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Association between MTHFR C677/APOB G10708B Polymorphisms and Excess Body Weight in Blood Donors

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ABSTRACT The researchers investigated MTHFR C677T/APOB G10708A polymorphisms as well as total antioxidant/oxidant status (TAS/TOS) in blood donors. Participants were stratified into two age groups: 18-39 years and 40-59 years and their body mass index: normal weight (<24.9) and obese (>25). The mutant homozygous MTHFR genotype was identified in 3 (12.5%) of normal weight and 4 (16.6%) of obese in 20-30 years while it was found in 1 (4.16%) of normal weight and 3 (12.5%) of obese in 40-59 years. The homozygous APOB genotype was found in all donors. TOS in obese donors was found higher than normal weight donors for both age groups. TAS in obese donors was found less than normal weight donors in 40-59 years. MTHFR was found significantly different between two age groups of normal weight donors. However, the researchers did not find any correlation between two polymorphisms and TAS/TOS for all groups.

INTRODUCTION

Obesity, characterized by an increase of body weight results in overabundant fat accumulation. It's a serious health and social problem that leads to several diseases including hypertension, diabetes, cardiovascular diseases, arthritis and mortality. Unfortunately, the numbers of obese children and adults have increased significantly over the last 25 years across all racial/ethnic and age groups (Gregg et al. 2005). Previous studies have shown that obesity induces systemic oxidative stress (Zhu et al. 2006; McMurray et al. 2016). Oxidative stress is defined as imbalance between oxidants and antioxidants. Several mechanisms are involved in generating oxidative stress in obesity such as superoxide generation from NADPH oxidases, oxidative phosphorylation, hyperleptinemia, tissue dysfunction, adipokines and adipocytokines function (Marseglia et al. 2015; Manna and Jain 2015). The reactive oxygen species (ROS) are highly reactive molecules that are generated by aerobic respiration and various other anabolic/catabolic processes (Schieber and Chandel 2014). According to their potential, ROS causes oxidative deterioration of macromolecules including DNA, lipid, protein, and it implies as one of the cause of aging (Cui et al. 2012). Aging is very complex and multifactorial process that progresses to the gradual deterioration in normal function and leads to degenerative diseases and death (Poljsak and Millisov 2013). Previous studies have demonstrated that aging is a major risk factor for vascular diseases. Also, aging increases the prevalence of vascular diseases and impaired responses to cardiovascular diseases (Chiao et al. 2016). However, cardiovascular diseases are not only the cause of death for people over 65 years old but are among the primary reason of death for all age groups worldwide (Lozano et al. 2012). The interaction of genetic factors and environment influences play important role in the development of vascular disease. Various gene polymorphisms have been discov-

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ered within genes encoding peptides directly and indirectly associated with hemostasis. Many of these genetic markers related to vascular disease have been investigated in defined patient groups; however, the possible impact of these risk factors on overall health and longevity are not fully understood in healthy population. Previous studies showed that MTHFR C677T and APOB G10708A polymorphisms are significant risk factors for vascular disease. MTHFR C677T plays a major role in the broad folate and homocysteine metabolism. The human MTHFR C677T gene is localized on chromosome 1p36.2 and codes for the enzyme MTHFR C677T consisting of 656 amino acids. MTHFR C677T converts 5, 10 methylene tetrahydrofolate (5, 10-methylene THF) irreversibly into 5-methyl tetrahydrofolate (5-methyl THF). It is known that extrinsic functions increase the risk of cardiovascular disease following increased homocysteine plasma levels (Antoniades et al. 2009). The incidence of homozygous individuals has been reported to be five to sixteen percent (Ocal et al. 1997). An APOB G10708A amphipathic glycoprotein is the major component of very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL) and low density lipoproteins (LDL) that are secreted in hepatocytes. These lipoproteins are responsible for the transport of cholesterol and triglycerides in the circulatory system and play an important role in atherosclerosis (Olofsson and Boren 2005).

Objectives

Comparison of genotype frequencies in healthy individuals may be advantageous strategy to define polymorphisms related to common human diseases, such as vascular. Therefore, in this study, the researchers investigated association between MTHFR C677T/APOB G10708B and excess body weight in blood donors.

METHODOLOGY

Study Population

This prospective study was carried out in the Regional Blood Center of Cukurova University. The study's protocol was approved by the Human Subject Ethics Committee of the Cukurova University (2016: 24) and was conducted in accordance with Helsinki Declaration. Peripheral blood was collected in disodium EDTA from 96 male Caucasian healthy blood donors living in South Anatolia Region of Turkey. Participants were stratified into two age groups: 18-39 years and 40-59 years and their body mass index: normal weight (<24.9) and obese (>25). First age group was selected according to legal blood donation age in Turkey and second age group was selected based on previous studies (Hessner et al. 2001). Genomic DNA was isolated from buffy coat using Qiagen DNA extraction kit (Germany).

Molecular and Biochemical Assays

The MTHFR C677T and APOB G10708A genotypes were determined by Real Time PCR (Roche, Cobas Z 480) using commercial kits from TIB, MOLBIOL, LightMix (CN: 40-0593-64, 40-0594-64). And the TAS/TOS levels were determined by Rel Assay Diagnostics kits (RLOO17, RL0024).

Statistical Analysis

The differences in genotype of MTHFR/APO B-100 and TAS/TOS were analyzed statistically using Fischer's exact test and chi-square test. The associations between allele frequencies in all groups were evaluated by computing the odds ratios (OR) and their confidence intervals using Hardy-Weinberg equilibrium. $P<0.05$ was considered statistically significant. All the variables were correlated with TAS/TOS and two polymorphisms using Spearman rank correlation coefficients (r) and statistical significance was computed.

RESULTS

The heterozygous and mutant homozygous MTHFR C677T genotypes in 18-39 age group were identified in 13 (54.16%), 3 (12.5%) of normal weight, 6 (25%) and 4 (16.6%) of obese donors, respectively. The heterozygous and mutant homozygous MTHFR C677 in 40-59 age group were found in 5 (20.83%), 1 (4.16%) of normal weight, 8 (33.33%) and 3 (12.5%) of obese donors, respectively (Table 1). No significant differences were observed in genotype or allele frequency of MTHFR C677 when these data were analyzed on the basis of body mass index ($p<0.129$ for 18-39 age group, $p<0.344$ for 40-59 age group) (Tables 1, 2 and 3). On the other hand,

there was significant difference between 18-39 and 40-59 age groups of normal weight donors. All donors were found to have homozygous for APOB G10708A.

Total antioxidant status in obese were found less than normal weight donors in 40-59 age group, whereas TAS did not have a significant difference between obese and normal weight donors in 18-39 age group (p<0.007, 0.212). In addition, TAS were found similar when this data were analyzed on the basis of age (p<0.671, 0.916). Total oxidant status were found higher in obese donors than normal weight donors for both age groups (p<0.01, 0.007). And then it was 1.5 times higher in 40-59 age group than 18-39 age group (p<0.01) (Table 4).

Further, the researchers correlated the TAS/TOS with the two genetic polymorphisms. APO

Table 1: Genotype frequencies of MTHFR gene in normal weight and obese donors of two age groups

	Age groups			
Genotype	*18-39 years (n/%)*		*40-59 years (n/%)*	
	Normal weight donors	*Obese donors*	*Normal weight donors*	*Obese donors*
CC	8 (33.33%)	14 (58.33%)	18 (75%)	13 (54.16%)
CT	13 (54.16%)	6 (25%)	5 (20.83%)	8 (33.33%)
TT	3 (12.5%)	4 (16.6%)	1 (4.16%)	3 (12.5%)

Table 2: The association between genotype and body weight of two age groups for MTHFR gene

	Age groups					
	18-39 years Normal and obese donors			*40-59 years Normal and obese donors*		
Genotype	*OR*	*95% CI*	*Chi-sq*	*OR*	*95% CI*	*Chi-sq*
CC/CT	0.26	0.07- 0.97	4.19	2.22	0.59- 8.34	1.41
CT/TT	2.89	0.49-17.17	1.41	1.88	0.15-23.4	0.24
CT/CC	0.76	0.13- 4.30	0.10	4.15	0.39-44.57	1.56

Table 3: Allele frequencies in peripheral blood MTHFR gene in normal weight and obese donors of two age groups

	Age groups			
Allele	*18-39 years (n/%)*		*40-59 years (n/%)*	
	Normal weight donors	*Obese donors*	*Normal weight donors*	*Obese donors*
C	29 (60.41%)	34 (70.83%)	41 (85.41%)	34 (70.83%)
T	19 (39.58%)	12 (29.16%)	7 (14.58%)	14 (29.16%)
	OR: 1.59 95% CI:0.77-4.45 P:0.166		OR: 0.41 95% CI:0.15-1.14 P:0.08	

Table 4: TAS (mmol Trolox Equv. /L) /TOS (µmol H_2O_2 Equiv./L) levels in normal and obese donors of two age groups

	Age groups			
Allele	*18-39 years (n/%)*		*40-59 years (n/%)*	
	Normal weight donors	*Obese donors*	*Normal weight donors*	*Obese donors*
TAS	1.22±0.06	1.11±0.05	1.19±0.01	1.11±0.02
TOS	5.30±0.20	7.27±0.32	6.72±0.29	11.41±0.26

B G10708A and MTHFR C677T polymorphism showed no correlation with TAS/TOS of all groups (p=0.247).

DISCUSSION

Although the most common ethology of vascular diseases is defined as multifactorial that are not yet fully understood (Ordovás and Smith 2010). Hereditary changes in the MTHFR C677T gene are considered as risk factors for some congenital anomalies and the development of cardiovascular diseases (Wang et al. 2013). Therefore, investigation of MTHFR C677T gene polymorphism in healthy population is important for understanding the ethology of these diseases. According to World Health Organization data, 17.7 million people die from cardiovascular diseases every year (WHO 2016). In Turkey, deaths due to circulatory system diseases are ranked first with a rate of 39.6 percent according to the data of 2016 TUIK report. Previous studies demonstrated that the prevalence of MTHFR C677T polymorphism varies in different geographical regions. The prevalence of the MTHFR C677T mutant homozygous genotype in Europe were reported to be 7.8 percent in Germany, 8.9 percent in Holland, 10.3 percent in Sweden, 13.2 percent in England, eighteen percent in Italy (Stevenson et al. 1997; Schneider et al. 1998; Pepe et al. 1998; Franco et al. 1998). The prevalence of MTHFR C677T mutant homozygous in Turkey was declared as 9.6 percent (Sazci et al. 2005) and three percent (Ozturk et al. 2016) in healthy population. In the researchers' study, the prevalence of C677T mutant homozygous was found 14.58 percent in 18-39 and 8.33 percent in 40-59 age groups. Similarly, Matsushita et al. (1997) reported that the MTHFR C677T genotype frequency decreased from nineteen percent to seven percent in healthy Japanese individuals <55 years *vs.* those >80 years. These results suggest that the mutant homozygous MTHFR could be a genetic factor that prevents accomplishment with old age. Also, this study showed that the prevalence of MTHFR C677T was higher in obese compared to normal weight donors for both ages but no significant differences were observed. At the same time, the prevalence of MTHFR C677T mutant homozygous in donors varied between 4.16-16.6 percent. Likewise, Hernández-Guerrero et al. (2013) reported that the frequency of the MTHFR C677T polymorphisms had no statistical difference between normal and obese individuals. Therefore, the researchers recommended that MTHFR C677 polymorphisms are mainly related to elaborate obesity rather than obesity alone as well as ethnic background. Furthermore, it could be also associated with ethnic background. Also, the researchers' results are consistent with other European countries except Italy, suggesting that mutation in MTHFR C677T gene is not unique to Turkish population.

Familial defective APOB G10708A is an autosomal dominant inherited hypercholesterolemia (Andersen et al. 2016). A common polymorphism is a G10708A transition in the APOB gene which results in R3500Q substitution (Teng et al. 2000). The mutation in the APOB G10708A gene is known as an increased risk factor in the early period of atherosclerosis. In the researchers' study, APOB G10708A gene polymorphism homozygous was found hundred percent. Similarly, Gialeraki et al. (2008) reported as hundred percent prevalence of homozygosity in the healthy Greek population. The rate of APOB R3500Q mutation frequency varies between 1/500-1/700 in Germany, England and USA (Brousseau et al. 1995). There were no mutations in Spain, Israel, Japan, Turkey, Lebanon and Iran populations (Eroglu et al. 2008; Friedlander et al. 1993; Sabbagh et al. 2007; Fard-Esfahani et al. 2005; Myant et al. 1997). In the researchers' study, the prevalence of APOB G10708A homozygous coincided with the hypothesis that the mutation in the APOB gene occurred in Central Europe 7000 years ago and spread to Europe (Awad and El-Tarras 2011).

Oxidative stress is known to play a key role in the development of cardiovascular diseases. The investigation of TAS/TOS provides information about the pro-oxidant and antioxidant status of the body. Research on these parameters in blood is important for monitoring the oxidative stress and oxidative stress response of the body (Bakhtiari et al. 2017). In the researchers' study, TAS was found less in obese than normal weight donors in 40-59 age group while there was no significant difference between obese and normal weight donors in the 18-39 age group. Total oxidant status was found higher in obese donors in both age groups. Turkdogan et al. (2014) reported that TAS/TOS levels did not change according to sex, but they found significant correlation between oxidative

stress index, cholesterol and LDL levels in healthy volunteers. Csont et al. (2007) reported increased cardiac superoxide production and NADPH oxidase expression and activity in cholesterol-rich APOB transgenic mice.

Furthermore, the researchers did not find any correlation between MTHFR C677T/APO B G10708A and total antioxidant/oxidant status. Similarly, Vijaya et al. (2011) found no correlation between MTHFR C677T and oxidative stress parameters such as malondialdehyde, protein carbonyl and glutathione however, they observed negative correlation of the plasma folate level with MTHFR C677T.

In the present study there were two limitations. First, the number of subjects was small because the samples were selected from donors who applied to the researchers' university during legal period of the project. Second status is the lacking of the folate and homocystein plasma level in the studied subject, and further studies will be performed addressing these questions.

CONCLUSION

According to this study, MTHFR C677T gene polymorphism was found to have significant difference between 18-39 and 40-59 age groups in normal weight ($p<0.010$) but APOB G10708A gene polymorphisms were not related to age and weight. Also, the researchers' results showed that there is high level of TOS together with low level of TAS detected in obese in 40-59 age group indicate the elevated oxidative stress. These findings suggested oxidative stress and MTHFR C677T/APOB G10708A polymorphisms in healthy population even though they have independent impact on the risk factor of vascular diseases; this interesting interrelationships between these two etiological factors depending on age and body mass index should be investigated with plasma folate or homocysteine level.

RECOMMENDATIONS

As a result, early lifestyle intervention against oxidative stress is expected to reduce the risk of cardiovascular disease in the future, especially when the lifestyle of people with familial predisposition to cardiovascular diseases is changed. However, in order to understand the ethology of the diseases and to develop alternative methods for treatment, it is necessary to carry out more extensive research in healthy individuals.

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