Original paper

Oxidative stress markers (MDA,SOD&GSH) and Proinflammatory Cytokine (interleukine-18)in Iraqi patients with Psoriasis vulgaris

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Abstract

Background: Psoriasis is a common chronic inflammatory skin disease with an unknown etiology. Psoriasis has been characterized by hyperproliferation accompanied by acanthosis andaberrant differentiation of keratinocytes. Several factors and cytokines, are assumed to be important. Recent studies indicate that various cytokines including tumor necrosis factor – α ,Interleukine-2R and Interleukine- 6 play an essential role in the induction and maintenance of psoriatic lesion.

Objectives: To evaluate oxidative stress markers (Malondialdehyde (MDA),Super oxide dismutase(SOD) & Glutathion (GSH)) and proinflammatory mediators Interleukine-18 (IL-18) in the sera of patients with active psoriasis (Psoriasis vulgaris) of mild-to-moderate and severe psoriasis compared to healthy controls,and to study correlation of the above markers with severity of psoriasis.

Subjects & Methods: one hundred and teen (110) psoriasis patients were recruited from the dermatology Outpatient clinicin Murjan Hospital in (Babylon city)during the period from November 2011 to March 2013.Fasting serum samples were obtained on enrolment. All the patientsdid not receive any treatment (locally or systemically), for at least 20 days before enrolment. Age& sex matched with fifty five(55) healthy controls were also recruited. Serum IL-18 level were estimated using an Enzyme-Linked Immunosorbant Assay (ELISA) technique. The patients group were subdivided to three groups according to the disease severity, into mild psoriasis group , moderate psoriasis group and severe psoriasis group. Serum MDA levels were assessed using thiobarbituric acid (TBA) method of Buege and Aust SOD and GSH was measured by Burtis and Ashwood, SOD levels using modified photochemical Nitroblue Tetrazolium (NBT) method utilizing sodium cyanide as peroxidase inhibitor.

Results & Discussion: Serum IL-18 shows statistically significant elevation in patients group compared to healthy controls($p< 0.05$). Levels of MDA were significantly increased ($p<0.001$) where as the GSH and SOD were significantly decreased ($p<0.001$) in patients with psoriasis compared to healthy control.Also they were all statistically significant increased in serum levels of IL-18 and MDA while a significant decreased in serum levels of SOD and GSH in patients with severe psoriasis compared to these with mild-to moderate psoriasis ($p <0.05$).

Conclusions: These data support the view that serum IL-18 , MDA, SOD and GSH areinvolved in the pathogenesis of psoriasis, possibly by induction and maintenance of psoriatic lesion. Its recommend a use of cytokine (IL-18) as a useful follow-up marker for monitoring of psoriatic patients and optimizing therapeutic strategies.

Keywords: Psoriasis vulgaris, Cytokines, IL-18 and oxidative stress.

Introduction

Psoriasis is relatively common, chronic, inflammatory and hyper proliferative skin disease that may appear at any age and affect any part of the skin. It affects 1.4 % to 2.0 % of the population and comprises 2.6% of skin related visits to primary care physicians, or between 0.3% and 1.6% of

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all visits to family physicians. It is a very troublesome disease with a high economic impact [1]. The disease often persists for life, and the patient has an increased risk of cardiovascular diseases and their complications. One out of five patients develops psoriatic arthritis. The clinical picture of psoriasis is highly variable with regard to lesional characteristics and the severity of disease [2].Psoriasis vulgaris is a multifactorial heritable disease character-ized by severe inflammation resulting in poorly differentiated, hyperproliferative keratinocytes. It is including genetic background, environmental factors, and vascular and immune system disturbances. Current research is dominated by the hypothesis that an immunological disorder with inflammatory reaction, mediated through T-lymphocytes, plays a key role in the pathogenesis of psoriasis [3]. The characteristic histological features of the disease are epidermal hyperproliferation and infiltration of both dermis and epidermis by inflammatory cells including neutrophils, lymphocytes, macrophages and mast cells. Interactions between infiltrating T cells and skin resident cells (keratinocytes, fibroblasts, endothelial cells) are often mediated by the synthesis and release of different proinflammatory cytokines [4].Recently, much attention has been directed towards the influence of cytokines in psoriasis, as they play an important role in inflammatory diseases. In addition, a number of studies have suggested that various cytokines released by keratinocytes and inflammatory leucocytes could contribute to the induction or persistence of the inflammatory processes in psoriasis; however, the precise mechanism of their involvement in psoriasis remains unclear. Few studies have been reported on serum cytokine levels that may be expected to alter if they are involved in the pathogenesis of psoriasis [5].

At the present time, one ofthe main areas of research in the psoriasis field concernsthe role of cytokines in the pathogenesis of this disease.Different cytokines play a part in sustaining the twomain characteristics of a psoriatic lesion; keratinocyte hyperproliferation and inflammation [6].Rocha-Perieraet al[7].have shown an association of psoriasis with inflammation, as indicated by higher levels of the inflammatory markers such as C-reactive protein (CRP), haptoglobin, fibrinogen, and C3 and C4, which increase with severity of disease. Those authors proposed that haptoglobin and CRP can be markers of psoriasis. It has also been proposed that the preferential association between dendritic cells (DCs) and psoriatic epidermal CD4+ T cells may lead to the stimulation and subsequent clonal expansion of epidermal CD8+ T cells. Along with CD4+ T cells and DCs, CD8+ T cells are key players in the production of pro-inflammatory cytokines that have been implicated in psoriasis. An extensive cytokine network including TNF-, interferon (IFN)-, and interleukin (IL)-12, IL-23 and IL-15 generated by activated DCs and T cells mediates the formation of psoriatic lesions.[8,9]. IFN- plays a key role in the stimulation and proliferation of T cells, and in the formation of psoriatic skin.[10]. IL-12 and IL-23 trigger T-helper cell activation and associated downstream responses within the type 1 pathway in psoriasis.[11]. IL-23 activates T-helper cells that subsequently produce IL-17 and IL-22,[12].and IL-15 is a pro-inflammatory cytokine that induces T-cell proliferation and skin hyperplasia.[8]. The presence of high levels of TNF-, IFN-, IL-2, IL-6, IL-8, IL-12 and leukaemia inhibitory factor (LIF)-1 and reduced levels of ofIL-1, IL-4, IL-5 and IL-10 in psoriatic skin lesions suggests that psoriasis is a type 1 immune-response disease[13].These immune-response parameters can be used as markers in the severity and management of the disease after further in-depth studies. Flisiaket al.[14].have confirmed an association between plasma IL-18 concentration and psoriasis severity, and have shown that combined measurement

of IL-18 and TGF- β1 in plasma can be considered as a possible biomarker of psoriasis activity. Another study has reported a significant correlation between the extent of skin lesions, Psoriasis Area Severity Index (PASI), and IL-18 levels in plasma of patients with psoriasis.[15].Some of the cytokines involved in pathogenic phenomena in psoriasis are known to be inducers of the acute-phase response. Of the large group of acute-phase reactants, CRP and fibrinogen may be of special interest in psoriasis, given their relationship with inflammatory cytokines involved in the development of skin inflammation.[16].

Malondialdehyde (MDA), is a marker of oxidative stress and specific enzymes that limit free-radical formation, such as glutathione peroxidase (GPX), superoxide dismutase (SOD) play an important role in the protection of cell membranes against oxidative damage and may be used as indicators of anti-oxidative status. There are several studies investigating the role of oxidant /antioxidant systems in the pathogenesis of psoriasis with discordant results [17,18].

Hence, this study was carried out to evaluate the oxidative stress markers (MDA,SOD&GSH) and Proinflammatory Cytokine (interleukine-18) in patients with Psoriasis vulgaris.

Material and Methods

Subjects

This study comprised one hundered sixteen consecutive patients ofpsoriasis were recruited from the dermatology outpatient clinic in Murjan Hospital in (Babylon city) during the period from November 2011 to March 2013.All thepatients were subjected to detailed examinationincluding the elicitation of dermatological andpsychiatric complaints. The diagnosis was madeclinically, based on the presence of characteristicplaque-type psoriatic lesions. All the patients wereasked to provide socio-demographic data, medicalhistory, and family history. Other questions included theduration of disease, age of onset of the disease, anytreatment taken and use of psycho-tropic drugs. Dermatological examination, hairs, mucosal involvement and nail changes were recorded. The patients group were subclassified to two groups

according to the diseases severity, severity index (PASI) into, mild-to-moderate psoriasis group andsevere psoriasis group. Fifty five healthy age and sexmatched volunteers with no family history of psoriasiswere included in the study as a control group. Thepurpose and nature of the study were explained to allsubjects. All included subjects have consented to beenrolled in this study.

Exclusion Criteria

liver disease, renal disease, recent history of cardiovascular disorder, hypertension, neurological disease, or diabetes mellitus,obese subjects with history of acute or chronicinfections, were excluded from thestudy. Moreover, patients who had received oral ortopical antipsoriatic therapy within one month were notincluded in the study.

Blood Sampling

Blood samples (10 ml) were collected frompatients and control subjects in serum separatorvacutainers (BDV acutainer Systems, Plymouth, UK). Sera were separated and immediately stored at − 20° Cuntil analysis.

Serum Cytokine Measurement

The quantitative determination of IL-18 level was conducted by an Enzyme-Linked Immunosorbant Assay (ELISA) technique, using a commercial available kit, RayBio_ Human IL-18 ELISA,Every sample was run induplicate, measurements differed by less than 10 %, andthe mean value was calculated and used for statisticalanalysis.

Serum Oxidative Stress Measurement

Serum levels were assessed for MDA using thiobarbituric acid (TBA) method of Buege and Aust [19], SOD and GSH was measured by Burtis and Ashwood [20], SOD levels using modified photochemical

Nitroblue Tetrazolium (NBT) method utilizing sodium cyanide as peroxidase inhibitor [21].

Statistical analysis

All data were coded and entered using the program statistical package for social sciences (SPSS) version 12 under windows XP. Descriptive data was summarized using mean, standard error (SE). Linear regression analysis was done to test for significant predictors for psoriasis severity as measured by PASI score. P values < 0.05 were considered statistically significant.

Results

Serum Oxidative stress markers (MDA, SOD & GSH), and Cytokine profile (IL-18) levels were estimated in 110 patients with Psoriasis patients, (50 sever psoriasis & 60 mild to moderate psoriasis) compared with 55 healthy control group, age and sex matched.

As expected, the patients had significantly higher level of IL-18 levels than the healthy controls, and a significant difference within psoriasis patients, as shown in figure 1.

The concentrations of serum level MDA, are presented in Table (1). Total Lipid peroxidation MDA are significantly higher in psoriasis patients as compared with normal subjects. As shown in figure 2, while a significant decrease in SOD and GSH in psoriasis patientsas compared with normal subjects,a significant difference was found within psoriasis patients. see figure 3 & 4.

The level of IL-18 and Oxidative stress (MDA, SOD & GSH) in normal healthy subjects and psoriasis subjects was depicted in Table 1.

Table1. The Anthropometric and biochemical variables among the three studied groups.

Parameters	Control	Mild to Moderate Psoriasis	Severe Psoriasis	P(ANOVA)-(T-Test)
NO.	**55**	**60**	**50**	……
IL-18(pg/ml)	**30.88±16.55**	**174.22±79.68**	**389.88 ± 170.16**	sever x mild-moderate: p< 0.01 psoriasis x C: P<0.0001
SOD(U/ml)	**6.85 ± 0.9**	**5.66±0.39**	**2.86±0.15**	sever x mild-moderate: p< 0.01 psoriasis x C: P<0.0001
MDA(µmol/l)	**1.32±0.55**	**2.73±0.67**	**5.58 ± 0.17**	sever x mild-moderate: p< 0.01 psoriasis x C: P<0.0001
GSH(µmol/l)	**3.38±0.61**	**2.57±0.19**	**1.29±0.82)**	sever x mild-moderate: p< 0.01 psoriasis x C: P<0.0001

Values are Mean ± SEM,X=VS.

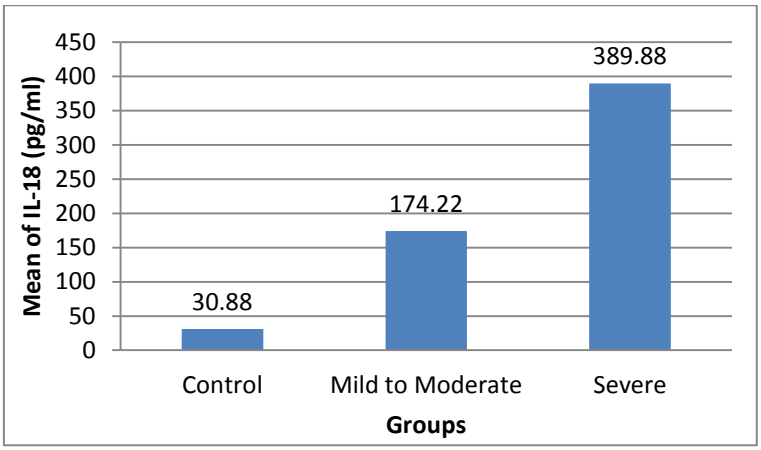

Figure 1. Serum levels of IL - 18 in patients with psoriasis vulgaris compared to healthy controls

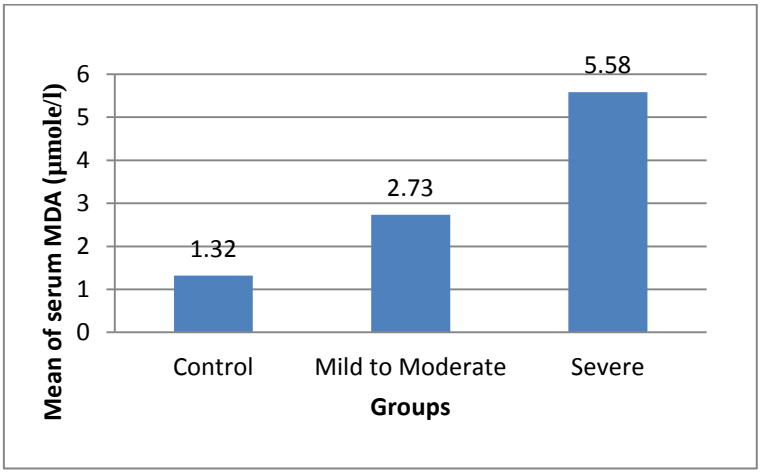

Figure 2. Serum levels of MDA in patients with psoriasis vulgaris compared to healthy controls

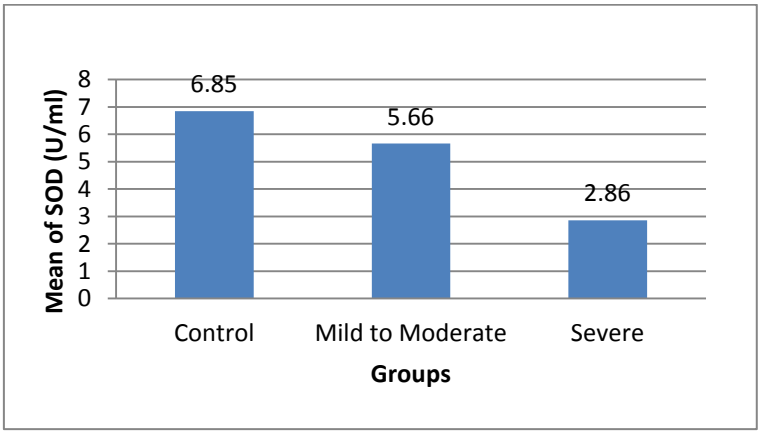

Figure 3. Serum levels of SOD in patients with psoriasis vulgaris compared to healthy controls

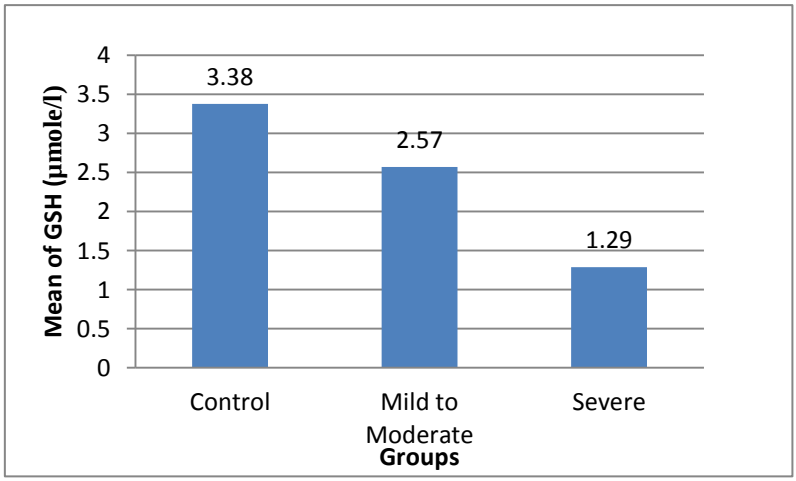

Figure 4. Serum levels of GSH in patients with psoriasis vulgaris compared to healthy controls

Discussion

In this study we focusedon the impact of serum levels of cytokine (IL – 18, MDA, SOD & GSH) in psoriasisvulgaris In Iraqi patients which are of major clinical relevance to the clinician. The results of this study shows an increment in the investigated Cytokine(IL-18), showed a significant increase in severe psoriasis than in mild-to-moderate ones which are notin agreement to the results obtained by(Vera M R Heydendael et al. in 2004) who found that there is no correlation between psoriasis severity assessed by PASI (Psoriasis Area and Severity Index) and levels of these mediators.this result is in agreement with studies demonstrated by previous report [22].found that serum levels of tumour necrosis factor (TNF)-alpha, interferon (IFN) -gamma, interleukin IL-2, IL - 6, IL - 7, IL - 8, IL - 12, IL - 17, IL - 18 and vascular endothelial growth factor (VEGF) were significantly increased in patients with psoriasis compared with those of healthy controls. And, increased serum levels of these cytokines were correlated with PASI. Furthermore, these cytokine levels were decreased after psoriasis treatment.

This study indicates an increase in the level of MDA (Table 1) in psoriatic patients as compared to healthy controls, which is in correlation with the studies of Gornicki A,[23] Rocha Pereira P et al [24] and Relhan V et al [25]. However, Yildrium et al [26] did not find any correlation in the levels of MDA in patients of psoriasis with that of controls. Increased production of free radicals may cause oxidative damage on biological biomolecules, cell membranes and tissues. The free radicals induced oxidation of polyunsaturated fatty acids results in the formation of lipid per-oxidation products such as MDA.

This study reveals a decrease in the levels of antioxidant enzyme SOD (Table 1). This is in concordance with the studies of Yildrium [27], Pujari [28], Kural [29], Drewa [30], and Kobayashi [31]. However, Utas [32] and Baz et al [18] found an elevated level of the antioxidant enzyme plasma SOD patients of psoriasis. In this study, the decrease in the levels of antioxidant SOD in patients of psoriasis is probably to counter act the stress caused by oxidation.

Cells contain enzymes GPx which change the hydroperoxide group to the much less toxic hydroxylmoiety., Kokcam [33] observed that GSH was found to be significantly decreased in patients with psoriasis as compared with the values from sex-age matched healthy controls this result Similar to the result of GSH in this study as shown in Table 1.

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