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Serum folic acid, vitamin B12 and homocysteine levels in Indian vitiligo patients.

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Abstract

Vitiligo is a common, acquired, often heritable, multifactorial, polygenic pigmentary disorder with a complex pathogenesis and prevalence of 1-2% of population. An association between vitiligo and reduced serum levels of vitamin B12 and folic acid has been found. Recently, it has been found that serum homocysteine (Hcy) is elevated in patients with vitiligo. Homocysteine level is mainly determined by levels of folic acid and vitamin B12. In this study we assessed the level of serum folic acid, vitamin B12 and Hcy and find data further supporting the role of oxidative stress in the pathogenesis of vitiligo.

Total of 275 subjects were enrolled for the study comprising of 200 patients with vitiligo and 75 healthy controls (HC) from the outpatient department of Dermatology and Venereology of Sir Sunderlal hospital, B.H.U., Varanasi, India. The statistical analysis of the data was done using SPSS for windows (version 16.0) statistical package (SPSS Inc., Chicago, IL) computer statistics program.

Mean value of serum folic acid (4.88 ± 1.52 Vs 6.25 ± 0.69) and vitamin B12 (428.46 ± 133.52 Vs 536.63 ± 111.43) was significantly decreased in vitiligo group as compared to controls. Regarding the sex of the patients, the mean Hcy level was higher in male than female patients (31.0 ± 7.8 Vs 22.0 ± 4.2 $p < 0.05$). While there was no significant difference in serum Folic acid (4.90 ± 1.52 Vs 4.87 ± 1.53) & Vitamin B12 (428.93 ± 134.11 Vs 428.14 ± 133.67) between male & female vitiligo patients. The mean Hcy level in patient with active vitiligo was significantly higher than controls (30.2 ± 6.5 Vs 23.1 ± 1.9 $p < 0.05$). While there was no significant difference between serum Folic acid, Vitamin B12 in active or stable vitiligo as compared to controls.

Introduction

Vitiligo is a common, acquired, often heritable, multifactorial, polygenic pigmentary disorder with a complex pathogenesis and prevalence of 1-2% of population [1]. The precise etiology of vitiligo still remains to be elusive. Free radical and immune mediated damage of melanocytes are the most probable pathological mechanism [2]. An association between vitiligo and reduced serum levels of vitamin B12 and folic acid has been found [3,4,5]. Improvement of this disease after treatment with vitamin B12 and folic acid has also been reported [6]. Recently, it has been found that serum Hcy is elevated in patients with vitiligo [7]. Homocysteine level is mainly determined by levels of folic acid and vitamin B12 [8] because both acts as cofactors by the enzyme Hcy methyl transferase for the regeneration of methionine from Hcy in the activated methyl cycle [9]. Thus, elevated Hcy level causes oxidative stress on melanocytes by producing reactive oxygen species [10]. Further, there are reports that Hcy inhibits tyrosinase activity by binding with copper in its active site, resulting in reversible hypopigmentation [11]. Pernicious anemia is induced by Vitamin B12 deficiency which is caused either by the absence of intrinsic factor from atrophy of the gastric mucosa or by autoimmune destruction of parietal cells of the stomach [12,13]. Vitamin B12 facilitates the conversion of folate to tetrahydrofolate, which is essential in various enzyme reactions [14,15]. There have been several conflicting reports on the blood levels of vitamin B12 and folate in vitiligo [6,16]. In this study we assessed the level of serum folic acid, vitamin B12 and Hcy and find data further supporting the role of oxidative stress in the pathogenesis of vitiligo.

Material and method

Total of 275 subjects were enrolled for the study comprising of 200 patients with vitiligo and 75 healthy controls (HC) from the outpatient department of Dermatology and Venereology of Sir Sunderlal hospital, B.H.U., Varanasi, India. Clinical diagnosis of the vitiligo patients was done by the dermatologist. Vitiligo patients included 128 cases (68%) of generalized vitiligo and 72 cases (32 %) of localized vitiligo. On the basis of stage, 110 cases (55%) are of stable stage (no change in the new lesions within the 2 months prior to the study as observed by the patients) and 90 cases (45%) are of active stage (new lesions within the 2 months prior to the study as observed by the patients). Patients with history of vitamin intake within the last six months were excluded from the study. Serum folic acid and vitamin B12 were measured in both patients and control by chemiluminescent assay using assay kit of IMMULITE, U. S. and Serum Hcy was done by Enzyme immunoassay (EIA), kit of Axis shield diagnostics ltd., Dundee, U.K., supplied by M/S OSB Agencies Delhi, India. IMMULITE folic acid is a solid, competitive, liquid phase, ligand-labeled, protein binding chemiluminescent assay with in situ immobilization, and with an anti-ligand detection system and IMMULITE vitamin B12 is a solid-phase, competitive chemiluminescent enzyme immunoassay.

Statistical analysis-The statistical analysis of the data was done using student's t-test for difference of mean on SPSS for windows (version 16.0) statistical package (SPSS Inc., Chicago, IL) computer statistics program. P values less than 0.05 was taken as significant.

Results

The mean age of vitiligo patients and controls were 33.23 ± 16.67 and 31.45 ± 11.02 year old respectively. Age in vitiligo varied from 12 to 48 years. The 200 patients included 82(41.0) men with age range 13-48 years and 118(59.0) women with age range 13-42 years. The mean

serum Hcy level in the patient group was significantly higher than in the control group (28.8 ± 7.7 Vs 23.1 ± 1.9 $p < 0.05$). Mean value of serum folic acid (4.88 ± 1.52 Vs 6.25 ± 0.69) and vitamin B12 (428.46 ± 133.52 Vs 536.63 ± 111.43) was significantly decreased in vitiligo group as compared to controls.

Groups	Folic acid	Vitamin B12	Homocysteine
	Mean \pm SD (ng/ml)	Mean \pm SD (pg/ml)	Mean \pm SD (μ mole/l)
Vitiligo (200)	4.88 ± 1.52	428.46 ± 133.52	28.8 ± 7.7
Control (75)	6.25 ± 0.69	536.63 ± 111.43	23.1 ± 1.9
P value	0.000*	0.000*	0.000*

Table 1: showing serum levels of folic acid, vitamin B12 and homocysteine in vitiligo and controls.

Regarding the sex of the patients, the mean Hcy level was higher in male than female patients (31.0 ± 7.8 Vs 22.0 ± 4.2 $p < 0.05$). While there was no significant difference in serum folic acid (4.90 ± 1.52 Vs 4.87 ± 1.53) & vitamin B12 (428.93 ± 134.11 Vs 428.14 ± 133.67) between male & female vitiligo patients.

Groups	Folic acid	Vitamin B12	Homocysteine
	Mean \pm SD (ng/ml)	Mean \pm SD (pg/ml)	Mean \pm SD (μ mole/l)
Male (82)	4.90 ± 1.52	428.93 ± 134.11	31.0 ± 7.8
Female (118)	4.87 ± 1.53	428.14 ± 133.67	22.0 ± 4.2
P value	0.882	0.968	0.000*

Table 2: showing serum levels of folic acid, vitamin B12 and homocysteine in male and female vitiligo patients.

The mean Hcy level in patient with active vitiligo was significantly higher than controls (30.2 ± 6.5 Vs 23.1 ± 1.9 $p < 0.05$). While there was no significant difference between serum

Folic acid, Vitamin B12 in active or stable vitiligo as compared to controls.

Groups	Folic acid	Vitamin B12	Homocysteine
	Mean \pm SD (ng/ml)	Mean \pm SD (pg/ml)	Mean \pm SD (μ mole/l)
Active (110)	4.08 \pm 1.49	417.93 \pm 133.11	30.2 \pm 6.5
Stable (90)	4.28 \pm 1.52	427.33 \pm 131.11	22.1 \pm 1.9
P value	0.782	0.978	0.007*

Table 3: showing serum levels of folic acid, vitamin B12 and homocysteine in active and stable vitiligo patients.

Correlation of disease duration with serum folic acid, vitamin B12 and Hcy showed that only vitamin B12 shows significant association while folic acid and Hcy are not associated with duration of disease.

Duration of disease (years)	Total (200)	Folic acid	Vitamin B12	Homocysteine
	No (%)	Mean \pm SD (ng/ml)	Mean \pm SD (pg/ml)	Mean \pm SD (μ mole/l)
<10	168 (82.5)	4.86 \pm 1.47	438.96 \pm 133.70	26.86 \pm 7.55
11-20	26 (13.0)	4.79 \pm 1.85	390.54 \pm 137.07	25.21 \pm 8.26
>20	9 (4.5)	5.44 \pm 1.40	345.56 \pm 59.61	26.22 \pm 9.21
P value		0.413	0.039*	0.532

Table 4: Correlation of folic acid, vitamin B12 and homocysteine with duration of disease.

Discussion

Vitiligo is a depigmenting disorder of the skin, which may derive from programmed melanocyte death or destruction due to inherent sensitivity to oxidative stress arising from

toxic intermediates of melanin, a melanocyte-specific protein or other sources [17]. Other sources included vitamin B12, folic acid and Hcy. 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 was more raised in active vitiligo patients than in stable vitiligo patients. These results can be substantiated by the pilot study of Shaker and El Tahlawi & Singh et al on 26 & 40 vitiligo cases respectively, who also reported high serum Hcy level in vitiligo patients [7,18]. The oxidation of Hcy produces reactive oxygen species which causes oxidative stress on melanocytes [10]. Hcy was also elevated in males as comparison to females and this may be explained by hormonal status, greater muscle mass in men and gender related lifestyle differences [19,20].

In our study we found that there was a significant decrease in serum folic acid and vitamin B12 levels in vitiligo cases as compared to controls. Sex and course of vitiligo (active or stable) did not affect the levels of both vitamins. This is in accordance with the study of Montes et al [3] and Batawi et al [5]. Hcy level is mainly determined by levels of folic acid and vitamin B12 [8] because both acts as cofactors by the enzyme Hcy methyl transferase for the regeneration of methionine from Hcy in the activated methyl cycle [9]. Thus, the nutritional deficiency in either of the vitamins results in elevated Hcy and decreased methionine in the circulation which interferes with normal melanogenesis [21,22]. Furthermore, hydroxylation of tyrosine in melanogenesis pathway requires an adequate supply of tetrahydrobiopterin (BH4) as a cofactor. Humans cannot synthesize the pterin ring and the formation of BH4 is dependent upon the dietary intake of folic acid [23].

Conclusion

Nutritional deficiency in these vitamins & elevated Hcy must be a precipitating factor in the pathogenesis of vitiligo in predisposed individuals and indirectly support the free radical mediated damage of melanocytes. Meanwhile we recommend the routine estimation of Hcy level in patients with vitiligo and the addition of Hcy lowering agents such as vitamin B6, B12 and folic acid to the vitiligo treatment protocol.

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