Correlation Between Serum Level of Homocysteine, Folate and Vitamin B12 in Elderly Patients with Primary Open-Angle Glaucoma

Dr. Rani Menon MS, DO, FRCS, Dr. Sobha Ramesh MS, DNB

Abstract
Elevated level of homocysteine (Hcy), a derived amino acid, is an independent risk factor for vascular diseases. The metabolism of Hcy requires enzymes with vitamin B12, and folic acid as coenzymes. High level of Hcy was suspected in glaucoma mainly pseudoexfoliative glaucoma.

Aim: The aim of this case-control study is to find out the level of serum Hcy, vitamin B12 and folic acid in elderly male and female (65-80 years) patients with primary open-angle glaucoma and their correlation.

Methods: Forty four patients with glaucoma (25 men of age 71 ± 4 and 19 women of age 72 ± 4) and 10 age matched controls (5 men of age 72 ± 6 and 5 women of age 69 ± 5) were included in this study. Levels of serum Hcy, vitamin B12 and folic acid were measured using enzyme immunoassay. The levels were statistically analyzed for their correlation.

Results: A higher mean value, statistically non-significant, for Hcy was observed in male glaucoma patients with that of control subjects. No significant difference could be observed between the level of folate and B12 in glaucoma patients with that of control. Hcy level in male and female patients were 21.7 ± 11.4 and 14.1 ± 4.6 μmol/l, respectively. However, 36 % (9/25) of male and 31.5 % (6/19) of female patients showed significantly higher level of Hcy. The levels were 33.5 ± 12.1 and 25.0 ± 5.8 μmol/l for male and female patients, respectively glaucoma group. Significant negative correlation found between the level of Hcy to both B12 (p < 0.02), and folic acid (p < 0.02) in male patients. In female patients, the negative correlation between Hcy and B12 (p < 0.01) was significant, whereas between Hcy and folate was non-significant.

Conclusion: The results of this study concluded that higher level of Hcy was found mainly in elderly men with glaucoma than that of the age matched control. The negative correlation between Hcy and folic acid suggests the necessity of supplementing folate to prevent further ocular vasculopathy.

Introduction
Nutrition is an important determinant of health in persons over the age of 65 and is often under diagnosed. Though there are no uniformly accepted definitions for malnutrition in the elderly people, specific vitamin deficiencies have been well described as reliable indicators. Homocysteine (Hcy) is a derived amino acid formed in trace amount during the metabolism of the essential amino acid, methionine and is found to be cytotoxic when present at elevated levels. Genetically, autosomal recessive inherited defects in the metabolism of Hcy are the most important primary determinants for its elevation. The metabolism of Hcy requires enzymes with vitamins such as vitamin B12 (B12), pyridoxine and folic acid as coenzymes. There is an inverse relationship observed between elevation of Hcy with the levels of B12 as well as folate. A direct dose-response association between hyperhomocysteinemia with vascular disease has been reported. Risk is mainly due to the inhibition of the endothelial synthesis of nitric oxide (NO) by asymmetric dimethylarginine which is increased by elevated Hcy. Hence, chronic elevation of plasma Hcy impairs endothelium dependent NO mediated vasodilatation. Treatment with folate and B12 reduces Hcy levels in subjects with or with out any defect in the enzymes involved in its metabolism. Willems et al. reported that supplementation with these vitamins can improve vascular function in hyperhomocysteinemic patients with evident coronary artery disease.

Glaucoma is one of the prominent causes of blindness. Intraocular pressure (IOP) is assumed to be the most significant risk factor in glaucoma. However, recent evidences indicate that vascular risk factors may also play a role. Apoptosis of retinal ganglion cell has been observed...
either due to impaired blood supply to the head of optic nerve or direct toxic action of various cytotoxic agents including Hcy.\textsuperscript{11,12} Previous prospective studies reported significant high level of Hcy in patients with pseudoexfoliative glaucoma (PEXG).\textsuperscript{13-16} However, it was unaltered among the other glaucoma patients.\textsuperscript{13-16} The mean serum folic acid level was found to be significantly low in subjects with PEXG. The increased risk of vascular disease among patients with PEXG glaucoma can be explained by the impaired endothelium dependent vasodilatation by the elevated Hcy.\textsuperscript{17} Although the positive correlation between the level of Hcy and PEXG glaucoma has been reported, its prevalence in Indian patients with glaucoma or its correlation with the level of folate and B12 has not yet been established. Further, a gender based study in glaucoma patients is required to rule out the prevalence of hyperhomocysteinemia. The outcome of the study may be beneficial to treat the hyperhomocysteinemia in order to prevent further damage to retinal ganglion cell in glaucoma patients. Since, B12, and folate are among the most common dietary factors to influence Hcy, this study is also aimed to determine the influence of folate and B12 on Hcy levels in elderly male and female patients with primary open-angle glaucoma.

Materials and Methods

Inclusion criteria

Forty four patients (25 men, age 71 ± 4 years and women, age 72 ± 4 years) with glaucoma and 10 age matched subjects (5 men, age 72 ± 6 years and 5 women, age 69 ± 5 years) as control, were randomly selected for this study. All patients underwent complete ophthalmic evaluation including visual acuity, slit lamp examination, gonioscopy, tonometry, fundoscopy and visual field examination. POAG was defined by the presence of an open angle on gonoscopy, IOP more than 22 mm of hg measured with applanation tonometer, typical glaucomatous cupping and visual field defect in at least one eye on standard automated peremanently (HFA 24-2). Fasting blood samples were collected from patients diagnosed with or with out primary open-angle glaucoma as a part of the routine laboratory investigations and further for the examination of serum levels of folate, B12 and Hcy. Informed consent was obtained from the subjects and the study was conducted according to the guidelines/ethics prescribed by Indian Council for medical Research for the experimental studies in human subjects.

Exclusion criteria

Patients with history of inflammatory diseases (ocular inflammation, autoimmune disease), cardiomyopathy, coronary artery disease, cerebrovascular disease, peripheral vascular diseases, retinal occlusive disease, vasculitis, renal or hepatic dysfunction, peptic ulcer, malabsorption or maldigestion, psychiatric illness, dementia, chronic alcohol abuse or tobacco consumption were excluded from the study. Patients with vitamin supplements, or known familial history of vascular diseases or inborn errors of metabolism were also excluded from the study. Age matched controls were selected from the subjects who visited the clinic for their vision check-up.

Determination of homocysteine, folate and vitamin B12

Blood samples were collected from all the subjects after an overnight fasting (8-10 hrs). Levels of serum Hcy were measured using immunoassay, and those of serum B12 and folic acid were measured using competitive chemiluminescent enzyme immunoassay by the referral laboratory.

Statistical analysis

Significant difference between the age, level of Hcy, folate and B12 in patients with the glaucoma and control were done using two-tailed student t test, whereas the correlation between the level of Hcy, folate and B12 in patients was done by Pearson correlation analysis using GraphPad InStat soft ware package (GraphPad Software Inc., San Diego, CA, USA). P value less than 0.05 was considered as statistically significant.

Results

Level of Hcy in patients with glaucoma is depicted in figure 1. A gender wise difference in the mean value of Hcy in elderly patients with that of control could be observed in this study (Table 1). The mean value of Hcy (21.7 μmol/l) was elevated in the elderly men with glaucoma when compared to that of the reference value (13.3 μmol/l). However, these values did not show any statistically significant difference. In female patients, the Hcy level was 14.1 μmol/l, which was again found to be non-significant with respect to the control

Figure 1. Level of homocysteine (Hcy) and folate in control and patients with glaucoma. Values are mean ± SD, (n = 10 in control and n = 44 in glaucoma patients). P > 0.05 (Student t test) non-significantly different from each other
Elevated Hcy with decreased folate and B12 found in the men age group 70-75, whereas in women a similar observation was seen in the 75-80 age group (Table 2).

Level of folate and B12 in both groups is depicted in figure 1 and 2. No statistically significant difference could be observed between these values with that of the control group.

Correlation analysis in elderly male patients indicates a negative correlation between the level of Hcy to that of folate and B12 which was found to be significant (p < 0.02) (Table 3). Similarly, correlation between the levels of Hcy to that of B12 in female patients was also found to be significant (p < 0.01). However, no such statistically significant correlation could be observed between the Hcy level and folate in the female patients.

Discussion

An elevation of mean Hcy level could be observed in the men with glaucoma than that of the control group. However, the values are consistently overlapping and hence a statistically insignificant difference obtained between them. On the other hand, Hcy level in female glaucoma patients did not show any such alteration. These findings are in agreement with the previous report that no significant elevation of Hcy could be found in open angle glaucoma patients when compared with the age matched control16.

Some of the vitamin deficiencies, particularly B12, B6, and folate, are associated with cognitive impairment, and may affect the perception of vision. B12 deficiency is expected to be higher in elder Indians owing to the strict vegetarian diet and prevalence of Helicobacter pylori infection. The atrophic gastritis may also be implicated in the impaired B12 adsorption in the elderly population. Since, B12 has direct role as coenzyme in the conversion of Hcy to methionine, B12 analysis has also been included in this study. In this study, level of B12 did not differ among the control and glaucoma patients. However, the level of B12 was found to be decreased with an increase in Hcy in both

Table 1. Levels of homocysteine (Hcy), folate and vitamin B12 in control and elder patients with glaucoma

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>Patient with glaucoma (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n = 5)</td>
<td>Female (n = 5)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>72 ± 6</td>
<td>69 ± 5</td>
</tr>
<tr>
<td><strong>Hcy (µmol/l)</strong></td>
<td>13.3 ± 1.8</td>
<td>15.25 ± 3.0</td>
</tr>
<tr>
<td><strong>Folate (ng/ml)</strong></td>
<td>7.5 ± 2.5</td>
<td>4.9 ± 1.2</td>
</tr>
<tr>
<td><strong>Vitamin B12 (pg/ml)</strong></td>
<td>382.6 ± 183.1</td>
<td>370.4 ± 239.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD, p > 0.05 (Student t test) non significantly differ from control group

Table 2. Levels of homocysteine (Hcy), folate and vitamin B12 in glaucoma patients with respect to age

<table>
<thead>
<tr>
<th>Age</th>
<th>Hcy (µmol/l)</th>
<th>Folate (ng/ml)</th>
<th>B12 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male glaucoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65-70 (n = 8)</td>
<td>22.4 ± 14.6</td>
<td>12.6 ± 7.9</td>
<td>428.1 ± 299.0</td>
</tr>
<tr>
<td>70-75 (n = 10)</td>
<td>24.9 ± 11.4</td>
<td>7.1 ± 4.6</td>
<td>393.8 ± 177.1</td>
</tr>
<tr>
<td>75-80 (n = 7)</td>
<td>16.4 ± 5.0</td>
<td>14.8 ± 9.4</td>
<td>1150.0 ± 798.2</td>
</tr>
<tr>
<td>Female glaucoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65-70 (n = 6)</td>
<td>12.8 ± 6.1</td>
<td>12.2 ± 9.3</td>
<td>801.8 ± 677.0</td>
</tr>
<tr>
<td>70-75 (n = 7)</td>
<td>13.9 ± 5.8</td>
<td>15.3 ± 10.1</td>
<td>971.8 ± 619.4</td>
</tr>
<tr>
<td>75-80 (n = 6)</td>
<td>14.9 ± 2.9</td>
<td>11.3 ± 7.4</td>
<td>416.3 ± 114.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD, p > 0.05 (Student t test) non significantly different from each other as well as from the control group

Table 3. Pairwise correlation analysis in old aged control and patients with glaucoma

<table>
<thead>
<tr>
<th>Pairwise correlation</th>
<th>Correlation coefficient (r)</th>
<th>Male glaucoma</th>
<th>Female glaucoma</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy with Folate</td>
<td>-0.45***</td>
<td>-0.39NS</td>
<td>-0.70*</td>
<td></td>
</tr>
<tr>
<td>Hcy with B12</td>
<td>-0.44**</td>
<td>-0.56*</td>
<td>0.28NS</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.01, **p < 0.02 considered significant and NS P > 0.05 not significant
In the elderly female patients, the decline of B12 with respect to Hcy was statistically significant. Cumurcu et al.16 and Turgut et al.21 have reported the levels of B12 does not statistically differ in patients with PEXG or open-angle glaucoma. The normal B12 level observed in this study also supports one of the previous reports by Shoba et al.22 that no deficiency of B12 is seen in elderly Indians. Since, a negative correlation could be observed between the B12 and Hcy in male patients in this study, the mechanism of hyperhomocysteinemia can be explained with the lowered B12 level.

The low folate level is a strong determinant of hyperhomocysteinemia in elderly age group 23. Decreased serum folic acid level was also found in PEXG group.21 However, no statistically significant difference in serum folate levels was reported in patients with other types of glaucoma. Results of this study also support that no significant decrease of folic acid was observed in both male and female patients. However, significant negative correlation found between the levels of Hcy to folic acid in male patients. This observation is supported by the findings of Cumurcu et al.16 in PEXG patients that, serum levels of Hcy in male patients in this study, the mechanism of hyperhomocysteinemia can be explained with the lowered B12 level.

A high level of serum Hcy is observed in disorders of Hcy metabolism, vitamin deficiencies, systemic arterial hypertension, chronic renal insufficiency, or in malignant neoplasms. Additionally, habitual smoking and coffee intake, some medications, alcohol consumption, and physical activity may also affect Hcy levels.24,25 In this study, the patient’s exclusion criteria could eliminate all these possible variants. Further, oral and dental issues, esophageal motility, and atrophic gastritis may also affect nutritional status. Some of the previous reports revealed that for some seniors, it may be difficult to meet daily micronutrient requirements with the reduced caloric intake.26,27,28 Therefore, a rapid weight loss as a result of malnutrition has been observed. It is unclear that alterations in normal physiological changes such as taste and smell are associated with aging might be contributed to decreased food intake.20,29 Other gastrointestinal changes occurring with age may also affect the oral intake of calories. Appetite after an overnight fast is often lower in the elderly. In this study, all the subjects were with no loss of body wt which was evidenced from the body weight at the time of their first consultation as well as during the subsequent follow-up (data not included). Nevertheless, results of this study could not make a correlation between hyperhomocysteinemia and the incidence of open-angle glaucoma.

The internationally accepted treatment for hyperhomocysteinemia involves the use of folic acid, B12, under fasting conditions and pyridoxine after meals. Sato et al.30 reported the effect of folate and B12 in Japanese patients with hyperhomocysteinemia. Folic acid and B12 alone was shown to reduce Hcy levels by 22 and 11%, respectively. But both can act synergistically to cause a reduction of 38.5% in the Hcy levels. Further, pyridoxine does not add to the effect of folate and B12 in the fasting state. Amongst Indians, a dietary deficiency of the Hcy lowering B vitamins is often present. Urban men were significantly more likely to have hyperhomocysteinemia than rural men.31 Study conducted in middle aged men concluded that 67% of the men had low vitamin B12 concentration and 58% had hyperhomocysteinemia.32 In general, high mean Hcy value (varying from 19.5 to 23.2 μmol/L) is observed in elder Indian population.31,32 This study failed to take the dietary habit of the subjects and the size of the aged control group population was too small to suggest a normal biological reference value.

An elevation of Hcy level may cause changes in the microvasculature in the optic nerve head and impair optic nerve blood flow and ocular vasculopathy via a vasoconstrictive effect, endothelial injury, smooth muscle proliferation, platelet activation, thrombogenesis, and apoptotic cell death in retinal ganglion cells.33,34 Therefore, aged subjects with elevated Hcy should be encouraged to include high folate food items in their diet. Dietary folic acid (0.5–5 mg/day) supplementation found to reduce the basal Hcy levels by 25%.17 However, a general recommendation for supplementing folate, B12 and other nutritional vitamins in elder subjects is outside the scope of this study. A multi-centric population based longitudinal study is further warranted for the establishment of relation between Hcy...
with glaucoma in our population.

**Conclusion**
The results of this study suggest that supplementation of folate and B12 is required in elder male patients with glaucoma to lower the Hcy level. Furthermore, this study may suggest the necessity of measuring the serum levels of Hcy, B12 and folate periodically in elderly subjects with glaucoma that may help to prevent further optic vasculopathy.

**Conflict of interest:** none to declare

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**References**


