BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 9(4)

Prevalence of hyperhomocysteinemia in healthy Indian doctors

Sandesh P Kamdi[1]* & Prashant Palkar[2]

[1]Medical Advisor, Akumentis Healthcare Ltd., 204, Second floor, G. Corp Tech Park, Kasarvadavali, Near Hyper City, Ghodbunder Road, Thane (W), 400 615, Maharashtra, India; [2]Head Medical Services, Akumentis Healthcare Ltd., 204, Second floor, G. Corp Tech Park, Kasarvadavali, Near Hyper City, Ghodbunder Road, Thane (W), 400 615, Maharashtra, India; Sandesh P Kamdi - Email: sandeshk@myakumentis.com; Tel: 022-61146060; Mobile: +91-8767451084; *Corresponding author

Received January 16, 2013; Accepted January 29, 2013; Published February 21, 2013

Abstract:
Currently, hyperhomocysteinemia is a well-known risk factor for variety of vascular diseases. Prevalence of hyperhomocysteinemia increases with age. Hence, the present study was aimed to investigate the prevalence of hyperhomocysteinemia in healthy upper socio-economic class population in India. Total homocysteine (tHcy) concentration was determined in 1243 (906 men & 337 women) healthy Indian doctors with different age group. Using Third National Health and Nutrition Examination Survey (NHANES III) study criteria, the prevalence of hyperhomocysteinemia was 92.85% among men (>11.4 µmol/L) and 81.60% among women (>10.4 µmol/L). The prevalence of hyperhomocysteinemia was higher among men with mean tHcy concentration (21.96 ± 0.38 µmol/L) significantly higher (P<0.0001) than women (15.90 ± 0.39 µmol/L) (95% CI, 4.733-7.376). Our study showed very high prevalence of hyperhomocysteinemia which may point to the future risk for various pathologies in the present subset of population. Further studies to look at the plasma levels of homocysteine lowering vitamins are warranted to prevent the future risk of vascular diseases.

Keywords: Homocysteine, Hyperhomocysteinemia, Prevalence.

Background:
Hyperhomocysteinemia is an independent risk factor in various vascular diseases **[1].** More than 100 epidemiological, case-control and longitudinal cohort studies have established that even mild hyperhomocysteinemia predicts and precedes the development of cardiovascular morbidity and mortality, and is an independent risk factor for cardio- and cerebrovascular diseases **[2].** Mean homocysteine (Hcy) levels increase throughout life by 3-5 µmol per liter and the level is higher in men than in women. Moreover, each 5 µmol/L increase in tHcy is associated with increased risk of vascular arteriosclerotic disease **[3].**

Increased levels of plasma homocysteine may be caused by several factors such as genetic polymorphism, deficiency of/disturbed distribution/increased catabolism of cofactor(s) **[4].** Moreover, there is often a cluster of factors including age & gender, genetics, vitamin status, lifestyle and ageing, etc. leading to hyperhomocysteinemia. The most frequent causes are an unhealthy lifestyle, low intake of vitamins, gastrointestinal malabsorption of vitamins, enzymatic defects and drug interactions. Folic acid, vitamin B12 and pyridoxine are the essential co-factors in Hcy metabolism are known determinants of plasma Hcy concentrations **[5, 6].** It is well established that, deficiency in homocysteine lowering vitamins such as, vitamin B12, folic acid and pyridoxine **[7-11]** is the dominant cause of hyperhomocysteinemia. Moreover, several studies have reported that methylenetetrahydrofolate reductase (MTHFR) polymorphism is also a risk factor causing hyperhomocysteinemia and associated complications **[12, 13].** There is an increasing mutation in the MTHFR C677T gene polymorphism in Indian population **[14].** Previously,

prevalence of hyperhomocysteinemia in India has been reported in young Asian Indians **[15],** adolescent population **[16]**, western Indians **[17],** in low socio-economic strata of north India **[18]** and in rural and urban areas of India **[12].** Although vitamin B12 deficiency and MTHFR polymorphism have been recognized in Indians for long period of time **[19],** there is little appreciation of this amongst Indian medical professionals and policy makers. This may be due to a number of reasons: 1) vitamin B12, MTHFR polymorphism and tHcy are not routinely measured in clinical practice 2) the majority of previous reports are clinic based and therefore may not represent community prevalence. However, there is no study has been carried out in upper economic class population. Because factors can be potentially reduced in the elderly, it is important to carry out epidemiologic studies of hyperhomocysteinemia. In this background of widespread nutritional deficiency and MTHFR polymorphism in Indians, we decided to estimate the prevalence of hyperhomocysteinemia in healthy Indian doctors.

Methodology:

An observational, simple blind study was carried out in 1254 healthy Indian doctors (age, 46.59±13.05) (men, N=912 & women, N=342). All healthy subjects were evaluated on the basis of a survey of symptoms related to coronary, cerebral, and peripheral diseases. Subjects having any acute or chronic disease were excluded from the study. Blood samples from fasting subjects were obtained using EDTA as anticoagulant by Super Religare Laboratories Ltd, India. Samples were immediately chilled at <4°C and centrifuged (2000 X g, 10 min) within 1 h of collection. Plasma was immediately frozen at -70°C until Hcy determination. Serum samples were obtained and stored at -70°C until analysis. Total plasma Hcy levels were determined by using the ADVIA Centaur® Immunoassay System (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). Sensitivity and Assay Range was < 0.50 – 65 µmol/L. Statistical methods ANOVA, unpaired t test was used to compare mean tHcy concentration in association with demographic factors, including age, BMI and gender. Results are expressed as mean ± SD/SEM if nothing else is specified.

Results:

Demographic characteristics of the population are shown in **Table 1 (see supplementary material).** Total plasma homocysteine concentration was measured in 1243 samples (men, N=906, & women, N=337) of total 1254 doctors. Mean Hcy concentration in total population of doctor was 19.67±11.25 µmol/L (range, 4.71 to >65.00 µmol/L). Mean tHcy concentration in men (n=879) was 21.96 ± 0.38 (95% CI, 4.73-7.37; range 5.88 to 65.00 µmol/L). In 27 (2.98%) men homocysteine level was > 65 µmol/L was taken separately.

Mean tHcy concentration in women (337) was 15.90 ± 0.39 µmol/L (95% CI, 4.73-7.37; range 4.71 to 64.02 µmol/L). As it was expected, the differences in tHcy between sexes were statistically significant (P<0.0001; 95% CI, 4.73-7.37; t=8.98; df=1214). Men were found to have significantly higher homocysteine levels compared to women. On the basis of Third National Health and Nutrition Examination Survey (NHANES III) criteria, 11.4 µmol/L in men and 10.4 µmol/L in women were considered as the cutoff values **[20].** Using these cutoff values for each age category, the prevalence of hyperhomocysteinemia was found to be 92.85% among men (>11.4 µmol/L) and 81.60% among women (>10.4 µmol/L). Moreover, the gender differences in plasma tHcy concentrations were present in the two major age groups. Plasma tHcy concentrations were positively associated with gender and at major age groups. **Table 2 (see supplementary material) shows** the tHcy level in three different age groups in both the genders. There was statistically significant higher tHcy levels in men than women in 20-39 years (P<0.0001; 95% CI, 8.59-14.67; t=7.50, df=37) and 40-59 (P<0.0001; 95% CI, 2.84-5.85; t=5.66, df=62) years age groups (P<0.0001). However, there was no statistically significant difference in the age group of >60 years (P=0.61; 95% CI, -1.86-3.15; t=0.50, df=21).

Discussion:

Because the association between hyperhomocysteinemia and risk of vascular events is gradual and continual, and tHcy levels are highly prevalent **[15, 18],** it is still difficult to establish reference values. Recently, the NHANES III study **[20]** established that higher values (95th percentile) in a healthy population with an adequate vitamin plasma level and without renal insufficiency can be considered having hyperhomocysteinemia. On the basis of these criteria, hyperhomocysteinemia was defined for values higher than 11.4 µmol/L in men and 10.4 µmol/L in women.

Taking into account the values considered normal by NHANES III in healthy population **[20],** our study showed a very high prevalence of hyperhomocysteinemia in healthy upper socioeconomic population. The proportion of hyperhomocysteinemia in men 92.85% was significantly higher than in women (81.60%). The prevalence observed in our study is quite similar to the previously reported prevalence in Indian subcontinent **[15, 18].** In earlier reports, prevalence of hyperhomocysteinemia was found to be 84% in healthy Indian population living in urban north India **[18].**

Several studies reported that plasma tHcy levels increase with age, depending on nutritional and metabolic factors, and on changes in vitamin B12 absorption and renal excretion. Folic acid and vitamin B12 deficiency or MTHFR polymorphism is considered the essential cause of high tHcy concentration **[21, 22]**. Moreover, vegetarianism and sedentary lifestyle are important etiological factors. Vegetarianism in India is multigenerational, lifelong and based on religious and cultural beliefs. In India, striking hyperhomocysteinemia has been commonly observed in markedly decreased intakes of folic acid and vitamin B12 in the vegetarians and urban middle class residents **[12, 18].** Moreover, low plasma concentrations of folate, vitamins B-12 and B-6, older age, being male, and living in urban areas were all independently associated with elevated tHcy, with low folate as the strongest determinant **[23].** In addition, there is a high prevalence of the thermolabile variant of the MTHFR enzyme in Indian population **[14, 24-27].** There is 54.5% prevalence of MTHFR polymorphism in Indian population **[28].** These may be the possible reasons for high prevalence of hyperhomocysteinemia in the present subset of population.

In accordance with the previous report, prevalence of hyperhomocysteinemia was found to be higher in men

compared to women **[29].** This may be due to the fact that, women had shown to be significantly higher remethylation rates than did men (P<0.005) and a tendency toward higher transmethylation which may results into differences between men and women in homocysteine concentrations **[30].**

As stated earlier, hyperhomocysteinemia is an independent risk factor for various cerebrovascular events. In the Rotterdam study **[31],** analyses carried out on a cohort of 7983 residents, all older than 55 years at the beginning of treatment, showed increases between 5% and 10% for the occurrence of different vascular events per each 1 µmol/L tHcy increase. In a 4-year follow-up, participants of the upper quintile with levels higher than 18 µmol/L showed a significant increase of myocardial infarction risk (odds ratio, 2.4; CI 95% 1.1–5.4) and cerebrovascular accident (odds ratio 2.5; CI 95% 1.2–5.4) when compared with those from the lower quintile with values less than 12.0 µmol/L. In a recent analysis on 10-year mortality in elderly people in Framingham, values of tHcy higher than 14.3 µmol/L were associated to a nonadjusted relative risk of cardiovascular death of 2.2 (95% CI 1.7–2.8) in respect of lower values **[32, 33].** Considering the above data, hyperhomocysteinemia may be the future risk for various pathologies in the present subset of population.

In the present study, the higher prevalence of hyperhomocysteinemia was observed in healthy upper socioeconomic class population in India. Hence, further studies to look at the plasma levels of folate and cobalamine and their association with hyperhomocysteinemia are warranted to prevent the future risk of vascular diseases.

Acknowledgement:
Authors acknowledged the Akumentis Healthcare Ltd. for the financial support. We wish to thank Mr. Virendra Dwivedi, Product Manager and the field staff of Akumentis Healthcare Ltd for coordinating with the volunteers. We also thank Super Religare Laboratories Ltd for assisting in the measurement of homocysteine levels in all healthy volunteers participated in the study.

Reference:

[1] McKinley MC, *Proc Nutr Soc.* 2000 **59:** 221 [PMID: 10946791]
[2] Bhargava S *et al. J Clin Biochem Nutr.* 2012 **50:** 222 [PMID: 22573925]
[3] Boushey CJ *et al. JAMA.* 1995 **274:** 1049 [PMID: 7563456]
[4] Kanth VV *et al. J Cardiovasc Dis Res.* 2011 **2:** 156 [PMID: 22022143].
[5] Selhub J *et al. JAMA.* 1993 **270:** 2693 [PMID: 8133587]
[6] Katre P *et al. Asia Pac J Clin Nutr.* 2010 **19:** 335 [PMID: 20805077]
[7] Jungers P *et al. Nephrol Dial Transplant.* 1999 **14:** 2903 [PMID: 10570095]
[8] Balasa VV *et al. J Pediatr Hematol Oncol.* 2002 **24:** 374 [PMID: 12142786]
[9] Green R & Miller JW, *Clin Chem Lab Med.* 2005 **43:** 1048 [PMID: 16197296]
[10] Remacha AF *et al. Int J Hematol.* 2011 **93:** 458 [PMID: 21475950]
[11] Mujawar SA *et al. Indian J Clin Biochem.* 2011 **26:** 257 [PMID: 22754189]
[12] Yajnik CS *et al. J Assoc Physicians India.* 2006 **54:** 775 [PMID: 17214273]
[13] Verkleij-Hagoort A *et al. Am J Med Genet A.* 2007 **143A:** 952 [PMID: 17431894]
[14] Mukherjee M *et al. Clin Genet.* 2002 **61:** 155 [PMID: 11940092]
[15] Carmel R *et al. Am J Hematol.* 2002 **70:** 107 [PMID:12111783]
[16] Anand P *et al.* Indian *J Pediatr.* 2009 **76:** 705 [PMID: 19381504]
[17] Refsum H *et al. Am J Clin Nutr.* 2001 **74:** 233 [PMID: 11470726]
[18] Misra A *et al. Eur J Nutr.* 2002 **41:** 68 [PMID:12083316]
[19] Banerjee DK & Chatterjea JB, *Br Med J.* 1960 **2:** 992 [PMID: 13796384]
[20] Gamble MV *et al. Am J Clin Nutr.* 2005 **81:** 1372 [PMID: 15941889]
[21] Castro R *et al. J Med Genet.* 2004 **41:** 454 [PMID: 15173232]
[22] Rozen R, *Thromb Haemost.* 1997 **78:** 523 [PMID: 9198208]
[23] Hao L *et al. J Nutr.* 2007 **137:** 407 [PMID: 17237319]
[24] http://www.iisc.ernet.in/currsci/feb102004/440.pdf
[25] Rai V *et al. Indian J Med Sci.* 2010 **64:** 219 [PMID: 22842321]
[26] Rai V *et al. Indian J Hum Genet.* 2012 **18:** 43 [PMID: 22754220]
[27] Rai V *et al. Cell Mol Biol (Noisy-le-grand).* 2012 **58:** OL1695 [PMID: 22762524]
[28] Nair KG *et al. J Assoc Physicians India.* 2002 **50:** 9 [PMID: 12186157]
[29] Naik S *et al. Int J Vitam Nutr Res.* 2011 **81:** 306 [PMID: 22419201]
[30] Fukagawa NK *et al. Am J Clin Nutr.* 2000 **72:** 22 [PMID: 10871556]
[31] Bots ML *et al. Arch Intern Med.* 1999 **159:** 38 [PMID: 9892328]
[32] Selhub J *et al. Ann Intern Med.* 1999 **131:** 331 [PMID: 10475885]
[33] Bostom AG *et al. Arch Intern Med.* 1999 **159:** 1077 [PMID: 10335684]

Edited by P Kangueane
Citation: Kamdi & Palkar, Bioinformation 9(4): 193-196 (2013)

BIOINFORMATION open access

Supplementary material:

Table 1: Baseline demographic data of enrolled eligible subjects

Group	Males	Females
Number of participants	906	337
Age (years)	46.58 ± 12.48	48.87 ± 11.55
Body weight (Kg)	74.37 ± 10.97	60.51 ± 8.84
Height (cm)	172.83 ± 9.53	162.66 ± 12.58
BMI (Kg / m^2)	25.08 ± 4.50	23.03 ± 3.55

- BMI, Body Mass Index.
- Values are mean ± SD.

Table 2: Serum tHcy levels by sex and age groups

Age group	Number of participants	tHcy (μmol/l)
	Men (tHcy >11.4 μmol/l)	
20-39	292	26.77 ± 0.82*
40-59	439	20.15 ± 0.44*
60+year	148	17.82 ± 0.70
Total	879	21.96 ± 0.38*
	Women (tHcy >10.4 μmol/l)	
20-39	86	15.15 ± 0.60
40-59	185	15.80 ± 0.54
60+year	66	17.18 ± 1.08
Total	337	15.90 ± 0.39

- Values are mean ± SEM.
- Participants included tHcy values and the difference between men's and women's with their respective age groups were determined by ANOVA, unpaired t test. Statistical significance was defined as $P<0.05$.
- tHcy values with superscript within a row within men group vs. women's group with respective age groups are statistically significant *$P<0.0001$.
- tHcy, total homocysteine; ANOVA, Analysis of variance.