Increased Level of Serum Homocysteine in Vitiligo

Suman Singh,* Usha Singh, and Shyam Sunder Pandey
Department of Pathology, Institute of Medical Sciences, Varanasi, India

Background: Vitiligo is an acquired depigmenting disorder caused by the destruction of melanocytes. The exact etiopathogenesis and mechanisms of vitiligo are not fully understood. Vitamin B12 and folic acid levels are decreased in vitiligo, which are the important cofactors required in the metabolism of Homocysteine (Hcy). Consequently, Hcy level increases in the circulation. Therefore, it is possible that increased Hcy plays a role in melanocytes destruction. The aim was to study for any association of vitiligo with serum Hcy level. **Method:** A total of 30 vitiligo patients of both sexes and 30 control subjects were enrolled in this study. Sera from patients and controls were assayed for Hcy by Enzyme immunoassay. The collected data were

analyzed by SPSS version-16. **Results:** The mean serum level of Hcy was significantly higher in patients with vitiligo as compared with healthy controls and its level was high in male patients as compared with female patients. The Hcy level in vegetarian patients was significantly higher as compared with nonvegetarian patients. The Hcy level was also significantly higher in active vitiligo patients as compared with stable vitiligo patients. **Conclusion:** An increased serum Hcy may be a precipitating factor for vitiligo in the predisposed individuals. Serum Hcy is related to the gender of patients, activity of disease, and dietary habits of vitiligo patients. *J. Clin. Lab. Anal.* 25:110–112, 2011. © 2011 Wiley-Liss, Inc.

Key words: vitiligo; homocysteine; melanocytes; dietary habits

INTRODUCTION

Vitiligo is one of the most common acquired pigmentary disorders with a prevalence of 1–2% of population (1). The exact etiopathogenesis and mechanisms of vitiligo are not fully understood. However, an association between vitiligo and reduced serum levels of vitamin B12 and folic acid has been found (2–4). Homocysteine (Hcy) level is mainly determined by the levels of folic acid and vitamin B12 (5) because both act as cofactors of the enzyme Hcy methyl transferase for the regeneration of methionine from Hcy in the activated methyl cycle (6). It has been reported that vitiligo is associated with elevated Hcy levels (7). A study from Egypt also showed that serum Hcy is increased in vitiligo patients as compared with healthy subjects (8). The oxidation of hcy produces reactive oxygen species, which causes oxidative stress on melanocytes (9). Tyrosinase is a 75 kD copper-containing enzyme that initiates the melanin biosynthesis in pigmented cells (10,11). Hcy also leads to inhibition of tyrosinase enzyme by binding with copper, at its active site resulting in reversible hypopigmentation (12). In this study we assessed the level of serum Hcy and compared it with healthy subjects in

order to find data further supporting the role of Hcy in the pathogenesis of vitiligo.

MATERIAL AND METHOD

A total of 30 vitiligo patients and 30 healthy age- and sex-matched subjects were taken from the outpatient department of Dermatology and Venereology of Sir Sunderlal hospital, B.H.U., Varanasi for a period of 6 months. Vitiligo patients included 16 (54%) cases of active vitiligo (new lesions within the 2 months before the study as observed by the patients) and 14 (46%) of stable vitiligo (no change in the vitiligo lesions during the 2 months before the study as observed by the patients). The 30 patients included 16 (54%) men with age range 13–48 years and 14 (46%) women with age range 13–42 years.

*Correspondence to: Suman Singh, C/O Dr. Usha, Department of Pathology, IMS, BHU, Varanasi 221005, Uttar Pradesh, India.
E-mail: sumanbiotechster@gmail.com

Received 26 October 2010; Accepted 15 December 2010

DOI 10.1002/jcla.20442

Published online in Wiley Online Library (wileyonlinelibrary.com).

Serum Hcy was performed by enzyme immunoassay (EIA), kit of Axis shield diagnostics ltd., Dundee, UK, supplied by M/S OSB Agencies Delhi, India. Before immunoassay the protein-bound Hcy was reduced to free Hcy and enzymatically converted to *S*-adenosyl-L-hcy(SAH). Dithiothreitol was used to reduce the Hcy, mixed disulfide and protein-bound forms of Hcy to free Hcy. Hcy in the test sample is converted to SAH by the use of SAH hydrolase and excess of adenosine. In the solid-phase EIA there is a competition between SAH in the sample and immobilized SAH bound to the walls of the microtiter plate for binding sites on a monoclonal anti-SAH antibody. After washing, the unbound anti-SAH antibody is removed and a secondary rabbit anti-mouse antibody labelled with the enzyme horse radish peroxidase is added. After washing, substrate is added to develop color which is stopped by sulfuric acid and the color is measured spectrophotometrically. Absorbance is inversely related to the concentration of Hcy in the sample.

STATISTICAL ANALYSIS

The statistical analysis of the data was done using Student's *t* test for difference of means on SPSS for windows (version 16.0) statistical package (SPSS Inc., Chicago, IL) computer statistics program. *P* values less than 0.05 were considered as significant.

RESULTS

The mean ages of vitiligo patients and controls were 28.5 ± 9.8 and 29.4 ± 7.6 year old, respectively. Age in vitiligo varied from 12 to 48 years. The disease was stable in 14(46%) patients and active in 16(54%) patients.

The mean serum Hcy level in the patient group was significantly higher than in the control group (26.8 ± 7.7 vs. 22.1 ± 1.9 , $P < 0.05$, Table 1). There was a significant relationship between Hcy level and activity of vitiligo. The mean Hcy level in patient with active vitiligo was significantly higher than stable vitiligo cases (30.2 ± 6.5 vs. 22.8 ± 7.4 , $P < 0.05$, Table 2).

Regarding the sex of the patients, the mean Hcy level was higher in male than female patients (31.0 ± 7.8 vs.

TABLE 1. Showing Serum Levels of Homocysteine Between Vitiligo and Controls

Groups	Range ($\mu\text{mol/l}$)	Mean $\pm$ SD ($\mu\text{mol/l}$)	<i>t</i>	<i>P</i>
<i>Homocysteine level</i>				
Vitiligo (30)	14–39.5	30.2 ± 6.5	3.1	0.03*
Controls (30)	18.5–28.5	26.8 ± 7.7		

Significant * $P < 0.05$.

22.0 ± 4.2 , $P < 0.05$, Table 3). There was a significant relationship between Hcy level and dietary habits of the patients; the Hcy level was significantly higher in vegetarian patients as compared to nonvegetarian patients (32.7 ± 5.7 vs. 21.6 ± 5.2 , $P < 0.001$, Table 4).

DISCUSSION

In our study, the serum Hcy level was significantly elevated in patients with vitiligo than in controls. After further analysis it was found that Hcy level was increased more in active vitiligo patients than in stable vitiligo patients. These results can be substantiated by the pilot study of Shaker and El Tahlawi on 26 vitiligo cases, who also reported high serum hcy level in vitiligo patients (8). Furthermore, as Hcy metabolism was dependent on folic acid and vitamin B12 and both of which are lowered in patients with vitiligo (2–4), this results in an increase in Hcy and decrease in methionine (13,14).

According to our study the hcy level was higher in male than in female patient within the patient group (31.0 vs. $22.0 \mu\text{mol/l}$). The gender disparity may be explained by hormonal status, greater muscle mass in men, and gender-related lifestyle differences (15).

TABLE 2. Showing Serum Levels of Homocysteine Between Active and Stable Vitiligo Patients

Groups	Range ($\mu\text{mol/l}$)	Mean $\pm$ SD ($\mu\text{mol/l}$)	<i>t</i>	<i>P</i>
<i>Homocysteine level</i>				
Active Vitiligo (16)	22–39.5	30.2 ± 6.5	2.9	0.007*
Stable Vitiligo (14)	14–35.5	22.8 ± 7.4		

Significant * $P < 0.05$.

TABLE 3. Showing Serum Levels of Homocysteine Between Male and Female Vitiligo Patients

Groups	Range ($\mu\text{mol/l}$)	Mean $\pm$ SD ($\mu\text{mol/l}$)	<i>t</i>	<i>P</i>
<i>Homocysteine level</i>				
Male (16)	22–39.5	31.0 ± 7.8	3.8	0.001*
Female (14)	36.5–14	22.0 ± 4.2		

Significant * $P < 0.05$.

TABLE 4. Showing Serum Levels of Homocysteine Between Vegetarians and Nonvegetarians Vitiligo Patients

Groups	Range ($\mu\text{mol/l}$)	Mean $\pm$ SD ($\mu\text{mol/l}$)	<i>t</i>	<i>P</i>
<i>Homocysteine level</i>				
Vegetarians (13)	29–39.5	32.7 ± 5.7	5.5	0.001*
Nonvegetarians (17)	14–39.5	21.6 ± 5.2		

Significant * $P < 0.05$.

Similarly, Hu et al. in another study also reported that Hcy levels differ significantly according to sex (16).

According to our study, serum hcy is significantly elevated in vegetarian as compared with nonvegetarian (32.7 vs. 21.6 $\mu\text{mol/l}$) within the patient group. Hcy level was significantly related to plasma B12 level and was lower in nonvegetarian compared with vegetarian (17). Vitamin B12 level is mainly present in animal proteins (18). Thus, dietary habit may influence Hcy level.

Contrary to this Balci and Yoden et al. found no significant difference in Hcy levels between the vitiligo and healthy controls (19). This discrepancy may be due to the fact that in Turkey mostly patients are nonvegetarian or it may be due to different ethnic groups between the two studies which may have affected the Hcy level. Furthermore, in our study most of the patients involved at least 25% of their body area. Thus, additional studies in larger series are suggested in order to investigate the potential role of Hcy in vitiligo.

CONCLUSION

Our study concludes that increased serum Hcy level might be a precipitating factor for vitiligo in the predisposed individuals. It has been found that serum Hcy is related to gender of the patients, activity of disease, and dietary habits of vitiligo patients.

REFERENCES

1. Dave S, D'Souza M, Thappa DM, Reddy KS, Bobby Z. High frequency of thyroid dysfunction in Indian patients with vitiligo. *Indian J Dermatol* 2003;48:68–72.
2. Montes LF, Dias ML, Lajous J, Garcia NJ. Folic acid and vitamin B12 in vitiligo: A nutritional approach. *Cutis* 1992;50:39–42.
3. Kim SM. Serum levels of folic acid and vitamin B12 in Korean patients with vitiligo. *Yonsei Med J* 1999;40:195–198.
4. El-Batawi MMY, El-Tawil NEA, El-Tawil AEA. Serum levels of vitamin B12 and folic acid in Egyptian patients with vitiligo. *Egypt J Derm Androl* 2001;21:77–80.
5. Robinson K. Homocysteine, B vitamins, and risk of cardiovascular disease. *Heart* 2000;83:127–130.
6. Mudd SH, Levy HL, Skerby LF. Disorders of transsulfuration. In: Scriver CR, Beudet AL, Sly WS, Valle D, editors. *The Metabolic and Molecular Basis of Inherited Disease*, 7th edition. New York: McGraw-Hill; 1995. p 1279–1327.
7. Gouaille CB. Homocysteine, the new marker of disease risk: An overview. *Eur Pharmacother* 2005;1–8.
8. Shaker OG, El-Tahlawi SM. Is there a relationship between homocysteine and vitiligo? A pilot study. *Br J Dermatol* 2008;159:720–724.
9. Guillard JC, Favier A, Potier de Courcy G, et al. Hyperhomocysteinemia: An independent risk factor or a simple marker of vascular disease? 1. Basic data. [In French]. *Pathol Biol (Paris)* 2003;51:101–101.
10. Baharav E, Merimski O, Shoenfeld Y, et al. Tyrosinase as an autoantigen in patients with vitiligo. *Clin Exp Immunol* 1996;105:84–88.
11. Manga P, Sheyn D, Yang F, Sarangarajan R, Boissy RE. A role for tyrosinase-related protein 1 in 4-tert-butylphenol-induced toxicity in melanocytes. *Am J Pathol* 2006;169:1652–1662.
12. Reish O, Townsend D, Berry SA, et al. Tyrosinase inhibition due to interaction of homocysteine with copper: The mechanism for reversible hypopigmentation in homocystinuria due to cystathionine beta-synthase deficiency. *Am J Hum Genet* 1995;57:127–132.
13. Guttormsen AB, Schneede J, Ueland PM, et al. Kinetics of total plasma homocysteine in subjects with hyperhomocysteinemia due to folate or cobalamin deficiency. *Am J Clin Nutr* 1996;64:194–202.
14. Minet JC, Bissé E, Aebischer CP, et al. Assessment of vitamin B12, folate and vitamin B6 status and relation to sulfur amino acid metabolism in neonates. *Am J Clin Nutr* 2000;72:751–757.
15. Kim MH, Kim E, Passen EL, et al. Cortisol and estradiol: Non genetic factors for hyperhomocyst(e)inemia. *Metabolism* 1997;46:247–249.
16. 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 [In Chinese]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2004;25:945–948.
17. Kalita J, Mishra UK, Srivastava AK. A study of homocysteine level in North Indian subjects with special reference to their dietary habit. *European society for clinical nutrition and metabolism* 2007;2:116–119.
18. Herbert V, Das KC. Folic acid and vitamin B12. In: Shills ME, Olson JA, Shike M, editors. *Modern Nutrition in Health and Disease*, 8th edition. Philadelphia: Lea & Febiger; 1994. p 402–425.
19. Balci DD, Yonden Z, Yenin JZ, Okumus N. Serum homocysteine, folic acid and vitamin B12 levels in vitiligo. *Eur J of Dermatol* 2009;19:382–383.