SERUM VITAMIN B12, FOLATE, AND HOMOCYSTEINE LEVELS IN VITILIGO PATIENTS: A CASE-CONTROL STUDY IN KURDISTAN POPULATION

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ABSTRACT

Background

Vitiligo is an acquired depigmentation disorder that significantly impacts a patient's quality of life. Its precise etiology is yet unknown, but it appears to be complex. Many studies have been conducted in the last decade to determine the role of hyperhomocysteinemia, vitamin B12, and folate deficiency in the etiology of vitiligo. Studying part of the aforementioned conditions may give insight into vitiligo pathogenesis.

Objectives

To assess the contribution of homocysteine, vitamin B12, and folate in the pathogenesis of vitiligo by measuring homocysteine (Hcy), B12, and folate levels in the sera of patients and controls. as well as to see if there are a link between them and the degree of severity and duration of the vitiligo.

Patients and Methods

In the current study, 50 vitiligo patients of both sexes and 50 age and sex-matched healthy persons were recruited for six months at the Sulaimani center for skin disease treatment. Serum B12 & folate were measured using electrochemiluminescence (ECL), while serum Hcy level was measured by colorimetric technique.

Results

The mean serum levels of Hcy, B12, and folate in the healthy controls did not differ significantly from those in the patient group. (13.75 ±2.32 vs. 14.31±6.14 µmol/l; P > 0.05), (324.01±131.93 vs 311.57±110.55 pg/ml; P > 0.05), and (8.82±3.09 vs. 8.70±3.20 ng/ml P > 0.05) respectively.

Furthermore, no link was discovered between the serum levels of these parameters and the clinical types, the activity of the disease, and the duration of vitiligo.

Conclusion

The findings don't support the theory that homocysteine, B12, and folate are contributing factors in vitiligo etiology. Thus, screening the importance of the above mentioned factors cannot be recommended.

Keywords: *Vitiligo, Homocysteine, Hyperhomocysteinemia, B12, Folate.*

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INTRODUCTION

Vitiligo is an acquired multifactorial, most frequent cause of depigmentation of epidermis and hair follicles, with an estimated prevalence of 0.5 to 2% worldwide [1]. Although vitiligo does not affect patients' survival, it can affect the quality of life. There are three types of vitiligo: localized, generalized, and universal. Localized vitiligo is further classified as focal, segmental, or mucosal. Generalized can be acrofacial, Vulgaris, or mixed. More than 80% of the skin is affected by universal vitiligo [2].

The precise mechanism by which melanocytes are destroyed has yet to be fully elucidated. Several theories have been proposed, including autoimmune disease, oxidative stress, neurohumoral disorders, and the cytotoxic hypothesis[2].

Homocysteine is a crucial intermediate metabolite in the folate cycle and is a metabolite of methionine. Its structure resembles that of amino acids. Folate and vitamin B12 are cofactors for homocysteine methyltransferase, which converts homocysteine to methionine. The levels of these three compounds appear to be linked [3]. Nutritional shortage in one of these two vitamins causes a rise in Hcy levels in the bloodstream, leading to hyperhomocysteinemia. Folate and vitamin B12 supplements have been shown to cause pigmentation in vitiligo lesions. [4,5].

Hyperhomocysteinemia has been linked to the development of vitiligo through various processes, including forming reactive oxygen species, which causes oxidative stress in melanocytes[6]. Hcy inhibits the tyrosinase enzyme in the skin by binding to the active copper site, causing reversible hypopigmentation[7]. Moreover, Methylenetetrahydrofolate reductase (MTHFR) is a critical regulatory enzyme that converts homocysteine to methionine. There is growing evidence that the MTHFR gene polymorphism and blood homocysteine levels are linked to autoimmune disorders like vitiligo. MTHFR C677T, a homozygous variation, may be a risk factor for both high homocysteine levels and the development of vitiligo [8]. Furthermore, a genetic basis for both vitiligo and hyperhomocysteinemia has been identified (11q23 is a susceptibility locus for both) [9].

Pigmentary dilution, a feature of fair skin and hair, is seen in both vitiligo and homocystinuria[10]. Metabolism of Hcy may be affected by a mutation in the catalase gene (CAT), and vitiligo patients have poor catalase activity [11].

Many studies found that serum Hcy level was higher among vitiligo patients than in healthy individuals[12–21]. On the other hand, other researchers report no variations in the mean serum Hcy level between vitiligo patients and healthy peopl[22–28]. Furthermore, homocysteine has been recommended as a biomarker for the severity of vitiligo [29].

Vitamin B12 and folate, which are cofactors for homocysteine methyltransferase, and responsible for remethylation of Hcy to methionine [3], are said to be low in vitiligo patients [15,18,20], causing Hcy serum levels to rise as they are significant determinants of Hcy level in the blood; however, some researchers has discovered the opposite [23,24,28].

There have been controversies over the contribution of serum levels of Hcy, B12, and folate in the pathogenesis of vitiligo, and the findings of studies in this regard are very conflicting. It appears that it is very variable among different ethnic groups. We did this study in a Kurdish population to better understand vitiligo and its relationship to Hcy, B12, and folate levels.

PATIENTS AND METHOD

This case-control study was conducted in Sulaimani, Iraq, at the Sulaimani center to treat skin diseases. From the 18th of May, 2021, for six months. Fifty vitiligo patients of both sexes (21 males and 29 females) and 50 healthy people of the same sex and age were recruited. Vitiligo patients aged 18-60 were involved, and subjects for the control group were chosen from those who visited the same center for cosmetic reasons. Any participants with conditions that affect serum Hcy levels were excluded from the study, such as renal failure, diabetes mellitus, hypertension, psoriasis, Behcet disease, cigarette smoking, thyroid dysfunction, alcohol consumption, pregnancy, and genetic disorders affecting the metabolism of amino acids and vitamin supplement intake in the last three months. The ethics committee of Kurdistan's Higher Council of Medical Specialties approved the research protocol. Before beginning the study, both groups provided written consent. Patients with vitiligo were diagnosed by senior dermatologists through clinical examination and woods light examination. Each studied case was subjected to thorough clinical history and analysis, including, clinical types of vitiligo, whether it was generalized (Vulgaris), acrofacial, focal, segmental, or universal (we had no cases of vitiligo patients with mucosal or mixed patterns). The distribution of vitiligo

lesions (whether unilateral or bilateral), having a family history of vitiligo or not.

Vitiligo cases were classified into three classes according to the duration of their illness, those who had vitiligo for five years or less, six years to 10 years, and more than ten years. Vitiligo activity was assessed using the VIDA score, a six-point scale used to evaluate vitiligo stability. It is determined by the patient's perception of current disease activity over time [31]. Stable vitiligo, no change in the lesions observed by the patient in the last year before the study. Progressive vitiligo, enlargement of the existing lesion and appearance of the new lesion in the previous year, and regressive vitiligo, Stable with spontaneous regimentation for one year or more of the vitiligo lesions.

After explaining the technique to the participants & under complete aseptic precautions each participant had fasting (5 ml) of venous blood drawn and sent to be tested for Hcy, B12, and folate serum levels. Serum B12 and folate concentrations were measured by the electrochemiluminescence (ECL) technique (Cobas 6000, e601 module), and serum Homocysteine concentrations were estimated by colorimetric (Cobas 6000, c501 module). The data was analyzed using IMB SPSS version 25. Mean, and standard deviation was calculated for continuous parameters. Student t-test/ANOVA tests were used to compare quantitative variables. The Chi-square test was used to compare qualitative variables. $P < 0.05$ was considered to be statistically significant.

Disease activity	VIDA score
Active in the past six weeks	+4
Active in the past three months	+3
Active in past six months	+2
Active in past year	+1
Stable for at least one year	0
Stable with spontaneous regimentation for one year or more	-1

RESULTS

The average age of vitiligo patients in the current study was (36±9.08) years, with 21 male and 29 female cases (1:1.38). The disease lasted anywhere from 2 months to 18 years. Table 1 shows no statistically significant differences in mean age and sex distribution between the two groups of patients and controls.

The most prevalent clinical variant (48%) was generalized vitiligo(Vulgaris), followed by acrofacial (22%), focal (20%), segmental (6%), and universal (4%).

The disease was stable in 19 (38%) of the cases, progressive in 27 (54%), and regressive in 4 (8 %). The most common VIDA score recorded was VIDA score 0, which denotes stable vitiligo, and the least common was VIDA score -1, which indicates regressive vitiligo. Family history of vitiligo among first-degree relatives was positive in 9 patients (18%). Vitiligo lesions were unilaterally distributed over the body in 11 patients (22%), and the rest were bilateral vitiligo.

The usual range of serum Hcy level in our study age group is (12-15 µmol/l). The mean serum Hcy levels in vitiligo patients (13.75±2.32µmol/l) were not significantly different from those of the control group (14.31±6.14µmol/l) (p-value 0.5).

Serum level of vitamin B12 in the vitiligo group (311.57±110.55pg/ml) was not significantly different from that of healthy individuals (324.01±131.93 pg/ml) (P- value 0.6). Similarly, the average value of serum folate in vitiligo group (8.70±3.20ng/ml) wasn't significantly different from those of control subjects (8.82±3.09 ng/ml) (p-value 0.8), the mean serum levels of all three parameters are within the standard references in both study groups as shown in Table 2.

Serum level of Hcy in male vitiligo patients (17.01±7.97) was significantly higher than in female counterparts (12.36±3.36) (p-value 0.02); there was no significant correlation between serum levels of measured biochemical parameters and patient age group as well as activity of the disease (p-value 0.12) as shown in Table 3 and 4 respectively.

Neither the laterality of the vitiligo lesions nor family history doesn't affect serum Hcy, B12, and folate levels, as shown in Table 5. Furthermore, the duration of the illness

did not affect serum Hcy level (p-value 0.69), Figure 1. Furthermore, the average serum levels of Hcy and folate have no relationship with clinical types of the disease (p values 0.36 and 0.24), respectively, whereas the mean serum level of B12 is affected by distinct clinical types, as shown in Table 6.

Table 1. Clinical characteristics of the study groups.

	Cases (n=50)	Controls (n=50)	P-value
Mean age (in years with SD)	35.68±11.08	36±9.08	0.87
sex	Male=21 Female-=29	Male=21 Female-=29	1:00
Family history	Positive=9(18%) Negative=41(82%)	NA	
Duration of the disease	<= 5 Years=25(50%) 6-10 Years=14(28%) > 10 Years=11(22%)	NA	
Disease activity	Stable=19(38%) Progressive=27(54%) Regressive=4(8%)	NA	
Clinical types	Generalized 24(28%) Focal 10(20%) Acrofacial 11(22%) Segmental 3(6%) Universal 2(4%)	NA	
Distribution of the lesions	Unilateral 11(22%) Bilateral 39 (78%)	NA	

NA: Not applicable, NS: No significant, SD: Standard deviation

Table 2. Comparison of serum levels of Homocysteine, B12 and folic acid between study groups

biochemical parameters	Mean with SD (cases)	Mean with SD (controls)	P-value
Serum Hcy (12-15µmol/l)	14.31±6.14	13.75±2.32	0.5 NS
Serum B12 (197-771pg/ml)	311.57±110.55	324.01±131.93	0.6 NS
Serum Folic Acid (4.2-16.1ng/ml)	8.70±3.20	8.82±3.09	0.8 NS

NS; not significant

Table 3. comparison between serum levels of Hcy, B12 and folate with different patient age groups.

	<=28 Years	29-38 Years	39-48 Years	49-60 Years	P value
HCY	13.7033	13.768	14.1538	17.0743	.652
B12	339.1333	301.4667	320.6931	257.2143	.428
Folic Acid	7.53	9.308	9.7108	7.9957	.246
N	15	15	13	7	

Table 4. Relation of serum levels of Hcy, B12 & folate with vitiligo activity.

biochemical parameters	Stable	Progressive	Regressive	P value
N	19	27	4	
HCY	16.26	12.67	16.18	0.12
B12	307.51	315.66	303.25	0.96
Folic Acid	8.37	8.98	8.32	0.80

Table 5. Relation of serum level with laterality of the lesions and family history of the disease.

	Mean			Mean		
	Unilateral	Bilateral	P value	Positive family history	Negative family history	P -value
N	11	39		9	41	
HCY	13.05	14.67	0.44	12.24	12.76	0.26
B12	261.39	325.72	0.08	297.07	314.75	0.66
Folic Acid	9.12	8.58	0.62	7.93	8.86	0.43

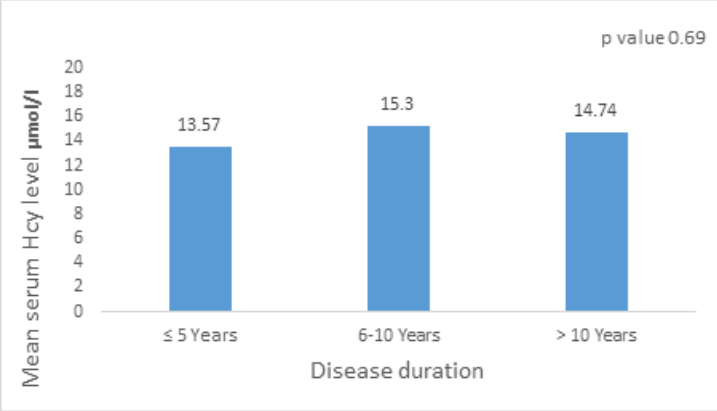

Figure 1. Correlation of mean Hcy level with the duration of vitiligo.

Table 6. Serum levels of vitamin B12, folic acid, and homocysteine in patients with different types of vitiligo.

	Generalized	Focal	Acrofacial	Segmental	Universal	P value
Hcy(SD)	16.01 (7.85)	13.79 (4.66)	11.61 (2.37)	12.17 (2.55)	14.60 (0.71)	0.36
B12(SD)	299.39 (102.44)	265.99 (78.63)	400.13 (123.21)	280.85 (51.13)	244.65 (149.69)	0.03
Folic Acid (SD)	7.97 (2.71)	10.12 (4.25)	8.89 (2.80)	7.17 (2.30)	11.50 (4.95)	0.24

DISCUSSION

Vitiligo is a chronic, multifactorial depigmentation disorder. It affects around 0.5-2% of the general population [1]. Although vitiligo is an asymptomatic disease, it can hurt patients' social interactions and self-esteem. There have been no curative therapeutic modalities for this chronically burdensome disease until now. Furthermore, unsatisfactory existing treatments necessitate further research into vitiligo pathogenesis [32].

Homocysteine is made from methionine in several stages before passing via one of two major metabolic pathways, remethylation or transsulfuration. Hyperhomocysteinemia is caused by a malfunction of enzymes and cofactors involved in the homocysteine production process. Excessive methionine consumption, certain disorders, and drug adverse effects are among others [33].

There is a growing body of research on the role of homocysteine in the pathogenesis of vitiligo. However, the results are contradictory. The current study looks at possible links between serum homocysteine, its cofactors B12 and folate levels, and vitiligo pathogenesis.

Hyperhomocysteinemia has been associated with the development of vitiligo by several mechanisms, including the production of IL-6 and the activation of NF-. B, the generation of reactive oxygen species in melanocytes causes oxidative stress and accumulation of melanocytotoxic products [6]. Hcy binds to the active copper site of the tyrosinase enzyme in the skin, inhibiting it and causing reversible hypopigmentation [7]. Furthermore, pigmentary dilution, a characteristic of fair skin and hair, is observed in both vitiligo and homocystinuria [10]. A mutation in the catalase gene (CAT) may affect Hcy metabolism, and vitiligo patients have low catalase activity [11].

Previous studies found that serum Hcy level is higher in vitiligo patients compared to healthy controls. Shaker & El-Tahlawi, concluded that hyperhomocysteinemia might be a precipitating factor in the pathogenesis of vitiligo. However, serum Hcy levels were higher only in progressive vitiligo than in controls but were comparable to healthy individuals in stable vitiligo [12]. Similarly, Singh et al., discovered that serum Hcy levels in vitiligo patients are higher than in the control group and active vitiligo cases had higher Hcy levels than stable vitiligo cases [13]. Other studies have found that serum Hcy levels are higher in vitiligo patients than controls [14–21].

Furthermore, Jonathan et al., strongly recommend screening serum Hcy level as a biomarker for vitiligo severity [29]. In a fascinating study, Anbar et al. revealed substantial differences in Hcy levels between suction-induced blisters fluid of vitiligo sufferers and healthy people. Hcy levels were higher in vitiliginous skin than in healthy skin. On the other hand, the serum levels of Hcy were not significantly different. They concluded that Hcy could play a role in the early stages of vitiligo pathogenesis, either as a primary event leading to the development of vitiligo or as a subsequent event in the process of vitiligo genesis [34].

In the process of Hcy remethylation, folate and vitamin B12 act as cofactors for homocysteine methyltransferase, which converts homocysteine to methionine. Any deficiency in these two cofactors results in trapped Hcy levels and, as a result, greater Hcy levels in the blood. These three chemicals' concentrations appear to be connected [3]. A nutritional deficiency causes hyperhomocysteinemia in one of these two vitamins, which produces an increase in Hcy levels in the bloodstream. Supplementing with folic acid and vitamin B12 has been proven to cause vitiligo lesions to pigment [4,5]. Park et al., performed a study among Korean vitiligo patients and healthy controls, and found that serum. B12 level is significantly higher in vitiligo cases than in controls [35].

A study conducted in Libya discovered that B12 and folate insufficiency were considerably prevalent among vitiligo cases, and dermatologists should check for B12 and folate deficiency in vitiligo patients [30]. Tsai et al. conducted the first systematic review on this topic. They discovered that vitiligo patients have higher serum Hcy and lower B12 levels, which were linked to vitiligo activity, but serum folate did not [21].

The outcome of our research differs from the findings of the above studies. Serum level of Hcy in male vitiligo patients (17.01±7.97) was significantly higher than in female counterparts (12.36±3.36) (p-value 0.02), Because of increased muscle mass, hormonal influences, and different lifestyle behaviors. This result is consistent with previous research. [12,13]. The mean serum level of Hcy in vitiligo patients was not significantly different from that of healthy individuals (13.75¬ ±2.32 vs. 14.31±6.14 µmol/l) (P value 0.5). Likewise, serum levels of B12 and folate were not

significantly different between vitiligo and healthy controls (311.57±110.55 vs. 324.01±131.93 pg/ml) (P value 0.6), (8.70±3.20 vs. 8.82±3.09 ng/ml) (P value 0.8) respectively.

Furthermore, activity, duration, and clinical types of vitiligo have no significant association with serum homocysteine level. Likewise, laterality of vitiligo lesions, family history, and age of participants had no association with serum levels of the above variables. This agrees with the finding of a study conducted among Iranian people by Ghiasi et al. [28]. Zaki et al., carried out a survey among the Egyptian population and their findings are consistent with our study; they found that there were no significant differences in the mean serum Hcy level between vitiligo patients and controls. They also found no significant correlation between vitiligo activity and duration of the disease with the mean level of Hcy[22].

Belucci et al., found the same results as the current study. They discovered that mean serum Hcy, B12, and folate levels did not differ significantly between the vitiligo and control groups. They also found that serum levels of Hcy, B12, and folate had no relationship with activity, duration of vitiligo, or patient age [23]. Our study revealed the same result as a study done in Saudia Arabia regarding serum levels of Hcy and B12 as there were no significant differences between vitiligo and controls. However, they found that serum level of folic acid is higher among vitiligo patients than in controls [24].

Hasibuan et al., carried out research among Indonesians, and they discovered no significant differences between serum Hcy levels of vitiligo patients and controls. They stated that there was no significant association between serum Hcy level and family history or disease duration. Still, They discovered a link between serum Hcy levels and patient gender, with males having greater Hcy levels than females, which is comparable to our findings [25]. Another study in Indonesia did not find a significant association between serum Hcy level and vitiligo activity[26].

The reason for these disparities in the outcomes of these studies is unclear; however, these differences can be partially explained firstly, the method of case selection and set inclusion and exclusion criteria; in one of the earliest studies performed in this context, which showed an association between Hcy level and vitiligo achieved by Shaker and El-Tahlawil, they selected severe vitiligo cases while excluding cases with less than 30% of vitiligo body involvement [12] in contrast to our study in which we included more comprehensive range of vitiligo activity scores and clinical types. Secondly, different studies, on the other hand, used different scoring systems; some researchers used the VASI score to measure the extent and severity of the disease [22], while others used the rule of nine to measure only the size of the depigmentation [13].

Thirdly, Methylenetetrahydrofolate reductase (MTHFR) is a critical regulatory enzyme that converts homocysteine to methionine. A recent study stated that the MTHFR C677T gene polymorphism and blood homocysteine levels are linked to autoimmune disorders like vitiligo, homozygous variation in this gene may be a risk factor for both high homocysteine levels and the development of vitiligo[36], thus, ethnicity might be a contributory factor for these disparities in the results, according to our knowledge till now, only two studies have been conducted in Iraq to investigate the relationship between serum Hcy level and vitiligo, the first of which was conducted among Arabic populations and found that there is an association & the current study, which was conducted among Kurdish populations and found no correlation between serum Hcy level and Vitiligo[16].

Finally, people from different nations, or even different regions within the same country, have varied dietary preferences, such as vegans and non-vegetarians, because cobalamin is mostly present in animal protein, hence dietary style has a direct impact on Hcy levels [20].

However, the current study is the first of its kind in the Kurdish population per our knowledge, and the sample size is not small, but it cannot represent the entire population due to the wide diversity of the Kurdistan community.

Conclusion & recommendation

Serum Hcy, B12 & folate is not significantly different between vitiligo and healthy controls. Serum Hcy level does not correlate with severity, duration, family history, laterality and clinical types of vitiligo. The finding does not support that, Hcy is the precipitating factor for development of vitiligo. Meanwhile, we do not recommend routine screening of serum Hcy level for vitiligo patients and addition of Hcy- lowering agents like vitamin B12 and folate in the protocol of vitiligo management

We recommend a comprehensive multicenter study with a larger sample size to investigate the relationship between vitiligo and serum Hcy levels.

REFERENCES

1. Krüger C, Schallreuter KU. A review of the worldwide prevalence of vitiligo in children/adolescents and adults. Int J Dermatol. 2012;51(10):1206–12.

2. Alikhan A, Felsten LM, Daly M, Petronic-Rosic V. Vitiligo: A comprehensive overview: Part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. J Am Acad Dermatol [Internet]. 2011;65(3):473–91. Available from: http://dx.doi.org/10.1016/j.jaad.2010.11.061

3. Blom HJ, Smulders Y. Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. J Inherit Metab Dis. 2011;34(1):75–81.

4. Kumar A, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW, Murthy SN. The metabolism and significance of homocysteine in nutrition and health. Nutr Metab. 2017;14(1):1–12.

5. Juhlin L OM. Improvement of vitiligo after oral treatment with vitamin B12 and folic acid and the importance of sun exposure. Acta dermato-venereologica. 1997;1;77(6):460–2.

6. Guilland JC, Favier A, Potier de Courcy G, Galan P HS. Hyperhomocysteinemia: an independent risk factor or a simple marker of vascular disease?. 1. Basic data. Pathol Biol (Paris). 2003;2(1;51):101-10.

7. Reish O, Townsend D, Berry SA, Tsai MY, King RA. Tyrosinase inhibition due to interaction of homocyst(e)ine with copper: The mechanism for reversible hypopigmentation in homocystinuria due to cystathionine β-synthase deficiency. Am J Hum Genet. 1995;57(1):127–32.

8. Bagheri Hamidi A, Namazi N, Mohammad Amoli M, Amani M, Gholami M, Youssefian L, et al. Association of MTHFR C677T polymorphism with elevated homocysteine level and disease development in vitiligo. Int J Immunogenet. 2020;47(4):342–50.

9. Tang XF, Zhang Z, Hu DY, Xu AE, Zhou HS, Sun LD, et al. Association analyses identify three susceptibility loci for vitiligo in the chinese han population. J Invest Dermatol. 2013;133(2):403–10.

10. Testai FD, Gorelick PB. Inherited metabolic disorders and stroke part 2: Homocystinuria, organic acidurias, and urea cycle disorders. Arch Neurol. 2010;67(2):148–53.

11. Góth L, Rass P, Páy A. Catalase enzyme mutations and their association with diseases. Mol Diagnosis. 2004;8(3):141–9.

12. Shaker OG, El-Tahlawi SMR. Is there a relationship between homocysteine and vitiligo? A pilot study. Br J Dermatol. 2008;159(3):720–4.

13. Singh S, Singh U, Pandey SS. Increased level of serum Homocysteine in vitiligo. J Clin Lab Anal. 2011;25(2):110–2.

14. Karadag AS, Tutal E, Ertugrul DT, Akin KO, Bilgili SG. Serum holotranscobalamine, vitamin B12, folic acid and homocysteine levels in patients with vitiligo. Clin Exp Dermatol. 2012;37(1):62–4.

15. Singh S, Singh U, Pandey S. Serum folic acid, vitamin B12 and homocysteine levels in Indian vitiligo patients. Egypt Dermatol Online J. 2012;8(1):1–7.

16. Salman MN, Alshalah HH, Alhattab MK. Effect of serum homocysteine in pathogenesis and activity of vitiligo. Res J Pharm Biol Chem Sci. 2016;7(6):1158–64.

17. Nahidi Y, Meibodi NT, Esmaili H. Serum homocysteine level in vitiligo patients. Iran J Dermatology. 2014;17(69):81–4.

18. Agarwal S, Mendiratta V, Chander R, Jain A, Yadav P. Study of serum levels of Vitamin B 12 , folic acid, and homocysteine in vitiligo . Pigment Int. 2015;2(2):76.

19. Sabry H, Sabry J, Hashim H. Serum levels of homocysteine, vitamin B12, and folic acid in vitiligo. Egypt J Dermatology Venerol. 2014;34(1):65.

20. Sundar PK, Chavala BM, Nagaraj SM, Reddy HK. A Study of Serum Levels of Vitamin B12, Folic Acid, and Homocysteine in Vitiligo Patients and Controls Attending a Tertiary Care Hospital in Bengaluru – An Analytical Cross-Sectional Study. J Evol Med Dent Sci. 2021;10(30):2271–5.

21. Tsai TY, Kuo CY, Huang YC. 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. J Am Acad Dermatol [Internet]. 2019;80(3):646-654.e5. Available from: https://doi.org/10.1016/j.jaad.2018.08.029

22. Zaki AM, Abdo HM, Ibrahim IM, Eldin A, Ibrahim K, Ch B. Serum homocysteine and vitiligo. gulf J dermatology Venereol. 2014;21(2):15–20.

23. Balci DD, Yonden Z, Yenin JZ ON. Serum homocysteine, folic acid and vitamin B12 levels in vitiligo. Eur J Dermatology. 2009;1;19(4):382–3.

24. AlGhamdi KM, Khurrum H, Moussa NA. Is there a real relationship between serum level of homocysteine and vitiligo? A controlled study on 306 subjects. J Cutan Med Surg. 2014;18(1):5–7.

25. Hasibuan DRU, Putra IB, Jusuf NK. Correlation between serum homocysteine and vitiligo area scoring index. Maced J Med Sci. 2017;5(3):332–4.

26. Asri E, Akhyar G, Gustia R. Correlation Between Homocysteine and Vitamin B12 Serum Level with Vitiligo Severity. 2019;10–5.

27. Hamza A, Farid C, El-sayed E, Kadeeb H. Assessment of serum homocysteine level in patients with nonsegmental vitiligo. Egypt J Dermatology Venerol. 2015;35(2):59.

28. Ghiasi M, Lajevardi V, Farahbakhsh A. Serum levels of vitamin B12, folic acid, and homocysteine in patients with vitiligo. Iran J Dermatology. 2015;18(2):45–50.

29. Silverberg JI, Silverberg NB. Serum homocysteine as a biomarker of vitiligo vulgaris severity: A pilot study. J Am Acad Dermatol. 2011;64(2):445–7.

30. Abdalhafid N, Sherif E, El-dibany SA, El A, Hussein S. Serum Vitamin B12 and Folic Acid in Vitiligo Patients : A Case Control Study. Int J Clin dermatology. 2020;3(2):19–21.

31. Bhor U PS. Scoring systems in dermatology. Indian J Dermatology, Venereol Leprol. 2006;72(4):315–21.

32. Chen J, Li S, Li C. Mechanisms of melanocyte death in vitiligo. Med Res Rev. 2020;(July):1–29.

33. Kim J, Kim H, Roh H, Kwon Y. Causes of hyperhomocysteinemia and its pathological significance. Arch Pharm Res [Internet]. 2018;41(4):372–83. Available from: https://doi.org/10.1007/s12272-018-1016-4

34. Anbar T, Zuel-Fakkar NM, Matta MF, Arbab MMI. Elevated homocysteine levels in suction-induced blister fluid of active vitiligo lesions. Eur J Dermatology. 2016;26(1):64–7.

35. Park HH, Lee MH. Serum levels of vitamin B12 and folate in Korean patients with vitiligo [3]. Acta Derm Venereol. 2005;85(1):66–7.

36. Chen JX, Shi Q, Wang XW, Guo S, Dai W, Li K, et al. Genetic polymorphisms in the methylenetetrahydrofolate reductase gene (MTHFR) and risk of vitiligo in Han Chinese populations: A genotype-phenotype correlation study. Br J Dermatol. 2014;170(5):1092–9.