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Role for Leptin and Prolactin in Human Juvenile Rheumatic Diseases

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Abstract: This study was done to evaluate the relation between the level of leptin, prolactin, IL-4 and IL-5 with the activity of Rheumatoid Arthritis (RA) and Lupus erythematosus (SLE). The study included 33 patients divided into two groups. Group 1 included twenty-one patients with Juvenile rheumatoid arthritis (13 males and 8 females) with age 11.9±3.6 years and twelve patients with systemic lupus erythematosus were enrolled as group 2 (2 males and 10 females) with age 15.8±2.9 years. Twenty-one healthy children with matched age, sex and anthropometrics measures were included in the study to serve as control group (group 3). There were significant increases in the levels of Leptin (<0.038), Prolactin ($p<0.021$) IL-4 ($p<0.005$) in Juvenile Rheumatoid Arthritis group with insignificant decrease in IL-5 ($p<0.724$) in comparison to control group. Systemic Lupus group show a significant increase in level of Leptin ($p<0.05$), Prolactin ($p<0.02$) and IL-4 ($p<0.000$) with an insignificant increase in IL-5 ($p<0.685$) in comparison to control group. RA patients show a positive significant correlation between Prolactin, IL-5 and activity with negative insignificant correlation between IL-4 and activity. Where in Lupus patients there was a positive significant correlation between Prolactin, IL-4 and activity with negative insignificant correlation between IL-5 and activity. There was no correlation between Leptin and activity in both diseases (RA, SLE). There's a highly significant positive correlation between serum Leptin levels and BMI among all patients of RA and Lupus ($p<0.000$, $p<0.003$), respectively. There was a difference in the Leptin level between male and female patients with a significant increase in the female than male ($p<0.05$). We can conclude from our results that Leptin cannot be used to assess disease activity in RA and SLE where Prolactin can be used to assess disease activity in RA and SLE.

Key words: Leptin, prolactin, RA, lupus, IL-4, IL-5

INTRODUCTION

Leptin (a cytokine-like 16 kDa peptide hormone produced by adipose tissue) has an important role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure through regulating the B-oxidation of fatty acids (Ahima and Flier, 2000; Fantuzzi and Faggioni, 2000). Leptin may also be involved in the control of local inflammatory events in the joint as it stimulates the proliferation of T cells in vitro; to promote T helper (Th1) responses and to protect T cells from corticosteroid induced apoptosis. It has been suggested that leptin potentates inflammation in patients with Rheumatoid Arthritis (RA) (Palmer and Gabay, 2003; Otero *et al*., 2006).

Prolactin a versatile hormone secreted not only by anterior pituitary gland but also by many extrapituitary sites including the immune cells. The endocrine/paracrine PRL has been shown to stimulate the immune cells by binding to PRL receptors. Increased PRL levels, frequently described in autoimmune diseases, could depend on the enhancement of coordinated bi-directional communications between PRL and the immune system observed in these diseases (De Bellis *et al*., 2005).

Pohlers *et al*. (2005) found that mina of IL-5 and IL-10 increase in acute phase of RA then there is overlapping of Th1-like cytokine in chronic state with decrease of IL-5. In RA the induction of pro-inflammatory cytokines in monocyte-macrophages are triggered by cellular contact interaction with stimulated T, lymphocytes; and the stimulus controls the choice of signaling pathway in monocyte-macrophage controlled or uncontrolled inflammatory processes (Burger, 2000).

The objectives of this study were to determine the serum levels of leptin, prolactin, IL-4 and IL-5 in patients with Juvenile rheumatoid arthritis and systemic lupus erythematosus, in order to compare them with healthy controls then to correlate these levels with Body Mass Index (BMI) and disease activity.

MATERIALS AND METHODS

The study included 33 patients who were recruited from the outpatient rheumatology clinic in children's

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(Abu El Riche) hospital, Kasr El Aini, Cairo University. An informed consent was obtained from their parents and from National Research Center Ethical committee before enrollments. The patients were divided into two groups. Group 1 included twenty-one patients with Juvenile Rheumatoid Arthritis (JRA). They were 13 males and 8 females with age 11.9±3.6 years classified according to the Durban criteria. 6. Fourteen had oligoarticular disease and seven with Polyarticular arthritis. Oligoarticular arthritis was defined as active in the presence of joint swelling with reduced range of motion in one to four joints. While Polyarticular arthritis was defined as active in the presence of (a) erythrocyte sedimentation rate >25 mm/1st h; (b) swelling with reduced range of motion in more than five joints. Patients were subdivided according to the clinical findings (fever, rash, joint inflammation) and Erythrocyte Sedimentation Rate (ESR) in a group of 6 patients with active disease and another of 15 patients with inactive disease. The 6 active cases were of the oligoarticular type. Twelve patients with Systemic Lupus Erythematosus (SLE) were enrolled as group 2. They were 2 males and 10 females with age 15.8±2.9 years. Patients were classified according SLEDAI into 4 cases with disease activity and 8 cases with inactive disease. All patients were subjected to thorough general and systemic clinical examination their body weights (kg) and heights (m) were recorded to calculate the Body Mass Index (BMI) (kg m^{-2}). Complete Blood Count (CBC), ESR, CRP and kidney functions together with calcium, phosphorus and alkaline phosphatase were determined for both groups. Twenty-one healthy children with matched age, sex and anthropometrics measures were included in the study to serve as control group (group 3). Blood samples were collected from the three groups to assess serum leptin, prolactin, interleukin 4 (IL-4) and interleukin 5 (IL-5) levels to compare cases' with controls' sera and to correlate these levels with Body Mass Index (BMI) and disease activity in groups 1 and 2 (i.e., in each group separately).

Body mass index: it was calculated as BW/L^2 (in which BW is expressed in kilograms and L in meters.)

Sample collection: Six milliliter of venous blood were collected under complete aseptic conditions and were used as follows:

- Two milliliter were added to EDTA anticoagulant and were used for ESR and CBC.
- Four milliliter were left to clot and the serum was obtained by flicking off after centrifugation for 15 min at 3000 rpm. This was used for assessment of ANA, CRP, Prolactin, Leptin, IL-4 and IL-5
- The erythrocyte sedimentation rate was measured by Westergren method after dilution of the EDTA blood sample with the standard 10^9 mmol/L (32 g L^{-1}) tri-sodium citrate in a ratio of 4 parts blood to 1 part citrate. Readings were obtained at room temperature (18-25°C) within 4 h of collection at 1 and 2 h interval.
- A complete blood count was performed using MaxM cell counter, Coulter electronics, Florida, USA.
- Serum ANA was assessed by indirect immunofluorescent microscopy (IMMCO Diagnostics, USA).
- CRP by latex agglutination.
- Blood urea Nitrogen and Creatinine by Express plus analyzer.
- Alkaline phosphatase by Express plus analyzer
- Calcium and phosphorus by Express plus analyzer.
- Prolactin was assayed using PRL_EASIA immunoenzymatic assay KAP1441 for quantitative measurement human prolactin from BIOSOURCE according to the manufacturer instructions. Interassay 6.1% intrassay 6.9% IU mL^{-1}.
- Leptin was assayed using leptin serum-EASIA an immunoenzymatic assay for quantitative measurement human leptin in serum and plasma from BIOSOURCE KAP 2281 according to the manufacturer instructions. Interassay 3.6% sensitivity 1 ng mL^{-1}.
- Human IL-5 was assayed by CYT Eliza. Human IL-5 Enzyme immunoassay for the detection of free Human IL-5 from CYTIMMUNE Sciences INC USA Maryland according to manufacturer instruction. Intrassay variation ±8.3% interssay variation ±10.0% sensitivity 0-92 pg mL^{-1}.
- Human IL-4 was assayed by CYT Eliza. Human IL-4 Enzyme immunoassay for the detection of free Human IL-4 from CYTIMMUNE Sciences INC USA Maryland according to manufacturer instruction. Intrassay variation ±7.7% interssay variation ±0.9% sensitivity 0-19 pg mL^{-1}.

Statistical analysis: Analysis of data was carried out on SPSS software (statistical Package for Social Sciences, window 8 version, USA) on IBM-PC microprocessor computer. Data was expressed as mean±SD. Normally distributed results were compared using student's t-test. Spearman's rank correlation test was used for correlations. Probability of less than 0.05 was considered significant.

RESULTS

Table 1 reveals the clinical and demographic data of patients.

Table 1: Demographic data of SLE and JRA patients

Clinical examination	JRA (group 1) No. (21)	SLE (group 2) No. (12)
Sex (M/F)	13/8	2/10
Age (years)	11.9±3.6	15.8±2.9
Height (m)	1.30±1.68	1.44±85
Weight (kg)	35.1±12.6	53.1±15.1
BMI (Kg m^{-2})	20.2±3.9	25.3±4.9
HB (g%)	12.6±2.3	11.6±1.9
Platelets 1000/cc	355.4±130.9	351.7±217.5
WBC's 1000/cc	6.9±2.2	6.1±2.5
ESR	56.7±33.9	39.3±45.4
Creatinin (mg dL^{-1})	0.6±0.2	0.9±0.6
BUN (mg dL^{-1})	9.3±2.8	12.7±9.8
Ca (mg dL^{-1})	8.2±0.5	8.8±0.6
Phosphorus (mg dL^{-1})	4.2±0.5	4.7±0.5
Alkaline phosphate (mg dL^{-1})	324.2±165.7	249.8±123
ANA positive	8	12
Active/Inactive	6/15	4/8

Results are shown as mean (±SD)

Table 2: Serum levels of leptin, prolactin, IL 4 and IL 5 in comparison to control group

Parameters	JRA group 1	SLE group 2	Control group	p-value group 1	p-value group 2
Leptin (ng mL^{-1})	8.79±866	21.920±30.422	2.758±0.72	<0.038	<0.050
Prolactin (ng mL^{-1})	375.00±131.32	424.167±119.58	284.170±96.24	<0.021	<0.020
IL-4 (pg mL^{-1})	31.06±6.58	40.380±8.01	20.770±4.69	<0.005	<0.000
IL-5 (Pg mL^{-1})	133.167±190.7	194.833±235.6	158.330±114.48	<0.724	<0.685

Results are shown as mean±SD, p-value <0.05 is significant

Table 3: Correlation between measured Lab parameters and disease activity of all cases

Parameters	JRA group 1	SLE group 2	p-value group 1	p-value group 2
Leptin	0.346	0.141	<0.271	<0.663
Prolactin	0.870	0.975	<0.000	<0.000
IL-4	-0.134	0.806	<0.679	<0.002
IL-5	0.631	-0.254	<0.028	<0.425

Pearson correlation (2 tailed) correlation is significant at the level 0.05

Table 2 presents serum levels of Leptin, Prolactin IL-4 and IL-5 in cases (group 1 and 2) and controls.

There were significant increase in the levels of Leptin (<0.038), Prolactin (p<0.021) and IL-4 (p<0.005) in Juvenile Rheumatoid Arthritis group with insignificant decrease in IL-5 (p<0.724) in comparison to control group.

Systemic Lupus group show a significant increase in level of Leptin (p<0.05), Prolactin (p<0.02) and IL-4 (p<0.000) with an insignificant increase in IL-5 (p<0.685) in comparison to control group.

Table 3 In RA patients shows a positive significant correlation between Prolactin, IL-5 and activity with negative insignificant correlation between IL-4 and activity. Liptin show no correlation with activity.

In Lupus patients shows a positive significant correlation between Prolactin, IL-4 and activity with negative insignificant correlation between IL-5 and activity. There was no correlation between Leptin and activity.

In cases with JRA (group 1) serum leptin showed significant positive correlation with BMI and age (p<0.000, p<0.05), respectively. Meanwhile Prolactin, IL-4 and IL-5 didn't show any significant relation with anthropometric data of the cases.

In cases with SLE (group 2) there's a highly significant positive relation between serum Leptin levels and BMI among all patients (p<0.003).

There was a difference in the Leptin level between male and female patients with a significant increase in the female than male (p<0.05).

DISCUSSION

In rheumatoid Arthritis (RA) it has been suggested that hypothalamic-pituitary dysregulation is an important pathogenic mechanism. A regulatory loop exists between the hypothalamus-pituitary axis and level of circulatory Leptin. Leptin levels are increased in patients with RA compared with healthy control (Bokrewa *et al*., 2003).

Present results showed that there was a significant increase in Leptin level compared to control in RA patients which agreed with results of Otero *et al*. (2006) and Bokarewa *et al*. (2003). On the other hand Tokarczyk-Kapik *et al*. (2002) reported that mean serum Leptin concentration in patients with RA was lower than control.

The results showed no correlation between Leptin level in RA and the disease activity which coincide with Aners *et al*. (1999), Nishiya *et al*. (2002) and Popa *et al*. (2005) who stated that Leptin serum levels were not correlated with disease activity as compared to the value of ESR, or CRP and disease stages of RA patients.

Nishiya *et al*. (2002), Bokarewa *et al*. (2003) and Toussirot *et al*. (2005) stated that Leptin in RA reflects

BMI but not joint inflammation, that agreed with our results as we found a positive correlation between Leptin level and BMI ($p<0.000$).

Perfetto *et al.* (2005) found a positive correlation between Leptin level and age of patients of RA ($p<0.0005$) which consistence with our results as we found a positive relation between Leptin and age of patients in RA ($p<0.05$).

Saad *et al.* (1997) reported that girls had higher Leptin concentrations than boys despite having similar BMIs. The reason for this difference between the sexes is unclear, but several studies report that girl's fat cells produce more Leptin than those of boys with a similar body mass which agree with which we found in our work, as there was a significant increase in the female level of Leptin in comparison to male patients ($p<0.05$).

Leptin level was found significantly higher inpatients with SLE than healthy control with no correlation with activity. This coincides with the results of Garcia-Gonzalez (2002) who reported that patients with SLE had higher Leptin levels than control. Perciaccant *et al.* (2006) reported that plasma Leptin was increased in SLE mice than control.

Hyperprolactinemia has been described in the active phase of some non-organ-specific autoimmune diseases, as systemic lupus erythematosus and Rheumatoid arthritis (De Bellis *et al.*, 2005).

Ram *et al.* (2004) showed that the increase of total serum Prolactin in patients of RA in comparison to healthy control is due to increased free Prolactin concentration.

These data is in consistence with our results which showed a significant increase in serum Prolactin in RA patients and this increase correlate positively with the activity of the disease.

On the other hand Rovensky *et al.* (2004) stated that there was no up-regulation in Prolactin in relation to RA patients.

Jara *et al.* (2001) experimental studies support the potential role of Prolactin as a Premotor of clinical activity and severity of SLE. They suggest that Prolactin participates in local and generalized immune and inflammatory processes and acts as a bridge between the neuro-endocrine and immune system in SLE. Alfredo (2006) and Rezaieyazdi and Hesamifard (2006) reported that serum free Prolactin concentrations in patients with SLE were associated with Lupus activity.

The anti-inflammatory cytokine IL-4 is believed to play a protective role in arthritis, it down regulates monocyte, macrophage cytotoxicity and cytokine production including that of TNF-alpha and TNF-alpha receptors, as well as IL-5 induced chemokine production. Notably, IL-4 decreases IL-1B production while increasing IL-1B receptor antagonist production, thus suggesting a coordinated anti-inflammatory approach (Hart *et al.*, 1996).

Kuroda *et al.* (1997) stated that IL-4 decrease the mRNA production of cyclooxygenase 2 and cytosolic phospholipase A2, by reducing the levels of prostaglandin E2 as it decrease the monokine production in synovial specimine, TNF-alpha receptor expression by synovial macrophages.

Kawashima and Miossec (2006) reported increase production of IL-4 in peripheral blood mononuclear cells from patients with rheumatoid arthritis than cells from healthy control.

Smith and Germolec (1999) reported that systemic autoimmune disorders were characterized by elevated levels of Th2 cytokines such as IL-5, IL-4 and IL-10.

Present results showed significant increase in IL-4 in RA patients, which coincide with the previous data.

In the other hand Hung *et al.* (2001) and Wong *et al.* (2000) stated that there was a decrease in IL-4 producing T helper cells and decrease IL-4 secretion in peripheral blood from subjects with JRA. Present results show insignificant decrease in IL-5 in RA patients with no correlation with activity which agree with Dirk Pohlers *et al.* (2005) who reported that mRNA of IL-5 and IL-10 increase in acute phase of RA then there is overlapping of Th1-like cytokin over Th2-cytokin in chronic state with decrease of IL-5.

Where Szodoray *et al.* (2006) reported an increase in IL-5 significantly in RA patients.

Wong *et al.* (2002) found a higher level of IL-4 in SLE patients than control and they propose that SLE is characterized by an elevation of both Th1 and Th2 cytokines.

Dean *et al.* (2002) found that a higher percentage TCR alphabeta + double negative T cells from patients with SLER contained IL-4 constitutively than did the same population of cells from healthy people or from those with RA.

The serum levels of IFN-gamma, IL-4 and IL-10 were higher than those of the healthy control ($p<0.05$) After treatment with corticosteroids, the level of IFN-gamma decrease but IL-4 and IL-10 of the patients with SLE were significantly higher than healthy control (Xie *et al.*, 2002).

These data were inconsistence with our results as we found a significant increase in the level of IL-4 in SLE patients compared to control.

In the other hand Viallard *et al.* (1999) and Roback *et al.* (2004) reported that IL-4 levels in serum were similar in SLE and healthy control.

AS regard IL-5 our results showed an increase but not significance in SLE patients in comparison to healthy control and this coincide with the data of Smith and Germolec (1999) as they stated that systemic autoimmune disorders are characterized by elevated levels of cytokines such as IL-5, IL-4 and IL-10.

We can conclude from our results that Leptin cannot be used to assess disease activity in RA and SLE where Prolactin can be used to assess disease activity in RA and SLE. In same time we need more research in immunological aspect of the autoimmune diseases to find an immune therapy for them.

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