

The Methylenetetrahydrofolate Reductase C677T Polymorphism in Patients with Esophageal Cancer

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Received date: January 04, 2019; Accepted date: February 01, 2019; Published date: February 06, 2019

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Abstract

Background: Esophageal cancer is the eighth most common cancer worldwide and the 6th cause of cancer related death. Genetic factors are also responsible in pathogenesis of esophageal cancer. This study aimed to investigate the association between methylenetetrahydrofolate reductase C677T polymorphism and esophageal cancer in Iranian population.

Methods and Materials: A cross-sectional study was conducted among patients diagnosed with esophageal cancer in Imam Reza hospital between June 2007 and June 2014, Tehran, Iran. Genotyping was performed using restriction fragment length polymorphism (RFLP)-PCR method using Hinf-1 restriction endonuclease enzyme.

Results: The frequencies of various genotypes of *MTHFR* gene were not statistically significant in cases and controls ($P=0.348$). There were no statistically significant difference in frequency of C and T alleles in patients with esophageal cancer and controls ($P=0.084$). Mean survival of patients with esophageal cancer was 31.25 ± 4.25 months in patients with CC genotype, 38.2 ± 4.11 months in CT genotype and 37.2 ± 6.44 months in patients with TT genotype ($P=0.459$). Allele frequency was not also associated with mean survival in patients and controls ($P=0.168$).

Conclusion: Methylenetetrahydrofolate reductase C677T polymorphism was not associated with esophageal cancer and did not impact on survival in this subset of Iranian patients.

Keywords: Methylenetetrahydrofolate reductase C677T polymorphism; Esophageal cancer; Iran

Introduction

Esophageal cancer is the eighth most common cancer worldwide and the 6th cause of cancer related death [1]. There is a geographic variation in incidence and even mortality of esophageal cancer with higher risk in eastern Asia and lower risk in Western Africa [2]. "Asian esophageal cancer belt" which extends from China to Middle East has an annual incidence of esophageal adenocarcinoma as high as 100 per 100000 populations [3]. In Iran, a higher incidence has been reported in areas around the Mazandaran Sea [4].

Histologically, squamous cell carcinoma (SCC) and adenocarcinoma are the two most common forms of esophageal cancer. Other rare forms of esophageal cancer include sarcoma, lymphoma and carcinoids [5]. While adenocarcinoma is associated with gastroesophageal reflux disease (GERD) and Barrett metaplasia, SCC risk factors include alcohol consumption, cigarette smoking and ingestion of nitrites [6,7]. However, the role of genetic predisposition should be considered just like other cancers [8].

Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in folate metabolism which catalyzes conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which is involved in conversion of homocysteine to methionine [9]. Methionine

is involved in DNA methylation process in many types of neoplasms [10]. MTHFR C677T polymorphism results in C to T transition at nucleotide 677 and conversion of alanine to valine at position 222 of MTHFR amino acid sequence [11]. These alterations leads to decreased MTHFR activity and increased homocysteine level [11].

MTHFR C677T polymorphism has been reported to be associated in several types of cancers including lung, gastric and colorectal cancers [12-14]. Several studies have also investigated MTHFR C677T polymorphism in patients with esophageal cancer in different populations [15,16]. However, there is lack of data among Iranian population. This study aimed to investigate MTHFR C677T polymorphism in Iranian patients with esophageal cancer and its impact on outcome of patients.

Methods and Materials

A cross-sectional study was conducted among patients diagnosed with esophageal cancer in Imam Reza hospital between June 2007 and June 2014, Tehran, Iran. Patients with confirmed adenocarcinoma or SCC of esophagus in pathology specimen were included in the study. Patients' information were collected using a data gathering form containing data regarding, age, sex, histologic type of cancer, presenting signs and symptoms, family history of cancer, opium consumption, cigarette smoking, drinking hot tea, Barrette esophagus,

site of involvement (1/3 upper, middle or lower) and survival of patients.

Genotype study

Genomic DNA was extracted from pathology specimen using phenol and chloroform method and stored in -20°C for polymerase chain reaction (PCR) study. Genotyping was performed using restriction fragment length polymorphism (RFLP)-PCR method using Hinf-1 restriction endonuclease enzyme. The DNA segment was amplified from the forward, 5'- TGA AGG AGA AGG TGT CTG CGG GA-3' and reverse, 5'- AGG ACG GTG CGG TGA GAG TG -3' primers. The PCR reaction yielded 198-bp PCR products. The restriction enzyme reaction was performed using 10x Hinf I buffer R (2mL), 1mL Hinf I restriction endonuclease (10 U/mL), 10mL PCR reaction product, and 7 mL sterile dH₂O (total volume of 20 µL). The reaction mixture was incubated at 37°C for 14 hours. The restriction products and genotypes were determined using Hinf I digestion products, yielded by 2.0% agarose gel electrophoresis (Figure 1).

Dyspepsia	13	
Odynophagia	11	
Cigarette smoking	26	
Opium consumption	11	
Drinking hot tea	31	
Obesity	11	
Barrette esophagus	4	
Site of involvement	1/3 Upper	14
	1/3 Middle	8
	1/3 Lower	21

Table 1: Baseline characteristics of patients with esophageal cancer.

The frequencies of three genotypes of *MTHFR* gene in patients with esophageal cancer and controls were outlined in Table 2.

<i>MTHFR</i> genotype	Esophageal cancer	Controls
CC (genotype 1)	28	18
CT (genotype 2)	18	20
TT (genotype 3)	4	6

Table 2: Frequency of 3 genotypes of *MTHFR* gene in patients with esophageal cancer and controls.

The frequencies of various genotypes of *MTHFR* gene were not statistically significant in cases and controls (P=0.348). There were no statistically significant difference in frequency of C and T alleles in patients with esophageal cancer and controls (P=0.084) (Table 3).

Allele frequency	Esophageal cancer	Controls
C	56	74
T	32	26

Table 3: Frequency of C and T alleles of *MTHFR* gene in patients with esophageal cancer and controls.

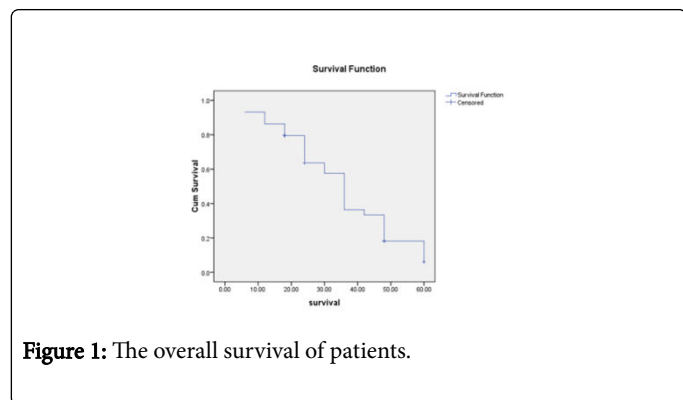


Figure 1: The overall survival of patients.

Statistical analysis was performed using SPSS software 16 (SPSS Inc, Chicago, IL, USA). Student T-test and Mann-Whitney tests were used for analysis when appropriate. A p<0.05 was considered statistically significant. The study protocol was confirmed by local Ethical committee of AJA University of Medical Sciences, Tehran, Iran.

Result

Forty four patients with esophageal cancer were included in the study. There were 17 males (38.63%) and 27 females (61.36%). Baseline characteristics of patients with esophageal cancer were outlined in Table 1. These were compared with 50 subjects (21 males and 29 females) without esophageal cancer as controls. There were no statistically significant difference in gender between cases and controls (P=0.452). Mean age of patients with esophageal cancer was 69.29 ± 10.76 years while mean age of control subjects was 56.02 ± 12.18 years (P=0.0001). In esophageal cancer group, 10 individuals had family history of cancer while none of the control subjects had such a history (P=0.0001).

Number	44
Mean age (year)	69.29 ± 10.76
Sex (male/female)	17/27
Family history of cancer	10
Dysphagia	42

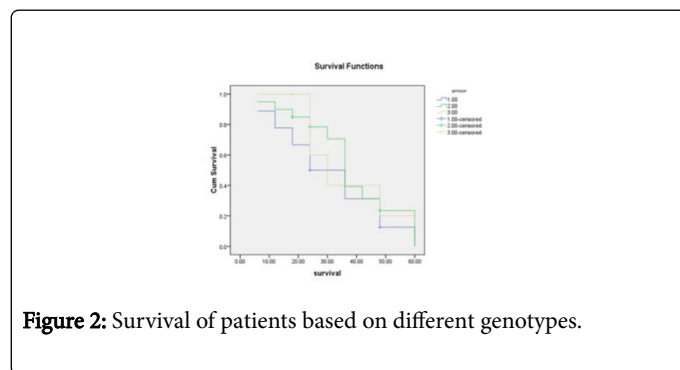
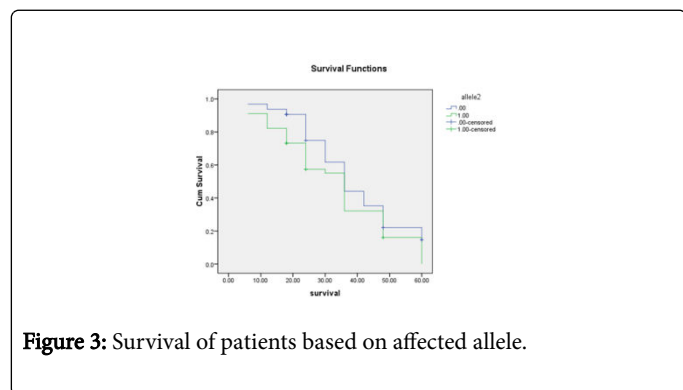


Figure 2: Survival of patients based on different genotypes.

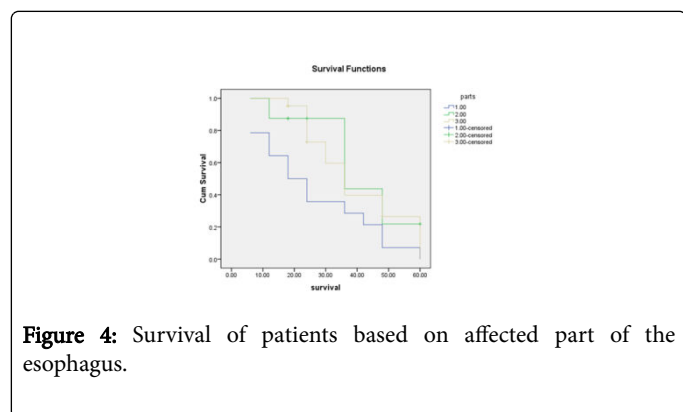
Figure 1 showed 1 to 5 year survival of patients with esophageal cancer.

Mean survival of patients with esophageal cancer was 31.25 ± 4.25 months in patients with CC genotype, 38.2 ± 4.11 months in CT genotype and 37.2 ± 6.44 months in patients with TT genotype ($P=0.459$) (Figure 2). Allele frequency was not also associated with mean survival in patients and controls ($P=0.168$) (Figure 3).



Mean survival of patients with esophageal cancer was significantly lower in those with involvement of cancer in upper third part of esophagus (25.71 ± 8.4 months) as compared with middle third part (40.87 ± 5.18 months) and lower third part (39.6 ± 3.71 months) ($P=0.042$) (Figure 4).

Patients were divided to <70 years ($n=23$) and >70 years ($n=21$) based on the age of cancer detection. Mean survival of patients in those who diagnosed with esophageal cancer less than 70 years was 38.22 ± 3.38 months while it was 31.14 ± 4.25 months in patients diagnosed after 70 years ($P=0.393$).



Discussion and Conclusion

Our findings failed to show any association between *MTHFR* gene polymorphism with esophageal cancer in Iranian population. Furthermore, neither C nor T alleles of *MTHFR* gene was associated with higher risk of esophageal cancer and did not impact on survival. Although patients with CC genotypes had lower mean survival in comparison with CT and TT genotypes, this difference was not reached statistical significance.

Since there is a great geographic variation in incidence of esophageal cancer, the role of genetic predisposition besides environmental factors should be investigated. In Iran *BRCA2* was the first gene found to be associated with esophageal cancer [17]. Other candidate genes confirmed to be involved in esophageal cancer in Iranian population were *ADH1B* and *CCND1* [18].

MTHFR gene is involved in folate metabolism and may affect theoretically risk of different types of cancers. *MTHFR* C677T polymorphism has been reported to increase the risk of gastric cancer in a cluster of Iranian population [19]. *MTHFR* CT+TT variant genotypes were strongly associated with microsatellite instability and colorectal cancer in Iranian population [20]. However, some other studies among Iranian population showed that TT genotype and T allele of *MTHFR* gene may be protective against gastric [21] and colorectal cancer [22]. Our study is in consistent with the only previous Iranian study that failed to show any association between *MTHFR* C677T polymorphism and SCC of esophagus in patients of a high risk Iranian region [18]. Like our findings, Song et al. could not find significant interaction of *MTHFR* CT genotype and esophageal cancer [23]. Despite our findings, several studies from different parts of the world confirmed the role of *MTHFR* C677T polymorphism in esophageal cancer. Wang et al. found increased risk of SCC of esophagus with *MTHFR* C677T variants [24]. A recent meta-analysis also showed a significant association between *MTHFR* 677 TT genotype and incidence of esophageal cancer [25]. This may refer to the folate status and *MTHFR* C677T polymorphism. Some nutrients riboflavin and methionine are involved in the folate metabolic pathway. Flavin adenine dinucleotide (FAD), a phosphorylated form of riboflavin, acts as a cofactor for *MTHFR*. Increased levels of riboflavin significantly decrease serum homocysteine in subjects carrying the *MTHFR* 677 TT genotype but not in subjects with other *MTHFR* 677 genotypes [26, 27].

Patients with esophageal cancer are usually asymptomatic until more than 2/3 of esophageal lumen is occluded by tumor. Subsequently, many of them are detected in advanced stages and long term survival is low despite aggressive treatment. Therefore, finding individuals that are genetically susceptible to this fatal cancer may lead to early surveillance and screening and result in better outcome after treatments. Further researches with larger sample size may be necessary to further investigate this issue.

Conflict of Interest

There is no conflict of interest.

Funding Source

This study was supported by a grant from AJA University of Medical Sciences, Tehran, Iran.

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