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Investigation of the Relationship Between the Frequency of MTHFRC677 Polymorphism and Smoking Behavior in An Iranian Population

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Abstract

Smoking is among the most important preventable risk factors for mortality in developed countries. Research has indicated that cigarette smoke is a strong environmental modifier of DNA methylation. Methylenetetrahydrofolate Reductase (MTHFR) gene codes MTHFR enzyme, which is associated with folate metabolism. Evidence has demonstrated a decrease in the activity of MTHFR enzyme and folate concentration among smokers, resulting in hyper-homo-cysteinemia that is a risk factor for stroke and cardiovascular diseases. The present study aimed to assess the frequency of MTHFR gene polymorphism among smokers in order to evaluate the relationship between this gene polymorphisms and smoking behavior. Our study also aimed to compare homocysteine level between smokers and non-smokers' people. Totally, 409 individuals were enrolled into the smoker group and 210 healthy nonsmokers into the control group, the genotypes were determined in two groups using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. Homocysteine concentration was also assessed in the separated sera using ELISA technique. The results of ELISA revealed higher homocysteine concentrations in smokers than in nonsmokers ($p<0.001$). The results of genotype determination showed that the frequency of TT genotype was significantly higher among smokers compared to nonsmokers, ($P<0.001$). However, significant difference was observed between the two groups regarding the frequency of C and T alleles, T alleles was significantly higher in smokers than non-smokers' group, ($P<0.001$). In conclusion, T alleles may be associated with the smoking behavior, and there is higher homocysteine concentration in smokers compared to nonsmokers.

Keywords: polymorphism; genotype; homocysteine; smoking behavior

Introduction

Based on the data obtained from the deaths resulting from cigarette smoking, it has been responsible for one-third of respiratory disorders, one- third of cancers, and one-third of cardiovascular diseases [1]. Cigarette smoke contains more than 4000 components, such as nicotine, cadmium, formaldehyde, methoprene, maltitol, polonium, arsenic, benzene, butane, carbon monoxide, naphthalene, methyl isocyanate, ammonia, and ethyl phorate [2]. Methylenetetrahydrofolate Reductase (MTHFR) gene is located on chromosome 1- p36.3, this gene codes MTHFR enzyme, which regulates homocysteine concentration. The most important polymorphisms of this gene are MTHFRC677 and MTHFRA1298C [2, 3]. In MTHFRC677 polymorphism, cytosine is replaced by thymine, which changes alanine to valine [4]. MTHFR gene plays a pivotal role in the uptake of folic acid and other folate forms by cells, hence, individuals with MTHFRC677 polymorphism are 40-60% unable to produce the most effective form of folate; i.e., methyl folate [3]. Methyl folate alongside methylcobalamin helps change homocysteine into methionine, and the cycle continues until MTHFR gene produces methyl folate. [5], this polymorphism changes the enzyme activity as well as the balance in modification of 5, 10-MTHFR to 5- MTHFR product, that is a risk for increasing homocysteine concentration. In individuals with TT genotype, folate rather than methylenehydrofolate is mostly in form of formyltetrahydrofolate [6]. Folate plays a key role in biosynthesis of purine and pyrimidine nucleotides. On the other hand, DNA repair mechanisms are affected by nucleotide access. Therefore, folate insufficiency can damage DNA and increase homocysteine concentration and cell

proliferation [7, 8]. Increased homocysteine level can be caused by many factors like, shortage of folic acid, pyridoxine, and cobalamin in the eating diet, Alzheimer's disease, kidney disorder, hypothyroidism, specific cancers, excessive alcohol consumption, cigarette smoking, and genetic factors such as MTHFR gene mutation [9, 10]. In addition, increase in homocysteine has been reported to be associated with reduction of folate in smokers with MTHFRC677 polymorphism [11]. Smoking increases homocysteine concentration through increasing 5, 10-MTHFR concentration [12]. The TT genotype of MTHFRC677 polymorphism plays a two- fold more important role in the incidence of hyper-homocysteinemia. The genetic relationship of this polymorphism in folate metabolism can be effective in smoking behavior. However, the mechanism of the effect of T allele on smoking behavior is still unknown. Although the impact of particular genetic factors on opioids consumption is quite evident in both humans and animals. Therefore, different MTHFRC677 polymorphisms can affect the biochemical pathways, explaining the relationship between this enzyme and smoking behavior [13]. In this study we aimed to investigate the association between MTHFRC677 polymorphism and smoking behavior. Also, we evaluated the association between MTHFRC677 polymorphism and hyper-homocysteinemia.

Materials and methods

This study was investigated in an Iranian population, all participant was male, unfortunately female smokers did not volunteer for the study because of social and cultural reasons. 409 smokers and 210 healthy controls from 2020 to 2021 were participated in this research. All of volunteers signed the consent form after we explained them the purpose of the study. Smoker individuals were classified to three groups according to the fagerstorm protocol: sever, moderate and low smokers. https://www.hse.ie/eng/about/who/tobaccocontrol/resources/fagerstrom-test-for-nicotine-dependence.pdf. Demographic information is shown in table 1.

Inclusion and exclusion criteria

All healthy adult cigarette smokers who smoked for at least two years were included in this study. The people who used opioid, were excluded.

PCR–RFLP

PCR reaction was performed in 25μL mixture including 2.5 μL 10 × PCR buffer, 10 mmol/L dNTP (0.5 μl), 25 mmol/L MgCl2 (1.5 μL),20 μmol/L of each primer (0.5 μl), template DNA 1.5 μl, Taq DNA polymerase 5 U/μl, (0.2 μl), and nuclease free water 17.8 μl. The reaction was 35 cycles, carried out at 94℃ for 5 min, 94℃ for 1 min, 61℃ for 1 min,72℃ for 1 min, final extension at 72℃ for 8 min. For RFLP, PCR products were digested with Hinf I at 37℃ for 13 h. The electrophoresis was done on a 2.0% agarose gel. Wild type CC homozygotes was identified via presence of 173bp fragment, CT heterozygotes were identified by two bands of 173bp, 125bp and 48bp fragments, T/T homozygotes were identified by the presence of 125bp and 48bp fragments.

Biochemical Analysis

For homocysteine levels, 2 ml fasting blood sample collected and was centrifuged at 10,000 rpm for 10 min and serum was separated. The sample was preserved at −40 °C till later determination. Total serum Hcy was estimated with ELISA assay, AXIS-SHIELD kit, England.

Statistical analysis

Genotype distributions were analyzed by Chi square test. The analysis of homocysteine plasma levels, MTHFR genotypes, smoking status and age, were done with multivariate linear regression analysis.

Ethical considerations

All research procedures were completely explained to the participants and their written informed consent forms were obtained.

Table 1: Demographic information of smokers and nonsmokers

	SmokersN=409			Healthy non-smokersN=210
	Low N (%) =99(24)	Medium N (%) =198(48)	High N (%) =112(27)	
BMI	22.3±2.3	22.8±5.1	23.1±4.7	22.4±6.4
Mean age at start moking±SD (16-87)	19±12.3	44.2±12.3	45.1±19.3	43.2±15.3
Alcohol using	81(19.8)	173(42.3)	111 (27.1)	51(24.2)
Huka using	81(19.8)	102(24.9)	92(22.5)	

Results

The present study aimed to explore the frequency of MTHFRC677 gene polymorphism in smokers to determine the relationship between this polymorphism and smoking behavior. Moreover, serum level of homocysteine was the other goal of this study. The frequency of MTHFRC677 gene polymorphism in three groups of low, moderate and high smokers are shown in table 2. The frequency of the gene polymorphism and alleles are shown in table 3. The results indicated significantly higher homocysteine concentrations in smokers than in non-smokers, table 4.

Table 2: MTHFRC677 gene polymorphism and the frequency in three groups of low, moderate and high smokers.

	Types of smoking	N (%)	Genotypes	N (%)
Smokers	Low	123 (30.07)	CC	35(28.45)
			TC	53(43.08)
			TT	35(28.45)
	Average	184 (44.98)	CC	56(30.43)
			TC	83(45.10)
			TT	45(24.45)
	High	102(24.93)	CC	14(13.72)
			TC	63(61.76)
			TT	25(24.50)

Table 3: MTHFRC677 gene polymorphism and the frequency of the three genotypes, C/T alleles and serum homocysteine concentration in two groups of the study. However, significant difference was observed between the two groups regarding the frequency TT genotype in two groups (P<0.001). Also, we found significant difference between C and T alleles, between smokers than nonsmokers' group, (P<0.001).

		Genotypes			Allele		Mean of homo- cysteine concentration (µmol/l)	P-value
		CC (n/%)	TC(n/%)	TT(n/%)	C(n/%)	T(n/%)		
Groups	Smokers N=409	195(47.67)	174(42.50)	40(9.77)	564(68.94)	254(31.05)	11.6225±5.679	
	NonsmokersN=210	182(86.66)	11(5.23)	17(8.09)	375(89.28)	45(10.71)	7.2661±4.958	0.001

Discussion

The present study aimed to investigate the MTHFRC677 gene polymorphisms and its association with smoking behavior and also serum homocysteine concentration. The results revealed significantly higher homocysteine concentration in smokers than in nonsmokers. The frequency of TT genotype and T allele were significantly higher among the smokers compared to nonsmokers. Therefore, it is probably T allele is associated with the incidence of smoking behavior and higher homocysteine concentration. In the research carried out by Linebank et al., T allele of MTHFRC677 polymorphism had a bolder relationship with the impact of the gene on homocysteine concentration. Additionally, the T genotype of this polymorphism was associated with smoking behavior. Yet, different genotypes of MTHFR gene could be related to homocysteine concentration [4]. However, I O Oliveira et al. conducted a study on the 3803 individuals from the Pelotas Birth Cohort. The results indicated that MTHFR 677TT genotype was higher in men in smokers and drinkers. highest concentration of homocysteine was detected among the smokers with the TT genotype of the polymorphism [14]. Suchita Rawat et al. showed that smoking not only increases homocysteine but also exacerbates the association of MTHFR C677T gene polymorphism with homocysteine [15]. Malhaba et al. have shown that MTHFR TT genotype was associated with higher water pipe dependence. Physiologically, nicotine activates the receptors of nicotinic acetylcholine (nAChRs) that trigger numerous dopaminergic neurons in the brain mesolimbic system, which itself will activate "reward pathways" through dopamine-releasing [16]. Some studies have shown that when smokers are exposed to micronutrient deficiency, the compound of cyanides and Sulphur dioxide from Tobacco smoke, inactivates Vitamin B12. On the other hand, the other compound of Tobacco smoke, Nitrous Oxide (N2O) inhibits methionine synthase activity, this reduces endogenous vitamin B12, resulting in folate deficiency, and folate getting trapped in the five methylenetetrahydrofolate forms [15, 17]. If

MTHRF677TT polymorphism will be accompanied by nutritional deficiency, therefore folate deficiency and hyper-homo-cysteinemia will be exacerbated. Epidemiological studies have demonstrated that the insufficiency of folate derivatives was effective in the risk of cardiovascular diseases, neural tube defects, and cancer [18-20]. Hence, folate can play a significant role in prevention of genomic damage. Moreover, scientists have previously proposed that some physiological metabolites and folate derivatives could be effective in the collection of free radicals in human body, which has been considered to be the response to their antioxidant activities. Since cigarette contains some xenobiotics, such as oxidants and free radicals, reduction of serum folate level and red blood cells has been reported among the individuals who smoke lots of cigarettes. Folate and vitamin B12 are antioxidant derivatives, this might be the reason for the higher homocysteine concentration among smokers. Cigarette smoking, reduction of folate and vitamin B12 levels, and increase of homocysteine concentration could in turn result in an increase in chromosomal damages [21, 22]. In conclusion, this study showed a significant association between MTHFR TT and smoking dependence, and also homocysteine level. more studies are necessary to confirm our results.

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