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COMPARATIVE STUDY OF RANKL LEVEL IN MALE PATIENTS WITH ACTIVE ACROMEGALY AND DM2

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ABSTRACT

Most of patients with acromegaly suffer from DM2. Excess GH production in active acromegaly indicate that inhibits osteoclasts throughout RANK-RANKL axis. Our aim is to determine the differences in the levels of RANKL in male patients with diabetic acromegaly and compare with male DM 2 patients and some related parameters, and if their level can possible use to follow up the patients with active acromegaly. Forty eight were enrolled in this study, (28) patients with DM2, (28) acromegaly patients with DM 2 and (28) healthy subject as a control group. Serum of RANKL, GH and insulin resistance were measured in all groups. RANKL level were showed significant decreased differences in patients with diabetes acromegaly compared with control group, non significant decreased differences in patients with DM2 when compared with control group and a significant negative correlation between RANKL and GH and IR. We conclude that serum RANKL levels were decrease in active acromegaly and its correlate with GH, so that possible RANKL can be used to follow up the male patients with active acromegaly and we suggest that it can be used therapeutically for better management of patients with active acromegaly.

Keywords: GH, RANKL, insulin resistance, active acromegaly, diabetes mellitus2.

INTRODUCTION

Acromegaly is a chronic hypersecretion of growth hormone (GH) most commonly due to pituitary tumor, other rare causes are increased growth hormone releasing hormone production from hypothalamic tumors, ectopic growth hormone-releasing hormone production, and ectopic GH secretion from non endocrine tumors (Giuseppina *et al*., 2011).

GH has role in the maintaining of bone mass by regulating bone remodeling through a complex interaction of circulating GH with the bone mass. GH stimulate bone turnover, by releasing molecules from activated marrow stromal cells and osteoblasts, which also lead to enhanced osteoclastogenesis and mature osteoclast activity (Ravn *et al.*, 1995; Thor, 2005).

GH stimulate osteoclastic bone resorption through direct and indirect effects on osteoclast differentiation and through indirect activation of mature osteoclasts (Nishiyama *et al*., 1996).

Receptor activator of nuclear factor-kB ligand (RANKL), which mediate the effects of many upstream regulators of bone metabolism, stimulates osteoclast differentiation, activates mature osteoclasts, and inhibits osteoclast apoptosis (Nishiyama *et al*.,1999).

Osteoprotegerin (OPG) and Receptor activator of nuclear factor-kB ligand (RANKL) are produced by osteoblasts, whichare the main mediators of osteoclastogenesis underosteoblast control. OPG inhibits osteoclast differentiation and activation by binding to RANKL, and thus preventing its bindingto the specific receptor RANK which is expressed on osteoclasts and their precursors, which leads to decreased osteoclastogenesis and osteoclast function (Hofbauer *et al.*, 2000).

Several hormones, insulin like growth hormone, growth factors, IL-6,IL-10, and prostaglandins regulate the OPG-RANKL-RANK system through their direct effects on bone cells (Cheung *et al*., 2003; Suda *et al.*, 2004). The aim of present study was to determine the differences in the levels of RANKL in patients with diabetic acromegaly and compare with diabetes mellitus type 2 patients and some related parameters , and if their level can possible use to follow up the patients with active acromegaly.

MATERIALS AND METHODS

Forty eight male (age 27-55) years were enrolled in this study, (28) patients with diabetes mellitus type 2, (28) active acromegaly patients with diabetes mellitus type 2

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who were attended the National Diabetes Centre, Baghdad from December 2013 to June 2014 and (28) healthy individual with matches as a control group. All patients were diagnosed by physicians and the study was approved by the center ethical committee. Patients and the volunteers involved in the study have given informed consent and were excluded osteoporosis, osteomalesia, cardiovascular disease, renal failure, hypertension alcoholics and smokers Blood was collected and the sera were separated. GH, insulin and RANKL levels were measured by enzyme-linked immune sorbet assay (ELISA) kits. Fasting serum glucose (FSG) was determined using enzymatic colorimetric method (Glucose oxidase-peroxidase). Mathematical formula was used to measure IR by, Fasting glucose×fasting Insulin/405 = IR, which used in HOMA-IR.BMI was calculated by (BMI)=mass (kg)/height^2 (m^2).

Statistical Analysis

Data were presented as mean ±SD using SPSS program version 20. The differences between two groups were analyzed by independent *t*-test, P-value equal or less than 0.05 considered significant. Pearson's correlation coefficient was used to examine between (RANKL) and other parameters.

RESULTS AND DISCUSSION

This is the first study to document, as far to knowledge, which depict the determination of RANKL levels in Iraqi male with diabetic acromegaly patients and compare with male patients with diabetic mellitus type 2. Mean of BMI in patients with DM and diabetic acromegaly shown in table 1, which was revealed that significantly difference higher than the control group($P<0.05$), BMI in patients with diabetic acromegaly was non significantly higher in comparison to that of the DM group ($P>0.05$). This increase was due to the body weight is positively correlated with bone mineral density not by obesity, the result in our study is agreement with the results obtained by (Reid, 2008) who showed that difference was observed between both acromegaly and control group ,and no difference between active acromegaly and controlled acromegaly ,but the result was disagreement with the results obtained by (Berg *et al.*, 2010) who found that no significantly difference as compared to physiological levels.

A very highly significant increase of serum GH level ($P= 0.0000$) was observed in the present study in patients with diabetic acromegaly when compared with DM and control groups, and non significant increase in patients with DM when compared with control group. Acromegaly is caused by hypersecretion of growth hormone (GH) due to pituitary tumor. The anabolic actions of GH on many organ systems are well documented. During the childhood GH stimulates longitudinal bone growth. During the adolescence and early adulthood GH stimulates skeletal maturation (Giuseppina *et al.*, 2011). The result is agreement with most researches such as the result which obtained by Johan *et al.* (2013).

Serum of Glucose, insulin and IR levels were measured in patients with DM and diabetic acromegaly in this study which was showed that highly significantly difference higher than the control group($P<0.01$), glucose , insulin and IR levels in patients with diabetic acromegaly were non significantly higher than in the DM group due to increase level of GH in patients with acromegaly ($P>0.05$), GH is often said to have anti-insulin activity, because it suppresses the abilities of insulin to stimulate uptake of glucose in peripheral tissues and enhance glucose synthesis in the liver, then growth hormone can induce IR and combined effects ,as in stress or infection (Daniela *et al.*, 2013; Berg *et al.*, 2010) this result is agreement with (Fieffe *et al.*, 2011) who was able to confirm in patients with active acromegaly a higher prevalence of diabetes type 2 and higher levels of fasting glycaemia (Colao *et al.*, 2011) who found that the newly diagnosed patients with acromegaly have higher insulin resistance.

Mean of serum RANKL level was measured in patients with diabetic acromegaly shown in table 1 which was revealed that significantly difference lower than the DM and control groups ($P<0.05$), while serum RANKL level in patients with DM was non significantly lower in comparison to that of the control group ($P>0.05$), this decrease was due to the high level of GH in patients with acromegaly, GH excess inhibits differentiation and activity of osteoclasts through the RANK-RANKL. RANKL produced by osteoblasts, which are the dominant mediators of osteoclastogenesis under osteoblast control (Hofbauer *et al.*, 2000). RANKL acts as an endogenous activator of osteoclast differentiation by binding to RANK, resulting in increased osteoclastogenesis. In acromegaly GH excess and then RANKL is decrease which inhibits the osteoclast function and excess on bone formation and longitude (Nakagawa *et al.*, 1998; Yasuda *et al.*, 1998), this result is agreement with Lacey *et al.* (1998) who show that RANKL stimulates osteoclast differentiation, activates mature osteoclasts, and inhibits osteoclast apoptosis.

Table 2 showed the correlation between RANKL and GH, insulin and IR for diabetic acromegaly patients and DM which were revealed that in male patients with diabetic acromegaly significant negative correlation between RANKL and GH , IR and non significant correlation with insulin. While non significant negative correlation between RANKL and GH, non significant positive correlation between RANKL and insulin, IR in male patients with DM.

Table 3 showed the correlation between IR and RANKL, GH and insulin for diabetic acromegaly patients and DM which were revealed that in male patients with diabetic acromegaly significant positive correlation between IR

Table 1. Mean±SD of BMI, FBG, GH, insulin, IR and RANKL.

Groups / Parameters	Mean ±SD			P-value		
	C(n=28)	D(n=28)	Acr(n=28)	C-D	C-Acr	D -Acr
BMI (Kg/m2)	29.6±6.4	32.2±6.8	32.3±5.3	0.047*	0.01*	0.9
Glucose(mg/dL)	97.1±9.4	193.6±62.5	176.2±77.5	0.00*	0.00*	0.56
GH (mU/L)	1.6±1.2	1.8±1.1	10.4±10.3	0.8	0.000*	0.000*
Insulin(µU/mL)	9.1±7.8	25.6±15.3	18.7±14.6	0.003*	0.004*	0.09
IR	1.95±1.7	8.4±4.5	6.8±7.3	0.000*	0.002*	0.08
RANKL(mg/dL)	118.5±21.4	117.3±23.4	91.7±18.2	0.87	0.01*	0.035*

Table 2. Correlation between RANKL and GH, insulin and IR for diabetic acromegaly patients and DM.

RANKL	(Acr) r	(Acr) P-value	(D) r	(D) P-value
GH	-0.67	0.001*	-0.21	0.5
Insulin	-0.31	0.058	0.35	0.12
IR	-0.55	0.03*	0.11	0.7

Table 3. Correlation between IR and RANKL, GHandinsulin for diabetic acromegaly patients and DM.

IR	(Acr) r	(Acr) P-value	(D) r	(D) P-value
GH	0.51	0.01*	0.33	0.08
Insulin	0.73	0.0001*	0.67	0.000
RANKL	-0.55	0.03*	0.11	0.7

and GH and insulin and significant negative correlation with RANKL. While non significant positive correlation between IR and RANKL, GH and insulin in male patients with DM. Our finding of the present study that the GH level in patients with DM is not affected by the disease, therefore, RANKL is in the normal state. IR which is present in patients with DM and acromegaly does not affect to RANKL level, because RANKL level in patients groups most possibly reflects the GH levels.

CONCLUSION

We conclude that the RANKL level can be used to follow up the patients with active acromegaly and diabetic acromegaly and we suggest that possible RANKL strategies that can be used therapeutically for better management of patients with active acromegaly to prevent the longitudinal bone growth in childhood or skeletal maturation during the adolescence and early adulthood.

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