Serum and Tissue Leptin Levels in Relation to Psoriasis vulgaris Severity

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Abstract

Background: Psoriasis is a chronic inflammatory T-cell-mediated skin disease, with a preponderance of type 1 cytokines that contribute in the induction and persistence of the inflammatory process. Leptin, a peptide hormone secreted mainly from the adipocytes of white fat is known to regulate a wide range of biological responses. Recently, leptin has been considered a cytokine -like hormone that modulates T-helper cell activity, it promotes type 1 cytokine synthesis and suppresses type 2 cytokine production. Accordingly, leptin was suggested to have a role in the pathogenesis of several chronic inflammatory diseases including psoriasis. However, the relationship between leptin and the severity of the disease still needs further studies.

Objective: This study aims at investigating the suggested relationship between leptin levels in both serum and tissue and the severity of psoriasis vulgaris.

Patients and Methods: This study included forty patients with chronic psoriasis vulgaris and forty healthy volunteers as a control group. Psoriasis area and severity index (PASI) was scored for all patients. Serum leptin levels were estimated using an Enzyme Linked Immunosorbant Assay (ELISA) technique. Skin biopsies were taken from lesional and non lesional skin of psoriatic patients as well as from normal skin of the control group. Tissue leptin levels were studied by both ELISA and reverse transcription-polymerase chain reaction (RT-PCR) in all patients and controls.

Results: Tissue leptin levels were found to be significantly higher in psoriatic lesions ($p < 0.05$) when compared to controls, as well as significantly higher when compared to the non lesional skin of the patients themselves ($p<0.05$) when studied by both ELISA and R-T PCR techniques. Moreover, tissue leptin levels were found to be significantly higher in the non lesional skin of psoriatic patients when compared to controls ($p<0.05$). Serum leptin levels, lesional and non lesional tissue leptin levels were found to be significantly higher in patients with severe psoriasis vulgaris than mild to moderate psoriasis than controls ($p<0.05$). A statistically significant correlation was found between serum leptin levels, lesional tissue leptin levels and PASI score in patients with severe psoriasis vulgaris ($r=0.68$, $p=0.001$, $r=0.793$, $p=0.000$).

Conclusion: Serum and tissue leptin levels are elevated in patients with severe psoriasis vulgaris and leptin level might serve as a marker of severity. [Journal of American Science 2010;6(10):412-422]. (ISSN: 1545-1003).

Keywords: Psoriasis, PASI, and leptin

1. Introduction

Psoriasis is a chronic cutaneous inflammatory skin disease. It is characterized by excessive, but controlled cellular proliferation, fibroblast activation, vascular expansion, leucocyte infiltration and alterations of cytokine production (Kapp, 1993). The disturbed keratinocyte proliferation and differentiation are a consequence of the abnormal immune activity with persistent T-lymphocyte activation (Prinz, 2003). These activated T-helper cells produce a variety of cytokines including INF-gama, TNF-alfa, and various interleukins that play an important role in its pathogenesis (Kristina and Krueger 2009). Recently, leptin an adipokine has been identified in the keratinocytes of psoriatic lesions suggesting its possible role in the pathogenesis of the disease (Cerman et al., 2008).

Leptin is a hormone synthesized and secreted by adipocytes. It is a 16 - kD non-glycosylated polypeptide that consists of 167 amino acids. The structure of leptin contains four interconnected anti-parallel α-helices, which is in high similarity to members of the long-chain helical cytokines such as interleukin-6 (IL-6), IL-11, IL-12, granulocyte colony stimulating factor (G-CSF), and others (Auwerx and Steals 1998 Hidetoshi et al. 2009).

Leptin regulates body weight through inhibiting food intake and stimulating energy consumption (Muoio et al., 2002). It has been also recognized as a key factor in regulating many biological responses including blood pressure, haematopoiesis, neuroendocrine function, angiogenesis, bone formation and reproduction (Lu and Li 2000). Leptin receptors are expressed primarily in the hypothalamus, but also expressed in other tissues as

keratinocytes, fibroblasts, endothelial cells, and peripheral blood mononuclear cells (Tartaglia,1997).

Leptin has an important role in inflammation and in immunoregulation. It activates monocyte/macrophage cells and potentiates production of the proinflammatory cytokines, tumor necrosis factor alpha (TNF - α), interleukin (IL) - 6, and directs T cell differentiation to Th1 phenotype, expressing interferon gamma (INF)-γ and IL-2 (Otero et al., 2005). On the other hand, it shows certain anti-inflammatory properties by releasing IL-1 receptor antagonist (Gabay et al., 2001). Thus it has been implicated in the pathogenesis of autoimmune inflammatory conditions such as chronic bowel disease, and rheumatoid arthritis (Everkhlioglu et al., 2002 and Lee et al., 2006). Recent studies have shown that leptin stimulates keratinocyte proliferation, expresses adhesion molecules and promotes angiogenesis as well as endothelial cell growth (Bernotiene et al., 2006 and Murad et al., 2003). Accordingly, a strong relation seems to exist between the immunopathogenesis of psoriasis and the proliferative and immunological effects of leptin.

Previous studies have suggested the role of leptin in the pathogenesis of psoriasis vulgaris (Cerman et al., 2008 and Wang et al., 2008) but its role in the severity of the disease still needs further studies. This study was performed in order to; investigate the suggested relationship between serum and tissue leptin levels with the severity of the disease.

2. Subjects and Methods

2. 1. Patients and Controls

This study included forty non obese patients with chronic stable psoriasis vulgaris recruited from the dermatology outpatient clinic of Cairo University Hospital. The diagnosis was made clinically, based on the presence of characteristic plaque-type psoriatic lesions. Forty healthy age and sex matched non obese volunteers with no family history of psoriasis were included in the study as a control group. The purpose and nature of the study were explained to all subjects. All included subjects have consented to be enrolled in this study.

2. 2. Exclusion Criteria

Obese subjects with history of acute or chronic infections, liver disease, renal disease, recent history of cardiovascular disorder, hypertension, neurological disease, or diabetes mellitus were excluded from the study. Moreover, patients who had received oral or topical antipsoriatic therapy within four weeks before taking samples were not included in the study.

2. 3. Method

2. 3.1. Clinical Assessment

All participants were subjected to thorough history taking and skin examination. The degree of severity of psoriasis was clinically assessed by psoriasis area and severity index (PASI) score for each patient **(Fredriksson and Petterson 1978).** It includes assessment and recording of erythema, infiltration, desquamation and extent of the disease (area %) by using numerical rating of 0 - 4 for each of the parameter: 0 for absent; 1 for slight (light pink, rare scales, no elevation with area involvement < 10 %) ; 2 for moderate (light red, poorly defined scales, slight elevation with area involvement 10 – 30 %); 3 for severe (red, defined scales, moderate elevation with area involvement 30 – 50 %); and 4 for very severe (very red, heavy scales, marked elevation with area involvement 50 – 70 %). Accordingly, mild to moderate psoriasis and severe psoriasis were defined as PASI <15 and PASI > 15, respectively.

2. 3. 2. Blood Sampling

10 ml venous blood samples were withdrawn from twelve hour fasting non obese patients and controls. Blood was collected in vacutainer tubes, left for 20 min at 37°C in order to clot and then centrifuged for 10 min at 3000 r.p.m.to separate the serum. Serum samples were divided into several aliquots and stored at - 80°C until subsequent analysis.

2. 3.3. Tissue Sampling

4 mm punch skin biopsy samples were taken from the active plaques and the non lesional skin of twelve hour fasting non obese patients with psoriasis vulgaris. Normal tissue samples were taken from the extensor surface of the extremities of the healthy fasting non obese volunteers who served as a control group. Tissue samples were stored under - 80°C till assessed.

2. 3.4. Laboratory Investigations

Routine laboratory investigations including; blood sugar, blood cell count, sedimentation rate, liver and renal function tests were done for all participants to exclude any organic disease or inflammation.

2. 3.5. Leptin Measurements

(A) Serum and tissue leptin analysis by ELISA

The quantitative determination of leptin was conducted in serum and tissue by an Enzyme Linked Immunosorbant Assay (ELISA) technique, using a commercial available kit, (DRG® ELISA EIA-2395). Every sample was run in duplicate, measurements

differed by less than 10%, and the mean value was calculated and used for statistical analysis.

Principle of the test

The DRG Leptin ELISA Kit is a solid phase Enzyme Linked Immunosorbant Assay based on the sandwich principle. The micro titer wells are coated with a monoclonal antibody directed towards a unique antigenic site on a leptin molecule. 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 colour developed is proportional to the concentration of leptin in the patient sample

(Considine *et al.*, 1996 and Guillaume and Björntorp 1996).

(B) Tissue leptin analysis by detecting the leptin gene by Reverse transcription-Polymerase Chain Reaction (RT-PCR)

1-RNA Extraction:

Total RNA was extracted from skin tissue homogenate by the acid guanidinum thiocyanate-phenol-chloroform method (Promega) R1. According to the manufacturers recommendation, the RNA obtained content and purity were measured by an UV spectrophotometer with absorbance at 260 nm

(Chomkczynski and Sacchi 1987).

2-RT –PCR Analysis:

RT-PCR was done using the extracted RNA for detection of leptin gene. For amplification of the target gene, RT and PCR were run in two separate steps. Reaction mixture of RT reaction containing 1 µg total RNA, 0.5 µg random primer, 5×RT buffer, 2.5 mmol/L dNTP, 20 U RNase inhibitor and 200 U moloney murine leukemia virus reverse transcriptase (MMLV) (Promega) in a total volume of 25 µl. The reaction was incubated at 37ºC for sixty minutes, and then heated to 95 ºC for 5 minutes to inactivate MMLV. PCR was carried out with 1.5 µl RT products, 10 × PCR buffer (without Mg^{2+}) 2.5 µl, 2.0 µl dNTP (2.5 mmol/L), 2.0 µl $MgCl_2$ (25 mmol/L), 0.5 µl each primer (20 µmol/L) of β-actin, 0.5 µl each primer of leptin gene (20 µmol/L) and 1 U of Taq DNA polymerase (Promega), in a final volume of 25 µl. The reaction profile consisted of an initial denaturation at 97°C for 5 minutes followed by 35 cycles of PCR at 96°C for 1.5 minutes (denaturation), 72°C for 1.5 minutes (annealing) and 72°C for 1.5 minutes (extension). A final extension cycle of 72 ^{o}C for 15 min was included. The fluorescence emitted was captured at the end of the extension step of each cycle at 530nm. Results were normalized to the expression of the housekeeping gene 18S ribosomal RNA. The appropriate primer pairs, annealing temperature and product size in are shown in table (2).

3-Agarose gel electrophoresis:

The amplified PCR products of leptin were electrophoresed on 2% agarose gel with size marker of 100bp, stained with ethidium bromide and visualized by an UV transilluminator fig (1, 2).

4- Semi-quantitative determination of PCR products:

Semi-quantitation was performed using the gel documentation system (BioDO, Analyser) supplied by Biometra. According to the following amplification procedure, relative expression of each studied gene (R) was calculated following the formula: R= Densitometrical Units of each studied gene/ Densitometrical Units of β-actin.

2. 3.6. Statistical analysis

All data were coded and entered using the program statistical package for social sciences (SPSS) version 15 under windows XP. Descriptive data was summarized using mean, standard deviation (SD) and range for quantitative variables and numbers and percent for qualitative variables. Inter group comparisons were conducted using Pearson Chi-square for qualitative variables and independent sample T test for normally qualitative variables. Non parametrical tests (Mann-Whitney U-test and Wilcoxon signed ranks test) were used for quantitative variables. Pearsons correlation co-efficient (r) test was used to test for linear relation between quantitative variables. Linear regression analysis was done to test for significant predictors for psoriasis severity as measured by PASI score. *P* values < 0.05 were considered statistically significant.

3. Results

3.1. Clinical Data:

The results of this study are demonstrated in tables (1 to 3) and figures (1 to 7). Forty non obese patients with psoriasis vulgaris were included in this study. Twenty of the patients had mild to moderate psoriasis (PASI <15), while the other twenty had severe psoriasis (PASI > 15). Of the forty patients, 26 were females (65%) and 14 were males (35%). Their age ranged between 18 – 62 years. The mean age and standard deviation (SD) was 38.50 ± 12.83 years. The duration of the disease ranged between 4 months to

180 months, with a mean ± SD 57.05 ± 54.08. The PASI score for clinical assessment ranged between 3.5 - 28.5, the mean ± SD was 14.61 ± 6.6. The control group included 29 females (74.5%) and 11 males (25.5%). Their age ranged between 18 - 54 years with mean ± SD 35.70 ± 9.09. Controls were age and sex matched.

Linear regression analysis was done, using constant predictors; age, gender, duration, serum leptin levels (ELISA), lesional tissue leptin levels (ELISA), and lesional tissue leptin expression (PCR) against PASI score as a dependent variable. Lesional tissue leptin levels tested by ELISA technique was presented as a model of specific predicator, p<0.00 as shown in (Table 3).

Estimation of serum leptin levels by ELISA technique:

The serum leptin level estimated in patients with psoriasis ranged between 12.5 - 98.2 ng/ml, with a mean ± SD of 40.9 ± 28.4. While in the control group it ranged between 18.6 - 56.7 ng/ml, with a mean ± SD of 33.85 ± 11.56. No statistically significant difference was found in the serum leptin level between patients and controls (p = 0.87). However, when serum leptin level was evaluated in relation to disease severity according to PASI score, serum leptin level was found to be significantly higher in severe psoriasis (PASI > 15), (62.1 ± 25.8ng/ml) than mild to moderate psoriasis (PASI <15) (19.72 ± 5.61), and also for controls (33.8 ± 11.56) (p < 0.05). Serum leptin level showed a statistically significant correlation with PASI score (r = 0.681, p =0.001). Moreover, there was a strong statistically significant correlation between serum leptin levels and tissue leptin levels in both lesional and non lesional skin of the patients
(r = 0.648, p = 0.002, r = 0.589 p = 0.006 respectively)) (Table 1).

Estimation of tissue leptin levels by ELISA technique:

The tissue leptin level in lesional and non lesional skin of patients, ranged between 11.1 - 19.7 ng/ml, with mean ± SD of 16.05 ± 2.53, and 8.6 -14 ng/ml with mean ± SD of 10.93 ± 1.5 respectively. In the control group, tissue leptin level ranged between 10.10 -19.8 ng/ml, with mean ± SD of 13.82 ± 3.1. A statistically significant difference was found between lesional tissue leptin level and controls (p=0.017). Also, a high statistically significant difference was found between lesional and non lesional tissue leptin levels (p< 0.001). When patients were evaluated according to disease severity, tissue leptins level were significantly higher in patients with severe psoriasis (17.8 ± 1.25) than patients with mild to moderate psoriasis (14.2 ± 2.13), and controls (13.82 ± 3.1) (p<0.05). Moreover, tissue leptin level in lesional skin showed a statistically significant correlation with PASI score (r = 0.79, 3 p=0.000) (Table 1).

Tissue leptin gene expression by RT-PCR technique:

Tissue leptin gene expression in both lesional and non lesional skin of patients ranged between 1.02 - 1.9 ng/ml, with mean ± SD of 1.49 ± 0.34, and 0.52 - 1.04 with mean ± SD 0.79 ± 0.15 respectively. In the control group tissue leptin levels ranged between 0.2-0.94 ng/ml, their mean ± SD was 0.62 ± 0.22. A high statistically significant difference was found between tissue leptin gene expression in lesional and non lesional skin (p< 0.001). Moreover, when patients were evaluated according to disease severity, leptin gene expression was significantly higher in patients with severe psoriasis than patients with mild to moderate psoraiasis, and control (p < 0.05) (Table1).

Table (1): Leptin levels in serum and tissue of psoriatic patients and controls in relation to disease severity.

	Mean ± SD	P Value
Serum leptin level ng/ml (ELISA)		
Control	33.85 ± 11.56	
Psoriatic patients	40.96 ± 28.41	0.087*
PASI < 15 (mild to moderate psoriasis)	19.72 ± 5.61	
PASI > 15 (severe psoriasis)	62.19 ± 25.88	0.001**
Tissue leptin level ng/ml (ELISA)		
Control	13.82 ± 3.10	
Lesional psoriatic patients skin	16.05 ± 2.53	0.017***
Lesional psoriatic patients skin PASI < 15	14.22 ± 2.13	0.001****
Lesional psoriatic patients skin PASI > 15	17.87 ± 1.25	
Non - Lesional psoriatic patients skin	10.93 ± 1.54	
Non - Lesional psoriatic patients skin PASI < 15	11.73 ± 1.24	0.001^{+}
Non - Lesional psoriatic patients skin PASI > 15	10.13 ± 1.42	0.018^{++}
Tissue leptin level (PCR)		
Control	0.62 ± 0.22	
Lesional psoriatic patients skin	1.49 ± 0.34	0.001^{-}
Lesional psoriatic patients skin PASI < 15	1.33 ± 0.30	
Lesional psoriatic patients skin PASI > 15	1.65 ± 0.32	0.028^{--}
Non - lesional psoriatic patients skin	0.76 ± 0.79	
Non - lesional psoriatic patients skin PASI < 15	0.76 ± 0.15	
Non - lesional psoriatic patients skin PASI > 15	0.83 ± 0.14	0.403^{---}

P value < 0.05 is significant.
** : Serum control versus psoriatic patients by ELISA.*
*** : Serum PASI < 15 versus PASI > 15 by ELISA.*
**** : Tissue control versus lesional psoriatic patients skin by ELISA.*
***** : Tissue lesional psoriatic patients skin PASI < 15 versus lesional PASI > 15 by ELISA.*
$^{+}$ *: Tissue control versus non - lesional psoriatic patients skin by ELISA.*
$^{++}$ *: Tissue non - lesional psoriatic patients skin PASI < 15 versus non - lesional PASI > 15 by ELISA.*
$^{-}$ *: Tissue control versus lesional psoriatic patients skin by PCR.*
$^{--}$ *: Tissue lesional psoriatic patients skin PASI < 15 versus lesional PASI > 15 by PCR.*
$^{---}$ *: Tissue non - lesional psoriatic patients skin PASI < 15 versus non - lesional PASI > 15 by PCR.*

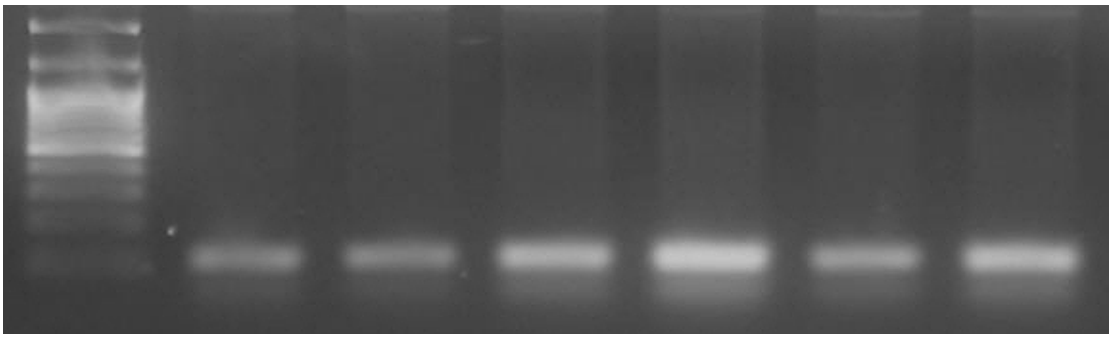
Table (2) :The oligonucleotide primers sequence of studied genes

	Primer sequence	Annealing temperature	Product size
Leptin	Forward primer : 5' CATTACTGAACAGTTCATTGTCTCC -3' Reverse primer : 5' 5'- CTTCCTTCATATTGTACTCTTTGCA -3'	65°C	126 bp
Beta actin	Forward primer : 5'TGTTGTCCCTGTATGCCTCT-3' Reverse primer : 5'TAATGTCACGCACGATTTCC 3'.	60°C	166 bp

Table (3): Leptin levels in lesional tissue (ELISA) as a model of specific predicator

Model of specific Predicator	Regression coeiffient (B) (B)	T	p value	95% Confidence Interval for B Lower Bound	Upper Bound
Leptin levels in lesional tissue (ELISA)	1.453	3.386	0.005	0.526	2.38

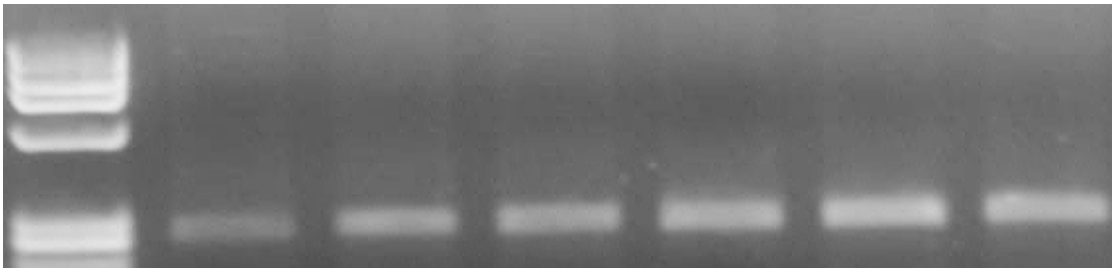
Fig.(1): An agarose gel electrophoresis shows PCR products of leptin gene in different studied groups



M 1 2 3 4 5 6

Lane M : PCR marker with 100 bp
Lane 1& 2: PCR products of leptin gene in control group
Lane 3 & 4: PCR products of leptin gene in psoriatic (lesional) group
Lane 5 & 6: PCR products of leptin gene in psoriatic (non lesional) group.

Fig. (2): An agarose gel electrophoresis shows PCR products of beta actin as housekeeping gene in different studied groups



M 1 2 3 4 5 6

Fig (3): The correlation of lesional tissue leptin levels with Psoriasis Area and Severity Index (PASI) as determined by ELISA.

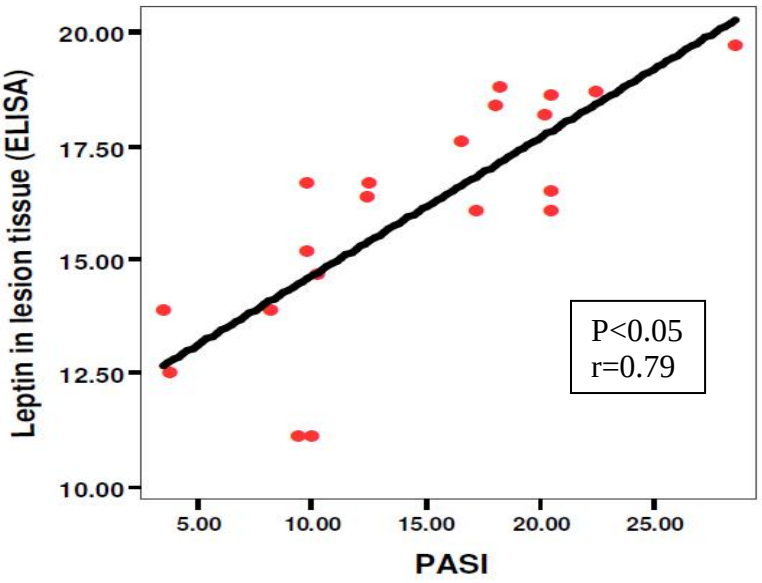


Fig (4): The correlation of lesional tissue leptin with Psoriasis Area and Severity Index (PASI) as determined by PCR.

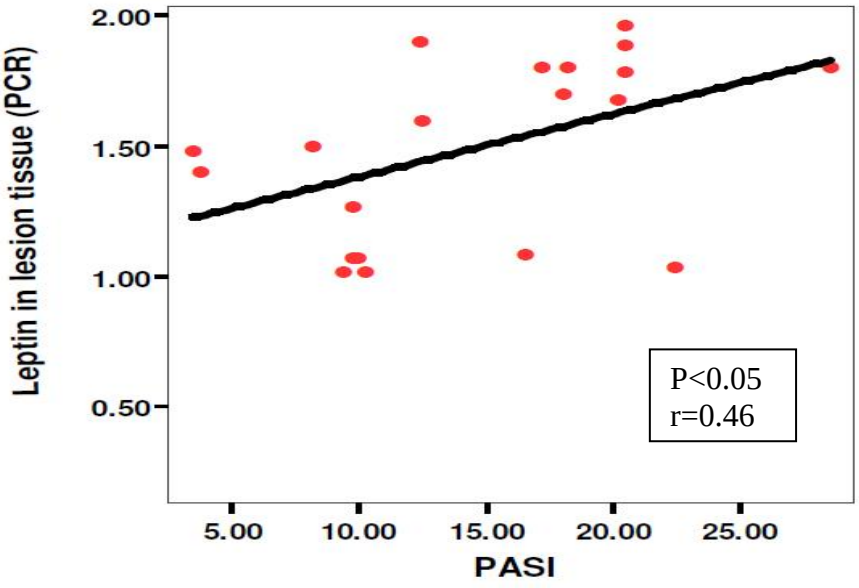


Fig (5): The correlation of serum leptin levels with Psoriasis and Area Severity Index (PASI) as determined by ELISA

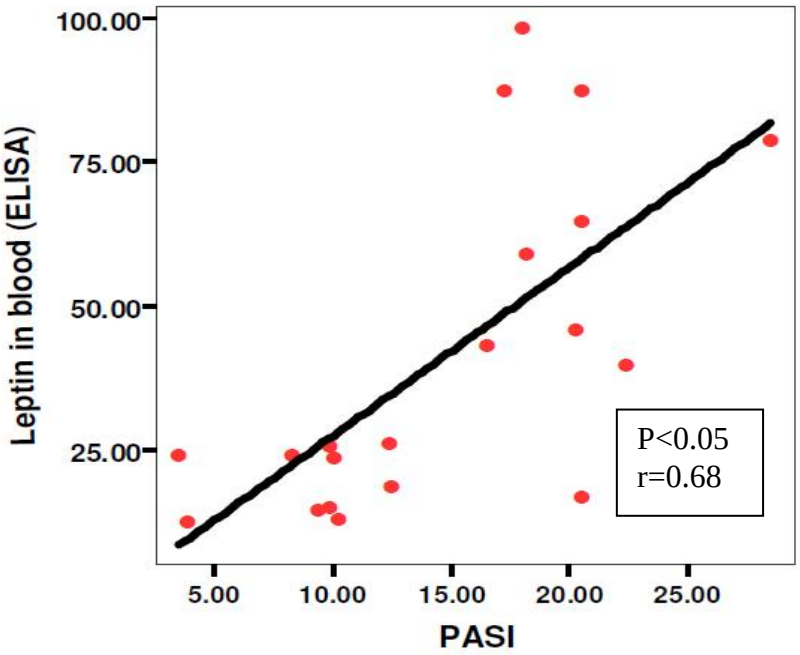


Fig (6): Tissue mean leptin levels ± SD in lesional and non - lesional skin as determined by PCR.

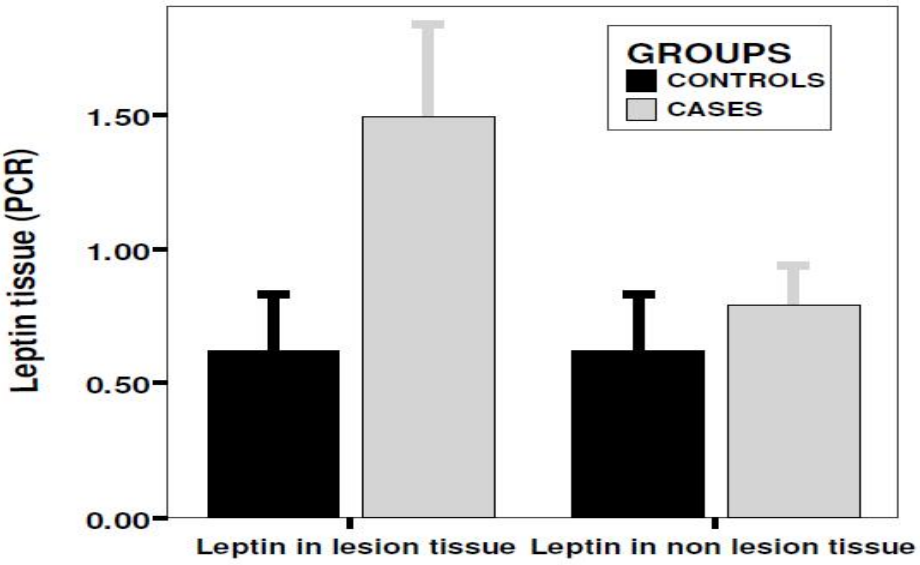
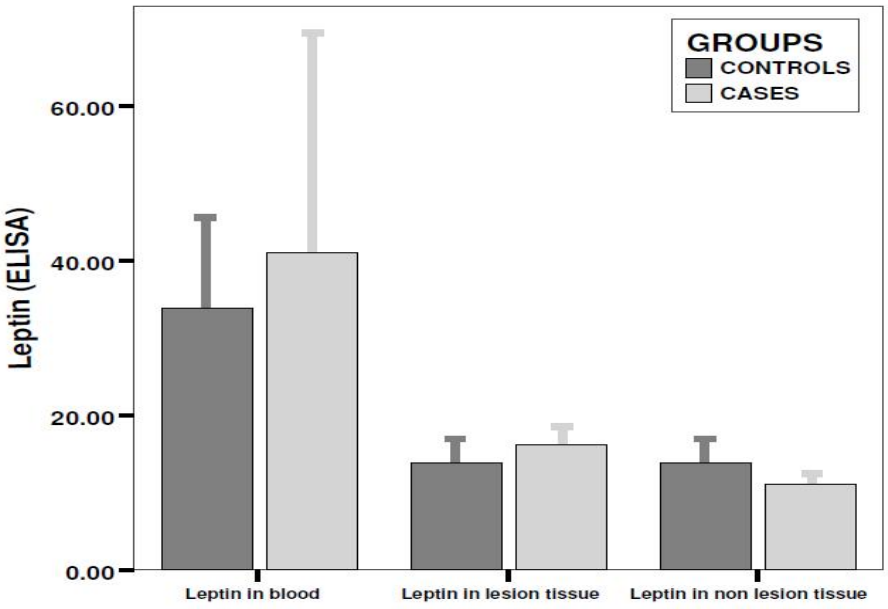


Fig (7): Mean leptin levels ± SD in blood, lesional and non - lesional skin as determined by ELISA.



4. Discussion:

In this study, both serum and tissue leptin levels were investigated by the ELISA technique aiming at studying the suggested relationship between leptin levels and the severity of psoriasis vulgaris. In addition, tissue samples from all subjects were subjected to RT-PCR examination for leptin gene expression. All patients included in the study did not receive any topical or systemic steroid therapy for four weeks before taking serum or tissue samples, in order not to disturb any cytokine production.

The results of this study showed that the mean serum leptin level in psoriatic patients was higher than the mean serum leptin level in the controls. However, the difference was not statistically significant (p = 0.87). When serum leptin levels were studied according to the severity of the disease, they were found to be significantly higher in patients with severe psoriasis than patients with mild to moderate psoriasis and also significantly higher than controls (p = 0.001). This could be explained on the basis of previous studies which demonstrated that leptin has an important role in the pathogenesis of immune - mediated inflammatory diseases (Gabay *et al.*, 2001and Otero *et al.*, 2005).

Serum leptin levels were reported to be elevated in patients with Behcets syndrome and rheumatoid arthritis and were correlated with the disease activity (Everkhlioglu *et al.*, 2002 and Lee *et al.*, 2006). Also, a previous study suggested that hyperleptinaemia is not a disease specific finding, but could be a marker for severity (Cerman *et al.*, 2008). Our findings seem to support the suggestions of that study.

Recently, it has been suggested that hyperleptinaemia may play an important role in cardiovascular diseases including atherosclerosis. Patients with severe psoriasis are known to be highly susceptible to cardiovascular diseases (Neimann *et al.*, 2006). Leptin, is known to induce endothelial dysfunction, stimulates platelet aggregation, and proliferation of vascular smooth muscle cells. Therefore, elevated serum leptin levels in patients with severe psoriasis may be one of the factors for the increased prevalence of cardiovascular diseases (Beltowski, 2006). Probably more studies should be made on psoriatic patients with cardiovascular disease, in order to further study any suggested relationship. This could be helpful in preventing serious

cardiovascular complications among patients with severe psoriasis.

Studies concerning the possible relationship between leptin levels (in tissue and serum) and psoriasis are still controversial. Aktan *et al*., in 2007 did not support this possible relationship. The measured serum leptin levels in the psoriatic patients in their study did not correlate with PASI score or duration of the disease. This may be due to the relatively small number of subjects with relatively low mean PASI scores included in that study. On the other hand, Wang *et* al., in 2008 reported that serum leptin levels were elevated in patients with severe forms of psoriasis (erythrodermic, pustular and arthropathic forms). Serum leptin levels were also correlated with body mass index but not with PASI score.

Few studies have been performed to investigate the possible role of tissue leptin levels in the pathogenesis of psoriasis (Cerman *et al.*, 2008 and Johnston *et al*., 2008). Using both ELISA and R-T PCR techniques in this study, we could demonstrate that the mean tissue leptin levels in lesional skin of psoriatic patients was higher than the mean tissue leptin level in the controls and the difference was statistically significant ($p<0.05$). This might be explained in view of previous studies (Otero *et al.*, 2005 and Bernotiene *et al.*, 2006), that showed that leptin modulates T-helper cell activity. In those studies, leptin was found to activate monocytes and macrophages, potentiating the production of various proinflammatory cytokines TNF - α, IL-6 and directing T- cell differentiation to Th1 phenotype (Bernotiene *et al.*, 2006). Also, leptin has been shown to stimulate keratinocyte proliferation, expression of adhesion molecules and angiogenesis (Cao *et al.*, 2001). Accordingly, it seems that the immunological role of leptin and the immunopathogenesis of psoriasis may overlap. Recently, it was found that high concentrations of leptin in psoriatic tissue may induce local production of amphiregulin, an endothelial growth factor which, together with leptin and resistin stimulates the production of chemokine (CXCL8) in cultured psoriatic cells. This was suggested to drive the keratinocyte proliferation which is characteristic for psoriasis (Johnston *et al*., 2008).

In the present study, tissue leptin levels in non lesional skin of psoriatic patients were found to be significantly higher than controls ($p<0.05$). This result may indicate that normally looking skin of psoriatic patients is possibly undergoing an immunological reaction. This seems in agreement with previous studies that considered psoriasis as a whole skin disease, and that the apparently normal skin may be in the pre-psoriatic stage in the form of immunological changes which are not yet detectable (Nickoloff *et al.*, 2000 and Fawzi and Shaker 2002).

Another remarkable result of this study is that , when the lesional tissue leptin levels were studied by both the ELISA and R-T PCR techniques according to the severity of the disease, they were found to be significantly higher in patients with severe psoriasis vulgaris than patients with mild to moderate disease and also higher than controls ($p<0.005$). It is noteworthy that our results showed this positive correlation between disease severity and serum leptin levels although we have excluded obese and cardiovascular patients.

In this study, serum and tissue specimens were taken from twelve hours fasting patients and controls in order to avoid fallacies, also all subjects were of average body weight and with no systemic disease. Other factors have been suggested to have influence on the severity of psoriasis such as persistent environmental stress and smoking (Naldi *et al.*, 2005). Moreover, it was suggested that serum leptin levels may vary between different populations (Cerman *et al.*, 2008) and consequently, tissue leptin levels might vary as well. This might explain the elevated leptin levels in our psoriatic patients in spite of absence of obesity and cardiac disease.

The results of this study seem to agree with Cerman *et al*., 2008 who used an immunohistochemical technique. They could demonstrate diffuse leptin receptor immunostaining in the epidermis and strong immunoreactivity in the dermal fibroblasts, of psoriatic patients and healthy controls. They suggested that the increased epidermal staining was due to an increased epidermal turnover. They also demonstrated that serum leptin levels showed a significant correlation with the PASI score. On the other hand, they were not able to demonstrate a correlation between the tissue leptin levels and leptin receptor expression with the PASI scores. This may be due to the less sensitive and semiquantitative technique of the immuneohistochemistry , which they used as compared to the more sensitive R-T PCR technique for expressing leptin gene which we used in this study.

In conclusion, the results of this study support the possible relationship between leptin levels in both serum and tissue and the severity of psoriasis vulgaris. Moreover, this study demonstrated that leptin might serve as a marker of severity as tested by linear regression analysis in psoriasis vulgaris patients. Further studies might be done on different types of psoriasis within obese patients with and without

cardiovascular disease for better understanding of the role of leptin in severe psoriasis vulgaris.

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