Serum levels of homocysteine, vitamin B12, and folic acid in vitiligo
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Background
Vitiligo is an acquired depigmenting disorder. The exact etiopathogenesis of vitiligo is not fully understood. Vitamin B12 and folic acid levels are decreased in vitiligo, which are important cofactors required for the metabolism of homocysteine (Hcy). Consequently, the Hcy level increases in the circulation. Therefore, it is possible that increased Hcy plays a role in the destruction of melanocytes.

Objective
To determine the role of Hcy, vitamin B12, and folic acid in the pathogenesis of vitiligo.

Patients and methods
Thirty-five patients of both sexes with vitiligo and 35 age-matched healthy controls were included in the study. After excluding factors that may affect serum Hcy levels, blood samples from patients and controls were obtained for Hcy, vitamin B12, and folic acid determination by an enzyme immunoassay.

Results
The mean serum level of Hcy was significantly higher in patients with vitiligo than in the controls (17.77 ± 7.72 vs. 11.81 ± 3.41 µmol/l; P < 0.05), whereas the mean level of vitamin B12 was lower in patients with vitiligo than in the controls (208.64 ± 66.73 vs. 304.7 ± 89.9 pg/ml; P < 0.05). There was no statistically significant difference in the folic acid level in patients and controls (8.42 ± 2.06 vs. 9.39 ± 2.38 ng/ml; P > 0.05).

Conclusion
Elevation of serum Hcy level might be a precipitating factor for vitiligo in predisposed individuals. Hcy level may represent a new biomarker of the extent of vitiligo. Elevation in Hcy is associated with relative deficiencies of vitamin B12, suggesting that aggressive supplementation may benefit vitiligo patients.

Keywords:
follic acid, homocysteine; vitamin B12, vitiligo

Introduction
Vitiligo is an idiopathic disorder characterized by depigmented patches in skin because of loss of melanocytes. Death of the pigment cells may be caused by factors from inside and/or outside the cell and there are many potential systems that could be involved. However, the exact cause of destruction of epidermal melanocytes is complex and not yet fully understood [1].

It is believed that patients with vitiligo are more likely to have pernicious anemia and vitamin B12 deficiency. Vitamin B12 and folic acid are major determinants of homocysteine (Hcy) levels, and a nutritional deficiency in either of these two vitamins results in hyperhomocysteinemia [2]. Hcy also leads to inhibition of tyrosinase enzyme by binding with copper at its active site, resulting in reversible hypopigmentation [3]. It has been reported that vitiligo is associated with elevated Hcy levels and Hcy may play a role in the destruction of melanocytes in vitiligo. Excess Hcy has been shown to induce oxidative stress and increased cellular destruction in vascular smooth muscle cells. Elevation in serum Hcy is associated with a wide variety of medical conditions, including systemic lupus erythematosus of childhood and psoriasis [4].

It is of interest to note that homocystinuria is associated with fair skin and hair, a phenomenon often described as ‘pigmentary dilution’ [5]. Hcy metabolism may be altered by mutations in the catalase gene (CAT), and low catalase activity is detected in vitiligo [6]. Furthermore, it was suggested that Hcy exerts an inhibitory action on histidase and tyrosinase activity of the skin [7]. Therefore, it is possible that an increase in local Hcy interferes with normal melanogenesis and plays a role in the pathogenesis of vitiligo.

The aim of this study was to determine serum Hcy, vitamin B12, and folic acid in patients with vitiligo and healthy controls to determine their possible roles in the disease pathogenesis.

Patients and methods
This study was carried out on 35 patients with vitiligo and 35 age-matched and sex-matched healthy...
controls. The patients were selected from among patients attending the Dermatology Outpatient Clinic of Benha University Hospital. Vitiligo was diagnosed clinically. Patients included in the study ranged in age from 20 to 50 years and had vitiligo involving at least 30% of their total body area.

Exclusion criteria were as follows: age younger than 20 and older than 50 years; cigarette smoking; extent of vitiligo less than 30% of the body area; intake of folic acid, vitamin B6, or B12; anemia, diseases known to affect the Hcy level including genetic disorders of amino acid metabolism, hypertension, diabetes mellitus, thyroid dysfunction, cardiovascular disease, renal failure, deep venous thrombosis, Behcet’s disease, and psoriasis; pregnancy; alcohol intake; and hormonal therapy. Exclusion criteria for controls were as follows: cigarette smoking; vitamin intake, especially folic acid, B6, and B12; anemia; hormonal therapy; and pregnancy.

All the individuals studied were subjected to a complete assessment of history and clinical examination including the extent of vitiligo, which was estimated by the rule of nines, distribution of the lesions, clinical type of vitiligo, and bilaterality of lesions. The activity of the disease was assessed as follows: stable disease—no change in the vitiligo lesions during the 2 months before the study as observed by the patient and progressive disease—enlargement of already present lesions and/or the appearance of new lesions within the 2 months before the study as observed by the patient.

After explaining the procedure and obtaining written informed consent from every patient and control, a fasting (5 ml) blood sample was drawn. After clotting, the sample was centrifuged for 10 min at 5000 rpm at the Clinical Pathology Department, Benha University Hospitals, and serum was separated and stored immediately at −20°C. Serum Hcy, vitamin B12, and folic acid levels were determined by an enzyme immunoassay. The kits were provided by Sytron Bioresearch Inc. (Carlsbad, California, USA).

Immolute/Immulate 1000 Hcy is a solid-phase, competitive chemiluminescent enzyme immunoassay with incubation cycles of 1 × 30 min. Immolute/Immulate 1000 vitamin B12 is a solid-phase, two-site chemiluminescent immunometric assay with incubation cycles of 1 × 60 min. The Immolute folic acid is a competitive analog immunoassay with incubation cycles of 2 × 30 min. These estimations depend on chemiluminescence reactions in which part of the chemical energy generated produces excited intermediates that decay to a ground state with the emission of photons. The emitted radiation is measured using a photomultiplier tube and the signal is converted into analyte concentration.

### Statistical analysis

The program used was SPSS version 16. Quantitative data were analyzed using mean and SD, whereas qualitative data were described a frequency and percentage. The Student t-test and the F-test were used to compare the means of different groups, whereas χ²-test was used to compare frequencies. Pearson correlation was used to determine relationships.

### Results

The mean age of the patient group and the control group was 37.03 ± 10.85 and 33.87 ± 8.09 years, respectively; the patient group included 13 (37.1%) men and 22 (62.9%) women, whereas the control group included 14 (40%) men and 21 (60%) women, with no statistically significant differences in age and sex.

There was a positive family history of vitiligo for first-degree relatives in four patients (11.4%). The duration of vitiligo ranged between one and 15 years (mean 4.32 ± 3.65) and the extent of disease ranged between 30 and 80% of BSA. The disease was progressive in 19 (54.3%) patients and stable in 16 (45.7%) patients.

In terms of the type of vitiligo, the acral type was found in six (17%) patients, acrofacial in eight (23%) patients, generalized in 16 (46%) patients, segmental in two (6%) patients, and truncal in three (8%) patients. Vitiligo was found unilaterally in seven (20%) patients and bilaterally in 28 (80%) patients.

The mean serum Hcy level was significantly higher in patients than in the controls (17.77 ± 7.72 vs. 11.81 ± 3.41 µmol/l; P < 0.05). The mean serum vitamin B12 level was significantly lower in patients than in the controls (208.64 ± 66.73 vs. 304.7 ± 89.9 pg/ml; P < 0.05), whereas the mean serum folic acid and hemoglobin levels were statistically nonsignificant between vitiligo patients and controls (8.42 ± 2.06 vs. 9.39 ± 2.38 and 13.66 ± 1.13 vs. 14.17 ± 1.02 ng/ml; P > 0.05, Table 1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Mean ± SD</th>
<th>Student’s t-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy (µmol/l)</td>
<td>Vitiligo</td>
<td>17.77 ± 7.72</td>
<td>2.86</td>
<td>0.006 HS</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>11.81 ± 3.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>Vitiligo</td>
<td>208.64 ± 66.73</td>
<td>4.193</td>
<td>0.001 HS</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>304.7 ± 89.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA (ng/ml)</td>
<td>Vitiligo</td>
<td>8.42 ± 2.06</td>
<td>1.47</td>
<td>0.149 NS</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>9.39 ± 2.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>Vitiligo</td>
<td>13.66 ± 1.13</td>
<td>1.5</td>
<td>0.141 NS</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>14.17 ± 1.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FA, folic acid; HB, hemoglobin; Hcy, homocysteine; HS, highly significant.

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Role of Hcy, vitamin B12, and folic acid

The mean Hcy level was higher in male patients than in female patients (21.84 ± 10.76 vs. 15.37 ± 3.74 µmol/l; P < 0.05), whereas in controls, the serum Hcy level was higher in men than in women, but with no statistically significant difference (12.67 ± 3.85 vs. 10.82 ± 2.77 µmol/l; P > 0.05, Fig. 1).

In terms of the extent of vitiligo, there were significant positive correlations with Hcy level (P < 0.05; r = 0.559), a significant negative correlation with serum vitamin B12 level (P < 0.05; r = −0.421), and a nonsignificant correlation with serum folic acid level (P > 0.05; r = −0.206, Table 2 and Fig. 2).

In terms of the activity of vitiligo, the mean Hcy levels in patients with progressive and stable disease were 20.71 ± 9.11 and 15.69 ± 3.15, respectively, with a significant relation between serum Hcy level and vitiligo activity. There was a nonsignificant relation between serum vitamin B12, folic acid levels, and vitiligo activity.

There was no significant relation between serum Hcy, vitamin B12, and folic acid levels and types of vitiligo (P > 0.05; Fig. 3).

Serum Hcy was found to be higher in the bilateral type than in the unilateral type of vitiligo (18.23 ± 8.49 vs. 11.39 ± 1.22 µmol/l; P < 0.05; Fig. 4). There was a statistically significant relation between serum Hcy and laterality of vitiligo, whereas there was no statistically significant relation between vitamin B12 and folic acid with laterality of vitiligo (Table 3).

There was no correlation between serum Hcy, vitamin B12, and folic acid levels and age of the patients (P > 0.05; r = 0.264, P > 0.05; r = −0.138 and P > 0.05; r = 0.155; Table 4).

There was no correlation between serum Hcy, vitamin B12, and folic acid levels and duration of disease (P > 0.05; r = 0.245, P > 0.05; r = −0.159 and P > 0.05; r = −0.283 Table 5).

**Table 2 Correlation between serum homocysteine, vitamin B12, folic acid levels, and extent of vitiligo**

<table>
<thead>
<tr>
<th>Vitiligo patients</th>
<th>Pearson correlation</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy (µmol/l)</td>
<td>Extent of vitiligo 0.559</td>
<td>0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>−0.421</td>
<td>0.012</td>
<td>S</td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>−0.206</td>
<td>0.234</td>
<td>NS</td>
</tr>
</tbody>
</table>

Hcy, homocysteine; HS, highly significant; S, significant.

**Table 3 Comparison between serum homocysteine, vitamin B12, folic acid levels, and vitiligo activity**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Vitiligo activity</th>
<th>Mean ± SD</th>
<th>F-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy (µmol/l)</td>
<td>Progressive</td>
<td>20.71 ± 9.11</td>
<td>3.54</td>
<td>0.041 S</td>
</tr>
<tr>
<td></td>
<td>Stable</td>
<td>15.69 ± 3.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>Progressive</td>
<td>208.91 ± 70.07</td>
<td>0.031</td>
<td>0.97 NS</td>
</tr>
<tr>
<td></td>
<td>Stable</td>
<td>211.6 ± 65.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>Progressive</td>
<td>8.56 ± 2.69</td>
<td>0.077</td>
<td>0.926 NS</td>
</tr>
<tr>
<td></td>
<td>Stable</td>
<td>8.32 ± 2.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hcy, homocysteine; S, significant.
There was a negative correlation between serum Hcy and both vitamin B12 and folic acid levels ($P < 0.05; r = \sim 0.349$ and $P < 0.05; r = \sim 0.53$, respectively; Figs 5 and 6).

### Discussion

The etiopathogenesis and mechanisms of vitiligo are not fully understood. An association has been suggested between vitiligo and pernicious anemia and/or folic acid deficiency; however, this issue remains controversial. Levels of folic acid and vitamin B12 are major determinants of Hcy levels [8].

In our study, the serum Hcy level was significantly elevated in vitiligo patients than in the controls. Serum vitamin B12 level was significantly lower in vitiligo patients than in the controls. Our result was in agreement with previous studies [3,8–11].

The present study showed that there was no statistically significant difference between vitiligo patients and controls in the serum folic acid level. This finding was in agreement with previous studies [3,8–11].

Gönül et al. [12], Balci et al. [13], and Kim et al. [14] reported that serum Hcy, vitamin B12, and folic acid levels showed no significant difference between vitiligo patients and controls. Balci et al. [13] carried out a Turkish age/sex-matched case–control study. They found no association between Hcy, vitamin B12, folic acid, and vitiligo. Their study did not control for vitiligo duration, age, or sex. This discrepancy may be because in Turkey, mostly patients are non vegetarian or it may be because of the inclusion of different ethnic groups.

The results of the present study indicated elevated serum Hcy levels in extensive vitiligo as most of the patients had at least 30% involvement of their body area.

### Table 4 Correlation between serum homocysteine, vitamin B12, folic acid levels, and age

<table>
<thead>
<tr>
<th>Vitiligo patients</th>
<th>Pearson correlation</th>
<th>$P$ value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy ($\mu$mol/l)</td>
<td>Age (years)</td>
<td>0.264</td>
<td>0.126</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>$-0.138$</td>
<td>0.429</td>
<td>NS</td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>$0.155$</td>
<td>0.375</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Table 5 Correlation between serum homocysteine, vitamin B12, folic acid levels, and duration of disease

<table>
<thead>
<tr>
<th>Vitiligo patients</th>
<th>Pearson correlation</th>
<th>$P$ value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy ($\mu$mol/l)</td>
<td>Duration</td>
<td>0.245</td>
<td>0.157</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>$-0.159$</td>
<td>0.362</td>
<td>NS</td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>$-0.283$</td>
<td>0.10</td>
<td>NS</td>
</tr>
</tbody>
</table>

Hcy, homocysteine.

The Hcy level was significantly higher in men than in women among patients and controls, a finding that was in agreement with previous studies [3,8,10,15]. The sex disparity may be attributed to hormonal status, greater muscle mass in men, sex-related lifestyle differences, and the effect of female sex steroid hormones on Hcy metabolism [14,15].
The present study showed that there was an association between serum Hcy and extent of vitiligo, suggesting Hcy as a new biomarker of the extent of vitiligo. Our result was in agreement with Silverberg and Silverberg [9]. In contrast to this, Shaker and El-Tahlawi [10] reported that no correlation was found between serum Hcy levels and extent of vitiligo.

Previous published studies [12–14] on the association between vitiligo and Hcy have yielded conflicting results. Elevation in Hcy was associated with relative deficiencies of vitamin B12, suggesting that aggressive supplementation may benefit vitiligo patients.

In our study, the Hcy level was increased in active than in stable vitiligo patients, pointing to a possible relationship with vitiligo activity as all possible known factors that may affect Hcy level were excluded. No correlation was found between Hcy, vitamin B12, folic acid level, and types of vitiligo. This finding was in agreement with previous studies [3,9].

Serum Hcy level was significantly correlated with laterality of vitiligo, but there was no significant correlation between vitamin B12, folic acid, and laterality of vitiligo.

No correlation was found between Hcy level and age in all patients. This finding was in agreement with Shaker and El-Tahlawi [10], but not in agreement with Hu et al. [15] and Guo et al. [16]. This difference may be related to our small number of patients. Genetic factors may also play an additional role.

Hcy level was correlated negatively with vitamin B12 and folic acid levels. This finding was in agreement with previous studies [3,9].

Elevation in serum Hcy levels might be a precipitating factor of vitiligo in predisposed individuals. The major determinants of Hcy levels are vitamin B12 and folic acid; this supports the fact that they play a major role in the pathogenesis of vitiligo in this sample.

Serum Hcy was related to the sex of the patients, extent, activity, and laterality of vitiligo. Therefore, Hcy levels may represent a new biomarker of the extent and activity of vitiligo. Elevation in Hcy is associated with relative deficiencies of vitamin B12, suggesting that aggressive supplementation may benefit vitiligo patients.

We strongly recommend the inclusion of Hcy as a severity and activity marker on initial examination for patients with vitiligo. More large studies are needed to evaluate Hcy as a risk factor and as an early marker for the vascular complication in extensive vitiligo. Aggressive supplementation of vitamin B12 may benefit vitiligo patients.

Acknowledgements

Conflicts of interest
None declared.

References