Original Article

High prevalence of mild hyperhomocysteinemia and folate, B_{12} and B_6 deficiencies in an urban population in Karachi, Pakistan

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ABSTRACT

Objective: To find out the prevalence of hyperhomocysteinemia, and deficiencies of folate, vitamin B6 and vitamin B12 in an urban population in Karachi, Pakistan.

Methodology: In a pre and post experimental study, eight hundred and seventy-two apparently healthy adults (aged 18-60 years; 355 males and 517 females) were recruited from a low-income urban locality in East of Karachi from February 2006 to March 2007. Fasting venous blood was obtained. Serum was analyzed for folate and vitamin B12. Plasma was analyzed for pyridoxal phosphate (PLP, coenzymic form of B6) and total homocysteine. A group of vitamin-deficient individuals (n=194) was given 3-week supplementation with folic acid (5mg/day), methycobalamin (0.5mg/day) and pyridoxine hydrochloride (vitamin B6, 50 mg/day). After supplementation, serum/plasma levels of folate, vitamin B12, PLP and homocysteine were again determined.

Results: Prevalence of hyperhomocysteinemia (>15µmol/l) was 32%. Similarly percent values of folate deficiency (<3.5ng/ml), vitamin B6 deficiency (PLP<20 nmol/l) and vitamin B12 deficiency (<200pg/ml) in the study population were 27.5%, 33.7% and 9.74%, respectively. Hyperhomocysteinemia was associated with male sex, folate deficiency, vitamin B12 deficiency [OR (95%CI), 8.3(5.7-12.1); 2.5(1.76-3.58); 2.6(1.5-4.5), respectively]. A 3-week supplementation with folic acid, methycobalamin and pyridoxine hydrochloride in vitamin-deficient subjects decreased plasma homocysteine levels by 37%.

Conclusion: High prevalence estimates of folate, vitamin B12, and vitamin B6 deficiencies appear to be the major determinants of hyperhomocysteinemia in a low income general population in Karachi.

KEY WORDS: Cardiovascular diseases, Folate deficiency, Hyperhomocysteinemia, Vitamin B_6 deficiency, Vitamin B_{12} deficiency.

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INTRODUCTION

Hyperhomocysteinemia is widely accepted as an independent risk factor for atherosclerosis.[1] Several epidemiological studies have revealed that mild hyperhomocysteinemia is generally asymptomatic and could have prevalence from 5 to 7% in general population.[2] However, the major determinants for hyperhomocysteinemia in a population include – nutritional and genetic factors (mutations in enzymes in homocysteine metabolism).[1,3]

Pakistan is having high prevalence rates of cardiovascular disease (CVD).[4] Previously, it has been reported that mild hyperhomocysteinemia is common among Pakistani healthy adults and patients with coronary artery disease (CAD).[5,6] However, hardly any studies have been carried out in this part of the world to find out the prevalence of hyperhomocysteinemia in general population and the major determining factors for this condition.

Present study was undertaken to find out the prevalence of hyperhomocysteinemia and folate, vitamin B6 and vitamin B12 deficiencies in an urban population in Karachi and to investigate whether deficiencies of these vitamins are the determining factors for hyperhomocysteinemia in this population group. Another objective was to find out whether supplementation with B-vitamins could reduce the high levels of plasma homocysteine in the study population.

METHODOLOGY

In a cross-sectional study, 926 (age 18-60 years; males and females) individuals were recruited from Sultanabad - a low-income urban locality in East of Karachi, from February 2006 to March 2007. A systematic random sampling was employed to select houses and subsequently individuals from selected houses as described in another publication.[7] Since a sampling frame of 4000 houses was available in this locality, every fourth house-hold was selected for the sample and the first house was selected randomly by computer draw. Only one individual was recruited from each randomly selected house-hold.

A detailed pre-coded questionnaire was used. Data was recorded on socio-demographic characteristics such as age, ethnic origin, gender, marital status, occupation, monthly income per person in a household, educational level, family history of CVD and diabetes mellitus, smoking, tobacco chewing with and without betel nuts, use of B-complex vitamins during the past 6 months and any known history of hypertension, diabetes, heart disease or any other major illness. Individuals with fasting serum glucose level above 125 mg/dl and serum creatinine above 1.3 mg/dl were excluded from the study. Moreover, individuals taking B-complex vitamins during the last 6 months, taking antiepileptics, using alcohol, suffering from any major clinical illness and pregnant women were also excluded. Of the 926 participants, 872 fulfilled the criteria and volunteered themselves to be included in the study with informed written consent. Anthropometric measurements including height and weight, were also recorded by trained research assistants. The clinical examination was carried out by a general physician.

Ten ml fasting blood was obtained from cubital vein and transferred to heparinized and plain tubes for plasma and serum. All the confounding factors which could lead to folate deficiency such as use of oral contraceptives, alcohol-intake, pregnancy etc. were taken into account. Plasma and serum samples were frozen at -70^{0}C for homocysteine, vitamin B12, pyridoxal phosphate (PLP; a coenzymic form of B6) and folic acid. Samples for folic acid were stored in ascorbate (5 mg/ml). Hemoglobin levels were determined within 8 hours. The experimental protocols and the process of obtaining informed consent were approved by the Ethics Review Committee of the Institution.

Serum samples were analyzed for folate and vitamin B12 using radioassays.[8,9] Plasma samples were screened for PLP and homocysteine. Plasma PLP concentration was chosen as standard for vitamin B6 status because this measure indicates tissue stores. For determination of PLP in plasma, a modification of the method by Camp et al was used.[10] Determination of plasma total homocysteine was carried out using an immunoassay based kit. (Abbott Laboratories, Ltd., Pakistan). Serum levels of lipids (total cholesterol, low density lipoprotein (LDL)–cholesterol, high density lipoprotein (HDL)–cholesterol and triglycerides were determined using kits (RANDOX, UK).

Vitamin B12 deficiency was defined as a serum concentration less than or equal to 200 pg/ml. Folate deficiency was defined as a serum concentration less than or equal to 3.5 ng/ml.[8] PLP deficiency was defined as a plasma level less than or equal to 20 nmol/l.

The vitamin-deficient individuals were supplemented with folic acid (5 mg/day), methy- cobalamin (0.5 mg/day) and pyridoxine hydrochloride (vitamin B6; 50mg/day) for a period of three weeks. Vitamin dosages were worked out keeping in view the guidelines delineated by Omenn et al.[11], and the regimens mostly used by hematologists in folate and B12 deficiencies. Three weeks after supplementation, fasting venous blood was again obtained and monitored for folate, vitamin B12, PLP and homocysteine. All quantitative variables were expressed as mean±standard deviation (SD). All statistical analyses were done with the help of an SPSS® (Statistical Package for Social Sciences) software version 13.0 for Windows®. The prevalences for all the dependent variables i.e. PLP, B12 and folic acid were

separately estimated. For comparison of variables, independent sample t-test, paired sample t-test, one way ANOVA and chi square analysis were used. The association of hyperhomocysteinemia with other variables was observed using simple and multiple binary logistic regression. A *p* value less than 0.05 was considered significant.

RESULTS

Demographic and clinical characteristics of normal healthy adults have been listed in Table-I. Nearly 88% of the subjects in Sultanabad were migrants from Northern Pakistan and belonged to the Hindko/Hazara ethnic group (53.6%) and Pathans (34.5%). More than half of the females (54.5%) had no education, while 10.6% had gone to college after 10 years of high school education. Among males, 14.1% were illiterate and 24.2% had college level education. Literacy rate was poor among Pathans as 53% of them had no education. In comparison, Hindko/Hazara group was relatively more literate with 29% illiterate individuals ($p<0.001$, data not shown). Nearly one quarter of males were smokers, while all recruited females were nonsmokers. There were no vegetarians among the recruited subjects.

Eighty-eight percent of study subjects had monthly household income less than Rs. 10,000/- (US$120). This indicates that a vast majority of them belonged to the low income group. Prevalence of hypertension was higher in females than males (7.5% vs 3.4%; $p=0.01$). Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90mmHg. Mean body mass index (BMI) was significantly higher among females compared to males ($p<0.001$). Females were significantly ($p<0.05$) more anemic than males (data not shown). Thirteen percent of both men and women were hypercholesterolemic (serum levels of total cholesterol >200 mg/dl), while 22.5% of males and 14.7% of females were found to have hypertriglyceridemia (serum levels of triglycerides > 200 mg/dl). Sixty-two percent of men and 42% of women had low HDL-cholesterol (serum levels < 40 mg/dl).

Mean homocysteine concentration among males was significantly higher ($p<0.001$) than females, while serum folate levels were significantly less in males compared to females ($p<0.001$). Plasma PLP levels were significantly less in females compared to males ($p<0.01$). However, no significant difference was observed regarding B12 values among males and

Table-I: Demographic and clinical characteristics of normal healthy adults in an urban population in Karachi.

Characteristics	*Male(n=355) Values (mean±SD)*	*Frequency (%)*	*Female (n=517)*	*Frequency (%)*
Age (years)	32.1±11.4	--	32.8±4.9	--
Ethnic origin				
- Hindko/Hazara	--	196(55.2)	--	271(52.4)
- Pathan	--	118(33.2)	--	183(35.4)
- Others	--	41(11.5)	--	63(12.2)
Education status				
- No education	--	51(14.1)	--	282(54.5)
- Middle (8th grade)	--	94(26.5)	--	101(19.5)
- Matric (10th grade)	--	124(34.9)	--	79(15.5)
- College & University	--	86(24.2)	--	55(10.6)
Smoking pattern				
- Never smoke	--	263(74.1)	--	517(100)
- Previous smoker	--	13(3.7)	--	--
- Smoker	--	79(22.3)	--	--
Blood pressure				
- Normotensive	--	343(96.6)	--	478(92.5)
- Hypertensive	--	12(3.4)	--	39(7.5)*
Body mass index (kg/m2)	22.9±4.8	-	25.44±6.1*	-
Plasma homocysteine (µmol/l)	19.7±12		11.4±5.3***	
Serum folate (ng/ml)	5.6±3.6		7.3±4.9 ***	
Serum vitamin B12 (pg/ml)	428±193		450±251	
Plasma PLP (µmol/l)	36.8±28		30.6±35.6 **	

Note: Independent sample t test was used to compare two mean values between males and females

* Significantly different at $p<0.05$

** Significantly different at $p<0.01$

*** Significantly different at $p<0.001$

Table-II: Prevalence of hyperhomocysteinemia, folate deficiency, vitamin B12 deficiency and PLP deficiency in normal healthy adults in an urban population in Karachi.

Variables	*All subjects (n=872)(%)*	*Males (n=355)(%)*	*Females (n=517)(%)*	*p-value**
Homocysteine (> 15µmol/l)	32	58	13.9	<0.001
Folate (≤3.5 ng/ml)	27.5	34	23	<0.001
Vitamin B12 deficiency (≤ 200 pg/ml)	9.74	9.85	9.7	0.96
PLP deficiency (20≤ nmol/l)	33.7	18.9	43.9	<0.001

*p-value compares percentages in males and females using chi-square analysis

females. As shown in Table-II, percent deficiencies of folate, B12 and PLP in total population were found to be 27.5%, 9.7% and 33.7%, respectively. However, folate deficiency was significantly more prevalent in males compared to females (p<0.001). PLP deficiency was more prevalent among females. Prevalence of hyperhomocysteinemia was found to be 32%. However, hyperhomocysteinemia was more prevalent in males compared to females (58% vs 13.9%, p<0.001).

Factors associated with hyperhomocysteinemia are shown in Table-III. Simple logistic regression analysis indicates strong association of hyperhomocysteinemia with male gender, folate deficiency, vitamin B12 deficiency and education. However, in multivariate analysis risk of having hyperhomocysteinemia with education was found to be nonsignificant. Males had 8.3-fold higher odds of hyperhomocysteinemia than females. Odds of having hyperhomocysteinemia with folate deficiency and vitamin B12 deficiency were found to be 2.5 and 2.6, respectively. It is worthwhile mentioning that odds of hyperhomocysteinemia increased 15-fold [95% CI (5.05-45.89)] in individuals with combined deficiency of folic acid and vitamin B12 compared to folate and vitamin B12 normal subjects. Females of menopausal age and above had nearly 3-fold higher odds of hyperhomocysteinemia than females in reproductive age group (data not shown). No association of hyperhomocysteinemia was found with B6 deficiency, BMI, and smoking (data not shown).

Two hundred sixty four vitamin-deficient individuals were offered 3-week supplementation with vitamins. One hundred ninety four subjects (74%) completed the trial. After three weeks of supplementation with vitamins, mean levels of serum folic acid, serum B12 and plasma PLP increased almost 100% (Table IV). This increase was accompanied with 37% decrease in levels of plasma homocysteine (19.1±2.9 µmol/l to 11.98±2.9 µmol/l; p=0.01). This shows that B vitamin supplementation can significantly reduce plasma levels of homocysteine in this population.

DISCUSSION

Some of the case-control studies related to CVD have revealed mild hyperhomocysteinemia to be quite prevalent among South Asians.[5,12,13] The controls in these studies represented a small group of normal healthy adults for comparison purposes. With the exception of few reports[14-16], no large studies have been carried out on healthy adults in the general population in South East Asia. The present study is one of the few large epidemiological studies in this region and the first in Pakistan to investigate whether

Table-III: Odds ratio (OR) and 95% confidence interval (CI) of hyperhomocysteinemia by gender, education and folate and B12 status.

Variable	*Subject with plasma homocysteine concentration*		*CrudeOR† (95%CI)*	*AdjustedOR‡ (95%CI)*
	(>15 µmol/l) (n)	*(≤15µmol/l)(n)*		
Gender				
- Male	206	149	8.5 (6-11.93)	8.3 (5.7-12.1)
- Female	72	445	1(ref)	1(ref)
Folate				
- Deficiency (≤3.5 ng/ml)	115	125	2.6 (1.94-3.6)	2.5(1.76-3.58)
- Normal (>3.5 ng/ml)	163	163	1(ref)	1(ref)
Vitamin B12				
- Deficiency (≤200 pg/ml)	40	45	2.05 (1.3-3.2)	2.6(1.5-4.5)
- Normal (>200 pg/ml)	238	549	1(ref)	1(ref)

†Crude OR and 95% CI from simple logistic regression analysis, for gender, folate, vitamin B12 and education
‡Adjusted OR and 95% CI from multiple logistic regression analysis for gender, folate, vitamin B12 and education

Table-IV: Plasma/serum levels of folate, B12, PLP and homocysteine before and after 3-week supplementation with B vitamins in normal healthy adults (n=194).

Mean±SD / Variable	*Concentration*		*p-value**
	Before supplementation	*After supplementation*	
Folate (ng/ml)	2.67±0.87	6.18±2.8	<0.001
Vitamin B12 (pg/ml)	146±48	293±141	<0.001
PLP (nmol/l)	14.0±3.92	33.5±30.4	<0.001
Homocysteine (µmol/l)	19.1±2.9	11.98±1.7	0.01

*p-value compares mean values before and after supplementation using paired sample t-test

B vitamins are among the major determinants of hyperhomocysteinemia.

The main findings of our study are three-fold. First, the study shows a high prevalence (58%) of mild hyperhomocysteinemia in males in a low-income general population in Karachi. Second, there are high percentages of folate and B6 deficiencies in our study population. Third, supplementation with B vitamins brings down plasma levels of homocysteine in vitamin deficient though apparently healthy adults in this population.

Study population represented migrants from North of Pakistan. Majority of the people belonged to the lower socio-economic group with house-hold income less than 100 US$ per month. There were no vegetarians among them. Literacy rates among males and females were not much different from the rates for urban population in Pakistan (66.9% in males and 42.2% in females). Prevalence of anemia among males (10.4%) and females (27.1%) was close to the values reported for the general population in Sindh province (16% for males and 31% for females) as reported in National Health Survey of Pakistan.[17]

Mean plasma homocysteine values of 19.7±12 µmol/l for men and 11.4±5.3 µmol/l for women in general population compare well with those reported earlier (19.4±8.7 µmol/l for males and 13.2±4.1 µmol/l for females) in normal healthy Pakistani adults working in health care institutions.[6] Similar high values of plasma homocysteine in normal healthy adults have been reported in some of the other populations in this region of South Asia. For example, Sastry et al have reported mean±SD homocysteine values to be 18±10.69µmol/l in a South Indian population[12], and Fakhrzadeh et al have shown Iranian men and women in Tehran having mean±SD homocysteine concentrations in plasma to be 19±12 µmol/l and 14±1.45 µmol/l, respectively.[14]

Using a cut-off value >15µmol/l for plasma homocysteine for mild hyperhomocysteinemia[3], a prevalence of 58% in Pakistani males in a general population is very high compared to several Western populations.[1,2] Similar high values of hyperhomocysteinemia (58% in males) have been reported in an Indian population.[16] This may be one of the reasons for high rates of CVD in South Asian men. Despite being a non-vegetarian population, prevalence of vitamin B12 deficiency was unexpectedly quite high (9.7%). Folate and vitamin B6 deficiencies were also very common (27.5% and 33.7%, respectively). However, males were more folate deficient compared to females. High prevalence of folate and B12 deficiencies in this population could be due to our urban dietary habits which include inadequate use of fresh fruits and green leafy vegetables and over cooking. Our observation that females with age greater than 46 years have nearly 3-fold higher odds of hyperhomocysteinemia than females in younger age group is suggestive that after menopause females too become equally vulnerable to developing hyperhomocysteinemia. Among the B vitamin deficient individuals, 264 consented to have vitamin supplementation and 74% of them completed the trial. Considering that it was a community-based trial, the compliance was quite encouraging. Two large-scale meta-analyses showed that 25% decrease in plasma homocysteine in population-based cohort studies resulted in 11-16% reduction in the risk of ischemic heart disease.[18] This shows that by supplementation not only homocysteine levels can be brought down, but the risk of CVD might also be reduced. In this Karachi population, 3-week supplementation in B vitamin deficient subjects resulted in a 37% decrease in plasma homocysteine concentration.

Homocysteine is known to promote injury to endothelial lining of blood vessels. In presence of hypercholesterolemia and hypertriglyceridemia, atherogenic potential of homocysteine increases significantly. In the population under study, 13% of men and women were hypercholesterolemic and 22.5% of males and 14.7% of females were suffering from hypertriglceridemia. In addition, prevalence of low HDL-cholesterol was 62% in men and 42% in women. These are very high proportions in apparently healthy adults indicating that

hyperhomocysteinemia along with dyslipidemia in our general population poses a greater risk to the development of atherosclerosis. Moreover, increased levels of homocysteine have been shown not only to stimulate production of cytokines, such as interleukin-8 and monocyte chemoattractant protein1,[19] which would attract inflammatory cells to the arterial wall and may cause rupture of the atherosclerotic plaque, but also lead to progression of coronary plaque burden.[20] Therefore, lowering of plasma levels of homocysteine could be of help in reducing the risk of CVD.

Although some of the meta-analyses and randomized trials have shown that supplementation of folate, vitamins B6 and B12 in patients with vascular disease did not reduce the risk of major cardiovascular disease[21-23], however, in a recently reported HOPE 2 trial lowering of homocysteine with folic acid and vitamins B6 and B12 was associated with reduced risk of overall stroke.[24] It is noteworthy that the above mentioned trials were carried out on patients, a vast majority of them might be having well developed atherosclerosis. Our contention is that the beneficial effects of B vitamin supplements in prevention of some of these diseases have never been questioned. In fact, a couple of small clinical trials using B vitamin supplementation on healthy siblings of patients with premature atherosclerotic disease showed decreased risk of coronary and cerebrovascular atherosclerotic events.[25,26]

To the best of our knowledge, there is hardly any large clinical trial using homocysteine-lowering treatment in general population. Therefore, it is imperative that such large clinical trials on normal healthy subjects in general population should be carried out to ascertain any reduction in the incidence of CVD. This might provide conclusive evidence regarding role of B vitamins in the prevention of CVD in our general population, hence paving way for consideration of mass supplementation. High prevalence of mild hyperhomocysteinemia in a low income urban Pakistani population along with dyslipidemia suggests that Pakistanis are at a greater risk of developing atherosclerosis, hence the focus primarily should be on preventive measures to reducing the burden of CVD in this country.

CONCLUSIONS

High prevalence estimates of folate, vitamin B12 and vitamin B6 deficiencies appear to be the major determinants of hyperhomocysteinemia in a low income general population in Karachi. Hyperhomocysteinemia along with dyslipidemia could be one of the major risk factors for the development of atherosclerosis in this population.

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Authors' Contribution:

MY, MPI, GNK, GR were involved in planning and design of the study, analysis and interpretation of data and write-up of the manuscript. YM, IA, NM, GH & SP were involved in implementation of the project at the community level and also participated in the analysis of the data and facilitated in write-up of the manuscript.

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