Serum total thiol level in Acne Vulgaris Patients and its relation to oxidative stress

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Abstract

Background:Acne vulgaris (AV): is the most frequent dermatologic illness and is the chronic, multifactoral, inflammatory disease of the pilosebaceous unit. Aim: to compare the dynamic balance between thiol/disulfide and the balance of healthy individuals in AV sufferers. Methods: This research has been performed on 40 individuals who have been clinically diagnosed with AV. Furthermore, 40 seemingly healthy people of matching sex and age are controlled. Each patient had a thorough history, a comprehensive clinical examination and a laboratory study for: Total Thiol and thiol disulphide serum level. Results & Findings: The age varied from 16 to 31 years with mean ± SD = (22.40±4.35) years, while the control age was between 16 and 31 years with mean ±SD = (23.83±4.99) years, with no significant statistical differences (p=0.288) between both groups. This age was considered to have been between 16 and 31 years. Comparative for serum total thiol, serum thiol disulfide and their ratio between the two groups examined. The serum total thiol (p<0,001) and serum thiol disulfide (p<0,001) in the AV group were significantly increased as compared to the control group. Although the total thiol/thiol disulfide ratio (p<0.001) in AV group has decreased considerably compared with the control group.

Keywords: Dynamic, Thiol, Disulfide, Balance, Acne Vulgaris.

1. Introduction

Acne vulgaris (AV) is the most prevalent dermatological disorder of the chronic, complex, inflammatory pilosebean unit, affecting 80 percent in the population 11-30 years [1].

There are many variables that play a part in AV development, the major causes of acnes, inflammation and immunological response, sebum hyperplasia and colonisation of Propionibacterium acnes, play a leading role in disease pathogenesis [2].

Thiols are chemical molecules containing sulphur, which form complexes and are necessary for the biological system with amino acids and proteins. Thiols are essential antioxidants that play a vital role in the elimination via enzyme and non-enzyme mechanisms of reactive oxygen species. The Thiol plasma pool is of low molecular weight. Thiols – include cysteine, homocysteine, glutathione and albumin – and protein thiol in particular. Thiols undergo an oxidation process forms a disulfide bond with oxidant molecules. For detoxification, the control of pathways of signalling, and apoptosis and enzyme activities dynamic thiol/disulfide homeostasis (TDH) is indispensable[3].

Studies have shown that the aetiology of several hair disorders, such as psoriasis, is linked with dysregulated TDH. In telogenetic effluvium occurs, for example, various conditions such as diabetes, cardiovascular illnesses, malignant tumours and Parkinson's disease[4] [5].

The research aimed at comparing the dynamic thiol/disulfide balance with that of healthy individuals in patients with AV.

2. Patients and methods

This was a case-control study, was conducted on 30 patients suffering from AV with various acne severity levels (Patient group) and 23 healthy individuals of matched age and sex as a (control group). Patients were recruited from the outpatient clinic of Dermatology and Andrology Department of Banha University Hospitals between 0ctober2020 and January 2021.

The local ethic committee approved the study of Banha Faculty of Medicine. Informed consent was obtained from each individual before enrollment in the study.

2.1. Inclusion Criteria

- Patients more than or equal 15 years old with different degrees of AV severity.
- Patients hadn't taken   any medications for acne for one month before taking samples.

2.2. Exclusion Criteria

Patients excluded from the study were those:
- Suffering from chronic inflammatory disease.
- Drinking alcohol or tobacco smoking.
- Pregnant or lactating female.
- Suffering of Diabetes mellitus, cancers, and hypertension.
- Taking any medication or oral contraceptive pills in the last four weeks before the study.

Each patient was subjected to full history taking, complete clinical examination, and   Laboratory investigation for: Serum level of Total Thiol and Thiol Disulphide by human total thiol Elisa Kit:(Cat No:E-02463hu.Langham Creek)and by human thiol disulfide Elisa Kit: (Cat NoE-02415hu.Langham Creek)**.**

2.3. Statistical analysis

Data was collected, coded then entered as a spread sheet using Microsoft Excel 2016 for Windows, Microsoft Corporation, United States. Data was

analyzed using IBM Statistical Package for Social Sciences software (SPSS), 21st edition, IBM, United States. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Continuous data was expressed as mean ± standard deviation, median & IQR while categorical data as numbers and percentage. Data was presented as tables and graphs. Results was considered statistically significant at a p-value of less than or equal 0.05 and highly statistically significant at a p-value of less than or equal 0.001. The used tests were

- Chi-square test For categorical variables, to compare between different groups
- Fisher's Exact test Correction for chi-square when more than 20% of the cells have expected count less than 5
- Student T-test For normally distributed quantitative variables, to compare between two studied groups
- Mann Whitney test For abnormally distributed quantitative variables, to compare between two studied groups

3. Results

This study was conducted on 40 patients Clinically diagnosed AV patient. In addition ,40 apparently healthy individuals of matched age and sex as a control group. This study was conducted on outpatient clinic of Dermatology, Andrology and Venereology department of Benha university hospitals. The age in AV patients group ranged from 16 to 31 years with mean ±SD = (22.40± 4.35) years while the in control group the age ranged from 16 to 31 years with mean ±SD = (23.83± 4.99) years with no statistical significant difference (p=0.288) between the two groups. There was no statistical significant difference between the two groups as regards gender, weight, height and BMI (p=0.698, 0.548, 0.472 and 0.723 respectively). Also, there was no statistical significant difference between the two groups as regards occupation, menstruation and marital status (p=0.577, 0.554 and 0.949 respectively) table (1).

Table (1) Demographic characteristics among the two studied groups.

		AV patients group (n = 40)		Control group (n = 40)		Test value	P-value	Sig.
		n	%	n	%			
Age (years)	**Mean± SD**	22.40± 4.35		23.83± 4.99		U= 286.0	0.288	NS
	Median (IQR)	22.5 (18.0 - 26.0)		25.0 (19.0 - 28.0)				
	Range	16.0 - 31.0		16.0 - 31.0				
Gender	**Male**	16	40.0%	14	35.0%	X^2= 0.151	0.698	NS
	Female	24	60.0%	26	65.0%			
Weight (Kg)	**Mean± SD**	70.80± 7.71		69.57± 6.89		T= 0.605	0.548	NS
	Median (IQR)	71.5 (65.00 - 76.00)		69.0 (64.0 - 75.0)				
	Range	56.0 - 87.0		57.0 - 84.0				
Height (cm)	**Mean± SD**	167.43± 7.80		165.65± 6.60		U= 305.0	0.472	NS
	Median (IQR)	165.0 (162.0 - 174.0)		164.0 (161.0 - 173.0)				
	Range	157.0 - 186.0		155.0 - 177.0				
BMI (Kg/m^2)	**Mean± SD**	25.22± 2.60		25.46± 2.23		T= 0.357	0.723	NS
	Median (IQR)	25.23 (23.53- 26.58)		25.14 (23.93 - 26.95)				
	Range	20.23 - 31.25		21.38 - 30.85				
Occupation	**Baker**	0	0.0%	1	2.5%	X^2= 11.41	0.577	NS
	Carpenter	1	2.5%	0	0.0%			
	Cooker	0	0.0%	1	2.5%			
	Dentist	1	2.5%	0	0.0%			
	Doctor	1	2.5%	4	10.0%			
	Driver	4	10.0%	1	2.5%			
	Farmer	0	0.0%	4	10.0%			
	Housewife	5	12.5%	5	12.5%			
	Nurse	6	15.0%	1	2.5%			
	Nurse supervisor	1	2.5%	0	0.0%			
	Pharmacist	1	2.5%	1	2.5%			
	Physical therapist	0	0.0%	1	2.5%			
	Student	16	40.0%	18	45%			
	Teacher	4	10.0%	3	7.5%			
Menstruation in females	**Regular**	17	42.5%	23	57.5%	X^2= 1.18	0.554	NS
	Irregular	7	17.5%	4	10.0%			
	Not detected or	16	40.0%	13	32.5%			

		AV patients group (n = 40)		Control group (n = 40)		Test value	P-value	Sig.
		n	%	n	%			
Marital status	**male** **Single** **Married**	17 23.	42.5% 57.5%	16 24	40.0% 60.0%	X^2= 0.004	0.949	NS

p≤0.05 is considered statistically significant, p≤0.01 is considered high statistically significant,

SD= standard deviation, -comparison between groups done by Independent Samples Student T test, Chi-Square Test and Mann- Whitney U test

Table (2) Clinical characteristics in AV patients group.

		AV patients group (n = 40)	
		n	%
Age of onset of AV (years)	**Mean± SD**		17.57± 1.72
	Median (IQR)		17.0 (16.0 - 19.0)
	Range		15.0 - 20.0
Duration of disease (years)	**Mean± SD**		4.93± 3.27
	Median		4.5 (2.0 - 7.0)
	Range		1.0 - 11.0
Distribution	**Face**	12	30.0%
	Face & Chest	9	22.5%
	Face &Back	3	7.5%
	Face& Back& Chest	16	40.0%
Course of disease	**Intermittent**	17	42.5%
	Progressive	23	57.5%
PIH	**No**	19	47.5%
	Yes	21	52.5%
Scar formation	**No**	21	52.5%
	Yes	19	47.5%
Scar type	**icepick**	10	25%
	Rolling	5	12.5%
	boxcar	3	7.5%
	hypertrophic	1	2.5%
GAGS score	**Mean± SD**		24.93± 6.55
	Median (IQR)		24.0 (21.0 - 27.0)
	Range		13.0 - 37.0
Severity according to GAGS score	**Mild**	5	12.5%
	Moderate	25	62.5%
	Severe	10	25%

SD= standard deviation, -comparison between groups done by Pearson Chi Square test and Mann- Whitney U test

Table (2) shows clinical characteristics in AV patients group. The mean (± SD) age of onset of AV was 17.57 (± 1.72) years while the mean (± SD) duration in those patients was 4.93 (± 3.27) years. The majority of cases had AV in face, back and chest (40%). Post-inflammatory hyperpigmentation (PIH) was found in 52.5% of cases and formation of scar was detected in 47.5% of the cases. 50% patients with scar had icepick type (fig. 9). GAGS score ranged from 13 to 37 with mean (± SD) 24.93(± 6.55). Most patients

(62.5%) had moderate degree of acne according to GAGS score.

On comparison between the two studied groups as regard serum total thiol, serum thiol disulfide and their ratio. There was high significant increase in serum total thiol (p<0.001) and serum thiol disulfide (p<0.001) in AV group compared to control group (fig. 1- 2). While there was high significant decrease in total thiol/thiol disulfide ratio (p<0.001) in AV group compared to control group fig. (3).
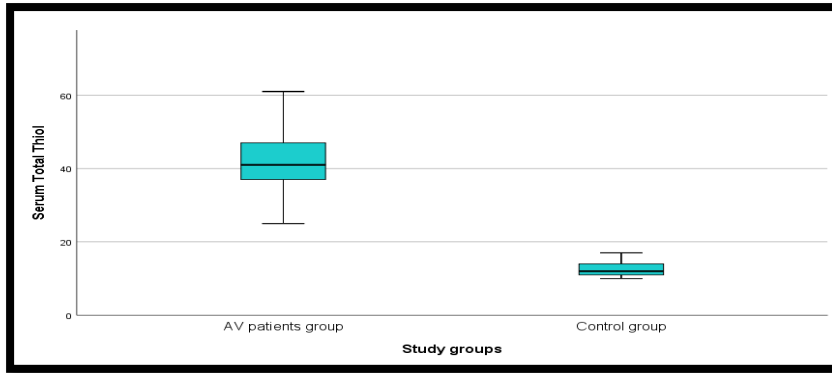
Fig. (1) Boxplot showing comparison between AV patients group and control group regarding serum total thiol.
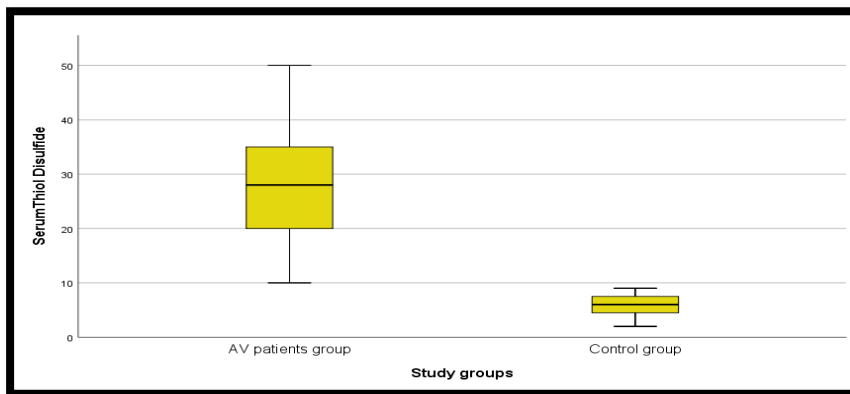

Fig. (2) Boxplot showing comparison between AV patients group and control group regarding serum thiol disulfide.
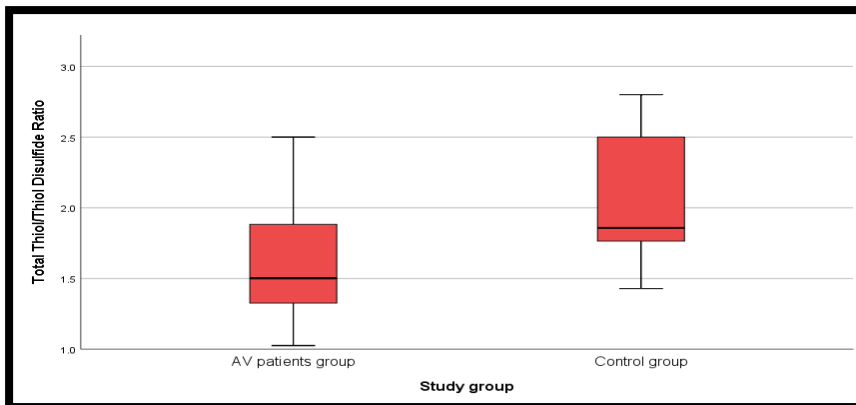

Fig. (3) Boxplot showing comparison between AV patients group and control group regarding total thiol/ thiol disulfide ratio.

Table (3) shows correlations analysis between total thiol/thiol disulfide ratio and other parameters in the studied groups. In AV patients, a significant negative correlation was found between total thiol/thiol disulfide ratio with AGA (r= -0.374, p=0.042), serum thiol disulfide (r= -0.86, p< 0.001) and serum total thiol (r= -0.581, p=0.001). While, there was significant positive correlation was found between total thiol/thiol disulfide ratio with BMI (r= 0.455, p= 0.01), scar formation (r= 0.54, p=0.002),

GAG score (r= 0.626, p< 0.001), stress and fatigue (r= 0.424, p=0.020) and serum thiol disulfide (r= 0.871, p< 0.001). In control group, it was noticed that there was positive correlation between total thiol/thiol disulfide ratio with weight (r= 0.488, p=0.018), height (r= 0.457, p= 0.028) and menstruation (r= 0.500, p= 0.015). while, a significant negative correlation was found between total thiol/thiol disulfide ratio with gender (r= -0.566, p=0.005), eating nuts (r= -0.621, p=0.002) and serum thiol disulfide (r= -0.77, p<0.001).

Table (3) Correlations analysis between total thiol/thiol disulfide ratio and other parameters in the studied groups.

| | Total Thiol/Thiol Disulfide Ratio | | | |
| | AV patients group (n = 40) | | Control group (n = 40) | |
	r	p- value	r	p- value
Age	-0.028	0.882	0.000	0.999
BMI	0.455	**0.011**	0.096	0.664
Age of disease onset	0.124	0.515	-	-
Course of disease (progressive)	0.031	0.870	-	-
Duration of disease	-0.183	0.333	-	-
PIH (+ve)	0.228	0.226	-	-
Scar formation (+ve)	0.540	**0.002**	-	-
GAG score	0.626	**<0.001**	-	-
Association with AGA(+ve)	-0.374	**0.042**	-0.008	0.971
Facial cleanser (frequency of use)	0.064	0.737	-0.129	0.559
Sunblock (frequency of use)	0.063	.0741	-0.053	0.810
Physical exercise (frequency)	0.088	0.642	0.286	0.185
Sleep hours	-0.147	0.438	-0.030	0.891
Stress and fatigue (+ve)	0.424	**0.020**	-0.043	0.846
Use of Scalp Oil / Bleaching cream	0.252	0.180	-0.146	0.506
Cosmetics use	0.127	0.505	-0.402	0.057
Milk Drinking	0.126	0.508	-0.382	0.072
Dairy products (frequency of use)	0.230	0.221	-0.241	0.269
Fruits (frequency of use)	0.203	0.283	-0.322	0.134
Vegetables (frequency of use)	-0.028	0.882	0.275	0.203
Sea food (frequency of use)	0.146	0.529	-	-
Chocolate (frequency of use)	0.064	0.735	-0.086	0.697
Nuts (frequency of use)	-0.086	0.650	-0.621	**0.002**
Oily food (frequency of use)	-0.054	0.776	-0.385	0.070
Serum Total Thiol	-0.581	**0.001**	-0.022	0.922
Serum Thiol Disulfide	-0.860	**<0.001**	-0.770	**<0.001**

p≤0.05 is considered statistically significant, p≤0.01 is considered highly significant

4. Discussion

The present research has shown that serum total thiol and serum thiol disulfide (p<0.001) have increased significantly in the AV group in comparison with the control group. Although the total thiol/thiol disulfide ratio was significantly changed to thiol disulfide (p<0.001) in AV group compared to the control group.

According to the present results, this major shift to thioldisulphide was observed in many dermatological disorders in the overall ratio of thiol/thiol-disulfide.

Emre et al. observed that in the blood of patients with active SD, the amount of thiol groups is greater than in healthy people, An increase in thiol levels in blood may be related to homeostasis in order to decrease the increasing burden of oxidative stress in individuals with SD, and higher thiol groups may lead to an enhanced proliferation of SD lesions[6].

In addition, Pektas et al., [7] showed in an alopecia areata (AA) research that oxidant stress and its impact on thiol/thiol total disulfide may be caused by widespread hair follicle loss in AA disease. In their research, the group of AA found that the amount of disulphide and the disulphide-total thiol ratio (shift to thiol-disulphide side) were much higher (p<0.01). It

was observed that the overall levels of thiol between the AA and control groups were comparable (p>0.05), the homeostasis of thiol/disulphide switched to oxidative stress, a reduction in thiols and rise in AA patients.

Aksoy et al. have shown [8], according to current research findings, that the balance of thiol/disulfide was changed to disulphide in psoriasis patients. This is essential to substantiate the involvement of oxidative stress in psoriasis aetiology.

Sener et al.[9] studies indicate that the thiol-disulfide balance in rosacea patients was seen in order to move towards disulfides, an indication of oxidative strain in the rosacea. The ROS produced by the variables that cause rosacea was believed to lead to disulfide bonds and thiol levels being reduced by oxidising thiol groups in the cell. Thiol/disulfide homeostasis may be considered as a biomarker reflecting the oxidative stress in rosacea.

Unlike the present findings of the research, Gürel et al.[10] have shown that the disulfide/total thiol proportion in AV patients is much lower than the control group (p=0.034) (shifting to total thiol side). Thiol levels and thiol-disulphide levels were shown to vary significantly between patients with AV and control individuals. The authors interpreted these

inverted results that the oxidative stress associated with AV may come from a disulfide-independent mechanism.

A substantial negative association was observed between total serum thiol and GAG score (r= -0.676, p<0.001) in the present research. A substantial positive connection between the thiol/thiol-disulfide total serum score ratio was observed with GAG (r=0.626, p<0.001). There was a substantial negative association between serum thiol- disulfide (r= 0.708, p< 0.001) and GAG score.

In accordance with the present research, in the study by Gürel et al.,[10], a GAG score of mild (n=25), moderate (n=24) and serious (n=25) was divided into three sub-groups. Total plasma concentrations of thiol and disulfide were not substantially different across subgroups and not significantly different between individuals with mild, moderate and severe disorders. The authors stated that oxidative stress associated with AV may be caused by an independent mechanism of disulfide levels, which means that there was no connection in the trial between the severity of A.V and total thiol/thiol–disulfide ratio.

The recent findings revealed a strong positive connection of serum total thiol with AGA (r= 0.463, p= 0.01) in both AV and control groups.

Significant positive connection was observed with the AGA relationship (r= 0.447, p= 0.013) between serum thiol disulfide. The total thiol/thiol disulfides ratio with AGA was shown to be significantly unfavourable in AV patients. (r= -0.374, p=0.042).

Prie et al. have discovered that total antioxidant activity has reduced in patients with AGA in line with current findings, therefore showing oxidative stress in such individuals. Declining significant superoxide dismutase activity (SOD), but no change in catalase, glutathione peroxidase, non-protein thiol levels and the overall antioxidant activity in erythrocytes are elements that suggest the presence of oxidative stress in these patients and a compensatory mechanical mechanism for SOD dysfunction of androgenetic alopecia patients in red blood cells.

There is also a strong positive link between the total thiol/thiol disulfide ratio and BMI (r= 0.455, p= 0.011) as shown in current studies.

According to the results, Söğüt et al.[12] showed that oxidative stress in groups with a BMI higher than normal is elevated (talk25).

While the overall Thiol/thiol disulfide ratio with scar development was significantly favourable (r=0.54, p=0.002).

Another research confirms the findings of recent studies by indicating that free radicals play a significant role in skin fibrotic diseases such as scleroderma, GVHD and hypertrophic scars[13]. Other writers were similarly supportive of the function of oxidative stress in skin scarring[14].

5. Conclusion

This research found that the total blood thiol and serum thiol-disulfide (p<0.001) were substantially greater than the control for AV cases, while the overall ratio of thiol/thiol-disulfide to thiol-disulfide (p<0.001) was much higher compared to the control group. Total thiol is a powerful antioxidant and total thiol and thiol-disulfide are regarded inflammatory initiators in the AV lesion. The thiol-thiol-disulfide total ratio may predict AV susceptibility to scar formation (p=0.002) and predict AV gravity by utilising the GAG scoring (p<0.001).

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