Inverse correlation between glutathione peroxidase activity in psoriatic cutaneous lesions and the severity of the disease

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Background: Psoriasis is a chronic-relapsing inflammatory skin disorder, in whose pathogenesis oxidative stress is suggested to be involved. Among different enzymes that play a role in maintaining the cellular redox balance, we aimed to assess the alteration of glutathione peroxidase (GPX) activity in cutaneous lesions and its correlation with the disease severity, firstly, to support the possible candidacy of this enzyme for future topical therapeutic regimens, and secondly, to move forward in understanding the etiology of the disease and the pathogenic mechanisms involved in cutaneous lesions so as to pave the way for further investigations.

Methods: The clinical severity of disease was determined according to Psoriasis Area and Severity Index (PASI) scoring system. The level of GPX activity in the skin biopsies from 20 psoriatic patients was measured using Cayman's glutathione peroxidase assay kit, and its association with disease severity was assessed in each patient.

Results: Tissue GPX activity was significantly higher in patients with mild psoriasis (149.02 ± 24.213 nmol/min/ml) compared to patients with moderate psoriasis (120.58±21.038 nmol/min/ml) (p-value < 0.05). There was a significant negative correlation between the activity of GPX and each PASI-associated criterion, including redness, scaling and thickness. Among all the criteria of PASI, scaling was independently correlated with the activity of GPX (p-value < 0.05).

Conclusion: The reduced activity of GPX in dermal lesions might be associated with the disease pathogenesis, having a valuable role in diagnosis and therapy.

Keywords: glutathione peroxidase, oxidative stress, psoriasis

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INTRODUCTION

Skin is an anatomical barrier performing a wide range of functions, including the regulation of body temperature, and preventing the loss of body fluids, and endocrinal and immunological roles [1,2]. Therefore, cutaneous diseases can adversely affect the quality of life. Psoriasis is a chronic-relapsing inflammatory cutaneous disorder, estimated to impact 0.2-2% of the population [3]. It is characterized by increased erythematous papules and rounded plaques, covered by silvery scales [4]. There is no agreement on the prevalence of psoriasis among men and women [5]. However, it has been evidenced that genetic and environmental factors such as drugs, trauma, infections, smoking and physical and psychological stresses are associated with the initiation of the disease [6]. However, the exact etiology of psoriasis is unclear, while, recently, oxidant-antioxidant imbalance has been suggested

as a triggering mechanism [7].

The imbalance between oxidants and antioxidants in favor of the former is defined as oxidative stress, caused by an insufficient antioxidant system and/or excessive generation of free radicals, particularly reactive oxygen species (ROS) [8]. ROS including molecular oxygen (O_2), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) are formed via endogenous mechanisms and exogenous stimuli such as ionizing radiation, alcohol consumption, injuries and inflammation [9]. The overproduction of ROS ensues lipid peroxidation, protein oxidation, DNA mutation/breakage and enzyme inactivation/activation along with the release of pro-inflammatory cytokines, all conducing to cell damage, and cardiovascular, neurodegenerative disorders, and autoimmune diseases [10-16].

To overcome oxidative stress, the body is equipped with antioxidant defense mechanisms generating non-enzymatic and enzymatic antioxidants such as thioredoxin, superoxide dismutase, catalase, and glutathione peroxidase (GPX) [17,18]. GPX is an important antioxidant enzyme in charge of catalyzing the reduction of lipid peroxides at the expense of reduced glutathione, hence protecting the body against oxidative stress. Moreover, some authors believe that GPX, in addition to being a catalase, has a basic role in degrading H_2O_2 to water, thereby significantly regulating cellular redox responses [19,20]. A low GPX serum level is reported to be correlated with a number of ROS-related diseases such as neurodegenerative diseases, cardiovascular disorders, type 2 diabetes, vitiligo, and so forth [21-24].

In the case of psoriasis, several researchers have evaluated the GPX activity of red blood cells (RBCs) in patients; however, as far as the authors of the present research are concerned, GPX activity has not been assessed in psoriatic skin lesions. Assessing GPX activity in skin lesions might conduce to shedding light on the underlying processes of skin, all of which result in presenting dermal lesions. As there exist limited data on GPX activity in psoriatic dermal lesions, we aimed to investigate the possible alteration of GPX activity in lesions and assess its correlation with disease severity and each of PASI criteria, firstly, to support the possible candidacy of this enzyme to be targeted in future therapeutic regimens, particularly topical ones, and secondly, to move forward in understanding the etiology of the diseases in order to pave the way for further investigations.

PARTICIPANTS AND METHODS

Study population

This cross-sectional study was carried out on 20 patients referred to the dermatology clinics of Imam Reza Hospital of Mashhad, between 23 September 2013 and 23 September 2014, who met the diagnostic criteria for psoriasis. Participants with a history of receiving antioxidant supplements, anti-inflammatory compounds and immunosuppressive drugs, and those with any systemic disorders were excluded.

Ethical Statement and Sample Preparation

Ethical approval was obtained from the Ethics Committee of the Research Council of Mashhad University of Medical Sciences, and adult participants and the parents or legal guardians of minors signed an informed consent; after that, skin samples were taken from skin lesions in a scale of 4 mm under sterile condition. These samples were divided into two parts by a scalpel; one part was utilized by the department of pathology to confirm the diagnosis of psoriasis, and the other was washed in PBS (phosphate buffer saline), pH 7.4, in order to remove red blood cells, and was homogenized in Tris-Hcl buffer (5 ml buffer per gram tissue) containing 50 mM Tris-Hcl, pH 7, 5 mM EDTA and 1 mM DDT. Supernatants were frozen at -70°C until enzyme activity measurement.

GPX Measurement

Glutathione Peroxidase Assay Kit (Item No 703102) was utilized for the measurement of GPX activity according to the instructions from the manufacturer based on a previously described method [25]. In brief, glutathione peroxidase activity was measured indirectly via the oxidation of $NADPH_2$ to $NADP^+$, accompanied by the reduction of absorbance at 340 nm. This method is based on a coupled reaction including the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG) by glutathione peroxidase (GPX) and subsequent oxidation of $NADPH_2$ to $NADP^+$

to recycle the reduced glutathione (GSH) by glutathione reductase (GR).

Assessment of the disease severity

The severity of the disease was determined based on the PASI criterion and scoring PASI chart for each patient [26]. After specifying the PASI score for each patient, they were classified into three groups of mild (PASI < 10), moderate (10 < PASI < 20), and severe (PASI > 20).

Statistical Analysis

The data were analyzed by Statistical Package for Social Sciences (SPSS 16), and were expressed as mean ± standard deviation (SD). The assessment of the normality of data was performed using the Kolmogorov–Smirnov test. The correlation between variables was assessed by Pearson's model. To compare categorical parameters and continuous parameters, Chi-square tests and independent-samples t-test were used, respectively. Stepwise linear regression model was employed to evaluate the correlation between each PASI-associated criterion independently with the level of GPX. P value of <0.05 was considered to be statistically significant.

RESULTS

A total of 20 psoriatic patients (11 men and 9 women) were included in this study, with a mean age of 38.85±12.57 years. Descriptive characteristics of the study participants are summarized in Table 1.

Determination of the Disease Severity Based on Psoriasis Area and Severity Index Scoring System

On the basis of the PASI scoring system, the disease severity ranged from 2-19 in the studied subjects. Twelve participants showed mild disease, and 8 presented moderate disease, but none of the participants had severe psoriasis (Table 2).

Correlation Between GPX Activity and the Disease Severity

As shown in Figure1, the average level of GPX was 149.02 ± 24.213 nmol/min/ml in subjects with mild psoriasis, while it was 120.58±21.038 nmol/min/ml in subjects with moderate disease.

An independent-sample t-test was used to compare the correlation between tissue GPX activity and PASI score. A statistically significant reverse correlation was observed between PASI score and the level of GPX in psoriatic patients (P-value = 0.015), meaning that in patients with a higher PASI score, a lower level of GPX activity was observed.

Correlation between GPX Activity and Each PASI Associated Criteria

Further assessed was the correlation between the tissue level of GPX and PASI-associated criteria using Pearson correlation coefficient test. The Pearson's r was 0.008 (Table 3), showing a significant negative correlation between the level of GPX and PASI-associated criteria (redness, scaling and thickness). Moreover, Stepwise regression model was used to evaluate the relationship between each PASI-associated criterion independently with the

Table 2. The psoriasis severity in participants according to the psoriasis area and severity index scoring system

Psoriasis severity	N (%)	X^2	Significance
Mild (0-10)	12 (60)	0.800	0.371
Moderate (10-20)	8 (40)		
Severe (>20)	0		

Table 1. Descriptive characteristics of the participants

Criteria	Number	Minimum	Maximum	Mean	Standard deviation
Age	20	23.00	64.00	38.8500	12.5709
GPX (nmol/min/ml)	20	93.00	193.00	137.6400	26.5840
Redness score	20	0.80	7.20	3.1500	1.6408
Scaling score	20	0.60	5.40	3.1150	1.5380
Thickness	20	0.60	6.40	3.0450	1.6996
Area score	20	3.00	9.00	6.20	1.7650
PASI	20	2.00	19.00	9.3000	4.6123

GPX: glutathione peroxidase, PASI: Psoriasis Area and Severity Index

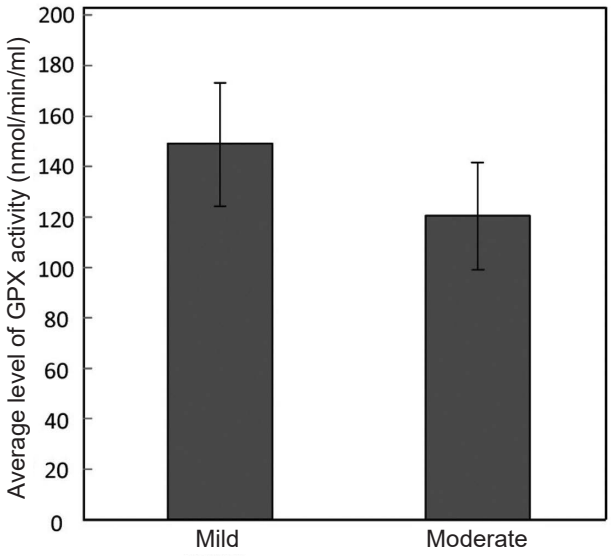

Figure 1. The average level of GPX in subjects with mild and moderate psoriasis. GPX activity was higher in patients with mild psoriasis compared with the moderate group (P = 0.015).

Table 3. The correlation between GPX activity and PASI-associated criteria

Variable	Level of GPX Activity	
	Coefficient correlation	Significance level
Redness	-0.575	0.008
Thickness	-0.572	0.008
Scaling	-0.688	0.008
PASI score	-0.648	0.002

PASI: Psoriasis area and severity index

level of GPX. It was observed that among all criteria of PASI, scaling had an independent correlation with the level of GPX as described in Table 4.

DISCUSSION

In this study, we assessed the level of GPX, an enzyme with a well-known antioxidant activity, in skin lesions of psoriasis patients with mild to moderate disease severity. In the case of psoriasis, several researchers have evaluated the GPX activity of red blood cells (RBCs) in patients; however, to the best of our knowledge, its activity in psoriatic dermal lesions has not been assessed. Our results showed that GPX activity in the psoriatic skin lesions was negatively correlated with the severity of the disease assessed by PASI. Further observed was a reverse correlation not only between GPX activity and PASI score, but also between the enzyme levels and each PASI criteria, including redness, scaling and thickness. This fact opens a new avenue for the underlying skin-pathogenic mechanisms involved in dermal lesions appearance, through showing that the increase in dermal GPX activity is correlated with a reduction in redness, scaling and thickness of the lesions. These findings are probably due to the reduction of the enzyme activity in oxidative stress, resulting in the oxidation of skin structural components and the loss of skin barrier's integrity.

Despite the remarkable progress in the understanding of the underlying pathogenic mechanisms of psoriasis, no definite cure has been reported yet. However, there are myriad therapeutic options that can be selected based on disease characteristics. Systemic therapies and phototherapy are commonly used for widespread or refractory psoriasis, while topical treatments such as corticosteroids, retinoids, and vitamin D analogues are effective in localized diseases [27,28]. Given that the preponderance of patients with psoriasis present mild-to-moderate severity of the disease, topical treatments are still the most frequently utilized approach [27,29]. Nevertheless, like other pharmacological therapies, side effects are of major concern. For instance, corticosteroids, as a widely used topical treatment, have shown some limitations due to side effects such as atrophy, traumatic purpura, perioral dermatitis, telangiectases, and hypertrichosis [27,30,31]. One approach to reducing the side effects is to accurately target the underlying fundamental changes. To that end, we decided to investigate the redox alteration in skin lesions so as to provide a better understanding of the basic changes responsible for the initiation or progression of lesions, to hopefully introduce a more valuable therapeutic target for future regimen.

Numerous studies have assessed the changes in oxidative stress markers such as malondialdehyde (MDA), and antioxidant scavengers including catalase (CAT), superoxide dismutase, and GPX in patients with psoriasis, underscoring the role of oxidative stress in the pathogenesis of

Table 4. Summary of linear stepwise regression analysis of independent correlation between GPX activity and scaling

Variable	Factor model	Standard error	Confidence interval	Significance level
Scaling	-11.886	2.958	(-18.100، -5.672)	0.001

the disease [7,32,33]. Although several studies have investigated GPX activity in the serum, erythrocytes and neutrophils of psoriasis patients [25,32-35], there exist limited data on GPX activity in the skin lesions of psoriasis patients.

GPX is known to be a pivotal antioxidant enzyme which protects the body against oxidative stress through degrading H2O2 to water, hence its crucial role in regulating cellular redox responses [19,20]. Several ROS-related diseases have been linked to low serum levels of GPX, such as neurodegenerative diseases, cardiovascular diseases, type 2 diabetes, and vitiligo [21-24]. Indeed, as mentioned earlier, several lines of evidence have reported depletion in GPX levels in psoriasis patients.

For instance, Kaur et al. noted that the levels of GPX in serum samples were significantly lower in psoriasis patients compared with healthy controls (p-value <0.001) [33]. Research has also found statistically significant decrease in the activity of erythrocyte and neutrophil GPX in psoriasis subjects [32,36]. Therefore, we deemed it logical to investigate GPX activity in the dermal lesions of psoriasis patients and assess any correlation between the enzyme activity and severity of the disease. Interestingly, an inverse correlation was seen between GPX activity and severity of psoriasis in dermal lesions of patients. Further observed was an inverse correlation between the level of GPX activity and each PASI criteria, including redness, scaling and thickness, signifying the involvement of GPX in the progression of lesions. GPX can therefore be recommended as a candidate target for future therapeutic regimens. Besides, it is worth noting that our obtained results presented an independent correlation between the scaling and activity of GPX. It is proven that ROS formation can be stimulated by epidermal growth factor [37,38]. In addition, ROS has been widely reported to be involved in the responses to cell growth and proliferation [38]. Studies have shown that GPx-1 diminishes cell proliferation through eliminating H2O2 which is necessary for certain signaling responses [39,40]. Therefore, inducing GPX activity in dermal lesions of psoriasis patients can possibly diminish the excess proliferation of cells and scaling. It is to be noted that studies have reported a significant correlation between the levels of GPX and the efficiency of treatment in psoriasis patients [34]. Disease improvement has also been observed in psoriasis patients taking a bioactive whey protein isolate powder (Immunocal) as a glutathione precursor [41]. Accordingly, targeting GPX activity in the dermal lesions of psoriasis patients might be a potential therapeutic strategy for increasing efficiency, particularly in mild-to-moderate psoriasis patients receiving topical treatment as the first-line therapy.

It can be concluded that the significant correlation between the enzyme activity and severity of the disease, and each PASI criteria in dermal lesions support its potential for eliminating the disease characteristics, namely thickness, redness, and scaling. The measurement of GPX activity in tissue samples might also assist in diagnosis as well as choosing an accurate therapeutic option. However, further studies with larger sample sizes might help explain the exact role of GPX in the etiology of the disease, and enable its use as a diagnostic factor and a therapeutic goal for a better disease management.

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Conflict of Interest: None declared.

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