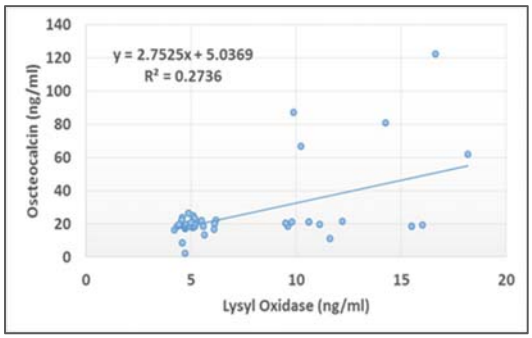

Figure (2): Correlation between LOX and OST in acromegaly patients

Conclusions

In conclusions, lysyl oxidase may be a novel biomarkers in endocrine diseases like acromegaly, and there was a positive highly significant correlation for lysyl oxidase with osteocalcin and a positive significant with bone alkaline phosphatase in acromegalic patients. Also, bone formation markers (osteocalcin and BALP) levels were highly significant increase in acromegalic patients, thus there were a relationship between osteoblast and growth of bone.

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