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Journal of Clinical & Experimental Dermatology Research

Research Article

Low Antioxidant Reserve in First Degree Relatives of Vitiligo Patients

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ABSTRACT

Background: Vitiligo is a chronic autoimmune skin condition characterized by the loss of melanocytes leading to a white patch on the skin. Oxidative stress is a significant key factor in the destruction of melanocytes however antioxidants help neutralize free radicals, which can damage cells and contribute to oxidative stress.

Aims: Our study explored how the role of demographics, lifestyle factors and total antioxidant reserves may interplay in the disease outcome of vitiligo. In this pilot study, we evaluated the total antioxidant reserve in spectrums of vitiligo patients, their first-degree family members and independent healthy individuals.

Methods: A total of 150 Indian study participants were recruited. Venous blood was collected from 59 Vitiligo Patients (VP), their corresponding 59 paired family members (VH) and 32 independent Healthy Controls (HC). Serum antioxidant capacity (TAC) was measured using the standard ELISA technique and correlated with clinicopathological findings.

Results: Our results reveal that lower TAC in both severe vitiligo and family members compared to unrelated HC shows a significant association (VP severe *vs.* HC; P=0.0005 and VH severe *vs.* HC; P=0.0003), indicating a familial predisposition to oxidative stress in this patient's group as a disease-modulating factor.

Limitations: Small sample size; assessments of oxidative stress as well as antioxidant reserves marker substantiate with large sample size in vitiligo patients and association with 1° and 2° degree family members.

Conclusion: Based on these findings, we concluded that oxidative stress should be monitored and treated in vitiligo patients and their family members as a preventive measure to reduce severe forms of vitiligo and many chronic diseases. Larger studies will further validate the approach and its broader application in other oxidative stress conditions.

Keywords: Vitiligo; Total antioxidant capacity; 1° family members; Severe; Stable

INTRODUCTION

Vitiligo also known as leukoderma is an autoimmune, non-contagious disease in which progressive loss of pigment from skin, overlying hair and oral mucosa consequences from the loss of melanocytes. The aetiology of the disease is still not clear. Many factors have been implicated in the aetiology and pathogenesis of the vitiligo including infections, stress, neural abnormalities, anti-melanocyte antibody, defective melanocyte adhesion and genetic susceptibility. It is the most common pigmentary disorder affecting 0.1%-2% of the world's population irrespective of race and gender and its incidence ranges from 0.1 to 8.8% in India although the states of Gujrat and Rajasthan have a highest dominance of 8.8%. It can develop at any age but approximately half of all vitiligo patients onset is before the age of 20 years. Though, vitiligo is usually not

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Received: 06-Nov-2024, Manuscript No. JCEDR-24-34981; **Editor assigned:** 08-Nov-2024, PreQC No. JCEDR-24-34981 (PQ); **Reviewed:** 22-Nov-2024, QC No. JCEDR-24-34981; **Revised:** 07-Feb-2025, Manuscript No. JBFBP-24-35147 (R); **Published:** 14-Feb-2025, DOI: 10.35841/2155-9554.25.16.688

Citation: Rajput Y, Mahilang D, Yadav A, Dhruve K, Singh MK, Koshley V, et al. (2025) Low Antioxidant Reserve in First Degree Relatives of Vitiligo Patients. J Clin Exp Dermatol Res. 16:688.

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incapacitating the patients physically, but still it has serious implication in patient's personal and social life. Vitiligo patients and their family are treated in different way in our community like low esteemed, causing serious psychological trauma and feeling of socially isolation to the patients [1].

Thus according to biochemical hypothesis, melanocyte destruction is due to the accumulation of toxic metabolites from melanogenesis, the breakdown of free radical defense and an excess of hydrogen peroxide. Recent study suggests that oxidative stress as the initial triggering factor in precipitating of vitiligo. Major Oxidative stress markers are Total Antioxidant Capacity (TAC), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Nitric Oxide (NO) synthetase and Malondialdehyde (MDA). Nowadays, in Chattisgarh there is gradual increase in pollutant level because of industrialization, civilization, emission of motor vehicles etc. which are important determinant for triggering the different kinds of stresses including oxidative, immunological and psychological. It causes several adverse effects on health like so many skin problems including vitiligo in Chattisgarh. Besides this, various other geographical factors may also contribute to increase in above factors [2].

There is no study reported exploring antioxidant capacity (TAC) in vitiligo in Indian patients group, correlation with first degree family member. In this study we will evaluate the Total Antioxidants (TAC) reserve rather than individual oxidative stress markers, in progressive vitiligo compare with stable group, subsequently based on the antioxidant level correlation with the clinicopathological parameters, through this pilot study we could find out the vital precipitation factors within the shortest time period. This study may help to formulate personalize treatment strategy in vitiligo patients and also formulate the actual reason of gradual increase in vitiligo patients in Central India [3].

MATERIALS AND METHODS

Study design

It was a cross sectional and case control study.

Ethical statement

This study was carried out at Department of Skin and Venereal Diseases (VD), B.R.A.M. Hospital and Multi-disciplinary Research Unit (MRU), Pt. Jawaharlal Nehru Memorial Medical College, Raipur, Chhattisgarh, India, following approval of the protocol by the institutional ethical committee, IEC (MC/Ethics/56). All procedures were carried out in accordance with the Declaration of Helsinki. Human samples were collected following informed written consent from the study subjects [4].

Study participants

Subjects who attended the Department of Skin and Venereal Diseases (VD), B.R.A.M. Hospital, Pt. J.N.M. Medical College, Raipur, Chhattisgarh, India with white patch had clinical diagnosis of vitiligo and were willing to participate in the study, were referred to Multi-Disciplinary Research Unit (MRU), Pt. Jawaharlal Nehru Memorial Medical College, Raipur, Chhattisgarh, India for enrollment. Normal course of clinical management of the patients was not hampered by the study protocol. Having systemic acute and chronic disease, Undertaken systemic or topical treatment within 3 month, undertaken drugs for any other reason in last one month were excluded from the study. Patients who had active and static vitiligo and also they should be <40 years of age as well as no previous history of systemic, acute and chronic diseases were recruited for the study. Also enrolled the attenders who were first degree family member and age and sex matched healthy volunteer [5].

Sample collection and processing

Relevant history to be taken before collecting samples *i.e.*, occupation, smoking, alcohol consumption, diet, migration (recent/past), chronic systemic diseases, drug history, recent or past infection.

Blood sample collection and storage: Venous blood samples collected from the vitiligo patients as well as paired control and independent healthy volunteers also. After collection of the whole blood, allowed the blood to clot by leaving it undisturbed at room temperature for 15-30 minutes. Centrifuging at 1,000-2,000 × g for 10 minutes in a refrigerated centrifuge and the resulting supernatants serum were kept at -20°C till further processing.

Measurement of Total Antioxidant Capacity (TAC) assay: Total Antioxidant Capacity (TAC) were assessed in stored serum samples by OxiSelectTM Total Antioxidant Capacity (TAC) assay kit (Cell Biolabs, Inc, San Diego, CA) as per the manufacturer's protocol using Multimode Microplate Reader (TECAN, Mannedorf, Switzerland) [6].

Statistical analysis

In our study group mean and standard deviation were performed in excel sheet. Difference between the observed means in two independent samples and its significance value (P-value) as well as 95% Confidence Interval (CI) were calculated by using software https://www.medcalc.org/calc/comparison_of_means.php. Kolmogorov-Smirnov test of normality was analyzed using social science statistics https://www.socscistatistics.com/tutorials/ttest/default.aspx. Statistical significance was considered if $p \leq 0.05$. The data were further evaluated by one-way Analysis of Variance (ANOVA) accounting for different groups of vitiligo patients and their corresponding paired controls (1° family member) compared with independent healthy control group with post-hoc Tukey-Kramer comparisons [7].

RESULTS

Demographic profile of vitiligo patients, paired control (1° family member) and independent healthy control

In the present pilot study total 59 vitiligo patients, their corresponding 59 paired control (1° family members) and 32 independent healthy control were recruited. Mean age of vitiligo patients, paired control and independent healthy control were 27.1 ± 18.4, 34.7 ± 11.4 and 27.8 ± 6.6 years respectively. Out of 59 vitiligo patients 18 (31%) were male and 41 (86%) female. Out of 59 patients 1° family members 25 (59%) were male and 24 (41%) female. Out of 34 independent healthy control 24 (71%) were male and 10 (29%) female (Table 1). Majority of vitiligo patients were below 20 years of age group (52.54%, n=31), detail age distribution of the vitligo patients are described in Table 2 [8].

Table 1: Demographic profile of vitiligo case (VP), 1° family member (VH) and Healthy Controls (HC).

Mean ± SD (in years)	Vitiligo Patient (VP)			Paired control (VH)			Healthy Control (HC)		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
	31.222± 18.65	25.341± 18.305	27.136± 18.453	36.419± 12.876	32.521 ± 9.205	34.720 ± 11.49	28.917 ± 7.046	25.3 ± 4.877	27.853 ± 6.624
VP *vs.* VH	t-test	2.68		VH *vs.* HC	t-test	0.218			
	P value	0.0084*	P value		0.8278				
	95% CI	1.9788 to 13.1892	95% CI		5.8107 to 7.247				

Note: VP: Vitiligo Patients; VH: Paired Control, HC: Healthy Control, the student's t test has been used for all sample, P is the significance level, CI is confidence interval in 95%, *=significance value

Table 2: Age wise distribution of vitiligo patients.

Age group distribution (in years)	Vitiligo Patient (VP)		
	Male% (n)	Female% (n)	Total% (n)
<20	11.86% (7)	40.67% (24)	52.54% (31)
20-40	8.47% (5)	11.86%(7)	20.33% (12)
40-60	8.47% (5)	13.55% (8)	22.03% (13)
60-80	1.69% (1)	3.39% (2)	5.08% (3)
Total (n)	30.50% (18)	69.69% (41)	59

Note: VP: Vitiligo Patients; VH: Paired Control; HC: sHealthy Control

Clinicopathological association with sociodemographic profile of vitiligo patients

The distribution of clinicopathological profile of patients group correlation with social and demographical status of participants were given in Table 3. Out of 59 patients, 33.89% had grouped in progressive vitiligo, followed by 30.20% of stationary vitiligo, 20.33% belongs to resistant vitiligo where as improved patients were only 13.55%. Maximum number of vitiligo patients were belongs to student category 55.93% (33/59) however 22.03% (13/59) participants were engaged in housebound work, 10.16% (6/59) were labour group, 8.47% recorded in office employee but only 3.38% (2/59) farmers were affected. We studied according to the food habit like vegetarian, non-vegetarian and eggetarian in different stage of vitiligo patients like progressive, stationary, improving and resistant, in which higher cases were undertaken the non-vegetarian category which includes fish, chicken and mutton 52.54% (31/59) where most of the participant were in progressive phase 16.95% whereas only 28.81% (17/59) patients had vegetarian dietary food habit and eggetarian patients population were 18.64% [9].

Table 3: Clinicopathological association with sociodemographic profile in vitiligo patients.

	Progressive % of total (n)	Stationary % of total (n)	Resistant % of total (n)	Improving % of total (n)	Total % of total (n)
Disease spectrum	33.89% (20)	30.20% (19)	20.33% (12)	13.55% (8)	59
Occupation profile					
Student	18.64% (11)	16.95% (10)	13.55% (8)	6.78% (4)	55.93% (33)
Housebound	6.78% (4)	6.78% (4)	5.08% (3)	3.39% (2)	22.03% (13)
Laborer	1.69% (1)	6.78% (4)	0% (0)	1.69 (1)	10.16% (6)
Office worker	3.39% (2)	1.69% (1)	1.69% (1)	1.69% (1)	8.47% (5)
Farmer	3.38% (2)	0% (0)	0% (0)	0% (0)	3.38% (2)
Dietary profile					
Vegetarian	6.78% (4)	6.78% (4)	8.47% (5)	6.78% (4)	28.81% (17)
Non vegetarian	16.95% (10)	23.72 (14)	6.78% (4)	5.08(3)	52.54% (31)
Eggetarian	10.17% (6)	1.69% (1)	5.08% (3)	1.69% (1)	18.64% (11)

Total antioxidant reserve in vitiligo patient as well as their 1° degree family members compared with independent healthy control

Total antioxidant reserve was evaluated in vitiligo patients group and also their paired control (1° degree family member) compared with independent healthy control details in Table 4. The mean value as well as standard deviation of Total Antioxidant Capacity (TAC) in vitiligo patients was 0.371 ± 0.107 mM, whereas 0.379 ± 0.124 mM in 1° degree family member but TAC level was high 0.442 ± 0.114 mM in random population. Evaluation of the blood antioxidant capacity of the subjects in relation to random population revealed a significant difference according to student's t-test (p=0.0034) among vitiligo patients (95% CI: 0.0241 to 0.1179) whereas correlation between 1° family member and random population also observed a significant difference (p=0.0171; 95% CI: -0.1145 to -0.0115), however there was no significance between patients group and 1° family member (paired control) p=0.7082 (Table 5).

Table 4: Total antioxidant reserve in vitiligo patient as well as their 1° degree family members compared with healthy control (mean ± SD, student's t test, p value and 95% confidence interval).

Study group		VP	VH	HC
Number of individual (n)		59	59	32
Serum TAC mean (mM) ± SD		0.371 ± 0.107	0.379 ± 0.124	0.442 ± 0.114
VP *vs.* HC	t-test	0.0047		
	P value	0.0034*		
	CI	0.0241 to 0.1179		
VP *vs.* VH	t-test	0.717		
	P value	0.7082		
	CI	-0.0342 to 0.0502		
HC *vs.* VH	t-test	0.0158		
	P value	0.0171*		
	CI	-0.1145 to -0.0115		

Note: VP: Vitiligo Patients; VH: Paired Control; HC: Healthy Control, the student's t test has been used for all sample, P is the significance level, CI is confidence interval in 95%, *=significance value

Table 5: TAC profile of different clinicopathological sub-groups of vitiligo patients.

Sub-groups	TAC (mM) Mean ± SD		t-test		
	VP	VH	VP *vs.* HC	VP *vs.* VH	HC *vs.* VH
Progressive (n=20)	0.344 ± 0.093	0.329 ± 0.080	0.0003*	0.5712	0.0022*
Stationary (n=19)	0.408 ± 0.111	0.429 ± 0.153	0.301	0.63	0.72
Improving (n=8)	0.363 ± 0.168	0.390 ± 0.130	0.11	0.72	0.27
Resistant (n=12)	0.388 ± 0.078	0.350 ± 0.095	0.13	0.29	0.016*
HC (n=34)	0.442 ± 0.114				

Note: VP: Vitiligo Patients; VH: Paired Control; HC: Healthy Control, P is the significance level, *=significance value, SD: Standard Deviation

Evaluation of total TAC profile of different clinicopathological subgroups of vitiligo patients

In this study, the level of TAC in progressive vitiligo group (n=20) were found to be statistically significant as compared with the independent healthy control (P=0.0003) and also shows significance relationship with 1° degree family members with healthy volunteers (P=0.016). Similarly resistant 1o family group also shows significant relation with the healthy control. No significant difference was found between stationary, improving and resistant vitiligo patients with healthy control as well as 1° family members details in Tables 6 and 7.

Comparison of TAC profile between stable and severe viligo patients and compared with independent healthy control

On the basis of severity of vitiligo, subject group is divided into two groups *i.e.*, stable and severe. Stable group belongs to improving as well as stationary group where as severe group includes progressive with resistant. One-way ANOVA analysis revealed that in our study groups severe as well as stable vitiligo patients as well as their corresponding paired control (1° degree family members) are showing significant correlation with independent healthy controls. The f-ratio value is 7.11216 and p-value is 0.000029. The result is significant at $p \leq 0.05$ (Table 6). p-value of distinct comparison of stable *vs.* severe vitiligo and their relative control (Table 7). VH-stable *vs.* VP-severe, VP-severe *vs.* HC and VH-severe *vs.* HC shows significant association. Supplementary Tables 1 and 2 predict the pair wise comparisons of stable *vs.* severe vitiligo and their paired control (1° family member) compared with healthy control within one way ANOVA data.

Table 6: TAC profile of stable *vs.* severe vitiligo patients.

Category	Stable vitiligo (n=27)	Severe vitiligo (n=32)	Healthy control (n=34)
	Mean ± SD (mM)	Mean ± SD (mM)	Mean ± SD (mM)
VP	0.395 ± 0.129	0.351 ± 0.083	0.442 ± 0.114
VH	0.417 ± 0.143	0.345 ± 0.092	
One-way ANOVA	f-ratio 7.11216; p-value 0.000029***. The result is significant at p<0.05		

Note: Whereas stable vitiligo means improving with stationary and severe vitiligo means progressive with resistant. VP=Vitiligo Patient, VH=paired control (1° family member), Healthy control. Data are presented as mean ± SD, n=total number in each individual group, *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.0001$ *vs.* healthy group with one-way ANOVA followed by post hoc turkey (HSD) test. Total ∑X=60.5307 and ∑X2=26.1358

Table 7: p-value of distinct comparison of stable *vs.* severe vitiligo and their relative control.

S. no.	Group of comparison	P value	Interpretation
A.	VP-stable *vs.* VP-severe	0.119	Non-significant
B.	VH-stable *vs.* VP-severe	0.0235*	Significant
C.	VP-stable *vs.* HC	0.136	Non-significant
D.	VP-severe *vs.* HC	0.0005*	Significant
E.	VH-stable *vs.* VP-severe	0.0313	Significant
F.	VP-stable *vs.* VH-severe	0.0881	Non-significant
G.	VH-stable *vs.* HC	0.45	Non-significant
H.	VH-severe *vs.* HC	0.0003*	Significant

Note: VP: Vitiligo Patients, VH: Paired Control; HC: Healthy Control, P is the significance level, *=significance value

Familial aggregation of TAC level with vitiligo patients

Out of 59 paired samples 46 were of 1° relatives (mother: 15, father: 10, son: 6, daughter: 4, sister: 3 and brother: 8) shows non significance correlation (P=0.595) with vitiligo patients and 1° grandfather and rest were non blood relatives (brother in-law, husband, wife) detailed profile in Table 8. Out of 9 blood relatives of VP patients husband: 4, wife 2 and 3 other relative (like brother-in-law, sister-in-law etc.) shows no significance (P=0.644). When we correlated with the antioxidant level of cumulative familial aggregation with patients, it's again reflects the non-significant association (P=0.708). However we observed highly significant correlation in Vitiligo Patient (VP) *vs.* independant Healthy Control (HC) and Vitiligo Paired control (VP) *vs.* Healthy Control (HC) were P=0.0034 and P=0.0171 respectively. As per our patients history, only one patient indicates the familial predisposition rest all the patients having no family history of vitiligo.

Table 8: Familial aggregation of TAC level with vitiligo patients.

S. no.	Relation with study subjects	Total (n)	VP (Mean ± SD)	VH (Mean ± SD)	P (<0.05)	Remark	Family history
Relation of 1° family member with patients							
1	Father	10	0.389 ± 0.103	0.420 ± 0.099	0.492	NS	N
2	Mother	15	0.313 ± 0.084	0.323 ± 0.068	0.743	NS	N
3	Brother	8	0.417 ± 0.124	0.405 ± 0.149	0.869	NS	N
4	Sister	3	0.382 ± 0.136	0.358 ± 0.144	0.838	NS	1
5	Daughter	4	0.438 ± 0.113	0.327 ± 0.098	0.192	NS	N
6	Son	6	0.429 ± 0.165	0.497 ± 0.125	0.443	NS	N
A	Total	46	0.394 ± 0.045	0.388 ± 0.066	0.595	NS	N
Non blood relation with patients							
1	Husband	4	0.379 ± 0.076	0.418 ± 0.068	0.481	NS	NA
2	Wife	2	0.256 ± 0.056	0.221 ± 0.033	0.534	NS	NA

3	Other	3	0.275 ± 0.150	0.327 ± 0.207	0.74	NS	NA
B	Total	9	0.303 ± 0.066	0.322 ± 0.098	0.644	NS	NA
No information							
C	Total	4	0.368 ± 0.033	0.407 ± 0.181	0.681	NS	NA
A+B+C	Grand total	59	0.371 ± 0.107	0.379 ± 0.124	0.708	NS	NA
HC	Healthy control	32	0.442 ± 0.114				
VP *vs.* HC	P=0.0034*					S	NA
VH *vs.* HC	P=0.0171*					S	NA

DISCUSSION

Vitiligo is the acquired pigmentary autoimmune disorder of the skin. We conducted a pilot study in vitiligo patient group and compared with the paired control (First degree family members) as well as independent healthy control.

In the present study blood samples of total 59 vitiligo patients, 59 paired control and of 34 independent healthy volunteers as independent control were collected. Average age of the vitiligo patients was observed 27.136 ± 18.453 years. Out of this, mean age group of male was 31.22 ± 18.65 years whereas 25.341 ± 18.305 years in female. Our finding was supported by Singh et al. 13 reported that the total average of the vitiligo patients was 27.35 ± 10.45 years in which mean age of male group was 30.75 ± 15.41 years whereas in female 26.07 ± 14.63 in Punjabian population of India. In the present study group maximum onset of symptom of vitiligo were below age of twenty. Research suggests that the onset of vitiligo symptoms typically occurs before the age of 20 in about 50% of cases. The incidence of vitiligo was higher in female patients group 69.69% (41/59) as compare to male group 30.50% (18/59). This finding is supported with the previous study with a predilection for women being affected two times more often than men where in few reports both genders almost with equal frequency of vitiligo.

Maximum number of vilitgo patients were belongs to student age group 55.93%, followed by house bound occupations 22.03%, labour 10.16%, Office worker 8.47% where as farmers were 3.38%. The occupational profile of vitiligo cases was similar to earlier published studies. Similar finding was observed in the study conducted in South Korea in which the most frequent occupation was house wife (33.0%), followed by office job (30.4%), production (7.4%), student (6.8%). Food habit and lifestyle were important predisposition factors for triggering the oxidative stress. Previous reports suggested that there is a correlation between junk and food with vitiligo. In our study group most of the patients follows the non-vegetarian food habit, same hypothesis establisheded that the incompatible diet (Viruddha ahara) is a potent cause of several diseases especially in present era when faulty dietary practice is in vague. Interestingly we reveal out that most of the patients who belong to progressive vitiligo clinical type having a non-vegetarian dietary habit.

Oxidative stress is one of the key factors in multifactorial pathogenesis of vitiligo. Oxidative stress occurs when there's an imbalance between the production of free radicals (unstable molecules) and the body's ability to neutralize them. In our study population Total Antioxidant Reserve (TAC) in vitliligo petients as well as their 1° family members was lower than independent healthy control. When we segregated the vitiligo patients group into progressive, stationary, improving and resistant, we observed that TAC level in progressive vitiligo as well as corresponding family members was significantly decreased compared with the independent healthy control, suggesting importance of antioxidant reserve for preventing progression of vitiligo (VP *vs.* HC; P=0.0003 and VH *vs.* HC; P=0.0022). Carrying out the one-way ANOVA in stable, severe vitiligo group with independent healthy control indicates highly significant association (P=0.000029). Interestingly when we combined the all the spectram of clinical presentation of vitiligo into two group stable (improving and stationary group) and severe (progressive and resistant groups), we observed lower TAC in both severe vitligo as well as 1° family members compared to unrelated HC (VP severe *vs.* HC ; P=0.0005 and VH severe *vs.* HC; 0.0003). Previous studies established the fact that oxidative stress plays vital role in the pathogenesis of vitiligo. Interestingly our study suggest that the prevalence of oxidative stress is high not only in severe form of vitiligo patients but also in their family members indicating familial predisposition of oxidative stress in this patient's group as disease modulating factor. Our findings also supported the previous research work that an enhanced oxidative stress status in the blood of the Egyptian vitiligo population as evidenced by a significant reduction in TAC along with a significant increase in plasma MDA levels of patients compared to controls however similar findings also observed in Indian population. To our knowledge, this is the first report of the anti-oxidant status of the family members of vitiligo patients. At a standstill, bigger sample size are required to establish the correlation. The association of low antioxidant reserve in severe vitiligo along with their family members could be due to common food habit, lifestyle or genetically determined.

Previously it was reported that vitiligo have genetic predisposition. However, in our disease spectrum of vitiligo patients, we could not show any genetic correlation between vitiligo patients and 1° family member. Only one patient had a genetic history, whereas in the rest of the vitiligo group, no family history was reported. There is conflicting evidence of genetic predisposition of vitiligo while some Egyptian study shows genetically non-significant association of vitiligo while the American study observed some significant association (Figures 1 and 2).

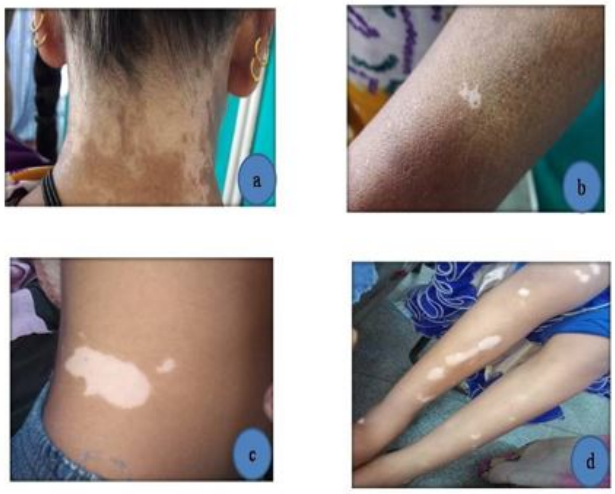

Figure 1: Vitiligo patients: A) patches of resistant condition in vitiligo; b) Patches of improving stage in vitiligo; c) Patches of stationary stage in vitiligo; d) Patches of progressive stage in vitiligo.

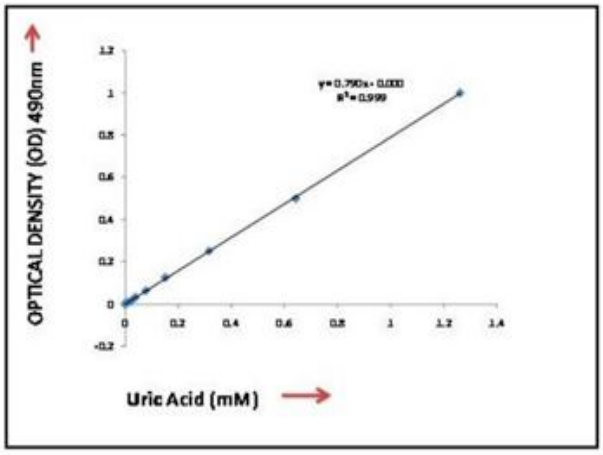

Figure 2: A linear regression curve of Total Antioxidant Capacity (TAC) assay with a correlation coefficient of 0.999 and regression equation of y=0.790x+0.0.

Limitations

Small sample size; assessments of oxidative stress as well as antioxidant reserves marker substantiate with large sample size in vitiligo patients and association with 1° and 2° degree family members.

CONCLUSION

Our study suggest that the prevalence of oxidative stress is high not only in severe form of vitiligo patients but also in their family members indicating familial predisposition of oxidative stress in this patient's group as disease modulating factor. Accordingly oxidative stress should be monitor and treated in vitiligo patients as well as their family members as preventive measure to reduce severe form of vitiligo and many chronic diseases.

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