Serum folic acid, vitamin B_{12}, vitamin D, and homocysteine levels in Iranian children with vitiligo

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Background: Hyperhomocysteinemia and vitamin D deficiency may be involved in the pathogenesis of vitiligo. This study compared the serum levels of vitamin D, homocysteine, vitamin B_{12}, and folic acid between vitiligo-affected children and healthy children.

Methods: Using a case-control design, 30 children with vitiligo and 30 age and sex-matched healthy children were enrolled from April 2018 to August 2020. Serum levels of vitamin D, homocysteine, vitamin B_{12}, and folic acid were analyzed in both groups during the same season of the year. Additionally, the association between serum levels of these factors with demographic and clinical features of the children (collected by interview and physical examination) was evaluated. Data were analyzed using the independent T-test, Fisher's exact test, and chi-squared test.

Results: The vitiligo group had significantly lower vitamin D and folic acid serum levels compared with the control group [95% CI -19.87 to -2.96 and -4.15 to -4.18, respectively]. Among patients, the vitamin D level was negatively correlated with age (r = -0.459, P = 0.011) and disease duration (r = -0.373, P = 0.042). Moreover, there was a significant association between vitiligo activity and serum homocysteine levels (P = 0.027).

Conclusion: Routine measurement of vitamin D and folic acid serum levels might be suggested, especially in children with long-standing disease. Monitoring the homocysteine level may be beneficial, particularly in children with progressive vitiligo.

Keywords: vitamin D, folic acid, homocysteine, vitiligo, children

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INTRODUCTION

Vitiligo is an acquired autoimmune dermatologic disorder that presents with depigmented lesions. The disease pathogenesis is not completely known, but several factors such as genetic background, autoimmunity, oxidative stress, neurohormonal etiology, and cytotoxicity of melanocytes may be involved [1-3]. Recently, the effect of hyperhomocysteinemia on impaired melanogenesis has been elucidated [4-6]. Decreased activity of the catalase enzyme can lead to hyperhomocysteinemia in vitiligo patients. Hyperhomocysteinemia results in an increased level of oxidative stress, cytokine activation (interleukin 6), nuclear factor κβ activation, and tyrosinase inhibition, destroying melanocytes and impairing melanogenesis [5-8]. On the other hand, low levels of B_{12} and folic acid in serum, as co-factors for homocysteine methylenetetrahydrofolatereductase (MTHFR) in homocysteine to methionine conversion, have been demonstrated in hyperhomocysteinemic patients [8-10].

Vitamin D protects melanocytes from apoptosis

by its antioxidant effect, activation of T regulatory cells, and immunomodulatory effects via shifting the immune response from T-helper (Th) 1 to Th 2 and inhibiting the maturation of dendritic cells. It also augments tyrosinase activity and melanogenesis, affecting the vitamin D receptor (VDR) of melanocytes [11-15].

To date, few have evaluated the serum levels of vitamin D, homocysteine, vitamin B_{12}, and folate in vitiligo patients, with paradoxical results due to confounding factors such as genetic polymorphism on MTHFR, diet habits, age, and sun exposure. Some authors recommend regular measurements of these factors in patients suffering from vitiligo [16-20].

Regarding the relatively high cost of laboratory tests and confounding results, we decided to check these factors in Iranian children with vitiligo in comparison with healthy children.

MATERIAL AND METHODS

The current study was performed on a case-control basis, where 30 vitiligo-affected children and 30 sex- and age-matched healthy children referred to the Pediatric Dermatology Clinic of Afzalipour Hospital in Kerman, Iran, from April 2018 to August 2020 were enrolled. Inclusion criteria were children less than 18 years old diagnosed with new cases of vitiligo that received no treatment. The control group included healthy age- and sex-matched children with negative history of autoimmune diseases, recruited from children who came to our clinic complaining of minor skin problems such as impetigo, herpes simplex, or nevus. Exclusion criteria consisted of patients with other systemic or cutaneous diseases, smoking, taking folic acid, vitamin D, vitamin B_{12}, and vitamin B6 since six months ago, and unwillingness to participate in the study. The current research was approved by the Ethics Committee of Kerman University of Medical Sciences (IR-KMU.REC.1395.763). According to Atas *et al.* [1], serum levels of homocysteine in the case and control groups were 16.9 ± 8.4 and 9.10 ± 3.4 µmol/l, respectively; therefore, a minimum sample size of 15 participants in each group was calculated (power = 80%, $\alpha = 0.05$). However, to increase the study's power, the sample was expanded to 30 in each group.

Parents provided written informed consent if their child was less than 12 years old, while it was obtained from both children and parents if children were older than 12. Vitiligo diagnosis was confirmed based on clinical examination and Wood's lamp assessment. Demographic features of the participants, including age, gender, and family history of vitiligo or other autoimmune disorders, were acquired by face-to-face interviews and questionnaires. Also, clinical features of vitiligo, including the site of the lesions, duration of the disease, and type of vitiligo, were recorded by history taking and physical examination. Based on the vitiligo disease activity (VIDA) score, the disease activity was classified using six points from -1 to +4. Furthermore, the area of involved skin was calculated using the vitiligo area scoring index (VASI) score (within the range of 10–100%) in five regions of the body according to the following equation (range: 0–100):

$VASI = \sum$ all body sites (hand units) × depigmentation [20].

After 12 hours of fasting, the serum level of vitamin D was assessed by a laboratory technician that was not aware of the two groups via the enzyme-linked fluorescence assay (ELFA) using Vidas kits (France), and homocysteine, vitamin B_{12}, and folic acid levels were assessed by chemiluminescence method using Siemens kits (Germany). The blood samples were obtained from all participants during the same season of the year to eliminate the confounding effect of weather on vitamin D serum levels. The analytical sensitivity for serum levels of vitamin D, homocysteine, vitamin B_{12}, and folic acid were 8.1 ng/ml, 0.5 µmol/l, 125 pg/ml, and 0.8 ng/ml, respectively. The normal ranges for vitamin D, homocysteine, vitamin B_{12}, and folic acid serum levels in pediatrics were 30-100 ng/ml, 0-15 µmol/l, 180-914 pg/ml, and 4.6-34.8 ng/ml, respectively.

Statistical analysis

SPSS 16 (software IBM, Armonk, NY, USA) was utilized for data analysis. Frequency and percentage were used to describe qualitative data, and mean ± standard deviation (SD) was used to describe quantitative data. Comparison of the two groups and association between clinical and demographic features of the case group with levels of vitamin B_{12}, homocysteine, folate, and vitamin D were evaluated using the independent T-test, Fisher's exact test, and chi-squared test.

RESULTS

Thirty children with vitiligo and 30 healthy age- and sex-matched children were enrolled in this study (Figure 1). Table 1 demonstrates the clinical features of children with vitiligo. The serum vitamin D in patients and controls were 19.34 ± 8.41 and 32.28 ± 15.41 ng/ml, respectively (95% CI -19.87 to -2.96, $P = 0.001$). The level of folic acid in the case and control groups was 12.62 ± 3.28 ng/ml and 14.60 ± 4.17 ng/ml, respectively (95% CI -4.15 to -4.18, $P = 0.046$).

A lower level of vitamin B_{12} and a higher level of homocysteine were revealed in the vitiligo group, but the results were not significant [95% CI -85.30 to 19.40 and -1.41 to 2.68, respectively, $P > 0.05$] (Table 2). Moreover, a significant negative association between vitamin D and the age of the patients (r = -0.459, $P = 0.011$) as well as disease duration (r = -0.373, $P = 0.042$) was observed, but there was no association between other factors and age or disease duration. Moreover, there was no significant association of the levels of vitamin B_{12},

Table 1. Clinical features of children with vitiligo

Variable	Number	Percentage
Site of the lesions		
Head & neck	23	53.4
Extremity	12	40
Trunk	9	6.7
Type of the lesion		
Segmental	3	10
Generalized	7	23.3
Localized	20	66.7
VIDA score		
Active in past 1 year	7	23.3
Active in past 6 months	13	43.3
Active in past 3 months	9	30
Active in past 6 weeks	1	3.3
VASI score		
< 30	24	80
> 30	6	20

VIDA: Vitiligo Disease Activity Score; VASI: Vitiligo Area Scoring Index

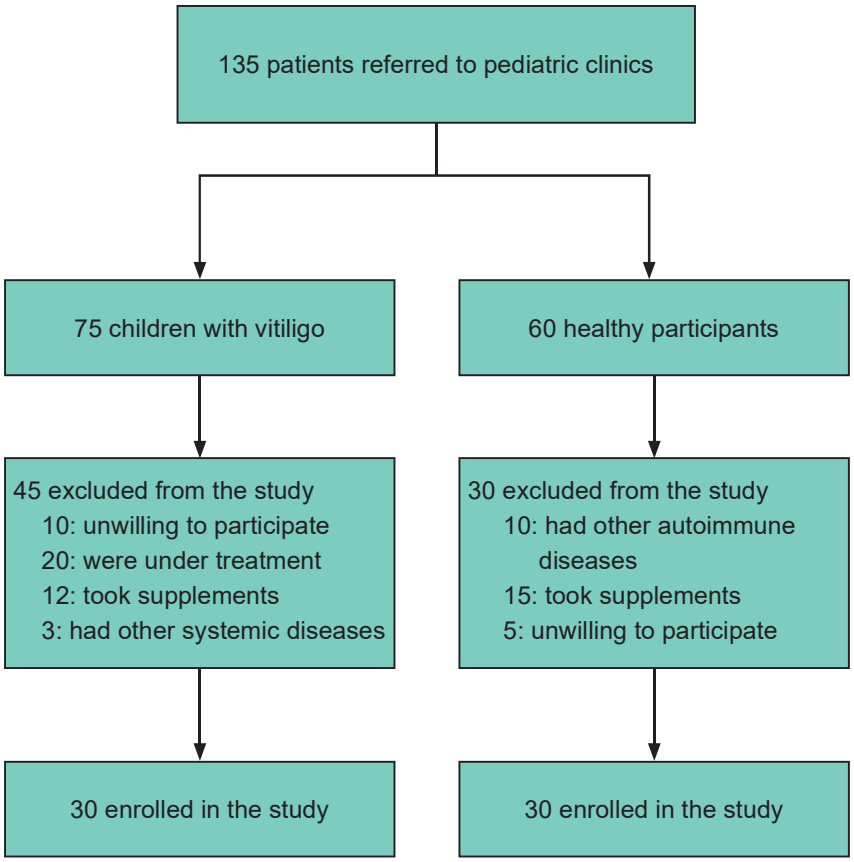

Figure 1. Flowchart of the study

Table 2. Demographic features and serum levels of homocysteine, vitamin B12, vitamin D, and folic acid in the two groups (mean ± SD, range)

Variable	Patients	Controls	*P*-value
Girls/boys (n)	16/14	15/15	0.796
Age (years)	6.90 ± 3.74 (2–15)	6.16 ± 2.98 (2–13)	0.4
Vitamin D (ng/ml)	19.34 ± 8.41 (5–35)	32.28 ± 15.41 11–66.6	0.001
Folic acid (ng/ml)	12.62 ± 3.28 (7.40–20.80)	14.60 ± 4.17 (5.12–19.91)	0.046
Vitamin B_{12} (pg/ml)	334.60 ± 108.56 (110–563.4)	373.19 ± 94.76 (171.4–536.8)	0.148
Homocysteine (µmol/l)	10.26 ± 3.62 (5.12–20.50)	9.63 ± 4.14 (1–17.1)	0.536

homocysteine, vitamin D, and folic acid with the sex of the participants, as well as the VIDA and VASI scores, except a positive association between VIDA score and homocysteine level ($P = 0.027$).

DISCUSSION

In this study, vitamin D and folic acid levels were significantly lower in children with vitiligo than in healthy controls, but the serum levels of homocysteine and vitamin B_{12} did not differ considerably between the two groups.

In a study by Singh and colleagues, folic acid/vitamin B_{12} levels were inferior, while homocysteine levels were superior in patients with vitiligo relative to the control group. In the aforementioned study, no significant association of homocysteine and folic acid levels with the disease duration was reported, validating the results of our study. However, in the present investigation, in contrast to Singh *et al.*, there was a significant association between homocysteine and VIDA score [16].

Zaki *et al.*, similar to this study, demonstrated a higher level of homocysteine in the serum of patients with vitiligo than in healthy individuals. But, in that study, a positive association between age and homocysteine level was reported, in contrast to our study. The lower age of our participants, higher level of serum homocysteine in older ages, and different genetic backgrounds can explain this difference [17].

Ghalamkarpour *et al.* revealed a significantly higher percentage of hyperhomocysteinemia in adults with vitiligo (64.2%) in comparison with healthy controls (36%). In the present study, hyperhomocysteinemia was evident in 13.3% and 6.7% of the children with vitiligo and healthy ones, respectively. But in contrast to Ghalamkarpour's study, the difference was not significant [18]. Several factors such as age, sex, lifestyle, genetic background, duration of disease, disease activity (VIDA score), and percentage of involvement (VASI) may have a role in modulating the serum levels of homocysteine, vitamin B_{12}, and folic acid. So, dissimilarity in the results between different studies can be due to differences in these factors [4-11].

Karaguzel *et al.* demonstrated higher vitamin D serum levels in children with vitiligo than in controls, contradicting our results [19]. Singla *et al.* reported significantly lower serum levels of vitamin D among individuals suffering from vitiligo compared to the healthy group, similar to the present study. Furthermore, there was no significant relationship between serum levels of vitamin D and VIDA and VASI scores, again compatible with the present study. In contrast to Singla *et al.*, our study indicated that the vitamin D level was negatively correlated with age and duration of disease [20]. Factors such as age, sex, body mass index (BMI), Fitzpatrick skin type, lifestyle, ethnicity, genetics, and sunscreen application affect the serum vitamin D level. This can explain the difference in results between the studies [21-23].

Lajevardi *et al.* revealed lower serum levels of vitamin D in male vitiligo patients than in male controls; nevertheless, the difference was insignificant in females. This can be explained by the type of dressing in females in Iran, an Islamic country, which can lead to lower serum vitamin D levels in both vitiligo and control groups. In the current investigation, there was no discernible association between sex and serum vitamin D level, which may be explained by the lower age of our patients than in Lajevardi *et al.'s* study and the

Table 3. Comparison of serum levels of homocysteine, vitamin B12, vitamin D, and folic acid in other studies

First author	Groups	Homocysteine (µmol/l)	Folic acid (ng/ml)	Vitamin B_{12} (pg/ml)	Vitamin D (ng/ml)
Singh [14]	Vitiligo	28.8 ± 7.7	4.88 ± 1.52	428.46 ± 133.52	NP
	Control	23.1 ± 1.9	6.25 ± 0.69	536.63 ± 111.43	NP
	P-value	< 0.01	< 0.01	< 0.01	NP
Zaki [15]	Vitiligo	11.35 ± 3.14	NP	NP	NP
	Control	10.49 ± 1.68	NP	NP	NP
	P-value	0.19	NP	NP	NP
Ghalamkarpour [16]	Vitiligo	12.5	883.38 ± 304.14	340.5	NP
	Control	17	939.87 ± 256.56	345	NP
	P-value	0.001	0.31	0.61	NP
Karaguzel [17]	Vitiligo	NP	NP	NP	26.6 ± 18.3
	Control	NP	NP	NP	22.8 ± 13.5
	P-value	NP	NP	NP	NS
Singla [18]	Vitiligo	NP	NP	NP	24.07 ± 3.79
	Control	NP	NP	NP	38.17 ± 9.54
	P-value	NP	NP	NP	< 0.001
Lajevardi [19]	Vitiligo	NP	NP	NP	10.24 ± 1.72
	Control	NP	NP	NP	18.31 ± 7.39
	P-value	NP	NP	NP	NS

NP: Not Performed

difference in the type of dressing between adults and children [21]. A comparison of the folic acid, vitamin D, vitamin B_{12}, and homocysteine levels in other studies is conveyed in Table 3.

Confounding factors such as dietary habits, socioeconomic status, lifestyle, physical activity level, culture, skin type, genetic background, and duration of outdoor activities can affect folic acid, vitamin D, vitamin B_{12}, and homocysteine serum levels [4-11,21-23]. The limitation of this study was the matching of case and control groups for only sex and age. Nonetheless, to eliminate the effect of sun exposure on the serum level of vitamin D, all subjects were sampled on the same day. Also, participants were recruited from a single center with the same socioeconomic status and skin type.

CONCLUSION

In this study, vitamin D and folic acid serum levels were significantly lower in children with vitiligo than in healthy ones. Furthermore, lower vitamin D serum levels were observed in pediatric vitiligo patients with longer disease duration and older age. Also, a higher homocysteine level was demonstrated in progressive vitiligo. Therefore, it might be suggested to regularly measure serum levels of these micronutrients in these children, especially in those with progressive and long-standing vitiligo.

Conflict of interest: None declared.

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