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Assessment of Serum Level of Vitamin B12 and Homocysteine in Vitiligo Patients and Their Correlation with Disease Activity and Severity

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ABSTRACT

Background: Vitiligo is a common idiopathic disorder featured by depigmented epidermis and hair follicles. It has been demonstrated that cases with vitiligo are often associated with vitamin B12 (cobalamin) deficiency. Vitamin B12 is a main determinant of homocysteine (Hcy) level. High values of Hcy are reported to be associated with toxic effects on melanocytes, which ultimately end in vitiligo. **Objective:** This study aimed to assess serum level of Hcy and vitamin B12 in vitiligo patients and to detect their correlation with disease activity and severity.

Patients and Methods: This was a case-controlled study conducted on 50 vitiligo patients and 50 healthy controls. General and dermatological examinations were made. Serum levels of vitamin B12 and Hcy were assessed. The severity of the disease was assessed by Vitiligo Area Scoring Index (VASI) score.

Results: There was a highly statistically significant increase in serum Hcy and a highly statistically significant decrease in vitamin B12 in the case group compared to the control group. There was a statistically significant relationship between serum Hcy and both VASI, duration of the studied cases, both sexes, and vitiligo type.

Conclusion: Our study revealed a significant correlation between serum Hcy and both disease activity and severity (VASI). This might indicate that Hcy can be utilized as a useful marker for activity and severity of vitiligo. There was a statistically significant negative correlation between vitamin B12 and severity of vitiligo (VASI score). This might indicate that vitamin B12 can be utilized as a useful marker for severity of vitiligo.

Keywords: Vitiligo, Vitamin B12, Homocysteine, Vitiligo area scoring index.

INTRODUCTION

Vitiligo is a common cutaneous lesion featured by depigmented epidermis and hair follicles. Its prevalence represents about 1% of the population globally. Loss of the pigment cells could be induced by intracellular and extracellular factors and there are a lot of possible systems, which could be comprised. Vitiligo has psychological devastation and negative impact on quality of life (QoL) [1]. It has been demonstrated that vitiligo cases are more susceptible to pernicious anemia and vitamin B12 deficiency [2]. Vitamin B12 is important in melanin synthesis via the stimulation of a lot of enzymatic pathways. Vitamin B12 has been considered as a primary determinant of homocysteine (Hcy) level, as it acts as cofactor of the enzyme methyltransferase for the regeneration of methionine from Hcy, so nutritional deficiency in this vitamin results in hyper homocysteinemia [3]. Increased Hcy values are supposed to have toxic actions on melanocytes which ultimately end in vitiligo. On the other hand, the actual reason of melanocytes destruction is complicated and not totally identified till now [4].

It has been recommended that Hcy exerts a suppressive action on histidase as well as on tyrosinase activity. Hcy has been demonstrated to be accompanied by suppression of tyrosinase enzyme by binding with copper, resulting in transient hypopigmentation [5]. As a result, it has been reported that increased Hcy could interfere with the process of melanogenesis with subsequent stimulation of vitiligo development [6]. Elevation in serum Hcy is accompanied by a broad spectrum of medical disorders, which include psoriasis [7]. Therefore, we aimed to assess the serum level, possible relation and the validity of utilization of Hcy and vitamin B12 in vitiligo patients as biomarkers of activity and severity of vitiligo.

PATIENTS AND METHOD

This was a prospective case-controlled study conducted on a total of 50 vitiligo patients attending the Outpatient Clinics of Dermatology Department of Mansoura University Hospital over a period of one year. They were divided into two groups: Group (A) included 50 patients with vitiligo and group B (control group) comprised 50 age- and sex-matched normal persons.

Exclusion criteria:

Patients with age younger than 20 and older than 50 years, smokers, treated by systemic or topical modalities within the 4 weeks preceding examination, patients took folic acid, vitamin B6, vitamin B12 or hormonal therapy 4 weeks preceding examination and who had diseases known to affect the Hcy levels.

Method:

All cases and the controls were subjected to history taking, which included personal history (age, smoking), present history, family history of vitiligo, medical history and past history of any medications or surgeries.

Complete general and dermatological examination such as the degree of vitiligo, which was estimated by the rule of nines, lesion distribution, types of vitiligo, and bilaterality of lesions. The severity of the disease was evaluated by VASI score [8]. The disease activity was evaluated as following: stable disease– no change in the lesion throughout the previous 60 days based on the patient's observation and progressive lesion– enlarged lesions and/or the development of new lesions within the 60 days before the study based on the patient's observation [9]. Investigations included

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assessment of serum Hcy and vitamin B12. A fasting (three ml) blood sample was drawn. Following clotting, the sample was centrifuged for ten min at 3000 rpm at the Clinical Pathology Department, Mansoura University Hospitals. Serum was separated and stored at -20 ◦C. Serum Hcy and vitamin B12 levels were detected by using ELISA in both groups.

Ethical approval: Our study was conducted after obtaining the approval form The Ethical Committee. Informed consents were taken from all cases and controls included in the study. Patient's privacy was respected. Acceptance of IRB of Mansoura Faculty of Medicine was obtained before starting of the research. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis: Data analysis was conducted by SPSS. Qualitative data were described by utilizing number and percent. Quantitative data were defined using median for non-normally distributed data and mean ± SD for normally distributed data. Chi-Square test was utilized for comparing qualitative data between groups. Mann Whitney U and Kruskall Wallis tests were used to compare between 2 studied groups and more than 2 studied groups, correspondingly for non-normally distributed data. The Spearman's correlation was utilized for detection of a linear correlation between 2 non-normally distributed continuous variables. In terms of previously used tests, p was considered significant when its value was ≤ 0.05.

RESULTS

Table **(1)** demonstrated comparison of sociodemographic features of the studied groups. There were no significant differences between both groups concerning age, gender and family history. Also, there was a highly statistically significant increase in serum Hcy and a highly statistically significant reduction in vitamin B12 in cases group in comparison with control group.

Table (1): Comparison of sociodemographic characteristics, serum Hcy and vitamin B12 of the studied groups

		Cases group N=50(%)	Control group N=50(%)	Test of significance
Age (years)	Mean ±SD	32.22± 11.92	31.62± 11.34	t=0.258 p=0.797
Sex	Male Female	23(46.0) 27(54.0)	27(54.0) 23(46.0)	χ^2=0.640 P=0.424
Family history	-ve +ve	38(76.0) 12(24.0)	43(86.0) 7(14.0)	χ^2=1.62 P=0.202
Serum HCY	(µmol/L)	18.98 (3.41-41.79)	2.83 (0.935-13.26)	Z=7.68 P<0.001*
Vitamin B12	(Pg/ml)	175.42 (36.54-415.64)	257.39 (180.16-900)	Z=4.64 P<0.001*

Median and range: non parametric test.

Table **(2)** revealed the distribution of the studied cases according to disease activity & characteristics. The median duration of the disease was 42 months. Regarding vitiligo type, most of the studied cases were vulgaris (38%), followed by acrofacial (36%) then focal (22%) and lastly segmental (2%) and mucosal (2%), which were recorded in a single patient for each. VASI score distribution among studied cases in which the median value of VASI score was 0.75.

Table (2): Distribution of the studied cases based on disease activity, characteristics and VASI score

		N	%
Duration (months)	Median (min-max)	42(2-360)	
Vitiligo type	Vulgaris	19	38.0
	Segmental	1	2.0
	Mucosal	1	2.0
	Focal	11	22.0
	Acrofacial	18	36.0
Course (Activity)	Stable	28	56.0
	Progressive	22	44.0
VASI score	Median (min-max)	0.75 (0.1-32.0)	

Table **(3)** demonstrated that there was a statistically significant positive correlation between serum Hcy and both VASI and duration of the studied cases (P<0.001). In contrast, no significant correlation was recorded between serum Hcy and age of the studied cases (P>0.05). Regarding the correlation between vitamin B12 and VASI score, duration and age of the studied cases, a significant negative correlation was detected between vitamin B12 and both VASI and duration of the studied cases (P<0.001). In addition, no significant correlation was recorded between vitamin B12 and age of the studied cases (P>0.05).

Table (3): Correlation between serum Hcy & vitamin B12 with VASI Score, duration and age of the studied cases

Cases	Serum Hcy(µmol/L)		Vitamin B12 (Pg/ml)	
	r	P	r	P
VASI	0.998	<0.001*	-0.398	0.006*
Duration (months)	0.949	<0.001*	-0.444	0.001*
Age / years	-0.176	0.221	-0.193	0.195

r: Spearman correlation coefficient, *statistically significant

Table **(4)** demonstrated relation between serum Hcy and sex, family history, disease activity and characteristics. There were statistically significant correlations between serum Hcy and both sex and family history. In addition, a highly statistically significant correlation was recorded between serum Hcy and vitiligo type (P<0.001). Also, a significant relation was recorded between serum Hcy and disease activity (P=0.04). Regarding the relation between vitamin B12, sex, family history, disease activity and characteristics, there were no statistically significant correlations between vitamin B12 and sex, family history, vitiligo type and disease activity (P>0.05).

Table (4): Relation between serum Hcy & vitamin B12 with sex, family history, and disease activity

	Serum Hcy (Umol/L) Median (min-max)	**Test of significance**	**Vitamin B12 (Pg/ml)** Median (min-max)	**Test of significance**
Sex				
Male	19.59(9.88-41.79)	Z=2.31 P=**0.02***	138.86(71.52-406.04)	Z=0.538 P=0.591
Female	15.25(3.41-37.01)		191.90(36.54-415.64)	
Family history				
-ve	17.05(3.41-37.01)	Z=2.45 P=**0.014***	189(70.87-415.64)	Z=1.42 P=0.157
+ve	23.33(9.12-41.79)		124.21(36.54-247.25)	
Vitiligo type				
Vulgaris	20.34(4.53-41.79)	KW=36.79 P<**0.001***	174.13(70.87-381.34)	Kw=9.61 P=0.142
Segmental	14.42(14.42-14.42)		228.67(228.67-228.67)	
Mucosal	12.62(12.62-12.62)		273.66(273.66-273.66)	
Focal	19.05(10.29-22.70)		165.64(84.17-415.64)	
Acrofacial	16.17(3.41-37.01)		145.12(36.54-406.04)	
Course (activity)				
Stable	18.98(3.41-39.19)	Z=1.97 P=**0.04***	185.81(36.54-381.34)	Z=0.043 P=0.966
Progressive	25.69(8.35-41.79)		166.05(71.52-415.64)	

Median and range: non parametric test.

DISCUSSION

Vitiligo, a common depigmented cutaneous lesion, has a measured prevalence of 1% of the population globally. It is featured by the loss of melanocytes, which has been demonstrated to be accompanied by classic non-scaly, chalky-white macules [1]. Of note, different hypotheses are suggested with regard to vitiligo pathogenesis, the typical hypotheses involve the autoimmune damage of melanocytes by chemical substances, and self-destruction. Such hypotheses is reinforced by the fact that the majority of vitiligo cases are often associated with other autoimmune diseases (AID) [8]. Autoimmune destruction of melanocytes is the most recognized theory and is reinforced by the fact that vitiligo cases often have other AID [10]. Different alteration in cytokine, such as increase in IL-6 and TNF-α and a drop in IFN-gamma, have been demonstrated, which are suggestive of its autoimmune nature [11].

Talking about the biochemical abnormalities happening in vitiligo, different authors have revealed increased Hcy, reduction in folic acid, and cobalamin in the serum of vitiligo cases [9, 12]. Vitamin B12 and folic acid are important co-factors needed in Hcy metabolism. Of note, vitamin B12 and folic acid deficiencies have been demonstrated to be accompanied by increased Hcy values, known to be a main predisposing factor in the generation of free radicals in melanocytes. In addition, it has been demonstrated that Hcy suppresses tyrosinase activity by binding with copper, which ultimately ends in transient hypopigmentation. In addition, improved vitiligo was recorded following the administration of cobalamin and folic acid in two studies [10, 13]. Therefore, our study aimed to assess serum level of Hcy and cobalamin in vitiligo cases and to detect their relationship with disease activity and severity.

Regarding comparison of sociodemographic characteristics, we displayed that there were no statistically significant differences between both groups concerning age, sex and family history. The mean age for cases group was 32.22 years and for control group was 31.62 years. The cases group included (46% males & 54% females) the control group included (54% males & 46% females). As regards family history, the cases group included 76% negative family history, while there were 24 % positive family history). **Memon *et al.*** [14] conducted their study on a total of 155 patients in which male to female (M/F) ratio was 42/58 with an average age of 28 years. **Choudhary *et al.*** [10] conducted a study on a total of seventy vitiligo cases in which male to female ratio was 23/47.

Regarding the distribution of the current study, we displayed that the median duration of the disease among patients was 42 months. Regarding vitiligo type, most of the studied cases were vulgaris (38%), followed by acrofacial (36%) then focal (22%) and lastly segmental (2%) and mucosal (2%) which were recorded in a single patient for each. In accordance, **Choudhary *et al.*** [10] have demonstrated that vitiligo vulgaris is the commonest form, demonstrated in 40% of cases, on the other hand vitiligo universalis was recorded in 1.4% of cases. Also, **Mahajan *et al.*** [15] have found that most of cases had involvement up to 10% BSA. The percentages of improvement were 59.5%, 18.7% and 3% in vitiligo vulgaris, focal vitiligo and segmental vitiligo respectively.

In terms of comparison of serum Hcy and vitamin B12, our study revealed that there was a significant elevation in serum Hcy and a significant reduction in vitamin B12 in cases group than in the control group (P<0.001). Likewise, **Agarwal *et al.*** [16] recorded that there was a significant increase in the mean serum Hcy level in vitiligo patients compared to controls (P<0.05),

while the mean serum vitamin B12 levels in vitiligo patients (157.18 ± 68.95 pg/mL) were significantly lower as compared to controls (306.6 ± 169.73 pg/mL) (P < 0.001) . **Karadag *et al.*** [3] also showed higher Hcy and lower vitamin B12 levels in the serum of patients with vitiligo compared to healthy subjects . Similarly, **Choudhary *et al.*** [10] have fond that mean serum Hcy level among vitiligo cases was significantly increased compared to normal subjects (14.4 versus 10.33 micromoles). In the same line **Anbar *et al.*** [17] have reported that fluid analysis of blister of active vitiligo cases was recorded to have increased Hcy values. Both vitamin B12 and folic acid are needed as co-factors by the Hcy methyltransferase for methionine regenerations. Additionally, it has been revealed that deficiency of vitamin B12 or folic acid may be accompanied by increased Hcy and decreased methionine values [18]. On the other hand, **El-Dawela and Abou-Elfetouh** [19] indicated that while serum Hcy levels were higher in vitiligo patients in comparison with healthy individuals, vitamin B12 and folic acid levels weren't different in both groups. In contrast, **Zaki *et al.*** [20] have displayed that no significant difference was detected between serum Hcy in vitiligo and control (P=0.91). Also, some studies reported that serum Hcy and vitamin B12 revealed no significant difference between vitiligo cases and controls [21-23]. The discrepancies among studies may be due to several factors, which could interfere with results such as dietary habits in certain countries (vegetarian subjects), also age and sex play a role in their value.

In the context of disease severity, we revealed that there were a significant positive correlation between serum Hcy and both VASI and duration of the studied cases (P<0.001). In contrast, no significant correlation was recorded between serum Hcy and age of the studied cases (P>0.05). Similarly, our study demonstrated that there were statistically significant negative correlations between vitamin B12 and both VASI and duration of the studied cases (P<0.05). In contrast, no significant relationship was recorded between vitamin B12 and age of the studied cases (P>0.05). This comes in agreement with **Agarwal *et al.*** [16] who revealed a highly significant positive relationship of serum Hcy levels with the duration of disease (P = 0.003) and VASI score (P<0.001). Serum Hcy levels were significantly greater in cases with VASI score ≥ 30 as compared to those with a VASI score of < 30 (P = 0.001). On the contrary there was no correlation between serum vitamin B12 levels and duration or extent (VASI score) of the disease. Also, **Sabry *et al.*** [24] have demonstrated that (as regards the extent) there were significant positive correlations with Hcy level (P<0.05; r=0.559) and a significant negative correlation with serum vitamin B12 level (P<0.05; r=-0.42). This is similar to another novel research conducted by **Choudhary *et al.*** [10] who revealed that serum Hcy levels had a significant correlation with the vitiligo extent. On the contrary, **Hanan and Ahmed** [25] have displayed that; no significant differences were recorded between levels of vitamin B12 in patients as regards their VASI score (p=0.169). Thus, no significant relationship was detected between vitamin B12 and vitiligo severity.

Regarding the correlation between Hcy with demographic and diseases characteristics, our study displayed that there were significant correlations between serum Hcy and both sex and family history. Based on the current study the hcy level was greater in males compared to females as regards the patient group (19.59 vs. 15.25Umol/l). Likewise, **Singh *et al.*** [9] recorded greater Hcy value in males compared to females (31 vs. 22Umol/l). This discrepancy as regards gender in the patient group could be clarified by hormonal changes, increased muscle bulk in males and lifestyle-related changes.

In addition, our study recorded a highly statistically significant relationship between serum Hcy and vitiligo type (P<0.001). Also, a significant correlation was recorded between serum Hcy and disease activity (P=0.04). On the other hand, regarding the correlation between vitamin B12 and demographic and diseases characteristics, we revealed that there were no statistically significant correlations between vitamin B12 and sex, family history, vitiligo type and disease activity (P>0.05). Also, **Singh *et al.*** [9] recorded a significant increase (p<0.05) in serum Hcy values in active vitiligo cases in comparison with stable vitiligo ones (30.2 versus 22.8 micromole/liter). Also, **Shaker and El-Tahlawi** [26] have reported a positive relationship between Hcy level and the disease activity. They recorded significantly greater values of Hcy in cases with advanced disease compared to controls (25.4µmol/L versus 13.1µmol/L). Also this current study is consistent with the study done by **Sabry *et al.*** [24] who have revealed that (as regards the activity) the mean Hcy levels in patients with progressive and stable disease were 20.71 ± 9.11 and 15.69 ± 3.15, respectively, with a significant relation between serum Hcy level and vitiligo activity. There was a non-significant relation between serum vitamin B12 level and vitiligo activity. In addition, they have displayed that there was no correlation between serum Hcy and vitamin B 12 values and age of the patients (P > 0.05; r = 0.264 and P > 0.05; r = −0.138). Also, **Hanan and Ahmed** [25] have demonstrated that there were no significant differences were recoded between values of vitamin B12 in cases with regard to the age (p=0.75) and activity (based on VIDA score) (p>0.05). In contrast, **Agarwal *et al.*** [16] have revealed that there was no significant correlation between serum Hcy or vitamin B12 level and the activity of the disease. This finding also is consistent with the study by **Karadag *et al.*** [3] who displayed that there was no significant difference between Hcy and cobalamin levels in patients with stable or progressive type of vitiligo. This comes in accordance with **Maryam *et al.*** [23] who displayed that among vitiligo cases, serum levels of Hcy and vitamin B12 were similar across various categories of vitiligo

disease activity. Also, they demonstrated that there were no associations between vitamin B12 and age, gender as well as type of vitiligo.

In contrast, different studies reported a significant correlation of serum Hcy level with the activity of disease, it is proposed that Hcy may be a contributory factor in addition to other factors (genetic susceptibility, autoimmune factors, neural, or melanocytorrhagy) in perpetuating a smoldering course of the disease, rather than acting as a trigger for sudden melanocytic death during periods of disease activity [9, 24].

The reason for these contradictory results is not clear. On the other hand, the differences in patient selection, particularly in terms of the severity, type, and duration of vitiligo, as well as their ethnicity, may account for the discrepancies [21]. Taken together, it seems that the presence of a relationship between vitiligo and serum Hcy and cobalamin values remains controversial and needs to be vigorously investigated. Having a clear empathy of the fundamental mechanism of melanocyte disappearance in vitiligo is crucial to finding treatment approaches for arresting further depigmentation of the skin. Increased serum Hcy level and decreased vitamin B12 level might trigger vitiligo in susceptible subjects and that change of serum Hcy seems to be accompanied by the severity and activity of the disease.

Limitations: Small sample size and single based center have been considered the main limitations. Hence, our results couldnot be generalized.

CONCLUSION

Our study displayed that vitiligo was accompanied by significant elevation in Hcy level, significant reduction in vitamin B12 level. These findings might indicate a possible relation between both Hcy & vitamin B12 and pathogenesis of vitiligo. Also, the current study revealed a significant correlation between serum Hcy and both disease activity and severity (VASI). This might indicate that Hcy can be utilized as a useful marker for activity and severity of vitiligo. There was a significant negative correlation between vitamin B12 and severity of vitiligo (VASI score). This might indicate that vitamin B12 can be utilized as a useful marker for severity of vitiligo.

RECOMMENDATIONS

Based on the current study, we recommned additional studies on large number of cases with broader geographical areas have to be conducted to verify our results with a special focus on therapeutic ones that could help in the eclaaution of increased Hcy values, based on vitiligo prognosis. Upcoming studies on vitiligo is required to detect and discover the therapeutic liklehood of Hcy-lowering plans.

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