Serum homocysteine, folate, and vitamin B_{12} levels in patients with vitiligo and their potential roles as disease activity biomarkers: A systematic review and meta-analysis

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Background: Hyperhomocysteinemia and folate and vitamin B_{12} deficiencies have been reported in patients with vitiligo. Investigating the role of these conditions might shed light on the pathogenesis of vitiligo.

Objective: To perform a systematic review and meta-analysis of studies assessing serum homocysteine, folate, and vitamin B_{12} levels in vitiligo patients.

Methods: Online databases were searched on May 15, 2018, to identify studies comparing serum homocysteine, folate, and vitamin B_{12} levels between patients with vitiligo and controls. A random effects model was used.

Results: Twenty-two studies involving a total of 1448 patients with vitiligo were included. Patients with vitiligo had significantly higher serum homocysteine levels (standardized mean difference [SMD] 0.550, 95% confidence interval [CI] 0.262-0.838; I^2 87.3%) and lower vitamin B_{12} levels (SMD −0.430, 95% CI −0.738 to −0.121; I^2 85.3%) than controls. Serum folate levels were not significantly different between the 2 groups (SMD −0.240, 95% CI −0.592 to 0.111; I^2 85.5%). A subgroup analysis revealed that these findings correlated with disease activity.

Limitations: The included studies were heterogeneous. Serum homocysteine levels could be influenced by various factors.

Conclusion: Patients with vitiligo have higher serum homocysteine levels and lower vitamin B_{12} levels than individuals without vitiligo. (J Am Acad Dermatol 2019;80:646-54.)

Key words: folate; homocysteine; hyperhomocysteinemia; vitamin B_{12}; vitiligo.

Vitiligo is an acquired, multifactorial pigmentary disorder characterized by depigmentation of the epidermis and hair follicles. The disease prevalence has been estimated to be 1% of the population worldwide.[1] The etiology of vitiligo

Abbreviations used:

CI: confidence interval
SMD: standardized mean difference
UVB: ultraviolet B

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and the exact mechanism for the destruction of melanocytes have not yet been fully elucidated. Various theories have been formulated, including autoimmune, oxidative stress, neurohumoral, and cytotoxic hypotheses.[1,2]

Hyperhomocysteinemia is involved in a variety of diseases.[3,4,5] Previous studies have reported hyperhomocysteinemia in vitiligo and postulated that homocysteine disrupts melanogenesis via various mechanisms, such as increased oxidative stress, activation of various cytokines, and inhibition of tyrosinase activity.[6-8] Folate and vitamin B_{12} serve as cofactors of homocysteine methyltransferase for the conversion of methionine from homocysteine.[9] The levels of these 3 compounds appear to be interconnected, with deficiencies in folate and vitamin B_{12} indicating an increase in the levels of homocysteine in the serum. Studies have shown that repigmentation of vitiliginous lesions is possible with supplementation of folic acid and vitamin B_{12}.[10,11] However, previous studies failed to demonstrate a consistent correlation between vitiligo and the serum levels of homocysteine, folate, and vitamin B_{12}.

The objective of this systematic review and meta-analysis was to investigate the serum levels of homocysteine, folate, and vitamin B_{12} in patients with vitiligo. In addition, a meta-regression analysis was performed to examine correlations between the levels of these compounds, disease duration, and other variables.

CAPSULE SUMMARY

- This study revealed that vitiligo was associated with higher serum homocysteine and lower vitamin B_{12} levels but not with lower folate levels. These findings correlated with disease activity.
- When treating patients with vitiligo, clinicians should be vigilant about potential elevated homocysteine and decreased vitamin B_{12} levels in the serum.

MATERIALS AND METHODS

This meta-analysis was registered in the international prospective register of systematic reviews (PROSPERO, registration number CRD42018093688). The recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement, explanation and elaboration document, and checklist were used to guide the methods used in the present analysis (http://www.prisma-statement.org/).

Data source and search strategy

Databases (PubMed, Cochrane Library, and Embase) were searched from inception to April 30, 2018. The last day of search was May 15, 2018. The search focused exclusively on clinical studies involving humans and reporting their results in English. The keywords used were "vitiligo," "leukodermia," "leucodermia," "leukoderma," "leucoderma," and "hypopigmentation" combined with "homocysteine," "2-amino-4-mercaptobutyric acid," "hyperhomocysteinemia," "folate," "folic acid," "vitamin B9," "vitamin B 12," "cyanocobalamin," or "cobalamin." Reference lists of screened articles were also reviewed.

Eligibility criteria and study selection

Studies comparing the levels of homocysteine, folate, and vitamin B_{12} in the serum between patients with vitiligo and healthy controls were included in the analysis. Review articles, case reports, case series and conference reports were excluded. The methodologic quality of the included articles was assessed using an adapted version of the Newcastle-Ottawa scale for case-control studies, with a maximum score of 9 points (Supplemental Table I; available at http://www.jaad.org). Two investigators (Dr Tsai and Dr Kuo) independently screened the titles and abstracts of articles. Full-text articles reporting relevant studies were assessed for eligibility. Articles irrelevant to the topic of this analysis on the basis of the title, abstract, and overall content were excluded. Quality assessment was performed independently. Any disagreement was resolved by the investigators discussing the issue and reaching a consensus. In case of studies that potentially had overlapping data sets, the study with the largest sample size was included.

Outcomes

The primary outcomes of the present study were the levels of homocysteine, folate, and vitamin B_{12} in the serum of patients with vitiligo and healthy controls. The secondary outcomes were the levels of homocysteine, folate, and vitamin B_{12} in the serum of patients with different disease activities of vitiligo and compared with those of the control group.

Data extraction

Two reviewers independently extracted and collected the data in tabular form. The accuracy of the resulting tables was reviewed by a third investigator. Any disagreement was resolved by the

investigators through discussion and reaching a consensus.

The following data was extracted: sample size; type of vitiligo; activity of vitiligo; severity of vitiligo; duration of disease; type of control group; quality scores; patient sex and age; and levels of homocysteine, folate, and vitamin B_{12} in serum (Table I and Supplemental Table II; available at http://www.jaad.org).[12-33] The value indicating the level of a compound in the serum was translated into a consistent measurement of unit.

Data analysis

A pooled estimate of the levels of homocysteine, folate, and vitamin B_{12} in patients with vitiligo and healthy controls was produced. Subsequently, data were stratified into different groups according to the disease activity of vitiligo (progressive, stable, or regressive) and type of control group (healthy control or disease control) for subgroup and sensitivity analyses. Continuous data were analyzed by using the standardized mean difference (SMD) to account for the varied and nonstandardized outcomes across studies. Heterogeneity testing was performed by using the I^2 test. Because of the large heterogeneity observed, a random effects model was used for all studies.

A random-effects meta-regression analysis was performed to determine the effects of age, disease duration, and quality score of studies on the levels of homocysteine in the serum of patients with vitiligo and controls. Publication bias was evaluated by using the Egger test. All analyses were performed using the software Comprehensive Meta-Analysis version 3 (Biostat Inc, Englewood, NJ).

RESULTS

Search results and trial characteristics

Of the 349 studies identified, 22 studies involving a total of 1448 patients with vitiligo met the inclusion criteria (Fig 1). Table I summarizes the characteristics of the studies, with quality scores ranging from 6 through 9. Most studies had low scores due to the absence of age-matched or sex-matched control groups and inclusion of patients with other dermatologic diseases. Levels of homocysteine, folate, and vitamin B_{12} were reported in 20 studies, 11 studies, and 14 studies, respectively.

Serum levels of homocysteine, folate, and vitamin B_{12}

The clinical data and levels of homocysteine, folate, and vitamin B_{12} are summarized in Supplemental Table II. In the vitiligo group, the mean level of homocysteine was 16.2 (range 9.4-36.1) μmol/L, folate 7.0 (range 4.2-8.9) ng/mL, and vitamin B_{12} 335.4 (range 157.2-630.3) pg/mL. In the control group, the level of homocysteine was 12.2 (range 2.6-22.4) μmol/L, folate 7.5 (range 5.4-10.2) ng/mL, and vitamin B_{12} 390.4 (range 103.5-627.2) pg/mL.

Statistical analysis results

The results of the meta-analysis are presented in Supplemental Table III (available at http://www.jaad.org). Compared with the control group, patients in the vitiligo group had significantly higher levels of homocysteine in the serum (SMD 0.550, 95% confidence interval [CI] 0.262-0.838; Fig 2, *A*) and lower levels of vitamin B_{12} (SMD −0.430, 95% CI −0.738 to −0.121; Fig 2, *B*). There was no significant difference in the levels of folate between the vitiligo and control groups (SMD −0.240, 95% CI −0.592 to 0.111; Fig 2, *C*).

The subgroup analysis showed that patients with progressive vitiligo had significantly higher levels of homocysteine in the serum than the control group (SMD 1.479, 95% CI 0.775-2.183). However, the levels of homocysteine were similar among patients with stable or regressive vitiligo and controls. The level of vitamin B_{12} in the vitiligo group was significantly lower than that observed in the control group, regardless of disease activity (progressive disease [SMD −0.787, 95% CI −1.005 to −0.570] or stable [SMD −0.720, 95% CI −0.989 to −0.452]). In addition, the levels of vitamin B_{12} were similar between those with progressive and stable vitiligo (progressive vs stable, SMD 0.121, 95% CI −0.075 to 0.317). The level of folate in the vitiligo group was similar to that observed in the control group, regardless of disease activity (Supplemental Table III). When disease controls were excluded, patients with vitiligo continued to have significantly higher levels of homocysteine, lower levels of vitamin B_{12}, and similar levels of folate (Supplemental Table III).

Meta-regression analysis of the comparison of the levels of homocysteine between the vitiligo and control groups showed that age, disease duration, and quality score of studies did not affect the SMD. There was no publication bias for studies in which the homocysteine, folate, and vitamin B_{12} levels were compared between patients with vitiligo and controls (Supplemental Fig 1, *A-C*; available at http://www.jaad.org).

DISCUSSION

This systematic review and meta-analysis revealed that patients with vitiligo had higher levels of homocysteine and lower levels of vitamin B_{12} in the serum compared with those in the control group. However,

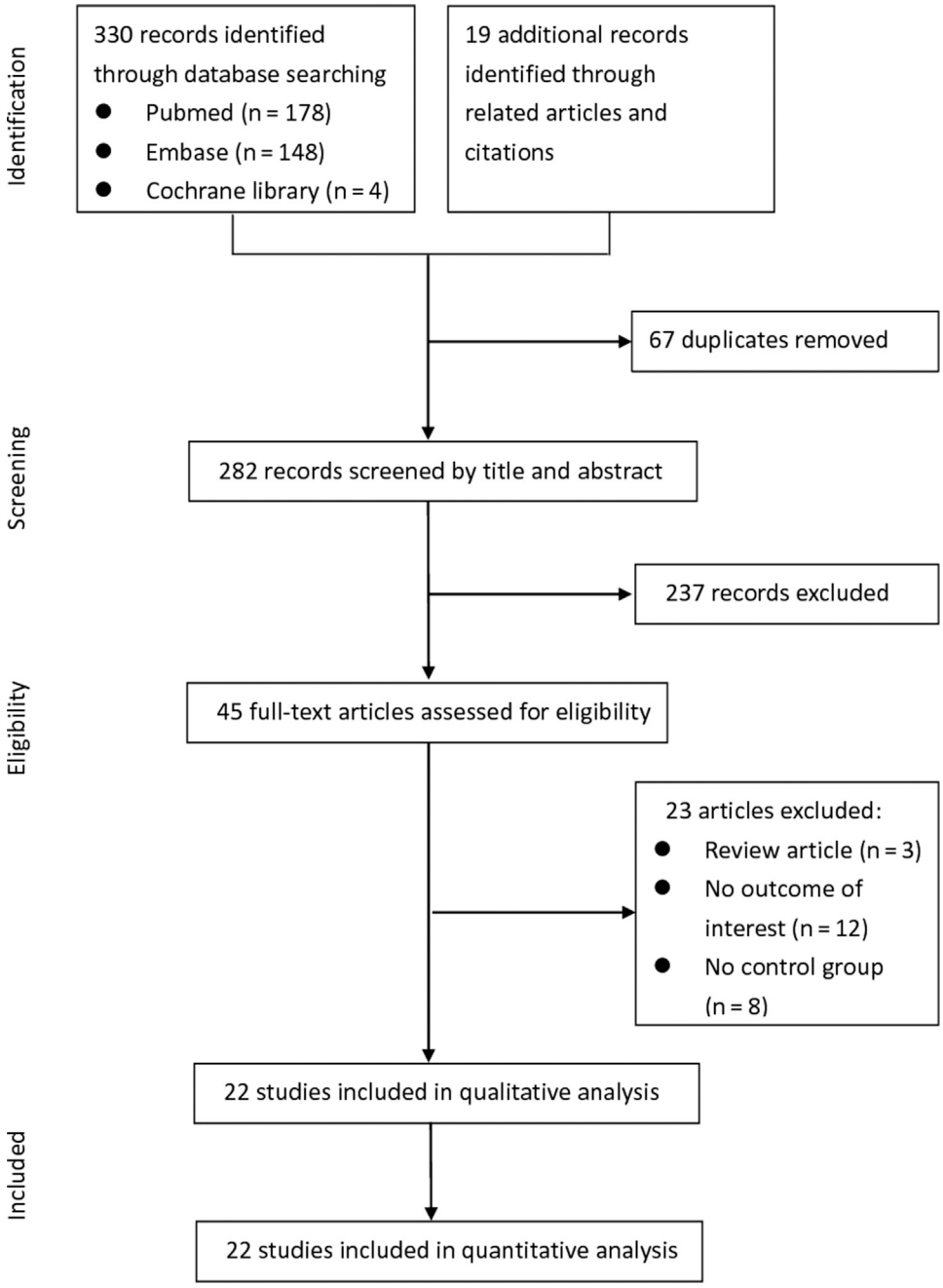

Fig 1. Flow diagram for study identification.

the levels of folate in the serum were not significantly different between the vitiligo and control groups.

Several plausible hypotheses could explain the occurrence of hyperhomocysteinemia in patients with vitiligo. First, homocysteine inhibits tyrosinase, a critical enzyme in melanin synthesis, by binding to copper at its active site.[34] Second, the metabolism of homocysteine is influenced by catalase and catalase gene mutations. In vitiligo, a decreased activity of catalase has been observed.[35,36] Third, the production of reactive oxygen species through the oxidation of homocysteine leads to increased oxidative stress and interferes with melanogenesis.[37-39] It is also postulated that, in vitiligo, oxidative stress induces the unfolded protein responses and activates chemokines in the endoplasmic reticulum, thereby making melanocytes targets for destruction.[40,41] Homocysteine has also been shown to increase endoplasmic reticulum stress in various cell types.[42,43] Finally, homocysteine can be converted by methionyl-tRNA synthetase into homocysteine thiolactone. Homocysteine thiolactone forms

Table I. Summary of included studies

Study	Type of vitiligo	Disease activity definition	VASI*	Disease duration*	Control	Sample size, n, vitiligo/control	Quality score†
Kim et al[12]	Segmental, focal, vulgaris, universal, mixed type	NA	NA	NA	Healthy	100/30	8
Park et al[13]	Localized, generalized	Active: enlargement of lesions during the month before examination or Koebner phenomenon; inactive: NA	NA	<1 y, 24 pt; >1 y, 53 pt	Healthy	77/80	7
Shaker et al[14]	NA	Within past 2 m, stable: no changes; progressive: enlargement of existing lesions or new lesions; regressive: lesion improved	NA	10.65 ± 8.33 y	Healthy	26/26	9
Balci et al[15]	Localized, generalized, universal	NA	8.48 ± NA	9.28 ± 9.32 y	Healthy	48/31	9
Karadag et al[16]	Focal, vulgaris, acrofacial, universalis, segmental	Stable: NA; progressive: NA	NA	25.5 ± 35.3 m	Persons with cosmetic problems	69/52	8
Yasar et al[17]	Focal, segmental, acrofacial, generalized	Within past 3 m, stable: no changes; active: enlargement of existing lesions or new lesions; regressive: reduced lesions	NA	NA	Healthy	40/40	9
Singh et al[18]	Localized, generalized	Within past 2 m, stable: no change; active: new lesions	NA	<10 y, 168 pt; 11-20 y, 26 pt; >20 y, 9 pt	Healthy	200/75	8
El-Dawela et al[19]	Segmental, nonsegmental	Within past 1 y, stable: no new lesions or progression of existing lesions, and no Koebner phenomenon; active: NA	9.5 ± 19.5	6.4 ± 6.05 y	Healthy	70/20	9
AlGhamdi et al[20]	Focal, generalized, acral, acrofacial	Stable: NA; active: NA; regressive: NA	NA	8.5 y	NA	153/153	8
Nahidi et al[21]	Localized (focal, segmental), generalized (vulgaris, acrofacial and mixed), universal	Stable: NA; active: NA; regressive: NA	NA	15 days to 20 y	Healthy	40/40	9
Chen et al[22]	Nonsegmental	Stable: NA; active: NA	NA	NA	Healthy	129/129	8

Continued

Table I. Cont'd

Study	Type of vitiligo	Disease activity definition	VASI*	Disease duration*	Control	Sample size, n, vitiligo/control	Quality score†
Zaki et al[23]	Localized, generalized, universal	Stable: NA; progressive: NA	3.75 ± 1.06 (stable); 13.33 ± 7.48 (progressive); 7.58 ± 3.87 (regressive)	NA	Healthy	30/30	8
Sabry et al[24]	Acral, acrofacial, generalized, segmental, truncal	Within past 2 m, stable: no change; progressive: enlargement of existing lesions or new lesions	NA	4.32 ± 3.65 y	Healthy	35/35	9
Ghiasi et al[25]	Generalized, focal, segmental, acrofacial, universal	Categorized as stable or progressive over the past 2 m without treatment	NA	44.5 m (median)	Healthy	30/30	9
Atas et al[26]	Acrofacial, segmental, universal, generalized	Within past 2 m, stable: no changes; active: new or enlargement of previous lesions	NA	4.6 ± 4.5 y	With minimal dermatologic problems	60/60	8
Ghalamkarpour et al[27]	Nonsegmental, acrofacial, mixed, generalized, universal, mucosal, segmental, focal	NA	1.62 (median)	5.5 y (median)	Healthy	50/53	9
Agarwal et al[28]	Acrofacial, focal, mucosal, segmental	Within past 6 m, stable: no change; progressive: enlargement of existing lesions or new lesions; regressive: lesion improved	16.62 ± NA	9.03 ± 9.55 y	Healthy	50/35	9
Gupta et al[29]	Localized, generalized, universal	Assessed as stable, progressive, or regressive based on the activity of disease over past 6 m	NA	2.09 ± 0.09 y	Dermatoses other than vitiligo	82/83	7
Anbar et at[30]	Nonsegmental	Active: new lesions within past 2 m	NA	NA	Healthy	30/30	8
Salman et al[31]	Vulgaris, segmental, acrofacial, focal	Within past 3 m, stable: no change; progressive: enlargement of existing lesions or new lesions; regressive: lesion improved	NA	10.73 ± 6.48 y	Persons without vitiligo	44/44	8
Hasibuan et al[32]	NA	NA	NA	NA	NA	30/30	5
Jadeja et al[33]	Generalized, localized	Within past 2 y, Sstable: no change; active: existing lesions spreading or new lesions	NA	7.17 ± 5.96 y	Healthy	55/60	9

NA, Not available; *pt,* patients; *VASI,* Vitiligo Area Scoring Index.
*Data are presented as mean ± standard deviation.
†The methodologic quality of the studies was rated by using an adapted version of the Newcastle-Ottawa scale for case-control studies with a maximum score of 9 points.

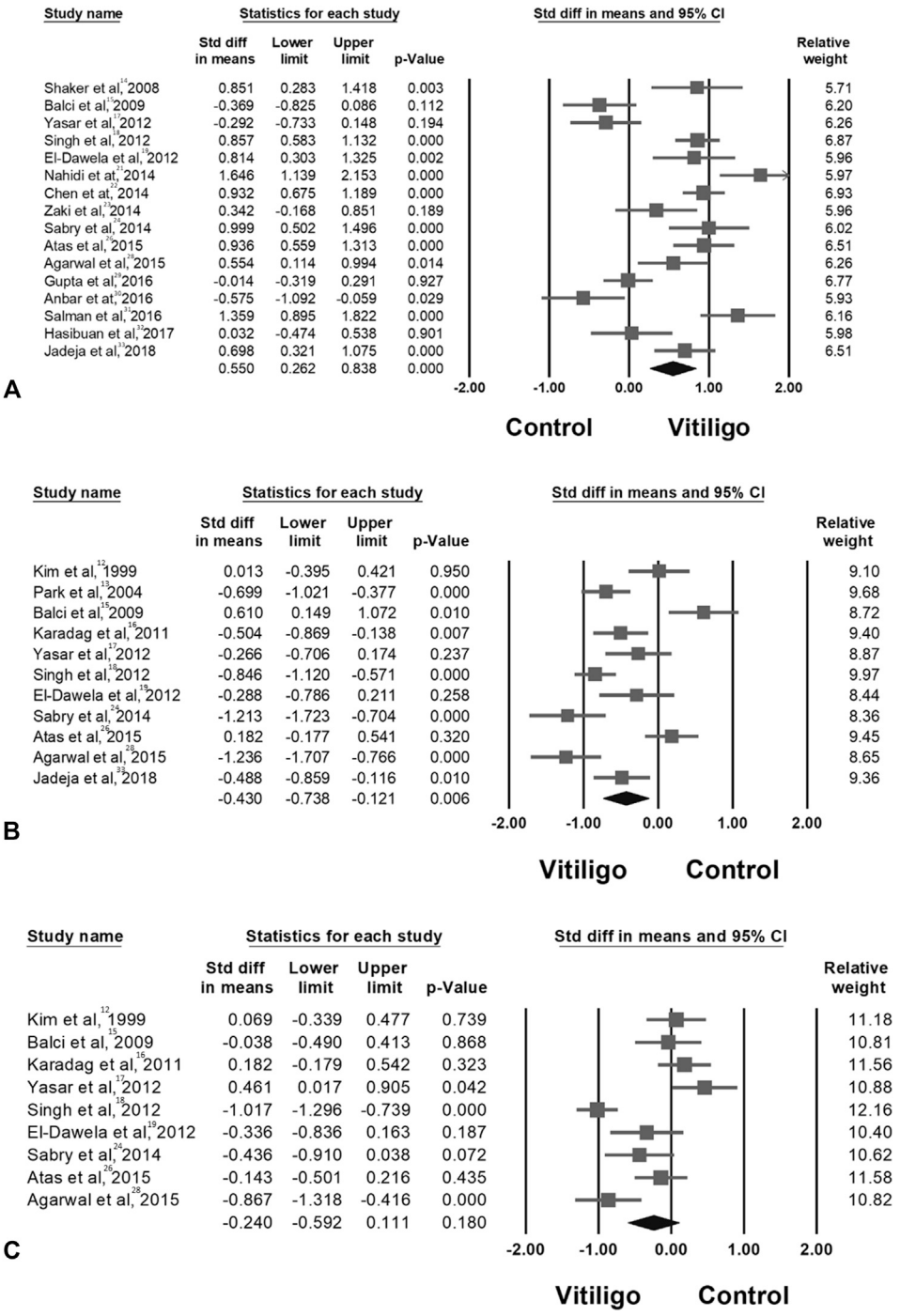

Fig 2. Forest plots comparing homocysteine (**A**), vitamin B_{12} (**B**), and folate (**C**) levels of patients with vitiligo and controls. **A** and **B**, Forest plots showed that compared with controls, patients with vitiligo had significantly higher serum homocysteine levels (standardized mean difference [SMD] 0.550, 95% CI 0.262-0.838; I^2 87.3%) and lower vitamin B_{12} levels (SMD −0.430, 95% CI −0.738 to −0.121; I^2 85.3%). **C**, Serum folate levels were not significantly different between the 2 groups (SMD −0.240, 95% CI −0.592 to 0.111; I^2 85.5%). *CI*, Confidence interval; *Std diff in means*, standardized mean difference.

N-homocysteine-protein adducts, which alter protein structure and function.[44,45] Furthermore, N-homocysteine-protein can be recognized as a neo-self antigen by antibodies, triggering autoimmunity.[45-47]

One of the highlights of our study was indicating the potential roles of homocysteine and vitamin B_{12} as disease activity biomarkers in vitiligo. Previous studies have discovered numerous circulating factors that correlated with the disease activity of vitiligo, including cytokines (interleukin 1β, interleukin 17, interferon γ, transforming growth factor β); chemokines (CXCL9, CXCL10); immune cells (regulatory T cells); soluble CDs (sCD25, sCD27); autoantibodies; and oxidative stress markers.[48] One of the advantages of using homocysteine and vitamin B_{12} as activity markers is that they are easy to measure. However, as the etiopathogenesis of vitiligo is extremely complicated and involves many pathways, it is difficult to find a single biomarker that reflects every aspect of the underlying mechanisms.

The therapeutic benefits of supplementation with folic acid and vitamin B_{12} in patients with vitiligo have not been thoroughly studied. Juhlin and Olsson reported that among 100 patients with vitiligo treated with vitamin B_{12} (1 mg by mouth twice daily), folic acid (5 mg by mouth twice daily), and sunlight or an ultraviolet B (UVB) lamp for 3 months, repigmentation was observed in 52 patients, while 6 patients achieved total repigmentation.[10] In a controlled trial conducted by Tjioe et al, 27 patients with stable vitiligo were randomly allocated to either a control group receiving only narrowband UVB therapy or the study group receiving UVB therapy combined with administration of vitamin B_{12} (200 mg/d by mouth) and folic acid (5 mg/d by mouth).[49] Although 92% all the participants showed 100% repigmentation after 1 year, there was no significant difference noted between the study and control groups.[49] Clinical trials with larger sample sizes and longer follow-up periods are warranted to determine the therapeutic roles of folic acid and vitamin B_{12} in treating vitiligo.

A major limitation of the present study was the substantial heterogeneity observed across the included studies. The investigators attempted to resolve this heterogeneity, by calculating the SMD instead of the mean difference and performing subgroup, sensitivity, and meta-regression analyses. However, for some analyses, the heterogeneity remained significant. It is speculated that differences in experimental methods, subtypes of vitiligo (segmental and nonsegmental), extent of body surface involvement, and ethnicity might be potential causes of heterogeneity. Furthermore, the genetic polymorphism of methylenetetrahydrofolate reductase mioght also affect the levels of homocysteine in the serum. However, it was not possible to perform subgroup analyses with these factors because of the limited data available. Another limitation was that the levels of homocysteine, folate, and vitamin B_{12} might be influenced by various uncontrollable factors, such as the baseline nutritional status, diet, smoking, concomitant use of certain medications, and comorbidities. Last, the present study demonstrated a correlation between hyperhomocysteinemia, vitamin B_{12} deficiency, and vitiligo but was unable to confirm causality.

Conclusion

In summary, this study revealed that vitiligo was associated with higher serum homocysteine and lower vitamin B_{12} levels and that these findings correlated with disease activity. Future research on vitiligo is needed to determine the underlying mechanisms of hyperhomocysteinemia and vitamin B_{12} deficiency and explore the therapeutic potential of homocysteine-lowering strategies.

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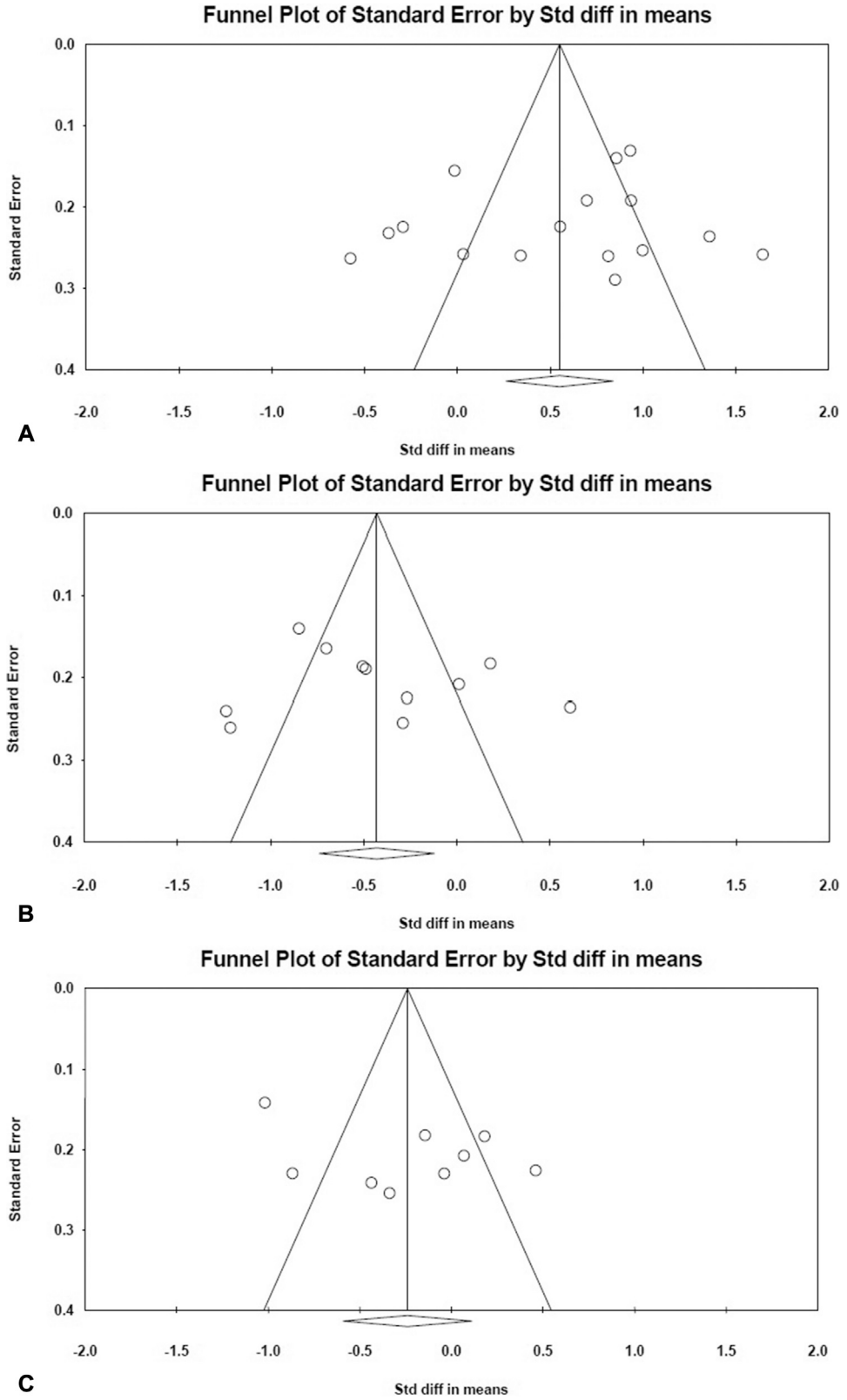

Supplemental Fig 1. Funnel plots. The funnel plots for analysis of serum homocysteine (**A**), vitamin B12 (**B**), and folate (**C**) levels in patients with vitiligo revealed no potential publication bias. The 2-sided *p* values of the Egger test for each analysis were 0.56, 0.57, and 0.21, respectively. *Std diff in means*, standardized mean difference.

Supplemental Table I. Adapted Newcastle-Ottawa scale for case-control studies

Category	Question, response
Selection	1. Is the case definition adequate? a) yes, with independent validation ◆ b) yes, record linkage or based on self-reports c) no description
	2. Representativeness of the cases a) consecutive or obviously representative series of cases ◆ b) potential for selection biases or not stated
	3. Selection of controls* a) healthy controls ◆ b) dermatology outpatient controls c) no description
	4. Definition of controls a) no history of disease (endpoint) ◆ b) no description of source
Comparability	1. Comparability of cases and controls on the basis of the design or analysis* a) study controls for sex◆ b) study controls for age◆
Exposure	1. Ascertainment of exposure* a) detailed description of the test about homocysteine, folate or vitamin B_{12} level◆ b) no description
	2. Same method of ascertainment for cases and controls a) yes ◆ b) no
	3. Nonresponse rate a) same rate for both groups ◆ b) nonrespondents described c) rate different and no designation

A study can be awarded a maximum of 1 star (◆) for each numbered item within the selection and exposure categories. A maximum of 2 stars (◆ ◆) can be given for comparability.
*Modified item for current study.

Supplemental Table II. Demographic characteristics and serum homocysteine, folate, and vitamin B_{12} levels of different groups

Study	Group	Age, y	Sex, n, M:F	Serum homocysteine level, μmol/L	Serum folate level, ng/mL	Serum vitamin B_{12} level, pg/mL
Kim et al[12]	Vitiligo	31 ± NA	50:50	NA	6.31 ± 2.82	630.25 ± 230.94
	Control	29 ± NA	14:16	NA	6.11 ± 3.11	627.16 ± 251.35
Park et al[13]	Vitiligo	34.5 ± 18	32:45	NA	NA	668 ± 290
	Control	35.8 ± 11.8	35:45	NA	NA	875 ± 302
Shaker et al[14]	Vitiligo	31.4 ± 8.09	12:14	21.61 ± 13.28	NA	NA
	Control	33.04 ± 6.37	9:17	13.1 ± 4.88	NA	NA
Balci et al[15]	Vitiligo	37.94 ± 16.27	27:21	10.79 ± 1.01	6.14 ± 2.45	211.69 ± 211.38
	Control	39.32 ± 13.15	14:17	11.14 ± 0.84	6.25 ± 3.44	198.32 ± 103.49
Karadag et al[16]	Vitiligo	34.1 ± 17.9	33:36	11.4 (median)	7.5 ± 3.1	250.6 ± 112.4
	Control	32.3 ± 16.7	17:35	9.4 (median)	7.0 ± 2.2	316.5 ± 152.0
Yasar et al[17]	Vitiligo	27.77 ± 13.44	17:23	9.35 ± 5.7	6.59 ± 2.78	212.9 ± 81.67
	Control	25.42 ± 4.48	18:22	10.96 ± 5.31	5.39 ± 2.41	241.15 ± 126.23
Singh et al[18]	Vitiligo	33.23 ± 16.67	82:118	28.8 ± 7.7	4.88 ± 1.52	428.46 ± 133.52
	Control	31.45 ± 11.02	NA	23.1 ± 1.9	6.25 ± 0.69	536.63 ± 111.43
El-Dawela et al[19]	Vitiligo	27.5 ± 15	21:49	15.2 ± 93	8.87 ± 2.9	297.28 ± 94.25
	Control	25.5 ± 14.2	9:11	8.4 ± 3.06	9.87 ± 3.22	323.8 ± 84.33
AlGhamdi et al[20]	Vitiligo	28 ± NA	87:66	12.73 (median)	10.0 (median)	351.6 (median)
	Control	NA	NA	12.94 (median)	7.6 (median)	356.85 (median)
Nahidi et at[21]	Vitiligo	24.68 ± 12.44	23:17	18.56 ± 5.69	NA	NA
	Control	24.5 ± 12.32	23:17	10.19 ± 4.4	NA	NA
Chen et at[22]	Vitiligo	24.9 ± 12.8	53.5:46.5	10.59 ± 1.75	NA	NA
	Control	25.9 ± 10.8	53.7:46.3	8.94 ± 1.79	NA	NA
Zaki et al[23]	Vitiligo	29.63 ± 9.91	NA	11.35 ± 3.14	NA	NA
	Control	29.37 ± 9.35	NA	10.49 ± 1.68	NA	NA
Sabry et al[24]	Vitiligo	37.03 ± 10.85	13:22	17.77 ± 7.72	8.42 ± 2.06	208.64 ± 66.73
	Control	33.87 ± 8.09	14:21	11.81 ± 3.41	9.39 ± 2.38	304.7 ± 89.9
Ghiasi et al[25]	Vitiligo	NA	18:12	9.5 (median)	12.7 (median)	302 (median)
	Control	NA	18:12	9.15 (median)	15.95 (median)	252 (median)
Atas et al[26]	Vitiligo	35.7 ± 11.2	30:30	16.9 ± 8.4	9.8 ± 2.9	372 ± 142
	Control	36.25 ± 7.8	30:30	10.9 ± 3.4	10.2 ± 2.7	348 ± 121
Ghalamkarpour et al[27]	Vitiligo	NA	28:22	12.5 (median)	NA	340.5 (median)
	Control	NA	30:23	17 (median)	NA	345 (median)
Agarwal et al[28]	Vitiligo	32.74 ± 10.52	19:31	15.39 ± 7.2	4.18 ± 3.55	157.18 ± 68.95
	Control	32.03 ± 10.19	14:21	11.88 ± 4.81	7.3 ± 3.67	306.6 ± 169.73
Gupta et al[29]	Vitiligo	30.56 ± 7.7	50:32	18.68 ± 89.65	NA	NA
	Control	31.27 ± 7.93	45:38	20.21 ± 121.99	NA	NA
Anbar et at[30]	Vitiligo	32.17 ± 9.15	17:13	2.33 ± 0.47	NA	NA
	Control	30.13 ± 7.6	18:12	2.64 ± 0.60	NA	NA

Continued

Supplemental Table II. Cont'd

Study	Group	Age, y	Sex, n, M:F	Serum homocysteine level, μmol/L	Serum folate level, ng/mL	Serum vitamin B_{12} level, pg/mL
Salman et al[31]	Vitiligo	27.82 ± 4.94	25:19	14.31 ± 6.68	NA	NA
	Control	27.56 ± 4.55	26:18	7.57 ± 2.14	NA	NA
Hasibuan et al[32]	Vitiligo	NA	NA	10.66 ± 2.89	NA	NA
	Control	NA	NA	10.58 ± 2.01	NA	NA
Jadeja et al[33]	Vitiligo	33.15 ± 14.13	26:29	36.13 ± 24.64	NA	252.8 ± 107.02
	Control	30.78 ± 11.15	28:32	22.44 ± 13.44	NA	311 ± 129.67

All data are presented as mean ± standard deviation.
NA, Not available.

Supplemental Table III. Results of meta-analysis of all studies, subgroup analysis, and sensitivity analysis

Analysis	Patient vs control (studies), n	Effect size	Effect estimate (95% CI)	*P*	I^2, %
Homocysteine					
All	969 vs 768 (16)	SMD	0.550 (0.262 to 0.838)	<.001	87.3
Healthy control	753 vs 551 (12)	SMD	0.542 (0.208 to 0.876)	.001	86.9
Activity of vitiligo					
Progressive	305 vs 380 (9)	SMD	1.479 (0.775 to 2.183)	<.001	93.5
Stable	223 vs 350 (8)	SMD	0.574 (−0.098 to 1.245)	.094	90.4
Regressive	22 vs 110 (3)	SMD	0.121 (−0.344 to 0.585)	.611	0
Folate					
All	672 vs 378 (9)	SMD	−0.240 (−0.592 to 0.111)	.180	85.5
Healthy control	612 vs 318 (8)	SMD	−0.252 (−0.654 to 0.149)	.218	87.1
Activity of vitiligo					
Progressive	181 vs 182 (4)	SMD	−0.401 (−1.551 to 0.750)	.495	95.7
Stable	193 vs 182 (4)	SMD	−0.442 (−1.407 to 0.522)	.369	94.3
Vitamin B_{12}					
All	804 vs 518 (11)	SMD	−0.430 (−0.738 to −0.121)	.006	85.3
Healthy control	744 vs 458 (10)	SMD	−0.494 (−0.802 to −0.185)	.002	83.4
Activity of vitiligo					
Progressive	220 vs 242 (5)	SMD	−0.787 (−1.005 to −0.570)	<.001	16.4
Stable	209 vs 242 (5)	SMD	−0.720 (−0.989 to −0.452)	<.001	37.6

CI, Confidence interval; *SMD,* standardized mean difference.