

Serum homocysteine level in vitiligo patients

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Conflict of interest: none to declare

Received: 28 April 2014

Accepted: 30 July 2014

Background: Vitiligo is a common cutaneous depigmentation disorder caused by the destruction of melanocytes. The exact etiopathogenesis of this disorder is not well known, but a complex of genetic, immunologic, inflammatory, and cytotoxic factors have been implicated. According to reports on the role of vitamin B12 and folic acid deficiency as important co-factors in the metabolism of homocysteine, we expected an increase in homocysteine levels in patients with vitiligo; therefore, our aim was to investigate the serum levels of homocysteine in Iranian patients with vitiligo.

Method: Forty patients with vitiligo and 40 healthy controls matched for age and sex were studied. After exclusion of cases with diseases that could affect the homocysteine level, serum homocysteine levels were measured by ELISA.

Result: Males comprised 57.5% of the participants in both groups and 42.5% were female. The mean age of the patients was 24.68 ± 12.44 years. The level of homocysteine in the case and control groups was 18.56 ± 5.69 and 10.19 ± 4.40 , respectively, which was significantly higher in patients with vitiligo ($P \leq 0.001$). There was no correlation between homocysteine levels and age, sex, history of previous treatment, duration of disease, and the extent of body surface involvement. Serum homocysteine levels in patients with regressive vitiligo (13.8) were lower than progressive (18.4) and stable (20.4) cases ($P = 0.05$).

Conclusion: We found that the increase in serum homocysteine level in patients genetically susceptible to vitiligo could be a predisposing factor for the development of vitiligo. The serum homocysteine level may be associated with disease activity, and may be used as a prognostic factor for disease activity.

Keywords: depigmentation, ELISA, homocysteine, vitiligo

Iran J Dermatol 2014; 17: 81-84

INTRODUCTION

Vitiligo is an acquired progressive depigmentation clinically characterized by white patches and macules. The inheritance pattern of vitiligo is polygenic with an incomplete penetrance. The exact pathogenesis of the disease is not yet known and is probably multi-factorial, including a combination of autoimmune theories, biochemical dysregulation, oxidative stress, and melanocytorrhagia ¹.

Homocysteine metabolism has been recently noted as a factor involved in the pathogenesis of vitiligo ². The decrease in catalase activity detected in vitiligo can be a factor that changes homocysteine metabolism in this disease ³. Homocysteine oxidation can lead to the accumulation of toxic melanocytotoxic compounds, oxidative stress on melanocytes, and their death ⁴. Homocysteine has an inhibitory effect on skin tyrosinase activity ⁵, resulting in hypopigmentation ⁶. Homocystinuria

is associated with blond hair and fair skin, a phenomenon known as pigmentary dilution⁷. There are some reports of reduced serum levels of vitamin B12 and folic acid in vitiligo patients and improvement of vitiligo after treatment with these vitamins. These two vitamins are required cofactors for homocysteine methyl transferase for the synthesis of methionine from homocysteine in the activated methyl cycle. Reduced levels of these vitamins will decrease the level of methionine and increase homocysteine in the circulation².

All these findings indicate the probable role of homocysteine in the pathogenesis of vitiligo as a new target for future therapies for this disease. Six studies have been conducted in this field up to now. Studies by Shaker² in Egypt, Singh⁸ in India, Karadage⁹ in Turkey, and Silverberg¹⁰ in New York showed a significant increase in serum homocysteine levels in vitiligo patients, but two studies from Turkey by Balci¹¹ and Yasar¹² did not confirm this finding. Based on the above-mentioned assumptions and since the serum homocysteine status has not been investigated in Iranian patients with vitiligo, we decided to conduct this study.

PATIENTS AND METHODS

Using non-probability convenience sampling, 40 patients with vitiligo referred to the Dermatology Department of Imam Reza Hospital were enrolled in this case-control study. The control group included 40 healthy age and sex matched subjects referred for cosmetic operation. A two-section questionnaire was filled for each patient. In the first section, demographic information such as age, sex, type of vitiligo, extent of involvement, disease duration, history of other diseases and previous treatments, drug use, and smoking was recorded. The second section included laboratory findings of the patients including the serum homocysteine level, fasting blood sugar, urea, creatinine, liver function tests, and thyroid hormones in order to exclude items that may alter the blood homocysteine level.

The extent of body involvement by vitiligo was calculated using the Nine Law. We determined the type of vitiligo by using the working clinical classification of vitiligo¹³ as follows: 1) localized (focal, segmental/uni-lateral), 2) generalized (vulgaris, acrofacial, mixed) and 3) universal (more than 80% of the body surface area). Exclusion

criteria included treatment for vitiligo in the past two months; smoking; alcohol, coffee, and animal proteins consumption 24 hours before drawing blood; treatment with folic acid, B12, and B6; hormone therapy; diseases affecting the homocysteine level such as hypertension and diabetes mellitus, thyroid dysfunction; cardiovascular disease; kidney failure; deep vein thrombosis; Behçet's disease; pregnancy; malignancy; consumption of drugs such as OCP, azathioprine, metformin, thiazide diuretics, anticonvulsants, D-penicillamine, theophylline, vitamins, phenytoin, carbamazepine, and methotrexate.

After interview with patients and obtaining their consent, 5 ml fasting blood was drawn from them. After 30 minutes at ambient temperature of 27°C and formation of clot, the serum was separated by centrifugation at 3000 rpm for 10 min and kept at -20°C until testing. Homocysteine was measured using ELISA DRG kit made in Germany. Using SPSS version 11.5, descriptive indicators of the community were determined. Given the descriptive nature of the study, statistical information including mean and standard deviation were calculated for the variables. T test, ANOVA, and Pearson's correlation were used for data analysis.

RESULTS

The mean age of the patients and controls was 24.68 ± 12.44 and 24.50 ± 12.32 years, respectively. Based on t-test, the two groups were matched for age ($P = 0.95$). Males comprised 57.5% of subjects in both groups and 42.5% were female patients; χ^2 test showed that the two groups were matched for sex ($P = 1$). Other demographic characteristics of the patients in the present study are summarized in Table 1.

The serum level of homocysteine in case and control groups was 18.56 ± 5.69 and 10.19 ± 4.40 , respectively. T-test (independent sample test) showed that the serum level of homocysteine was significantly higher in patients with vitiligo than normal subjects ($P \leq 0.001$).

The serum level of homocysteine was 17.50 ± 6.12 in male patients and 20.00 ± 4.85 in female patients. T-test indicated no significant relationship between the mean serum homocysteine level and sex ($P = 0.157$). According to the normal distribution of quantitative data, Pearson correlation indicated

Table 1. Characteristics of the vitiligo patients

Age (Mean \pm SD)	24.68 \pm 12.44
Gender	
Male	23 (57.5%)
Female	17 (42.5%)
Type of vitiligo	
Localized	12 (30%)
Local	4 (33.3%)
Segmental	8 (66.6%)
Generalized	28 (70%)
Vulgaris	17 (42.5%)
Acrofacial	8 (20%)
Mix	3 (7.5%)
Body surface Area Involvement mean \pm SD Min 5% Max 70%	18.89 \pm 16.39
Duration of vitiligo	15 days to 20 years
History of previous treatment	
Yes	27 (67.5%)
No	13 (32.5%)
Disease activity	
Regressive	6 (15%)
Active	18 (45%)
Stable	16 (40%)

no significant relationship between the serum homocysteine level and age ($P = 0.624$). The serum homocysteine level was 18.14 ± 5.89 and 19.44 ± 5.36 in patients with no history of treatment and those with a treatment history, respectively. T test showed that a history of treatment had no significant effect on the serum homocysteine level in patients ($P = 0.50$). Pearson correlation showed no relationship between the serum homocysteine level and the extent of body involvement with vitiligo or with disease duration [$r = 0.089$, $P = 0.607$] and [$r = 0.101$, $P = 0.53$], respectively]. Based on ANOVA, the serum homocysteine level in patients with regressive vitiligo (13.8) was lower than progressive (18.4) and stable (20.4) cases ($P = 0.05$). T test showed no difference between the serum homocysteine level in stable and progressive vitiligo. ($P = 0.38$) According to ANOVA, the serum homocysteine level was not significantly different among different subtypes of localized and generalized vitiligo ($P = 0.47$, $P = 0.60$, respectively). The serum level of homocysteine was not different in generalized and localized forms of vitiligo ($P = 0.85$).

DISCUSSION

In the present study, the serum homocysteine

level was significantly higher in patients with vitiligo (18.56 ± 5.69) than the healthy control group (10.19 ± 4.40) ($P < 0.001$). As the two groups were matched for age and sex and considering the elimination of other factors affecting the serum level of homocysteine in both groups following examination, history, and complementary tests, the high level of homocysteine appears to be related with the pathogenesis of vitiligo. Studies by Shaker ² in Egypt, Singh ⁸ in India, Karadage ⁹, and Silverberg ¹⁰ all showed significant increases in the serum homocysteine level in vitiligo patients, but two studies from Turkey by Balci ¹¹ and Yasar ¹² did not confirm this finding and attributed it to racial variations and polymorphism in the gene involved in homocysteine metabolism and the diet rich in meat which is common in Turkey. Increased homocysteine causes destruction of melanocytes through production of IL-6 and activation of NF-kB and reactive oxygen species ^{4,10}. Homocysteine has been suggested to have an inhibitory effect on the skin activity of histidinase and tyrosinase, which have a vital role in melanin synthesis ⁵. Homocysteine inhibits tyrosinase by binding copper in the active site, leading to hypopigmentation ⁶. The increase in the homocysteine level can interfere with normal melanogenesis and plays a role in the pathogenesis of vitiligo. The possible role of homocysteine in the development of metabolic disorders and increased risk of cardiovascular disease in vitiligo patients has also been addressed ^{9,14}.

There was no difference in serum homocysteine levels between male and female patients in our study, while the studies by Singh, Shaker, and Balci indicated higher levels of homocysteine in men than women ^{2,8,11}. Studies on general population have attributed higher levels of homocysteine in men than women due to hormonal factors ¹⁵. But regarding the influence of other factors such as age, extent and activity of disease, as well as genetic and ethnic differences on the homocysteine level of patients along with small sample size of our study, it could be justified for the lack of difference in the homocysteine level between genders in our study.

Similar to the study performed by Shaker ², a history of previous treatment had no impact on serum level of homocysteine in our patients, indicating that current treatments have no effect on the level of homocysteine, and newer therapies

with a homocysteine-lowering mechanism should be sought after. Like the study by Shaker, age had no effect on the serum level of homocysteine ².

Similar to the studies by Shaker ² and Balci ¹¹, the type of vitiligo and the extent of body involvement did not have any effects on the serum level of homocysteine in our patients, whereas a direct relationship was reported between the serum homocysteine level and generalized and localized vitiligo in the study conducted by Silverberg, and the serum homocysteine level was introduced as a new biomarker for the severity of vitiligo ¹⁰. Possible reasons for the absence of such a finding in the present study can be due to racial differences and characteristics of patients in the two studies. Serum homocysteine levels in our patients were lower in the regressive type than active and stable types similar to the studies by Singh ⁸ and Shaker ², indicating a possible role of homocysteine in the activity of vitiligo.

Based on the findings of this study, we found that the increased serum homocysteine levels in genetically susceptible Iranian patients can be one of the factors predisposing to the development of vitiligo. Increased serum homocysteine levels are associated with the disease activity and may be used as a prognostic factor for the disease activity. New studies are recommended to evaluate the therapeutic effect of the agents reducing the serum homocysteine level such as folic acid and vitamin B12 in vitiligo.

REFERENCES

1. Lee BW, Schwartz RA, Hercogová J, et al. Vitiligo road map. *Dermatol Ther* 2012;25 Suppl 1:S44-56.
2. Shaker OG, El-Tahlawi SM. Is there a relationship between homocysteine and vitiligo? A pilot study. *Br J Dermatol* 2008;159:720-4.
3. Góth L, Rass P, Páy A. Catalase enzyme mutations and their association with diseases. *Mol Diagn* 2004;8:141-9.
4. Guillard JC, Favier A, Potier de Courcy G, et al. Hyperhomocysteinemia: an independent risk factor or a simple marker of vascular disease? 2. Epidemiological data. *Pathol Biol (Paris)* 2003;51:111-21.
5. Kurbanov Kh, Burobin VA, Berezov TT. Histidase activity of the skin in relation to the state of melaninogenesis. *Biull Eksp Biol Med* 1974;78:57-60.
6. Reish O, Townsend D, Berry SA, et al. Tyrosinase inhibition due to interaction of homocyst(e)ine with copper: the mechanism for reversible hypopigmentation in homocystinuria due to cystathionine beta-synthase deficiency. *Am J Hum Genet* 1995;57:127-32.
7. Brenton DP, Cusworth DC, Dent CE, Jones EE. Homocystinuria. Clinical and dietary studies. *Q J Med* 1966;35:325-46.
8. Singh S, Singh U, Pandey SS. Increased level of serum Homocysteine in vitiligo. *J Clin Lab Anal* 2011;25:110-2.
9. Karadag AS, Tatal E, Ertugrul DT, et al. Serum holotranscobalamine, vitamin B12, folic acid and homocysteine levels in patients with vitiligo. *Clin Exp Dermatol* 2012;37:62-4.
10. Silverberg JI, Silverberg NB. Serum homocysteine as a biomarker of vitiligo vulgaris severity: a pilot study. *J Am Acad Dermatol* 2011;64:445-7.
11. 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.
12. 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.
13. Hercogová J, Schwartz RA, Lotti TM. Classification of vitiligo: a challenging endeavor. *Dermatol Ther* 2012; 25 Suppl 1:S10-6.
14. Pietrzak A, Bartosińska J, Hercogová J, et al. Metabolic syndrome in vitiligo. *Dermatol Ther* 2012;25 Suppl 1:S41-3.
15. Hu CP, Shao JM, Yan JT, et al. Study on the distribution of serum homocysteine and on multi-stepwise regression analysis of the associated factors in the population of community areas in Wuhan. *Zhonghua Liu Xing Bing Xue Za Zhi* 2004;25:945-8.