Estimation of serum leptin in female patients with nodal osteoarthritis

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Summary:

Background: Nodal osteoarthritis is one of the most common arthropathy worldwide, the etiology is uncertain but many biochemical markers are recognized. Many studies have shown that leptin might have a role in the pathogenesis of osteoarthritis, but little is known about the relation between serum leptin and nodal osteoarthritis.

Subjects and method: 52 women with nodal osteoarthritis and 40 apparently healthy women as a control were included in the study; serum leptin was measured in all subjects. Student t-test was applied to find out the significance of difference in the mean v

Results: There was a significant difference in the mean of serum leptin between patients and control groups.

Conclusion: the results of the current study suggest that leptin might have a role in the pathogenesis of nodal osteoarthritis

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Introduction:

Osteoarthritis (OA) is a heterogeneous condition showing a wide spectrum of clinical manifestations. The hand is commonly involved, and polyarticular interphalangeal OA is taken as the marker for predisposition to OA at multiple sites ('generalised OA').Nodal OA (NOA) ,a subset of OA , is characterized by polyarticular interphalangeal and thumb base OA , Heberden's and Bouchard's nodes formation ,a predominance in women , and a clear genetic predisposition(1).

The estimated prevalence of OA in the hands varies depending on the definition. Although the prevalence of radiographic OA is reported to be as high as 29 – 76% in population based studies in subjects with age more than 55 years, the prevalence of symptomatic hand OA is much lower, the prevalence of OA, usually, increases with age (2). The pathogenesis of OA appears to be the result of a complex interplay between mechanical, cellular, and biochemical forces. Cytokines such as tumor necrosis factor-α and interleukin-1, lipid mediators, free radicals, and even fragments of the cartilage itself induce chondrocytes to undifferentiated (3). As a result, these cells increase their synthesis of proteinases, especially the family of matrix metalloproteinases (MMPs) that cause the breakdown of proteoglycans. At the same time, a decrease in Tissue Inhibitors of Matrix Metalloproteinases (TIMPs) occurs (4). Leptin is a 16 kDa adipocyte-secreted hormone that regulates weight centrally and links nutritional status with neuroendocrine and immune Function (5). Its name is derived from the Greek root Leptos, meaning thin.Leptin may have important biological effects on chondrocyte, on both growth factor synthesis and anabolism, and also on catabolism. Leptin expression is strongly up regulated in various articular tissues that undergo strong structural and biochemical changes during OA (for example: cartilage, osteophytes and subchondral bone) when compared with normal tissues (6).

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Subjects and method:

Subjects: Fifty two female patients with NOA and 40 asymptomatic apparently healthy women matched for age, and weight have been studied. The patients studied in this case-control study have been selected from patients attended Rheumatology and Rehabilitation Out-Patient Clinic, Medical City, Baghdad Teaching Hospital during the period from May to July 2008. They were randomly selected, diagnosed clinically and radiologically. Many laboratory tests have been done for each patients to exclude other possible causes of arthritis, these tests were: ESR, CRP, RF and serum uric acid. Regarding the control group, 40 age and weight matched apparently healthy women have been selected from subjects attended a private laboratory for routine checking. A pre-tested questionnaire was designed to obtain information from both patients and control group about past medical and drug history.

Normal values of serum leptin:
Female 7.36 ± 3.73 ng/ml
Male 3.84 ± 1.79 ng/ml (7)

Both groups were further subdivided according to BMI into obese and non-obese groups; (table 1)

BMI = weight (kg)/ square height (m^2)

Subjects with BMI > 30 kg/m^2 were classified as obese, while those with BMI <25 kg/m^2 were classified as non obese.

Table (1): Number and percentage of studied groups and subgroups

Group		Obese	Non-obese
Patients	number	31	21
	percentage	59.6	40.4
Controls	number	23	17
	percentage	57.5	42.2
Total	number	54	38
	percentage	58.6	41.4

Blood Collection: After an overnight fasting, about seven milliliters of venous blood was aspirated using disposable syringes and needles. Samples were collected between 09.00-12.00 am. The blood was allowed to clot in plain tubes for 15 minutes, serum was obtained by centrifugation at 3000 rpm for 10 minutes and transferred into plain plastic tubes and kept frozen at -20°C until the time of assay.

Methods: Measurement of Leptin in serum: The Leptin Enzyme immunoassay kit provides materials for the quantitative determination of leptin in serum and plasma. This assay is intended for in vitro diagnostic use only.The leptin (sandwich) ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal anti-leptin antibody. An aliquot of patient sample containing endogenous leptin is incubated in the coated well with a specific rabbit anti leptin antibody. A sandwich complex is formed. After incubation the unbound material is washed off and an anti rabbit peroxidase conjugate is added for detection of the bound leptin . Having added the substrate solution, the intensity of color developed is proportional to the concentration of leptin in the patient's sample read at 450nm with the microtiterplate reader within 10 minutes after adding the stop solution.
Normal values:
Female 7.36 ± 3.73 ng/ml
Male 3.84 ± 1.79ng/ml (7)
RayBio® Human Leptin ELISA Kit was used to determine serum leptin level in subjects involved in the study.

Results:
The study showed that the mean of serum leptin level in patients group was significantly higher compared to control group P <0.05. There was no significant difference in the body mass index between the two groups (table 2).

Table (2): Statistical data of patients and control groups

Variable	Patients	Control	P value
Number	31	23	-
BMI (kg/m^2)	31.9±2.8	32.2±2.4	NS
Leptin (ng/ml)	24.8±4.3	13.8±5.2	<0.05

Serum leptin level was significantly higher in the obese patients group compared with the obese control group P<0.05 (table 3).

Table (3): Statistical data of obese group

Variable	Patients	Control	P value
Number	52	40	-
Age (year)	50.6±7.6	50.8±6.8	NS
BMI(kg/m^2)	28.4±3.2	28.3±2.9	NS
Leptin (ng/ml)	22.2±4.1	11.9±1.8	<0.05

There was a significantly higher leptin level in the non obese patient group compared to non obese control group P<0.05 (table 4).

Table (4): Statistical data of the non obese group

Variable	Patients	Controls	P value
Number	21	17	-
BMI (kg/m^2)	23.4±2.3	23.1±1.9	NS
Leptin (ng/ml)	18.6±3.7	8.8±3.4	<0.05

Discussion:
Leptin regulates overall metabolism , including food intake , energy balance and body temperature by signaling satiety and decreasing the sensation of hunger at the hypothalamic level (8).At the peripheral level , the hormone stimulates the process of oxidation of fatty acids in muscles , inhibits the accumulation of triglycerides in hepatic cells (9) and has potent lipid-lowering effects in peripheral tissues and plasma that are thought to be essential for the prevention of cellular lipotoxicity and insulin resistance (10).During the last years, It was increasingly evident that adipose tissue secretes a large variety of highly active proteins including cytokines, chemokines, and hormone like factors, such as leptin which have an important role in the modulation of inflammatory and immune responses (11). The recent studies on OA have focused more on evaluation of biochemical markers in serum

and/or synovial fluid. Many studies have shown the positive relation between serum leptin level and OA (12), Takeda S. *et al* confirmed in his study that leptin has a strong regulatory effect on bone metabolism& this effect occurs independently of its effect on weight. Thus leptin acts on bone metabolism& weight regulation through different pathways (13). A possible role of disregulated leptin production in OA of the knee is also suggested by the report of S. Juma & colleagues (14). Because leptin has been observed to be present in synovial fluid and in chondrocytes of articular cartilage of patients affected by osteoarthritis as well as in osteophytes, it has been postulated that leptin might exert a modulatory effect on chondrocyte metabolism and consequently on the pathogenesis of OA (15). A specific study about the relation between serum leptin level and NOA has not been found. In Iraq, the relation between serum leptin level and knee OA has been studied and higher serum leptin levels have been found in patients with knee OA compared to control group (16). The present study showed that the level of serum leptin in patients with NOA was significantly higher in comparison to control group; it was significantly higher in obese patients and non-obese patients compared to obese and non-obese controls respectively. These results give a clue that elevation of serum leptin level might be related to the presence of NOA. Many investigators have described Leptin alterations only in obese or overweight patients (17). Serum leptin concentration is increased in obese subjects and is closely related to fat mass and BMI;the present study has shown a strong relation of serum leptin and BMI .

Conclusion:

The results of the current study suggest that leptin might have a role in the pathogenesis of NOA; this could explain the increasing suggestions that the relation between obesity and OA is due to biochemical factor as well as mechanical one.

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