

## Serum Homocysteine: Is it a Biomarker for Vitiligo?

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### Abstract

**Background:** Vitiligo is a common, multifactorial, polygenic pigmentary disorder with a complex pathogenesis. Free radical and immune mediated damage of melanocytes are the most probable pathological mechanism. There have been several conflicting reports on the blood levels of vitamin B12, folate and homocysteine in vitiligo and its severity. Because of relation between vitamin B12, RBC folate and homocysteine, we checked serum level of vitamin B12 and RBC folate as well.

**Methods:** In this study a total of 50 patients and 53 age and sex matched healthy controls were enrolled. Serum vitamin B12, homocysteine and RBC folate are checked. Disease activity was assessed by Vitiligo Disease Activity (VIDA) score and disease severity assessed by VASI score.

**Results:** The two groups did not differ significantly in RBC folate concentrations and serum levels of vitamin B12. Patients with vitiligo had significantly lower levels of homocysteine compared to healthy controls. Hyperhomocysteinaemia was detected in 34 (64.2%) healthy control but only in 18 (36%) patients with vitiligo.

**Conclusions:** Our study showed that serum homocysteine level did not affect the vitiligo severity and homocysteine level was not higher in majority of vitiligo patients comparing to healthy controls. But vitamin B12 had significant association with disease severity and a negative correlation was found.

**Keywords:** Vitiligo; Homocysteine; Vitamin B12; RBC folate

**Abbreviations:** Hcy: Homocysteine; Vit: Vitamin

### Introduction

Vitiligo is a common, multifactorial, polygenic pigmentary disorder with a complex pathogenesis and prevalence of 1-2% of worldwide population [1]. The etiology of vitiligo still remains unknown. Different theories concerning autoimmune, cytotoxic, biochemical, neural, and oxidant-antioxidant mechanisms have been suggested in the pathogenesis of vitiligo [2]. Free radicals and immune mediated damage of melanocytes are the most probable pathological mechanism [3]. An association between vitiligo and reduced serum levels of vitamin B12 and folic acid has been suggested [4], and recently, it has been found that serum Hcy is elevated in patients with vitiligo [5]. Elevated Hcy level causes oxidative stress on melanocytes by producing reactive oxygen species [6]. Furthermore, there are reports that Hcy inhibits tyrosinase activity by binding to copper in its active site, resulting in reversible hypopigmentation [7]. There have been several conflicting reports on the blood levels of vitamin B12 and folate in vitiligo [8-11]. In this study we tried to assess the serum level of Hcy, vitamin B12 and RBC folate and evaluate our data for supporting the role of oxidative stress in the pathogenesis of vitiligo.

### Materials and Methods

A total of 50 patients and 53 age and sex matched healthy controls were enrolled in this study from the outpatient department of Dermatology of Shohada-e- Tajrish Hospital from Shihid Beheshti University Of Medical Sciences, Tehran, Iran. The healthy controls were selected from patient's family to eliminate the difference of nutritional habits. Clinical diagnosis of the vitiligo patients was done by the dermatologist. Patients and controls with history of vitamins, methotrexate, oral contraceptive pills, phenytoin, carbamazepine, theophyllin, metformin, diuretics, nitric oxide, DOPA, retinoids, statins, immune suppressor drugs, fibrates and niasin intake within the last six months, smoking, hypertension, genetic amino acid metabolism disorders, chronic liver or kidney disease, diabetes mellitus, metabolic syndrome, systemic lupus erythematosus and other rhomatologic disease, deep vein thrombosis, poly cystic ovary syndrome,

inflammatory bowel disease, other skin diseases, hypothyroidism, cancers, low HDL or teriglycerid level, BMI<20 or BMI> 27, sleeping disorders and pregnancy were excluded from the study.

The local ethics committee approved the study design and all the participants signed an informed consent letter.

Venous blood samples were drawn after a 12-hour fasting. RBC folate and vitamin B12 were measured in both patients and control by electrochemiluminescent assay using assay kit Elecys (Roche, Germany), and Serum Hcy was done by Enzyme Callorimetry, kit of Axis shield kit by Cobas Mira (Roche, Germany). Normal range of Vit B12 is 160-970 pg/ml and folate is 1.5-17 ng/ml. Normal range of Hcy is: subjects<15y=<10 µl/L, adults (15-60y) = 5-15 µl/L, subjects>60y = 5-20 µl/L.

Disease activity was assessed by Vitiligo Disease Activity (VIDA) score [12] and disease severity assessed by VASI score [13]. According to distribution pattern, the patients were classified as non-segmental (acrofacial, mucosal (more than one site affected), generalized or common, universal, mixed (associated with segmental vitiligo), segmental and unclassified or indeterminate [14].

### Statistical analysis

Statistical analysis was performed using the statistical software SPSS 18.0.0. (SPSS Inc. Chicago, IL, U.S.A.). P-values less than 0.05 were considered statistically significant.

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Continuous variables are reported as mean  $\pm$  SD or as median with interquartile range (25th-75th percentiles). Categorical data are expressed as number (percentage). Shapiro-Wilk's W-test was used to examine the normality assumption of continuous variables.

Mann-Whitney U-test was applied for comparison between serum levels of homocysteine and vitamin B12 and t-test was used for comparison of RBC folate concentrations of the two groups. Pearson chi-square test was applied whenever the expected cell frequencies were at least 5. With small expected frequencies, Fisher's exact test was employed. Spearman's correlation coefficients were reported for the association between continuous variables.

In addition, multiple linear regression analysis was applied to determine the parameters most predictive of the serum homocysteine level. A step-wise forward regression algorithm was used to choose variables entering in the final standard least square model. All variables that were significant in univariate analysis and biologically plausible to affect serum homocysteine level were selected for examining in this algorithm. The logarithmic transformation of serum homocysteine level was used to improve the fit of the model. Analysis of the residuals was performed to detect violations in regression modelling assumptions.

## Results

This study is comprised of 50 patients with vitiligo and 53 age and sex frequency-matched healthy controls. Baseline demographics and clinical characteristics of patients and controls are presented in Table 1. There were no significant differences between these two groups according to age, BMI and gender of participants (Table 1).

The two groups did not differ significantly in RBC folate concentrations and serum levels of vitamin B12 (Table 2). There was no significant difference in mean RBC folate concentrations of males and females ( $909.76 \pm 249.16$  ng/mL for males vs.  $915.91 \pm 319.73$  ng/mL for females,  $p=0.91$ ). However, the median serum levels of vitamin B12 was significantly higher in female subjects compared to the males (median (IQR): 375 (281.25-444.50) pg/mL for females vs. 319 (231-395.25) pg/mL for males,  $p=0.03$ ). Low RBC folate was observed in one patient and none of the healthy controls ( $p=0.48$ ). Low vitamin B12 was detected in one of the patients and one of the healthy controls ( $p=1.00$ ).

Patients with vitiligo had significantly lower levels of homocysteine than healthy controls ( $p=0.001$ , Table 2). Our findings demonstrated significantly lower levels of homocysteine in female subjects compared to the male individuals (Median (IQR): 11 (10-16.15)  $\mu$ mol/L in females vs. 18 (15-22)  $\mu$ mol/L in males,  $p<0.0001$ ). Hyperhomocysteinaemia was detected in 18 (36%) patients with vitiligo and 34 (64.2%) healthy controls ( $p=0.006$ ). Forty out of 58 (69%) male subjects and 12 out of 45 (26.7%) female subjects had hyperhomocysteinaemia ( $p<0.0001$ ).

Serum homocysteine level was negatively associated with vitamin B12 level in both groups of study ( $r=-0.38$ ,  $p=0.01$  in patients and  $r=-0.34$ ,  $p=0.01$  in controls). Also, homocysteine was inversely associated with RBC folate in patient group ( $r=-0.36$ ,  $p=0.01$ ). When the two groups were combined, serum homocysteine concentration was negatively related to serum vitamin B12 level ( $r=-0.33$ ,  $p=0.001$ ).

According to our findings, no significant association was detected between serum homocysteine level and severity of disease (VASI score) ( $r=0.25$ ,  $p=0.08$ ), disease activity (VIDA score) ( $r=0.19$ ,  $p=0.18$ ), body surface area involved ( $r=0.22$ ,  $p=0.12$ ) and duration of disease ( $r=0.08$ ,  $p=0.56$ ). Serum vitamin B12 level was inversely associated with VASI score ( $r=-0.33$ ,  $p=0.02$ ) and body surface area involved ( $r=-0.32$ ,  $p=0.02$ ). However, there was no significant association between

serum levels of vitamin B12 and both VIDA score ( $r=-0.14$ ,  $p=0.33$ ) and duration of the disease ( $r=-0.27$ ,  $p=0.06$ ). Furthermore, RBC folate concentration was not significantly associated with VASI score ( $r=-0.26$ ,  $p=0.07$ ), VIDA score ( $r=-0.19$ ,  $p=0.18$ ), body surface area involved ( $r=-0.19$ ,  $p=0.19$ ) and duration of disease ( $r=0.09$ ,  $p=0.55$ ).

Characteristic	Patients with vitiligo (n=50)	Healthy controls (n=53)	P-value
Gender, no. (%)			0.95
Female	22 (44%)	23 (43.4%)	
Male	28 (56%)	30 (56.6%)	
Age, years	28.5 (21.8-37.8); (15-62)	27 (23-39.5); (20-62)	0.62
BMI	24.5 (21.6-26.8); (20.1-27.3)	24.4 (21.5-26.8); (20-27.2)	0.92
Duration of disease, year	5.5 (2.8-13); (0.33-44)		
Positive family history of vitiligo	18 (36%)		
Positive history of UV-therapy*	30 (60%)		
Type of vitiligo			
Non-segmental	48 (96%)		
Acrofacial	3		
Mixed	0		
Generalized or Common	43		
Universal	2		
Mucosal(more than one site)	0		
Segmental	0		
Unclassified or indeterminate	2 (4%)		
Focal	2		
Mucosal(only one site affected)	0		
Site of involvement			
Hands	36 (72%)		
Upper extremities	36 (72%)		
Lower extremities	37 (74%)		
Trunk	32 (64%)		
Feet	30 (60%)		
Head & neck	32 (64%)		
BSA involvement, %	2.5 (1-7.6); (0.1-93)		
VASI score	1.62 (0.49-5.54); (0.04-89.3)		
VIDA			
-1	8 (16%)		
0	2 (4%)		
+1	4 (8%)		
+2	10 (20%)		
+3	9 (18%)		
+4	17 (34%)		

The values are expressed as no. (%) or median (IQR); range.

\* Positive history of UV-therapy within the last two months.

Abbreviations: BMI, Body Mass Index (calculated as weight in kilograms divided by height in meters squared); IQR, Interquartile range (25th -75th percentiles); VASI: Vitiligo Area Scoring Index; VIDA score, vitiligo disease activity score

Note: Grading of VIDA score is as follows: +4, Activity of 6 weeks or less duration; +3, Activity of 6 weeks to three months; +2, Activity of 3-6 months; +1, Activity of 6-12 months; 0, Stable for one year or more; and -1, Stable with spontaneous repigmentation since one year or more. A low VIDA score indicates less activity.

**Table 1:** Baseline demographics and clinical characteristics of the study participants.

	(n=50)	Healthy controls (n=53)	P-value
Serum vitamin B12 (pg/mL)	340.5 (260-399.25)	345 (240.5-438.5)	0.61
RBC Folate (ng/mL), Mean $\pm$ SD	883.38 $\pm$ 304.14	939.87 $\pm$ 256.56	0.31
Serum Homocysteine ( $\mu$ mol/L)	12.5 (10-18)	17 (14.4-21.95)	0.001
Low vitamin B12 #			
	1 (2%)	1 (1.9%)	1
Low RBC Folate†	1 (2%)	0	0.48
Hyperhomocysteinaemia*	18 (36%)	34 (64.2%)	0.006

Abbreviations: IQR, Interquartile range (25th -75th percentiles); RBC Folate, Red Blood Cell folate

# Low vitamin B12 is defined as values less than 160 ng/mL.

**Table 2:** Laboratory findings of the patients with vitiligo and healthy controls.

	Beta	Standard	t-value	p-value
Predictors				
Constant	3.09	0.15	20.87	<.0001
Age	0.008	0.003	2.72	0.008
Gender (males vs. females)	0.33	0.06	5.22	<.0001
Serum B12 level	-0.001	0.0001	-3.18	0.002
RBC folate	-0.0004	0.0001	-3.72	0.0003
Group (patients vs. controls)	-0.25	0.06	-4.11	<.0001
Adjusted R-squared=0.43, F=16.18, p<.0001				
Note: Serum homocysteine level was log-transformed to improve the fit of the model. In addition, three outliers were excluded from the statistical analysis.				

**Table 3:** Summary of multiple regression analyses to identify factors associated with serum homocysteine level.

Multiple regression analyses were applied including all subjects (patients with vitiligo and healthy controls) and introducing “group of study” to the model as a dummy variable. According to the results of step-wise forward regression, age, gender, serum vitamin B12 level, RBC folate and group of study were significantly associated with log-transformed homocysteine levels (adjusted  $R^2=0.43$ ,  $F=16.18$ ,  $p<.0001$ ). Summary of multivariable regression model is presented in Table 3. Based on the results of step-wise forward regression in patients with vitiligo, gender and serum levels of vitamin B12 were the only significant predictors of log-transformed homocysteine levels (adjusted  $R^2=0.28$ ,  $F=20.27$ ,  $p<.0001$ ).

## Discussion

Vitiligo is a commonly acquired, idiopathic, heritable depigmentary disorder of skin [15]. Poor body image due to cosmetic disfigurement leads to low self-esteem and psychological trauma and therefore poor quality of life [16]. The generalized pattern of vitiligo was the most common subtype our study, as other studies [17].

The pathogenesis of vitiligo is not fully understood yet. Vitiligo may derive from programmed melanocyte death or destruction due to inherent sensitivity to oxidative stress arising from toxic intermediates of melanin, a melanocyte-specific protein or other sources that included vitamin B12, folate and Hcy [18]. An association has been suggested between vitiligo and pernicious anemia and/or folic acid deficiency [19]. Park et al. [19] reported lower vitamin B12, folate levels in the vitiligo patients. There are studies that show no differences in vitamin B12 and folate levels between vitiligo and control groups [4,20,21]. However, this issue remains controversial. In this study we evaluate the RBC folate as opposed to serum level for having more accurate data. Furthermore unlike previous study we use the VASI and VIDA score simultaneously with body surface area involved to assess the correlation of Hcy with disease severity and activity, respectively.

In our study, serum Vit B12 and RBC folate levels differences between two groups were not statistically significant. There was no significant relation between the disease activity and Vit B12 and folate levels. We found Vit B12 levels significantly higher in the female patients. Sex, activity and severity of vitiligo did not affect the levels of RBC folate, but Vit B12 had significant association with disease severity and serum vitamin B12 level was inversely associated with VASI score.

Yasar and et al. [11] found that Hcy levels were not altered in vitiligo, on the other hand Shaker and et al. [5] and Balci and et al. [20] reported significantly higher Hcy levels in vitiligo patients. Shaker and et al. [5] also reported significantly higher Hcy levels in patients with especially progressive vitiligo. They recommended routine determination of Hcy levels in patients with vitiligo and addition of Hcy lowering agents such as Vit B12 and folic acid to the vitiligo treatment protocol. But contrary to their finding in our study the median Hcy level in patients was lower than control group. Hyperhomocysteinemia was detected in 34 (64.2%) healthy controls but only in 18 (36%) patients with vitiligo ( $p=0.006$ ). The Hcy level in female patients was lower than male patients and this may be explained by hormonal status, greater muscle mass in men and gender related lifestyle difference [21,22].

Eighteen (36%) of our patients had hyperhomocysteinaemia, but overall there was no significant statistical association between Hcy level and disease severity, disease activity, body surface area involved and duration of disease was lower than. Like Yasar and et al. [11] we suggested that ethnic differences might have effects on Hcy levels. Another limitation was that, in Shaker and et al. study, vitiligo patients had a more severe disease classification and may have affected the Hcy levels. In addition other oxidative stress may be implicated in the pathogenesis of vitiligo as Jain et al. [23] revealed that malondialdehyde (an oxidative stress marker) levels were significantly raised while those of vitamin E, uric acid and seroluplasmin were significantly lowered in blood of vitiligo patients.

After further analysis we did not find significant association between Hcy and severity and activity in vitiligo patients, unlike Shaker et al. [5] and Suman et al. [10] in their studies.

Considering our finding, nutritional deficiency in Vit B12 and folic acid and serum Hcy level did not affect the vitiligo severity and did not have role the pathogenesis of this disease, but despite these results we suggested to perform another study with a large sample size to evaluate this role.

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