Original Article

Serum Homocysteine in Indian Adolescents

Pratima Anand, Shally Awasthi, Abbas Mahdi[1], Manoj Tiwari[2] and GG Agarwal[2]

Departments of Pediatrics, [1]Biochemistry and CSM Medical University, Lucknow, and [2]Department of Statistics, Lucknow University, Lucknow, India

ABSTRACT

Objective. To assay serum homocysteine levels and examine its association with conventional risk factors for cardiovascular disease (CVD) in Indian adolescents. **Methods**. This was a cross-sectional study conducted in tertiary care hospital in northern India in apparently healthy adolescents aged 10 – 19 yr. A pre-designed questionnaire was used to assess conventional risk factors. Serum homocysteine levels of ≥ 12μmol/L, serum triglycerides ≥ 150 mg% and serum cholesterol ≥ 200 mg% were taken as hyperhomocysteinemia, hypertriglyceridemia and hypercholesterolemia, respectively. Serum high-density lipoprotein (HDL) ≥ 40 mg% was considered protective for CVD. **Results.** In 103 subjects, 36.87 % females, mean serum homocysteine level was 11.649 ±0.416μmol/L. Hyperhomocysteinemia was present in 46 (44.6%, 95% CI: 34.965-54.75) subjects. Dietary deficiency of vitamin B12 and folic acid, body mass index (BMI) > 84th percentile and altered lipid profile were associated with hyperhomocysteinemia on univariate analysis. After multivariate adjustment for BMI and vegetarian diet, low serum HDL (OR: 23.81, 95% CI: 2.86-200; p =0.003) and serum hypertriglyceridemia (OR: 4.17, 95% CI: 1.51 – 13.51; p = 0.022) had independent association with hyperhomocysteinemia. **Conclusion.** Since we have also found an association between hyperhomocysteinemia and low serum HDL levels and hypertriglyceridemia, which are conventional risk factors for CVD, interventional strategies are urgently needed among adolescents for prevention of CVD. **[Indian J Pediatr 2009; 76 (7) : 705-709]** *E-mail: sawasthi@sancharnet.in*

Key words: *Adolescents; India; Serum HDL; Serum homocysteine; Serum triglycerides*

Cardiovascular disease (CVD) is a major cause of global morbidity and mortality and in India, in past five decades, its rate among urban population has risen from 4 % to 11%.[1] Evidence is accumulating that besides the conventional risk factors like obesity, hypertension, dyslipidemia, diabetes, poor diet and smoking, several newer risk factors may contribute to cardiovascular disease. Some of these are C- reactive protein (CRP), homocysteine, fibrinogen and lipoprotein A.[2] Reports of association of homocysteine with CVD have been conflicting as studies have either found[3] or not found an association between the two.[4] Our hypothesis is that for primary prevention of CVD, it is essential to identify risk factors for CVD in children and adolescents so that remedial steps can be taken early. Hence, this study was undertaken to assess association between newer factor (homocysteine) and conventional risk factors for CVD.

Correspondence and Reprint requests : Prof. Shally Awasthi Department of Pediatrics, CSM Medical University, Lucknow-226003, (UP) India.

[DOI-10.1007/s12098-009-0116-z]

[Received April 17, 2008; Accepted August 26, 2008]

MATERIAL AND METHODS

The study objective was to assess the serum homocysteine levels in apparently healthy Indian adolescents and to find its association with conventional risk factors for cardiovascular disease. This has been based on studies conducted in India where 10% of young adults have elevated homocysteine levels (above 10μmol/L).[5-6] To detect 10% prevalence of elevated homocysteine with a precision of 7 % and confidence level of 95%, a sample size of 100 subjects was needed.

This was a cross -sectional study conducted in a tertiary care hospital in northern India. It was approved by ehics committee. Included were apparently healthy children aged 10 – 19 years with informed, written consent from the parents /guardians and assent from subject. These were healthy siblings of patients admitted in Pediatric ward of the hospital. Excluded were those who suffered from any illness that could potentially affect the serum lipid profile or serum homocysteine levels like nephrotic syndrome, renal failure, liver disease, moderate to severe anemia, diabetes mellitus, hypothyroidism, chronic infections (tuberculosis) and malignancies. Also excluded were

those who were on medications affecting the lipid profile or serum homocysteine such as antiepileptics (phenobarbitone, phenytoin, carbamazepine), isoniazid, methotrexate, cyclosporine, thiazide diuretics, trimethoprim and multivitamins.

A pre- designed questionnaire was used to assess conventional risk factors for cardiovascular disease i.e. demographic (age, sex, residence (urban/rural), religion), documented birth weight, socioeconomic status and lifestyle factors, family history of vascular diseases, diet and biochemical parameters like serum triglycerides, serum cholesterol and serum high density lipoprotein (HDL) levels.

Socioeconomic stratification was assessed according to modified Kuppuswamy scale—by individual assessment of the education, occupation of the head of the family and per capita income per month. Scoring system based on the above three criteria, was done with a maximum score of 27 (7 for education and 10 each for income and occupation). Lifestyle factors considered were the level of physical activity, frequency of fast food consumed and number of sleeping hours.

Dietary assessment of energy, protein, vitamin B_{12} and folic acid was done using Food Frequency Questionnaire (FFQ). FFQ contained 121 food items selected according to the local food preferences. The food items were divided into 11 groups , comprising of (1) cereals and whole grains, (2) milk and milk products, (3) pulses and legumes, (4) flesh foods, (5) fish and sea food, (6) drinks and beverages, (7) leafy vegetables, (8) roots and tubers (9) other vegetables, (10) nuts and oilseeds and (11) fruits. The intake of each food item was assessed by dividing the frequency of consumption into 9 categories (1) never or < 1 per month , (2) 1-3 per month, (3) once a week, (4) 1-3 per week ,(5) 4- 6 per week ,(6) once a day, (7) 2-3 per day , (8) 4-5 per day and (9) > 6 per day. Subjects and their attendants were shown standardized measuring cups and spoons. From these weights, the raw equivalent of the cooked food consumed was computed and nutritive value of the raw foods consumed was determined using the Food Composition Table by United States Department of Agriculture (USDA). The nutrient intake of the subjects was compared with the Dietary Reference Intakes (DRI).[7]

For clinical assessment, systemic and anthropometric examination was done. Weight was measured by using the SECA Integra 815 portable scale with accuracy of 0.01kgs. Height was measured by using a portable stadiometer with an accuracy of 0.1cm. Body Mass Index (BMI) was defined as weight (kg) divided by square of height (meter). BMI of < 5^{th} percentile was taken as undernourished, 50-84 percentile as normal, 85-94 percentile as over weight and = 95 percentile as obese, according to NCHS criteria.

Five ml of fasting venous blood sample was taken from the subjects, immediately centrifuged at 1600 rpm and kept at – 20°C for biochemical analysis. Blood samples were thawed in water bath just prior to analysis. The analytical biochemist was totally blind to the medical history and final diagnosis of the participant. The reagents for serum homocysteine estimation were procured from AXIS HOMOCYSTEINE EIA manufactured by Axis Shield Diagnostics Ltd the technology park, United Kingdom. The test was a solid phase enzyme immunoassay. Protein bound homocysteine was reduced to free homocysteine by dithiothreitol and enzymatic ally converted to S – Adenosyl – L – homocysteine (SAH). Assay was based on competition between SAH in the sample and immobilized SAH bound to the walls of the microtitre plate. A secondary rabbit anti mouse antibody labeled with the enzyme horseradish peroxidase (HRP) was added. The peroxidase activity was measured spectrophotometrically and absorbance was inversely related to concentration of homocysteine in the sample. Final solution was shaken and the color read at 450 nm. A four-parameter logistic curve fit was used for preparing the calibration curve and calculation of unknown samples.

Serum triglyceride, serum cholesterol and serum HDL cholesterol were estimated by enzymatic method and reagents were procured from Anamol Laboratories Pvt., Maharashtra, India. (GPO – POD Code LT2). Hypertriglyceridemia (serum triglyceride = 150mg %), Hypercholesteremia (serum cholesterol =200 mg %) and protective level of serum HDL cholesterol (serum HDL cholesterol = 40 mg %) were defined according to National Cholesterol Education Program (Adults), USA 2001.[8] Serum homocysteine levels of = 12 µmol/L were taken as hyperhomocysteinemia in the present study.[3, 8]

The distribution of baseline characteristics as well as serum homocysteine levels in the enrolled subjects was assessed. For continuous variable mean and standard deviation (SD) are expressed as mean ± SD. Difference between means was analyzed using Student's t test and differences in distribution on categorical variables were analyzed using chi square test. All statistical tests were two-tailed, and a P value of <0.05 was considered significant. Clinically relevant statistically significant variables from univariate analysis were used to create a logistic regression model to assess the difference between hyperhomocysteinemia and normal adolescents. Analysis was done using SPSS Version 11 SPSS Inc., Chicago, IL.

RESULTS

Of the 2014 patients admitted in Pediatric ward from September 2006 to August 2007, 724 had adolescent siblings, of which 205 refused participation due to

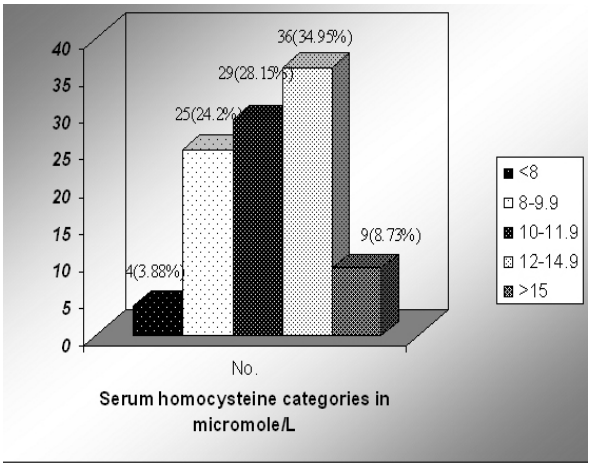

Fig. 1. Distribution of serum homocysteine in study group.

TABLE 1. Comparison of Baseline Characteristics in Adolescents with Hyperhomocysteinemia

Characteristics	Hyperhomo cysteinemia (n=46)	Normal (n=57)	OR (95%CI)	P value
Age (in years)				
<12 (72)	32 (69.56%)	40 (70.17%)	0.97(0.38-2.47)	0.946
≥ 12 (31)	14 (30.44%)	17 (29.83%)		
Sex				
Male (64)	24 (52.17%)	40 (70.17%)	0.46(0.19-1.12)	0.061
Female (39)	22 (47.83%)	17 (29.83%)		
Religion				
Hindus (83)	32 (69.56%)	51(89.47%)	0.27(0.08-0.85)	0.011
Muslims (20)	14 (30.44%)	6 (10.53%)		
Residence				
Rural (53)	33 (71.73%)	20 (35.08%)	4.70(1.87-11.96)	0.002
Urban (49)	13 (28.27%)	37(64.92%)		
Education				
0-5 yrs (69)	28 (60.86%)	41(71.92%)	0.61(0.24-1.50)	0.235
>5 yrs (34)	18 (39.14%)	16 (28.08%)		
Socioeconomic Status				
Lower Class	28 (60.86%)	9(15.78%)	8.30(3.01-23.49)	<0.001
Middle/Upper	18 (39.14%)	48 (84.22%)		
Type of Diet				
Vegetarian (59)	34(73.91%)	25 (43.85%)	3.62(1.21-6.61)	0.009
Non Vegetarian (44)	12(26.08%)	32 (56.1%)		
Energy (Kcal)/day				
<RDA (44)	32 (69.56%)	12 (21.05%)	10.99(4.28-27.79)	<0.001
≥ RDA (59)	14(30.44%)	45 (78.95%)		
Protein (gm)/day				
<RDA (56)	37 (80.43%)	19 (33.33%)	7.556(3.016-18.925)	<0.001
≥ RDA (57)	9 (19.57)	38 (66.67%)		
Diet vitamin B12 (µgm/day)				
<0.5 (43)	29 (63.04%)	14 (24.56%)	5.24(2.07-13.49)	<0.001
≥ 0.5 (60)	17 (36.96%)	43 (75.44%)		
Diet Folic Acid (µgm/day)				
< 100 (92)	44(95.65%)	48 (84.21%)	4.13(0.76-29.36)	0.061
≥ 100 (11)	2 (4.35%)	9 (15.79)		
BMI (Percentile)				
≥85th Percentile	22 (47.82%)	15 (26.31%)	2.57 (1.04-6.39)	0.023
< 85th Percentile	24 (52.18%)	42 (73.69%)		
S. Triglyceride (mg %)				
≥ 150	38 (82.60%)	15 (26.31%)	13.30 (4.63-39.62)	<0.001
< 150	8 (17.40%)	42 (73.69%)		
S. Cholesterol (mg %)				
≥ 200	36 (78.26%)	11 (19.29%)	15.05 (5.25-44.84)	<0.001
< 200	10 (21.74%)	46 (80.71%)		
S. HDL (mg %)				
< 40	38 (82.60%)	14 (24.56%)	14.59 (5.03-43.97)	<0.001
≥ 40	8 (17.40%)	43 (75.44%)		

various reasons. Of the remaining 519, 416 had one or more exclusion criteria. Here we present assessment of 103 healthy adolescents who were enrolled in the study, 39 (37.87%) were females. Mean age of the group was 11.76 ± 1.65y. Documented birth weights were available in 49(47.57%) of which 8(16.3%) weighed < 2.5kg at birth. Serum homocysteine level of study group was 11.649 ± 2.42 µmol/L. Hyperhomocysteinemia was found in 46 (44.6%, 95% CI: 34.965-54.75) in the study group. Distribution of serum homocysteine in the study group is shown in Fig 1. There was no statistical difference in serum homocysteine levels among 39 females (11.742 ± 2.64 µmol/L) as compared with 64 males (11.515± 2.08 µmol/L). However, serum homocysteine level was statistically significantly higher in 53 rural residents (12.549 ± 2.21 µmol/L) when compared with 49 urban residents (10.53 ± 2.21 µmol/L) (p = <0.0001), as well as in 59 vegetarians (12.639 ± 2.032 µmol/L) as compared with 44 non vegetarians (11.140 ± 2.453 µmol/L) (p <0.0001) .

Mean BMI of the study group was 22.57 ± 2.62 kg/$m^{2,}$ which was similar among boys and girls. Thirty-one subjects (30.02%) were overweight, 6 (5.92%) obese and 10 (9.70%) undernourished while the rest of 56 (54.4%) were normal. Mean level of serum triglyceride was 148.19 ± 40.16 mg% and 53 (51.4%) had hypertriglyceridemia. Mean serum cholesterol was 152.92 ± 49.32 mg% with 47 (45.6%) children having serum hypercholesterolemia. Mean serum HDL was 33.455 ± 6.88 mg % and 51 (49.5%) had serum HDL levels in the protective range. Univariate association between various study group characteristics and hyperhomocysteinemia is shown in table 1. Religion, place of residence, socioeconomic status, dietary intake

TABLE 2. Logistic Regression Model Assessing Association of Hyperhomocysteinemia with Certain Conventional Risk Factors for Cardio-vascular Disease

Sl. No	Risk factor (Coding)	OR (95% CI)	p value
1	Type of diet (1= Vegetarian, 0= non-vegetarian)	1.49 (0.51-4.37)	0.463
2	BMI (kg/m^2) (1= ≥85th percentile, 0 = <85th percentile)	1.06 (0.13-33.16)	0.920
3	S. Cholesterol (mg/dL) (1= ≥200 mg%, 0= < 200 mg%)	1.67 (0.52-5.43)	0.398
4	S. Triglyceride (mg/dL) (1= ≥150 mg%, 0= < 150 mg%)	4.17 (1.51 – 13.51)	0.022
5	S. HDL Cholesterol (mg/dL) (1= <40 mg%, 0= ≥ 40 mg%)	23.81 (2.86 – 200)	0.003

of energy, protein, folic acid and vitamin B_{12}, BMI and altered lipid profile were found to be statistically significantly associated with hyperhomocysteinemia in univariate analysis. Results of logistic regression are given in table 2.

DISCUSSION

In the present study, conducted on 103 apparently healthy adolescents from northern India, we found that about half the subjects (n= 46, 44.6%, 95% CI: 34.965-54.75) had hyperhomocysteinemia, which may possibly be one important, though preventable, cause of rising morbidity and mortality due to CVD in South Asia[1-3]. In logistic regression model hyperhomocysteinemia was associated with serum hypertriglyceridemia and low serum HDL levels which are conventional risk factors for CVD. In addition, univariate association of hyperhomocysteinemia was found with inadequate intake of energy, protein, vitamin B12 and folic acid.

Mean homocysteine levels of 11.649 ± 2.416µmol/L found in the present study are slightly higher than those reported from USA (8.5 µmol/L) [9] and Belgium (10.2 µmol/L)[10] Studies on adults have also shown that plasma homocysteine concentrations are higher in immigrant ethnic Indians compared to North Americans and European whites.[11] American College of Cardiology defines normal range of homocysteine as 5 – 15 µmol/L with 12 µmol/L being the upper reference range in North America.[12] Therefore, serum homocysteine level of = 12 µmol/L was defined as hyperhomocysteinemia in the present study. Hyperhomocysteinemia seen in the current study may be explained by genetic and ethnic variations[13] as well as observed dietary deficiencies of Vitamin B12 and folic acid which could in turn result in subclinical deficiency of these vitamins. In last 25 years, several studies have demonstrated association of serum homocysteine with future atherosclerosis. [2, 3, 9, 11, 14] As found by us, other studies have noted a positive correlation between altered lipid profile and hyperhomocysteinemia.[9,14-16] Homocysteine in range of 5 – 10 mmol/ L inhibits apoA A-1 transcription and HDL C synthesis in hepatocytes and hence induces atherosclerotic CVD.[14] Studies have also shown a direct association between serum homocysteine and triglycerides [15, 16] but the mechanism is still under study.

Age and sex were not found to be associated with serum homocysteine in the present study, as reported from US.[17] Indian studies on such comparison are lacking. As reported from US,[17] none of the lifestyle factors studied and positive family of vascular event were associated with hyperhomocysteinemia in the present study. Dietary deficiency of vitamin B_{12} and folic acid are known risk factors for hyperhomocysteinemia. Dietary supplementation with folic acid has shown to decrease the serum homocysteine levels and future risk of CVD in a study in adults.[18] We found the prevalence of dietary vitamin B12 and folic acid deficiency to be 38 % and 89.1% respectively, consistent with a report for Hyderabad [19] and we were able to demonstrate an association between these and hyperhomocysteinemia on univariate analysis.

Association of BMI with homocysteine has been widely studied[16,17]. Prevalence of overweight varies from 8.5% to 29% while that of obesity from 1.5% to 7.4% is in India.[20] The 5.09% prevalence of obesity found by us is comparable with the above report . Unlike others[10, 16], we did not find BMI to be associated independently with hyperhomocysteinemia in multivariate analysis adjusted for serum lipid profile, although it showed association on univariate analysis. The present study possibly gives initial data on homocysteine levels in apparently healthy Indian adolescents. We also report homocysteine levels in rural *vs* urban, vegetarians *vs. non-vegetarians*. We have also assessed association of hyperhomocysteinemia with conventional cardiovascular risk factors. Our results can form the basis of future large-scale studies in India. However, since it was based on siblings of children self-referred for admission to our hospital; it is likely to have selection bias. Also, since dietary intake was not prospectively observed, there is a possibility of recall bias.

CONCLUSION

In apparently normal healthy adolescents in northern India, we have found a high prevalence of hyperhomocysteinemia, which was associated with low serum HDL levels and hypertriglyceridemia, the conventional risk factors for CVD. Hence interventional strategies are urgently needed among adolescents for prevention of CVD.

Contributions: Dr Pratima Anand had primary responsibility for patient screening, enrollment, outcome assessment, preliminary data analysis, and writing the manuscript. Prof. Shally Awasthi had primary responsibility in the development of the protocol and analytical framework of the study, preliminary data analysis and contributed to the writing of the manuscript. Prof Abbas Ali Mahdi had primary responsibility for the biochemical analysis, participated in the analytical framework of the study, and contributed to the writing of the manuscript. Mr. Manoj Tiwari participated in the development of analytic framework of the study and preliminary data analysis. Prof G.G.Agarwal had primary responsibility of the final data analysis, supervised the design of the study and contributed to the writing of the manuscript.

Conflict of Interest: None

Role of Funding Source: No External Funding

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