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Circulating Preptin as a Marker for Osteoblast Inhibition in Rheumatoid Arthritic Patients Treated with Corticosteroids

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Abstract
Preptin is a newly isolated 34 amino acid peptide hormone co-secreted with insulin and amylin from pancreatic β-cells as a regulatory element in bone metabolism with an unclear yet mechanism
Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints
Two classes of medications are used in the management of RA: fast-acting non-steroidal anti-inflammatory drugs and corticosteoids, and slow-acting drugs. Corticosteroids are well known to have several adverse effects on bone metabolism.
Aim
The aim of the present study is to assess the association of corticostroids when used in the treatment of rheumatoid arthritic patients, with circulating preptin, in an attempt to shed a light on the mechanism of induced osteoporosis in such patients.
Subjects and methods
Ninety subjects were enrolled in this study. Divided into three groups:
G1= Thirty RA lean patients taking DMARDs + corticosteroids
G2= Thirty RA lean patients taking DMARDs without corticosteroids
G3= Thirty healthy weight and aged matched controls
Circulating serum preptin was measured in all groups using ELISA technique.
Results
Results showed that circulating serum preptin was elevated in patients with RA. However it was lower in G1 than in G2
In conclusion
Results showed that preptin was affected in such patients when compared to arthritic patients not treated with corticosteroids. This suggests that this newly discovered hormone could be considered as a new marker for bone mineral density and osteoporosis.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints. It is linked initially to immunity against an unknown antigen and later to self-maintained inflammatory processes (1). RA pathogenesis involves complex humoral and cellular reactions one of which is infiltration of lymphocytes and monocytes into the synovium. These infiltrating cells and synoviocytes release pro-inflammatory mediators, including IL-6, which perpetuate inflammation and destruction through effects on other cell types in the synovium and peri-articular structures (2) . There is no known definite cure method for RA. However, the goal of treatment is to reduce joint inflammation and pain, maximize joint function, and prevent joint destruction and deformity (3).

Two classes of medications are used in the management of RA: the first class include drugs that which promote disease remission and prevent progressive joint destruction these are either fast-acting non-steroidal anti-inflammatory drugs, or slow-acting drugs such as antimetabolite or antimalarial drugs such as. Methotrexate, leflunamide, sulphasalazine, hydroxychloroquine, and biologic agents (TNF-alpha inhibitors and B-cell depleting agents). The second class of drugs include those that help reduce inflammation by decreasing the action of the body's immune response. Although this effect can help relieve pain and swelling, it may make you more susceptible to infection examples are corticosteroids. These are well known to have several adverse effects on bone metabolism. One of many is direct inhibition of osteoblast function and may be differentiation (4).

Bone cells are classified into three primary classes: osteoblasts, osteocytes, and osteoclasts. Osteocytes are terminally differentiated osteoblastic lineage cells that are embedded into a matrix of deposited minerals (5). Osteoblasts localize on bone surfaces and promote bone formation by secreting proteins, such as osteocalcin and osteopontin, to anchor serum minerals within the bone matrix (6). The mesenchymal bone marrow cells are the osteoblast precursor cells, although these pluripotent cells can also differentiate into other cell types like adipocytes, chondrocytes, and muscle cells (7).

Preptin is a newly isolated 34 amino acid peptide hormone co-secreted with insulin and amylin from pancreatic β-cells as a regulatory element in bone metabolism with an unclear yet mechanism (8). It is a novel

peptide with a direct effect on the action of RUNX2 (a transcriptional factor associated with osteoblasts differentiation and marrow stroma cells).(3). Preptin corresponds to Asp69-Leu102 of pro-IGF-IIE peptide (9). The prime functions of preptin include regulating carbohydrate, lipid and protein metabolisms by moderating glucose-mediated insulin release.(10)Preptin, similar to other products of the pancreatic cell, insulin and amylin, stimulate osteoblast proliferation (11).

To date very few articles have been published on the significance of preptin in 2.humans (12); however none of these were conducted on RA patients.

2. Aim

The aim of the present study is to assess the association of corticostroids when used in the treatment of rheumatoid arthritic patients, with circulating preptin, in an attempt to shed a light on the mechanism of induced osteoporosis in such patients.

3. Subjects and methods

This study was conducted at Baghdad Teaching Hospital (Rheumatology Clinic and Teaching Laboratories) during the period from September 2014 to February 2015.

Sixty patients were enrolled in this study. Their consent and approval were taken after through description of the nature of the research. All patients were identified and diagnosed as having RA according to the ACR criteria.

The patients were selected according to specific criteria designed by the researchers in which they all must be on therapeutic medication for a period not less than 12 months and not more than 18 months. All should have lean body weight.

These patients were divided into two groups according to their drug administration:

1. Group 1 (G1)= patients on non-steroidal anti-inflammatory drugs
2. Group 2 (G2)= patients on corticosteroids

Thirty healthy volunteers were also included in this study. They were recruited from staff members and colleagues and were subjected to a thorough physical examination to exclude any diseased individual. They were all aged and weight matched. These were Group3 (C).

Three milliliters of venous blood were aspirated using disposal syringes. Samples were collected between(09.00 am-12.00 pm).The blood was allowed to clot in plain tubes for (30 to 45) minutes at room temperature and the serum recovered by centrifugation at (3000 rpm) for (10 minutes) and transferred into plain plastic tubes and kept frozen at (-20c^{o}) until time of assay.

Serum preptin assay:

Principle of the Assay: The ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in the kit is pre-coated with an antibody specific to Preptin. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Preptin and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Preptin, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm +/- 2 nm. The OD value is proportional to the concentration of Preptin.

The kit was supplied by MyBioSource, Inc. located in San Diego, California, USA.

Descriptive analysis is used to show the mean, standard deviation for BMI, and preptin in all groups.

4. Results

Sixty RA patients were included in this study. The age range was between 45-75 years.

Thirty healthy volunteers' age and gender matched were included as the control group. There was no significant difference between the groups regarding gender, age or their body mass index (BMI).

RA patients had significantly higher preptin serum level than the controls as seen in Table (1).

Table (1): Body mass index and serum preptin level in the studied groups

	Patients	Control	P value
Preptin (ng/dl)	423.65	106.8	< 0.05
Body mass index	24.45	23.9	>0.05

No significant differences were found in serum preptin levels between patient groups subdivided according to their therapeutic medications as seen in Table (2) & Figure (1).

Table (2): Serum preptin level in the patients sub groups.

	G1	G2	P value
Preptin (ng/dl)	441.6	405.7	>0.05

G1= patients on non-steroidal anti-inflammatory drugs, G2= patients on corticosteroids

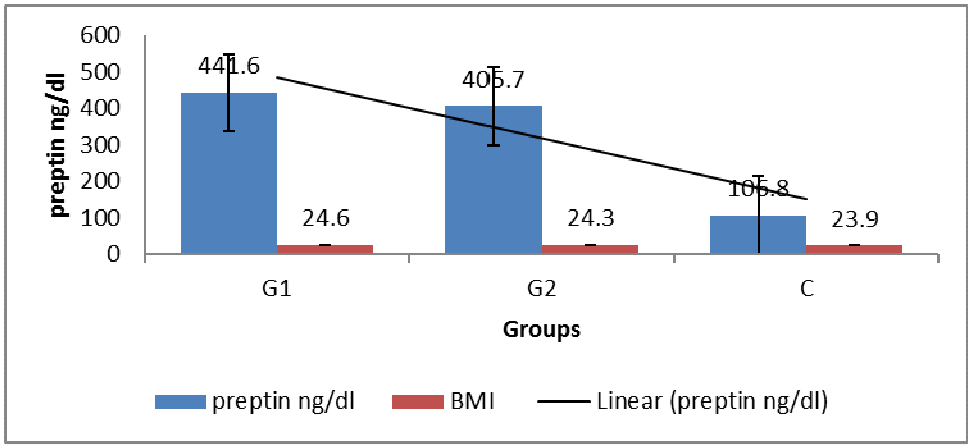

Figure (1): Body mass index and serum preptin level in the studied groups. BMI = Body mass index, G1= patients on non-steroidal anti-inflammatory drugs, G2= patients on corticosteroids

5. Discussion

Rheumatoid arthritis is a systemic chronic inflammatory disease. A certain correlation has been observed between chronic inflammatory conditions and resistance to insulin actions (3).

None of the therapies used in treatment of RA showed any correlation studies as to their impact on insulin resistance (13).

However some studies agreed with the hypothesis that corticosteroids are responsible for glucose intolerance (14).

On the other hand several other studies showed that there is only a discrete or a nonexistent relationship between corticosteroids and the presence of insulin resistance in patients with RA. These data suggest that their beneficial effect on inflammation counteracts their effect on insulin resistance (15).

This study showed a highly significant increase in serum preptin in diseased groups compared to the healthy ones (106.8 vs 423.65 ng/dl). This may be due to the inflammatory effect of RA on the pathogenesis of insulin resistance.

Inflammatory blockade improves pancreatic islet function and the proinflammatory cytokines associated with RA induce beta cell toxicity and predispose to insulin resistance (16). Preptin increases glucose-mediated insulin secretion (Preptin derived from proinsulin-like growth factor II (proIGF-II) is secreted from pancreatic islet beta-cells and enhances insulin secretion (17).

However comparing serum preptin levels in the two RA patients groups, G2 showed higher preptin level than G3. This might be due to the fact these patients have impaired preptin secretion that will overall effect bone remodeling. Preptin signals osteoblast proliferation through a G protein-coupled receptor that activates Gi-dependent phosphorylation of p42/44 MAP

Kinases. Activation of p42/44 MAP kinases (ERKs) is a common feature of proliferative signals initiated by a variety of extracellular agents (18).

A prevalence of osteoclastic bone resorption over osteoblastic bone formation increases a risk of development of osteopenia or osteoporosis. Osteoporosis is well known as a bone metabolic disease associated with high risks for bone fractures that often lead to prolonged incapacitation, although patients may be unaware of the disease because of a lack of clear symptoms (19). In aging population both genders have risk developing osteoporosis (20). Osteoporosis may be also caused by a side effect of glucocorticoid treatment of autoimmune diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (21).

As it is well known that GC exposure alters the fragile balance between osteoclast and osteoblast activity in bone metabolism. GC stimulates osteoclast-mediated bone resorption and reduces osteoblast-mediated bone formation, which results in increased overall net bone resorption (4).

One of the risk factors associated with glucocorticoid-induced osteoporosis is the activity of the 11β-hydroxysteroid dehydrogenase (11β-HSD) system, a prereceptor modulator of glucocorticoid action (22). Two isoenzymes are involved, 11β-HSD1 and 11β-HSD2, catalyze conversion between hormonally active glucocorticoids (e.g., cortisol or prednisolone) and inactive glucocorticoids (e.g., cortisone or prednisone). The 11β-HSD1 enzyme is an activator, and the 11β-HSD2 enzyme is an inactivator. The increased risk of fracture with glucocorticoid administration in the elderly may be explained in part by the increase in 11β-HSD1 that occurs with aging (21). The risk of glucocorticoid-induced osteoporosis appears to be similar in men and women and among various ethnic groups (23). Impaired preptin could add to this equation or it could be involved indirectly in the mechanism of osteoblast inhibition by glucocorticoid treatment

6. Conclusion

This study was designed to investigate the possibility of considering preptin as a potential marker for bone disorders in patients with RA treated with corticosteroids. Results showed that preptin was affected in such patients when compared to arthritic patients not treated with corticosteroids. This suggests that this newly discovered hormone could be considered as a new marker for bone mineral density and osteoporosis.

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