Serum levels of vitamin B12, folic acid, and homocysteine in patients with vitiligo

Maryam Ghiasi, MD [1,2]
Vahide Lajevardi, MD [1,2]
Abbas Farahbakhsh, MD [1,2]

1. Department of Dermatology, Tehran University of Medical Sciences, Tehran, Iran
2. Autoimmune Bullous Diseases Research Center, Tehran University of Medical Sciences, Tehran, Iran

Corresponding author:
Maryam Ghiasi, MD
Address: Department of Dermatology, Razi Hospital, Vahdat-e-eslami Square, Tehran, Iran
Email: mghiasi@tums.ac.ir

Conflict of Interest: None to declare

Received: 24 January 2015
Accepted: 5 May 2015

Background and Aim: Vitiligo is a hypopigmentation disorder of the skin that is associated with depression and an impaired quality of life. There has been conflicting reports on the association between the disease and the serum levels of homocysteine, vitamin B12, and folic acid.In this study,serum levels of homocysteine, vitamin B12, and folic acid were evaluated in patients with vitiligo.

Methods: Thirty patients with vitiligo and 30 age- and sex-matched healthy controls were recruited. Venous blood samples were obtained from the study subjects and the levels of homocysteine, vitamin B12, and folic acid were measured. Data were analyzed using non-parametric statistical tests.

Results: No significant differences were found in the levels of serum homocysteine, vitamin B12, and folic acid between vitiligo patients and healthy controls. Moreover, there were no associations between these factors and age, body weight, gender, as well as the extent, duration, and type of vitiligo.

Conclusion: It seems that vitiligo is not related to serum levels of homocysteine, vitamin B12, and folic acid.

Keywords: vitiligo, homocysteine, vitamin B12, folic acid

Iran J Dermatol 2015; 18: 45-50

INTRODUCTION

Vitiligo is a pigmentary disorder of the epidermis and hair follicles manifested by hypopigmentation lesions of the skin. It is a common dermatological disease with a reported prevalence of 0.1–4% worldwide [1]. Although there are no other textural changes besides the loss of color [2], vitiligo has been associated with mild to moderate depression and an impaired quality of life [3,4]. The key event in the pathogenesis of vitiligo is destruction of melanocytes in the skin lesions due to a still not fully characterized etiology [5]. However, vitiligo is considered a multifactorial disorder involving a complex interplay between genetic susceptibility, immunological responses, and environmental insults. Oxidative stress has been also suggested to critically implicate in the melanocyte loss in the depigmented macules [6,7].

It has been proposed that local increases in homocysteine may interfere with normal melanogenesis and take part in the disappearance of melanocytes from the skin [8]. This idea was raised from the observation that homocystinuria shares the common manifestation of the skin and hair hypopigmentation with vitiligo [9]. Serum homocysteine levels appear to be higher in patients with vitiligo than in healthy subjects [8,10-12], although other studies indicate a lack of such difference [13,14]. Nevertheless, serum homocysteine has been suggested as a biomarker of vitiligo severity [15]. Moreover, serum levels of vitamin B12 and folic acid [11,16,17], co-enzymes essentially involved in homocysteine remethylation to methionine [18], have been claimed to be lower in patients with vitiligo [12], which implicates them in the presumed excess of homocysteine. However, other studies in this regard have reported no association [13,14,19,20]. It has

also been shown that adding vitamin B12 and folic acid to the treatment regimen of vitiligo patients improves the disease and causes repigmentation of the lesions [17, 21].

Since the data on the serum levels of homocysteine, vitamin B12, and folate in patients with vitiligo are mostly conflicting and there have been controversies over the role of these factors in the pathogenesis of vitiligo, we conducted this study in an Iranian population of vitiligo patients to further investigate the likely association between the disease and the homocysteine, B12, and folate levels.

PARTICIPANTS AND METHODS

This case-control study was carried out in Razi Hospital, Tehran, Iran in 2013. Thirty patients of both sexes with vitiligo and 30 age- and sex-matched healthy controls were recruited to the study. The study protocol followed the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Tehran University of Medical Sciences. Written consent forms were signed by all participants. None of the study subjects had conditions affecting homocysteine, vitamin B12, and folic acid balance, including genetic disorders related to impaired amino acid metabolism, hypertension, diabetes mellitus, thyroid dysfunctions, renal failure, Behcet's disease, psoriasis, pregnancy, cigarette smoking, alcohol consumption, and vitamin supplements intake [8]. Only one patient was on ultraviolet therapy when recruited to the study. Healthy controls were selected from people who were admitted to Razi Hospital for cosmetic reasons.

Personal health information was obtained from the subjects before sample collection using a questionnaire on the demographic profile, medication history, established diseases, and other health problems. Details about the vitiligo condition of the patients were collected by precise dermatological assessments including the extent of vitiligo defined as the area of the skin with hypopigmented lesions and measured by the rules of nine, the disease activity categorized as stable or progressive over the last two months without treatment, the type of vitiligo as generalized, focal, segmental, acrofacial, or universal, and the duration and the family history of the disease. Then, 5 ml of venous blood were drawn from each participant. The serum levels of vitamin B12 and folic acid were measured using the electrochemiluminescence method (ECL, Roche, Germany). Homocysteine levels were measured via enzyme immunoassay (Axis-Shield, UK). The whole blood cell count, hemoglobin, hematocrit, and RBC indices were also assayed using standard laboratory protocols.

Data was analyzed using IBM SPSS Statistics (IBM, Armonk, NY, USA) version 20. The goodness of normal fitting across the data was evaluated using Shapiro-Wilk W test. Since the distributions of serum vitamin B12, folic acid, and homocysteine deviated from the normal fitting, non-parametric tests were used for statistical analysis of the data. The Mann-Whitney U test was applied to compare the differences between healthy controls and vitiligo patients, as well as patients with stable and progressive vitiligo and patients with different genders. The Kruskal-Wallis test was used to compare data across different types of vitiligo. Correlations between numerical variables were evaluated with Spearman rho test. The difference in the distribution of subjects with abnormal vitamin B12 levels between the healthy and vitiligo groups was assessed by the Fisher's exact test. Data are presented as median (interquartile range). *P* values less than 0.05 were considered significant.

RESULTS

Thirty patients with vitiligo, 18 men and 12 women, aged [median, interquartile range] 30.50, 22.75–38.25 years were included in the study. For the control group, 30 sex- and age-matched healthy individuals were recruited (18 men and 12 women, aged 29.50, 22.50–38 years). Basic clinical and laboratory characteristics of study subjects are summarized in Table 1. There was a significant difference between the control and case groups in terms of MCV ($P = 0.01$) as patients with vitiligo had lower MCV. However, there was no difference in other measures.

From 30 patients with vitiligo, 5 (17%) had a positive family history for vitiligo. With respect to the type of vitiligo, 12 patients (40%) had generalized, 8 (26.7%) had acrofacial, 7 (23.3%) had focal, 2 (6.7%) had segmental, and 1 (3.3%) had universal vitiligo. The disease was stable in 11 (36.7%) patients and progressive in 19 (63.3%). The duration of the disease was 44.5, 24–94 months,

Table 1. Basic clinical and laboratory characteristics of the participants.

	Healthy subjects	Vitiligo patients	*P* value
Number of subjects	30	30	–
Gender (men)	18	18	–
Age (year)	29.50, 22.50–38	30.50, 22.75–38.25	–
Weight (kg)	70, 59.50–77	66, 55–80	0.59
WBC (× 10^3/mm^3)	6.20, 5.72–7.25	6.20, 4.95–7.25	0.99
RBC (× 10^6/mm^3)	4.77, 4.35–5.15	4.82, 4.67–5.32	0.78
Platelets (× 10^3/mm^3)	218, 175–259	227, 182–279	0.78
Hemoglobin (g/dl)	13.30, 12.45–15.17	13.70, 12.30–14.92	0.78
Hematocrit (%)	41.80, 37.12–44.75	41.40, 38.05–44.30	0.41
MCV (fl)	87.00, 84.65–90.30	83.25, 80.77–86.67	0.01*
MCH (pg)	28.75, 27.10–31.23	27.65, 25.75–28.92	0.17
MCHC (g/dl)	32.60, 31.55–33.97	32.75, 32.07–33.75	0.82

Data are presented as median, interquartile range. *P<0.05

and vitiligo affected 3, 1.37–8.5 percent of the total body surface. The disease involvement was bilateral in 25 (86.2%) and unilateral in 4 (13.8%) patients.

There were no significant differences in serum levels of vitamin B12, folic acid, and homocysteine between healthy individuals and vitiligo patients (table 2). Among patients with vitiligo, serum levels of vitamin B12, folic acid, and homocysteine were similar across various categories of gender, vitiligo type, laterality, and disease activity (tables 3–6). There were no significant correlations between vitamin B12, folic acid, or homocysteine levels and age, body weight, extent of vitiligo, and duration of the disease (table 7). However, the extent of vitiligo was significantly associated with the duration of the disease (Spearman *rho* =

Table 2. The serum levels of vitamin B12, folic acid, and homocysteine in study groups.

	Healthy subjects	Vitiligo patients	*P* value
Vitamin B12 (pg/ml)	252, 166.5–320.5	302, 204.25–357.25	0.10
Folic acid (ng/ml)	15.95, 11.97–19.10	12.70, 9.50–19.35	0.15
Homocysteine (nmol/ml)	9.15, 5.10–13.70	9.50, 7.22–14.25	0.38

Data are presented as median, interquartile range.

Table 3. Serum levels of vitamin B12, folic acid, and homocysteine in male and female vitiligo patients.

	Men	Women	*P* value
Vitamin B12 (pg/ml)	295, 204–344	307, 220–426	0.66
Folic acid (ng/ml)	12.15, 9.47–19.35	16.40, 9.90–20.00	0.57
Homocysteine (nmol/ml)	10.15, 6.42–15.50	9.00, 7.30–10.50	0.91

Data are presented as median, interquartile range.

Table 4. Serum levels of vitamin B12, folic acid, and homocysteine in patients with different types of vitiligo.

	Generalized	Focal	Acrofacial	Segmental	Universal	*P* value
Vitamin B12 (pg/ml)	295, 218–500	301, 231–364	303, 206–335	288, 185–392	307	0.90
Folic acid (ng/ml)	12.30, 10.37–19.65	10.10, 9.25–20.00	14.45, 8.95–19.52	16.65, 16.4–16.9	17.1	0.87
Homocysteine (nmol/ml)	13.25, 7.15–17.75	8.20, 5.17–10.37	8.45, 7.30–11.02	17.00, 9.00–25.00	10.0	0.48

Data are presented as median, interquartile range, except for the segmental and universal vitiligo. Since there were only two cases with segmental vitiligo, their data are presented as median, minimum to maximum. In the universal vitiligo group, there was only one case, whose data are presented as they were.

Table 5. The serum levels of vitamin B12, folic acid, and homocysteine in patients with unilateral and bilateral vitiligo.

	Unilateral vitiligo	Bilateral vitiligo	*P* value
Vitamin B12 (pg/ml)	345, 206–381	302, 212–364	0.69
Folic acid (ng/ml)	12.65, 9.50–16.25	13.10, 9.65–19.90	0.56
Homocysteine (nmol/ml)	9.35, 7.72–10.37	10.00, 6.85–16.00	0.73

Data are presented as median, interquartile range.

Table 6. The serum levels of vitamin B12, folic acid, and homocysteine in patients with stable and progressive vitiligo.

	Stable vitiligo	Progressive vitiligo	*P* value
Vitamin B12 (pg/ml)	296, 198–342	303, 220–413	0.23
Folic acid (ng/ml)	11.90, 8.72–17.20	13.40, 9.90–20.00	0.17
Homocysteine (nmol/ml)	9.45, 7.12–17.75	10.00, 7.00–12.50	0.89

Data are presented as median, interquartile range.

Table 7. Correlations between vitamin B12, folic acid, or homocysteine levels and age, body weight, extent of vitiligo, and duration of the disease.

		Age	Weight	Extent of vitiligo	Duration of the disease
Vitamin B12	Correlation Coefficient*	- 0.19	- 0.04	0.10	0.16
	P value	0.13	0.73	0.58	0.40
Folic acid	Correlation Coefficient	0.09	- 0.13	0.21	0.09
	P value	0.47	0.33	0.25	0.63
Homocysteine	Correlation Coefficient	0.05	0.09	0.17	- 0.03
	P value	0.69	0.51	0.35	0.98

*Correlation coefficient was measured using Spearman *rho* test.

0.57, $P = 0.001$). Serum homocysteine levels were below the upper limit of the standard reference range (<37 nmol/ml) in all healthy individuals and patients with vitiligo. None of the subjects in the case and control groups had a serum folic acid level below the normal range (<5.7 ng/ml). Four healthy individuals had serum vitamin B12 levels below the normal values (<126.5 pg/ml). No significant difference was found in the distribution of subjects with vitamin B12 deficiency between the two groups.

DISCUSSION

In the present study, the probable connection between vitiligo disease and homocysteine levels, as well as two co-enzymes involved in the metabolism of homocysteine, namely vitamin B12 and folic acid, was evaluated. There are indications that serum homocysteine levels are higher in vitiligo patients compared to healthy individuals. In this regard, Shaker *et al.* reported that serum homocysteine levels were significantly higher in patients with progressive vitiligo than healthy sex- and age-matched controls. In patients with stable vitiligo, however, the mean homocysteine level is comparable to that in controls [8]. Similarly, Singh *et al.* showed that homocysteine levels were higher in sera obtained from vitiligo patients than those from healthy controls. The homocysteine level was related to gender and disease activity; it was higher in males than in females as well as in patients with active vitiligo versus those with a stable disease [10]. Similar results were also obtained in other studies [11,12,22]. Homocysteine may provoke oxidative damage to melanocytes, increase interleukin-6 production, and activate nuclear factor κB, leading to melanocyte destruction [17]. Homocysteine is a sulfur-containing amino acid derived from the metabolism of methionine. Its balance is dependent on its production, its irreversible catabolism to other compounds, and its remethylation to methionine. The latter process uses folate as the methyl donor and involves vitamin B12-related enzymes [23]. Consequently, low plasma levels of vitamin B12 and folic acid have been implicated in elevated concentrations of homocysteine [24], hence the presumed contribution to the pathogenesis of vitiligo [16,17]. In this regard, Singh *et a.l* reported that in comparison with healthy individuals, patients with vitiligo had significantly lower levels of serum vitamin B12 and folic acid but higher levels of serum homocysteine [12]. Karadag *et al*. also showed higher homocysteine and lower vitamin B12 levels in the serum of patients with vitiligo compared to healthy subjects, but failed to show any difference in the serum folic acid levels [11]. However, El-Dawela and Abou-elfetouh indicated that while serum homocysteine levels were higher in vitiligo patients when compared to healthy individuals, vitamin B12 and folic acid levels were not different in the two groups [22]. Moreover, besides being suggested as a predisposing factor for vitiligo, homocysteine has also been proposed to be a biomarker of the disease [15].

However, the results obtained from our study are

in marked contrast to these findings. We showed that serum levels of homocysteine, vitamin B12, and folic acid in patients with vitiligo were similar to their levels in healthy individuals. Furthermore, these factors were not associated with the extent, duration and activity of the vitiligo, and the family history of the disease. Consistent with our results, several previous studies also indicated that patients with vitiligo had the same levels of serum homocysteine, vitamin B12, and folic acid as the healthy population. In this regard, Kim *et al* reported that serum vitamin B12 and folic acid levels were similar in vitiligo patients and healthy individuals [19]. Gonul *et al*. also found the same results [20]. Moreover, Balci *et al*. found no significant differences in the serum levels of homocysteine, vitamin B12, and folic acid between patients with vitiligo and healthy subjects. No association was also found between these factors and the type and activity of vitiligo, and the duration and severity of the disease [13]. Similarly, Yasar *et al.* found no significant difference in the serum homocysteine and vitamin B12 in vitiligo patients compared to healthy individuals [14].

The reason for these contradictory results is not clear. However, 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 [13]. Taken together, it seems that the presence of a relationship between vitiligo and the serum homocysteine, vitamin B12, and folic acid levels remains controversial and needs to be vigorously investigated. Having a clear understanding of the underlying mechanisms of melanocyte disappearance in vitiligo is crucial to finding treatment approaches for arresting further depigmentation of the skin. In this condition, repigmentation would be rather simple to accomplish [25].

The limitation of our study was its small sample size and therefore it might not be representative of all Iranian vitiligo patients because of the diversity of the Iranian population in factors like ethnicity and ecological and genetic factors.

Acknowledgment

This study was funded and supported by Tehran University of Medical Sciences (TUMS); Grant no. 91-04-30-20151

REFERENCES

1. Rodríguez-Martín M, Sáez M, de Paz NM, et al. When are laboratory tests indicated in patients with vitiligo? Dermato-Endocrinol 2012; 4: 53-7.
2. Shankar DK, Shashikala K, Madala R. Clinical patterns of vitiligo and its associated co morbidities: A prospective controlled cross-sectional study in South India. Indian Dermatol Online J 2012; 3: 114.
3. Ghajarzadeh M, Ghiasi M, Kheirkhah S. Associations between skin diseases and quality of life: a comparison of psoriasis, vitiligo, and alopecia areata. Acta Medica Iranica 2012; 50: 511-5.
4. Arýcan O, Koç K, Ersoy L. Clinical characteristics in 113 Turkish vitiligo patients. Acta Dermatovenerol Alp Panonica Adriat 2008; 17: 129-32.
5. Laddha NC, Dwivedi M, Gani AR, et al. Tumor Necrosis Factor β (TNF-β) Genetic Variants and Its Increased Expression Are Associated with Vitiligo Susceptibility. PLoS One 2013;8.
6. Laddha NC, Dwivedi M, Mansuri MS, et al. Vitiligo: interplay between oxidative stress and immune system. Exp Dermatol 2013;22:245-50.
7. Passeron T, Ortonne JP. Activation of the unfolded protein response in vitiligo: the missing link & quest. J Investig Dermatol 2012;132:2502-4.
8. Shaker O, El-Tahlawi S. Is there a relationship between homocysteine and vitiligo? A pilot study. Br J Dermatol 2008;159:720-4.
9. Testai FD, Gorelick PB. Inherited metabolic disorders and stroke part 2: Homocystinuria, organic acidurias, and urea cycle disorders. Arch Neurol 2010;67:148-53.
10. Singh S, Singh U, Pandey SS. Increased level of serum Homocysteine in vitiligo. J Clin Lab Analysis 2011;25:110-2.
11. Karadag A, Tutal E, Ertugrul D, Akin K, Bilgili S. Serum holotranscobalamine, vitamin B12, folic acid and homocysteine levels in patients with vitiligo. Clin Exp Dermatol 2012;37: 62-4.
12. Singh S, Singh U, Pandey SS. Serum folic acid, vitamin B12 and homocysteine levels in Indian vitiligo patients. Egyptian Dermatol Online J 2012;8:2.
13. Balci DD, Yonden Z, Yenin JZ, Okumus N. Serum homocysteine, folic acid and vitamin B12 levels in vitiligo. Eur J Dermatol 2009;19:382-3.
14. Yasar A, Gunduz K, Onur E, Calkan M. Serum homocysteine, vitamin B12, folic acid levels and methylenetetrahydrofolate reductase (MTHFR) gene polymorphism in vitiligo. Dis Markers 2012;33:85-9.
15. Silverberg JI, Silverberg NB. Serum homocysteine as a biomarker of vitiligo vulgaris severity: A pilot study. J Am Acad Dermatol 2011; 64: 445-7.
16. Park H-H, Lee M-H. Serum Levels of Vitamin B 12 and Folate in Korean Patients with Vitiligo. Acta Dermato-Venereol 2005;85:66-7.
17. Montes LF, Diaz M, Lajous J, Garcia N. Folic acid and vitamin B12 in vitiligo: a nutritional approach. Cutis 1992;50:39.

18. Selhub J. Homocysteine metabolism. Ann Rev Nutr 1999; 9:217-46.
19. Kim SM, Kim YK, Hann SK. Serum levels of folic acid and vitamin B12 in Korean patients with vitiligo. Yonsei Med J 1999;40:195-8.
20. Gonul M, Cakmak SK, Soylu S, Kilic A, Gul U. Serum vitamin B12, folate, ferritin and iron levels in Turkish patients with vitiligo. Indian J Dermatol Venereol Leprol 2010;76:448.
21. Juhlin L, Olsson MJ. Improvement of vitiligo after oral treatment with vitamin B12 and folic acid and the importance of sun exposure. Acta Ddermato-Venereol 1997;77:460-2.
22. El-Dawela RE, Abou-elfetouh S. Relationship between Homocysteine, Vitamin B12, Folic acid Levels and Vitiligo. J Appl Sci Res 2012;8.
23. Schalinske KL, Smazal AL. Homocysteine imbalance: a pathological metabolic marker. Adv Nutr 2012;3:755-62.
24. Stanger O, Herrmann W, Pietrzik K, et al. Clinical use and rational management of homocysteine, folic acid, and B vitamins in cardiovascular and thrombotic diseases. Z Kardiol 2004;93:439-53.
25. Parsad D. A new era of vitiligo research and treatment. J Cutan Aesthet Surg 2013;6:63-4.

