Polymorphisms in *MTHFR, MS* and *CBS* genes and premature acute myocardial infarction in a Pakistani population

Mohammad Perwaiz Iqbal[1]*, Khalida Iqbal[1], Asal Khan Tareen[2], Siddiqa Parveen[1], Naseema Mehboobali[1], Ghulam Haider[1] and Saleem Perwaiz Iqbal[3]

[1]Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan
[2]National Institute of Cardiovascular Diseases, Karachi, Pakistan
[3]Department of Community Health Sciences, Aga Khan University, Karachi, Pakistan

Abstract: High prevalence of premature coronary heart disease in Pakistanis compared to other populations points towards the genetic predisposition of this population to develop this disease. Since no investigations have been carried out in Pakistan to study the relationship of polymorphisms in genes involved in homocysteine cycle, the objective of the present study was to find out if there is any association of methylenetetrahydrofolate reductase *(MTHFR)* C677T, A1298C; methionine synthase *(MS)* A2756G; cystathionine-β-synthase *(CBS)* 844ins68, G919A polymorphisms with premature acute myocardial infarction (AMI) in a population of Pakistani patients with this disease. In a cross-sectional study, DNA samples of 143 AMI patients (age <45 years) and 153 healthy controls were genotyped for the above mentioned polymorphisms using PCR-RFLP methods. Plasma/serum samples of both patients and healthy controls were screened for homocysteine, folate and vitamin B12. One way ANOVA and chi-squared test were used for analysis of data. Mean plasma homocysteine levels in premature AMI patients and healthy controls were found to be 23±17.2 and 23±13.4 µmol/l, respectively which are higher than the upper normal limit of this biomarker (15µmol/l). *MTHFR* 677 CT genotype in healthy controls and *MTHFR* 677 TT genotype in AMI patients were found to have significantly increased levels of plasma homocysteine (p value <0.05), while all other polymorphisms did not show any significant difference in mean levels of homocysteine between AMI patients and healthy controls. Moreover, no association was observed between *MTHFR* C677T, A1298C; *MS* A2756C; *CBS*844ins68 polymorphisms and premature AMI in this population. This indicates that common polymorphisms in *MTHFR, MS* and *CBS* genes have no role in premature AMI in Pakistani population.

Keywords: Acute myocardial infarction; Cystathionine beta synthase; Methylenetetrahydrofolate reductase; Methionine synthase; Polymorphism; Pakistani population

INTRODUCTION

There is a high prevalence of premature coronary artery disease in Pakistani population. In a previous study conducted at the Aga Khan University, it was shown that sixteen percent of all the acute myocardial infarction (AMI) patients admitted at its hospital during the 3-year period between 2000 to 2002 were less than 45 years old (Saleheen and Frossard, 2004). A comprehensive survey carried out in Pakistan showed that average age of Pakistani patients with myocardial infarction (MI) is 10 years lower compared to Caucasian MI patients in the West (Jafary *et al.,* 2007). Recent reports from South Asian region have indicated hyperhomocysteinemia to be associated with young AMI patients in Bangladesh (Karim *et al.,* 2015) and India (Kaur *et al.,* 2016). Another study showed hyperhomocysteinemia to be very common amongst young (below 40 years) AMI patients from Western India and folate deficiency appeared to be the major cause for higher levels of fasting plasma homocysteine (Ghosh *et al.,* 2007). This clearly showed an association of plasma homocysteine with AMI in young South Asian populations and vitamin B deficiencies appeared to be causing hyperhomocysteinemia. This association of plasma homocysteine with AMI has also been reported in a Turkish population (Akyürek *et al.,* 2014). While B vitamin deficiencies and hyperhomocysteinemia have been shown to be associated with AMI in Pakistani adults (Iqbal *et al.,* 2005), only a few investigations have been carried out in South Asia to study the association of polymorphisms in genes of enzymes involved in homocysteine metabolism with AMI (Iqbal *et al.,* 2005; Ghosh *et al.,* 2007; Iqbal *et al.,* 2011). However, no study has been carried out to find out the relationship of polymorphisms in methylenetetrahydrofolate reductase (MTHFR), methionine synthase *(MS)* and cystathionine beta synthase *(CBS)* genes and AMI in Pakistani population. The present study aims at investigating the relationship of polymorphisms in these three genes of homocysteine cycle with premature AMI in a Pakistani population in Karachi.

MATERIALS AND METHODS

Two hundred and three consecutive adult patients with first episode of AMI (age range from 18-45 years)

**Corresponding author:* e-mail: perwaiz.iqbal@aku.edu

admitted at the National Institute of Cardiovascular Diseases (NICVD), Karachi were recruited with written informed consent. They had the confirmed diagnosis of AMI. The details of the WHO criteria for diagnosis and the inclusion and exclusion criteria have been published previously (Iqbal *et al.,* 2013). Similarly, 205 healthy controls who had been matched for gender and age (within 3 years) were also enrolled with written informed consent. Controls were recruited from the personnel of the Aga Khan University and other health care institutions in Karachi. Inclusion and exclusion criteria of these controls have been mentioned in detail in a previous paper (Iqbal *et al.,* 2013). The study had been approved by the Ethical Review Committee of the Aga Khan University.

Ten ml of venous blood with at least 10 hours of fasting was collected and divided into two equal parts. One part was transferred to a heparinized tube and the other part into a plain tube. Plasma/serum was obtained for determination of homocysteine and folate using kit methods (Roche Diagnostics, USA), while serum concentration of vitamin B12 was determined using a radio assay (Quadros, 2000). DNA was extracted from leukocytes using standard protocols (Sambrook *et al.,* 1989). Genotyping of *MTHFR* C677T, A1298C; *MS* 2756; *CBS* 844ins68, G919A polymorphisms was done using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods. The details of primers and restriction enzymes have been mentioned in a previous paper (Yakub *et al.,* 2012).

All analyses were conducted using the software SPSS® (Statistical Package for Social Sciences) version 13 for Windows® (Apache Software Foundation, USA). All the continuous variables such as homocysteine, folate, vitamin B12, age and body mass index (BMI) were expressed as mean ±SD. Hardy-Weinberg proportions of genotype distributions were tested by using Hardy-Weinberg equilibrium calculator (Rodriguez *et al.,* 2009). The mean differences in the values of homocysteine, folate and vitamin B12 across various genotypes were assessed using one way ANOVA. A post-hoc test was carried out for multiple pair-wise comparisons. All p values less than 0.05 were considered significant.

RESULTS

Although we had recruited 203 patients with premature AMI and 205 healthy subjects as controls, yet the complete genotyping for all 5 polymorphisms could be obtained for 143 AMI patients and 154 healthy controls. Therefore, all our results are with this sample size. As shown in table 1, no significant differences were found in age, BMI, and plasma/serum concentrations of homocysteine and vitamins folate and B12 between premature AMI patients and healthy controls. Frequency distribution of genotypes of SNPs in AMI patients and healthy controls indicates that all polymorphisms investigated in this study, except for *CBS* 844ins68 in AMI patients, were found to occur in Hardy-Weinberg equilibrium (table 2).

Genotyping of *CBS* G919A polymorphism revealed that all AMI patients and healthy controls were GG genotype and, therefore, were not subjected to analysis. The percentages of genotypes for the 4 polymorphisms (*MTHFR* 677CC, CT, TT; *MTHFR* 1298AA, AC, CC; *CBS* 844ins68 WW, WI, II; *MS* 2756 AA, AG, GG) in AMI patients and healthy controls when compared by chi-squared analysis showed no significant difference indicating that there is no association of these polymorphisms with premature AMI in this population.

Mean concentrations of plasma homocysteine with respect to various genotypes of healthy controls and AMI patients have been shown in table 3. Significant differences in mean levels of homocysteine were observed in *MTHFR* C677T polymorphism. *MTHFR*CT genotype and *MTHFR* TT genotype in healthy controls and AMI patients, respectively were found to have significantly increased levels of plasma homocysteine ($p<0.05$). This indicates that *MTHFR* 677T allele is the determinant of plasma homocysteine. However, no significant difference was found among the genotypes of the other 3 polymorphisms (*MTHFR* A1298C, *CBS* 844ins68, *MS* A2756G). Serum folate and vitamin B12 levels were not found to be associated with any genotype of the four polymorphisms investigated in this study (table4).

DISCUSSION

High prevalence of AMI in young Pakistani population points towards a possible genetic predisposition of this population to developing coronary artery disease (CAD). Association of certain polymorphisms in paraoxonase gene cluster with MI in Pakistani population has already been reported (Saeed *et al.,* 2007). Moreover, evidence regarding association of the 9p21.3 locus with risk of AMI in Pakistanis lent further support to genetic predisposition of this population to CAD (Saleheen *et al.,* 2010). Since hyperhomocysteinemia, an established risk factor for atherosclerosis is very much prevalent in Pakistani general population (Yakub *et al.,* 2010), it is conceivable that polymorphisms in genes (such as *MTHFR, MS* and *CBS*) involved in homocysteine metabolism could be contributing to increased risk of premature AMI in this population.

Results of the present study however, show that none of these polymorphisms has any association with premature AMI in Pakistani population. Mean plasma homocysteine levels were high both in AMI patients as well as healthy controls. The percentage of *MTHFR* TT genotype – a major factor for hyperhomocysteinemia in most

Table 1: Demographic and clinical characteristics of patients with premature AMI and age and gender-matched healthy controls

Variable	AMI patients (n=143)	Controls (n=154)	p value
Age (years)	41.3±5.0	41.0±5.0	0.61
Body Mass Index (BMI)	25.5±4.3	25.1±4.0	0.396
Homocysteine (µmol/l)	23.0±17.2	23.0±13.4	0.985
Vitamin B12 (pg/ml)	320±189	333±168	0.536
Folate (ng/ml)	6.71±3.7	6.11±2.84	0.120

Mean ± SD

Table 2: Frequency distribution of genotypes of polymorphisms in genes associated with homocysteine metabolism in healthy controls and AMI patients

SNP	Genotype	N (%)					
		Healthy controls (n=154)	X^2	HWE_P	AMI patients (n=143)	X^2	HWE_P
MTHFR C677T	CC	118(76.6)			103(72.0)		
	CT	33(21.4)	0.15	NS	34(23.7)	2.03	NS*
	TT	3(1.9)			6(4.2)		
MTHFR A1298C	AA	37(24.0)			32(22.4)		
	AC	86(55.8)	2.17	NS	63(44.1)	1.66	NS
	CC	31(20.1)			48(33.6)		
CBS 844ins68**	WW	132(85.7)			127(88.8)		
	WI	21(13.6)	0.03	NS	14(9.8)	4.13	<0.05
	II	1(0.6)			2(1.4)		
MS A2756G	AA	75(48.7)			88(61.5)		
	AG	67(43.5)	0.31	NS	45(31.5)	1.55	NS
	GG	12(7.8)			10(7.0)		

Note: Hardy-Weinberg equilibrium (HWE) testing is shown in this table. Except for *CBS* 844ins68 polymorphism in AMI patients, all other polymorphisms (*MTHFR* C677T; *MTHFR* A1298C and *MS* A2756G) were in HWE in both AMI patients and control groups. *NS indicates "not significant". **WW: Homozygous wild (ancestral); WI: Wild (ancestral)/Insertion heterozygous; II: Insertion homozygous variant.

populations was found to be very low in both AMI patients and healthy controls (4.2% and 1.9%, respectively). These findings are in line with previous reports that TT variant does not appear to play any significant role in high prevalence of hyperhomocysteinemia in South Asian populations (Markan *et al.,* 2007; Michael *et al.,* 2009; Yakub *et al.,* 2012). A study in Cyprus showed that even in populations with high prevalence of *MTHFR* TT genotype in both AMI patients and healthy controls (17.7% vs. 19.2%, respectively), *MTHFR* polymorphism appeared to have no association with AMI in young Cypriots (Eftychiou *et al.,* 2012).

The percentages of *CBS* 844ins66 heterozygotes among AMI patients and healthy controls (9.8% and 13.6%, respectively) merit some discussion. These values of heterozygosity are similar to the values observed in Iranian population (12.3%), Indian population (7.97%), Italian population (7.8%), US population (11.2%) and Turkish population (11.8%), (de Franchis *et al.,* 2000; Dutta *et al.,* 2005; Senemar *et al.,* 2009; Koc and Akar, 2009), but different from Japanese, Chinese and Indonesian populations where it has been found to be absent (Dutta *et al.,* 2005). Kluijtmans *et al.,* (1997) have reported this insertion to be present in nearly equal proportions in both patients with premature arterial occlusive disease and controls. Furthermore, the insertion variants were not associated with hyperhomocysteinemia. Our results corroborate these findings and the insertion variants were neither associated with AMI nor with increased levels of plasma homocysteine.

Lack of association of *CBS* 844ins68, *MS* A2756G and *MTHFR* C677T polymorphisms with AMI in Pakistani population observed by us is also supported by results of a

Table 3: Plasma concentration of homocysteine with respect to various genotypes of healthy controls and premature AMI patients

SNP& Genotype	Healthy controls (n=154)		AMI patients (n=143)	
	Homocysteine (µmol/l)	ANOVA p value	Homocysteine (µmol/l)	ANOVA p value
MTHFR 677				
CC	21.4±9.9		22.2±14.3	
CT	28.3±21.2*	0.03	21.6±10.3	0.006
TT	23.0±13.4		44.0±53.9**	
MTHFR 1298				
AA	23.4±12.4		21.3±10.4	
AC	23.9±14.8	0.363	22.9±14.5	0.752
CC	19.9±10.2		24.2±23.2	
CBS 844ins68				
WW	23.0±14.1		23.4±18.0	
WI	22.8±8.9	0.937	19.3±7.5	0.703
II	27.8		23.7±9.0	
MS 2756				
AA	23.1±14.8		23.9±18.4	0.593
AG	22.1±10.8	0.402	22.1±16.1	
GG	27.8±17.7		18.5±7.8	

*Significantly different from CC genotype when analyzed by Tukey's HSD test for multiple pair-wise comparison (p=0.026). **Significantly different from CC and CT genotypes when analyzed by Tukey's HSD test for multiple pair-wise comparison (p<0.01).

Table 4: Serum concentrations of folate and vitamin B12 with respect to various genotypes of healthy controls and AMI patients

SNP	Healthy controls (n=154)				AMI patients (n=143)			
	Folate (ng/ml)	ANOVA p value	B12 (pg/ml)	ANOVA p value	Folate (ng/ml)	ANOVA p value	B12 (pg/ml)	ANOVA p value
MTHFR 677								
CC	6.2±2.9		340±173		6.5±3.3		324±203	
CT	5.6±2.6	0.57	303±150	0.298	7.2±4.9	0.61	308±156	0.96
TT	5.9±2.9		440±199		7.4±3.4		324±114	
MTHFR 1298								
AA	6.5±2.8		323±173		7.5±3.9		310±249	
AC	5.9±2.8	0.539	329±162	0.735	6.4±3.6	0.401	327±163	0.918
CC	6.0±2.9		354±199		6.5±3.8		318±180	
CBS 844ins68*								
WW	6.1±2.9		333±173		6.7±3.7		328±192	
WI	5.9±2.4	0.84	339±146	0.79	6.3±4.1	0.93	253±168	0.39
II	4.8±0		220±0		6.8±3.8		280±57	
MS 2756								
AA	6.1±0.3		342±181		6.7±3.7		339±210	
AG	6.1±2.4	0.98	331±149	0.6	6.3±3.0	0.16	281±141	024
GG	5.9±3.4		291±190		8.7±6.0		330±173	

*WW: Homozygous wild (ancestral); WI: Wild (ancestral)/Insertion heterozygous; II: Insertion homozygous variant.

study carried out on Southern Chinese Han population in which no relationship was found between these 3 polymorphisms and ischemic cardiovascular disease (Dai and Zhang, 2001). These observations support the notion that plasma homocysteine alone does not appear to play any significant role in increasing the risk of AMI in Pakistani population.

Our results should be viewed in the context of a limitation of this study. Information about the use of medications by the recruited subjects was not part of our questionnaire. In a large clinical trial, it has been shown that statin treatment is protective against coronary heart disease in subjects with *MTHFR* 677 CC genotype (0.71(95% CI 0.58-0.87) and *CBS* 844ins68 insertion variants

(0.58(95% CI 0.44-0.78) (Maitland-van der Zee *et al.,* 2008). Therefore, statin users with the above mentioned genotypes could have been protected from having AMI episode and, hence, were not included as AMI patients. This notion is supported by the observation that the proportions of *MTHFR* 677 CC genotype and the *CBS* 844ins68 insertion variants are less (though non-significant) in AMI patients compared to healthy controls (72.0% vs. 76.6.0% and 9.8% vs.13.6%, respectively) in the study population.

Large prospective studies with complete information of medications used by the participants would be required to ascertain the relationship of these polymorphisms with AMI in Pakistani population.

CONCLUSION

Most common polymorphisms in *MTHFR, MS* and *CBS* genes are not associated with premature AMI in a Pakistani population.

ACKNOWLEDGEMENTS

The study had been supported by a grant from the Pakistan Academy of Sciences/Higher Education Commission. We gratefully acknowledge the help of Ms. Shaheena Anwar for refining the data.

REFERENCES

Akyürek Ö, Akbal E and Günes F (2014). Increase in the risk of ST elevation myocardial infarction is associated with homocysteine level. *Arch. Med. Res.,* **45**(6): 501-506.

Dai C and Zhang G (2001). Study on homocysteine metabolism related enzymes gene mutations in Chinese patients with ischemic cardiovascular and cerebrovascular diseases. *Zhonghua. Xue. Ye. Xue. Za. Zhi.,* **22**(9): 484-487.

de Franchis R, Fermo I, Mazzola G, Sebastio G, Di Minno G, Coppola A, Andria G and D′Angelo A (2000). Contribution of the cystathionine beta-synthase gene (844ins68) polymorphism to the risk of early-onset venous and arterial occlusive disease and of fasting hyperhomocysteinemia. *Thromb. Haemost.,* **84**(4): 576-582.

Dutta S, Sinha S, Chattopadhyay A, Gangopadhyay PK, Mukhopadhyay J, Singh M and Mukhopadhyay K (2005). Cystathionine beta-synthase T833C/844INS68 polymorphism: a family-based study on mentally retarded children. *Behave. Brain Funct.,* **26**(1): 25.

Eftychiou C, Antoniades L, Makri L, Koumas L, Costess PA, Kyriakou E, Nicolaides E and Papadogiannis D (2012). Homocysteine levels and MTHFR polymorphisms in young patients with acute myocardial infarction: A case control study. *Hellenic J. Cardiol.,* **53**(3): 189-94.

Ghosh K, Khare A and Shetty S (2007). Fasting plasma homocysteine levels are increased in young patients with acute myocardial infarction from Western India. *Indian Heart J.,* **59**(3): 242-245.

Iqbal MP, Ishaq M, Kazmi KA, Yousuf FA, Mehboobali N, Ali SA, Khan AH and Waqar MA (2005). Role of vitamins B6, B12 and folic acid on hyperhomocysteinemia in a Pakistani population of patients with acute myocardial infarction. *Nutr. Metab. Cardiovasc. Dis.,* **15**: 100-108.

Iqbal MP, Fatima T, Parveen S, Yousuf FA, Shafiq M and Mehboobali N (2005). Lack of association of methylenetetrahydrofolate reductase 677C>T mutation with coronary artery disease in a Pakistani population. *J. Mol. Genet. Med.,* **1**: 26-32.

Iqbal MP, Mehmood S, Parveen S, Mehboobali N and Haider G (2011). Polymorphisms in methylenetetrahydrofolate reductase gene are not associated with acute myocardial infarction in a Pakistani population. *Pak. J. Biochem. Mol. Biol.,* **44**(1): 32-35.

Iqbal MP, Mehbooali N, Tareen AK, Yakub M, Iqbal SP, Iqbal K and Haider G (2013). Association of body iron status with the risk of premature acute myocardial infarction in a Pakistani population. *PLoS ONE.,* **8(**6): e67981.

Jafary MH, Samad A, Ishaq M, Jawaid SA, Ahmad M and Vohra EA (2007). Profile of acute myocardial infarction (AMI) in Pakistan. *Pak. J. Med. Sci.,* **23**: 485-489.

Karim MA, Majumder AA, Islam KQ, Alam MB, Paul ML, Islam MS, Chowdhury KN and Islam SM (2015). Risk factors and in-hospital outcome of acute ST segment elevation myocardial infarction in young Bangladeshi adults. *BMC Cardiovasc. Disord.,* **15**:73.

Kaur R, Das R, Ahluwalia J, Kumar RM and Talwar KK (2016). Genetic polymorphisms, biochemical factors, and conventional risk factors in young and elderly North Indian patients with acute myocardial infarction. *Clin. Appl. Thromb. Hemost.,* **22**(2): 178-183.

Kluijtmans LA, Boers GH, Trijbels FJ, van Lith-Zanders HM, Van den Heuvel LP and Blom HJ (Year). A common 844INS68 insertion variant in the cystathionine beta-synthase gene. *Biochem. Mol. Med.,* **62**(1): 23-25.

Koc YL and Akar N (2009). Single nucleotide polymorphisms that affect homocysteine levels in Turkish population. *Clin. Appl. Thromb. Hemost.,* **15**(6): 701-704.

Markan S, Sachdeva M, Sehrawat BS, Kumari S, Jain S and Khullar M (2007). *MTHFR* 677 CT/*MTHFR* 1298 CC genotypes are associated with increased risk of hypertension in Indians. *Mol. Cell Biochem*., **302**: 125-131.

Maitland-van der Zee AH, Lynch A, Boerwinkle E, Arnett DK, Davis BR, Leiendecker-Foster C, Ford CE and Eckfeldt JH (2008). Interactions between the single nucleotide polymorphisms in the homocysteine pathway (MTHFR 677C>T, MTHFR 1298 A>C and CBSins) and the efficacy of HMG-CoA reductase inhibitors in preventing cardiovascular disease in high-risk patients of hypertension: the GenHAT study. *Pharmacogenet Genomics.,* **18**(8): 651-666.

Micheal S, Qamar R, Akhtar F, Khan MI, Khan WA and Ahmed A (2009). *MTHFR* gene C677T and A1298C polymorphisms and homocysteine levels in primary open angle and primary closed angle glaucoma. *Mol. Vis.,* **15**: 2268-2278.

Quadros E (2000). Vitamin B12. *In*: Song WO, Beecher GR, Eitenmiller RR, editors. Modern analytical methodologies in Fat and water-soluble vitamins. Chemical analysis series. John Wiley & Sons, Inc., New York, pp.311-326.

Rodriguez S, Gaunt TR and Day INM (2009). Hardy-Weinberg Equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am. J. Epidemiol*., **169** (4): 505-514.

Saleheen D and Frossard P (2004). CAD risk factors and acute myocardial infarction in Pakistan. *Acta. Cardiol.,* **59**(4): 417-424.

Salaheen D, Alexander M, Rasheed A, Wormser D, Soranzo N, Hammond N, Hammond N, Butterworth A, Zaidi M, Haycock P, Bumpstead S, Potter S, Blakburn H, Gray E, Di Angelantonio E, Kaptoge S, Shah N, Samuel M, Janjua A, Sheikh N, Haider SR, Murtaza M, Ahmad U, Hakeem A, Memon MA, Mallick NH, Azhar M, Samad A, Rasheed SZ, Gardezi AR, Memon NA, Ghaffar A, Memon FU, Zaman KS, Kundi A, Yaqoob A, Cheema LA, Qamar N, Faruqui A, Jooma R, Niazi JH, Hussain M, Kumar K, Saleem A,, Kumar K, Daood MS, Memon F, Gul AA, Abbas S, Zafar J, Shahid F, Memon Z, Bhatti SM, Kayani W, Ali SS, Fahim M, Ishaq M, Frossard P, Deloukas P and Danesh J (2010). Association of the 9p21.3 locus with risk of first-ever myocardial infarction in Pakistanis: Case-control study in South Asia and updated meta-analysis of Europeans. *Arterioscler Thromb. Vasc. Biol.,* **30**(7): 1467-1473.

Sambrook J, Fritsch EF and Maniatis T (1989). Molecular cloning a laboratory manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring.

Saeed M, Iqbal MP, Yousuf FA, Parveen S, Shafiq M and Sajid J (2007). Interactions and associations of paraoxonase gene cluster polymorphisms with myocardial infarction in a Pakistani population. *Clin. Genet.,* **71**: 238-244.

Senemar S, Doroudchi M, Pezeshki AM, Bazragar M, Torab-Jahromi A and Ghaderi A (2009). Frequency of cystathionine beta-synthase 844INS68 polymorphism in Southern Iran. *Mol. Biol. Rep.,* **36**(2): 353-356.

Yakub M, Moti N, Parveen S, Chaudhry B, Azam I and Iqbal MP (2012). Polymorphisms in *MTHFR, MS* and *CBS* genes and homocysteine levels in a Pakistani population. *PLoS ONE.,* **7**(3): e33222.

Yakub M, Iqbal MP, Kakepoto GN, Rafique G, Memon Y and Azam I (2010). High prevalence of mild hyperhomocysteinemia and folate, B12 and B6 deficiencies in an urban population in Karachi, Pakistan. *Pak. J. Med. Sci.,* **26**: 923-929.