Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.4, 2016

Impact of Methotrexate on Serum Vascular Endothelial Growth Factor and Reduced Glutathione in Patients with Vulgaris Psoriasis

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Abstract
To evaluate the effect of methotrexate on serum of vascular endothelial growth factor (VEGF) reduced glutathione (GSH) and disease severity in psoriatic patients.Aim: To estimate the impacts of methotrexate on levels of vascular endothelial growth factor and reduced glutathione (GSH) in serum of patients with psoriasis and to assess the efficacy and impact of methotrexate on severity of psoriasis which recognized by psoriasis area and severity index (PASI). This is a follow up study conducted on a total 80 subjects; forty patients (25 male and 15 female) with plaque psoriasis compared to forty healthy persons (21male and 19 female). Venous blood collected from the clinic of dermatology in Merjan Teaching Hospital in Hilla city/Iraq. Serum was prepared from collected blood, and used to investigate the effect of methotrexate on serum vascular endothelial growth factor and reduced glutathione concentrations. The present study findings revealed a significant elevation in serum vascular endothelial growth factor concentration and significant decrease in reduced glutathione concentration in patients group when compared to the control group. On the other hand, the study showed significant reduction in serum vascular endothelia growth factor level and PASI score after three month's duration of treatment with methotrexate, and significant elevation of serum reduced glutathione level after the same period of treatment.
Keywords: Psoriasis vascular endothelial growth factor, reduced glutathione, psoriasis area and severity index and methotrexate.

Introduction

Psoriasis is a chronic, recurring inflammatory skin disease that affects 1-3% of the world's population. Plaque psoriasis manifests itself as raised, well-isolated, erythematous and painful plaques with silvery, white scales. The plaques are most commonly found on the elbows, knees, and lower back as well as in cosmetically sensitive regions such as the scalp, face, hands, and feet (Yeilding *et al*.2011). Hereditary predisposition, environmental factors and immunological mediators are all involved in the pathogenesis of psoriasis (Di Nuzzo *et al*.2014). There are several types of psoriasis: chronic plaque, guttate, inverse, pustular and Erythrodermic psoriasis. Each of these types of psoriasis and the various forms within certain types can come in three levels of severity, mild, moderate, and severe. Gender and ethnicity are two factors that are also involved in this disease. The disease occurs with equal frequency in both genders (James et at., 2011). Accurate evaluation and objective of disease severity in all patients with plague psoriasis is very importing for any designing therapeutic strategies , and assessing psoriasis patient progress and is invaluable for comparing and evaluating the efficacy of new treatments give to psoriasis patients in clinical trials . Traditionally, assessments of psoriasis severity in plaque psoriasis patient depended on the treating physician's appraised of the signs of disease that be visible, can be done by using psoriasis area and severity index (PASI) (Krueger ,, 2000). In addition to topical treatment, there are various types of systemic agents used for psoriasis treatment include methotrexate; acitretin; cyclosporine and biologics therapy as a common anti-psoriatic drugs. Methotrexate (MTX) is an antimetabolite and antifolate drug used in treatment of cancer, autoimmune diseases and It is also used in the treatment of psoriasis (Bertino,2000 and Thomas &Aithal,2005).

Many inflammatory molecules like cytokines, chemokines, and growth factors were shown to have a role in psoriasis pathogenesis. 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(Coimbra et al.2010 and Luo *et al* .2010).

Angiogenesis process is the physiological process which formation of newly capillaries form established blood vessels, and it is an important process in growth and development (Risau *et al*.1992). Angiogenesis is a key feature in psoriatic skin, which is associated with local and systemic elevation of angiogenic cytokines including vascular endothelial growth factor (VEGF) which fluctuates in line with disease activity. Psoriasis is a skin condition where skin becomes red and irritated. The disease also involves inflammation, angiogenesis, and vascular remodeling. In 2006, a study was conducted to see if VEGF played a role in psoriasis, and the researchers found that overexpression of VEGF, HIF-1α, and MMP-2, leads to the

inflammation and angiogenesis seen in patients with psoriasis(Simonetti *et al*.2006).

The worsening of psoriasis has been linked with oxidative stress (Rocha-Pereira *et al*.2004).

Disorders in the antioxidant defense mechanisms are known to be involved in the pathogenesis of psoriasis (Wozniak *et al.* 2007 and Zhou *et al*. 2009). Antioxidants are the body's premier resource for protection against the diverse free radical and other oxidative stressors to which it invariably becomes exposed(Cross *et al*. 1987).The antioxidant defense system is sophisticated and adaptive,and GSH is a central constituent of this system(Kidd,1991).

Materials and Methods:

Materials

Subjects:

This a follow up study was conducted through the period from October 2014 till August/ 2015. A total of 80 age-sex matched subjects were enrolled, 40 apparently healthy individuals(21 male and 19 female) and 40 patients (25 male and 16 female) presented with moderate to severe plaque psoriasis attending to the dermatology outpatient clinic of Merjan Medical Teaching Hospital in Hilla City.

Every patients had been asked about name , address, age, onset of disease, site of lesions, smoking and alcoholism(questionnaire). Exclusion criteria include: hypertension, diabetes, patients on specific medications, pregnancy, hepatitis or other liver diseases and any other medical or dermatological diseases.

Blood Sampling:

Five milliliters of venous blood sample were drawn (at the baseline or zero time sample) from each patient by vein puncture and poured slowly into plain tubes. The same quantity of blood was collected from control subjects, and a second sample was withdrawn from the patients group after three months from the starting MTX treatment. The samples were left at room temperature for thirty minutes to two hours for clotting, then centrifugation of samples were done for 10-15 minutes at 1000 xg, and then serum were separated into several parts:

Aliquot of serum is transfer into 1ml Eppendorf tube, which was used to measure vascular endothelia growth factor and another amount of serum into1ml Eppendorf reduced glutathione . The tubes were stored at -20 centigrade until analysis.

Methods:

Serum vascular endothelial growth factor concentration was measured by **Cusabio(China)** kit. Serum levels of was reduced glutathione determined by **Cusabio (**China**)** kit too. The effect of methotrexate drug on psoriatic lesions was appreciated or calculated by PASI score.

Statistical analysis:

Statistical analysis was performed by using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). The normal distribution was conformed correlation analysis, independent t-test was used to estimate differences between two groups in continuous variable as well as the repeated measurement used to estimate the differences between before treatment with MTX, after one month and after three month from treatment levels of the measured parameter among patient group. Result are reported as mean and stander deviation (mean±SD) unless otherwise indicated. A p-value of ≤ 0.05 was considered as a lowest limit of significant. (Daniel, 1999).

1-Age and body mass index (BMI) Differences among psoriasis Patients and Control:

Forty adults with plaque psoriasis disease was taken as patients group (25 male and 15 female), and forty apparently healthy adults (21male and 19 female), as control group were enrolled in this study. Found that the mean ± SD of age in psoriasis group (male and female) and control group (male and female) as shown in table (1). Which show there was no significant difference between the two groups as P-value of ≤ 0.05.

Table (1): The number of subjects (patients group and control group), gender and mean age.

Groups	Total No.	Male	Female	Mean ±SD Age(years)	
				Male	Female
Patients	40	25	15	42.72±10.25	41.33±10.37
Controls	40	21	19	41.29±9.93	36.11±9.98
P-value				0.63	0.14

P-value ≤ 0.05 is significant.

In this study table (2), show significant increase ($p \leq 0.05$) in body mass index of psoriasis group (male and female) compared with those of the control group (male and female).

Table (2): The means and standard deviation of body mass index (BMI) (kg /m^2) for psoriasis and control groups according to sex.

Body mass index (kg /m^2)	Subjects	Total Number (female)/(male)	Mean ± SD	
			Male	Female
	Psoriasis group	40 (25/15)	A 28.53±2.75	A 27.39±1.34
	Control group	40 (21/19)	B 25.65± 2.39	B 25.61±1.86

- Values are mean ±SD.
- The value with different capital letter are significant at P ≤ 0.05 level.

2-The values difference of psoriasis area severity index (PASI) score of psoriasis patients before and after (one month's and three months) of administration of methotrexate drug:

The results in table (3) show, there was a significant decrease values difference of psoriasis area and severity index (PASI) score of forty plaque psoriasis patients (male and female) between before treatment and after treatment with MTX (after one month's and after three month's from administration of methotrexate therapy). Finally, the values of patients after one month's compared with after three month's from administration of methotrexate therapy, considered statistically significant decrease at P-value ≤ 0.05.

Table (3): The values difference of psoriasis area and severity index (PASI) score by patients before and after one month's, after three months of administration of methotrexate drug according to sex.

Parameter	Gender	Patients before treated with methotrexate	Patients after one month of treated with methotrexate	Patients after three month of treated with methotrexate
Psoriasis area and severity index (PASI) Score	Male	(a) 38.09± 8.77	(b) 17.53±4.18	(c) 7.18±2.81
	Female	(a) 32.12±7.95	(b) 15.44±5.31	© 5.55±2.22

- Values are mean ±SD.
- The value with different small letter significant at P≤ 0.05 level.

3-Serum vascular endothelial growth factor (VEGF) concentration (pg/ml):

The mean of VEGF in the patient group in all follow up study period at before treatment and after (one months and after three months) from administration of methotrexate was significantly higher than the control group at (p≤ 0.05),as described in table (4). During this period compared the mean of VEGF among patient at baseline and after (one month and after three months) found significantly decrease at (p≤ 0.05).as shown in table (5).

Table (4): Mean difference of vascular endothelial growth factor (pg/ml) of patients before and after (one month's and after three months) from administration of methotrexate drug and control groups according to sex.

Parameter	Gender	Patients before treated with methotrexate	Patients after one month of treated with methotrexate	Patients after three month of treated with methotrexate	Healthy control
Vascular endothelial growth factor (pg/ml)	Male	(A) 604.31±29.54	(A) 403.69±38.79	(A) 258.65 ±43.73	(B) 186.29±18.14
	Female	(A) 614.64±39.40	(A) 428.93±29.91	(A) 265.59 ±30.29	(B) 204.96±19.52

- Values are mean ±SD.
- The value with different capital letter significant at P ≤ 0.05 level between each patients before treatment, after one and three months of treatment as compared with control.

Table (5): Means difference of vascular endothelial growth factor (pg/ ml) to patients before and after (one month's and after three months) from administration of methotrexate drug.

Parameter	Gender	Patients before treated with methotrexate	Patients after one month of treated with methotrexate	Patients after three month of treated with methotrexate
Vascular endothelial growth factor (pg/ml)	Male	(a) 604.31±29.54	(b) 403.69±38.79	(c) 258.65 ±43.73
	Female	(a) 614.64±39.40	(b) 428.±29.91	(c) 265.59 ±30.29

-Values are mean ±SD.
-The values with different small letter significant at P ≤ 0.05 level between patients before treatment and after (one and three months) of treatment.

4-Reduced glutathione concentration (GSH) (µ/ml):

Significant decrease P ≤ 0.05 between patients group (male and female) during follow up period and healthy control (male and female) group (table 6). As well as, when compared the patients before treatmet and after (one months and three months) from admistreation of MTX drug ,there is significant increase at P ≤ 0.05 between them ,that show in table (7).

Table (6): The results difference of reduced glutathione (µ/ml) of patients between each of before and after (one month's and after three months) from administration of methotrexate drug and control groups.

Parameter	Gender	Patients before treated with methotrexate	Patients after one month of treated with methotrexate	Patients after three month of treated with methotrexate	Healthy control
Reduced Glutathione (µ/ml)	Male	(A) 0.70±0.37	(A) 1.00 ±0.46	(A) 1. 77±1. 19	(B) 4.02± 2.55
	Female	(A) 0.60±0.54	(A) 1.31±0.40	(A) 1.70 ±0.48	(B) 3.99±2.83

- Values are mean ±SD.
- The value with different capital letter significant at P ≤ 0.05 level between patient before treatment and after (one and three months) of treatment with healthy control group.

Table (7): Means difference of reduced glutathione (µ/ml) by patients before, after one month's and after three months from administration of methotrexate drug according to sex.

Parameter	Gender	Patients before treated with methotrexate	Patients after one month of treated with methotrexate	Patients after three month of treated with methotrexate
Reduced glutathione (µ/ml)	Male	(a) 0.70±0.37	(b) 1.00 ±0.46	(c) 1. 77±1. 19
	Female	(a) 0.60±0.54	(b) 1.31±0.40	(c) 1.70 ±0.48

- Values are mean ±SD.
- The values with different small letter mean significant at P ≤ 0.05 level between patient before treatment and after (one and three months) of treatment as compared with control.

Discussion:

Age:

In this cohort study, the values of mean and standard deviation of age for patients (male and female) was (42.72±10.25 and 41.33±10.37). While the mean and stander deviation of age for control group (male and female) was (41.29±9.93 and 36.11±9.98), table (1). However, there was no significant (P>0.5) difference in age between patients with plaque psoriasis as compared with healthy control, this results agrees with study done by Nilgon et al., 2007). A specific study for psoriatic patients from Japan presented that the mean age of onset of psoriasis of their study population was 40 years(Takahash *et al*.2010). On this study, onset of psoriasis is before the age of 40 years, and in about one-third of cases, it was before the age of 20 years. Psoriasis can present at any age, the disease is less common in children than adults, there were two peaks for the age of onset: one between the ages

of 30 and 39 years and another between the ages of 50 and 69 years (Parisi *et al*.2014).

Body mass index (BMI):

Table (2) showed the results of (BMI) of this study and there was a significant increase ($p \leq 0.05$) in body mass index of plaque psoriasis group compared with those of the healthy control group. Body mass index provided a simple numeric measure of person adiposity.The risk of psoriasis increased with the increasing levels of adiposity, indicating a strong consistent relationship. The results of the present study indicated that psoriasis was associated with increased body weight. This present study was in agreement with Naldi *et al*., (2005). During this study, the increased body mass index indicates as a risk factor for triggering psoriasis. The chronic, low-grade inflammatory states associated with adiposity explain the increased risk of incident psoriasis among obese individuals (Boehncke and Schon, 2005).

Obesity has serious health consequences including hypertension, vascular disease and type 2 diabetes mellitus. Psoriasis was first associated with obesity in several large European epidemiologic studies. Studies from the United States also show an elevated BMI in patients with psoriasis (Herron *et al* .2005).The Nurses Health Study II, which contains prospective data from 78,626 person followed up during a 14-year period, indicates that obesity and weight gain are strong risk factors for the development of psoriasis (Setty *et al*.2007).

Clinical assessment of the response to treatments by psoriasis area and severity index (PASI):

It was found during this study, there was a significant improvement in skin lesion of psoriasis that signified by reduction in PASI score in all patients as compared to the baseline values (before treatment with MTX). The mean of baseline value of PASI score in psoriasis pa¬tients (male and female) assigned to receive methotrexate (15 mg/week) was (38.09± 8.77, 32.12±7.95), respectively. The values of PASI score started to decline as treat¬ment continued, at the end of one and three months of treatment, the mean values of PASI score became (17.53±4.18, 15.44±5.31) and (7.18±2.81, 5.55±2.22), respectively; These values were significantly different from that of the baseline , table (3). The result of this study agree with an Iraqi study done by Haider Abd Jabbar Alammar (2015), in Baghdad University/ Medical College in Baghdad City, whose findings from forty three patients with plaque psoriasis that indicate a decrease of psoriasis area and severity index (PASI) score and the effect of methotrexate on psoriasis was appreciated by measuring the PASI scores in psoriatic patients, both, before and after taking MTX . It was clearly that, the data of the present study reflects good response of patients and improvement, sometimes with complete resolution of psoriatic skin lesions after taking the drug. Another study done by Elghandour and his colleagues (2013) found that ,there was significant reduction in PASI score for patients treated with methotrexate as well as significant reduction for patients treated with narrow band UVB phototherapy alone . In addition, research work in India also plays an important role in studies of psoriasis, see the efficacy of methotrexate, the use of which supported by immunopathogenic basis of origin of psoriasis in the treatment of psoriasis, 40 patients of psoriasis having more than 20% body surface involvement during a one-year period treated with methotrexate for three months. The severity of disease evaluated by PASI, the results show effective response to treatment of psoriasis. In addition, methotrexate leads to a faster clearance of disease in early course of treatment (Gupta *et al*.2005). PASI score has been used for the assessment of severity of psoriasis and as a tool to monitor response to treatment. The use of markers in combination with clinical measures like PASI will help in better understanding the disease as well as to develop treatment strategies and monitor responses. Serum markers like cytokines have been instrumental in understanding the pathology of skin diseases like psoriasis (Leveque *et al*.2004). Treatment basis and therapeutic response experience strongly supports the use of immunomodulators as important modalities in the treatment of psoriatic arthritis and plaque psoriasis. Studies with these therapeutic agents, which act in different steps of the psoriatic inflammatory cascade, have also shown significant efficacy (Scarpa *et al*. 2010). Severity and progression of psoriasis, as well as its response to treatment, is variable among patients (Sua´rez-Farin˜as *et al*.2010). So, methotrexate has been shown to be efficacious as a systemic therapy in the treatment of psoriasis, with controlled studies showing an improvement in symptoms of psoriasis in approximately 60% of patients treated (Flytström *et al*. 2008).

Vascular endothelial growth factor level (VEGF):

The results of the present study revealed a significant difference in the values of VEGF levels between psoriatic patients (male and female) and healthy controls (male and female), and between patients before and after therapy at the end of the follow-up period (table.4 and.5). These results are compatible with the finding of several authors, who reported that its levels are significantly high in serum in the active stage of the disease (Coimbra *et al*.2010). Vascular endothelial growth factor could also be a biomarker of the disease. Indeed, VEGF found in higher concentration in serum of psoriatic patients in comparison to healthy control group (Nofal *et al*.2009). Flisiak and his co-worker (2012) reported that the increment of the serum of VEGF became significant only in patients with medium and sever activity of the disease. On the other hand, Shimauchi and his coworker (2013) found, there was no significant differences between psoriatic patients and control group in the level of the VEGF.

Because of this discrepancy, they believed that VEGF serum levels could not be a useful monitor of psoriasis activity and/or treatment response that disagree with our present study.

The precise role of VEGF in the evolution of psoriatic lesions is to promote vascular permeability that enhances leucocyte traffic into the dermis of psoriatic lesions, induces capillary dilatation that help to nourish the hyperplasic epidermis (Ellis and Kreuger,2009) . The previous findings suggest that VEGF is likely a key factor in the link between inflammation and angiogenesis in psoriasis (Simonetti *et al*.2006)

When compared between the patients group before treatment and after (one and three months) from administration of MTX (Table4.5), that was a decrease in the level of the VEGF. This result was consist with the result of Flisiak and his coworker (2012), who found that there was decrease in the VEGF during treatment. Methotrexate first used for its effects on keratinocytes such as inducing cellular differentiation in vitro. Low dose methotrexate also exerts anti-proliferative effects on endothelial cells. It later suggested that the efficacy of low dose methotrexate resided in its anti-inflammatory action on T lymphocytes. Indeed, methotrexate inhibits the expression of adhesion molecules by both T lymphocytes and endothelial cells .It demonstrates that although angiogenesis by itself is important, the expression of adhesion molecules by endothelial cells is a crucial step in the recruitment of leukocytes. Therefore the perpetuation of the skin inflammation (Yazici *et al*. 2005 and Stinco *et al*.2007).

Reduced glutathione:

Skin is a major target of oxidative stress due to reactive oxygen species (ROS) that originate in the environment and in the skin itself. Reactive oxygen species generated during normal metabolism and are an integral part of normal cellular function, and are usually of little harm because of intracellular mechanisms that reduce their damaging effects. Antioxidants attenuate the damaging effects of ROS and can impair and/or reverse many of the events that contribute to epidermal toxicity and disease. However, increased or pro-longed free radical action can overcome ROS defense mechanisms, contributing to the development of cutaneous diseases and disorder (Trouba *et al* .2002). As regard to antioxidant status, which represented in the present study by reduced glutathione, which is the master antioxidant in the body. There was a significant decrease in the levels of GSH in psoriatic patients (male and female) when compared to healthy controls group (male and female) table (6). A similar finding has reported by Bariaa Ali Makii AL- Jubori (2010)in Babylon University /College of Medicine in Hilla city whose findings were decrease of glutathione level in sixty psoriasis patients when compared with control group. The decrease in the levels of this non-enzymatic antioxidant parameter may be due to the increased turnover, for preventing oxidative damage in these patients suggesting an increased defense against oxidant damage in psoriasis. Similar reports of decreased GSH has been reported in psoriatic patient (Leveque *et al*.2004).

In follow up of this study and during treatment period with methotrexate, there was, significant increase of gultathione level frome basline (betore treatment) compared to the first and third month from treatment with MTX in psoriasis patients (male and female) (Table.7). Similar results were reported by Saadia Saleh Mehdy Al-zeiny (2012), who found significant increase in the level of GSH in the rats group that treated with MTX. Nevertheless, disagree with Madhyastha *et al.,* (2008), that approved decrease in GSH level after MTX administration.

Conclusions:

1- The vascular endothelial growth factor have play important role in the evaluating of psoriasis severity, oral methotrexate therapy have efficacy upon serum levels of vascular endothelial growth factor.
2-Efficacy of oral methotrexate therapy on psoriatic patient's treatment, so have faster remission of psoriatic lesions and this reflect the immune-modulatory anti-inflammatory role of The MTX in psoriasis.
3- Low level of serum GSH before treatment suggest that psoriasis may be an oxidative stress condition.

Recommendations:

1- Further follow up studies to psoriatic patients treated vitamin A derived like Neotigasone and study their effects on vascular endothelial growth factor and lipid profile.
2- Study the effect of methotrexate on liver enzymes in psoriatic patient treatment with low dose of MTX.
3- Study the effects systemic treatment of on antioxidants in patients with psoriasis.
4- Folic acid is an important supplementation in psoriasis patient has treated with MTX, so it is important to assess folic acid levels in those patients.

Acknowledgement:

The authors are too gratefull and thankfull to psoriatic patient and healthy control for their help to complete our research, hopping a quick recovery for patient.

References:

Bariaa Ali Makii AL-Jubori (2010). Oxidants and antioxidants status in psoriasis. Babylon University, College of Medicine.

Bertino, J. R. (2000). Methotrexate: historical aspects. In Cronstein BN, Bertino JR. Methotrexate. Basel: Birkhäuser.

Coimbra S., Oliveira H., Reis F.(2010). Interleukin (IL)-22, IL-17, IL-23, IL-8, vascular endothelial growth factor and tumour necrosis factor-α levels in patients with psoriasis before, during and after psoralen-ultraviolet A and narrowband ultraviolet B therapy. Br J Dermatol; 163: 1282-1290.

Coimbra, s.; Oliverira, H.; Reis, F.; Belo, L. and Rocha, S. (2010). Interleukin (IL)-22, IL- 17. IL-23, IL-8 vascular endothelial growth factor and tumor necrosis factor-alpha level in patient before, during and after ultraviolet A and narrowband ultraviolet B therapy. Br J Dermatol; 163: 1282-1290.

Cross CE, Halliwell B, Borish ET. Oxygen radicals and human disease(proceedings of a conference). Ann Intern Med.1987;107:526-545.

Danial, W. (1999). Probability and t distribution Biostatistics, 7th ed. A foundation for analysis in the health sciences. 83-123.

Di Nuzzo S.; Feliciani C., Cortelazzi C., Fabrizi G. and Pagliarello C. (2014). Immunopathogenesis of Psoriasis: Emphasis on the Role of Th17 Cells. Internatio. Tre. Immun.; 2 (3): 111-115.

Elghandour, T.; El Sayed M., Sahar, M. and Abdel Moneim M (2013). Effect of Narrow Band Ultraviolet B Therapy versus Methotrexate on serum Level of Interleukin-17 and Interleukin-23 in Egyptian Patients with Sever Psoriasis. Br J Dermatil; 27(7): 564-576.

Flisiak I, Zaniewski P, Rogalska-Taranta M & Chodynicka B (2012). Effect of psoriasis therapy on VEGF and its soluble receptors serum concentrations. Journal of the European Academy of Dermatology and Venereology; 26: 302-307.

Flytström I, Stenberg B, Svensson A & Bergbrant IM (2008). Methotrexate vs. cyclosporine in psoriasis: effectiveness, quality of life and safety. A randomized con- trolled trial. Br J Dermatol; 158: 116-121.

Gupta, S.K., Dogra, A. & Kaur, G. (2005). Comparative efficacy of methotrexate and hydroxyurea in treatment of psoriasis. Journal of Pakistan Association of dermatologists; 15: 247-251.

Haider Abd Jabbar Alammar (2015). The impact of methotrexate on some serum adipokines and oxidized low-density lipoprotein in patients with vulgaris psoriasis. Baghdad University, College of Medicine.

Herron MD, Hinckley M, Hoffman MS, Papenfuss J, Hansen CB, Callis KP & Krueger GG (2005). Impact of obesity and smoking on psoriasis presentation and management. Arch Dermatol; 141: 1527-34.

James, W. D., Berger, T. G., & Elston, D. M. (2011). Seborrheic dermatitis, psoriasis, recalcitrant palmoplantar eruptions, pustular dermatitis, and erythroderma. In R. Gabbedy, & S. Pinczewski (Eds.), Andrews' Diseases of the Skin Clinical Dermatology (11th ed., pp. 190-199). : Saunders Elsevier.

Kidd PM. Natural antioxidants-first line of defense. PMK Biomedical Nutritional Consulting.1991;115-142.

Kreuger, G. & Ellis, C. N. (July, 2005). Psoriasis-recent advance in understanding its pathogenesis and treatment. Journal of the American Academy of Dermatology, July .53 (1), Pp 94- 100.

Krueger G.(2000)Two consideration for patients with psoriasis and their clinicians :what define mild ,moderate ,and sever psoriasis .What constitutes a clinical significant improvement when treatment psoriasis ? Am Acad Dermatal .43:281-285.

Leveque N, Robin S, Muret P, Mac-Mary S, Makki S, Berthelot A, Kantelip JP & Humbert P (2004). In vivo assessment of iron and ascorbic acid in psoriatic dermis. Acta Derm Venereol; 84(1): 2-5.

Leveque N, Robin S, Muret P, Mac-Mary S, Makki S, Berthelot A, Kantelip JP & Humbert P (2004). In vivo assessment of iron and ascorbic acid in psoriatic dermis. Acta Derm Venereol; 84(1): 2-5.

Luo J, Wu SJ, Lacy ER. (2010). Structural basis for the dual recognition of IL-12 and IL-23 by ustekinumab. J Mol Biol; 402: 797-812.

Madhyastha, S.; Prabhu, L. V.; Saralaya, V. & Rajalakshmi R. (2008). A comparison of vitamin A and leucovorin for the prevention of methotrexate-induced micronuclei production in rat bone marrow. Clinics (63) N.6.

Nilgon Solak Tekin, Ishak Ozal Tekin, Figen Barut & Sipahi EY (2007). Accumulation of oxidized low-density lipoprotein in psoriatic skin and changes of plasma lipid levels in psoriatic patients. Hindawi Publishing Corporation, Mediator of Inflammation; Article ID 78454, 5 pages.

Nofal A, Al-Makhzangy I, Attwa E, Nassar A & Abdalmoati A (2009). Vascular endothelial growth factor in psoriasis: an indicator of disease severity and control. J Eur Acad Dermatol Venereol 23: 803-806.

Parisi, R.; Symmons, D.P.M.; Griffiths, C.E.M. and Ashcroft, D.M. (2013). The Identification and Management of Psoriasis and Associated Comorbidity project team. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. J. Investigat. Dermatol.; 133 (2): 377-385.

Risau W, Drexler H, Mironov V(1992). Platelet-derived growth factor is angiogenic in vivo. Growth Factors; 7:261–266.

Rocha-Pereira P, Santos-Silva A, Rebelo I, Figueiredo A, Quintanilha A, Teixeira F. The inflammatory response in mild and in severe psoriasis. Br J Dermatol 2004; 150 : 917-28.

Saadia Saleh Mehdy Al-zeiny (2012). The role of antioxidant (vit-A and glutamine) in ameliorating methotrexate induced hepatic toxicity in rats. Kufa Journal For Veterinary Medical Sciences Vol. (3) No. (1).

Scarpa, R., Altomare, G., Marchesoni, A., Balato, N., Matucci Cerinic, M., Lotti, T., Olivieri, I., Vena, G. A., Salvarani, C., Valesini, G. & Giannetti, A. (2010). Psoriatic disease: concepts and implications. J Eur Acad Dermatol Venereol Vol. 24, No. 6, pp. 627-630, ISSN. 1468-3083.

Schon M. and Boehncke W. (2005). Psoriasis. N Engl J Med; 352(18): 1899-1912.

Sci Monit 2007; 13 : CR30-3.

Setty AR, Curhan G & Choi HK (2007). Obesity, waist circumference, weight change, and the risk of psoriasis in women: nurses' health study II. Arch Intern Med; 167: 1670-5.

Shimauchi, T; Hirakawa, S.; Suzuki, T.; Yasuma, A.; Majima, Y.; Tatsuno, K.; Yagi, H.; Ito, T. and Tokura, Y. (2013). Serum interleukin-22 and vascular endothelial growth factor serve as sensitive biomarkers but not as predictors of therapeutic response to biologics in patients with psoriasis .J Dermatol; 40: 805-812.

Simonetti O, Lucarini G, Goteri G, Zizzi A, Biagini G, Lo Muzio L, Offidani (2006) VEGF is likely a key factor in the link between inflammation and angiogenesis in psoriasis: results of an immunohistochemical study. International Journal of Immunopathology and Pharmacology, 19(4): 751-760.

Simonetti O, Lucarini G, Goteri G, Zizzi A, Biagini G, Lo Muzio L & Offidani (2006). VEGF is likely a key factor in the link between inflammation and angiogenesis in psoriasis: results of an immunohistochemical study. Int J Immunopathol Pharmacol; 19: 751-760.

Stinco G, Lautieri S, Valent F & Patrone P (2007). Cutaneous vascular alterations in psoriatic patients treated with cyclosporine. Acta Derm Venereol; 87: 152-154.

Sua´rez-Farin˜as M, Shah KR, Haider AS, Krueger JG & Lowes MA (2010). Personalized medicine in psoriasis: developing a genomic classifier to predict histological response to Alefacept. BMC Dermatol; 10: 1.

Takahashi, H.; Tsuji, H.; Hashimoto, Y.; Ishida-Yamamoto, A & Iizuka, H. (2010). Serum cytokine and growth factor level in Japanese patients with psoriasis. Clin Exp Dermatol; 35: 645-649.

Thomas, J. A. and Aithal, G. P. (2005). Monitoring liver function during methotrexate therapy for psoriasis: are routine biopsies necessary. Am. J. Clin. Dermatol., 6 (6): 357-363.

Trouba KJ, Hamadeh HK, Amin RP & Germolec DR (2002). Oxidative Stress and Psoriasis. Antioxid Redox Signal; 4 (4): 665-73.

Wozniak A, Drewa G, Krzyzynska-Maliniowska E, Czajkowski R, Protas-Drozdf, Mila-Kierzenkowska C,. Oxidant-antioxidant balance in patients with psoriasis. Med

Yazici AC, Tursen U, Apa DD, Ikizoglu G, Api H, Baz K & Tasdelen B (2005). The changes in expression of ICAM-3, Ki-67, PCNA, and CD31 in psoriatic lesions before and after methotrexate treatment. Arch Dermatol Res; 297: 249-255.

Yeilding, N., Szapary, P., Brodmerkel, C., Benson, J., Plotnick, M., Zhou, H., Guzzo, C. (2011). Development of the IL-12/23 antagonist ustekinumab in psoriasis: past, present, and future perspectives - an update. Annals of the New York Academy of Sciences, 1222(1), 30–39.

Zhou Q, Mrowietz U, Rostami-Yazdi M. Oxidative stress in the pathogenesis of psoriasis. Free Radic Biol Med 2009; 47 : 891-905.