



Vitiligo: The role of oxidative stress test as a marker in Sulaimani

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Abstract

To evaluate oxidative stress test and to assess the prevalence and common treatment pattern of vitiligo patients. An observational, cross-sectional descriptive study was carried out for a period of three months among the patients visited the dermatology teaching center in Sulaimaniyah. The study included 50 patients and 40 controls. Results revealed that prevalence of vitiligo was found to be (8%), and females represented (55%) of the vitiligo patients. Of the 50 patients, most of the patients were within the age group of 11-20 years (28%). The commonest type of vitiligo was generalized vitiligo (66%). And the most common treatment was corticosteroid (42%) followed by psoralen combined with UVA (PUVA) (34%), also the most common combination of treatments was corticosteroid with PUVA (8%). MDA and GSR in vitiligo significantly ($P < 0.05$) higher than controls. In conclusion, patients with vitiligo had high plasma level of MDA and GSR at diagnosis.

Keywords: Vitiligo, Prevalence, Common treatments.

Introduction

Vitiligo is a chronic skin disease, characterized by pigmentation disorder, due to selective loss of melanocytes that appear as white macules on the skin (Allam *et al.*, 2013). It is rapid in onset and slow in progression (Martin, 2011). It affects all ages, races and both genders (Huggins *et al.*, 2005). The macules can appear anywhere, but the firstly affected parts are the area of sun-exposed, like face and hands, also the areas around the orifices and genitals may be affected (Reghu, 2011). It is not life-threatening and asymptomatic (Reghu, 2011), but it may lead to a psychological stress and affect quality of life, especially in darker-skinned patients, and because it is rapidly noticed by others, especially in the sensitive years of adolescence, as half of the patients are affected before the age of 18 years (Huggins *et al.*, 2005), also in certain culture due to confusion with leprosy, and it is poorly responsive to treatment, needs a long time and non-curable.

There are many potential pathophysiological theories involving genetic, autoimmunity, autocytotoxicity, and oxidative stress hypotheses:

- Genetics: gene expression studies in vitiligo were done to analyze the changes in the expression pattern of several genes associated with immunomodulation, melanogenesis, and regulation of the development and survival of melanocytes. An imbalance in cytokines expression

may have a role in the pathogenesis of vitiligo and decreased expression of TNF due to treatment with topical tacrolimus was associated with repigmentation (Allam *et al.*, 2013).

- Autoimmunity: there is a strong evidence that vitiligo is preferentially an autoimmune disease, due to the association with autoimmune conditions such as Addison's disease, hypothyroidism and pernicious anemia, the presence of some alleles of MHC II antigens and other autoimmune susceptibility genes; couple to the detection of organ-specific antibodies in the sera of patients with vitiligo, the positive response to topical immunosuppressive therapy (topical steroids and tacrolimus), the participation of immune cells especially the demonstration of autoreactive T cells, and the presence of antibodies against different antigens in melanocytes. All these several aspects support this hypothesis (Allam *et al.*, 2013).

- Autocytotoxicity: toxic metabolites from direct environmental or occupational exposure to certain chemicals, principally phenols and catechols or through accumulation of byproducts due to inhibition of enzymes involved in melanin pathway can damage melanocytes of genetically susceptible individuals (Allam *et al.*, 2013).

- Oxidative stress: the tripeptide glutathione (GSH) is essential for metabolic and cell-cycle related functions in virtually all cells. Its ability to

directly scavenge free radicals and to act as a co-substrate in the glutathione peroxidase catalyzed reduction of H₂O₂ and lipid hydroperoxides makes GSH central to defense mechanisms against intra- and extracellular stress (Ozturk *et al.*, 2008).

It can be diagnosed by physical examination, medical history and laboratory tests (Allam *et al.*, 2013). The diagnosis of vitiligo is mostly dependent on the clinical finding. Also some laboratory tests may be helpful like antinuclear antibody, thyroid stimulating hormone, and complete blood count (Halder *et al.*, 2009).

Materials and Methods

It was a non-experimental (observational), and cross sectional study done in a dermatology teaching center, Sulaimaniyah, Kurdistan, for a period of three months from Jan 15th to Apr 15th 2015. The study sample consists of fifty vitiligo patients and forty controls (total of ninety sample). The data included questionnaire list; age, sex and occupation of the patients, duration of vitiligo, presence of autoimmune diseases in association with vitiligo (thyroid, diabetes, Addison's disease, pernicious anemia, arthritis), and family history of vitiligo.

Vitiligo patients were diagnosed clinically by a dermatologist in the center and the precise distribution of lesions (types of vitiligo) were classified into six groups according to the standard working classifications of clinical type of vitiligo like (focal, mucosal, acrofacial, generalized, universal, and segmental).

All patients underwent blood sampling; 5ml of blood was drawn from median cubital vein of the patients and control group into test tube. The blood samples were immediately centrifuged at 1000 Xg for 10 minutes. Upper plasma phase was drawn with pipette and tested by ELISA based on biotin double antibody sandwich technology for evaluating of human level of GSR and MDA using total GSR and MDS kits.

Evaluating therapeutic management pattern of the individual patients, including drug and non-drug therapy, were obtained by the prescriptions and questionnaire list, and some of the patients were called to assess the therapeutic management pattern, because they bought treatments outside the center, in the community pharmacies, due to unavailability in the center.

Complete information was obtained from all the patients included in this study, and the results were assessed for inclusion and exclusion criteria based on the title.

Results and Discussion

Oxidative stress test: The $P < 0.05$ for MDA and GSR in vitiligo patients as a result is statistically significant. The MDA and GSR were higher in vitiligo patients compared to controls (Table 1).

Table (1): Comparison of mean \pm SD of total glutathione reductase (GSR) and malondialdehyde (MDA) serum level.

		MDA nmol/L	GSR μ mol/L
Patient n = 50	Mean	2.3	6.87
	SD	0.580	0.9663
	SE	0.082	0.1366
Control n = 40	Mean	1.24	3.63
	SD	0.2599	0.4876
	SE	0.0411	0.770

n = number of patients, $P < 0.05$

Prevalence of vitiligo: of the 14,779 visits to the dermatology teaching center over the period of three months, 1236 visits were diagnosed as vitiligo. This showed the prevalence of (8%). The number of females visited the center was 674 visits (55%), while the number of males was 562 visits (45%), which is giving a male to female ratio of 0.8:1, as shown in (Figure 1).

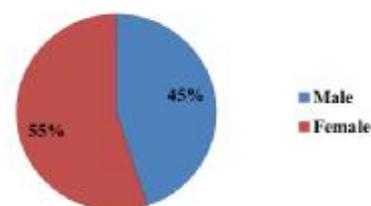


Figure (1): Gender distribution among vitiligo patients.

Fifty patients were interviewed randomly with diagnosed vitiligo. The females showed 70% (35/50) of the patients, which was more than males 30% (15/50).

Of the 50 patients, majority of patients were within the age group of 11-20 years 28% (14/50), with only 2% (1/50) over sixty years old. The youngest patient was 5 years old and the oldest was 63 years old. The Mean age was 27.7 ± 15.8 years. The distribution of age and sex of vitiligo patients are shown in (Table 2 and Figure 2).

Occupation: Of the 50 patients, 46% (23/50) were student, followed by housewife 28% (14/50), as shown in (Table 3 and Figure 3).

Table (2): Distribution of vitiligo patients according to age and sex.

Age group (Yrs)	Male		Female		Total	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
0-10	2	4%	5	10%	7	14%
11-20	5	10%	9	18%	14	28%
21-30	2	4%	8	16%	10	20%
31-40	3	6%	5	10%	8	16%
41-50	2	4%	5	10%	7	14%
51-60	1	2%	2	4%	3	6%
>60	0	0%	1	2%	1	2%
Total	15	30%	35	70%	50	100%

n = number of patients, χ^2 P < 0.05

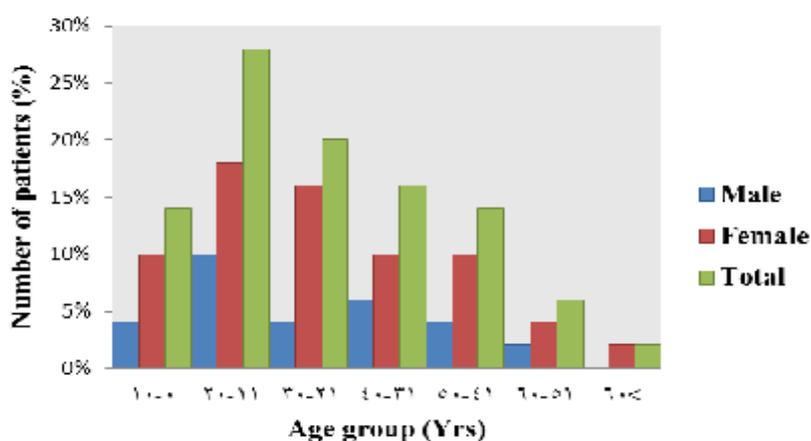


Figure (2): Graphical representation of distribution of patients according to age.

Table (3): Distribution of vitiligo patients according to occupation.

Occupation	Total	
	<i>n</i>	%
Student	23	46%
Housewife	14	28%
Free job	3	6%
Teacher	3	6%
Medical staff	2	4%
Employer	1	2%
Farmer	1	2%
Driver	1	2%
Engineer	1	2%
Pre-school	1	2%
Total	50	100%

n = number of patients, χ^2 P < 0.001

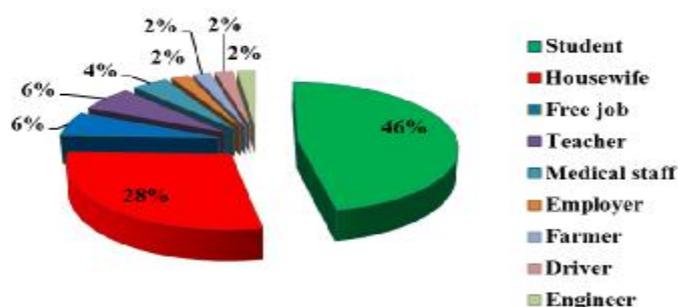


Figure (3): Graphical representation of distribution of patients according to occupation.

Type of vitiligo: The prevalent clinical form was generalized vitiligo, with 66% (33/50) of the studied patients, followed by the acrofacial form affected 32% (16/50) of the patients. Other types

of vitiligo (universal, mucosal, and segmental) were not observed in the study, as shown in (Table 4 and Figure 4).

Table (4): Distribution pattern of clinical types of vitiligo.

Type of vitiligo	Total	
	<i>n</i>	%
Generalized	33	66%
Acrofacial	16	32%
Focal	1	2%
Total	50	100%

n = number of patients, χ^2 P < 0.001

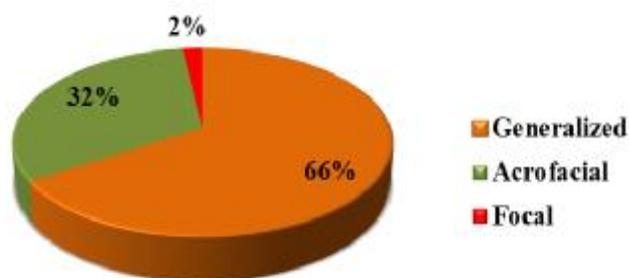


Figure (4): Graphical representation of distribution of patients according to type of vitiligo.

Duration of vitiligo: Of the 50 patients, duration of vitiligo in the majority of the patients 52% (26/50) was between 0-5 years, while minority of the patients 4% was over than 20 years. The minimum duration of the vitiligo was 1 week, while the

maximum duration was 26 years, with the mean duration of 6.7 ± 6.4 years, as shown in (Table 5 and Figure 5).

Table (5): Distribution of vitiligo patients according to duration of vitiligo.

Duration group (Yrs)	Total	
	<i>n</i>	%
0-5	26	52%
6-10	15	30%
11-15	3	6%
16-20	4	8%
>20	2	4%
Total	50	100%

n = number of patients, χ^2 P < 0.001

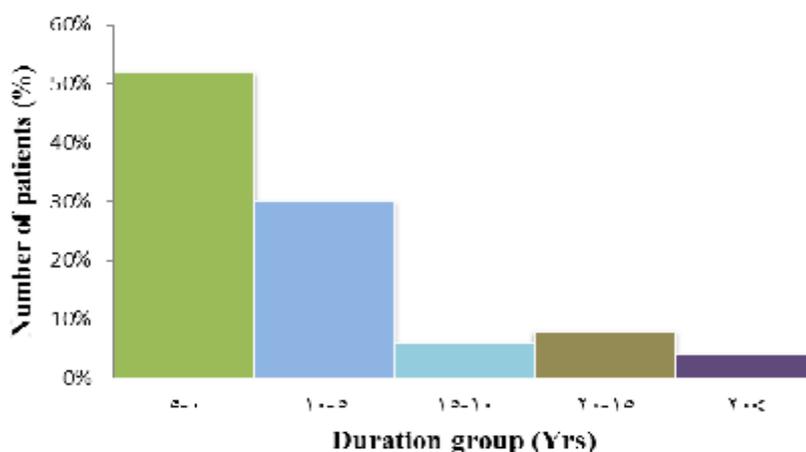


Figure (5): Graphical representation of distribution of patients according to duration of vitiligo.

Family history: Positive family history of vitiligo was present in 18% of the patients. The details

regarding family history are shown in (Table 6 and Figure 6).

Table (6): Distribution of vitiligo patients according to family history of vitiligo.

Family history	Total	
	<i>n</i>	
Positive	9	Positive
Negative	41	Negative
Total	50	Total

n = number of patients, χ^2 P < 0.001

Associated autoimmune diseases: Associated autoimmune disorders were present in 32% of the

patients, and the details are shown in (Table 7 and Figure 6).

Table (7): Distribution of vitiligo patients according to associated autoimmune diseases.

Associated disease	Total	
	<i>n</i>	%
Positive	16	32%
Negative	34	68%
Total	50	100%

n = number of patients, χ^2 P < 0.05

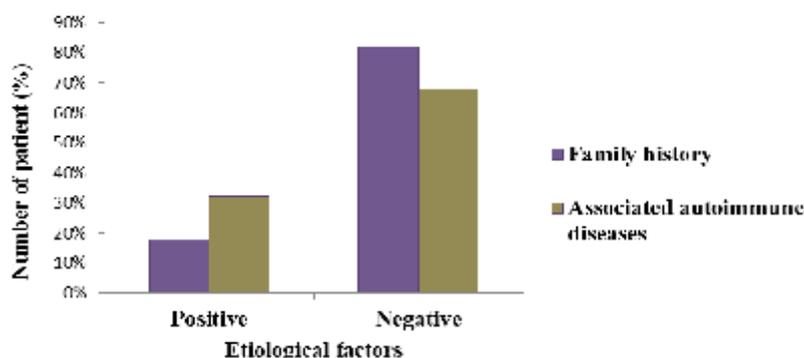


Figure (6): Graphical representation of vitiligo according to presence of etiological factors.

Treatments: The most common treatment was corticosteroid (%42), followed by PUVA (%34). Combination therapy were used for some patients, and the most common one was corticosteroid plus

PUVA (8%), and only one patient did not take any treatment, other details regarding the common treatments can be found in (Table 8 and Figure 7).

Table (8): Distribution of vitiligo patients according to treatments.

Treatment	Total	
	<i>n</i>	%
Corticosteroid	21	42%
PUVA	16	34%
Tacrolimus	3	6%
Corticosteroid + PUVA	4	8%
Corticosteroid + tacrolimus	1	2%
PUVA + tacrolimus	1	2%
Corticosteroid + PUVA + tacrolimus	1	2%
Corticosteroid + sunscreen	1	2%
No treatment	1	2%
Total	50	100%

n = number of patients, χ^2 *P* < 0.001

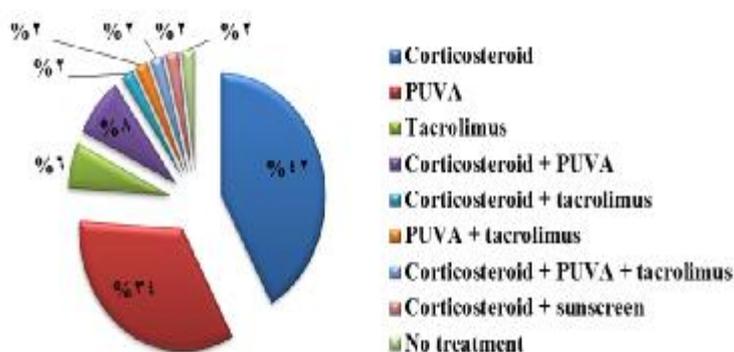


Figure (7): Graphical representation of common treatments.

The role of free radicals and oxidative damage in the pathophysiology of vitiligo has been shown (Schallreuter *et al.*, 1991; Maresca *et al.*, 1997). The relationship between increased oxidative

stress due to the accumulation of radicals and reactive oxygen species (ROS) and associated changes in blood and epidermal component of vitiliginous skin have been reported many times

(Maresca *et al.*, 1997; Courtney *et al.*, 2002). An increased epidermal H₂O₂ level has been described both in vivo and in vitro in the active phase of vitiligo. High H₂O₂ level is possibly associated with reduced catalase, glutathione peroxidase activities and selenium levels (Schallreuter *et al.*, 1991; Schallreuter *et al.*, 1999). The consequence of increased free radicals via H₂O₂ generation and imbalances in oxidant/antioxidant balance is oxidative stress, which leads to oxidative damage, resulting in increased MDA levels, which is the end product of lipid peroxidation. Koca *et al.* has reported increased serum MDA levels in vitiligo.

Although vitiligo occurs worldwide, it is known that its prevalence varies between races and regions. In this study, a dermatology teaching center was selected in Sulaimaniyah, and the 'prevalence' was expressed as the number of visits, which was 8%.

Howitz *et al.* reported that the prevalence of vitiligo in Denmark was 0.38%. Mehta *et al.* reported that the prevalence was 0.49% in rural areas of Indian and 1.78% in urban areas. Abdel-Hafez *et al.* performed a survey in Upper Egypt and found the prevalence of vitiligo to be 1.2%. In the USA, the prevalence was 0.74% (Rose *et al.*, 1998). This result expresses a higher prevalence in comparison to the published data, because there is no register for each patient in the center, instead the number of visits was recorded in the registration office. So the exact prevalence of the visited patients was not available in the registration office.

The results also demonstrated a higher prevalence of vitiligo in females than in males. The male to female ratio was 08:1, which is similar to the result reported by Boisseau-Garsaud *et al.* Women are usually more concerned about pigmentation changes of their skin and may be more diligent in seeking treatment. This may be a possible reason for the greater number of female patients in the studies (Rose *et al.*, 1998). However, this result is different from that reported by other studies (Alkhateeb *et al.*, 1998; Lu *et al.*, 2007; Handa *et al.*, 1999), which showed that males and females were equally affected. In contrast to the results, McBurney found that men were more affected than women.

In this study vitiligo was found to more common in the age group of 11-20 years (28%) which is in agreement with other reports (Boisseau-Garsaud *et al.*, 2000; Alkhateeb *et al.*, 2003; Nordlund, 1997), which indicates that vitiligo predominantly affects a younger population.

The duration of the disease varied widely from 1 week to 26 years. Most of the patients 52% (26/50)

were less than 5 years in duration regardless of sex, which in agreement with the study done by Hann *et al.*

Regarding the occupation, most of the patients were student (46%), as half of the patients are affected before the age of adolescence (Huggins *et al.*, 2005), and followed by housewife (28%) that are usually more concerned about pigmentation changes of their skin (Rose, 1998).

Most displayed generalized vitiligo, vitiligo vulgaris, which was the most common clinical type in this study, including more than half of the patients (66%). Kovacs and Handa and Kaur also reported that generalized vitiligo was the commonest type seen. Acrofacial vitiligo affected (32%) of the studied group. The frequency of distribution of clinical types of vitiligo varies in different studies. However, it is difficult to comprehend the mechanisms and determinants underlying varying clinical patterns of vitiligo seen in different patients (Mutairi *et al.*, 2006).

Several epidemiological studies have suggested that a genetic predisposition might be a risk factor for vitiligo (Alkhateeb *et al.*, 2003; Nanda *et al.*, 1999; Zhang *et al.*, 2004). In this study, a positive family history was present in 18% of patients with vitiligo, whereas other studies reported 13%²¹ 18.9%²³ and even 1.6% (Liu *et al.*, 2005). So positive family history is considered to be a poor predisposing factor for vitiligo.

Association of vitiligo with other autoimmune diseases has also been a subject of great interest. Presence of autoimmune diseases in association with vitiligo was observed in (32%) of patients. Positive result also was reported by Liu *et al.*

Conclusion

In this study, the treatment modalities used including corticosteroid, PUVA, tacrolimus, and sunscreen. The most common treatments used were corticosteroids (42%) and PUVA (34%). Among the combinations, the most common one was corticosteroid with PUVA (8%). Most of the studies reported that topical corticosteroids are the most commonly used treatment option (Martis *et al.*, 2002; Mutairi *et al.*, 2006; Chanda *et al.*, 1996; Tawade *et al.*, 1997).

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