Original Article

DOI: 10.7860/JCDR/2023/61400.17476

Biochemistry Section

Association of C677T Polymorphism of Methylenetetrahydrofolate Reductase with Metabolic Syndrome among Eastern Indian Women: A Case-control Study

Kazi Ashique Firdoush[1], Rituparna Maji[2], Ritam Banerjee[3], Anindya Dasgupta[4]

ABSTRACT

Introduction: Polymorphisms of Methylene Tetrahydrofolate Reductase (MTHFR) gene have been associated with hyperhomocysteinaemia, which in turn may lead to hypertension, insulin resistance and abnormality of lipid metabolism. All these abnormalities are also components of Metabolic Syndrome (MetS). Thus, there is possibility of an association between MetS and MTHFR polymorphism. Literature search revealed paucity of data on this association, particularly from India.

Aim: To evaluate the association of C677T polymorphism of MTHFR gene with MetS, among women of eastern Indian population.

Materials and Methods: A hospital-based case-control study was conducted in the Department of Biochemistry, Calcutta National Medical College, Kolkata, West Bengal, India, between December 2016 to June 2018. Anthropometric and biochemical profiling of all 417 study subjects were done. Genetic work-up was done by Polymerase Chain Reaction (PCR), using suitable primer, followed by restriction fragment length polymorphism analysis using *Hinf 1* enzyme to identify MTHFR C677T polymorphism. Subjects were divided into two groups according to presence or absence of MetS as per International Diabetic Federation worldwide definition and compared with Mann-Whitney U Test. Logistic regression analysis was also performed.

Results: Out of enrolled 417 female subjects, 243 were categorised as MetS group. Mean age (in years), of MetS group (48.70±9.03) was found to be significantly higher ($p<0.001$) than that of control group (42.83±10.90). A total of 45 (25%) females with MetS exhibited the presence of heterozygous CT genotype; that was significantly higher ($p=0.026$) than the non MetS group 21 (14.9%). The mutant T allele frequency was also significantly higher among the subjects suffering from MetS; 45 (12.5%) compared to 21 (7.4%) in the control group ($p=0.036$). Multiple logistic regression analysis revealed the odds of developing MetS among subjects with heterozygous CT genotype was 7.721 times (CI: 2.38-25.05) compared to those with wild genotype.

Conclusion: C677T polymorphism of MTHFR gene was associated with the occurrence of MetS among woman.

Keywords: Abdominal obesity, Hyperhomocysteinaemia, Hypertension, Insulin resistance

INTRODUCTION

The MetS, a major non communicable health hazard of the modern world, is not a single disease but a constellation of cardiovascular risk factors: hypertension, dyslipidaemia, insulin resistance and abdominal obesity [1]. Individuals with MetS have 1.64 times increased risk of cardiovascular mortality, as well as, 1.45 times increased risk of all-cause mortality in comparison to persons without MetS [1]. Recent metanalytical data suggests that 30% of the Indian population suffers from MetS, with females having a higher prevalence (35%) compared to males (26%) [2]. A complex interplay of genetic and environmental factors may be involved in the development of MetS. Research endeavours have been directed in identifying the genes associated with this syndrome, so that they can serve as future screening tools for susceptible individuals. MTHFR gene may be one such candidate gene related to MetS [3].

The MTHFR is a crucial enzyme in folate metabolism and plays significant role in remethylation of homocysteine into methionine. Various polymorphisms of MTHFR gene have been related to higher plasma homocysteine levels, the most notable being the C→T mutation at nucleotide position 677 in exon 4. This causes alanine to valine transition at the catalytic site of the MTHFR protein, resulting in production of a thermolabile enzyme with reduced activity, causing enhanced plasma homocysteine concentration [4]. Hyperhomocysteinaemia has been proven to be an integral component of MetS in rats [5]. Also, studies have demonstrated the association of MTHFR C677T polymorphism with obesity [6], hypertension [7], insulin resistance or type 2 diabetes mellitus [8,9], and lipid disorders [10]. So, authors hypothesised that, there might be a role of MTHFR C677T polymorphism in the development of MetS.

There is a paucity of literature studying this association all over the world, and almost none found from the Indian subcontinent. Moreover, the results of these studies remain inconclusive and differs from one population to another [3,11,12]. The present study is a part of authors' previously published study, where authors studied the association of MTHFR polymorphism with hypertension [13]. Hence, present study was conducted to explore the association between MTHFR polymorphism and MetS from already available data.

MATERIALS AND METHODS

A hospital-based case-control study was conducted in the Department of Biochemistry, Calcutta National Medical College, Kolkata, West Bengal, India, between December 2016 to June 2018. All the guidelines of the Helsinki declaration as revised in 2000 were followed and the study was initiated, only after obtaining the approval of Institutional Ethics Committee (No. CNMC/7; dated 15-03-2016).

Inclusion criteria: Total 417 female patients aged between 18-65 years, who gave written informed consent, were enrolled in the study.

Exclusion criteria: Subjects with history of severe infections, renal disease, hepatic dysfunction, endocrine disorders, stroke, coronary artery disease, peripheral vascular disease, regular consumers of oral contraceptive pills or multivitamin, were excluded.

Sample size calculation: The minimum required sample size (n) was calculated by using online sample size calculator tool for case control studies [14] putting the following values: expected proportion in controls=0.32 [11], assumed odds ratio=2, confidence level 95%, power 80%. The calculated minimum sample size was 135 per group and total sample size (both groups) was 270.

Study Procedure

After a brief interview, weight and height of the participants were measured after an overnight fast, using a standard scale with light clothing and barefoot. Body Mass Index (BMI) was calculated as weight (kg) by height squared (m^2). Waist Circumference (WC) and Hip Circumference (HC) were also measured and Waist to Hip Ratio (WHR) were calculated. After 15 minutes rest, blood pressure was measured in sitting position, preferably at the right arm. The average of three measurements was recorded.

A 10 mL venous blood sample was collected from each participant following universal precautions for venepuncture. A 5 mL was collected in the plain clot vial, allowed to stand for 30 minutes, centrifuged at 3000 rpm for 10 minutes and serum separated for biochemical analysis of serum urea, serum creatinine, serum cholesterol, serum Triglyceride (TG), and serum High Density Lipoprotein (HDL). These parameters were estimated by standardised methods in a commercial autoanalyser. A 2 mL was used for Fasting Plasma Glucose (FPG) estimation. Rest 3 mL blood was collected in EDTA vial for genetic analysis. All samples were analysed within eight hours, on the day of collection or stored at -20°C.

Identification of MTHFR C677T polymorphism: DNA was isolated from 3 mL of whole blood by phenol-chloroform extraction method [15]. After assessment of quality and quantity of isolated DNA, amplification of exon 4 of MTHFR gene was done using forward primer 5'-TTT GAG GCT GAC CTG AAG CAC TTG AAG GAG-3' and reverse primer 5'-GAG TGG TAG CCC TGG ATG GGA AAG ATC CCG-3' [16]. The following reaction conditions for polymerase chain reaction were used: primary denaturation at 94°C for seven minutes, followed by 35 cycles of denaturation at 94°C, annealing at 61.5°C and extension at 72°C for 30 seconds each; final extension was conducted at 72°C for 7 minutes. The 173bp long PCR product, thus, obtained was digested with *Hinf I* restriction enzyme according to the protocol supplied by the manufacturer. C677T polymorphism creates a new restriction site for *Hinf I* that leads to cleavage of the 173bp product into 125bp and 48bp and fragments. The digestion products were electrophoretically run in 3% agarose gel and visualised with ethidium bromide for identification of the polymorphism. However, the visualisation of 48bp was not consistent and authors relied on following protocol for genotypic identification: single band of 173bp as CC, two bands of 173bp and 125bp as CT and single band of 125bp as TT.

Reanalysis plan: The study population of 417 female subjects were reclassified into two groups according to presence or absence of MetS using the new International Diabetic Federation worldwide definition of MetS (2006) as mentioned below [17]:

WC 80 cm or more (all female subjects) and atleast two of the following minor criteria:

a. Raised TG: Serum TG level 150 mg/dL or more and/or on medication for dyslipidaemia
b. Low HDL: HDL level less than 50 mg/dL and/or on medication for dyslipidaemia
c. High BP: Systolic Blood Pressure (SBP) 130 mmHg or more and/or Diastolic Blood Pressure (DBP) 85 mmHg or more or on medication for HTN
d. Elevated FPG: FPG level 100 mg/dL or more

A total of 243 subjects were included in the MetS group. Control group comprised of 174 subjects, who did not meet the above criteria for MetS. However, genetic data were available for only 180 (74.07%) subjects with MetS and 141 (81.03%) subjects without MetS. Poor yield in DNA extraction process in the remaining cases, lead to negative PCR to the standardised protocol despite three attempts.

STATISTICAL ANALYSIS

Data were analysed using principles of descriptive and inferential statistics by Microsoft excel and Statistical Package for the Social Sciences (SPSS), version 20.0. Results were expressed in terms of percentage, mean and standard deviation. Data were represented in tabular form. Normality test of data were done by Kolmogorov-Smirnov test. For the non parametric data Mann-Whitney U test was performed to analyse intergroup variation of each parameter. The Chi-square test was performed to identify departure from the Hardy-Weinberg equilibrium, and to compare the differences between the two groups regarding allelic and genotypic frequencies. Bivariate and multivariate logistic regression was used to analyse the effect of MTHFR C677T gene polymorphisms on MetS and the components in the MetS group. For all purposes, p-value <0.05 was taken as significant.

RESULTS

Mean age of MetS group was (48.70±9.03) and of non MetS group was (42.83±10.90) years; the difference was significant (p<0.001). The BMI, WHR, SBP, DBP, FPG, serum cholesterol and serum TG were all significantly higher while serum HDL was significantly lower in the MetS group compared to the control group [Table/Fig-1].

	MetS (n=243)	Non MetS (n=174)	
Characteristics	Mean±Std. Dev.	Mean±Std. Dev.	p-value
Age (years)	48.70±9.03	42.83±10.9	<0.001
Body mass index (kg/m^2)	27.49±4.94	24.68±4.16	<0.001
Waist hip ratio	1.00±0.06	0.94±0.07	<0.001
Systolic blood pressure (mmHg)	133.42±15.94	115.10±20.03	<0.001
Diastolic blood pressure (mmHg)	87.54±9.99	79.34±9.59	<0.001
Mean arterial pressure (mmHg)	102.83±11.21	91.26±12.79	<0.001
Fasting plasma glucose (mmol/L)	109.28±35.40	91.37 ±13.36	<0.001
Serum cholestrol (mg/dL)	203.11±52.27	193.17 ±38.18	0.033
Serum rides (mL)	177.48±74.66	113.14±32.41	<0.001
Serum HDL (mg/dL)	51.32±12.13	59.69±7.38	<0.001

[Table/Fig-1]: Comparison of baseline characteristics between MetS and non MetS group.
Mann-Whitney U Test

The two groups did not differ significantly with respect to their dietary habits (p=0.247) and their physical activity status (p=0.708) [Table/Fig-2].

Genotype distributions of the C677T polymorphism were consistent with Hardy-Weinberg equilibrium in both the MetS (p=0.27) and the control group (p=0.92). No TT genotype was found among the study population. Heterozygous CT genotype was found in 25%

of MetS group compared to 14.9% in non MetS group and the difference was significant (p=0.026). The mutant T allele frequency was also significantly higher among the women suffering from MetS; 12.5% compared to 7.4% in the control group (p=0.036) [Table/Fig-3].

Characteristics		MetS (n=243)	Non MetS (n=174)	p-value
		Number (percentage)	Number (percentage)	
Dietary habit	Veg	93 (38.3%)	57 (32.8%)	0.247
	Non veg	150 (61.7%)	117 (67.2%)	
Physical activity	Sedentary	189 (77.8%)	138 (79.3%)	0.708
	Moderate	54 (22.2%)	36 (20.7%)	

[Table/Fig-2]: Comparison of epidemiological characteristics between MetS and non MetS group.
Chi-square test

Frequency		MetS (n=180)	Non MetS (n=141)	p-value	Odds ratio	95% Confidence interval	
						Lower	Upper
Genotype*	CC	135 (75.0%)	120 (85.1%)	0.026†	1.905	1.074	3.380
	CT	45 (25.0%)	21 (14.9%)				
Allele	C	315 (87.5%)	261 (92.6%)	0.036†	0.563	0.327	0.970
	T	45 (12.5%)	21 (7.4%)				

[Table/Fig-3]: Distribution of C677T genotype and allele frequency among MetS and non MetS group.
Allelic distributions were consistent with Hardy-Weinberg equilibrium in MetS group (p=0.27) and Non MetS group (p=0.92). *Numbers of TT genotype were zero in both groups; †Significant

Bivariate logistic regression analysis showed that, presence of MTHFR C677T polymorphism, BMI, WC, HC, SBP, DBP, mean arterial blood pressure, FPG, serum cholesterol, serum TG and serum HDL were all significantly associated with the presence of MetS [Table/Fig-4].

Parameters	Odds ratio	95% Confidence interval		p-value
		Lower limit	Upper limit	
C677T Polymorphism (CT)	1.905	1.074	3.380	0.028
BMI	1.146	1.093	1.201	<0.001
Waist circumference	1.079	1.056	1.103	<0.001
Hip circumference	1.044	1.020	1.068	<0.001
Age	1.063	1.041	1.086	<0.001
Physical activity	1.095	0.681	1.762	0.708*
Systolic BP	1.062	1.047	1.077	<0.001
Diastolic BP	1.092	1.066	1.118	<0.001
Mean arterial pressure	1.087	1.065	1.109	<0.001
Serum triglyceride	1.022	1.016	1.027	<0.001
Serum cholesterol	1.005	1.000	1.009	0.035
Serum HDL	0.923	0.902	0.944	<0.001
Fasting plasma glucose	1.043	1.029	1.057	<0.001

[Table/Fig-4]: Bivariate logistic regression analysis showing association of genotype and clinical parameters with the presence or absence of MetS.
*Non significant

Multiple logistic regression analysis was done to build a prediction model for MetS, taking all the significant independent factors and gradually eliminating the factors that became non significant during analysis. Though FPG became non significant during analysis, it was kept in the model considering its importance in MetS. Authors' proposed model was able to correctly predict 78% cases (Nagelkerke R square value is 0.780). The odds of developing MetS among subjects with heterozygous CT genotype was 7.721 times (CI: 2.38-25.05) compared to those with wild genotype [Table/Fig-5].

Parameters	Beta	Adjusted odds ratio	95% CI of odds ratio		p-value
			Lower	Upper	
C677T Polymorphism (CT)	2.044	7.721	2.380	25.051	0.001
Age	0.057	1.059	1.014	1.105	0.009
Waist circumference	0.153	1.166	1.102	1.233	<0.001
Mean arterial pressure	0.089	1.093	1.047	1.142	<0.001
Serum triglyceride	0.031	1.032	1.019	1.044	<0.001
Serum cholesterol	0.019	1.020	1.004	1.036	0.015
Fasting plasma glucose	0.025	1.026	0.992	1.061	0.138*
Constant	-38.384				

[Table/Fig-5]: Multivariate logistic regression model for prediction of MetS.
Nagelkerke R square value is 0.780; *Non significant

DISCUSSION

The objective of the present study was to find out the genotype and allelic distribution of MTHFR C677T polymorphism among women fulfilling the criteria of MetS and among those, who were not. Among the 180 women in the MetS group, 25% were heterozygous mutant (CT) genotype while the rest 75% was homozygous wild type. In the control group, 14.9% was heterozygous mutant (CT) while 87.5% was homozygous wild type. None of the women in either group had the homozygous mutant (TT) genotype. This might be because of an extremely low prevalence of TT genotype in the Indian subcontinent.

A study by Kumar J et al., reported the prevalence of TT genotype in Indian population to be just 2.9% [18]. A recent study on North Indian population by Yadav U et al., showed that, out of 1000 blood samples analysed, frequency of T allele and TT genotype was 11% and 1%, respectively. The same study also included a metanalysis which documented that the T allele frequency of south Asian countries like India were much less (11.4%) compared to the north Asian countries like China or Korea (40%) [19]. In this population, the authors found the mutant T allele frequency to be 12.5% in MetS group and 7.4% in control group, which were comparable to the findings of the above studies.

As per the results, both the frequencies of CT genotype and T allele in the MetS group were significantly higher than those of the control group. Also, the women, who carried the CT genotype were 7.7 times more likely to develop MetS than women with the wild CC genotype. These findings suggest an association between C677T polymorphism and MetS in the eastern Indian population. Hyperhomocysteinaemia might be the missing link, which could explain this association. MTHFR is located on the short arm of chromosome1 (position 36.3). A total of 11 exons join to form a mature mRNA that encodes the 77 kDa MTHFR protein. The MTHFR protein, active in dimer form, catalyses the conversion of 5, 10 methylene tetrahydrofolate to 5 methyl tetrahydrofolate and hence, plays vital role in the re-methylation of Homocysteine to Methionine. Any polymorphism affecting MTHFR protein function may lead to hyperhomocysteinaemia, especially in suboptimal folate status. Out of the different polymorphisms of MTHFR gene, C677T has been studied extensively and found to contribute to hyperhomocysteinaemia [20].

In 677T (rs 1801133) there is Ala to Val substitution at codon 222 on exon 4. This leads to synthesis of a thermolabile enzyme with dissociation of dimer and loss of FAD binding capacity. Hence, there is around 30% reduction of MTHFR enzyme

activity in heterozygotes (CT) and 60% in homozygotes (TT) [21]. This loss of enzyme activity could lead to higher homocysteine concentrations, which in turn may produce oxygen-free radicals, thus, stimulating proliferation of vascular smooth muscle cells, inducing insulin resistance, and causing endothelial dysfunction. Hyperhomocysteinaemia has also been associated with increased plasma C-reactive protein levels, promotion of lipid peroxidation, decrease in Apo-A1 expression and accelerated lipoprotein oxidation [22,23].

The deficiency of MTHFR activity may also cause impaired DNA methylation and activation of repair mechanism that may result in chromosomal breakage, decreased nitric oxide formation, elevated production of reactive oxygen species and the production of proinflammatory cytokines [12,23]. All these factors may lead to hypertension, diabetes mellitus and abnormal lipid metabolism: the various determinants of MetS.

As the loss of FAD has been recognised as a major cause of decrease in MTHFR 677T enzyme activity, riboflavin supplementation may lead to reduction of homocysteine levels in hypertensive patients with the variant of MTHFR [24]. Current literature has shown that riboflavin supplementation [25] and folate and vitamin B12 supplementation [26] can also attenuate insulin resistance and development of MetS. A recent study from Italy, also states that MTHFR genetic variations analysis would be an innovative tool for the nutritional assessment in MetS [27]. This underlines the importance of determining MTHFR polymorphism in MetS and delineates the scope of personalised medicine and personalised diet, based on an individual's genetic make-up and nutritional status.

The findings of the present study are consistent with the previous study from Hubei province, China which documented that the risk of MetS was higher for the TT genotype and T allele carriers than for the CC genotype and C allele carriers [12]. Another study from northern Chinese Han population showed, MTHFR 677T allele, may contribute to an increased risk of MetS [3]. A study on Greek population found that, the 677T allele increased the risk of MetS by 4.02 times [28]. However, there are also studies, which refute this association [11]. Different frequencies of C677T mutation in different populations, using diverse diagnostic criteria for MetS, varied dietary habits of each population and overlap with other genetic polymorphisms, may be the cause of such conflicting reports.

Limitation(s)

The major limitations of present study is that, sampling was done from a single centre, inability to measure plasma homocysteine and folate levels and confounding effect of other polymorphisms or candidate genes of MetS. Age matching was also not possible among the two groups, due to sample size constrain, also controls were less than cases.

CONCLUSION(S)

The present study found a significant association between MTHFR C677T polymorphism and MetS among eastern Indian population, thus supporting the null hypothesis. This implied that C677T polymorphism could be a potential risk factor for development of MetS. However, larger prospective population-based studies are required to corroborate these findings. Future avenues of research could be the genetic interaction of MTHFR with other candidate genes of MetS, role of MTHFR polymorphism in nutritional assessment and supplementation of individuals with MetS and the effect of MTHFR polymorphism, in relation to cardiovascular morbidity and mortality in MetS.

REFERENCES

[1] Guembe MJ, Fernandez-Lazaro CI, Sayon-Orea C, Toledo E, Moreno-Iribas C. Risk for cardiovascular disease associated with metabolic syndrome and its components: A 13-year prospective study in the RIVANA cohort. Cardiovasc Diabetol. 2020;19(195). https://doi.org/10.1186/s12933-020-01166-6.

[2] Krishnamoorthy Y, Rajaa S, Murali S, Rehman T, Sahoo J, Kar SS. Prevalence of metabolic syndrome among adult population in India: A systematic review and meta-analysis. PLoSONE. 2020;15(10):e0240971.

[3] Yang B, Fan S, Zhi X, Wang D, Li Y, Wang Y, et al. Associations of MTHFR C677T and MTRR A66G gene polymorphisms with metabolic syndrome: A case-control study in Northern China. Int J Mol Sci. 2014;15(12):21687-702.

[4] Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet .1995;10:111-13. Doi: 10.1038/ng0595-111. PMID: 7647779.

[5] Oron-Herman M, Rosenthal T, Sela B. Hyperhomocysteinemia as a component of syndrome X. Metab. 2003; 52(11):1491-95. Doi: 10.1016/s0026-0495(03)00262-2. PMID: 1462441.

[6] Lewis SJ, Lawlor DA, Nordestgaard BG, Tybjaerg-Hansen A, Ebrahim S, Zacho J, et al. The methylenetetrahydrofolate reductase C677T genotype and the risk of obesity in three large population-based cohorts. Eur J Endocrinol. 2008;159(1):35-40.

[7] Yang B, Fan S, Zhi X, Li Y, Liu Y. Associations of MTHFR gene polymorphisms with hypertension and hypertension in pregnancy: A meta-analysis from 114 studies with 15411 cases and 21970 controls. PLoS ONE. 2014;9(2):e87497.

[8] Zhu B, Wu X, Zhi X, Liu L, Zheng Q, Sun G. Methylenetetrahydrofolate reductase C677T polymorphism and type 2 diabetes mellitus in Chinese population: A meta-analysis of 29 case-control studies. PLoS One. 2014;9:e102443.

[9] Chen AR, Zhang HG, Wang ZP, Fu SJ, Yang PQ, Ren JG, et al. C-reactive protein, vitamin B12 and C677T polymorphism of N-5,10-methylenetetrahydrofolate reductase gene are related to insulin resistance and risk factors for metabolic syndrome in Chinese population. Clin Invest Med. 2010;33 E290-97.

[10] Real JT, Martinez-Hervas S, Garcia-Garcia AB, Chaves FJ, Civera M, Ascaso JF, et al. Association of C677T polymorphism in MTHFR gene, high homocysteine and low HDL cholesterol plasma values in heterozygous familial hypercholesterolemia. J Atheroscler Thromb. 2009; 16: 815-20.

[11] Fakhrzade H, Mirarefin M, Sharifi F, Ghotbi S, Rezaei Hemami M, Mohammad Amoli M, et al. Association of methylenetetrahydrofolate reductase gene polymorphism (C677T) with metabolic syndrome in an Iranian population: Tehran homocysteine study. Iran J. Diatebes Lipids Disord. 2009;8:37-46.

[12] Wang J, Xu L, Xia H, Li Y, Tang S. Association of MTHFR C677T gene polymorphism with metabolic syndrome in a Chinese population: A case-control study. J Int Med Res. 2018;46(7):2658-69.

[13] Maji R, Firdoush KA, Dasgupta A. Study of association of Methylenetetrahydrofolate reductase C677T polymorphism with essential hypertension in eastern India. National Journal of Medical Research. 2019;9(4):149-53.

[14] Ausvet. Epitools Epidemiological Calculators [Internet]. 2018. Available at: http://epitools.ausvet.com.au.

[15] Blin N, Stafford DW. A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res. 1976;3:2303-08.

[16] Devi AR, Govindaiah V, Ramakrishna G, Naushad SM. Prevalence of methylene tetrahydrofolate reductase polymorphism in South Indian population. Current Science. 2004;10:440-43.

[17] Alberti KGMM, Zimmet P, Shaw J. Metabolic syndrome-A new world-wide definition. A consensus statement from the International Diabetes Federation. Diabet Med. 2006;23:469-80.

[18] Kumar J, Das SK, Sharma P, Karthikeyan G, Ramakrishnan L, Sengupta S. Homocysteine levels are associated with MTHFR A1298C polymorphism in Indian population. J Hum Genet. 2005;50(12):655-63.

[19] Yadav U, Kumar P, Gupta S, Rai V. Distribution of MTHFR C677T Gene polymorphism in healthy North Indian population and an updated meta-analysis. Indian J Clin Biochem. 2017;32(4):399-410.

[20] Raghubeer S, Matsha TE. Methylenetetrahydrofolate (MTHFR), the one-carbon cycle, and cardiovascular risks. Nutrients. 2021;13(12):4562.

[21] Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in Methylenetetrahydrofolate Reductase (MTHFR) associated with decreased enzyme activity. Molecular Genetics and Metabolism. 1998;64(3):169-72. Doi: 10.1006/mgme.1998.2714. PMID: 9719624.

[22] Araki A, Hosoi T, Orimo H. Association of plasma homocysteine with serum interleukin-6 and C-peptide levels in patients with type 2 diabetes. Metabolism. 2005;54:809-14.

[23] Hayden MR, Tyagi SC. Homocysteine and reactive oxygen species in metabolic syndrome, type 2 diabetes mellitus, and atheroscleropathy: The pleiotropic effects of folate supplementation. Nutr J. 2004;3:4.

[24] McNulty H, Dowey LRC, Strain JJ, Dunne A, Ward M, Molloy AM, et al. Riboflavin lowers homocysteine in individuals homozygous for the MTHFR 677C→T polymorphism. Circulation. 2006;113(1):74-80.

[25] Mazur-Bialy AI, Pocheć E. Riboflavin reduces pro-inflammatory activation of adipocyte-macrophage co-culture. Potential application of vitamin b2 enrichment for attenuation of insulin resistance and metabolic syndrome development. Molecules. 2016;21(12):1724.

[26] Ashok T, Puttam H, Tarnate VCA, Jhaveri S, Avanthika C, Trejo Treviño AG, et al. Role of vitamin B12 and folate in metabolic syndrome. Cureus. 2021;13(10):e18521.

[27] Di Renzo L, Marsella LT, Sarlo F, Soldati L, Gratteri S, Abenavoli L, et al. C677T gene polymorphism of MTHFR and metabolic syndrome: Response to dietary intervention. J Transl Med. 2014;12:329.

[28] Vasilopoulos Y, Sarafidou T, Bagiatis V, Skriapa L, Goutzelas Y, Pervanidou P, et al. Association between polymorphisms in MTHFR and APOA5 and metabolic syndrome in the Greek population. Genet Test Mol Biomarkers. 2011;15:613-17.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, Maharaja Jitendra Narayan Medical College, Coochbehar, West Bengal, India.
2. Assistant Professor, Department of Biochemistry, Calcutta National Medical College, Kolkata, West Bengal, India.
3. Associate Professor and Head, Department of Biochemistry, Maharaja Jitendra Narayan Medical College, Coochbehar, West Bengal, India.
4. Professor and Head, Department of Biochemistry, Jhargram Government Medical College, Jhargram, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Kazi Ashique Firdoush,
Assistant Professor, Department of Biochemistry, Maharaja Jitendra Narayan Medical College, Pilkhana, Coochbehar-736101, West Bengal, India.
E-mail: ashiquefirdoush@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 25, 2022
- Manual Googling: Dec 29, 2022
- iThenticate Software: Jan 06, 2023 (12%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Nov 12, 2022**
Date of Peer Review: **Dec 07, 2022**
Date of Acceptance: **Jan 10, 2023**
Date of Publishing: **Feb 01, 2023**