



Original Research Article

Evaluation of Some Serum Adipokines and Oxidized Low Density Lipoprotein Before and After Methotrexate Treatment in Patients with Chronic Plaque Psoriasis (A Case-Control Study)

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Abstract

Psoriasis is a common inflammatory autoimmune skin disease that affects 2–4% of the world population. T-cells infiltration, interleukins and cytokines including adipocytokines like visfatin, high molecular weight-adiponectin (HMW-adiponectin) and retinol binding protein-4 (RBP-4), together with oxidized low density lipoprotein (ox-LDL) have been implicated in psoriasis pathogenesis.

A total of 86 subjects (43 with Psoriasis and 43 healthy controls) were enrolled in this study. Their mean age was (41± 14.37) for patients, and (35.81± 18.66) for the control group. The study conducted in dermatology outpatient clinic in Merjan Teaching Hospital in Al –Hilla City and investigations were done in laboratory units of the hospital through the period from June 2013 to September 2014. The sera obtained from blood were used to determine the level of serum visfatin, HMW-adiponectin, RBP-4 and oxidized-LDL by enzyme-linked immunosorbent assay (ELISA). Assessment of psoriasis disease activity and methotrexate efficacy was done by psoriasis area and severity index (PASI) score.

Results of this study showed a significant increase ($p < 0.05$) in serum visfatin, HMW- adiponectin, and oxidized-LDL concentrations, and a significant decrease ($p < 0.05$) in retinol binding protein-4 (RBP-4) concentrations in sera of patients with chronic plaque psoriasis when compared with control group. There was a significant correlation between oxidized-LDL and visfatin, HMW-adiponectin and retinol binding protein-4 ($r = 0.81, 0.68, -0.77$) ($p \text{ value} < 0.001$) respectively .

Finally, the impact of systemic methotrexate on above adipocytokines and oxidized-LDL was determined by significant reduction in the levels of visfatin, HMW-adiponectin and ox-LDL and a significant increase in levels of RBP – 4 after treatment and by a significant mean difference in PASI score before and after treatment in psoriatic lesions.

Results obtained by this study indicate that some adipocytokines including serum visfatin, HMW–adiponectin and RBP-4 levels, in addition to oxidized-LDL measured in patients with chronic plaque psoriasis were closely associated with disease severity and could be used to assess the successful treatment.

To the best of our knowledge, this is the first study in Iraq to evaluate serum levels of these adipokines and Ox – LDL in patients with psoriasis and study the effect of methotrexate treatment on these parameters.

Key words: Psoriasis, Adiponectin, Visfatin, Rbp- 4, Ox – LDL, Methotrexate.

الخلاصة

يعتبر داء الصدفية من الأمراض الالتهابية الشائعة التي تصيب الجلد وهو ذاتي المناعة ويصيب الناس بمعدل (٢-٤) % من مجموع سكان العالم. خلافاً T المختزلة لأنسجة الجلد، الانترلوكنات، والساييتوكينات مثل (visfatin) ، وهرمون high molecular weight adiponectin ذات أوزان جزيئية كبيرة ، وبروتينات رابطة ريتونولية رباعية (RBP-4) مع البروتين الشحمي الواطئ الكثافة المؤكسد ox-LDL جميعها تؤثر بداء الصدفية.

الدراسة الحالية قامت بإجراء اختبارات لقياس التغيرات الحاصلة في مستويات انزيم الفيزفاتن ، وهرمون ساييتوكين دهني ذات أوزان جزيئية كبيرة HMW-adiponectin ، وبروتينات رابطة ريتونولية رباعية (RBP-4) مع البروتين الشحمي الواطئ الكثافة المؤكسد ox-LDL وكذلك اقتران هكذا تغيرات مع مدى نشاط أو استفعال المرض.

اشتملت الدراسة على ست وثمانون شخص (٨٦) من الحالات المطابقة في الجنس والعمر المطابقة للدراسة الحالية (٤٣ مصابين بالصدفية و ٤٣ أشخاص أصحاء). متوسط العمر كان (41±14.37) للمرضى، بينما كان (35±18.66) لمجموعة الضبط. تم اجراء هذه الدراسة في استشارية الجلدية في مستشفى مرجان التعليمي في مدينة الحلة، وتم اجراء الاختبارات في مختبرات المستشفى خلال الفترة بين (حزيران ٢٠١٣) الى (اب ٢٠١٤). تم سحب عينات المصل المأخوذ من الدم لاستخدامه من اجل إيجاد مستوى وإجراء اختبارات لقياس التغيرات الحاصلة في مستويات الفيزفاتن visfatin، والساييتوكينات الدهنية ذات أوزان جزيئية كبيرة HMW-adiponectin، وبروتينات رابطة ريتونولية رباعية (RBP-4) مع البروتين الشحمي الواطئ الكثافة المؤكسد ox-LDL. تم قياس هذه الساييتوكينات في جميع الحالات بواسطة طريقة الفحص المناعي ELISA. تم تقييم نشاط داء الصدفية وكفاءة الميثوتريكسيت بواسطة مؤشر شدة المرض (PASI) وتأثير الميثوتريكسيت على مستويات الساييتوكينات الدهنية المذكورة أعلاه والبروتينات الدهنية المؤكسدة ذات الكثافة الواطئة ox-LDL في المصل.

أظهرت نتائج هذه الدراسة زيادة واضحة ($p < 0.05$) في الفيزفاتن visfatin، الاديبونكتن ذات أوزان جزيئية كبيرة HMW-adiponectin، ونسبة تركيز البروتين الدهني المؤكسد الواطئ الكثافة (ox-LDL) ، ونقصان واضح ($p < 0.05$) في نسبة تركيز البروتين الريتونيولي الرباعي الارتباط (RBP-4) في مصل مرضى الصدفية عندما تقارن مع مجموعة الضبط. أظهرت الدراسة ارتباطا كبيرا بين (ox-LDL) والفيزفاتن visfatin، وبين الاديبونكتن ذو اوزان جزيئية كبيرة HMW-adiponectin وكذلك البروتين الرباعي الارتباط الريتونيولي RBP-4. أخيرا أوضحت الدراسة تأثير الميثوتريكسيت على مستوى الساييتوكينات و (ox-LDL) من خلال الانخفاض الكبير في أغلب مستوياتها بعد المعالجة وبواسطة متوسط الاختلاف الكبير في قيم (PASI) قبل و بعد المعالجة لمرض الصدفية. تشير نتائج هذه الدراسة إلى أن بعض الساييتوكينات الدهنية في مصل الدم ومنها انزيم الفيزفاتن visfatin، الاديبونكتن ذات أوزان جزيئية كبيرة HMW-adiponectin، والبروتين ذو رابطة ريتونولية رباعية (RBP-4) مع البروتين الشحمي الواطئ الكثافة المؤكسد (ox-LDL) في مرضى داء الصدفية يمكن أن تكون مؤشرات جيدة للتنبؤ بشدة مرض الصدفية وكيفية متابعة العلاج.

الكلمات المفتاحية: الصدفية ، هرمون الساييتوكاين الدهني، الفيزفاتن ، البروتين ذي الرابطة الريتونيولي الرباعية ، البروتين الشحمي الواطئ الكثافة المؤكسد، الميثوتريكسيت.

Introduction

Pсориаز represents a complex, chronic, systemic, T-cell immune-mediated inflammatory dermatosis characterized by skin and joint manifestations, and presented commonly with erythematous, scaly plaques on various surfaces of the body [1].

The prevalence of psoriasis ranged from 2% -3% of the world population and is characterized by epidermal hyperplasia, dilated micro-vessels in the dermis and leukocytes infiltrations mainly in the dermal layer [2].

Many inflammatory molecules like cytokines, chemokines and growth factors were shown to have a role in psoriasis pathogenesis[3,4]. Actually, it was known that several immune cells such as T-helper1 (Th-1) and T-helper17 (Th-17) were involved in the development of psoriasis, and anti-psoriatic drugs were directed to inhibit T-cells and interleukins to achieve partial or complete remission of psoriatic lesions [3,4].

The psoriatic lesions usually start in scalp and elbow and may remain localized to the same site for years. The course of the

disease is unpredictable and psoriasis disease affects both sexes equally [5].

Additionally, the moderate and severe form of psoriasis have greater risk to develop insulin resistance, ischemic heart disease and other co-morbidities. Psoriasis disease will affect life quality of patients on all directions [6].

The abnormal adipokine levels reported in psoriasis suggest that the systemic inflammation associated with the disease may be associated with adipose tissue inflammation just similar to what happened in obesity [7]. Fat tissue in human can produce several biochemical active substances termed as adipocytokines which exert many functions [8].

Visfatin was found to be identical to pre-B-cell colony-enhancing factor 1 (PBEF1) which is the precursor of B- lymphocyte lineage cells and also to the enzyme nicotinamide phosphoribosyl transferase (NAMPTase or Nampt). It is a conserved 52-kDa protein found in living species from bacteria to humans [9].

There are two different forms of visfatin/PBEF/Nampt have been described at present time. On one side, intracellular visfatin plays a central role in maintaining the activity of NAD-dependent enzymes and is implicated in the regulation of cellular metabolism in response to nutrient availability, maturation and survival. On the other side, extracellular visfatin can be synthesized and released to the extracellular milieu, not only by adipocytes but also by many different cell types, where it can exert a wide range of actions in a paracrine or endocrine manner. Indeed, extracellular visfatin shows a slightly higher molecular weight than the intracellular isoform and seems to undergo post-translational modification [10]. Visfatin appears to mediate vascular endothelial inflammation by inducing the expression of vascular cell adhesion molecule -1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM- 1) via

oxidative stress-dependent NF-kB stimulation. Visfatin also can mediate the inflammatory responses in monocytes by inducing the pro- inflammatory cytokines IL-1B, IL-6 and TNF- α . However, the higher the concentration of visfatin, the more augmented expression of anti-inflammatory cytokines (e.g. IL-10) [11].

Several studies have observed no difference in visfatin mRNA expression in visceral and subcutaneous adipose tissue in humans [12]. However, other studies confirmed an increased level of circulating visfatin whereas results from other studies were contradictory in that they showed lower levels of plasma visfatin in obese subjects [13].

Visfatin is not only an adipocyte-specific protein; its expression gene was originally found in human peripheral blood lymphocytes. It increases the effect of IL-7 and stem-cell factor on pre-B cell colony formation [14].

The production of several pro-inflammatory adipocytokines were not the only function of adipose tissues but has the ability to secrete a small amount of anti-inflammatory cytokines, including adiponectin [15]. Adiponectin level was negatively associated with body fat percentage in adults, while in infants and young children, the correlation was obscured [16].

The most biologically active isoform of adiponectin is the high-molecular weight one. The adiponectin exhibits several functions such as anti-inflammatory, anti-atherogenic, insulin-sensitizing, cardio-protective and the distinctive effects in lipid metabolism [17]. Like the cytokine leptin, the weight reduction effects of adiponectin were mediated by the central nervous system, and the two hormones perform complementary actions, and can have additive effects [18]. The important anti-inflammatory actions of adiponectin were achieved by inhibiting the inflammatory cytokine network and down-regulating

TNF- α , which in turn induce the expression of endothelial adhesion molecules [19].

RBP-4 is a 21-kDa protein responsible for transportation of vitamin-A in the blood circulation. It was produced and secreted by adipocyte and hepatocyte cells and the main source of RBP-4 was the visceral adipocyte cells [20].

The elevated serum level of RBP-4 can lead to systemic insulin resistance. The state of insulin resistance can be induced in normal mouse by increasing the serum level of RBP-4 about three times above the normal level during a period of 9-12 days. It was estimated that human serum concentration of RBP-4 was elevated in obese diabetic subjects and obese non-diabetic persons [20].

When the oxidative stress develops, it leads to the oxidative damage of lipids and proteins. High titers of auto-antibodies against ox-LDL have been reported in patients with psoriasis. Oxidation of the low density lipoproteins (LDL) results in the production of modified LDL. One of the major and early lipid peroxidation products is oxidized low-density lipoprotein (ox-LDL) [21], so high titers of auto-antibodies against ox-LDL have been reported in patients with psoriasis. The level of auto-antibodies against ox-LDL has been suggested to reflect the in vivo oxidation of LDL [22].

In basic term, ox-LDL is an important marker of oxidative stress and lipid peroxidation process. Importantly, ox-LDL, by itself, may induce inflammation. This capability may directly affect psoriatic epidermis and they believe that the accumulation of ox-LDL in the psoriatic skin may have an important role in initiation and maintaining the inflammatory process together with macrophage in psoriatic patients [23].

MTX was thought to affect cancer and inflammatory conditions by two different pathways. In malignant disease, MTX is competitively inhibits dihydrofolate

reductase (DHFR), that participates in the tetrahydrofolate synthesis. The affinity of methotrexate for DHFR is about 1000 fold that of folic acid. MTX, therefore, inhibits the synthesis of DNA, RNA and proteins [24]. In inflammatory diseases, inhibition of DHFR by MTX is not the only main mechanism, but rather the drug will inhibit the enzymes that incorporated in purine metabolism [25]. It is primarily used in treatment of psoriasis but later on, was described to have anti-inflammatory properties exhibited by its effects on gene expression of T-cells [26].

The aim of Study was to evaluate serum levels of these adipokines and Ox – LDL in patients with psoriasis and study the effect of methotrexate treatment on these parameters.

Materials and Methods

A total of 86 subjects, 43 healthy individuals (30 males and 13 females) and 43 patients (23 males and 20 females) presented with typical lesions of moderate to severe chronic plaque psoriasis attending to the dermatology outpatient clinic of Merjan Teaching Hospital in Al-Hilla City. Every patient had been asked about name, address, age, occupation or job, onset of disease, site of lesions, smoking and alcoholism (questionnaire). Exclusion criteria included the followings: hypertension, diabetes, patients on specific medications, pregnancy, liver diseases and any other medical or dermatological diseases.

In patients group, serum adipocytokines: Visfatin, HMW–adiponectin, Retinol binding protein – 4 and Ox LDL were measured at zero time (base line) and after 3 months from taking MTX. Patients were seen regularly every one month for three months to be investigated for liver function tests, renal function tests, blood glucose and lipid profile. Regarding control subjects only one blood sample had been taken and the measurement of serum

adipocytokines and oxidized-LDL proceeded just at the same time of analysis. The response to methotrexate drug was assessed by special measure called psoriasis area and severity index (PASI) score that measure the intensity of erythema, scales and thickness of plaques. Patients with estimated reduction in psoriasis area and severity index score equal or more than 50% regarded as good response group, while patients with a reduction in PASI score < 25% regarded as poor response group [27].

Anthropometric Measurement & BMI

A diagnosis of obesity is established by determining the body mass index (BMI) of patients and healthy subjects by using specific equation formulated as weight in kilograms divided by the square of the height in meters. Generally, the body mass index used routinely to estimate the degree of obesity state. Excess body weight simply classified into two categories; the first one named overweight when the measured BMI more than 24.9 kg/m^2 and the second named obesity when the estimated BMI more than 29.9 kg/m^2 [28].

Assessment of psoriasis severity (PASI score)

Several scales exist for measuring the severity of psoriasis. Generally, Psoriasis Area and Severity Index (PASI) score is the main scale used to measure the severity of plaque psoriasis. The range of PASI score has been between zero and this means no disease and 72 represents the highest score in severity of disease. In general, PASI less than 10 defines as mild case, PASI score from 10-20 defines as moderate case while PASI more than 20 is considered severe plaque psoriasis [29]. The benefit beyond calculation of PASI score is to estimate the disease severity and to achieve careful follow up of various treatment responses [30].

Blood Sampling

Blood samples were collected from fasting psoriatic and control subjects by using disposable syringes while subjects in the sitting position. Five to seven mls of blood were withdrawn from each group by vein puncture and poured slowly into a clean plane tubes, left at room temperature for 30 minutes for coagulation, then centrifugation of samples was done for 10-15 minutes and then serum was separated into several parts (1ml Eppendorf tube) to be stored at - 20 centigrade until time of analysis.

Statistical Analysis

Data analysis was performed by using SPSS version 18. Categorical variables were presented as frequencies and percentages. Independent sample t-test has been done to compare between two groups. Meanwhile One Way Analysis of Variance (ANOVA) was done to compare among more than two groups. Pearson's correlation coefficient was done to find the association between two continuous variables. A p -value of ≤ 0.05 was considered as a lowest limit of significant. Each patient was treated by methotrexate tablets 15 mg/ week through the periode of study (2 tablets every 12 hours in one day within a week), each tablet equal to 2,5 mg. Folic acid one tablet per day was given for patients also, missing the days of methotrexate intake.

Results

The age characteristics

The present study findings demonstrate that, the mean age of psoriatic patients was 41 ± 14.3 and about 34.9 % of them were < 35 years old and 65.1 % were >35 years old. Meanwhile, the mean age of control subjects was 35.8 ± 18.6 and about 46.5 % of them were <35 years old, while about 53.5 % of them were >35 years old as seen in figure (1).

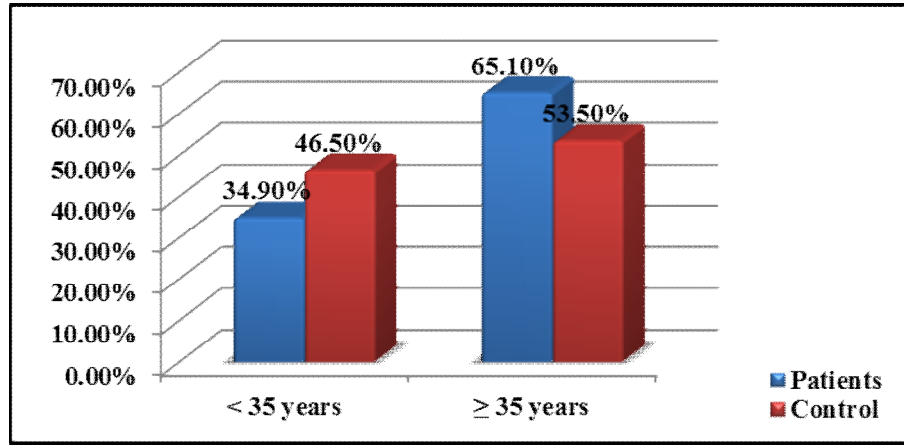


Figure 1: Distribution of patients and control by age

The Sex characteristics

Figure (2) showed that, the distribution of patients and control groups by sex. There were about 23 males (53.5%) and 20

females (46.5%) in patients with chronic plaque psoriasis and there were about 30 males (69.8%) and 13 females (30.2%) in control group respectively.

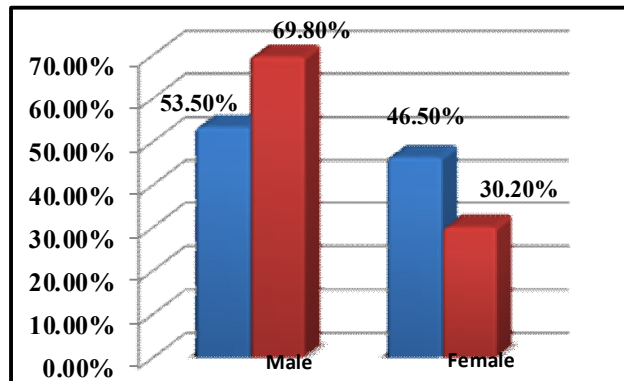


Figure 2 : Distribution of patients and control by sex

Distribution by Body Mass Index (BMI)

The study results regarding BMI as shown in figure (3) about (53.5%) of patients and (62.8%) of control were overweight, their BMI range between 25-29.9 kg/m², respectively. Meanwhile, 18.6%

of psoriatic patients, and 9.3% of control group were obese (BMI ≥ 30 kg/m²). The body mass index of the remainder of both groups was lying between (18.5- 24.9 kg/m²) and they were regarded as a normal weight individuals.

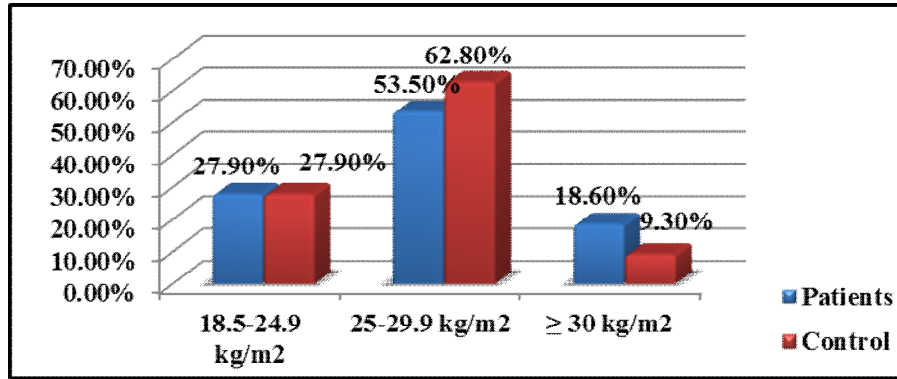


Figure 3 : Distribution of patients and control by BMI

Age, Sex and BMI Differences There was no significant differences of age,

sex and BMI by patients and control group, p value > 0.05, **table (1)**

Table 1 : Age, gender and BMI differences among patients and control subjects

Variable	Patients	Control	P value
Age years (Mean ±SD)	41.00±14.37	35.81±18.66	0.153
Sex (M/F)	(23/20)	(30/13)	0.183
BMI kg/m ² (Mean ±SD)	27.76±3.39	26.49±2.25	0.662

difference of PASI Score in patients before and after administration of oral Methotrexate

There was a significant mean difference of PASI Score between before (14.17±2.65) and after oral MTX treatment (4.18±1.21), p value <0.001, as shown in **figure (4)**.

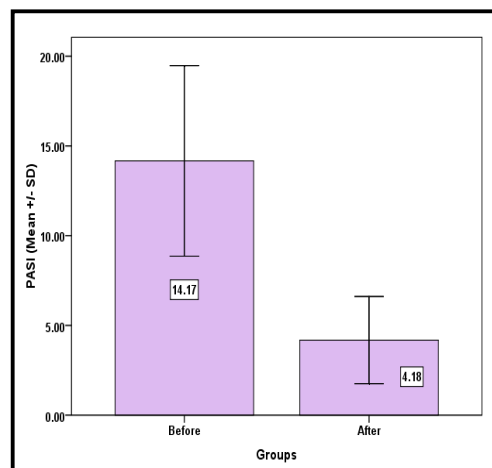


Figure 4 : Mean difference of PASI Score in patients before and after oral MTX therapy

Mean Differences of Visfatin, HMW Adiponectin, RBP-4 and Oxidized-LDL

Table (2) and figures (5,6,7,8) showed the mean differences of serum visfatin (23.75 ± 4.03 ng/ml), HMW-adiponectin (113.21 ± 14.27 ng/ml), RBP-4 (6.81 ± 2.34 μ g/ml) and oxidized LDL (48.68 ± 8.27 mU/ml) in patients before MTX treatment while they were about 13.65 ± 3.13 ng/ml for visfatin, 81.52 ± 17.28 ng/ml for HMW-adiponectin, 14.6 ± 2.83 μ g/ml for RBP-4 and was

22.82 ± 5.82 mU/ml for oxidized-LDL after oral methotrexate therapy and the serum concentrations of visfatin (3.45 ± 1.4 ng/ml), HMW-adiponectin (9.11 ± 3.25 ng/ml), RBP-4 (18.82 ± 3.87 μ g/ml) and oxidized-LDL (5.27 ± 1.93 mU/ml) for control group respectively. There were significant mean differences of visfatin, HMW-adiponectin, RBP-4 and oxidized-LDL in patients before and after oral MTX and control groups, p value <0.001 .

Table 2: Mean differences of Visfatin, HMW-adiponectin, RBP-4 and ox-LDL in patients (before and after MTX) and control groups by ANOVA test

Variable	No.	Mean $\pm$ SD	P value
<u>Visfatin ng/ml</u>			
Before	43	23.75 ± 4.03	<0.001
After	43	13.65 ± 3.13	
Control	43	3.45 ± 1.40	
<u>HMW-adiponectin ng/ml</u>			
Before	43	113.21 ± 14.27	<0.001
After	43	81.52 ± 17.28	
Control	43	9.11 ± 3.25	
<u>RBP-4 μg/ml</u>			
Before	43	6.81 ± 2.34	<0.001
After	43	14.60 ± 2.83	
Control	43	18.82 ± 3.87	
<u>Ox-LDL mU/ml</u>			
Before	43	48.68 ± 8.27	<0.001
After	43	22.82 ± 5.82	
Control	43	5.27 ± 1.93	

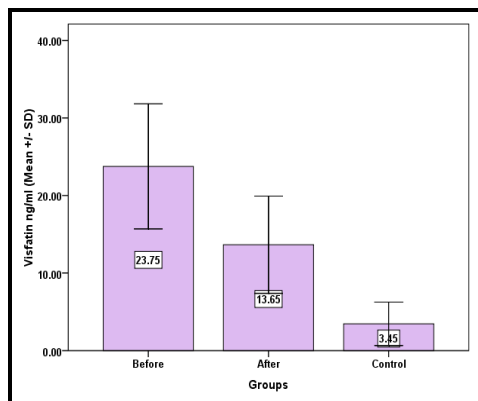


Figure 5 : Mean difference of Visfatin

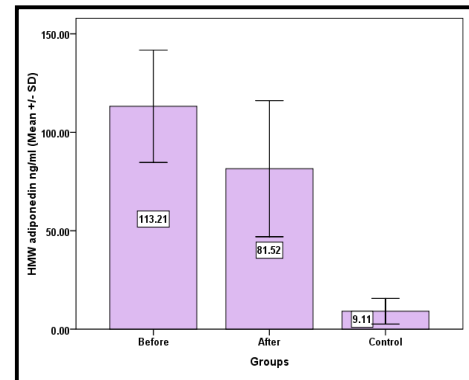


Figure 6 : Mean difference of HMW

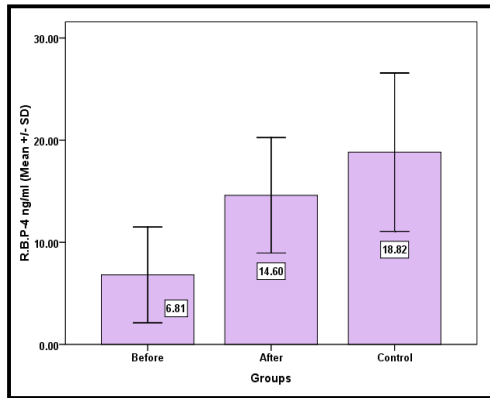


Figure 7: Mean difference of RBP-4 in patients and control

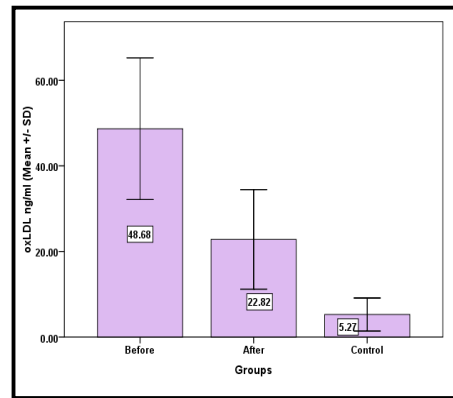


Figure 8: Mean difference of ox-LDL in patients and control

Correlation of BMI with serum Visfatin, HMW-adiponectin, RBP-4 and oxidized-LDL among patients and control group:

The study results regarding body mass index(BMI) were conflicting and query in that, there were no significant correlations between body mass index(BMI) and Visfatin ($r = 0.121$ and $p < 0.05$), HMW-adiponectin ($r = 0.043$ and $p > 0.05$),

Retinol binding protein-4 ($r = -0.17$ and $p > 0.05$), and oxidized- LDL ($r = 0.049$ and $p > 0.05$) among patients while the findings of control groups were ($r = 0.15$ and $p > 0.05$), ($r = 0.108$ and $p \text{ value} > 0.05$), ($r = -0.023$ and $p > 0.05$) and ($r = 0.137$ and $p \text{ value} > 0.05$) respectively for the same parameters as shown in table [3].

Table 3: Correlation of BMI with serum Visfatin, HMW-adiponectin, RBP-4 and oxidized-LDL among patients and control group

Variable	BMI among Patients		BMI among Control	
	r	P value	r	P value
Visfatin ng/ml	0.121	0.440	0.150	0.337
HMW Adiponectin ng/ml	0.043	0.784	0.108	0.489
R.B.P-4 $\mu\text{g/ml}$	-0.170	0.276	-0.023	0.882
ox-LDL mU/ml	0.049	0.753	0.137	0.381

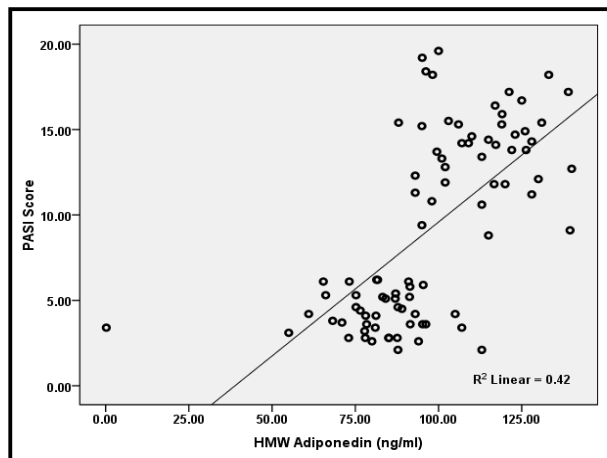
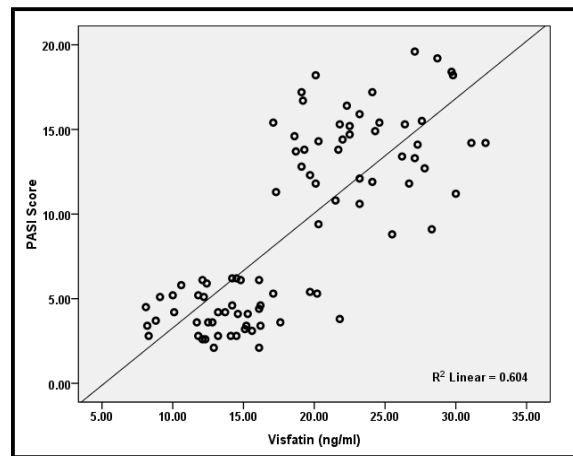
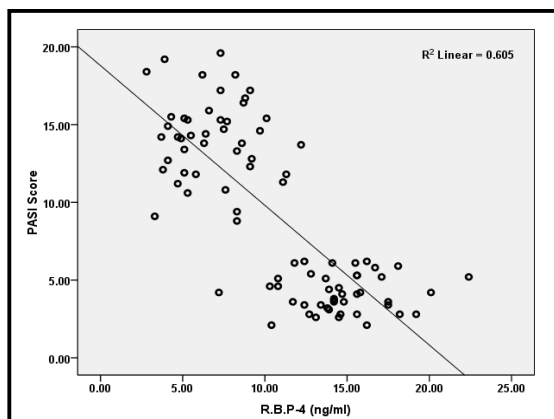
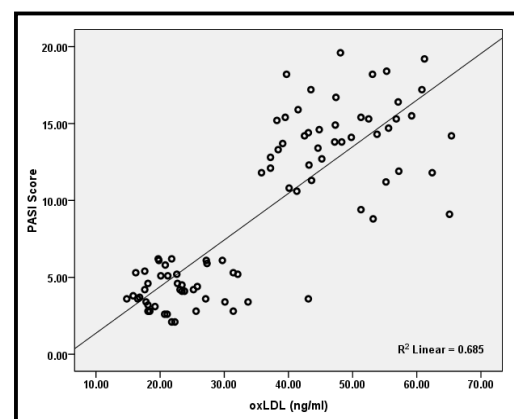
Correlations of PASI Score with serum Visfatin, HMW-adiponectin, RBP-4 and oxidized-LDL:

Table (4) and figures (9), (10), (11), and (12) showed the association of PASI Score with serum visfatin, HMW-adiponectin, RBP-4 and oxidized-LDL. There were direct perfect significant correlations

between PASI Score with visfatin, HMW-adiponectin, and oxidized-LDL ($r = 0.777$, 0.648 , 0.728) and ($p \text{ value} < 0.001$) respectively, meanwhile, there was indirect perfect significant correlation ($r = -0.778$) ($p \text{ value} < 0.001$) between PASI Score with RBP-4 as shown in figure (11).

Table 4 : Correlation of PASI Score with Visfatin, HMW Adiponectin, RBP-4 and oxidized-LDL

Variable	Mean $\pm$ SD	r	P value
Visfatin ng/ml	18.70 $\pm$ 6.21	0.777	<0.001
HMW Adiponectin ng/ml	97.36 $\pm$ 22.41	0.648	<0.001
RBP-4 μ g/ml	10.70 $\pm$ 4.69	-0.778	<0.001
Ox-LDL mU/ml	35.75 $\pm$ 14.82	0.728	<0.001

**Figure 9:** Correlation of PASI Score with Visfatin**Figure 10:** Correlation of PASI Score with HMW-adiponectin**Figure 11:** Correlation of PASI Score with RBP-4**Figure 12:** Correlation of PASI Score with ox-LDL

Correlation of oxidized-LDL with serum Visfatin, HMW-adiponectin and RBP-4 in patient group:

Table (5) and figures (13), (14) and (15) showed the correlation of serum oxidized-LDL with serum visfatin, HMW-adiponectin and RBP-4. There were direct

perfect significant correlations between oxidized-LDL with visfatin and HMW-adiponectin ($r= 0.818, 0.682$) ($p<0.001$) respectively, meanwhile, there was indirect perfect significant correlations between oxidized-LDL with RBP-4 ($r= -0.775$) ($p <0.001$).

Table 5: Correlation of oxidized-LDL with serum Visfatin, HMW-adiponectin and RBP-4 in patients group

Variable	Mean $\pm$ SD	r	P value
Visfatin ng/ml	18.70 $\pm$ 6.21	0.818	<0.001
HMW - adiponectin ng/ml	97.36 $\pm$ 22.41	0.682	<0.001
RBP-4 μ g/ml	10.70 $\pm$ 4.69	-0.775	<0.001

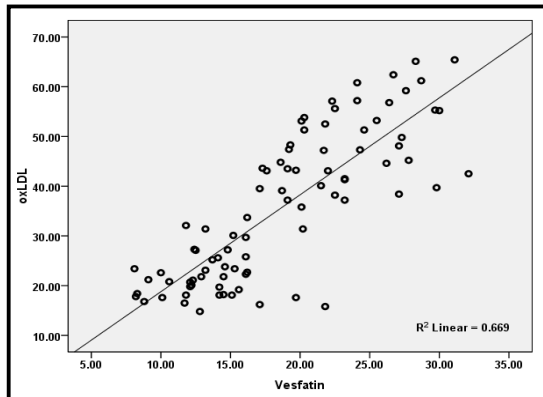


Figure 13 : Correlation of ox-LDL with visfatin

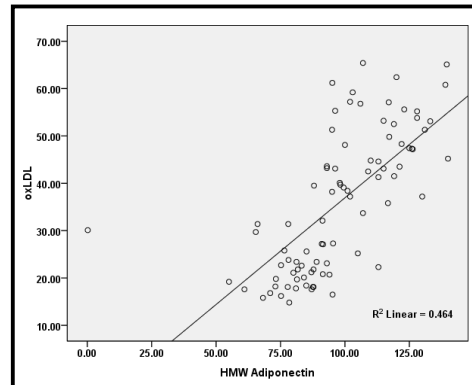


Figure 14: Correlation of ox-LDL with HMW-adiponectin

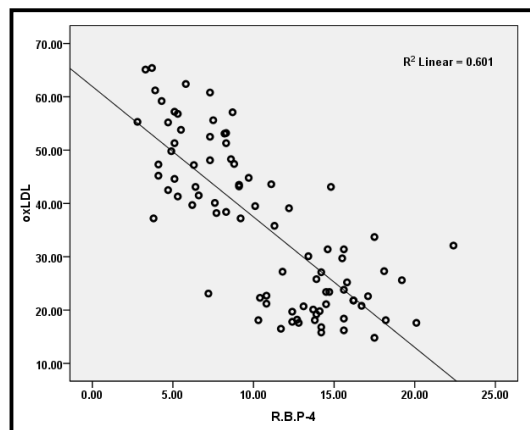


Figure 15 : Correlation of oxidized-LDL with RBP-4

Discussion

Age:

The mean age of patients was (41.00 ± 14.37) years old, meanwhile, it was (35.81 ± 18.66) years old for control group and the majority of both groups nearly (59.3%) were older than 35 years, as seen in figure (1). Although, there were two peak age of incidence (the first between 20-30, the second between 50-60 years old), this study does not find any significant statistical difference between the age of patients and severity of the psoriasis disease. The study findings regarding the age of the patient were in agreement with results of other studies [31,32].

Gender:

The frequency of male persons group was higher than that of female in this study both in patients (23 males and 20 females) and control group (30 males and 13 females), as shown in Table (1) and figure (2). Yet, there was no statistically significant differences between the two sex groups ($p < 0.05$). This means that the disease could affect both sexes equally. The present study results are in agreement with other studies [31,32].

Body Mass Index and Psoriasis Area and Severity Index:

Adipose tissue is principally divided into two types, subcutaneous adipose tissue and intra-abdominal one or what is called visceral fat (white adipose tissue): the central one includes visceral fat sources such as omental fat and adipose tissue that covers the mesentery. The visceral fat, is thought to be more metabolically active than subcutaneous adipose tissue [33].

Actually, Panagiotakos, et al. clarify that obesity is associated with a state of chronic low-grade inflammation that is manifested by elevated serum concentrations of adipocytokines including: visfatin, HMW-adiponectin and Oxidized-LDL. Obesity was positively correlated with BMI and PASI score. The risk of occurrence or development of psoriasis in obese persons

was estimated to be increased and thought to be directly correlated with body mass index. These findings are against the results of the current study regarding BMI as indicated in table (3) that showed weak positive correlation between serum visfatin, HMW-adiponectin and oxidized-LDL levels and BMI ($r = 0.121, 0.043, 0.049$) (p value > 0.05) respectively, meanwhile there was negative non-significant correlation between BMI and retinol binding protein-4 (RBP-4) ($r = -0.170$) (p value > 0.05). The findings of the present study were certainly against the most findings obtained by other studies [34,35].

Visfatin and serum HMW-adiponectin in addition to ox-LDL showed a significant positive correlation with PASI score, conversely, the study findings regarding RBP-4 indicate a negative association with PASI score as that shown in table (4) ($r = 0.777, 0.648, -0.778, 0.728$, respectively) (p value < 0.001). There was a clear association between zero time levels (base line) of these adipocytokines in addition to oxidized-LDL and PASI score and this is due to white adipose tissue dysfunction and because of the impact of psoriatic inflammatory process on the adipocytokines network.

Visfatin:

This pro-inflammatory adipocytokine had been discovered to be increased in chronic inflammatory diseases, so it is highly expected to be elevated in the psoriatic patients with plaque type, especially severe psoriatic cases. The main objective for measurement of serum visfatin is to establish the role and the effect of this pro-inflammatory adipocytokine in the pathogenesis of psoriasis. The study results obtained by Ismail and Mohamed reported that skin visfatin levels were elevated in psoriatic patients when compared to healthy donors (skin specimen) [36]. Visfatin present in the blood circulation may trigger the mRNA and protein expression of antimicrobial peptides in

epidermal keratinocytes of psoriatic lesions. Therefore, visfatin may exacerbate psoriatic skin lesions by stimulating the expression of antimicrobial peptides and chemokines [37]. These findings suggest that visfatin may lead to acceleration of psoriasis process. So that, by regulating the systemic visfatin levels, it may be possible to control the exaggerated production of antimicrobial peptides in psoriatic skin lesions and this will alleviate the symptoms and reduce the lesion size. The findings present in table (4) showed a significant positive correlation and direct association between visfatin and PASI index ($r = 0.777$). These findings agreed with the results obtained by (Gerdes, et al. (2012) [38] and Al-Suhaimi and Shehzad [39].

The visfatin serum levels in this study was up-regulated in psoriatic patients with plaque type before and after oral MTX therapy as shown in table (2). Therefore, it is reasonable and logical to consider that visfatin should have a role and participates in the process of underlying mechanism in the psoriasis pathogenesis and in fact potentially increases the disease severity.

High molecular weight-adiponectin (HMW-adiponectin)

It is well known that, the adiponectin has a strong anti-inflammatory and athero-protective effects in the vascular system in addition it reduces the tissue insulin-threshold, so make them highly sensitive to insulin hormone so that, indirectly it deals with glucose metabolism [40].

High molecular weight-adiponectin is considered to be the active part of total adiponectin, and was estimated to be an excellent bio-marker in the measurement of metabolic abnormalities than total serum adiponectin [41].

HMW-adiponectin concentrations were highly increased in patients with plaque psoriasis in the present study in comparison to the control group as shown in table (2) (p value < 0.001). Unlike reports from other studies (which reported a low adiponectin

levels in psoriasis) and against the theory mentioned above, it is fair to believe that serum HMW-adiponectin concentrations seems to be regulated independently in psoriatic patients and these results go well with findings obtained by El-Haggar, *et al.* [42] and Shibata *et al.* [43].

The findings of the present study indicates a direct significant correlation between serum concentrations of HMW-adiponectin and PASI score ($r = 0.648$) (p value < 0.001) as shown in table (4).

An Iraqi study in Kufa University/ College of Medicine in Al- Najaf City on 60 male patients with plaque psoriasis showed a lowered serum concentration of total adiponectin in patients with a plaque psoriasis when compared to healthy persons which explained by increased body fat mass [44].

The explanation regarding high serum level of HMW-adiponectin in thin or normal weight psoriatic patients, in part may be due to increased secretion of adiponectin from sources other than visceral white adipose tissue (WAT) like subcutaneous fat by the effect of psoriasis inflammatory process or resulted from increasing activity of WAT to produce and secrete more adiponectin in patients with normal weight but with moderate/severe form of psoriasis. But, still further and extended prospective studies (large sample size and over long period) are requested to exhibit the precise correlation between serum adiponectin and the severity of disease process.

The present study suggested that, the increased serum HMW – adiponectin concentrations seen in the psoriatic patients were rather to the unique effect of psoriasis on adiponectin signaling pathway.

The up-regulation of serum HMW-adiponectin, that is manifested in present study as shown in table (2), might represent a protective mechanism against the chronic inflammatory state that present in psoriasis.

Retinol Binding Protein-4 (RBP-4)

Retinol binding protein-4 (RBP-4) is another adipocytokine secreted by white adipose tissue (visceral fat); and the abnormality in serum RBP-4 levels has a major impact in the development of insulin resistance [45].

The findings of the present study showed a lowered serum level of this adipocytokine in patients with moderate to severe psoriasis in comparison with the control group as shown in table (2) (p value <0.05). The data presented in figure (11) and table (4) showed a negative statistical significant correlation between RBP-4 and PASI index ($r = -0.778$) (p value <0.001). These findings were similar to that found by Rollman and Vahlquist [46].

The high serum RBP-4 which might be responsible for the development of insulin resistance or diabetes mellitus in psoriatic patients could not be emphasized. Instead, the down-regulation of RBP-4 could be a protective mechanism to prevent the affected person from developing insulin resistance in a low-grade chronic inflammatory conditions including chronic plaque psoriasis.

Oxidized-Low Density Lipoprotein (Ox LDL)

Abnormalities in serum lipid compartments concentrations might reflect an oxidative stress state which is responsible for psoriatic lesion development and progression by one way or another [47].

By itself, oxidized-LDL, which is found in the upper layer of epidermis of psoriatic skin, is responsible for the initiation of inflammatory process and affects the rate of adhesion and the anti-oxidant state of endothelial cells. This fact was thought to be the reason behind the incorporation of oxidized-LDL in early steps of atherosclerotic process in psoriasis [47].

The present study showed that serum concentrations of oxidized-LDL were significantly increased in patients with

plaque psoriasis compared with control group, both at the baseline level and after three months period of systemic oral methotrexate therapy as shown in table (2). Results of the present study, showed no association between serum level of oxidized-LDL and BMI as demonstrated in table 3), both in psoriatic patients and control subjects group, meanwhile, the present study results indicates a perfect direct significant association between oxidized-LDL and PASI score ($r = 0.728$) (p value < 0.001) as that shown in table (4). Additionally, results of the present study confirm a significant direct correlation between serum level of oxidized-LDL and serum concentrations of visfatin, HMW-adiponectin, but showed a negative significant association with serum concentration of RBP-4 in psoriatic patients as seen in table (5). The study results were in agreement with other findings obtained by Tekin, *et al.* [48].

It can be postulated the increased LDL levels could be the trigger factor in the deposition of oxidized-LDL in the body organs including skin; so it may be the key risk factor in the patho-physiology of psoriasis disease.

Low dose methotrexate (MTX dose less than 15-20mg per week) was an effective drug especially for severe types of psoriasis in which the percentage of involved skin surface area more than 20% and if the psoriatic cases were carefully chosen and the follow-up (monitoring) was regular, with continuous screening by liver chemistry test and bone marrow toxicity (by doing complete blood count and blood film) [49].

Methotrexate therapy is relatively safe drug, and this is according to the world health organization (WHO) guidelines and till now has been used in the management of psoriasis and other inflammatory diseases with more than 50 years of accumulated data regarding its effect and safety [49].

The findings of the present study, regarding the impact of MTX on adipocytokines (visfatin, HMW-adiponectin and retinol binding protein-4) and oxidized-LDL and also on psoriatic lesions were acceptable and expectable. It was manifested by psoriatic lesions improvement and sometimes disease remission. This might be achieved, through the reduction of pro-inflammatory and anti-inflammatory cytokines levels, especially, when patients remain restricted to the medical instructions about methotrexate drug like taking the medicine at low doses and in combination with folic acid and in exact time [50].

The effect of methotrexate on psoriasis was appreciated by measuring the PASI scores in psoriatic patient, both, before and after taking MTX. It was clearly that, the data of the present study reflect good response of patients as revealed by decreasing of serum levels of most adipokines studied and improvement and sometime complete resolution of psoriatic skin lesions after taking the drug as shown in table (2) and figure (4).

Conclusion

This study indicates that psoriasis is associated with increased serum visfatin, HMW- adiponectin and Ox – LDL levels and decreased RBP-4 levels. The association between psoriasis and increased HMW-adiponectin level and low RBP-4 level might postulate an independent association between psoriasis and adipose tissue dysfunction. While, the elevated serum visfatin and oxidized-LDL levels regarded as key players in initiation, proceeding and maintenance of psoriasis pathogenesis.

This study also showed a significant positive correlation between visfatin, HMW-adiponectin, in addition to oxidized-LDL and PASI score, while a significant negative correlation between RBP-4 and PASI score, but there was no significant association between these adipocytokines

and BMI. So that, the estimation of serum concentrations of these adipokines together with oxidized LDL, may be helpful in evaluating psoriasis severity, treatment success and risk of comorbidities.

The results showed a good effect of oral methotrexate therapy upon serum levels of adipocytokines and oxidized-LDL in psoriatic patients, in addition to better and faster remission of psoriatic lesions and this reflects the immune-modulatory role of this drug in psoriasis.

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