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Impact of psoriasis disease and its treatment with Etanercept on serum and saliva Thiol- disulfide homeostasis

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Abstract

Psoriasis is a chronic inflammatory immune-mediated skin disease that causes red dry patches of thickened skin with reactive abnormal epidermal differentiation in which genetic and environmental factors have a significant role. Therapeutic agents either modulate the immune system, or normalize the differentiation program of psoriatic. Etanercept (ETN) is a new, safe and effective treatment for moderate to severe psoriasis. This study aimed to investigate the situation of thiol/disulfide homeostasis in saliva and serum of Iraqi psoriasiatic patients compared to that of healthy individuals, as well as study the impact of its treatment with Etanercept on this homeostasis in saliva and serum of these patients.

Keyword: Total thiol, native thiol, dynamic thiol, psoriasis, biological treatment, follow up;

1. Introduction

Psoriasis, is a common, chronic inflammatory with autoimmune aspects dermatitis skin disease with an important genetic component involvement in its pathogenesis [1, 2, 3]. It is as result of disorder in proliferation and differentiation of the keratinocytes and is characterized by involvement of a complex network of cells including dendritic cells, macrophages, T cells (Th-1 and Th-17 lymphocytes), in addition to different cytokines such as tumor necrosis factor (TNF)- alpha and interleukins (IL-23 and IL-17) [4, 5].The chronic inflammation in this disease is associated with keratinocytes excessive proliferation, In-complete differentiation, accompanied with reduced apoptosis and alteration of adaptive immune as well as innate immune responses which participate to the pathogenicity of this disease[6].Considerable pathogenesis of psoriasis extends to the psychosocial impact on the patient [7].Sometimes this disease develops into serious diseases like psoriatic arthritis, metabolic syndrome, cardiovascular diseases and mental illness [8, 9, 10, 11 and 12].Therefore psoriasis can be considered a multifactorial condition with complex pathogenesis [4, 5]. Throughout the last few years different chemicals such as aldehydes, phenols, hydrocarbons, nitric oxide (NO), Quinone and semi Quinone radical, which are directly or indirectly lead to the formation of free radical, are recognized to be present in the patients psoriasis [13]. And one of the remarkable features, which few recent researches have pointed to in psoriatic patients, is the involvement of oxidative stress (OS) in this disease pathogenesis [11, 14, 15]. The OS can be defined as in-balance between the oxidants and antioxidants present within the body which results in accumulation of free radicals in different forms of reactive species [16, 17]. Under normal physiological condition the presence of these species at low concentration is critical for the different body cells process like cell proliferation, differentiation, and

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apoptosis, While their presence in high concentration cause different pathological conditions as a result accumulation of various mutagen compounds, abnormal expression of oncogenes, as well as chronic inflammation that caused by the oxidative damage of the different cell biomolecules [18–20, 21]. Generally to prevent the occurrence of the oxidative stress the body has a defense system known as antioxidants which are of two classes: enzymatic and none enzymatic, these antioxidants are nucleophile molecules that interact with the present reactive species to counteract their toxic effects on the different bio-components and thus prevent cell damage [22, 23]. Among the non- enzymatic antioxidants is the thiols that represent very potent antioxidants through acting as electron donors thus reducing unstable free radicals [24].Thiol homeostasis is increasingly implicated in many disorders and psoriasis is one of them[14, 15, 23].Now-days, there has been a rapid rise in using thiol/disulfide measurements to evaluate the free radical status in an organism both at physiological and pathological conditions, therefore determination of this homeostasis can provide valuable information on various normal, or abnormal biochemical processes [25, 26, 27, 28]. Treatment of psoriasis is based on the disease severity and includes topical therapies for milder patients, phototherapy for mild to moderate disease, and oral systemic biological agents in patients with moderate to severe skin disease [29]. These therapeutic strategies can be used as immunotherapy in various combinations. Biological therapies have revolutionized the management of psoriasis and are suggested to be the better choice in the treatment of psoriasis [30]. Where anti-tumor necrosis factor therapy has showed efficiency in treating psoriatic skin lesions, joint pain and swelling, plus its ability to improve mobility, reduce radiographic progression of disease, and influence quality of life parameters [29].Among such treatment; Etanercept drug (ETN, its trade name is Enbrel) is the first TNF-α inhibitor to be approved by FAD for use in psoriasis. Chemically it is a dimeric, soluble fusion protein, with a molecular weight of 150 kDa, consisting of the extracellular ligand binding portion of the TNF receptor linked to the Fc portion of human immunoglobulin1 (IgG1) by 3 disulfide bonds. It is capable of binding and neutralizing soluble TNF and transmembrane TNF [7].Previous researches on the level of thiol in patients with psoriasis have reported discrepant results with clear disagreement [14, 31, 32, and 33]. Meantime no literatures have been found that investigate this homeostasis in saliva of psoriatic patients, or about the effect of ETN treatment on the different thiol homeostasis parameters in both serum and saliva of these patients. Therefore the present research did shield the light on these two important points where the measured thiol- disulfide homeostasis parameters included the levels of total thiol, native, dynamic thiol, and the ratios of dynamic thiol/ total thiol, native thiol/ total thiol and dynamic thiol/native thiol in sera and saliva of psoriatic Iraqi patients compared to healthy normal individuals, as well as follow up the effect of thebiological drug ETN on these thiol/disulfide homeostasis parameters in both type of fluids of Iraqi patients with psoriasis.

2. Materials

2.1. Materials

All chemicals used in this study were of analytical grade.

2.2. Participants Criteria.

A total of 30 of aprioristic patients, as well as 30 gender matched healthy individuals with about the same ages as the patients were enrolled in the present study. The patients were attending Baghdad Teaching/ Hospital. Department of Floral & Skin, and diagnosed by the specialists in the hospital. Blood and unstimulated saliva samples were collected twice from these patients, one of them after diagnosis and before receiving any treatment (pre-treated group), while the other was after two and a half month of starting the treatment with Enbrel (four doses of 50 mg injection/ week) (post-treated group). The detailed information of these participants is illustrated in (Table 1).

2.3. Exclusion criteria

Patients and controls individuals who had vitiligo, liver disease, active inflammatory conditions, chronic pancreatitis, chronic renal failure, chronic or acute inflammatory disease as well as those patients who were taking any type of drugs, and those who had a history of smoking, lipid-lowering therapy, or alcohol drinking were excluded. The study protocol conforms to the ethical guidelines, endorsed by the

College of Science, University of Baghdad ethics committee.

2.4. Serum & saliva collection

Blood and unstimulated saliva samples were collected from all studied groups; following overnight fasting. Before collected the unstimulated saliva, all participants were asked to rinse their months with saline and the collected saliva samples were centrifuged at (2400×g) for 15 minutes, then the supernatant was kept frozen to be used for the desired measurements. At the same time venous blood were withdrawn from the same individual in plain tubes and kept for 15 minutes at room temperature then centrifuged at (3000 x g) for 5 minutes. The sera samples were collected and kept frozen at (-20Cº) to be used for the determinations of the different parameters.

3. Methods

3.1. Determinationof Thiol-disulfide homeostasis in serum and saliva samples:

Erel and Neselioglu method was used for determination of, total thiol, native thiol, and dynamic thiol [6].To calculations of total and native thiol-disulfide as follow equation below.

$$\text{Total thiol and Natived thiol} = \frac{\text{AbsorbanceX D.FX10}^6\ (\mu\text{ M/L})}{\varepsilon}$$

Where:
d=1 cm, ε = Extinction coefficient (DTNB) = 14150 M^{-1} cm^{-1}, and D.F =Dilution factor=14

3.2. Determinationof disulphide level in serum and saliva samples:

The disulphide level (Dynamic thiol) is a value which can be calculated by following equation and as reported by Erel&Neselioglu [6].

$$\text{Dynamic thiol} = \frac{\text{total thiol} - \text{native thiol}}{2}$$

All statistical analysis was performed using SPSS program, version 25 under Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean ± SD of variables. The significance of difference between mean values was estimated by student paired samples T-test p-value < 0.05 was considered as significant and p-value < 0.01 was considered as a highly significant.

4. Result and desiccation

The mean value of age of the patients group and the healthy control group was as shown in Table 1. The demographic characteristic including gender, age and body mass index.

Table 1: The demographic characteristics of the aprioristic patients groups and the control group.

Characteristic	Pre-treated patients	Post-treated patients	Controls
Number (male: female)%	30 (52: 48) %	30 (52: 48) %	30 (51: 45)%
Age (year) Range Mean value ± SD	35-15 28 ± 2	35-15 28 ± 2	36-13 29 ± 8
Period of the treatment	------	two and half months	-----
Dose of treatment	-----	Receiving four doses of Enbrel injection (50 mg/week)	-----

4.1. Total thiol in serum and saliva samples

Total thiol was measuredin both serum and saliva samples before and after ETN treatment and the results are illustrated in Figure 1.

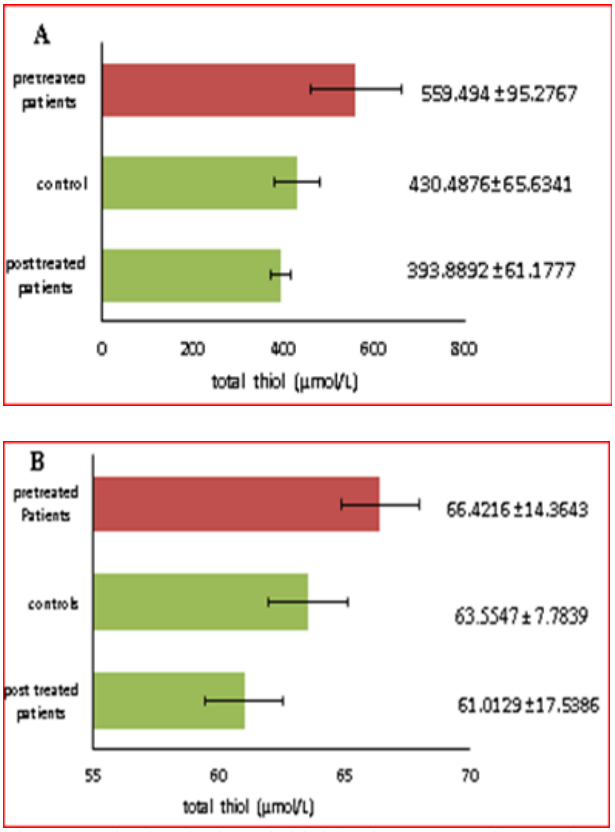

Figure .1:The level of total thiol in the groups of control, psoriatic patients before and after ETN treatment.

A: in sera samples

The comparison between pre-treated patients with healthy controls (p=0.000) using independent sample T-test.

The comparison between post treated patients with pre-treated patients (p=0.000) using paired sample T-test.

B: in saliva samples

The comparison between pre-treated patients with healthy control (p=0.000) using independent sample T-test.

The comparison between post treated patients with pre-treated patients (p=0.000) using paired sample T-test.

As it is obvious from the above results a highly significant increase (p< 0.00) was observed in total thiol level in both tested fluids of pretreated in comparison to that of the control group. While a highly significant decrease (p< 0.00) was observed in this level in both tested fluids of the pretreated group compared to that of the post treated and this level significantly reduced even to lower that of control group.

4.2. Native thiol in serum and saliva samples

The results in Figure 2 represented the level of native thiol in both serum and saliva of the control and the pretreated patients, as well as the effect of ETN treatment on the native thiol levels in both fluids of the pretreated patients group.

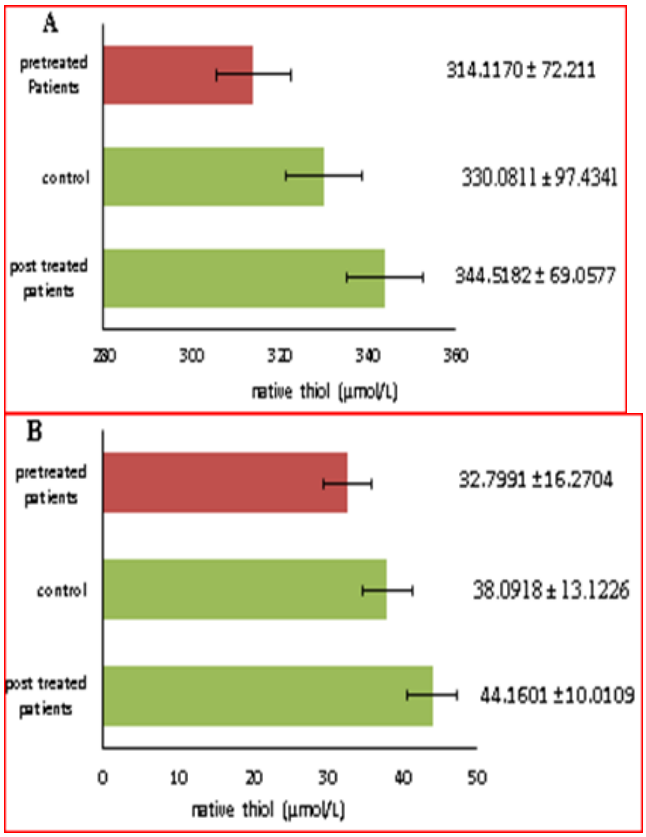

Figure .2:The level of native thiol in the control and the psoriatic patients before and after treatment with ETN.

A: in sera samples

The comparison between pre-treated patients with healthy control (p=0.000) using independent sample T-test.

The comparison between post treated patients with pre-treated patients (p=0.000) using paired sample T-test

B: in saliva samples

The comparison between pre-treated patients with healthy control (p=0.000) using independent sample T- test.

The comparison between post treated patients with pre-treated patients (p=0.000) using paired sample (T- test).

The above results showed a highly significant decrease in native thiol level (p<0.01) is obvious in both fluids of the pretreated patients compared with that of control. As well as a highly significant

increase in native thiol level (p<0.01) is obvious in both fluids: serum and saliva of the post treated patients compared with that of the pretreated patients and ETN treatment seems to raise the level of native thiol even higher than that of control.

4.3. Dynamic thiol in serum and saliva samples

The results in Figure 3 showed the effect of ETN treatment on dynamic thiol concentration in serum and saliva of the pretreated patients, healthy control and post treated patients.

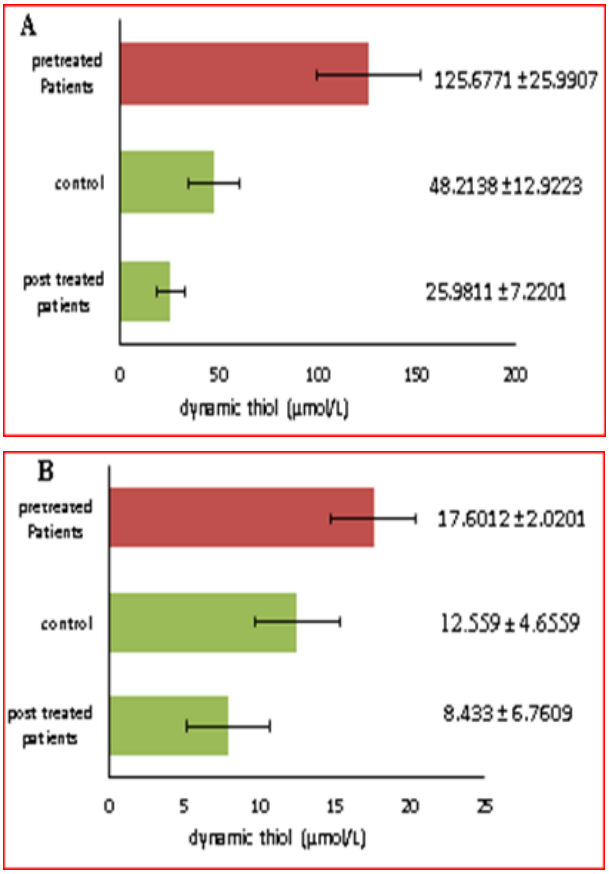

Figure.3: The level of dynamic thiol in the control group and in psoriatic patients before and after ETN treatment.

A: in sera samples

The comparison between pre-treated patients with healthy control (p=0.000) using independent sample T-test.

The comparison between post treated patients with pre-treated patients (p=0.000) using paired sample T-test.

B: in saliva samples

The comparison between pre-treated patients with healthy control (p=0.000) using independent sample T-test.

The comparison between post treated patients with pretreated patients (p=0.000) using paired sample T-test.

The comparison between post treated patients with pretreated patients (p=0.000) using paired sample T-test.

A highly significant elevation (p<0.00) was obvious in serum and saliva dynamic thiol level in the pretreated patients group compared with that of the controls group. And the ETN therapy affect the dynamic thiol in serum and saliva samples, where a highly significant reduction (p<0.00) was obvious in serum and saliva dynamic thiol level in the post-treated patients group compared with that of pretreated patients group and as it is obvious the dynamic thiol is reduced after the treatment, to lower than its normal level in the healthy individuals.

The results in Table 2 illustrated the comparison between the different ratios that were calculated in the sera and saliva samples of the three studied groups.

Table 2: Comparison between the Mean value ± SD of the thiol ratio in the sera & saliva samples of the control, pre and post treated patients groups.

Parameters Group		Sera samples	*P*-values	Saliva samples	*P*-values
D-SH /N-SH	Pretreated patients	0.3978 ± 0.0226	0.000** **a, b**	0.5366 ± 0.0682	0.000** **a, b**
	Control	0.1460 ± 0.0701		0.3277 ± 0. 0621	
	Post treated patents	0.1009 ± 0.0232	0.099 **c**	0.1909 ± 0.0611	0.100 **c**
D-SH/ T-SH	Pretreated Patients	0.2266 ± 0.0566	0.000** **a, b**	0.2646 ± 0.0553	0.000** **a, b**
	Control	0.1129 ± 0.0210		0.1976 ± 0.0105	
	Post treated patients	0.0884 ± 0.0133	0.054 **c**	0.1322 ± 0.0221	0.09 **c**
N-SH/ T-SH	Pretreated patients	0.5819 ± 0.1662	0.000** **a, b**	0.4938 ± 0.0191	0.000** **a, b**
	Control	0.7965 ± 0.0664		0.5933 ± 0.1011	
	Post treated patients	0.8332 ± 0.1120	0.101 **c**	0.7233 ± 0.1211	0.100 **c**

**Correlation is a highly significant at 0.01 levels.
a: represents the comparison between post treated patients with pretreated patients using paired sample T-test.
b: represents the comparison between pretreated patients with healthy control using independent sample T-test.
c: represents the comparison between post treated patients with healthy control using independent sample T-test.

The results of the biological treatment on different ratios concerning thiol homeostasis in both serum and saliva samples showed that the dynamic thiol/total thiol ratio and dynamic/native thiol ratio are highly significantly decreased (p<0.01) in the post treated patients compared with that of the pretreated patients. Whereas the comparison of the native thiol/total thiol ratio showed a highly significant increase (p<0.01) in these parameters in serum & saliva of the post treated patients compared with that of the pretreated patients group.

To check if the saliva can be used as instead of blood serum to look for the changes in thiol-disulfide homeostasis, Pearson correlation was used and the results presented in Table 3.

Serum / Saliva	Controls r: Pearson correlation P: P-Value	Pretreated-patients r: Pearson correlation P: P-Value	Post-Treatment patients r: Pearson correlation P: P-Value
[T-SH] µmol. /L	r = -0.454**; P= 0.000	r = -0.354**; P= 0.000	r = - 0.235*; P= 0.011
[N-SH]µmol. /L	r = -0.170; P= 0.104	r = -0.280**; P= 0.000	r = - 0.703**; P= 0.002
[D-SH]µmol. /L	r = -0.029; P= 0.781	r =0.152*; P=0.032	r = 0.357*; P= 0.050
D-SH/N-SH	r = 0.015; P= 0.887	r = 0.062; P= 0.539	r = 0.359*; P= 0.050
D-SH/T-SH	r = 0.076; P= 0.168	r = -0.159*; P= 0.013	r = 0.422*; P= 0.020
N-S-S/T-SH	r = 0.153; P= 0.143	r = 0.121; P= 0.229	r = 0.422*; P= 0.020

Table 3: Pearson correlation between [T-thiol], [N-thiol], [D-thiol], Dynamic/Native ratio, Dynamic/Total ratio and Native/Total ratio parameters in saliva andserum.

4.4. Discussion

It has been reported that oxidative stress (OS) play an important role in the pathogenesis of many diseases including inflammatory diseases and some new studies have reported the role of oxidative stress in the pathogenesis of psoriasis [14, 18]. The chemotactic effect of ROS on neutrophils contributes to the accumulation of the various inflammatory cells in psoriasis lesions [34], and the ROS generated by TNF- alpha in keratinocytes are responsible for initiating and maintaining chronic inflammation by regulation of certain pathways that are involved in the inflammatory process [15, 34]. Generally human body are supplied with their own antioxidant system which includes enzymatic and non- enzymatic machineries which prevent the accumulation of different free radicals that cause oxidative damage of the different cellular biomolecules and inhibit them from doing their normal functions [35]. Thiols are good nucleophiles and good reductant, they can react by one & two electrons mechanisms, and they are susceptible to reversible & irreversible changes [6]. Andthe thiol homeostasis has been reported to have a critical role in many processes such as antioxidant protection, detoxification, cellular signaling mechanism, apoptosis and regulation of many enzymes activities. Thus thiol/disulfide homeostasis acts as a major modulator of intracellular redox homeostasis [23]. The disulfide bond represents dynamic- redox-sensitive covalent bonds between two thiol groups and it participate in various functional properties of many enzymes [29, 36]. The oxidation of thiols to disulfides can occur through various reactions. The formed disulfides are converted back into thiols by the cleavage of S-S bonds [37, 38]. The Dynamic thiol/ disulfide homeostasis is responsible for many processes such as antioxidant defense, regulation of enzyme functions, signal transduction, apoptosis, and other processes [19] and the alteration in the thiol/disulfide balance appears to be involved in the pathogenesis of many diseases, especially diseases that are characterized by chronic inflammation [39-41].

Meanwhile the tumor necrosis factor (TNF-α) has been known to be -the major mediator in the pathogenesis of psoriasis [29]. Baek& lee [37] showed that TNF-α increased pro-inflammatory cytokine expression through the effect on oxidants. Thiol/ disulphide homeostasis is one of the most important systems which enable optimal redox balance in humans. In the present study, disulphide level in untreated psoriatic patients group was found to be higher than that of the control group, and the difference was statistically highly significant ($p< 0.001$). The thiol- homeostasis parameters results measured in the present study agreed with the results of a study carried on in Turkey in 2017 by Solak.,*et.al.* [42],who suggested that Total thiol / Disulfide can be used as a marker for inflammation and with that of Emre *et al*.[43]who investigated this homeostasis using Turkish smoker prostatic patients and they reported that Thiol/disulfide balance is important in pathogeneses and development of psoriasis. Also it agreed with Ustiner, *et al* [44] results that were obtained from their study in the same country. Meantime the results of the current study disagreed with Aksoy*et al* [45], results who observed that the level of serum dynamic thiol was unchanged in psoriatic patients compared to their used healthy group. Fass reported that the proteins eliminate most of the plasma free radicals species and most of the antioxidant ability of proteins is attributed to their contents of thiol containing amino acid residue: cysteine [46]. The measured high thiol levels in psoriatic patients suggested that molecules-containing -SH groups may be effective in boosting cell proliferation [38]. The mechanism of how this elevated thiol levels act is not clear, but it might be through its responsibility of the increased keratinocyte proliferation in the psoriasis disease [42]. The present study results measured an increase in blood disulfide levels indicating that a shift in thiol/disulfide imbalance is significant in psoriasis thus causes a highly significant increased oxidative stress as a result of tissue inflammation [39]. Disulfide bridges play an important role in maintaining the stability of mature keratin [47]. Since psoriasis is characterized by excessive proliferation of keratinocytes, high serum disulfide levels can be detected in these patients. Increased amounts of pro-oxidant molecules promote the consumption of antioxidants resulting in low serum levels of thiols [32].

The other part of the study was performed to evaluate the effects of ETN on total thiol, including dynamic thiol, which is considered one of the causes of oxidative stress, that cause damage to DNA, and lipid peroxidation [47].It is clear that this biological treatment reduced the levels of dynamic thiols to a level lower than the level in the normal state, as shown in the (Figure 3). Serum thiols levels were reduced after three months of the receiving initial treatment with ETN, such results demonstrate that this type of treatment, acts as a regulator against OS formation. This effect of ETN seems to resemble to that of another biological drug (Inflimab) which was reported to reduce the protein carbonyl groups and increase native thiols [48]. ETN is a receptor protein that reversibly binds to TNF factor and reduces the inflammatory response [49]; furthermore, it alters neutrophil migration, dendritic cell and T-cell maturation and migration, thus decreasing the local and systemic production of pro inflammatory cytokines and their subsequent effects [30].

Moreover, ongoing inflammation and enhanced oxidative stress in psoriasis patients continuously cause the formation of pro-oxidants that are neutralized by antioxidants. Therefore, the consumption of ETN treatment may contribute to the reduction in total thiol levels. However, these findings should be interpreted carefully as their power is limited by the relatively small cohort size and lack of prospective data. Further research is warranted to clarify how thiol-disulphide homeostasis and inflammatory markers are altered in patients with psoriasis in the pre and post state. Etanercept acts as a competitive inhibitor of TNF, a naturally occurring pro-inflammatory cytokine produced by many different cell types and acts as a key inflammatory processes mediator in the psoriasis pathogenesis. By competitively binding of ETN to TNF, it inhibits TNF action and thus preventing activation of the inflammatory cascade. Thiol-disulfide balance shifted toward disulfides in psoriasis patients resulting in an increase in the total oxidant status that may promote chronic inflammation [44].

5. Conclusion

The results of the present study indicated that the thiol-disulfide status play an important role in the oxidative stress measured previously in the patients included in the study (14) and using the biological drug ETN seems to improve the in- balance observed in this status. Moreover the current data provide evidence of the possibility of using saliva, as an alternative fluid instead of blood serum, to detect changes in the level of some thiol homeostasis related parameters, as well as to follow the impact of ETN treatment on these parameters. More experimental research is needed to check this point more in which a larger sample size are used, as well as follow the impact of treatment after different treatment periods.

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