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ORIGINAL ARTICLE

A study of homocysteine level in North Indian subjects with special reference to their dietary habit

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Summary
Background & aim: Dietary habit may influence homocysteine (Hcy) level and there is paucity of such studies from Northern India. This study was carried out to study Hcy levels in normal volunteers and compare these with dietary habits, plasma B12 and folic acid.
Methods: In a cross-sectional study, 200 normal volunteers were subjected to evaluation of demographic (age, gender, dietary habit and physical activity), clinical (body mass index, blood pressure, smoking and alcohol) and laboratory (plasma B12, folic acid and Hcy) parameters. The Hcy levels were compared in vegetarians and non-vegetarians and correlated with various demographic, clinical and laboratory findings.
Results: The mean age of the subjects was 39.4(18–73) years and 76 were females. A total of 27 were sedentary, 36 smokers and 30 took alcohol. Body mass index was high in 36 and 99 were lacto-vegetarians. Plasma Hcy level in vegetarians was significantly higher ($20.42 \pm 12.98\,\mu mol/L$) compared to non-vegetarians (16.43+10.66 μmol/L). Plasma B12 and folic acid levels, however, were insignificantly lower in vegetarians. On multivariate analysis hyperhomocysteinemia was related to low plasma vitamin B12 level only.
Conclusion: In vegetarians Hcy levels were higher than non-vegetarians and were related to low plasma B12 level.

Introduction

Homocysteine (Hcy) is a sulfur containing amino acid and is formed during metabolism of methionine, an essential amino acid derived from dietary protein. Hcy is regarded as a risk factor for coronary artery disease, stroke, dementia and peripheral vascular disease.[1] In the metabolism of Hcy, B12

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and folic acid in remethylation pathway and vitamin B6 in trans-sulfuration are crucial. Deficiency of these vitamins can result in varying degree of hyperhomocysteinemia.[2] Elevated Hcy level may be due to genetic defects and dietary factors including low intake of vitamin B12, B6 and folic acid. A number of studies have found that Indians and South East Asians including those living in USA have higher levels of Hcy compared to Americans and Europeans, which translates into 2–4 times higher risk of cerebrovascular disease.[3,4] Hyperhomocysteinemia in Asian Indians (people from Indian subcontinent) has been attributed to relatively normal plasma folate and low vitamin B12 levels.[5] High Hcy concentration compared to Caucasian have also been reported in Asian Indians living in various geographical areas.[3,6–8]

A substantial proportion of the North Indian population adheres to vegetarian diet because of cultural and religious reasons. Moreover the non-vegetarians in India also consume much less animal-derived proteins than in a usual Western diet. The strict vegetarian diet has been reported to be deficient in cobalamin. Daily requirement of 1 μg cobalamin would necessitate about 1 l of milk or milk products, which is generally not consumed by most vegetarians. In a study from Pune, high frequency of Hcy occurring in 76% subjects were reported and attributed to an extent by vegetarian diet.[9]

In North India, about 50% of people are vegetarian; therefore, it allows an opportunity to evaluate the role of dietary factors in hyperhomocysteinemia. We report the results of Hcy level in normal persons with special reference to their dietary habits.

Subjects and methods

In a cross-sectional study, healthy relatives of patients attending neurology out-patient department, and hospital employees volunteering to participate in the study were included. Subjects taking vitamin supplementation or fortified food were excluded. The project was duly approved by Institutional Ethics Committee. Patients with any neurological or medical disease or on any drug were excluded. The subjects underwent a detailed medical history including dietary habits. To determine vitamin B12 and folic acid consumption, daily recall method was used. The patients were shown standard 150 mL cup and 5 mL tea spoon and asked to compare their consumption of vitamin B12 and folic acid sources such as milk, milk products, eggs, meat, pulses and green leafy vegetables in terms of these standards. A food frequency was formulated and frequency of consumption of these food items was also determined. The average daily consumption of vitamin B12 and folic acid was calculated from consumption in 1 week by comparing with per 100 gm or mL values given in Indian Council of Medical Research (ICMR) guideline.[10]

The subjects' height and weight were measured and body mass index (BMI) was calculated as weight in kg/height in m^2. Then, 10 mL fasting blood sample was collected in EDTA-coated vial between 8 and 9am after 12 h fasting. Blood sample was centrifuged at 10,000 rpm for 10 min and plasma was separated. The sample was preserved at $-40\,^{\circ}C$ till analyzed. Total plasma Hcy was estimated by enzyme-linked immunosorbent (ELISA) assay (Axis Shield Diagnostic Ltd., UK). Folic acid and vitamin B12 were estimated by radioimmunoassay (DPC, USA). The B12, folic acid, and Hcy levels in our subjects were considered abnormal as per the norms given with the diagnostic kits.

Statistical analysis

Hcy levels were compared in vegetarians and non-vegetarians using independent *t*-test. Demographic, dietary, and low plasma vitamin B12 and folic were compared with Hcy level using χ^2 and Fisher's exact tests. To study the factors contributing to hyperhomocysteinemia dependent variable Hcy was categorized into 0 if the level was $>13.9\,\mu mol/L$, and 1 if values were 13.9 μmol/L or below. The independent variables were categorized as age ($\leqslant 40$ years $= 1$, >40 years $= 0$), B12 intake ($<1\,\mu/day = 0, \geqslant 1\mu = 1$), sex (female $= 1$, male $= 0$), BMI ($>25 = 0$, $\leqslant 25 = 1$), smoking and alcohol (yes $= 0$, no $= 1$), B12 ($<211\,pg/mL = 0$, $\geqslant 211\,pg/mL = 1$) and folic acid ($<3\,ng/mL = 0$, $\geqslant 3\,ng/mL = 1$). The best predictors of hyperhomocysteinemia were evaluated by logistic regression analysis using SPSS 10 version software.

Results

There were 200 subjects whose mean age was 39.4 (18–73) years; 124 were males and 76 females. 83% belong to rural area. 27(13.5%) subjects were sedentary, 36(18%) smokers and 30(15%) consumed alcohol. BMI was high in 36(18%) subjects. Mean systolic blood pressure was 129 ± 5 mm of Hg and diastolic blood pressure 81 ± 4 mm of Hg. 99 subjects were lacto-vegetarians and 111 non-vegetarians. Hyperhomocysteinemia was present in 113 (56.5%) subjects which was mild (14−39 μmol/L) in 91 and moderate (31–100 μmol/L) in 22. Severe hyperhomocysteinemia exceeding 100 μmol/L was not found in any subject. Hyperhomocysteinemia was more frequent with low plasma B12 level (68 vs. 31, $P = 0.001$). However, on comparing the frequency of hyperhomocysteinemia in vegetarians and non-vegetarians, there was borderline significance ($P = 0.05$). Daily vitamin B12 intake was significantly lower in vegetarians ($0.60 \pm 0.49\,\mu g$) compared to non-vegetarians ($0.75 \pm 0.43\,\mu g$, $P = 0.02$). In non-vegetarians, vitamin B12 intake was below 1 μg/day in 59% as opposed to 76% in vegetarians which was significant ($P = 0.02$). In most of the subjects the folic acid intake was nearly normal ($>100\,\mu g/day$). Plasma folic acid and B12 levels were insignificantly lower in vegetarians. Plasma B12 levels were low in 99 subjects and of them 55 were vegetarians. Plasma folic acid level was also low in 99 subjects and of them 53 were vegetarians. Evaluation of other demographic and clinical variables such as age, sex, alcohol, smoking sedentary habits and BMI did not reveal significant difference in subjects with normal or high Hcy level. The details are presented in Table 1. Comparison of age, vitamin B12 and folic acid levels and daily vitamin B12 intake in vegetarians and non-vegetarians are presented in Table 2.

Hcy levels were marginally higher in males ($19.4 \pm 12.6\,\mu mol/L$) compared to females ($16.8 \pm 10.90\,\mu mol/L$) ($P = 0.13$). These is no significant correlation of age and Hcy level ($r = 0.03$). Comparison of vitamin B12, folic acid