Evaluation of Soluble P-selectin and Leptin Serum Levels in Sera of Patients of Psoriasis and their Possible Role in the Increase in the Cardiovascular Risks in Psoriatic Patients

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Abstract

Background: Psoriasis is a chronic immune mediated disease that is considered to be a multisystem disease that extends beyond the limits of the skin to affect other tissues and organs, possibly associated with enhanced atherosclerosis and risk of coronary artery disease.

Objective: The aim of this work is to evaluate the serum level of soluble p-selectin and leptin serum levels in patients with psoriasis and their possible relation to the cardiovascular risk factors in those patients also the relation between their levels and the clinical severity of the disease

Patients and methods: This study was carried on seventy patients with chronic moderate to severe psoriasis. Forty of them (group A) were excluded from disorders affecting platelet activity, and compared to 20 healthy age and sex matched control subjects (control A), both groups were studied for serum soluble P-selectin level. The other thirty patients (group B) were excluded from disorders affecting serum leptin level, and compared to thirty healthy age and sex matched control subjects (control B) both groups were studied for serum leptin level.

Results: soluble P-selectin level is significantly increased in psoriatic patients in the group A in comparison to control A subjects, and there was a significant increase in serum leptin level psoriatic patients in the group B in comparison to the control B subjects. Serum leptin and soluble P-selectin levels showed a positive correlation with Psoriasis Area and Severity Index.

Conclusion: Soluble p-selectin is a marker of platelet activation and has an impact on psoriasis activity; also it can be used as a marker of psoriasis severity. Leptin is an adipose tissue derived hormone that was suggested to have a role in pathogenesis of psoriasis whether through its inflammatory role or through its permissive effect on the development of psoriasis associated metabolic risk factors.

Keywords: Psoriasis; Leptin; Soluble P-selectin; Metabolic syndrome; Body mass index

Abbreviations: BMI: Body Mass Index; PASI: Psoriasis Area and Severity Index

Introduction

Psoriasis is a chronic, autoimmune, noncontagious, multisystem, hyperproliferative, inflammatory disorder with significant comorbidities that affects 1% to 4% of the world’s population. It is a complex, multifactorial disease that appears to be influenced by genetic, environmental factors, vascular and immune-mediated components [1].

Compared with the general population, Psoriasis is associated with increased risk of major adverse cardiac events, specifically, patients with psoriasis appear to be more likely to develop hypertension, diabetes mellitus, obesity, and dyslipidemia compared with the general population [2-4]. However, the mechanisms underlying this association remain poorly understood. Systemic mechanisms have been implicated in: (1) metabolic and adipokine derangement leading to an insulin resistant (IR) state; (2) lipoprotein particle dysfunction; (3) increased generation of cell membrane vesicles, or microparticles (MP) which are resistant (IR) state; (2) lipoprotein particle dysfunction; (3) increased

P-selectin is a 140 kDa protein which belongs to the selectin family of adhesion molecules [7]. Selectins are carbohydrate-binding molecules that bind to fucosylated and sialylated glycoprotein ligands, and are found on endothelial cells, leukocytes and platelets.

P-selectin is stored in a-granules of platelets and in Weibel-Palade bodies of endothelial cells, on activation of these cells it will be translocated to their surfaces [8].

After establishing interactions with endothelial cells, activated platelets secrete IL-1b and PF4, triggering further inflammatory effects in the endo-thelium, including NF-kappaB activation and the deposition of mediators such as IL-6 or MCP-1 [9]. Taken together, P-selectin expressing platelets can trigger inflammatory reactions in endothelium, increase formation of leucocyte-platelet aggregates and increase local recruitment of leucocytes from blood into the tissue. Hence, P-selectin plays a pivotal role in regulating an inflammatory response.

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An additional function of platelet P-selectin is in the recruitment of monocyte-derived microparticles, which are a rich source of the blood-clotting element ‘tissue factor’, to the forming thrombus [10].

On the other hand a high ratio of obesity in psoriasis patients is reported, and obesity leads to a higher risk of developing psoriasis and a poorer long-term clinical outcome. Obesity is associated with metabolic syndrome; several studies proved the high incidence of metabolic syndrome in psoriatic patients, but the precise underlying mechanism of psoriasis and metabolic syndrome is not fully elucidated [11].

Leptin, the product of the OB (obese) gene, is an adipocyte-specific secretory 16 kDa protein whose levels increase with an increase in body mass index (BMI). Leptin acts primarily through specific receptors in the hypothalamus, regulates appetite and the balance of energy in the body. It has an important role in regulating a wide range of biological responses including energy homeostasis, haematopoiesis, neuroendocrine function and immune responses [12].

Leptin exerts its biologic actions through the activation of its cognate receptors which belong to the type 1 cytokine receptor superfamily [13]. In addition to being a hypothalamic modulator of food intake, body weight and fat stores, leptin has a dual role in inflammation: it activates monocytes and macrophages, potentiates production of the proinflammatory cytokines TNF-α, IL-6 and IL-9, and directs T-cell differentiation to Th1 phenotype [14]. Additionally, leptin has been shown to stimulate keratinocyte proliferation, expression of adhesion molecules and angiogenesis [15]. Regarding this information, immunological and proliferative effects of leptin and immunopathogenesis of psoriasis have many overlapping features [16].

**Aim of Work**

The aim of this study is to estimate the level of soluble P-selectin as one of the markers of platelet activation and leptin level in sera of patients with chronic plaque psoriasis and to investigate the possible role of these two mediators to the increase in cardiovascular risks in patients with psoriasis. Furthermore to examine the relationship between the level of these markers and a severity score of psoriasis also to examine if there is any changes in these marker levels after using narrow band UVB as a line of treatment.

**Subjects and Methods**

**Patients and control**

This study was carried on seventy patients with chronic moderate to severe psoriasis. Inclusion criteria of Patients PASI ≥ 10 evaluated by the same dermatologist No systemic antipsoriatic therapy (acitretin, ciclosporin, methotrexate or biologics), natural or artificial UV sources, by the same dermatologist. No systemic antipsoriatic therapy (acitretin, ciclosporin, methotrexate or biologics), natural or artificial UV sources, dietary regimen. This group was compared to three healthy age and sex matched control subjects free from psoriasis (Control A). Group B included thirty patients 21 males (70%) and 9 females (30%), with a mean age of 44.03 ± 14.83 years, mean weight of 72.40 ± 10.74 kg, mean height of 164.95 ± 10.63 cm and with a mean BMI of 26.50 ± 2.09. mean PASI score was 15 (range=3.40-56.20), this group were excluded from disorders affecting serum leptin level; chronic renal or liver disease, hypothyroidism, polycystic ovary, eating disorders or dietary regimen. This group was compared to 30 healthy age and sex matched control subjects free from psoriasis (Control B).

All patients and controls were recruited from the outpatient clinic of the dermatology department of the Alexandria university hospital. The study protocol conformed to the ethical guidelines of Alexandria University and was approved by the local ethical committee of scientific research. Prior to initiation, every subject was informed about the aim of the study and they gave consent.

**Methods**

Every patient was subjected to history taking, general and dermatological examination. Body mass index (BMI) was determined using the following equation: weight (kg)/ height (m)^2_. Subjects with BMI 19-25 kg/m^2_ were considered normal, 26-29 kg/m^2_ as overweight and ≥30 kg/m^2_ as obese [17].

Identification of metabolic syndrome was done using updated National Cholesterol Education Programme criteria [18]. Diagnosis was made when three or more criteria were present, including waist circumference of more than 102 cm in men or more than 88 cm in women, blood pressure level of 130/85 mmHg or higher or use of antihypertensive medication, fasting plasma glucose levels of 100 mg/dL or higher or use of oral hypoglycaemic medication, fasting triglyceride levels of 150 mg/dL or higher, and fasting high density lipoprotein cholesterol (HDLc) levels of <40 mg/dL in men or <50 mg/dL in women.

Patients were classified according to PASI score into: Mild psoriasis: PASI<10%, Moderate psoriasis PASI=10-50% and severe psoriasis: PASI>50. The degree of improvement is classified after treatment into mild improvement (<30%), moderate improvement (30-70%), while marked improvement (>70%).

**Treatment**

Group (A) and group (B) patients were treated with NB-UVB radiation for at least 3 months or till improvement of psoriatic lesions. The radiation was administered using a Waldmann 7002 cabin (Waldmann Medizintechnik, Villigen-Schweibingen, Germany) providing the average irradiance 1.4 mw/cm^2_ measured by a calibrated UVB meter. A conventional therapeutic schedule was employed in this study, MED was estimated from the patients according to their skin types:

Initial dose was dependent on Fitzpatrick skin phototype, and dosage increases followed the guidelines of the Spanish Photobiology Group. Ultraviolet irradiation regimen; three times per week. Starting with approximately 50% of MED then increment given each time based on percentage of previous dose and erythema response taking into consideration mild, transient erythema responses were usually attained with this suberythrogenic dosing schedule. NB (MED means the dose that causes barely perceptible ecrema 24 hour after irradiation)

We usually calculate the MED by exposing eight 1.5 by 1.5 cm sites on unaffected skin of upper back to NB-UVB (50, 70, 100, 140, 200, 250)
Serum analysis

Venous samples were taken both groups on two occasions: After

<table>
<thead>
<tr>
<th>Group A patients</th>
<th>Control A</th>
</tr>
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<tbody>
<tr>
<td><strong>Soluble p selectin</strong></td>
<td>Before NB-UVB</td>
</tr>
<tr>
<td>Min.-Max</td>
<td>55.11-235.05</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>114.96 ± 43.55</td>
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<tr>
<td><strong>Median</strong></td>
<td>101.54</td>
</tr>
<tr>
<td><strong>p,</strong></td>
<td>&lt;0.001*</td>
</tr>
</tbody>
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p1: p value for Wilcoxon signed ranks test between before and after NB-UVB.

p2: p value for Mann Whitney test between control with before and after NB-UVB.

*: Statistically significant at p ≤ 0.05.

Table 1: Comparison between the two studied groups according to soluble p-selectin before and after NB-UVB and their relations to control A patients.

Results

In group A patients as shown in Table 1 and Figure 1. The plasma levels of sP-selectin were significantly higher (114.96 ± 43.55 ng/mL) compared to control A subjects (46.38 ± 16.71 ng/mL) (p<0.001*). These data suggest that blood platelets are activated in patients with psoriasis.

We also compared the plasma levels of soluble P-selectin in that group of patients before and after treatment for maximum of 12 weeks with narrow band phototherapy or after the skin lesions had improved. The mean PASI score had decreased from a mean of 20.32 ± 11.93 before NB-UVB to a mean of 10.69 after NB-UVB (p<0.001*). It was found that there was a statistically significant decrease in the level of soluble p-selectin with a mean of 114.96 ± 43.55 ng/mL before treatment with NB-UVB to a mean of 52.39 ± 26.44 ng/mL after treatment (p<0.001*).

According to Figure 2, our results proved that there is a significant relation between the severity of the disease (PASI) and soluble p selectin serum level (p=0.042). The mean of soluble p-selectin in patients with mild psoriasis was 104.84 ± 49.58 while in patients with moderate psoriasis was 109.37 ± 46.36 and the mean in patients with severe psoriasis was 140.16 ± 16.09.

After treatment with NB-UVB there was a significant relation between serum level of soluble P-selectin and the degree of improvement. The mean of soluble p-selectin after NB-UVB in patients that showed marked response was 49.93 ± 22.07, while the mean of soluble p-selectin in patients that showed moderate response was 53.96 ± 23.59 and the mean in patients with minimal response was 93.93 ± 25.19 (p<0.001).

Subjects have fasted 8 hours for measurement of fasting blood sugar. After subjects have fasted 12-14 hours for measurement of serum high density lipoprotein (HDL) cholesterol, triglycerides, LDL cholesterol using Synchron CX-7 autoanalyzer (Beckman, Coulter, Nyon, Switzerland).

In group A and control A patients Collection of samples for measurement of sP-selectin blood was taken from the immediately mixed with 1/10 the volume of acid citrate dextrose. The blood was then centrifuged at 2500 x g for 20 minutes at 48°C. The top third of the platelet-poor plasma was stored at 80°C until use in enzyme-linked immunosorbent assays for sP-selectin. (R&D Systems, Minneapolis, MN)

Venous blood samples were drawn from the participants in group B and control B group a 12 h fasting period. Following centrifugation of the blood sample at 1500 x g for 10 min, Serum was isolated after clotting and preserved at -80°C. Serum levels of leptin were measured using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Data were analyzed using SPSS software package version 18.0 (SPSS, Chicago, IL, USA). Quantitative data was expressed using Range, mean, standard deviation and median while Qualitative data was expressed in frequency and percent. Qualitative data was expressed using Chi-square test and Fisher exact was applied to compare the two groups. Not normally distributed quantitative data was analyzed using Mann Whitney test for comparing the two groups. Pearson coefficient was used to analyze correlation between any two variables. p value was assumed to be significant at 0.05. Multivariate analysis was done for the assessment of multiple risk factors affecting serum leptin level.
Discussion

Psoriasis is a common chronic immune mediated disease that affects 1-3% of the population. Although the exact cause of psoriasis remains unknown, the evolving evidence suggests that psoriasis is a complex disorder caused by the interaction of multiple genes, the immune system and environmental factors [19].

Recent advances in understanding the immunopathogenesis and genetics of psoriasis have shifted the focus from a single organ disease confined to dermal structures to a systemic inflammatory condition. Several previous studies in the literature suggest a high prevalence of cardiometabolic risk factors among psoriasis patients as obesity, smoking, diabetes, hypertension and hyperlipidemia [2,20,21]. Others proved a high incidence of occlusive vascular diseases such as cardiovascular events, thrombophlebitis, cerebrovascular accidents, and pulmonary embolism in patients with severe psoriasis [22,23]. Activated platelets have been shown to circulate in patients with coronary artery disease and they are directly involved in atherosclerotic plaque formation and plaque destabilization. As the increased cardiovascular risk of patients with psoriasis is now readily recognized [24,25].

Other studies have suggested a possible link between platelet activation and cutaneous inflammatory diseases like atopic dermatitis.
and psoriasis [6,26-28]. Inflammation is intimately linked to the process of leucocyte extravasation. Its initial step, leucocyte tethering and rolling, is facilitated by activated platelets expressing P-selectin [29].

On the other hand recent studies suggest that hyperleptinaemia may play an important role in obesity-associated cardiovascular diseases including atherosclerosis [2]. Leptin induces endothelial dysfunction, and stimulates inflammatory reaction, oxidative stress, platelet aggregation, and migration, hypertrophy and proliferation of vascular smooth muscle cells [30]. Therefore, elevated leptin in patients with severe psoriasis may be a contributing factor for the increased prevalence of cardiovascular disease.

Leptin is an adipocyte-derived hormone associated with the metabolic syndrome. It has recently been implicated in the development of metabolic dysregulation in psoriasis. High serum leptin levels have been demonstrated in obesity and hypertension. Moreover, psoriasis has been shown to be associated with leptin levels independently of the metabolic syndrome, and obesity [16,31-34].

The aim of this study was to evaluate the soluble p-selectin and leptin level in sera of psoriatic patients. As these mediators may have a possible relation to the increase cardiovascular risk factors in psoriasis. The first part of the study aims at establishing a clearer explanation of the association between platelet activation and psoriasis severity by investigating the level of one of the markers of platelet activation that is soluble p-selectin in patients with psoriasis. Furthermore to examine the relationship between the level of this marker and a severity score of disease severity. The later proved statistically significant correlation between the change in platelet P-selectin expression and the change in erythema followed by infiltration whereas no such correlation was observed with a scaling. Thus, platelet P-selectin expression reflects those components of the PASI which directly mirror severity of inflammation.

Taken together, these observations suggest that blood platelets are in a state of activation in patients with psoriasis and platelet-derived mediators may contribute to leukocyte recruitment to psoriatic skin lesions to sites of cutaneous inflammation through formation of platelet-leukocyte aggregates via P-selectin in peripheral blood and secretion of chemokines at inflamed sites [26,27].

The second part of this study aim at estimating the serum level of leptin in psoriatic patients and its correlation to the disease severity and the change in this level after treatment with phototherapy.

Results in this part of the study proved a significant increase in incidence obesity, hypertension, and type 2 diabetes mellitus (p=0.021) in psoriatic patients in group B in relation to control B group, and these findings were in agreement with the study of Al-Mutairi et al. [38] Solomon et al. [39] Brauchi et al. [40] Cohen et al. [41] of Chen et al. [30] and Qureshi et al. [42].

High incidence of obesity in psoriatic patients could be explained by the expansion of adipose tissue during weight gain that leads to recruitment of macrophages which are the chief source of adipose tissue-derived tumor necrosis factor (TNF)-α, IL-6 and CXCL8. These cytokines are abundant in psoriasis skin and their role in psoriasis pathogenesis has been established. Additionally, Leptin, an adipose-derived cytokine, appears to contribute to Th1 and suppresses Th2 immune responses and thus provides a potential link between adipose tissue and psoriatic inflammation [32].

On the other hand increased risk of hypertension in psoriatic patients can be explained by the effect of the chronic systemic inflammatory state in psoriasis and the systemic use of medications which are known to increase the risk of hypertension such as CSA [42]. Additionally, the serum level of angiotensin converting enzyme (ACE) is elevated in psoriasis with a subsequently increased production of angiotensin II which increases the vascular tone. Lastly, the lack of exercise due to physical or social discomfort can also increase that risk of hypertension in psoriasis patients [43].

The potential association between psoriasis and type 2 DM can be explained by TNF-α, a principal component of the inflammation accompanying psoriasis, which is known to regulate insulin function.
It down-regulates insulin signaling activity. Furthermore, insulin-mediated glucose uptake was inhibited in humans infused with TNF-α [44].

On the contrary, the study of Gisondi et al. [45] reported insignificant difference between chronic plaque psoriasis patients and controls neither regarding diabetes nor hypertension.

As regards dyslipidaemia, the current study found that serum TG levels were significantly elevated in group B than control B group (P=0.042). However, there was insignificant difference in the serum HDL levels between the two groups (P=0.347). These findings were in agreement with the study of Akhyani et al and Gisondi et al. [45].

The possible association between psoriasis and dyslipidaemia may be a direct consequence of inflammatory activity as TNF-α alters the gene expression profile of adipocytes and liver leading to increased release and production of FFAs, cholesterol and VLDL. Elevated IL-6 levels have been shown to be associated with decreased levels of HDL cholesterol. Dyslipidaemia may be also related to systemic medications that are known to cause lipid abnormalities such as Acitretin and CSA [46,47].

On the contrary the study of Toker et al. [48] reported that lipid profile changes in psoriasis were totally insignificant, but it excluded all obese, pustular and erythrodermic psoriasis patients.

Metabolic syndrome is characterized by a state of chronic low grade inflammation with increased production of certain inflammatory mediators or adipocytokines such as TNF-α, IL-6 and PAI-1 which are similar to the mediators induced by the chronic systemic Th 1 mediated inflammation characteristic for psoriasis. These cytokines augment the effects of pro-inflammatory cytokines characteristic of psoriatic inflammation (such as TNF-α and IL-6) and create a possible link between psoriasis and metabolic syndrome [49].

In this part of the study, metabolic syndrome was more significantly observed in group B than control B patients (P=0.021). This finding was in agreement with the study of Nisa et al. [50] Al-Mutairi et al. [38] and Love et al. [4]. The study of Gisondi et al. [45] reported a significant increase in metabolic syndrome in psoriasis patients after the age of 40. Meanwhile, the study of Sommer et al. [11] reported a significant association between metabolic syndrome and moderate to severe psoriasis.

On the contrary, the study of Chen et al. [31] reported insignificant association between metabolic syndrome and psoriasis. This was explained by the authors by the missing data on lipid profiles and BMI values in the study.

In the present study, the serum leptin levels were significantly higher in group B than control B group (P=0.022). This finding was in agreement with the study of Takahashi et al., Wang et al. [51-53], Cerman et al. [16] and Chen et al. [31].

In an attempt to clarify the exact association between psoriasis and hyper-leptinemia and to assess whether this association is dependent on psoriasis or its metabolic co-morbidities, multivariate analysis was done. Interestingly, it revealed that psoriasis as just a dependent risk factor of hyper-leptinemia (P=0.158).

On the contrary, the study of Chen et al. [31] found that psoriasis was independently associated with hyper-leptinemia. This difference may be related to the limited sample size in the present study or the different ethnic group.

Johnston et al. [33], Kaur et al. [52] and Aktan et al. [32] reported insignificant association between serum leptin levels and psoriasis. The former study reported that all the patients and controls were matched as regards their body weights, waist circumferences and serum lipid profiles. The later study excluded diabetic, hypertensive and dyslipidemic subjects.

The present study didn’t find any significant relation between serum leptin and clinical type, duration, age of onset or clinical severity of psoriasis. These findings were in agreement with the study of Chen et al. [31] Takahashi et al. [53] and Aktan et al. [32].

In contrast to that finding, Cerman et al. [16] reported that serum leptin concentration was significantly higher in patients with severe psoriasis than in those with mild to moderate psoriasis. This could be explained by the finding that most patients with severe psoriasis in this study were females and obese.

In the present study, there was a significant relation between serum leptin and BMI. Multivariate analysis revealed that obesity was an independent risk factor of hyper-leptinemia. This was in agreement with the study of Chen et al. [31], Wang et al. [51], Aktan et al. [32], Johnston et al. [33] and Al-Maskari et al. [53].

Conclusion

Current literature proved that leptin might serve as a marker for psoriasis and in other studies it a marker for the severity and chronicity. At the same time it has a positive correlation with diabetes, hypertension and hypertriglyceridaemia and BMI, those serve separately as a cardiovascular risk factor. The present study proved also that soluable P-selectin might serve as factors for cardiovascular risks in psoriatic patients. And so patients with psoriasis may have a higher prevalence of cardiovascular (CV) risk factors and might experience worse CV outcomes compared with the general population. It is worth investigating leptin levels and soluable P-selectin levels in different psoriasis with and without cardiovascular complications. Furthermore both leptin and its antagonists are being studied in various conditions and their use as an adjuvant therapy in obese patients with psoriasis may be another issue for further research.

References
