Antioxidants Use of Vitamin C and vitamin E in Patients with Vitiligo

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Abstract:

Background: The etiology of vitiligo is still unknown, but the autocytotoxic theory suggests that increased melanocytes activity leads to its own destruction. One of the proposed mechanisms by which autocytotoxicity may occur is that the destruction take place through reactive oxygen species.

Objectives: The present work was arranged to evaluate the effect of antioxidants in the form of vitamin C and vitamin E in patients with vitiligo in the view of their effects on the alterations in oxidative stress as measured by plasma and red blood cells Malondialdehyde (MDA) and the changes in antioxidant mechanism as measured by plasma and red blood cells Glutathione (GSH)..

Patients & Methods: Twenty-three patients with vitiligo were included in this study. The severity of the disease was graded according to the rule of nine into three grades (mild, moderate, and severe). The oxidative stress (plasma and red blood cells Malondialdehyde) and the antioxidants (plasma and red blood cells Glutathione) were measured in all patients. All patients included in this study received a combination of vitamin C and E (vitamin C 500 mg, vitamin E 100 mg twice a day) for 2 weeks and then the oxidative stress (plasma and red blood cells Malondialdehyde) and the antioxidants (plasma and red blood cells Glutathione) were measured again.

Results: The results showed that only the mild group showed a statistically significant reduction in mean plasma MDA of 0.86 micro mole/L after two weeks of treatment $P<0.02$. The changes observed in mean plasma MDA in the remaining 2 groups and in the total cases were small and not statistically significant, while in erythrocytes MDA, the changes observed in mean erythrocytes MDA in the 3 severity groups of vitiligo and in the total cases were small in magnitude and not significant statistically. Also, the changes observed in mean Plasma GSH in the 3 severity groups of vitiligo and in the total cases were small in magnitude and not statistically significant.

Conclusion: The results showed that the oxidative stress decreased significantly after treatment with antioxidant drugs in the mild group (i.e. early in the course of the disease when the disease is still active) and this may have a therapeutic applications suggesting that antioxidants may help to decrease the oxidative stress if it was given early in the course of the disease.

Keywords: Vitiligo, Glutathione, Malondialdehyde, Antioxidants, Oxidative stress.

Introduction:

Vitiligo is an acquired pigmentary disorder of the skin manifested by depigmented white patches surrounded by a normal or hyperpigmented border [1].

The destruction of melanocytes is the cause of these depigmented macules. The aetiology is still unknown, but various theories such as the autoimmune, autocytotoxic and neural hypothesis have been proposed [2].

The autocytotoxic theory suggests that increased melanocyte activity leads to its own destruction [3, 4].One of the proposed mechanisms by which autocytotoxicity may occur is through inhibition of thioredoxin reductase, a free radical scavenger located on the membrane of melanocytes. Levels of catalase, an enzyme that reduces superoxides to water, have also been shown to be reduced in involved and uninvolved skin in patient with vitiligo, causing cell death [5, 6].

These findings may support the pathogenesis of vitiligo as an auto-immune disease, and this is supported by previous studies that showed that the progress of auto-immune diseases may be related to oxidative modification of cellular structures by free radicals, and at least partly depends on the concentration of antioxidants at critical cellular sites, such as mitochondria, nuclear and plasma membranes [7].

The reactive oxygen species (ROS) generate under physiological conditions as a consequences of aerobic metabolism and there is a balance between ROS and antioxidant defense mechanisms [8]. An oxidative stress is apparent when the antioxidant defense is insufficient to cope the pro-oxidant production [9]. ROS toxicity includes mutation, protein degeneration, and lipid peroxidation which can severely disturb membrane permeability and alter intracellular calcium and pH [10].

Glutathione is a major endogenous antioxidant scavenging free radicals and toxins such as lipid peroxides that would otherwise damage the cells. Low GSH level is associated with most of immune and degenerative diseases in aging and one of these is vitiligo [11].

In the past there were several studies concerned with the role of free radicals and antioxidant

mechanisms in vitiligo [4, 5, 6, 12, 13, 14]. One of these studies showed that there is a highly significant reduction in the levels of antioxidants and an increase in the levels of free radicals in patients with vitiligo compared to control [15]. The body has a very good defense mechanism against free radicals, but these might not be enough to cope with the oxidative stress that exogenous antioxidants like vitamin E and C are needed to help the defense mechanism [15]. This encouraged us to use the antioxidants in patients with vitiligo and to watch any decrease in the free radicals level.

The present work was arranged to evaluate the effect of antioxidants in the form of vitamin C and vitamin E in patients with vitiligo in the view of their effects on the oxidative stress as measured by plasma and red blood cells Malondialdehyde (MDA) and the changes in antioxidant mechanism as measured by plasma and red blood cells Glutathione (GSH).

Patients & methods:

This study was conducted in the Department of Dermatology and venereology in Baghdad Teaching Hospital from period between March, 2001 and January 2002.

Twenty-three patients with vitiligo were included in this study, their ages ranged between 12-65 years, with mean ± SD (34.47±15.05).Eleven patients (47%) were males and twelve patients (53%) were females. A detailed history about the onset of the disease, distribution, severity, family history and associated disease was taken and thorough medical examination was done. Patients with associated medical problems like diabetes mellitus, psoriasis, alopecia areata and others were excluded from this study. The severities of the disease were graded according to the rule of nine into three grades.

Mild group: from 1 –10 % of the surface area of the skin involved.

Moderate group: from 11–25% of the surface area of the skin involved.

Severe group: from 26 - > of the surface area of the skin involved.

Eight patients (35%) had mild vitiligo; nine patients (39%) had moderate vitiligo and six patients (26%) Patients had severe vitiligo

Sample Collection: -

Blood samples were collected from vitiligo patients. From each patient 10 ml of venous blood were collected in heparinized tubes using disposable syringes with 22 x u/4-G, hypodermic needles. Part of blood were left to clot and centrifuged at 3000 pm for 10 minutes. The separated plasma sample was stored at 20C° until biochemical analysis performed.

Measurement of the Basal Level of Erythrocytes and Plasma Malondialdehyde (MDA)

Erythrocytes were separated from plasma by centrifugation at 3000 pm for 10 minutes at 4C°. Buffy coat was carefully removed, and erythrocytes were washed twice with isotonic saline containing 2 ml sodium azide to inhibit catalaze activity [16].

Erythrocytes (MDA) Assay.

Measurement of erythrocyte MDA (which is the by-product of lipid peroxidation), based on the reaction of thiobarbituric acid (TBA) forming TBA. MDA adduct, was carried out using the modified method of stocks and Dormandy [16]. The results were expressed as mole/g Hob based on the molar extinction coefficient of MDA is $1.56x10^5$ M^{-1}. CM^{-1}. A one-ml aliquot of ten percent suspension of erythrocyte in salineazide was pre-incubated for 5 min at 37C°. Preoxidative challenge was induced by the addition of an equal Volume (1.0) ml of various concentration of H_2O_2 in Isotonic saline solution (final H_2O_2 concentration 10, 15, 20m M). Basal level of MDA was obtained without addition of H_2O_2 (i.e. H_2O_2 concentration zero). Following 30 min. Incubation at 37C°, the reaction was terminated by addition of 1-0 ml 28% (TCA- 0.1 M Sodium arsenate). The mixture was centrifuged and (2.0) ml of supernatant was combined with 0.5 ml distilled water 1.0 ml of 0.5% TBA in 0.05 M sodium hydroxide. Color development was achieved by boiling for 15 minutes. The tubes were cooled under tap water and the extent of MDA production was estimated from the absorbencies at 532 and 453 nm [16].

Plasma (MDA) Assay:

To 0.25 ml of plasma, a 0.75 ml saline–azide was added, and then the procedure is the same as that described for erythrocytes MDA. The results were expressed as µmole/L that also based on the molar extinction coefficient of MDA.

Glutathione Assay

Erythrocytes Glutathione (GSH) Assay: -

Erythrocytes GSH content was determined to the method of Godin et al [17]. Aliquots of 0.1 ml packed erythrocytes were used, and combined with 0.1 ml distilled water and 0.65 ml of 5% TCA-1mM Na_2 EDTA. Then centrifuged and the supernatant analyzed for sulfhydryl group content at 412 nm using 3 mM DTNB in phosphate buffer. The assay mixture contained 2.6 ml of 0.1 M phosphate buffer (pH=8.0), 0.3 ml supernatant and 0.1 ml DTNB. The light absorbance of the solution at 412 nm is measured after 2 minutes waiting. Known amount of GSH were assayed by the same method and used for calculation of GSH quantities in erythrocytes.

Plasma Glutathione (GSH) Assay:

Plasma was used 05. Ml was taken to assay GSH with 0.5 ml 5% TCA, 1 mM Na_2 EDTA as described above.

Antioxidant Therapy

All patients included in this study were received a combination of vitamin C and E, (vitamin C 500 mg, vitamin E 100 mg twice a day) for two weeks and then the oxidative stress (plasma and red blood cells Malondialdehyde) and the antioxidants (plasma and red blood cells Glutathione) were measured again.

The response to two weeks of treatment with antioxidants (vitamin C and E) was assessed through observing the mean change in 4 response variables (indicators). The magnitude of response to treatment was stratified by severity group of vitiligo and finally presented for the whole group of vitiligo cases.

Results:

Plasma MDA

As shown in table (1), only the mild group showed a statistically significant reduction in mean plasma MDA of 0.86 micro mole/L after two weeks of treatment. The changes observed in mean plasma MDA in the remaining 2 groups and in the total cases were small and not significant statistically.

Table 1: The changes in plasma MDA concentration (micro mole/L) after two weeks of treatment by severity group of vitiligo.

		Plasma MDA concentration. (micro-mole/L)	
		Baseline	**2 weeks after intervention**
1.	Mild vitiligo		
	Minimum	1.061	.953
	Maximum	4.461	2.358
	Mean	2.35	1.49
	SE	.448	.193
	N	8	8
	P (paired t-test) = 0.02		
2.	Moderate vitiligo		
	Minimum	.384	.538
	Maximum	4.330	2.840
	Mean	1.51	1.61
	SE	.406	.255
	N	9	9
	P (paired t-test) = 0.79[NS]		
3.	Severe vitiligo		
	Minimum	.461	.584
	Maximum	1.784	2.061
	Mean	1.20	1.36
	SE	.219	.216
	N	6	6
	P (paired t-test) = 0.63[NS]		
4.	Total Cases		
	Minimum	.384	.538
	Maximum	4.461	2.840
	Mean	1.72	1.50
	SE	.243	.129
	N	23	23
	P (paired t-test) = 0.32[NS]		

Erythrocytes MDA

As shown in table (2), the changes observed in mean erythrocytes MDA in the 3 severity groups of vitiligo and in the total cases were small in magnitude and not significant statistically.

Table 2: The changes in erythrocytes MDA concentration (nano mole/gm Hb) after two weeks of treatment by severity group of vitiligo.

		Erythrocytes MDA conc. (nano mole/gm Hb)	
		Baseline	**2 weeks after intervention**
1.	Mild vitiligo		
	Minimum	21.050	7.185
	Maximum	44.701	48.878
	Mean	28.75	23.84
	SE	2.986	4.592
	N	8	8
	P (paired t-test) = 0.43*[NS]*		
2.	Moderate vitiligo		
	Minimum	7.431	6.758
	Maximum	57.460	34.817
	Mean	19.80	21.21
	SE	4.990	2.796
	N	9	9
	P (paired t-test) = 0.71*[NS]*		
3.	Severe vitiligo		
	Minimum	4.403	11.023
	Maximum	26.081	25.944
	Mean	15.00	18.58
	SE	3.514	2.671
	N	6	6
	P (paired t-test) = 0.29*[NS]*		
4.	Total		
	Minimum	4.403	6.758
	Maximum	57.460	48.878
	Mean	21.66	21.44
	SE	2.578	2.014
	N	23	23
	P (paired t-test) = 0.93*[NS]*		

Plasma GSH

As shown in table (3), the changes observed in mean Plasma GSH in the 3 severity groups of vitiligo and in the total cases were small in magnitude and not significant statistically. The smallest change observed was -0.01 in the mild group and the highest change was +0.1 in the severe group.

Table 3: The changes in plasma GSH concentration (micro mole/L) after two weeks of treatment by severity group of vitiligo.

		Plasma glutathione conc. (micro-mole/L	
		Baseline	**2 weeks after intervention**
1.	Mild vitiligo		
	Minimum	.092	.127
	Maximum	.323	.208
	Mean	.18	.16
	SE	.028	.011
	N	8	8
	P (paired t-test) = 0.65*[NS]*		
2.	Moderate vitiligo		
	Minimum	.042	.150
	Maximum	.346	.935
	Mean	.16	.25
	SE	.033	.086
	N	9	9
	P (paired t-test) = 0.38*[NS]*		
3.	Severe vitiligo		
	Minimum	.080	.102
	Maximum	.265	.902
	Mean	.17	.27
	SE	.034	.127
	N	6	6
	P (paired t-test) = 0.51*[NS]*		
4.	Total		
	Minimum	.042	.102
	Maximum	.346	.935
	Mean	.17	.23
	SE	.018	.046
	N	23	23
	P (paired t-test) = 0.29*[NS]*		

Erythrocytes GSH

As shown in table (4), only the mild group showed a statistically significant reduction in mean erythrocytes GSH concentration of 3.65 nano mole/gm Hb after two weeks of treatment. The changes observed in mean erythrocytes GSH in the remaining 2 groups and in the total cases were small and not significant statistically.

Table 4: The changes in erythrocytes GSH concentration (nano mole/gm Hb) after two weeks of treatment by severity group of vitiligo.

		Erythrocytes glutathione conc. (micro-mole/gm Hb)	
		Baseline	**2 weeks after intervention**
1.	Mild vitiligo		
	Minimum	5.400	2.700
	Maximum	24.430	14.820
	Mean	10.13	6.48
	SE	2.124	1.296
	N	8	8
	P (paired t-test) = 0.02		
2.	Moderate vitiligo		
	Minimum	3.276	2.812
	Maximum	9.110	11.719
	Mean	6.68	6.71
	SE	.670	.959
	N	9	9
	P (paired t-test) = 0.98*[NS]*		
3.	Severe vitiligo		
	Minimum	.784	.907
	Maximum	5.860	7.375
	Mean	3.77	4.88
	SE	.938	1.130
	N	6	6
	P (paired t-test) = 0.43*[NS]*		
4.	Total		
	Minimum	.784	.907
	Maximum	24.430	14.820
	Mean	7.12	6.15
	SE	.946	.647
	N	23	23
	P (paired t-test) = 0.25*[NS]*		

Discussion:

Vitiligo is a common skin disease characterized clinically by hypo and /or depigmented macules and patches, and histologically by the absence of identifiable melanocytes. The cause of depigmentation in vitiligo was attributed to the destruction of melanocytes [1].

There are 3 theories concerned with the cause of vitiligo; the auto-immune theory, the auto-cytotoxic theory and neural theory. Reactive oxygen species seem to play a crucial role in the destruction of melanocytes in all of these theories [2].

In the past there were several studies concerned with the role of free radicals and antioxidant mechanisms in vitiligo [4, 5, 6, 12, 13, 14, 15]. A recent Iraqi study showed that there is a highly significant reduction in the levels of antioxidants and an increase in the levels of free radicals in patients with vitiligo compared to control [15].

In human body, there is a critical equilibrium between the levels of free radicals and the antioxidant mechanisms in human body that can prevent ameliorate or limit the damage caused by free radicals. Sometimes this equilibrium became exhausted and necessitates the use of exogenous antioxidant to help the antioxidant systems in the body [15].

This encouraged us to use the antioxidants in patients with vitiligo and to watch any decrease in the free radicals level.

The response to two weeks of treatment with antioxidants was interesting. The results showed that only the mild group showed a statistically significant reduction in mean plasma MDA of 0.86 micro mole/L after two weeks of treatment $P<0.02$. The changes observed in mean plasma MDA in the remaining 2 groups and in the total cases were small and not statistically significant, while in erythrocytes MDA, the changes observed in mean erythrocytes MDA in the 3 severity groups of vitiligo and in the total cases were small in magnitude and not significant statistically. Also, the changes observed in mean Plasma GSH in the 3 severity groups of vitiligo and in the total cases were small in magnitude and not statistically significant.

These results showed that the oxidative stress decreased significantly after treatment with an antioxidant drugs in the mild group, i.e. early in the course of the disease when the disease is still active and this may have a therapeutic applications suggesting that antioxidants may help to decrease the oxidative stress if it was given early in the course of the disease. However, the changes in plasma and erythrocyte GSH was not significant after 2 weeks of treatment, and this may be attributed to the short period of treatment, (i.e. if we give the treatment for a longer period we may reach a significant difference in plasma and erythrocyte GSH).

These 2 weeks of therapy with antioxidant drugs is not enough to assess the clinical response and repigmentation in vitiligo, but if the antioxidants are used for a longer time, they might probably help in the regeneration of melanocytes causing recovery of the disease, or it might act as a prophylactic agent preventing new lesions appearance.

This trial of therapy might support the new topical antioxidant (superoxide dismutase"SOD" and Catalaze complex) in patients with vitiligo [17].

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