Correlation between Total Plasma Homocysteine Levels and Oxidative DNA Damage in Healthy Indian Adults

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ABSTRACT

Introduction: Association of serum folate, Vitamin B_6 and B_{12} deficiencies with hyperhomocysteinemia are well-documented. Several studies on Indian population, address the association of hyperhomocysteinemia with numerous diseases, but population-specific baseline levels of homocysteine in healthy population have not been elucidated till date.

Aim: To establish Indian-population specific reference ranges for total plasma homocysteine in healthy adults in accordance to variation in dietary factors, serum B12 and folate status. The study also aimed to assess the contribution of cofactors in modulating homocysteine levels, and to evaluate the impact of higher homocysteine levels on the oxidative DNA damage.

Materials and Methods: In this retrospective study, 151 apparently healthy non-diabetic, non-hypertensive individuals with normal thyroid, renal and cardiac function were recruited. Vitamin B_{12}, Folate, and Homocysteine assays were performed on ADVIA Centaur® XP Immunoassay system. Plasma 8-oxo-2deoxyguanosine levels were estimated by using commercially available ELISA kit. Student's t-test, ANOVA, Spearman rank correlation was used to establish the associations between variables. The $p < 0.05$ was considered statistically significant.

Results: The data was stratified according to age (Group 1: ≤45 yr, n=58 and Group 2: >45 yr, n=93) and gender. In both the age groups, homocysteine and 8-oxo-2deoxyguanosine levels were found to be elevated in men (p=0.003) while no gender-specific differences were observed in the levels of folate and B_{12}. Post-menopausal women had higher oxidative DNA damage as compared to the pre-menopausal women. Positive correlation was observed between total plasma homocysteine and plasma 8-oxo-2deoxyguanosine (r=0.35, p=0.03). An inverse correlation was observed between plasma B_{12} levels and total plasma homocysteine in men (r=-0.24, p=0.02) while no such correlation was observed in women (r=0.22, p=0.10). No significant correlation was observed between plasma folate levels and total plasma homocysteine in both genders (male: r=-0.10, p=0.33; female: r=-0.23, p=0.09). Non-vegetarians had higher plasma B_{12} levels (p<0.05) and lower homocysteine levels (p=0.03) than vegetarians.

Conclusion: Healthy Indian adults have higher homocysteine levels than their counterparts due to vegetarian diet resulting in B_{12} deficiency. A positive correlation was observed between total plasma homocysteine and oxidative DNA damage. Higher oxidative DNA damage was observed in post-menopausal women than pre-menopausal women, which might predispose them to many multifactorial disorders.

INTRODUCTION

One-carbon metabolic pathway plays a crucial role in synthesis, repair and methylation of DNA thus perturbations in this pathway are associated with multiple diseases [1]. Homocysteine serves as a surrogate marker of perturbations in the re-methylation of homocysteine to methionine and transsulfuration of homocysteine to cysteine through cystathione. Defect in re-methylation process is accompanied by impaired synthesis of S-adenosyl methionine thus affects the cellular methylation status [2]. Defect in transsulfuration process is accompanied by elevation of S-adenosyl homocysteine and lowered cysteine levels [3]. Lower levels of cysteine were reported to be associated with reduced levels of glutathione in liver and skeletal muscles [4]. The re-methylation process requires 5-methyl tetrahydrofolate as the substrate, methyltetrahydrofolate homocysteine Methyltransferase (MTR) as the catalyst and methylcobalamin as the cofactor. The reductive methylation of cobalamin is catalyzed by methyltetrahydrofolate homocysteine Methyltransferase Reductase (MTRR) [5]. Cystathionine β-synthase is the first enzyme executing the transsulfuration pathway, which is allosterically activated by S-adenosy lmethionine and uses pyridoxal phosphate as the cofactor for the conversion of homocysteine to cystathionine [6]. Cystathionine β-lyase catalyses cleavage of cystathionine to cysteine through β-elimination of homocysteine

moiety [6]. Several putatively functional polymorphisms were reported in the genes regulating one-carbon metabolism, which influence homocysteine levels [7,8]. Apart from genetic contributors, deficiencies of folate, B_6 and B_{12} are also associated with hyperhomocysteinemia [9].

In the year 1997, the incidences of anemia, B_6 deficiency and folate deficiency among Indian women were reported to be 15%, 84% and 24.5%, respectively [10]. High prevalence of cobalamin deficiency (47%) and moderate prevalence of folate deficiency (5%) were reported among Asian Indians [11]. A study comprising of 40 normal men from South India reported 19.83±1.25 μmol/L as the mean homocysteine with RBC and plasma folate showing inverse association with homocysteine [12]. Various studies on Indian population suggest association of hyperhomocysteinemia with multiple diseases [13-15]. Population-specific baseline levels of homocysteine in relatively healthy population have not been elucidated till date. Only one study established reference ranges of homocysteine in Indian newborns and no such studies are available on the adult population [16]. At present, reference ranges for homocysteine in Indian population are based on western data. In view of variations in dietary factors, B_{12} and folate status, genetic and environmental factors, reference ranges established from the Western population cannot be used to predict Indian scenario.

Therefore, the rationale of the current study was to establish Indian-population specific reference ranges for total plasma homocysteine in healthy adults, to elucidate the contribution of folate and B_{12} levels in modulating homocysteine; and to assess the impact of elevated homocysteine levels on the oxidative DNA damage.

MATERIALS AND METHODS

The retrospective study was conducted from June 2018 to January 2019 in the Outpatient Department of Nizam's Institute of Medical Sciences, Hyderabad, India. This study was approved from the Institutional ethics committee (EC/NIMS/1931/2017) of Nizam's Institute of Medical Sciences, Hyderabad, India. A total of 151 healthy controls (95 men and 56 women) above 20 years of age who were non-diabetic, non-hypertensive and had normal thyroid, renal and cardiac function, were enrolled after obtaining an informed written consent. The study participants included institutional staff and volunteers accompanying the patients. Subjects taking S-adenosyl-methionine, methotrexate, nicotinic acid, theophylline, nitrous oxide, or L-dopa were excluded from the study.

About 5 mL of fasting venous blood sample was collected in EDTA anticoagulant tube. Plasma was obtained by centrifugation at $2000 \times g$ for 10 min at room temperature. Homocysteine level was estimated immediately after plasma collection, and one plasma aliquot of each sample was stored at -20°C until 8-oxo-2deoxyguanosine measurement. Vitamin B_{12}, Folate, and Homocysteine assays were performed on ADVIA Centaur® XP Immunoassay system using kits manufactured by Simens Healthcare Diagnostics Inc., USA. Plasma 8-oxo-2deoxyguanosine levels were estimated by using commercially available ELISA kit (Cat. No. ab201734) obtained from Abcam, MA, USA. All the estimations were performed as per manufacture's manual.

STATISTICAL ANALYSIS

The unpaired student's t-test was used for assessing mean differences in continuous variables between two groups. Analysis of variance (ANOVA) was used when groups were more than two. Spearman rank correlation coefficient was used as index of association between two variables. For all the statistical analysis, $p < 0.05$ was considered statistically significant.

RESULTS

The biochemical parameters namely homocysteine, folate, B_{12} and 8-oxo-2-deoxyguanosine were studied and segregated based on age and gender. The homocysteine data was stratified in two groups based on age i.e., Group 1: 20-45 years (n=58) and Group 2: >45 years (n=93) and the mean age of this cohort was 49.8±13.3 years. The plasma homocysteine levels in males were higher than females (20.7±9.2 vs. 15.5±5.8 µmol/L) irrespective of the age [Table/Fig-1].

Variable	Mean±standard deviation		p-value
	Men (n=95)	Women (n=56)	
Homocysteine (µmol/L)			
Group 1	19.2±6.3	14.3±2.8	0.003*
Group 2	21.9±10.7	16.0±6.7	0.003*
B12 (pg/mL)			
Group 1	378±232	398±195	0.80
Group 2	454±208	466±190	0.69
Folate (ng/mL)			
Group 1	11.4±8.5	11.1±10.4	0.91
Group 2	10.3±6.1	9.5±7.3	0.56
8-oxo-2-deoxyguanosine (ng/mL)			
Group 1	6.1±3.7	4.7±2.0	<0.05*
Group 2	8.9±3.7	6.7±4.2	0.04*

[Table/Fig-1]: Distribution of biochemical variables among the study cohort.
Group 1: ≤45 years age group: Group 2: >45 years age group. ANOVA test *p<0.05 statistically significant

An inverse correlation was observed between plasma B_{12} levels and total plasma homocysteine in men (r=-0.24, p=0.02) while no such correlation was observed in women (r=0.22, p=0.10) [Table/Fig-2]. Plasma B_{12} levels were higher in non-vegetarians than vegetarians (444±254 pg/mL vs. 239±118 pg/mL, p=0.05).
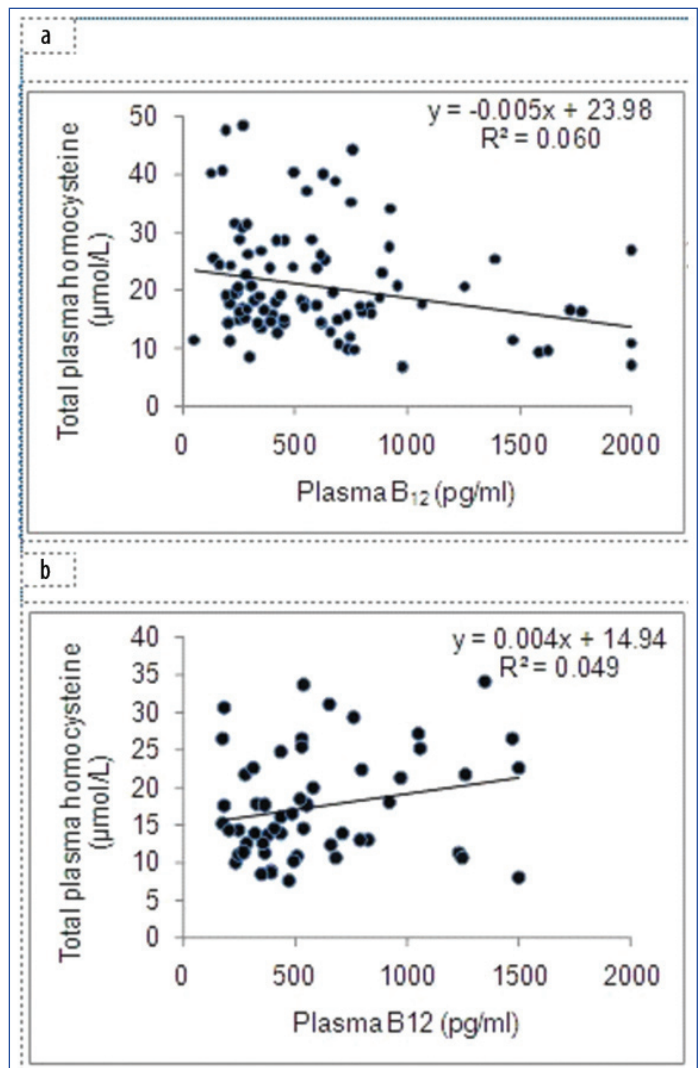
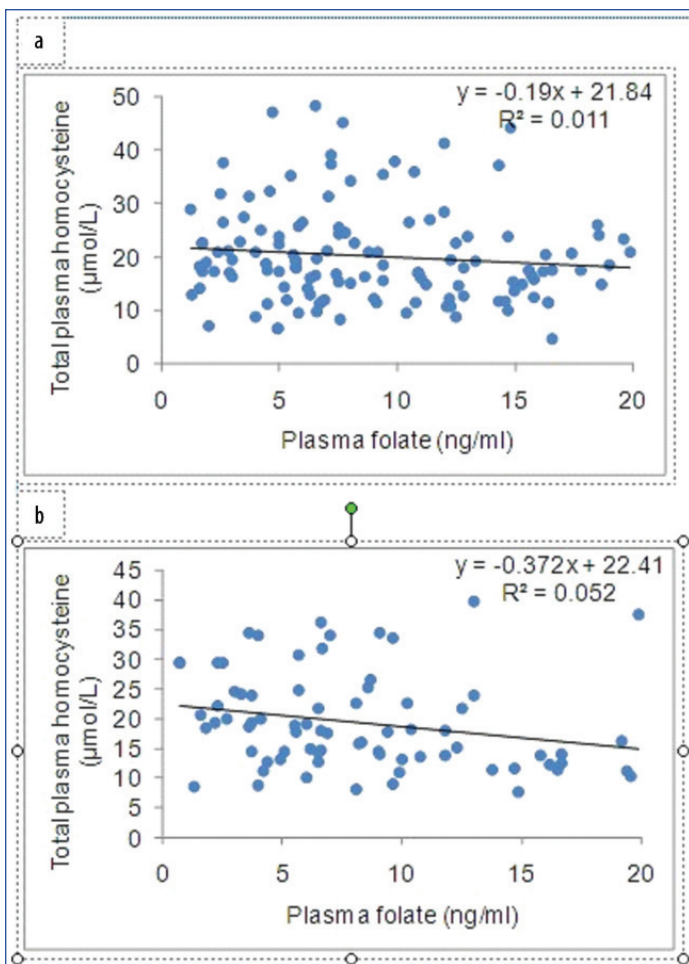[Table/Fig-2]: Correlation of plasma B_{12} with homocysteine in men (a) and women (b).

No significant correlation was observed between plasma folate levels and total plasma homocysteine in both genders (male: r=-0.10, p=0.33; female: r=-0.23, p=0.09) [Table/Fig-3].

Homocysteine levels were observed to be lower in non-vegetarians compared to vegetarians. (13.1±6.5 µmol/L vs. 20.9±18.9 µmol/L, p=0.03). A positive correlation was observed between total plasma homocysteine and plasma 8-oxo-2deoxyguanosine (r=0.35, p=0.03) [Table/Fig-4].

A literature-based comparison with ten studies across the globe on homocysteine levels in adult population revealed that homocysteine levels were higher in Indian, Algerian and Ivorian populations [Table/Fig-5] [17-25].

DISCUSSION

The current study established baseline reference values of homocysteine for the adult Indian population. Exclusion of diabetes, hypertension, dyslipidemia and other co-morbid cardiac and renal conditions makes this data more authentic. In addition, the micronutrient status in terms of plasma folate and B_{12}; impact of homocysteine elevation on oxidative DNA damage were measured. No gender differences were observed in the distribution of vitamin B_{12} and folate. Homocysteine levels were lower in women in both age groups. Women had lower levels 8-oxo-2-deoxyguanosine compared to men suggesting lower oxidative DNA damage in women. An inverse correlation was observed between

[Table/Fig-3]: Correlation of plasma folate with homocysteine in men (a) and women (b).
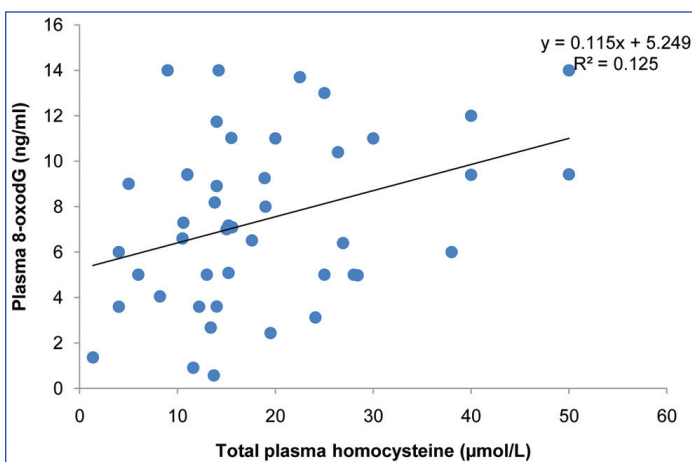
[Table/Fig-4]: Correlation of total plasma homocysteine with plasma 8-oxodG.

homocysteine and vitamin B_{12} in men while no such correlation was observed in women. No statistically significant correlation was observed between plasma homocysteine and folate. The plasma homocysteine was reported to have positive correlation with fat free mass and testosterone and an inverse correlation with estradiol [20]. In the presence of adequate estradiol levels, homocysteine mediated oxidative DNA damage was reported to be quenched which is consistent with lower oxidative stress in women, specifically pre-menopausal women since post-menopausal women experience more oxidative DNA damage due to lower levels of estradiol [26].

Reduced homocysteine levels were observed in non-vegetarians compared to vegetarians. The plasma B_{12} levels were higher in non-vegetarians than vegetarians. Post-menopausal women had higher homocysteine levels and higher oxidative stress than pre-menopausal women. These results conclude that homocysteine levels are higher in Indian adults compared to their counterparts in other populations, which is attributed partly to vegetarian diet resulting in B_{12} deficiency.
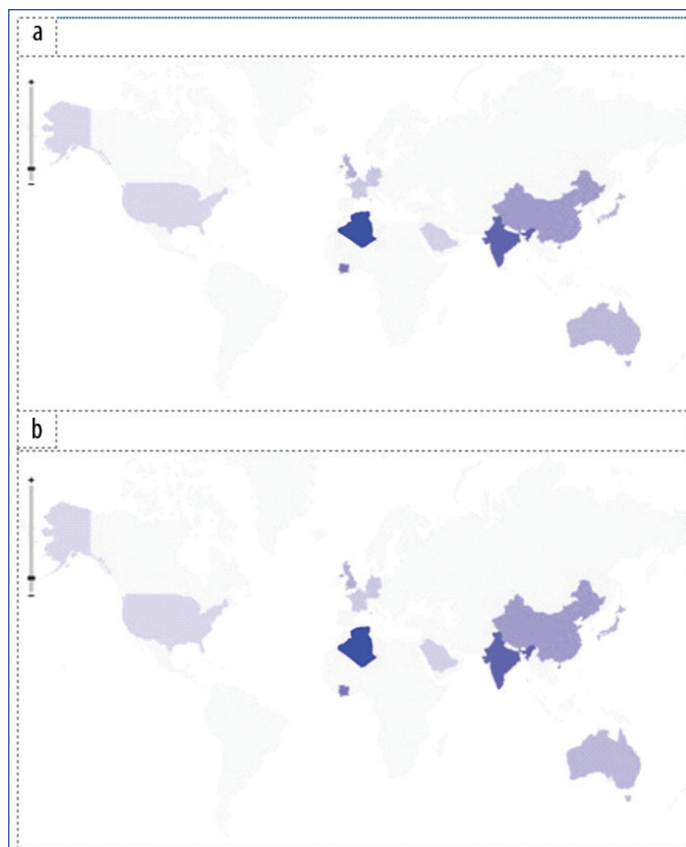
[Table/Fig-5]: Population-specific distribution of homocysteine in men (a) and women (b) [17-25].

This observation is consistent with an earlier study, which reported higher homocysteine levels in vegans who avoid all foods of animal origin when compared to high meat eaters [27]. Apart from non-vegetarian diet, daily consumption of milk was reported to increase plasma B_{12} levels thus reducing homocysteine [28].

Apart from the dietary determinants of homocysteine, several genetic determinants were identified among which Methylene Tetrahydrofolate Reductase (MTHFR) C677T, 5-methyltetra-hydrofolate homocysteine methyltransferase (MTR) A2756G and 5-methyltetrahydrofolate homocysteine methyltransferase reductase (MTRR) A66G were shown to be positively associated with homocysteine, while hydroxymethyltransferase 1 (SHMT1) C1420T and TYMS 5′-UTR 28 bp tandem repeat were reported to have negative association with homocysteine [29].

The present study has observed that the population-level differences in the distribution of homocysteine are consistent with a multi-ethnic study conducted in United states whereas in Asian Indians exhibited higher homocysteine levels compared to other ethnic groups [11]. It has been observed from the heatmap diagram of homocysteine developed for this study that Indians, Africans and certain Asian populations have higher homocysteine levels while those far from the equator have relatively less homocysteine levels [Table/Fig-5]. Hence, the environmental factors such as climate, cooking patterns and dietary habits might be influencing the variation in homocysteine levels globally. MTHFR, one of the major rate limiting enzyme of homocysteine metabolic pathway was found to be thermolabile in certain subjects who harbor MTHFR 677 C>T polymorphism, which induces enhanced propensity to dissociate into inactive monomers with loss in FAD-binding capacity. Apart from this, prolonged cooking habits result in loss of major nutrients such as folate and B_{12} from the diet. Vegetarian diet also is a major contributor to elevated homocysteine levels.

Despite the study being conducted in apparently healthy population, a positive association of homocysteine with oxidative DNA damage was observed. This is consistent with our previous study on breast cancer where in hyperhomocysteinemia was reported to induce BRCA1 methylation and thus affecting the formation of BRCA1-

associated genome surveillance complex, which plays a pivotal role in the maintenance of genomic integrity [30].

To the best of our knowledge, this is the first study from India conducted exclusively on healthy adult population to establish baseline homocysteine reference ranges. The key dietary determinants such as plasma folate and B$_{12}$, known to modulate homocysteine were included in the study. Induction of oxidative DNA damage was also observed in subjects with elevated homocysteine levels. However, future studies are warranted to investigate the clinical utility of folate or B$_{12}$ supplementation in reducing homocysteine levels and decreasing oxidative DNA damage.

LIMITATION

The major limitation is lack of follow-up of subjects for possible evaluation. Further, no supplementation was advised as it was the initial study for assessing the true micronutrient status, and the contribution of genetic factors towards homocysteine could not be established.

CONCLUSION

The reference ranges of homocysteine in Indian adults are higher than their counterparts. The deficiency of B$_{12}$ is likely contributor for this elevation, specifically in men. Regular consumption of meat was shown to lower homocysteine levels by correcting B$_{12}$ deficiency. Homocysteine is positively correlated with oxidative DNA damage, which might increase the susceptibility towards geriatric disorders, including cardiovascular disorders and cancer.

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