

## Serum Level of Vitamin B12 in Patients with Vitiligo and its Potential Role as a Disease Biomarker

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### Abstract

**Background:** Vitiligo is an acquired depigmenting cutaneous disorder characterized by the loss of melanocytes from the epidermis. It affects approximately 0.5% of the population worldwide.

**Aim of Study:** To determine serum vitamin B 12 level in patients with vitiligo and healthy controls to determine its possible role as a disease biomarker.

**Patients and Methods:** This study was conducted on 40 patients with vitiligo and 40 age and sex matched healthy controls recruited from El-Demerdash Hospital, Ain Shams University.

**Results:** Serum level of vitamin B12 decreased in cases in comparison to control, and there is an inverse relation between vitamin B 12 and affected body parts.

**Conclusion:** This study confirms that vitamin B12 is a very efficient and simple biomarker for vitiligo cases. Thus we recommended the use of vitamin B 12 in investigation to improve our diagnostic capabilities in vitiligo patients.

**Key Words:** Serum vitamin B12 – Vitiligo – Disease biomarker.

### Introduction

**VITILIGO** is an acquired pigmentation disorder of the skin in which there is destruction of melanocytes. It is characterized by white patches on the skin. Around 0.1-2% of the world's population is affected by the disease, irrespective of race and sex. The disorder has been reported to have a high incidence in the second and third decades of life [1].

Vitiligo is classified into segmental and non-segmental vitiligo. Non-segmental vitiligo is usually bilateral and symmetrical in distribution. Segmental vitiligo is unilateral and focal in distribution [2]. The etiopathogenesis of vitiligo involves various

factors, which include oxidative stress [3], autoimmune destruction of melanocytes, neural hypothesis through an accumulation of a neurochemical substance that decreases melanin production, and sympathetic nervous system activity through direct cytotoxic effect and indirectly through the generation of free radicals [4]. Moreover, vitiligo may co-occur with other autoimmune disorders, such as hypo- or hyper-thyroidism, diabetes, adrenocortical insufficiency, rheumatoid arthritis, and pernicious anemia. Other triggering factors include sunburn, environmental or industrial chemicals, and stress [5].

Vitamins are known to play important roles in the process of skin pigmentation. Vitamin B12 inhibits the production of homocysteine, a homologue of amino acid cysteine. Homocysteine down regulates the activity of tyrosinase, an enzyme responsible for melanin production, as well as generates free radicals, leading to impaired melanin synthesis and destruction of melanocytes. In this whole process, folic acid works in tandem with vitamin B12 as a methyl group donor. This is why it is always recommended to take these two vitamins together in order to treat vitiligo. According to some scientific studies, a combination of vitamin B 12 and folic acid supplementation and sun exposure is a good strategy to regain natural skin color [6].

It is believed that patients with vitiligo are more likely to have pernicious anemia and Vitamin B 12 deficiency. Vitamin B12 and folic acid are major determinants of Hcy levels and a nutritional deficiency in either of them results in hyperhomocysteinemia. A lot of studies showed that, the serum level of Hcy was significantly increased in vitiligo patients than in the control and serum Vitamin B 12 level was significantly lower in vitiligo patients than in the controls [7].

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Biomarkers analysis can be useful to follow patients over time and even predict the chance of future disease progression [8].

#### *Aim of the Work:*

The aim of this study is to determine serum vitamin B12 level in patients with vitiligo and healthy controls to determine its possible role in the disease biomarker.

### **Patients and Methods**

The study included 40 patients suffering from different grades, mild, moderate and severe of vitiligo during the period between 2019 till 2020 and forty age matched healthy volunteers were included as controls. The diagnosis was confirmed via clinical diagnosis, dermatological findings.

The study included patients more than 16 years old affected by vitiligo. While patients with hormonal therapy, pregnancy and anemia and patients and volunteers with vitamin intake especially vitamin B12 were excluded from the study.

All included patients were subjected to full history taking including the personal history including: Name, age, sex, marital status, address, occupation, telephone number, special habits of medical importance, past medical history for the presence of any chronic illness, family history for the presence of vitiligo and detailed history for the vitiligo condition including the onset of vitiligo, the course and duration of current incident of vitiligo. All data were filed and documented.

Clinical examination included general and dermatological examination: Careful and dermatological examination were performed to detect any signs of systemic disease or dermatological disease.

#### *Local examination:*

Local examination was aimed to confirm the diagnosis of vitiligo and assess the severity of the disease. The extent and severity of the disease was graded according to the vitiligo area scoring index.

#### *Photographic documentation:*

Standard digital photographs were taken during the first visit for the affected area before taking samples.

#### *Methods:*

##### *Detection of vitamin B12:*

The level of vitamin B 12 in the study participants was assessed using Enzyme linked immunosorbent assay (ELISA) Technique. The vitamin

B 12 kit was purchased from CALBIOTECH company, catalog No:VB369B.

#### *Vitiligo severity:*

The severity of vitiligo as measured by VASI, the VASI score is determined by summing the vitiligo area in the hands unit and the degree of depigmentation in each hand unit examined.

*Measuring method:* Physical examination.

*Measuring instrument:* Study sheet status, VASI score Measuring Result: Score 0-100.

#### *Sample collection and storage:*

*Serum-samples:* The serum sample was taken by venipuncture and was coagulated at room temperature 10-20 minutes, then centrifuged 20 minutes at the speed of 5000 r.p.m, then the serum was separated and stored at 20c.

#### *Reagent preparation:*

##### *1- Extraction agent:*

Before use, dilute the TCEP solution 1:40 with extraction buffer.

##### *2- Sample extraction:*

Label test tube then add 50ul of each sample to be tested, then 25ul of extraction reagent to each sample and allowed to proceed for 15 minutes. After that 25ul of neutralization buffer was added to samples and stand for 5 minutes.

#### *Prepare wash buffer:*

By adding the content of bottle (25ml, 20 X) to distilled water at room temperature.

#### *Assay procedure:*

All reagent and specimens must be at room temperature, and all reagent must be gently mixed without foaming. Then dispense 50ul of extracted vitamin B12 into tubes and 50ul of intrinsic factor reagent followed by shaking gently for 20-30 second to mix.

All was incubated for 45 minutes at room temperature. Then 50ul of enzyme conjugate was added and shaken gently for 20 second to mix, and incubated for 30 minutes. After that we rinse by wash buffer three times and 100ul of TMB Substrate was added into them. After 15 minutes, stop solution was added and gently mix until uniform color was obtained. Then we start reading the absorbance in each sample to get the result.

#### *Ethical considerations:*

The study protocol was approved by Faculty of Medicine, Ain Shams University, Research

Ethics Committee. The patients gave written consents to participate in the work after explanation of the technique, expectation and possible side effects.

*Statistical methods:*

The collected data were coded, tabulated, then statistically analyzed using IBM SPSS statistics (V. 26.0, IBM Corp., USA, 2019). Data were expressed as median and percentiles for quantitative non-parametric measures and both number and percentage for categorized data. The probability of error at 0.05 was considered sig., while at 0.01 and 0.001 are highly significant.

**Results**

The age of patients ranged from 19 to 62 years with median age of 37.5, with different duration of disease ranging from 0.06 to 20 years with median of 4.

Our patient group has different area affected ranging from 0.5 to 14 with median 1 and different in last activity time and stability from 0 to 4 with median of 3. Vitamin B12 level ranged from 0.629 to 509 with median 63.71 as shown in Table (1).

The healthy volunteers age ranged from 24-64 years old with median of 43 and they have vitamin B12 level ranged from 204.8 to 963 with median 519.9 as shown in Table (2).

Table (1): Descriptive statistics for patient group.

	N	Median	Min.	Max.	Percentile	
					25	75
Age	40	37.5	19	62	30.25	45
Dur	40	4	0.067	20	2	9.5
VASI. Score	40	1	0.5	14	0.75	2.375
VIDA. Score	40	3	0	4	1	4
Vit. B 12	40	63.71	0.629	509	51.89	80.835

Table (2): Descriptive Statistics for control group.

	N	Median	Min.	Max.	Percentile	
					25	75
Age	40	43	24	64	33	50
Vit. B12	40	519.9	204.8	963	404.05	625.525

*Comparison between case and control:*

The mean age and gender distribution were similar in case and control with no statistically significant difference. Moreover patients suffered from vitiligo has highly significant decreased vitamin B 12 among them in comparison to healthy group with (median 519.9 for control Vs 63.71 for cases) ( $p < 0.001$ ) as shown in Table (3).

Table (3): Comparison between control and cases as regards gender.

		Case	Control	Total
Sex: Female	Count	23	23	46
	%	57.5%	57.5%	57.5%
Male	Count	17	17	34
	%	42.5%	42.5%	42.5%
Total	Count	40	40	80
	%	100.0%	100.0%	100.0%
Chi-Square Tests				
		Value	<i>p</i>	
Pearson Chi-Square		0.000 <sup>a</sup>	1.000	

Table (4): Comparison between control and cases as regards age and vitamin B12.

	N	Median	25 Perc	75 Perc	Z	<i>p</i>	Sig.
<i>Age:</i>							
Control	40	43	33	50	-1.887	0.059	NS
Cases	40	37.5	30.25	45			
<i>Vit. B12:</i>							
Control	40	519.9	404.05	625.525	-7.467	0	HS
Cases	40	63.71	51.89	80.835			

*Correlation between vitamin B12 and all studied parameter:*

There were no significant differences between levels of vitamin B12 in patients regarding their Age ( $p=0.756$ ), Duration ( $p=0.19$ ), VASI SCORE ( $p=0.169$ ) and VIDA SCORE ( $p=0.234$ ). But in our study there is an inverse relation between Vitamin B12 level and affected parts in patients with vitiligo with ( $r=-0.363$ ,  $p=0.021$ ) as shown in Table (5).

Table (5): Correlation study between vitamin B 12 and all studied parameters. Ranked Sperman Corre hlation Test:

	Vit. B 12		Sig.
	<i>r</i>	<i>p</i>	
Age	-0.051	0.756	NS
Dur	-0.211	0.19	NS
VASI. Score	-0.222	0.169	NS
VIDA. Score	-0.193	0.234	NS
Aff.B. Parts	-0.363	0.021	S

Also, distribution of lesions in vitligo patients not affected with vitamin B 12 level as No-significant change were found between segmental and non segmental subgroups with (median 64.895 for non segmental Vs 61.8 for segmental ( $p=0.755$ )). No significant change found between vitamin B 12

level and different types of vitiligo as (median 67.14 for focal Vs 73.655 for generalized vs 68.155

for acro facial vs 59.205 for acral vs 52.31 for facial ( $p=0.886$ ) as shown in Table (6).

Table (6): Comparison between segmental and non-segmental subgroups as regards all studied parameters.

	Types	N	Median	25 Perc	75 Perc	Z	p	Sig.
Age	Non-Seg.	30	36.5	29.25	43.5	-0.703	0.482	NS
	Seg.	10	39	29.5	49.75			
Dur	Non-Seg.	30	4.5	2	10	-0.754	0.451	NS
	Seg.	10	3	2	8.5			
VASI. Score	Non-Seg.	30	1.5	1	3.25	-2.996	0.003	HS
	Seg.	10	0.75	0.5	1			
VIDA. Score	Non-Seg.	30	4	2	4	-1.3	0.194	NS
	Seg.	10	2	1	4			
Vit. B 12	Non-Seg.	30	64.895	53.8575	82.8675	-0.312	0.755	NS
	Seg.	10	61.8	50.7575	74.02			
Aff.B. Parts	Non-Seg.	30	2	2	3	-1.517	0.129	NS
	Seg.	10	1.5	1	2.25			

## Discussion

Vitiligo, a depigmenting skin disorder, is characterized by the selective loss of melanocytes, which in turn leads to pigment dilution in the affected areas of the skin. Considerable recent progress has been made in our understanding of the pathogenesis of vitiligo, and it is now clearly classified as autoimmune disease, associated with genetic and environmental factors together with metabolic, oxidative stress and cell detachment abnormalities [9].

It is believed that patients with vitiligo are more likely to have pernicious anemia and vitamin B 12 deficiency. Vitamin B12 and folic acid are major determinants of homocysteine (Hcy) levels, and a nutritional deficiency in either of these two vitamins results in hyperhomocysteinemia. Hcy also leads to inhibition of tyrosinase enzyme by binding with copper at its active site, resulting in reversible hypopigmentation [10].

Our study recruited forty patients with vitiligo and we found that vitamin B 12 level in the serum, is highly decreased. In concordance with our study, Tsai et al., [11] investigated the serum level of vitamin B12 in twenty-two studies involving a total of 1448 patients with vitiligo and showed that, patients with vitiligo had significantly lower vitamin B12 levels (SMD -0.430, 95% CI -0.738 to -0.121;  $I^2$  85.3%) than controls.

There is inverse relation between vitamin B12 and affected body parts. In line with our findings, Agarw et al., [12], did a cross-sectional, observa-

tional study consisting of 50 patients with vitiligo, and 35 age and sex matched controls was conducted. Serum Vitamin B 12 levels were estimated by chemiluminescence using the Access Immunoassay System.

According to the results of the present study, the mean serum Vitamin B12 was found to be significantly lower in vitiligo patients than controls ( $157.18 \pm 68.95 \text{ pg/mL}$  vs.  $306.6 \pm 169.73 \text{ pg/mL}$  as compared to controls ( $p < 0.05$ ). It was concluded that Long standing vitiligo (especially universal and generalized variants) may show deranged serum Vitamin B 12.

Park and Lee [13], made a comparison between the subclasses divided according to the distribution of lesions showed that the levels of vitamin B 12 was significantly lower in the generalized type of the disease than in those with the localized type ( $p < 0.05$ ). These results seem to imply that decrease in the serum levels of vitamin B 12 may play a role in the depigmentation process, especially in generalized vitiligo.

These results were similar to that of Karadag et al., [10], who tested the hypothesis that vitamin B 12 and folate metabolism might have an influence on the pathogenesis of vitiligo. Full blood count and levels of folic acid, vitamin B 12 were examined for 69 patients with vitiligo and 52 controls. The vitiligo group had lower levels of vitamin B12 ( $p < 0.01$ ) than the control group. Folic acid levels were similar for both groups. In a risk analysis, vitamin B 12 deficiency ( $< 200 \text{ pg/mL}$ ,  $p < 0.01$ ) was significant risk factors for vitiligo. Also, Park and

Lee [13], reported a significantly lower mean level of vitamin B 12 in Korean vitiligo patients.

There was no significant correlation between vitamin B12 and vitiligo severity. Although mean vitamin B12 level was lower, there is a weak correlation between serum vitamin B 12 level with VASI score. Also this result was obtained by Ghalamkarpour et al., [14].

Also, No correlation was found between vitamin B12 and types of vitiligo. This finding was in agreement with Karadag et al., [10] and Silverberg and Silverberg [15].

We observed that, no statistically significant relation between vitamin B12 and VADI score as in Ghalamkarpour et al., [14] study they found that, 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 ( $r=-0.27$ ,  $p=0.06$ ).

Taieb et al., [16] mention that screening to vitiligo patients for vitamin B12 deficiency may be warranted. Physicians, should also consider requesting a complete blood count as patients might have microcytic anemia. Guidelines published by the European dermatology forum consensus in 2013 on the management of vitiligo.

In conclusion, vitamin B12 deficiency is significantly more prevalent in vitiligo patients and lower vitamin B 12 was detected with more affected area in body of patients. Vitamin B12 level was not related to disease severity, duration and activity, so vitamin B 12 has a role as disease biomarker.

### Conclusions:

Vitamin B 12 was decreased in vitiligo patients and it is a simple way in diagnosis of vitiligo. There is inverse relation between vitamin B 12 and affected body parts so it facilitates prediction of course of the disease and helps in choosing the way of treatment. Screening vitiligo patients for vitamin B12 deficiency is important step in the diagnosis and treatment.

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## مستوى فيتامين ب١٢ فى مصل الدم لدى مرضى البهاق ودوره المحتمل كمؤشر حيوى للمرض

الخلفية: يُعتبر البهاق أحد أنواع الاضطرابات الجلدية، ويُعرف بإزالة الصبغة المكتسب ويتميز بفقدان الخلايا الصباغية من البشرة. ويصيب البهاق ما يقرب من ٠.٥٪ من سكان العالم.

الهدف من الدراسة: تحديد مستوى فيتامين ب١٢ فى مصل الدم لدى مرضى البهاق والضوابط الصحية لتحديد دوره المحتمل فى المرقم الحيوى للمرض.

المرضى وطرق البحث: تم إجراء هذه الدراسة على ٤٠ مريضاً يعانون من البهاق و ٤٠ سنة من المتطابقين فى العمر والجنس من الأصحاء كمجموعة، وقد تم تجميعهم من مستشفى الدمرداش، جامعة عين شمس.

النتائج: انخفض مستوى فيتامين ب١٢ فى الدم فى مجموعة المرضى مقارنة بالمجموعة الضابطة، وهناك علاقة عكسية بين فيتامين ب١٢ وأجزاء الجسم المصابة.

الخلاصة (الاستنتاج): تؤكّد هذه الدراسة أن فيتامين ب١٢ علامة بيولوجية فعالة وبسيطة جداً لحالات البهاق. لذلك أوصينا باستخدام فيتامين ب١٢ فى الاستقصاء لتحسين قدراتنا التشخيصية لدى مرضى البهاق.