Association between Leptin 2548 G/A gene polymorphism and serum Leptin in patients with psoriasis

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Abstract

Background: Psoriasis is a disorder with genetic and immunologic background. Leptin can involve in development of psoriasis by regulation the T-helper response.

Objective: Our primary goal is to study the effect of polymorphism (G-2548A) of the leptin (LEP) gene as a risk factor for predisposition of psoriasis, and relation between the leptin serum levels and leptin 2548 gene polymorphism.

Methods: The study involved 100 patients with psoriasis and 80 healthy controls. Analysis of G-2548A polymorphism of the LEP gene was made by the PCR and restriction fragment length polymorphism technique. The relationship between LEP gene polymorphism and the clinical features of the patients was analysed. Serum leptin levels and proportions of comorbidities in patients vs controls were compared.

Results: In controls, the GA, AA and GG frequencies were 50%, 30% and 20%, respectively, while in patients, the distribution of genotypes was 48%, 20% and 32%, respectively, with significant difference between patients and controls.

There were a significant differences in the genotype distribution of Leptin -2548 cytokines examined with respect to its serum levels in patients group. the GG genotype mean of serum level was(23.4 ± 4.1ng/ml), and GA were 20.5 ± 6.84 and AA was (16.2 ± 5.86 ng/ml).

Conclusion: LEP G-2548A polymorphism could be a predictor for higher plasma leptin and increased risk of psoriasis and could be used as a marker for psoriasis-related comorbidity risk.

Key words: gene polymorphism – leptin – metabolic syndrome – psoriasis

Introduction

Psoriasis is a common and complex disease affecting approximately 2% to 3% of the world population. This disease can manifest itself in the skin, as well as affect the nails and joints of patients[(1)]. In addition, psoriatic patients have been found to have increased risk of a distinct pattern of other chronic disorders including coronary artery disease, metabolic syndrome, fatigue, and depression[(2)].

As is the case of many autoimmune diseases its real cause remains poorly defined, also the pathogenesis of psoriasis it still not understood perfectly [(3)] . Cells in the dermis and epidermis including dermal dendritic cells, epidermal Langerhans cell, melanocytes, and migrating lymphocytes, are considered important in this aspect and are known to produce a great variety of cytokines. The possibility that psoriatic epidermal hyperplasia results from increased local cytokine that produce by keratinocytes or by activated cellular elements in the inflammatory infiltrate has drawn increasing attention .

As psoriasis is a disease with genetic and immunologic background, and leptin has been suggested to provide a link between T-cell function and inflammation in psoriasis , our primary goal is to investigate whether the functional polymorphism (G-2548A) of the LEP gene is involved in the genetic predisposition in a sample of Iraqian patients with psoriasis, and our secondary goal is to examine relation between serum levels and Leptin 2548 G/A gene polymorphism[4] .

Leptin (OB protein) is a 16 kDa adipokine, which was initially described as an adipocyte-derived hormone. It is coded by obesity gene localized on the 7q31 chromosome. The adipose tissue is a major source of leptin and its circulating concentrations indirectly reflect body fat stores. Leptin is an important signal in the regulation of food intake and energy balance and its levels usually correlate with BMI [5] . It links nutritional status with neuroendocrine and immune functions, and its role in the modulation of immune response and inflammation has recently become increasingly evident. The increase in leptin production that occurs during infection and inflammation strongly suggests that leptin is a part of the cytokine network that governs the inflammatory-immune response and the host defense mechanisms. Leptin plays an important role in inflammatory processes involving T cells and has been reported to modulate T-helper cell activity in the cellular immune response [6] . Hence, leptin has a dual role in inflammation: it activates monocyte/macrophage cells and potentiates production of the proinflammatory cytokines, TNF-α, interleukin (IL)-6 and 9, and directs T cell differentiation to Th1 phenotype, expressing interferon (INF)-γ and IL-2. On the other hand, it expresses certain anti-inflammatory properties by releasing IL-1 receptor antagonist [7] . Several studies have implicated leptin in the pathogenesis of autoimmune inflammatory conditions, such as rheumatoid arthritis (RA). In patients with RA, it is reported that fasting leads to an improvement in disease activity, which was associated with a marked decrease in serum leptin and a shift towards Th2 cytokine production [8].

Despite the fundamental role of leptin in energy balance regulation, only few obese patients present leptin deficiency as a result of rare mutations that cause severe monogenic syndromes [9]. Actually, most obese individuals have increased leptin concentrations in blood , which led to the concept of leptin resistance . This phenomenon has been related to genetic mutations [10], leptin self-regulation , limited tissue access due to saturation of leptin transport by LEPR-a and cellular or circulating molecular regulation Leptin resistance explains why exogenous administration of this hormone is not efficient to control body weight in the majority of obese patients [11].

people with leptin deficiency are very obese . On the other hand, obese patients have higher leptin concentration. However, they do not react to higher leptin levels by a decrease in their appetite [12] . Therefore, it is supposed that obese patients are resistant to leptin, just as in type 2 diabetes, where insulin resistance is observed . Furthermore, hyperleptinemia caused by obesity is an important risk factor leading to the development of type 2 diabetes [13] . Hyperleptinemia may lead to the development of atherosclerosis in obese patients . Other authors suggest that leptin is involved in the formation of atheromatous plaques [14] . The suggestion has been made to regard elevated leptin levels as an independent factor indicating

future coronary disease and cardiovascular complications [15] .

An assumption, based on the articles mentioned above, can be made that leptin is correlated with metabolic syndrome, and moreover it may be involved in the pathophysiology of psoriasis.

One of the earliest attempts to try and identify patterns of cytokine expression that would have an immunologic context was provided by Mossmann and colleagues who proposed the T-Helper (TH)-1 and TH-2 cytokine network theories.[16] . According to this proposal, immune responses were identified in various murine models that appeared to be mutually exclusive and self-reinforcing. In the TH-1 cytokine network immune response, the net effect of producing IL-2, IFN-γ, and TNF-α was to promote a T cell–mediated reaction. Conversely, in the TH-2 cytokine network response, the net immune response included IL-3, IL-4, IL-5, and IL-10 contributing to a humoral or B cell–mediated immune reaction. Of course, in psoriasis, because no one has consistently demonstrated or identified a specific antigen, the question arose whether psoriatic plaques would be characterized as either a distinct TH-1 or TH-2 type of immune response.[1]

Cytokines are small polypeptides produced in response to antigens, microorganisms or other non-infectious stimuli.[17] .They are capable of regulating immune and inflammatory reactions and interacting with the endocrine and nervous systems. Because they are not stored, they are released in small amount to induce a self-limited events as a result of cellular activation after synthesis initiated by gene transcription.[18] Cytokines generally mediate interactions between cells that are in close proximity via engagement of specific receptors acting in an either autocrine or paracrine fashion. Cytokines are generally appreciated for their ability to influence the proliferation, differentiation, or secretion of proinflammatory or anti-inflammatory factors by resident and recruited cell types.[19] .

It is known that leptin is influenced by proinflammatory cytokines , patients with an acute-phase response have significantly higher serum leptin levels. Leptin increases IL-2 and interferon-γ (IFN-γ) production and decreases IL-4 levels. Thus, leptin may play an important role in the regulation of the T-helper (Th1/Th2) balance towards the Th1 type.[20]. Several studies have reported correlations of different variations throughout the length of the *OB* gene, such as promoter, noncoding, and coding regions, with different diseases. [21].

The secretion of TNF-α and IL-6 from keratinocytes and secretion of IL-6, IL-8, IL-17, IL-22, TNF-α and IFN-g from T lymphocytes are mostly stimulated by leptin. Recent studies have demonstrated that increased serum leptin level positively correlates with the psoriasis disease severity [22]. Furthermore, other reported that leptin regulates naive and memory T cell proliferation followed by increased Th1 and reduced Th2 cytokine production. These results are consistent with the view that leptin affect psoriatic pathophysiology through the regulation of cytokines including IL-6, IL-8, IL-17, IL-22 and TNF-α [23].

Single nucleotide polymorphisms (SNPs) are the most frequent occurring manifestation of genetic variation in the human genome. Approximately one out of every 1000 nucleotides in the human genome is expected to be a SNP site, accounting for more than 90% of all differences between humans. SNPs contribute to the variation in human phenotypes, such as disease susceptibility, responses to drugs and environmental chemicals, and susceptibility to infection [24].

The leptin (*LEP*) gene, the human homolog of the rat obese (*OB*) gene, has been cloned and sequenced . It is located at 7q31.3 and expresses a 4.5-kb mRNA in adipose tissue. Both the structure of leptin and that of its receptor suggest that leptin should be classified as a cytokine. It is known that leptin is influenced by proinflammatory cytokines[25].

Several studies have reported correlations of different variations throughout the length of the *OB* gene, such as promoter, noncoding, and coding regions, with different diseases. The single nucleotide polymorphism (SNP) (*G*→A) in the 5′flanking region at –2548 has been shown to be involved in *LEP* gene regulation[26] .

There is a high variability in the 5 ′ -flanking region in the leptin gene, and a bulk of studies has been conducted on the association between leptin gene variants and the obesity risk. Among the variants studied, a common single-nucleotide polymorphism identified in the 5 ′ -untranslated region of the leptin gene (LEP –2548 G/A polymorphism) is the most studied one. Substantial data indicated that the LEP –2548 polymorphism was associated with the variations in plasma leptin and body mass index (BMI) in both obese and non-obese individuals [27] .

The mechanism may be that the LEP –2548 G/A polymorphism influences leptin expression, possibly at the transcriptional level, and therefore also adipose secretion levels of the hormone . However, the direct association between the –2548 G/A polymorphism and obesity remains vague. While most current published studies have failed to identify a significant association in various populations [28] , 3 studies found that the current variant was significantly associated with the risk of obesity (defined as BMI ≥ 30) in subjects of mixed race (South America) and Caucasians (Turkish and Tunisian) . Additionally, while most pieces of evidence have indicated that the frequency of the GG genotype or G allele is higher in the obesity or overweight group compared to that of the control in various populations [29] , found a sharply contrasting outcome, that the homozygosity for the A allele is significantly associated with an increased risk of obesity. All these inconsistencies in the magnitude or direction of the association may lead to a poor understanding of the true association between the LEP –2548 G/A polymorphism and obesity[30].

Material and Methods

patients with psoriasis who had not received any prior local or systemic treatment within two months were included in the study. The diagnosis was made clinically, based on characteristics of psoriatic . patients with psoriatic arthritis were excluded. The severity of psoriasis was assessed by the psoriasis area and severity index (PASI) for each patient. The control group was comprised of healthy, non psoriatic volunteers with no family history of psoriasis. A total of 100 patients (41 males, 59 females), and 80 healthy subject (39 male ,41 female) were included in this study , 10 ml blood sample were taken from patient and control group.

Sample collection and Leptin measurements of Blood samples were prospectively collected with the appropriate Ethical Committee permissions, from patients attending dermatology Outpatients Clinics at Tikrit Teaching Hospital in Iraq. Each sample had been collected in a two tubes first one for ELISA where serum was separated within 1 h of blood collection after spinning for 15 min at 1500 g. The serum was stored without preservative at _20 C and then thawed just prior to testing second tube for DNA polymorphism.

Psoriasis was diagnosed according to the its standard criteria. Relevant data collected from all subjects included age, sex, weight, height, BMI, waist

circumference, smoking habit, duration of psoriasis onset, age of psoriasis onset , and severity of psoriasis, was measured according to PASI score . BMI was calculated as weight (kg)/height (cm2).

Serum Leptin concentrations were determined using the commercially available enzyme-linked immuno-sorbent assay (ELISA Ultra sensitive), kit supplied by BOSTER Immunoleader (USA). The assays employ the quantitative sandwich enzyme immunoassay technique using recombinant human Leptin with antibodies raised against the recombinant proteins .

DNA was isolated from the whole peripheral blood taken into ethylenediaminetetraacetic acid (EDTA) tubes with the use of Omega DNA Isolation Kit (Omega , USA).

The product of DNA extracted had been calculated ***by Thermo Scientific Nanodrop 2000*** to determine the quantity and quality of DNA. the samples were send to LJMU and saved in -80 in life science building.

Molecular study of the LEP gene For molecular study of SNP in the LEP gene 2548G/A and genotyping, genomic DNA was extracted and then genotyped by polymerase chain reaction / restriction fragment length polymorphism (PCR & RFLP) according to the method performed by Iciek et al. (16). Genomic DNA was isolated from EDTA anticoagulated whole peripheral blood samples using the E.Z.N.A blod DNA kit (Omega). G-2548A polymorphism in *lep* gene promoter was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Briefly, a 242 bp fragment of *lep* gene promoter was PCR amplified from genomic DNA (100 ng) using 25 picomols of each specific forward (5'-TTTCCTGTAATTTTCCCGTGAG-3') and reverse (5'-AAAGCAAAGACAGGCATAAA-3') primers.

we were used Touchdown (TD) PCR that offers a simple and rapid means to optimize PCRs, increasing specificity, sensitivity and yield, without the need for lengthy optimizations and redesigning of primers. TD-PCR employs an initial annealing temperature above the projected melting temperature (T_m) of the primers had been used, then progressively transitions to a lower, more permissive annealing temperature over the course of successive ten cycles that been used in this method . Any difference in T_m between correct and incorrect annealing will produce an exponential advantage of twofold per cycle. We were used this technique to avoid confusing between the small segments with 61 base paired which more similar the size of primer dimers that may be produce so by such method we were overcome to this problem an addition to run these product with agarose electrophoresis to confirm our result so we start with annealing temperature 61°C and gradually decrease with cycling process (1°C for each step) by the end of 10 cycles annealing temperature become below that of primer 51 °C and continue for the next 30 cycles. PCR was performed at 94°C for 5 min, 94°C for 30 s, 51°C for 45 s and 72°C for 60 s, for 30 cycles, with a final extension step at 72°C for 5 min in the presence of DNA polymerase SYBR mix (Qiagen).

All Primers were purchased from an established oligonucleotide manufacturer(Eurofins MWG Operon, Anzingerstraße 7a D- 85560 Ebersberg order ID: 3235890), Lyophilized primers dissolved in TE stock solution of 100 µM . Primer stock solutions were stored in aliquots at –20°C until used.

PCR products (0.5 µg DNA) were then treated with 1 U Cfo I (Promega corporation R 6241 USA) for 4 h at 37°C. Restricted samples were resolved by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The recognition sequence of restriction enzyme at the site of polymorphism so its produce DNA segment with different length size according to the genotype they have .

Two fragments of 181 and 61 bp were expected for homozygotes GG, three fragments of 242, 181 and 61 bp for heterozygotes GA and a unique 242 bp fragment for homozygotes AA

Mix gently by pipetting , close the tube and centrifuge for a 15 seconds in a microcentrifuge . incubate at the optimum temperature for 4 hours . Add 4 µl of 6X loading buffer and proceed to gel analysis. The ladder that used in electrophoresis was the hyperladder IV (BIOLINE)10 band 50bp and 100bp-1000bp

Results

There were no significant differences in age and BMI and levels of cytokines between male and female in patients and control gruop (P >0.05).table(1) .

In this study we observed a significant increase in The mean levels of Leptin of the patients than those of the control. Leptin serum levels were19.93 ± 6.69 ng/ml , 14.62 ± 4.2 ng/ml in patients and control group respectively as shown in table (2) .

Association of G/A SNP in Leptin -2548 with psoriasis: The genotype distribution of G/A SNP in Leptin -2548 among psoriasis patients and healthy control was shown in Table (3). The observed genotype frequencies in both groups were similar to the expected according the Hardy-Weinberg equilibrium . There was no association between the genotypes of G/A SNP in Leptin -2548 and psoriasis in the investigated Iraqian population. The distribution of genotypes among patients was similar to that observed in the control group Genotypes distribution of Leptin -2548 promoter gene were GG 0.32 and 0.20 , GA 0.48 and 0.50 , AA 0.20 and 0.30 in patient and control group respectively as shown in table (3).

Our results confirmed that There were a significant differences in the genotype distribution of Leptin -2548 cytokines examined with respect to its serum levels in patients group. the GG genotype mean of serum level was(23.4 ± 4.1ng/ml), and GA were 20.5 ± 6.84 and AA was (16.2 ± 5.86 pg/ml). As show in table (4). Also There were a significant difference in genotype distribution of Leptin -2548 cytokines examined with respect to its serum level in control group GG was 16.5 ±2.75 , GA was 15.4 ± 4.22 and AA was 12.0 ± 4.16. table (3).

Since *lep*G-2548A polymorphism could be affecting *lep* transcription and therefore leptin synthesis, our result indicate that there were a significant increase in serum levels of Leptin in obese patients who had a GG genotype than non obese, while there were no significant differences with other genotypes (GA,AA) table (5) .

the cytokines serum levels have a significant relation with obesity an addition to it is relation with psoriasis . Our result indicate that the there were a significant higher level of Leptin in sever cases than that in mild cases of psoriasis in obese patients table (6) . Our result appeared that there were no significant differences in Leptin 2548 genotypes distribution depends on severity of psoriasis (PASI score) table (7).

Discussion

Our study observed that the serum level of leptin was significantly higher in patient than that of control group. This result is totally agree with others who confirm that leptin serum level are significantly increase in patients of psoriasis than healthy control group[31].

The adipose tissue is a major source of leptin and its circulating concentrations indirectly reflect body fat stores. Leptin is an important signal in the regulation of food intake and energy balance and its levels usually correlate with BMI . It links nutritional status with neuroendocrine and

immune functions, and its role in the modulation of immune response and inflammation has recently become increasingly evident. The increase in leptin production that occurs during infection and inflammation strongly suggests that leptin is a part of the cytokine network that governs the inflammatory-immune response and the host defense mechanisms. Leptin plays an important role in inflammatory processes involving T cells and has been reported to modulate T-helper cell activity in the cellular immune response [(32).] Hence, leptin has a dual role in inflammation: it activates monocyte/macrophage cells and potentiates production of the pro-inflammatory cytokines, TNF-α, interleukin (IL)-6 and 9, and directs T cell differentiation to Th1 phenotype, expressing interferon (INF)-γ and IL-2. On the other hand, it expresses certain anti-inflammatory properties by releasing IL-1 receptor antagonist [(33).] Several studies have implicated leptin in the pathogenesis of autoimmune inflammatory conditions, such as rheumatoid arthritis (RA). In patients with RA, it is reported that fasting leads to an improvement in disease activity, which was associated with a marked decrease in serum leptin and a shift towards Th2 cytokine production .

Epidemiological studies suggest that, worldwide, populations of patients with psoriasis are not only burdened with its symptoms, but also have a high prevalence of metabolic disorders, including obesity, insulin resistance, dyslipidemia, hypertension and the associated cardiovascular consequences .

Also this study found that there is no significant differences of serum level of leptin between mild and sever cases of psoriasis even thought there were a significant relation between serum levels of leptin and PASI score that represent the severity of psoriasis. this findings were in agreement with a multiple studies have indicated that an association exists between leptin level and psoriasis . this study approved that there is a significant relation between PASI score and BMI the same that approved by a other studies have indicated that an association exists between psoriasis and obesity [(34)] . This finding confirm that there are other factors effects the Leptin levels . Two prospective studies indicated that a higher body mass index (BMI) and weight gain are risk factors for incident psoriasis [(35)] . Zhang et al. reported that the severity of psoriasis, as measured by the Psoriasis Area and Severity Index (PASI), was significantly correlated with BMI in a hospital-based study in the Chinese Han population [(36)] . Dermatologists from Taiwan also observed a significant linear trend between clinically more severe psoriasis and increased BMI, and reported that moderate-to-severe obesity was associated with a significantly increased risk of clinically more severe psoriasis, compared to patients with a normal BMI .

Epidemiological studies indicate that there is a high prevalence of metabolic disease in patients with psoriasis and that patients with psoriasis and other metabolic comorbidities have a poor long-term clinical outcome. In our clinical evaluation of 100 adult patients with psoriasis , we confirmed that being overweight or obese was a risk factor for psoriasis, consistent with previous research. Zhang et al. reported that the PASI correlated with BMI [(35)] .this is the first study highlighting the possible role of LEP G-2548A polymorphism as a predictor for psoriasis and relation between psoriasis and obesity among a sample of Iraqi population. This study showed that people carrying LEP 2548G allele had significantly more prevalence of psoriasis than other subject , suggesting that LEP G-2548A polymorphism could be a psoriasis predictor.

We found that LEP 2458GG genotype was significantly associated with increased plasma leptin and prevalence of psoriasis . The relationship between LEP 2548G allele and increased plasma leptin

concentrations was previously described in obese and diabetic individuals [37] .

Our results were in agreement with many studied who found that an association between the G-2548A polymorphism of the LEP gene with psoriasis in deferent populations . also our result were not in agreement with Kara et al. [38] who found lack of association between the G-2548A polymorphism of the LEP gene with psoriasis in a Turkish population.

leptin concentrations were significantly higher in obese having the GG variant in *lep* promoter in comparison with the AA genotype, with $p<0.05$ according to ANOVA-TUKEY test . this finding in agreement with others [39-40] suggesting that *LEP* G-2548A polymorphism is a strong obesity predictor. An association of *LEP* G-2548A polymorphism and increased BMI was also found in overweight Europeans and in a sample of Taiwanese Aborigines with extreme obesity .

Rreferences

1- Brian J. Nickoloff, MDa, Hong Xin, MDa, Frank O. Nestle, MDb, Jian-Zhong Qin, MDa The cytokine and chemokine network in psoriasis Clinics in Dermatology (2007) 25, 568–573.

2- Sommer D, Jenisch S, Suchan M, Christophers E, Weichenthal M. Increased prevalence of the metabolic syndrome in patients with moderate to severe psoriasis. Arch Dermatol Res 2006;298:321-8.

3- Gelfand JM, Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB. Risk of myocardial infarction in patients with psoriasis. JAMA 2006;296:1735–41.

4- Conus C, Bruno A, Simon HU. Leptin is an eosinophil survivor factor. J Allergy Clin Immunol 2005; 116: 1128-1134.

5- Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996; 334: 292-295.

6- Gabay C, Dreyer M, Pellegrinelli N, Chicheportiche R, Meier CA. Leptin directly induces the secretion of interleukin 1 receptor antagonist in human monocytes. J Clin Endocrinol Metab 2001; 86: 783-791.

7- Faggioni R, Fantuzzi G, Gabay C, Moser A, Dinarello CA, Feingold KR et al. Leptin deficiency enhances sensitivity to endotoxininduced lethality. Am J Physiol 1999; 276: R136-R142.

8- Takahashi H, Tsuji H, Takahashi I, Hashimoto Y, Ishida- -Yamamoto A, Iizuka H. Plasma adiponectin and leptin levels in Japanese patients with psoriasis. Br J Dermatol. 2008;159:1207–1208.

9- Farooqi, I.S. and S. O'Rahilly, 2000. Recent advances in the genetics of severe childhood obesity. Arch. Dis. Child., 83: 31-34. DOI: 0.1136/adc.83.1.31

10- Scarpace, P.J., M. Matheny and N. Tumer, 2001 Hypothalamic leptin resistance is associated with impaired leptin signal transduction in aged obese rats. Neuroscience, 104: 1111-1117. DOI: 10.1016/S0306-4522(01)00142-7

11- .Scarpace, P.J., M. Matheny, N. Tumer, K.Y. Cheng and Y. Zhang, 2005. Leptin resistance exacerbates dietinduced obesity and is associated with diminished maximal leptin signalling capacity in rats. Diabetologia, 48: 1075-1083. DOI: 10.1007/s00125- 005-1763-x

12- . Poeggeler B, Schulz C, Pappolla MA et al. Leptin and the skin: a new frontier. *Exp Dermatol*. 2010;19:12–18.
13- . Moran O, Phillip M. Leptin: obesity, diabetes and other peripheral effects — a review. *Pediatr Diabetes.* 2003;4:101–109.
14- . Koh KK, Park SM, Quon MJ. Leptin and cardiovascular disease: response to therapeutic interventions. *Circulation.* 2008;117:3238–3249.
15- . Beltowski J. Leptin and atherosclerosis. *Atherosclerosis*. 2006;189:47–60.
16- Arles Martins Brotas[I]; José Marcos Tellas Cunha[II]; Eduardo Henrique Jorge Lago[III]; Cristiane Chaves Nascentes Machado[IV]; Sueli Coelho da Silva Carneiro[V]; Tumor necrosis factor-alpha and the cytokine network in psoriasis; An. Bras. Dermatol. vol.87 no.5 Rio de Janeiro Sept/Oct. 2012
17- Zalewska A, Głowacka E, Wyczołkowska J, Tchorzewski H, Narbutt J, Sysa-Jędrzejowska A. Interleukin 6 and 8 Levels in Plasma and Fibroblast Cultures in Psoriasis. Mediators Inflamm. 2006;2006:81767.
18- Goodman WA, Levine AD, Massari JV, Sugiyama H, McCormick TS, Cooper KD. IL-6 Signaling in Psoriasis Prevents Immune Suppression by Regulatory T Cells1. J Immunol. 2009;183;3170-6.
19- RICHARD J. SIMPSON,' ANNET HAMMACHER,' DAVID K. SMITH: JACQUELINE M. MATI'HEWS,' AND LARRY D. WARD'x3; Interleukin-6: Structure-function relationships; Protein Science (1997), 6929-955. Cambridge University Press.
20- Hamminga EA, van der Lely AJ, Neumann HA, Thio HB. Chronic inflammation in psoriasis and obesity: implications for therapy. Med Hypotheses 2006;67:768-73.
21- Alsufyani MA, Golant AK, Lebwohl M. Psoriasis and the metabolic syndrome. Dermatol Ther. 2010;23:137–143.
22- Prinz JC: Psoriasis vulgaris--a sterile antibacterial skin reaction mediated by cross-reactive T cells? An immunological view of the pathophysiology of psoriasis. Clin Exp Dermatol 2001, 26(4):326-332.
23- Valdimarsson H, Thorleifsdottir RH, Sigurdardottir SL, Gudjonsson JE, Johnston A: Psoriasis--as an autoimmune disease caused by molecular mimicry. Trends Immunol 2009, 30(10):494-501.
24- Skibola CF, Holly EA, Forrest MS, Hubbard A, Bracci PM, Skibola DR, Hegedus C, Smith MT: Body mass index, leptin and leptin receptor polymorphisms, and non-Hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev 2004; 13: 779–786.
25- Wang TN, Huang MC, Chang WT, Ko AM, Tsai EM, Liu CS, Lee CH, Ko YC: G-2548A polymorphism of the leptin gene is correlated with extreme obesity in Taiwanese aborigines. Obesity 2006; 14: 183–187.
26- Duarte SFP, Francischetti EA, Genelhu VA, Cabello PH, Pimentel MMG: LEPR p.Q223R, beta 3-AR p.W64R and LEP c.2548G > A gene variants in obese Brazilian subjects. Genet Mol Res 2007; 6: 1035–1043.
27- Bienertova-Vasku J, Bienert P, Tomandl J, Forejt M, Vavrina M, Kudelkova J, Vasku A: No association of defined variability in leptin, leptin receptor,adiponectin, proopiomelanocortin and ghrelin gene with food preferences in the Czech population. Nutr Neurosci 2008; 11: 2–8.
28- Ben Ali S, Kallel A, Ftouhi B, Sediri Y, Feki M, Slimane H, Jemaa R, Kaabachi N: The –2548G/A LEP polymorphism is associated with blood pressure in Tunisian obese patients. Blood Press 2008; 17: 278–283.
29- Constantin A, Costache G, Sima AV, Glavce CS, Vladica M, Popov DL:

Leptin G-2548A and leptin receptor Q223R gene polymorphisms are not associated with obesity in Romanian subjects. Biochem Biophys Res Commun 2010; 391: 282–286.

30- Portoles O, Sorli JV, Frances F, Coltell O, Gonzalez JI, Saiz C, Corella D: Effect of genetic variation in the leptin gene promoter and the leptin receptor gene on obesity risk in a population-based case-control study in Spain. Eur J Epidemiol 2006; 21: 605–612.

31- Otero M, Lago R, Lago F, Casanueva FF, Dieguez C, Gomez-Reino JJ et al. Leptin from fat to inflammation: old questions and new insights. FEBS Lett 2005; 579: 295-301.

32- Glasow A, Kiess W, Anderegg U, Berthold A, Bottner A, Kratzsch J. Expression of leptin (Ob) and leptin receptor (Ob-R) in human fibroblasts: regulation of leptin secretion by insulin. J Clin Endocrinol Metab 2001; 86: 4472-4479.

33- Sakai R, Matsui S, Fukushima M, Yasuda H, Miyachi Y. Prognostic factor analysis for plaque psoriasis. Dermatology 2005; 11: 103-106.

34- Takahashi H, Iizuka H. J Dermatol 2012: 39: 212–218.

35- Kumar S, Han J, Li T et al. J Eur Acad Dermatol Venereol 2012: doi: 10.1111/jdv.12001

36- . Zhang C, Zhu K J, Zheng H F et al. J Eur Acad Dermatol Venereol 2011: 25: 87–91.

37- .Le Stunff C, Le Bihan C, Schork N J et al. Diabetes 2000: 49: 2196–2200.

38- Kara N, Aydin F, Senturk N et al. Int J Dermatol 2007: 46: 1271–1274.

39- Ren W, Zhang S H, Wu J et al. Chin Med J 2004: 117: 558–561.

40- Ahmed E L-S F, Seliem M K, El-kamel M F et al. Egypt J Dermatol and Androl 2009: 29: 91–100.

Table show(1) mean and St.D of age , BMI and cytokines in both male and female of patients groups.

Gender	N	Mean	Std.D	P value
AGE Female	59	38.2	12.46	

years Male	41	33.9	12.97	≥0.05
PASI Female score Male	59 41	11.07 9.56	6.14 3.65	≥0.05
BMI Female Kg/m^2 Male	59 41	27.04 26.3	4.38 5.24	≥0.05
Leptin Female ng/ml Male	59 41	20.25 19.46	6.56 6.94	≥0.05

Table(2) show the serum level of IL-6, TNF-alpha and Leptin between patients with psoriasis and control group.

Cytokines	**Psoriasis patients No: 100 mean ± SD**	**Control N0: 80 mean ± SD**	**value**
Leptin	**19.93 ± 6.69 ng/ml**	**14.62 ± 4.2 ng/ml**	**≤ 0.001**

Table(3) show the Allelic frequency and genotypes disruption of cytokines polymorphism in patients and control groups.

		Psoriasis patients N 100 %	**Healthy control N 80 %**	**P valuve**
Leptin 2548				
Alleles	G	112(56%)	74(46.25%)	
	A	88(44%)	86(53.75%)	
Genotypes	GG	32(32%)	16(20%)	**≥ 0.1**
	GA	48(48%)	40(50%)	
	AA	20(20%)	24(30%)	

Table(4) show a Plasma leptin levels (ng/ml) in psoriatic patients and control gruops with the different genotypes of 2548 G/A polymorphism

	GG	GA	AA	P value
Psoriasis patients Leptin mean +SD	23.4 ± 4.1	20.5 ± 6.84	16.2 ± 5.86	≤ 0.05
Control Leptin mean +SD	16.5 ± 2.75	15.4 ± 4.22	12.0 ± 4.16	≤ 0.05

Tabe(5) show the Effect of G-2548A polymorphism in the leptin promoter gene on leptin serum level in obese and normal weight of patient with psoriasis.

Leptin gene -2548 G/A polymorphism	Serum level of Leptin in ng/ml		P value
	Normal weight BMI <25	Obese BMI ≥30	
GG	21.09 ± 4.40	25.32 ± 1.90	0.019
GA	19.51 ± 7.60	23.02 ± 5.25	0.171
AA	16.43 ± 7.06	17.9 ± 4.97	0.722

Table(6). show the serum level of leptin in obese patient depend on psoriasis severity.

		Leptin serum levels ng/ml Obese Patients BMI ≥ 30kg/m^2			P value
		N	Mean	Std. Deviation	
Psoriasis severity	Mild PASI <10	7	20.24	7.05	0.08
	Sever PASI ≥ 10	17	24.04	3.34	

Table (7) show the Effect of G-2548A polymorphism in the leptin promoter on mean leptin serum level in obese and normal weight of patient with psoriasis

Leptin gene -2548 G/A polymorphism	Serum level of Leptin in ng/ml		P value
	Mild psoriasis PASI <10	Sever psoriasis PASI ≥	
GG	23.43 ± 4.39	22.77 ± 3.99	0.664
GA	17.95 ± 7.89	24.02 ± 2.16	0.002
AA	14.77 ± 6.39	17.92 ± 4.93	0.242

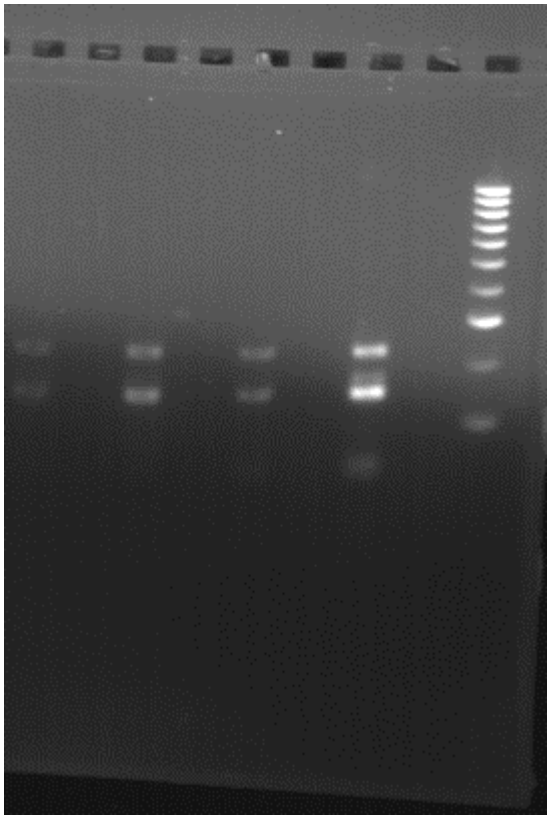

Figure (6) Restriction fragments length polymorphism (RFLP) distribution pattern of LEP G2548A polymorphism. Line 1 Ladder of 100pb line 2 G and A allele.