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Original Research Article

Genetic Variants of Methylenetetrahydrofolate Reductase (MTHFR) in Head and Neck Cancer

B. Papa Kusuma[1*], V. Lakshmi Kalpana[2], A. Anuradha[3], H. Uma Bharathi[1]

[1]Research Scholars, Department of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh, India
[2]Associate Professor, Department of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh, India
[3]Women Scientist -B, Department of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh, India

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***Corresponding author:** B. Papa Kusuma

Abstract

Background: Head and neck squamous cell cancer" (HNSCC) is a term collectively describes the malignant tumours of oral cavity, pharynx and larynx. Globally, the total burden of HNSCC is estimated to be around 22 millions and males outnumber females by 4.1 to 2. Folate Metabolic pathway is complex and crucial process that contributes to folate levels and DNA methylation. The genes behind the enzymes in the pathway are polymorphic in nature and a specific combination of genetic variants may implicates differential susceptibility to different cancers. Methylenetetrahydrofolate reductase (MTHFR), a key enzyme for intracellular folate homeostasis and metabolism provides methyl groups for the methylation of homocysteine to methionine**.** ***Aim:*** The present study aimed to identify genetic variants of MTHFR C677T (rs1801133) and MTHFR A1298C (rs1801131) among HNSCC patients and controls, gene – environment interactions in North Coastal Andhra Pradesh. ***Methods:*** A total of 220 samples (110 HNSCC patients and 110 controls) were included in the study and genotyping was accomplished by using PCR - RFLP technique and analyzed by SPSS. ***Results:*** The chi square p values revealed that MTHFR (C677T) polymorphism has association with HNSCC and MTHFR (A1298C) polymorphism does not shows association with HNSCC. For MTHFR (C677T) polymorphism, the odds ratio p value of TT genotype and CC genotype of MTHFR (A1298C) was found to be statistically significant. ***Conclusions:*** In conclusion, the MTHFR C677T (rs1801133) polymorphism shows association and A1298C (rs1801131) polymorphism does not shows association with HNSCC.

Key words: Head and neck squamous cell cancer, Methylenetetrahydrofolate reductase, Gene Polymorphism, Restriction Fragment Length Polymorphism.

INTRODUCTION

Head and neck cancer is identified by diverse group of malignant tumors that can develop in or all around the mouth, throat, nose and sinuses [1]. Head and neck cancer may collectively termed as malignant tumours of different etiologies that develops mainly from the surface layers of upper aero digestive tract (UADT). Upper aerodigestive tract is comprised of larynx, pharynx and nasopharynx and mouth [2, 3].

Squamous cell carcinomas circumscribe over 90% of all head and neck cancer because of the involvement of mucus linings of UADT **[**4, 5]. Head and neck cancers represent the sixth most common cancer worldwide with approximately 630,000 new patients diagnosed annually resulting in more than 350,000 deaths every year **[**6]**.** Squamous cell cancer of the head and neck is one of the most common cancers worldwide, with occurrence of more than 30 per 100000 population in India (oral cancer) and in France and Hong Kong (nasopharyngeal cancer). It constitutes about 4% of all cancers in the United States and 5% in the United Kingdom.

Epidemiologic studies have indicated a multifactor etiology for this cancer; these predisposing factors include alcohol use, smoking, human papilloma virus (HPV) infection, and genetic factors [7-11]. There is a geographic difference in the prevalence of cancer of the head and neck among different countries of the world and among different regions within a country (Hakulinen et al., 1986). These specify that environmental factors may play an essential role in the pathogenesis of HNSCC.

For all types of head and neck cancer, like most other cancers, risk increases with age. Most cases

of head and neck cancer are found in people 50 years or older. In Europe, in 98% of the patients are over 40 years of age [13]. Head and neck cancer is more common in men, with 66% to 95% of cases by site. Particularly in the developed countries men are affected more often than women by 10:1 [14].

Folate is important in deoxynucleoside synthesis to provide methyl groups and in intracellular methylation reactions [15]. Low folate levels can result in uracil misincorporation during DNA synthesis, leading to chromosomal damage, breaks in DNA strands, impaired DNA repair, and DNA hypomethylation [16]. Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate metabolism. Epidemiological evidence indicates that the genetic variants encoding the enzymes involved in folate metabolism may increase the risk of HNSCC by altering DNA methylation synthesis and genomic stability. Genetic mutations in MTHFR gene alter folate level and DNA methylation that may provide to hereditary diseases and cancer development [17]. Genetic polymorphisms leading to folate deficiency appear to facilitate the onset and growth of head and neck squamous cell carcinoma and other types of cancers.

Folate is part of DNA methylation, in which methyl groups (CH3) are transferred to the 5‟ position of cytosine residues on cytosine-guanine (CpG) dinucleotides in reactions catalyzed by proteins (DNA methyltransferases). Due to its role in nucleotide synthesis, the enzyme that converts folate into its active form of methylene tetrahydrofolate reductase (MTHFR), may have a role in carcinogenesis, as variants with different activities can alter purine metabolism and patient prognosis [18]. The methylene tetrahydrofolate reductase (MTHFR) polymorphisms C677T and A1298C have reduced enzymatic activity, altering folate bioavailability and purine metabolism [19] and the reduced enzyme activity may cause uncontrolled gene expression, genomic instability and induce carcinogenesis [20].

Based on the above evidence, the present study aimed to identify genetic variants of MTHFR C677T (rs1801133) and MTHFR A1298C (rs1801131) among HNSCC patients and controls and interaction of different known risk factors with these genetic variants has any impact on HNSCC.

MATERIALS AND METHODS

The present retrospective case control study was carried out with 110 head and neck cancer patients (72 males and 38 females) from Pinnacle cancer hospital and Mahatma Gandhi cancer hospital and Research Institute, Visakhapatnam and 110 age and sex matched controls (70 males and 40 females) above 30 years from North Coastal Andhra Pradesh during the period 2017-2018. The study was approved by the Institutional Ethics Committee for blood sample collection from the patients. The informed consent was obtained from each and every participant before collecting blood sample for the evaluation of MTHFR (C677T) and MTHFR (A1298C) polymorphisms.

MOLECULAR ANALYSIS

DNA extraction:

The genomic DNA was extracted from peripheral blood by using salting out method. Genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique.

Genotyping of MTHFR C677T (rs1801133)

One set of forward "5'-TGAAGGAGAAGGTGTGCGGGA-3' and reverse: 5'-AGGA CGGTGCGGTGAGAGTG-3" primers were used for amplification of fragments of 198 base pairs and then amplified fragments was digested with HInfi enzyme. The PCR profile was: initial denaturation at 94°C, 5 minutes, And denaturation: 94°C, 1 minute, annealing: 62°C, 1 minute followed by 30 cycles each of 1 minute, and 72°C, then final extension at 72°C for 5 minutes. The amplification product was visualized in a 2% agarose gel under UV light.

The CC (homozygous type allele) genotype produces a single band of 198bp and CT (heterozygous type allele) genotype produces three bands of 198, 175 and 23bp. The TT (homozygous mutant type allele) genotype produces two bands of 175 and 23bp.

Genotyping of MTHFR A1298C (rs1801131)

One set of forward 5'-CTTTGGGGAGCTGAAGGACTAC- 3'and reverse 5'-ACTTTGTGACCATTCCGGTTTG -3'primers were used for amplification of fragments of 163 base pairs and then amplified fragments was digested with Mbo II enzyme. The PCR profile was: initial denaturation at 94°C, 5 minutes, And denaturation: 94°C, 1 minute, annealing: 62°C, 1 minute followed by 30 cycles each of 1 minute, and 72°C, then final extension at 72°C for 5 minutes. The amplification product was visualized in a 2% agarose gel under UV light.

The AA (homozygous type allele) genotype produces a single band of 250bp and AC (heterozygous type allele) genotype produces three bands of 250, 163 and 87. The CC (homozygous mutant type allele) genotype produces two bands of 163 and 87 bp.

STATISTICAL ANALYSIS

The data was analyzed by using Statistical Package of Social Sciences Software program (SPSS) 19 was used for calculating genotype and allele frequencies. Chi-square analysis was used to test for allele frequencies and odds ratio analysis was used to test for genotype frequencies in comparison of patients

and healthy control groups. P value less than 0.05 considered statistically significant.

RESULTS

Table-1: Genotype and allele frequencies of MTHFR (C677T) (rs1801133) and MTHFR (A1298C) (rs1801131) polymorphism in HNSCC patients and controls

	Genotype and alleles	HNSCC patients N=110 (%)	HNSCC Controls N=110 (%)	Odds ratio	95%CI	P-value
MTHFR (C677T)	CC	37 (33.63%)	59 (53.63%)	1	_	_
	CT	54 (49.09%)	39 (35.45%)	0.874	0.380 – 2.009	0.752
	TT	19 (17.27%)	12 (10.90%)	2.524	1.099 – 5.798	0.029**
	C	128 (58.18%)	157 (71.36%)			
	T	92 (41.81%)	63 (28.63%)	8.377		0.003**
MTHFR(A1298C)	AA	44 (40%)	49 (44.54%)	1	_	_
	AC	47 (42.72%)	54 (49.9%)	0.320	0.123 – 0.829	0.019**
	CC	19 (17. 27%)	7 (6.36%)	3.022	1.103 – 7.874	0.023*
	A	135 (61.36%)	152 (69.09%)			
	C	85 (38.63%)	68 (30.90%)	2.896		0.088

Table 1 describes the genotype frequencies of MTHFR C677T in HNSCC patients and controls. The genotype frequencies of CC was higher in controls (53.63%) than HNSCC patients (33.63%), whereas the genotype frequencies of CT (49.09%) and TT (17.27%) were higher in HNSCC patients than genotype frequencies of CT (35.45%) and TT (10.90%) in controls. The odds ratio of TT genotype was found to be showing higher risk when compared to CC and CT genotypes. The odds ratio p values of genotypes CC and TT were statistically significant whereas CT genotype was found to be statistically insignificant.

The frequency of C allele was (58.18%) in HNSCC patients and (71.36%) in controls. The T allele frequencies in HNSCC patients and controls were (41.81%) and (28.63%) respectively. The chi-square p value reveals that MTHFR C677T gene shows association with HNSCC.

The genotype frequencies of MTHFR A1298C in HNSCC patients and controls were represented in table 1. The genotype frequencies of AA (44.54%) and AC (49.9%) were higher in controls than genotype frequencies of AA (40%) and AC (42.72%) of HNSCC patients, whereas the genotype frequency of CC (17.27%) was higher in HNSCC patients than controls (6.36%). The odds ratio of CC genotype was found to be showing higher risk when compared to AA and AC genotypes. The odds ratio p values of genotypes AC and CC were statistically significant whereas AA genotype was found to be statistically insignificant.

The frequency of an allele was (61.36%) in HNSCC patients and (69.09%) in controls. The C allele frequencies of HNSCC patients and controls was (38.63%) and (30.90%) respectively. The chi-square p value reveal that MTHFR A1298C gene does not shows association with HNSCC.

Table-2: Distribution of MTHFR C677T (rs1801133) genotype frequencies in relation to demographic variables in HNSCC patients and controls

Variables	MTHFR C677T genotype	HNSCC patients N= 110 (%)	Controls N= 110 (%)	Chi – Square P- value
Smoking	CC	26 (23.63%)	02 (1.81%)	0.019*
	CT	45 (40.90%)	11 (10%)	
	TT	12 (10.90%)	08 (7.27%)	
Non smoking	CC	11 (10%)	57 (51.81%)	0.002**
	CT	09 (8.18%)	28 (25.42%)	
	TT	07 (6.36%)	04 (3.63%)	
Tobacco chewing	CC	20 (18.18%)	09 (8.18%)	0.209
	CT	43 (39.09%)	11 (10%)	
	TT	14 (12.72%)	09 (8.18%)	
Tobacco non chewing	CC	17 (15.45%)	50 (45.45%)	0.090
	CT	11 (10%)	28 (25.45%)	
	TT	05 (4.54%)	03 (2.72%)	
Alcohol consumption	CC	13 (11.81%)	23 (20.90%)	0.000***
	CT	42 (38.18%)	08 (7.27%)	
	TT	17 (16.45%)	07 (6.36%)	
Alcohol non consumption	CC	24 (21.81%)	36 (32.72%)	0.419
	CT	12(10.09%)	31 (28.18%)	
	TT	02(1.81%)	05 (4.54%)	

Table 2 shows the distribution of MTHFR C677T genotype frequencies in relation to demographic variables in HNSCC patients and controls. In smoking, the genotype frequencies of HNSCC patients were CC (23.63%), CT (40.90%) and TT (10.90%) and in controls it was CC (1.81%), CT (10%) and TT (7.27%), whereas in nonsmoking, the genotype frequencies of HNSCC patients were CC (10%), CT (8.18%) and TT (6.36%) and in controls it was CC (51.81%), CT(25.42%) and TT (3.63%).

In tobacco chewing, the genotype frequencies of HNSCC patients were CC (18.18%), CT (39.09%) and TT (12.72%) and in controls it was CC (8.18%), CT (10%) and TT (8.18%), where as in tobacco non chewing, the genotype frequencies of HNSCC patients were CC (15.45%), CT (10%) and TT (4.54%) and in controls it was CC (45.45%), CT (25.45%) and TT (2.72%).

In alcohol consumption, the genotype frequencies of HNSCC patients were CC (11.81%), CT(38.18%) and TT (16.45%) and in controls it was CC (20.90%), CT (7.27%) and TT (6.36%), whereas in alcohol non consumption, the genotype frequencies of HNSCC patients were CC (21.81%), CT (10.09%) and TT (1.81%)and in controls it was CC (32.72%), CT (28.18%) and TT (4.54%).

The chi-square p value shows that smoking, non-smoking and alcohol consumption variables were found to be significantly associated with MTHFR C677T gene polymorphism.

Table-3: Distribution of MTHFR A1298C (rs1801131) genotype frequencies in relation to demographic variables in HNSCC patients and controls

Variables	MTHFR A1298C genotype	HNSCC patients N= 110 (%)	Controls N= 110 (%)	Chi Square P- value
Smoking	AA	34 (30.90%)	7 (6.36%)	0.642
	AC	38 (34.54%)	12 (10.90%)	
	CC	11 (10%)	02 (1.81%)	
Non smoking	AA	10 (9.09%)	42 (38.18%)	0.002**
	AC	09 (8.18%)	42 (38.18%)	
	CC	08 (7.27%)	05 (4.54%)	
Tobacco chewing	AA	25 (22.72%)	11 (10%)	0.846
	AC	39 (35.45%)	14 (12.72%)	
	CC	13 (11.81%)	04 (3.63%)	
Tobacco non chewing	AA	19 (17.27%)	38 (34.54%)	0.005**
	AC	08 (7.27%)	40 (36.36%)	
	CC	06 (5.45%)	03 (2.72%)	
Alcohol consumption	AA	26 (23.63%)	07 (6.36%)	0.629
	AC	34 (30.90%)	15 (13.63%)	
	CC	12 (10.90%)	05 (4.54%)	
Alcohol non consumption	AA	18 (16.36%)	42 (38.18%)	0.006**
	AC	13 (11.81%)	39 (35.45%)	
	CC	07 (6.36%)	02 (1.81%)	

Table 3 shows the distribution of MTHFR A1298C genotype frequencies in relation to demographic variables in HNSCC patients and controls. In smoking, the genotype frequencies of HNSCC patients were AA (30.90%), AC (34.54%) and CC(10%) and in controls it was AA(6.36%), AC(10.90%) and CC(1.81%), whereas in non-smoking, the genotype frequencies of HNSCC patients were AA (9.09%), AC(8.18%) and CC (7.27%) and in controls it was AA (38.18%), AC(38..18%) and CC(4..54%).

In tobacco chewing, the genotype frequencies of HNSCC patients were AA (22.72%), AC (35.45%) and CC (11.81%) and in controls it was AA (10%), AC(12.72%) and CC (3.63%), whereas in tobacco non chewing, the genotype frequencies of HNSCC patients were AA (17.27%), AC (7.27%) and CC (5.45%) and in controls it was AA (34.54%), AC (36.36%) and CC (2.72%).

In alcohol consumption, the genotype frequencies of HNSCC patients were AA (23.63%), AC(30.90%) and CC (10.90%) and in controls it was AA (6.36%), AC (13.63%) and CC (4.54%), whereas in alcohol non consumption, the genotype frequencies of HNSCC patients were AA (16.36%), AC(11.81%) and CC (6.36%) and in controls it was AA (38.18%), AC (35.45%) and CC (1.81%).

The chi-square p value shows that nonsmoking, tobacco none chewing and alcohol non consumption variables were found to be significantly associated with MTHFR A1298C gene polymorphism.

Table-4: MTHFR C677T (rs1801133) polymorphism and its interaction with MTHFR A1298C (rs1801131) gene polymorphism in HNSCC patients and controls MTHFR C677T CC v/s

Gene	Genotypes	HNSCC patients N=110	Control N=110	Odds ratio	95% CI	p- value
MTHFR (A1298C)	AA	25	27	1	-	-
	AC	23	29	0.226	0.042 – 1.196	0.080
	CC	07	02	3.780	0.716 – 19.939	0.117

MTHFR C677T CT+ TT v/s

MTHFR (A1298C)	AA	19	22	1	-	-
	AC	24	25	0.400	0.122 – 1.307	0.129
	CC	12	05	2.778	0.828 – 9.323	0.097

Table 4 represents the MTHFR C677T polymorphism and its interaction with MTHFR A1298C polymorphism in HNSCC patients and controls. From the above it is evident, that the CC genotype of MTHFR C677T polymorphism was statistically insignificant with AA, AC and CC genotypes of MTHFR A1298C polymorphism.

The CT and TT genotypes of MTHFR C677T polymorphism was statistically insignificant with AA, AC and CC genotypes of MTHFR A1298C polymorphism.

Table-5: MTHFR polymorphisms in head and neck cancer

Study groups	Geographical region	MTHFR			
		C677T		A1298C	
		Significant	Non significant	Significant	Non significant
Vairaktaris et al., 2006	Greece (Athens)	✓		Ǿ	
Suzuki et al., 2007	Japan (Nagoya)	✓		Ǿ	
Ni et al., 2008	China (Beijing)	✓			✓
Siraj et al., 2008	Saudi Arabia (Riyadh)	✓		✓	
Solomon et al., 2008	India (Madurai)	✓		Ǿ	
Boccia et al., 2009	Italy (Rome)	✓		✓	
Cao et al., 2010	China (Guangzhou)	✓		✓	
Krsuszyna et al., 2010	Italy (Rome)		✓		✓
Rodrigues et al., 2010	Brazil		✓	Ǿ	
Fard-Esfahani et al.,2011	Iran (Tehran)	✓		Ǿ	
Sailasree et al., 2011	India (Kerala)	✓		Ǿ	
Supic et al., 2011	Serbia (Belgrade)	✓		Ǿ	
Prasad et al.,2011	India (Hyderabad)		✓	Ǿ	
Tsai et al., 2011	Taiwan	✓		Ǿ	
Galbiatti et al., 2012	Brazil (Sao Paulo)	✓		✓	
Present study, 2019	India (Visakhapatnam)	✓			✓

Ǿ - Unstudied

(Table from Muzeyygen Izmirli, 2013)

DISCUSSION

The etiology of cancer is not well understood, but research has linked risk to certain genes. MTHFR is one such gene, but this association requires consideration of environmental factors such as geographical region. The studies identified in the table 5**,** provide a snapshot of investigations associated with C677T and A1298C polymorphisms of the MTHFR gene.

To date, large numbers of studies have evaluated the association between the MTHFR C677T polymorphism and cancer risks, but the results are incompatible. The MTHFR C677T variant is a possible risk factor of pancreatic [21] esophageal [22] and breast cancers [23].

In the present study**,** the chi-square p value reveals that MTHFR C677T gene shows association with HNSCC. The odds ratio p values of genotypes CC and TT were statistically significant whereas CT genotype was found to be statistically insignificant.

Upto now [24] have been conducted four studies for examining the two MTHFR polymorphisms, three in a high-risk area in China [25, 26] and one in Germany [27]. The Chinese studies reveal the MTHFR 677T allele to increase esophageal cancer risk [25, 26], while risk change was not observed among Caucasians

in Germany [27]. The results of [24], MTHFR genotype alone showed a tendency for lowered risk in individuals harboring the 677TT genotype. Regional dissimilarity in folate consumption among populations may be a possible explanation for this incompatibility [25].

The study of [28] found no association between MTHFR C677T polymorphism and HNSCC risk in a Puerto Rican population. Since then, many studies have evaluated this association, but have obtained inconsistent results.

The study of [29] reveals that the MTHFR C677T genotype showed a non-significantly reduced risk of HNSCC among subjects with an adequate intake of folate. This trend is consistent with previous studies [30, 31, 32].

The studies of [33, 34, 35] did not observe an association between MTHFR C677T polymorphic variant with the HNSCC cases. The overall data of the [36] meta-analysis did not divulge an association of MTHFR C677T polymorphism with HNSCC risk, the subgroup analysis suggested that MTHFR C677T alleles might increase HNSCC risk in individuals who have a heavy drinking history.

For potential association of MTHFR C677T gene polymorphism with HNSCC, a number of studies have been carried out [28, 37, 38, 39, 40]. Previous studies reported the association of MTHFR C677T genotype combination as a risk factor for HNSCC in the South Indian population [37] and non-Hispanic whites [39, 41].

In the present study**,** the chi-square p value reveal that MTHFR A1298C gene does not shows association with HNSCC. The odds ratio p values of genotypes AC and CC were statistically significant.

The correlations between A1298C mutation and head and neck cancer risk have been studied, but the results remain controversial. The studies of [33, 34] did not find an association between MTHFR A1298C polymorphic variant with the HNSCC cases.

In the study of [42] in Brazilian, the dominant model also indicated increased risk for head and neck cancer with age over 49 years, tobacco and alcohol habits, especially in oral cancer. But the other studies, included [43-48], did not find any significant association between MTHFR A1298C polymorphism and head and neck cancer risk.

The case control study of [33] provide evidences that MTHFR A1298C polymorphism, MTHFR combined genotypes (at least 2 risk alleles) significantly contribute to increased risk for HNSCC.

CONCLUSIONS

The study population found significant association only with the MTHFR C677T (rs1801133) but not with MTHFR A1298C (rs1801131) variant. Although, genetic and environmental risk factors are experientially recognized as sharing a role in increasing the risk for certain types of cancers, there is very limited data on gene-environment interactions. The present study concludes that the risk factors and genes confer risk individually and found statistically insignificant interaction between gene polymorphisms and environment. Also gene-gene interaction has not been observed in the etiology of HNSCC.

Since the International Human Genome Sequencing Project and the International HapMap Project have generated a very large amount of data on the location, quantity, type, and frequency of genetic variants in the human genome, large-scale case-control studies are required to demonstrate not only the candidate genetic variants association with cancer risk but also the potential gene-environment and gene-gene interactions between gene polymorphisms and head and neck cancer.

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