Lidia Maria Rebolho Batista da Silva

VARIANTES GENÉTICAS ENVOLVIDAS NO METABOLISMO DO FOLATO: IMPACTO NA CARCINOGÊNESE DE CABEÇA E PESCOÇO

Dissertação apresentada à Faculdade de Medicina de São José do Rio Preto para obtenção do Título de Mestre no Curso de Pós-graduação em Ciências da Saúde, Área de Concentração: Medicina e Ciências Correlatas.

Orientadora: Prof[a]. Dr[a]. Eny Maria Goloni-Bertollo

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São José do Rio Preto, 07/10/2010.

Sumário

Dedicatória

À minha mãe Marina

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Epígrafe

"Para adquirir conhecimento é preciso estudar, mas para adquirir sabedoria é preciso observar" Marilyn vos Savant

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5-MTHF	5-metiltetrahidrofolato (5-*methyltetrahydrofolate*)
5,10-MTHF	5,10-metilenotetrahidrofolato (*5,10-methylenetetrahydrofolate*)
BHMT	Betaína-homocisteína metiltransferase (*Betaine-homocysteine methyltransferase)*
Cb	Cobalamina
CβS	Cistationina β-sintase (*Cystathionine β-synthase*)
CCP	Câncer de cabeça e pescoço
CEP	*Research Ethics Committee*
CH_3	Metil (*Methyl*)
CI 95%	*Confidence interval*
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico (*National Council for Scientific and Technological Development*)
CONEP	Comitê Nacional de Pesquisa (*National Research Commission*)
DHFR	Dihidrofolato redutase (*Dihydrofolate reductase*)
DNA	Ácido desoxirribonucléico (*Desoxirribonucleic acid*)
dTMP	Timidina monofosfato (*Deoxythymidine monophosphate*)
dUMP	Deoxiuridina monofosfato (*Deoxyuridine monophosphate*)
FAMERP	Faculdade de Medicina de São José do Rio Preto (*São José do Rio Preto Medical School*)

FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo (*São Paulo State Research Foundation*)
FUNFARME	Fundação Faculdade Regional de Medicina de São José do Rio Preto
HB	Hospital de Base
Hcy	Homocisteína (*Homocysteine*)
HNC	*Head and neck câncer*
HNSCC	*Head and neck squamous cell carcinoma*
HPV	*Human Papiloma Virus*
INCA	Instituto Nacional do Câncer (*Brazilian National Cancer Institute*)
L-MM-Coa mutase	L-metilmalonil coenzima A mutase (*L-methylmalonyl coenzyme A mutase*)
M	Metástase á distância
MMA	Ácido metilmalônico (*Methylmalonic acid)*
MTHF	Metilenotetrahidrofolato (*Methylenetetrahidrofolate*)
MTHFD1	Metilenotetrahidrofolato desidrogenase 1 (*Methylenetetrahydrofolate dehidrogenase 1*)
MTHFR	Metilenotetrahidrofolato redutase (*Methylenetetrahydrofolate reductase*)
MTR	Metionina sintase (*Methionine synthase*)

MTRR	Metionina sintase redutase (*Methionine synthase reductase*)
N	Envolvimento de linfonodos
OR	*Odds ratio*
PB	Pares de base
PCR	Reação em Cadeia da Polimerase (*Polymerase chain reaction*)
PCR-RFLP	*Polymerase chain reaction-restriction fragment length polymorphism*
RFC1	Carregador de folato reduzido 1 (*Reduced folate carrier 1*)
RNA	Ácido ribonucléico (*Ribonucleic acid)*
SAH	S-adenosilhomocisteína (*S-adenosylhomocysteine*)
SAM	S-adenosilmetionina (*S-adenosylmethionine*)
SHMT	Serina hidroximetiltransferase (*Serine Hydroxymethyltransferase*)
SISNEP	Sistema Nacional de Informação sobre Ética em Pesquisa (*National Information System on Research Ethics*)
T	Tamanho do tumor
TC2	Transcobalamina 2 *(Transcobalamin 2)*
THF	Tetrahidrofolato *(Tetrahydrofolate)*
TNM	Classificação dos Tumores Malignos (*TNM classification*)
TYS	Timidilato sintase (*Thymidilate synthase*)
UICC	*International Union of Cancer Control*
UPGEM	Unidade de Pesquisa em Genética e Biologia Molecular (*Genetics and Molecular Biology Research Unit*)

RESUMO

Introdução: Câncer de cabeça e pescoço é um termo coletivo definido por bases anatômicas e topográficas para descrever tumores malignos do trato aerodigestivo superior. Esta região anatômica inclui a cavidade oral, faringe e laringe, tendo como principais fatores de risco o tabagismo e o etilismo. O tipo histológico mais representativo de todos os cânceres de cabeça e pescoço é o carcinoma espinocelular (HNSCC), com mais de 500 mil casos novos no mundo todos os anos. Deficiência de folato no organismo está associada ao aumento do risco de vários tipos de câncer e alterações neste metabolismo podem contribuir para o processo de carcinogênese por influenciar as reações de metilação do DNA e a estabilidade genômica. Polimorfismos em genes que codificam enzimas envolvidas no metabolismo do folato podem alterar a atividade enzimática e interferir nas concentrações de homocisteína, S-adenosilmetionina e outros produtos do metabolismo, importantes para a síntese de DNA e reações de metilação celular. **Objetivos:** Avaliar a influência dos polimorfismos *MTHFD1* G1958A, *BHMT* G742A, *TC2* C776G e *TC2* A67G em pacientes com carcinoma espinocelular de cabeça e pescoço e em indivíduos controle sem história da neoplasia, além de verificar a associação entre os polimorfismos e os sítios primários de ocorrência, extensão do tumor, comprometimento de linfonodos, e o prognóstico da doença. **Pacientes e Métodos:** Foram incluídos no estudo 694 indivíduos (240 pacientes com câncer de cabeça e pescoço e 454 controles). Foi feita análise molecular através de extração de DNA genômico de sangue periférico e as alterações genéticas foram investigadas por meio das técnicas de Reação em Cadeia da Polimerase (PCR) em tempo real e Análise de Polimorfismo de Comprimento de Fragmento de Restrição (PCR-RFLP). Os dados sócio-demográficos foram obtidos através do prontuário dos

pacientes e entrevista dos indivíduos controles. **Resultados:** Regressão logística múltipla mostrou que tabagismo, etilismo e idade superior a 42 anos foram preditores da doença (P<0,05). As distribuições genotípicas estiveram em equilíbrio de Hardy-Weinberg em ambos os grupos em todos os polimorfismos estudados. Os genótipos *MTHFD1* 1958GA ou AA associados ao tabagismo (P=0,04) e etilismo (P=0,03) aumentaram o risco de carcinoma espinocelular de cabeça e pescoço. Estes mesmos genótipos estiveram presentes em maior proporção em pacientes com tumores em estadios mais avançados T3 e T4 (P=0,04) e em pacientes com menor sobrevida (P=0,01). O polimorfismo *TC2* C776G (P=0.03) esteve presente em menor frequência em pacientes com idade superior a 52 anos e o polimorfismo *TC2* C776G (P=0.03) em pacientes com idade entre 52-63 anos. O polimorfismo *TC2* C776G não foi relacionado ao risco da doença, porém esteve presente em alta proporção em pacientes que tiveram a faringe como sítio primário de ocorrência do tumor. **Conclusões:** São preditores para o câncer de cabeça e pescoço, independentemente da variável genética o uso de tabaco, álcool e idade superior a 42 anos. A presença do polimorfismo *MTHFD1* G1958A associado aos hábitos tabagista e etilista podem modular o risco para o desenvolvimento da doença.

Palavras chave: Polimorfismo genético, câncer de cabeça e pescoço, genes *MTHFD1, BHMT* e *TC.*

ABSTRACT

Introduction: Head and neck cancer is a collective term defined by anatomical and topographical basis to describe malignant tumors of the upper aerodigestive tract. This anatomical region includes the oral cavity, pharynx and larynx, having as the main risk factors smoking and alcoholism. The most representative hystologic type from head and neck cancer was squamous cell carcinoma (HNSCC), with more than 500,000 new cases worldwide every year. Folate deficiency is associated with increased risk of several types of cancer and alterations in folate metabolism may contribute to the process of carcinogenesis by influencing DNA methylation and genomic stability. Polymorphisms in genes encoding enzymes involved in this pathway may alter enzyme activity and consequently interfere in concentrations of homocysteine and S-adenosylmethionine that are important for DNA synthesis and cellular methylation reactions. **Objectives:** Investigate *MTHFD1* G1958A, *BHMT* G742A, *TC2* C776G and *TC2* A67G polymorphisms involved in folate metabolism on head and neck cancer risk, and the association between these polymorphisms with primary site, tumor extension, lymph node involvement and prognosis of the disease. **Patients and Methods:** Were included in the study 694 individuals (240 patients with head and neck cancer and 454 controls). Molecular analysis was made by genomic DNA from peripheral blood and genetic alterations were investigated by Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP) and Real Time-PCR. Socio-demographic data were obtained from patient´s medical records and interview of the controls. **Results:** Multiple logistic regression showed that tobacco, alcohol and age over 42 years were predictors for the disease ($P<0.05$). Hardy-Weinberg equilibrium showed that the genotypic distributions were in equilibrium for both groups in all

polymorphisms studied. The *MTHFD1* 1958GA or AA genotypes associated with tobacco (P=0.04) and alcohol (P=0.03) consumption increase the risk for head and neck cancer (HNSCC). These same genotypes were found in higher proportion in patients with advanced stage tumors (P=0.04) and in patients with lower survival (P=0.01). *TC2* C776G polymorphism (P=0.03) were less frequent in patients with age over 52 years and *TC2* A67G polymorphism (P=0.04) were less frequent in patients with 52-63 years. *TC2* C776G polymorphism was not associated to HNC, however was present in higher proportion in patients with pharynx as primary site of tumor (P=0.02). **Conclusions:** Are predictors for head and neck cancer, regardless of the gene, tobacco and alcohol consumption and age over 42 years. The presence of *MTHFD1* G1958A polymorphism associated to tobacco and alcohol consumption may modulate the risk for disease development.

Key words: Genetic polymorphism; head and neck cancer; *MTHFD1*, *BHMT* and *TC2* genes.

1 INTRODUÇÃO

1. INTRODUÇÃO

"Câncer de cabeça e pescoço" é um termo coletivo definido por bases anatômicas e topográficas para descrever tumores malignos do trato aerodigestivo superior. Esta região anatômica inclui a cavidade oral, faringe e laringe. Cerca de 40% dos cânceres de cabeça e pescoço ocorrem na cavidade oral, 15% na faringe e 25% na laringe. [1-3] É considerado o quinto tipo mais comum no mundo, está associado à baixa taxa de sobrevivência e alta taxa de mortalidade, quando diagnosticado em estágios avançados. [4] O tabagismo e o etilismo são os principais fatores de risco estabelecidos para o câncer de cabeça e pescoço. [4-12]

No Brasil ocupa o 5º lugar entre todas as neoplasias, com estimativas de 14.120 casos novos para o câncer de cavidade oral no ano de 2010, sendo 10.330 para o gênero masculino e 3.790 para o gênero feminino. [3] O comportamento desta neoplasia é bastante agressivo, apresentando metastatização cervical e contralateral precoce e, sobretudo na orofaringe, e em nódulos linfáticos cruzam a linha média da região cervical. [5]

O tipo histológico mais representativo de todos os cânceres de cabeça e pescoço é o carcinoma espinocelular (HNSCC), com mais de 500 mil casos novos no mundo todos os anos, [9] desenvolve-se a partir de um epitélio sujeito a um campo de cancerização, com mais agressividade na laringe. [1,13] Os fatores que promovem este campo de cancerização incluem exposições ambientais ao tabaco e álcool, infecções virais, especialmente com o vírus Epstein-Barr e Papiloma Vírus Humano dos subtipos 16 e 18 e deficiências ou desequilíbrios de vitaminas e micronutrientes, tais como ácido fólico, vitaminas A, C, E, zinco e selênio. [14-17]

Deficiência de folato no organismo está associada ao aumento do risco de câncer de cólon, [14,18] colorretal, [19-21] mama, [19-24] pulmão, [19-21,25] ovário, [19-21] esôfago, [26] colo de útero, [26] orofaringe, [27-29] estômago, [27-29] pâncreas, [27-29] rins, [30] e cabeça e pescoço. [31] Dietas ricas em frutas e legumes, que são fontes de ácido fólico e outros nutrientes antimutagênicos, são fortes proteções contra a maioria dos tipos de cânceres.

O folato possui importante papel na oncologia, principalmente a partir de sua ação na metilação do DNA e na síntese de purinas e pirimidinas. [27] Alterações genéticas ou de deficiência dessa vitamina foram relacionadas ao câncer em vários estudos, incluindo o de cabeça e pescoço. [18,21,22,26,27,31-35]

Existem três mecanismos pelos quais as alterações no metabolismo do folato podem contribuir com a carcinogênese: (1) hipometilação de DNA e subsequente ativação dos proto-oncogenes; [27,36] (2) erro de incorporação da uracila durante a síntese de DNA que leva à instabilidade genômica; [14,27,36] e (3) um aumento na desaminação de citosina nos sítios de metilação de DNA. [14,27]

O folato está envolvido na formação de grupos metil (CH3) durante a interconversão de um carbono no metabolismo intermediário de S-adenosilmetionina (SAM), que serve como um doador de grupos metil nas reações de metilação celulares. [27,37-40] A metilação do DNA é a transferência de grupos metil para a posição 5 de resíduos de citosinas localizadas em dinucleotídeos citosina-guanina (CpG), por meio de reações catalisadas por proteínas denominadas DNA metiltransferases. [40,41] Esta modificação epigenética do DNA possui vários papéis funcionais, incluindo controle da expressão gênica, estabilidade da estrutura da cromatina e manutenção da estabilidade genômica. [27,38,41-46]

Níveis adequados de folato são essenciais para a biosíntese de purinas e pirimidinas, necessárias para a síntese e reparo do DNA. Portanto, alterações na via metabólica do folato estão associadas à redução na capacidade de reparo do DNA. A enzima Timidilato sintase catalisa a conversão de deoxiuridina (dUMP) a monofosfato de deoxitimidina (dTMP), utilizando 5,10 metilenotetrahidrofolato como doador de grupos metil. Em caso de deficiência de folato, o acúmulo de dUMP pode induzir a incorporação de uracila ao DNA ao invés de timina. As uracilas erroneamente incorporadas são removidas das fitas de DNA por enzimas da maquinaria de reparo, o que pode levar a quebras temporárias na molécula, posteriormente ligadas pela enzima DNA ligase. Entretanto, se a disponibilidade de folato é continuamente limitada, um ciclo de reparo descontrolado pode causar quebras frequêntes na molécula de DNA e danos cromossômicos, resultando em alteração celular maligna. [27,38,46-49] Outros nutrientes, como metionina, vitamina B_6 e vitamina B_{12}, que interagem com genes envolvidos no metabolismo do folato, contribuem para a síntese adequada de DNA, e também podem influenciar o risco de desenvolvimento do câncer. [14,27,37,38,46]

Alterações em genes que codificam enzimas envolvidas na via do folato têm sido investigadas como fatores de risco para susceptibilidade ao câncer, uma vez que podem interferir nas concentrações de Hcy e SAM. [32-35,50-52]

Na Figura 1 são apresentadas as enzimas que participam do metabolismo do folato. A enzima Metilenotetrahidrofolato redutase (MTHFR) catalisa a conversão do 5,10 metilenotetrahidrofolato para 5-metiltetrahidrofolato (5-MTHFR), a principal forma circulante de folato, que atua como doador de grupos metil para a remetilação da homocisteína (Hcy) para metionina. Esta reação de remetilação é catalisada pela enzima Metionina sintase (MTR), que requer a vitamina B_{12} (metilcobalamina) como cofator, e

resulta na formação de SAM. Participando também deste metabolismo, a enzima Cistationina β-sintase (CβS), dependente de vitamina B_6, desenvolve papel crucial no metabolismo do folato, convertendo a Hcy em cistationina na chamada via de transsulfuração. [53,54]

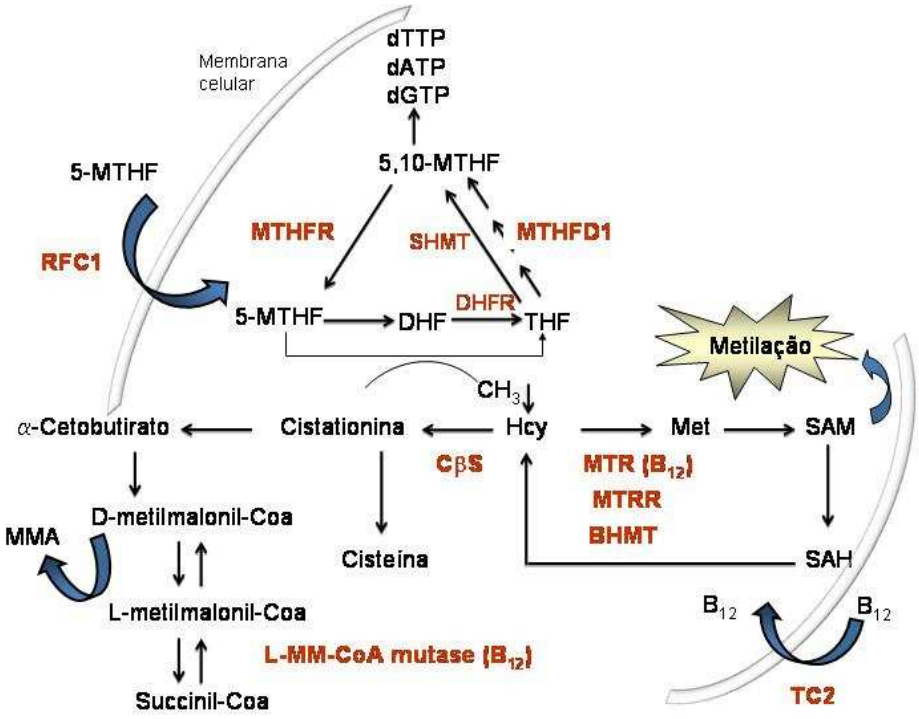

Figura 1. Esquema representando o metabolismo do folato com as principais enzimas envolvidas. BHMT = Betaína-homocisteína metiltransferase, CβS = Cistationina β- sintase, dATP = Desoxiadenosina 5'-trifosfato, dGTP = Desoxiguanosina 5'-trifosfato, DHFR = Dihidrofolato redutase, dTTP = Desoxitimidina 5'-trifosfato, CH_3 = Metil, 5-MTHF = 5-metiltetrahidrofolato, 5,10-MTHF = 5,10-metilenotetrahidrofolato, Hcy = Homocisteína, L-MM-Coa mutase = L-metilmalonil coenzima A mutase, MMA= Ácido metilmalônico, MTHFD1 = Metilenotetrahidrofolato desidrogenase 1, MTHFR = Metilenotetrahidrofolato redutase, MTR = Metionina sintase, MTRR = Metionina sinstase redutase, RFC1 = Carregador de folato reduzido 1, SAH = S-adenosil-homocisteína, SAM = S-adenosil-metionina, SHMT = Serina hidroximetiltransferase, TC2 = Transcobalamina 2 THF = Tetrahidrofolato.

1.1 Polimorfismos genéticos envolvidos no metabolismo do folato

O gene *Metilenotetrahidrofolato desidrogenesase 1* (*MTHFD1*) codifica uma proteína trifuncional cistólica, que compreende 5,10-metileno-THF dehidrogenase, 5,10-metenil-THF ciclohidrolase, e 10-formil-THF sintase. As enzimas metileno-THF desidrogenase e da metenil-THF ciclohidrolase, que residem no mesmo domínio da proteína, catalisam a oxidação do 5,10-metileno-THF a 5,10-metenil-THF, que é então convertido para 10-formil-THF. [55] Estas três reações sequenciais estão envolvidas na interconversão de derivados do carbono-1 do THF, que são substratos para a síntese de metionina, timidilato e purinas. [38,56]

O gene *MTHFD1* apresenta-se polimórfico no nucleotídeo 1958 (G→A) resultando na substituição de uma alanina por uma glicina no códon 653, localizado no domínio 10-formil-THF sintase da enzima. [38,57] Pacientes pediátricos com leucemia linfoblástica aguda, portadores dos alelos *MTHFD1* 1958A e timidilato sintase 2R (TS 2R), mostraram maior tempo de sobrevida quando tratados com MTX. [52,57]

Também envolvido no metabolismo do folato, o gene Betaína-homocisteína metiltransferase (*BHMT*) apresenta-se polimórfico no nucleotídeo 742 (G→A), levando à substituição de arginina por glutamina na proteína produzida. [58] A avaliação do impacto deste polimorfismo nas propriedades funcionais da proteína BHMT resultante da variante polimórfica, não mostrou diferença na termoestabilidade e atividade catalítica em relação à enzima do tipo selvagem. [35] Xu e colaboradores (2008)[59] associaram o alelo polimórfico 742A com a redução dos casos de morte por câncer de mama. Por outro lado, Koushik e colaboradores (2006)[60] observaram um aumento do risco de desenvolvimento de câncer colorretal para portadores dos genótipos variantes

(742G/A e 742A/A) em relação aos portadores do genótipo homozigoto selvagem (742G/G).

Vitamina B_{12} (cobalamina) e vitamina B_6 são nutrientes essenciais para o metabolismo do folato, uma vez que atuam como co-fatores de algumas enzimas envolvidas. A cobalamina (Cbl) possui um papel importante em 2 reações metabólicas: (1) conversão de L-metilmalonil-CoA a succinil-CoA e (2) remetilação da homocisteína para metionina. Assim, o transporte deste nutriente para a célula é importante para a manutenção do status de vitamina B_{12} intracelular. [61,62]

Para a absorção celular de cobalamina é necessário que esta esteja ligada a uma proteína carregadora, a trancobalamina 2 (TC2). [61,62] A proteína TC2 é sintetizada no endotélio vascular da vilosidade intestinal e liga-se à vitamina B_{12} livre no fluido intersticial. A proteína TC2 ligada à vitamina B_{12} (complexo TC2-vitamina B_{12}) passa, então, a microcirculação da vilosidade intestinal e, por meio da veia portal, alcança a circulação sistêmica. [63]

A presença de polimorfismos no gene *TC2* pode influenciar a quantidade de vitamina B_{12} disponível no organismo. Estudos anteriores sugerem que um polimorfismo no nucleotídeo 776 (C→G) do gene *TC2* pode levar a alterações da proteína, influenciando sua afinidade e capacidade de transporte da cobalamina aos tecidos. [61,62] Concentração do complexo TC2-vitamina B_{12} significantemente mais alta foi observada na presença do polimorfismo *TC2* C776G em homozigose para o alelo selvagem (776G/G). Além disso, concentrações médias de ácido metilmalônico (MMA), um indicador do *status* de vitamina B_{12} [48] foram significantemente mais baixas na presença dos genótipos TC2 776C/C e 776C/G em relação ao genótipo 776G/G. [47]

O polimorfismo C776G leva a substituição de uma prolina por uma arginina no códon 259 (P259R) da transcobalamina. O polimorfismo C776G, também denominado de P259R (nomenclatura segundo alteração na proteína), altera a concentração plasmática da transcobalamina 2 livre (apo-TC2). Indivíduos portadores do genótipo PP apresentam maior concentração de apo-TC2 em comparação aos indivíduos com genótipos RR. Segundo Namour e colaboradores (2001), [64] a presença do alelo polimórfico em homozigose pode interferir na disponibilidade de Cbl intracelular e consequentemente no metabolismo da homocisteína.

O gene *TC2* também apresenta-se polimórfico no nucleotídeo 67 (A→G), localizado no éxon 2, resultando na substituição de uma isoleucina por uma valina no códon 23 (I23V). [61] Este polimorfismo foi associado com concentrações mais baixas da proteína produzida na presença do genótipo heterozigoto *TC2* 67A/G quando comparado ao genótipo tipo selvagem 67A/A, [61] entretanto, este genótipo também foi associado à proporção maior da proteína produzida ligada à vitamina B_{12}.

Ainda não existem estudos que relacionam os polimorfismos do gene *TC2* ao câncer; porém estudo de Biselli e colaboradores (2008)[65] suportam evidências da relação entre esta variante genética em mães e a incidência de crianças portadoras de síndrome de Down. Este polimorfismo também foi associado à formação de aneurismas intracraniais. [66]

Dessa forma, o estudo de alterações em genes que participam do metabolismo do folato poderia auxiliar no esclarecimento dos processos que levam ao desenvolvimento de tumores, principalmente o câncer de cabeça e pescoço, colaborando na busca de novas estratégias de tratamento e prognóstico.

1.2 Objetivos

Considerando as evidências apresentadas, este estudo teve como objetivos:

1. Avaliar a associação dos polimorfismos *MTHFD1* G1958A, *BHMT* G742A, *TC2* A67G e *TC2* C776G no risco do câncer de cabeça e pescoço, em um estudo caso-controle.

2. Avaliar a associação dos polimorfismos com os hábitos tabagista e etilista, gênero e idade (fatores de risco) no desenvolvimento do câncer de cabeça e pescoço.

3. Verificar a associação entre os polimorfismos e os sítios primários de ocorrência, extensão do tumor, comprometimento de linfonodos e o prognóstico da doença.

2 ARTIGOS CIENTÍFICOS

2. Artigos Científicos

Os resultados referentes aos objetivos dessa dissertação estão apresentados na forma de artigo. No total estão apresentados 03 artigos submetidos para publicação.

Artigo 1

Título: Head and neck cancer: impact of MTHFD1 G1958A polymorphism

Periódico: Revista da Associação Médica Brasileira (RAMB), aceito para publicação.

Artigo 2

Título: *MTHFD1* G1958A, *BHMT* G742A, *TC2* C776G and *TC2* A67G polymorphisms and head and neck squamous cell carcinoma risk

Periódico: Molecular Biology Reports, submetido para publicação.

ARTIGO CIENTÍFICO 1

Title: Head and neck cancer: impact of MTHFD1 G1958A polymorphism

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RESUMO

INTRODUÇÃO: Alterações no metabolismo do folato podem contribuir para o processo de carcinogênese por influenciar as reações de metilação do DNA e a estabilidade genômica. Polimorfismos em genes que codificam enzimas envolvidas nesse metabolismo podem interferir nas concentrações de homocisteína, S-adenosilmetionina e outros produtos importantes para a síntese de DNA e reações de metilação celular. **OBJETIVOS:** Investigar o polimorfismo *MTHFD1* G1958A envolvido no metabolismo do folato no risco para o câncer de cabeça e pescoço e verificar a associação entre esse polimorfismo com fatores de risco e características clínico-histopatológicas. **PACIENTES E MÉTODOS:** Estudo retrospectivo que avaliou o polimorfismo *MTHFD1* G1958A em 694 indivíduos (240 pacientes e 454 controles), por meio da técnica de Análise de Polimorfismo de Comprimento de Fragmento de Restrição. Para análise estatística foram utilizados os testes de regressão logística múltipla e qui-quadrado. **RESULTADOS:** Tabagismo e idade superior a 42 anos foram preditores da doença (P<0,05). Os genótipos *MTHFD1* 1958GA ou AA associados ao tabagismo (P=0,04) e etilismo (P=0,03) foram preditores da doença. Estes mesmos genótipos estão presentes em maior proporção em pacientes com tumores em estadios mais avançados (P=0,04) e em pacientes com menor sobrevida (P=0,03). **CONCLUSÃO:** A presença do polimorfismo *MTHFD1* G1958A associada aos hábitos tabagista e etilista aumenta o risco para desenvolvimento de câncer de cabeça e pescoço.

Palavras-Chave: Polimorfismo genético; Neoplasias de cabeça e pescoço; Gene *MTHFD1*.

ABSTRACT

INTRODUCTION: Alterations in folate metabolism may contribute to the process of carcinogenesis by influencing DNA methylation and genomic stability. Polymorphisms in genes encoding enzymes involved in this pathway may alter enzyme activity and consequently interfere in concentrations of homocysteine and S-adenosylmethionine that are important for DNA synthesis and cellular methylation reactions. **AIM:** Investigate *MTHFD1* G1958A polymorphism involved in folate metabolism on head and neck cancer risk and the association between this polymorphism with risk factors and clinical-histopathological parameters. **PATIENTS AND METHODS:** A retrospective study in *MTHFD1* G1958A polymorphism investigated in 694 individuals (240 patients and 454 controls) by Polymerase Chain Reaction-restriction Fragment Length Polymorphism. Multiple logistic regression and chi-square were used for the statistical analysis. **RESULTS:** Multivariable analysis showed that tobacco and age over 42 years were predictors for the disease ($P<0.05$). The *MTHFD1* 1958GA or AA genotypes associated with tobacco ($P=0.04$) and alcohol ($P=0.03$) consumption were predictors of the disease. This polymorphism were more frequently in patients with advanced stage tumors ($P=0.04$) and patients with lower survival ($P=0.03$). **CONCLUSION:** The presence of *MTHFD1* G1958A polymorphism associated to tobacco and alcohol consumption increase the risk for head and neck cancer.

Key words: Genetic polymorphism; Head and neck cancer; *MTHFD1 g*ene.

INTRODUÇÃO

As neoplasias de cabeça e pescoço são responsáveis por uma grande incidência de óbitos em todo o mundo, sendo considerado o 6º tipo mais comum[1]. A região anatômica afetada por esse tipo de tumor inclui principalmente a cavidade oral (40%), faringe (15%) e laringe (25%)[2]. Dados do Instituto Nacional do Câncer[2] mostraram que na população brasileira, há uma proporção de três casos no gênero masculino para cada caso no gênero feminino, localizados com maior incidência na cavidade oral.

O câncer de cabeça e pescoço tem como principais fatores de risco o tabagismo e o etilismo[1]. Infecções virais especialmente com o vírus Epstein-Barr e Papiloma Vírus Humano dos subtipos 16 e 18 e deficiências ou desequilíbrios de vitaminas e micronutrientes, tais como ácido fólico, vitaminas A, C, E, zinco e selênio também foram associados à ocorrência de neoplasias em câncer de cabeça e pescoço[3-5].

O folato possui importante papel na oncologia, principalmente a partir de sua ação na metilação do DNA e na síntese de purinas e pirimidinas[6]. Alterações genéticas ou de deficiência dessa vitamina foram relacionadas ao câncer em vários estudos, incluindo o de cabeça e pescoço[6-15].

O gene *Metilenotetrahidrofolato desidrogenesase 1* (*MTHFD1*) é responsável pela formação do 10-formil-THF, essencial para a síntese de DNA. Este apresenta-se polimórfico no nucleotídeo 1958 (G→A) resultando na substituição de uma alanina por uma glicina no códon 653, localizado no domínio 10-formil-THF sintase da enzima[16]. Se a disponibilidade de folato é continuamente limitada, um ciclo de reparo descontrolado pode causar quebras frequentes na molécula de DNA e danos cromossômicos, o que resulta em alteração celular maligna, contribuindo para o desenvolvimento do câncer[6].

Poucos estudos avaliaram esse polimorfismo em câncer e os resultados são contraditórios. Kruszyna *et al.*[17] não encontraram diferenças estatísticas significantes na freqüência genotípica e alélica do polimorfismo *MTHFD1* A1958G em pacientes com câncer de laringe. Matakidou *et al.*[18] e Chen *et al.*[19] não associaram o mesmo polimorfismo as neoplasias de pulmão e colorretal, respectivamente. Por outro lado, Li *et al.*[20] encontraram associação do polimorfismo *MTHFD1* G1958A com o câncer de mama.

Assim, os objetivos desse estudo foram investigar a freqüência do polimorfismo *MTHFD1* G1958A em pacientes com carcinoma espinocelular de cabeça e pescoço e comparar com indivíduos sem história familiar da doença, e verificar se há associação entre esse polimorfismo e os fatores de riscos (tabagismo e etilismo) e características clínico-histopatológicas dos tumores (sítio primário de ocorrência, comprometimento de linfonodos e extensão tumoral).

PACIENTES E MÉTODOS

A amostra desse estudo foi constituída de 694 indivíduos, 240 pacientes com câncer de cabeça e pescoço (grupo caso) e 454 indivíduos sem história de neoplasia (grupo controle), após obtenção do Termo de Consentimento Livre e Esclarecido (parecer 5566/2005 da Comissão de Ética em Pesquisa – CEP da Faculdade de Medicina de São José do Rio Preto – FAMERP).

Os pacientes foram incluídos no estudo após o diagnóstico histopatológico de carcinoma espinocelular realizado pelo Serviço de Otorrinolaringologia e Cirurgia de Cabeça e Pescoço do Hospital de Base de São José do Rio Preto/SP. Os tumores foram classificados de acordo com os parâmetros da *Union International Control Cancer* (IUCC), 2002 e *American Joint Commitee for Cancer* (AJCC), 2002 em três critérios: tamanho do tumor (T), presença de linfonodos regionais comprometidos (N) e presença de metástase à distância (M). Quanto à localização anatômica do sítio primário do tumor, foram classificados em cavidade oral, faringe, laringe e sítio primário desconhecido[21,22]. O DNA das amostras de sangue foram provenientes do banco de amostras do laboratório e foram coletadas no período de março de 2000 a outubro de 2009.

O grupo controle consistiu em 454 indivíduos sem história de neoplasia e, por serem oriundos de um serviço de doação de sangue, são isentos de vinte tipos de doenças, conforme determina legislação brasileira (http://www.hemonline.com.br/portarias/rdc153/indexframe.htm). Os critérios para inclusão e exclusão foram, respectivamente, idade acima de 40 anos e história de neoplasia na família. Todos os participantes foram submetidos a uma entrevista para obtenção de variáveis, como idade, gênero e hábitos tabagista e etilista. Foram

considerados tabagistas indivíduos que consumiram cerca de 100 cigarros durante toda a vida e etilistas aqueles que ingeriram mais do que quatro drinques por semana[23,24].

Para análise molecular, o DNA genômico foi extraído a partir de sangue periférico de acordo com a técnica de Miller *et al.*[25] com modificações. A técnica de Análise de Polimorfismo de Comprimento de Fragmento de Restrição (PCR-RFLP) foi utilizada para determinar os genótipos do polimorfismo *MTHFD1* G1958A. Os *primers* utilizados foram descritos por Hol *et al.*[26] (Sense: 5' – CACTCCAGTGTTTGTCCATG – 3'; Anti-sense: 5' – GCATCTTGAGAGCCCTGAC – 3'). A amplificação foi obtida com desnaturação inicial a 95°C por 5 minutos, seguida por 35 ciclos de 30 segundos para desnaturação do DNA a 95°C, 50 segundos de anelamento dos *primers* a 53°C e 90 segundos de extensão a 72°C. A extensão final foi realizada por 5 minutos a 72°C. O produto de 331pb foi submetido a digestão enzimática com a enzima *Msp*I por 3 horas a 37°C. Os fragmentos de 166pb e 70pb foram gerados quando o alelo G esteve presente e o fragmento 266pb foi gerado quando o alelo A esteve presente.

A análise estatística foi realizada utilizando-se os programas computacionais Minitab/Windows - Versão 14.0, para avaliar os efeitos das variáveis analisadas em câncer de cabeça e pescoço e Bio Estat versão 3.0 para verificar se as distribuições enotípicas estavam em equilíbrio de Hardy-Weinberg. O teste de regressão logística múltipla foi utilizado para determinar o efeito das variáveis analisadas em câncer de cabeça e pescoço, que incluiu idade (referência: < 42 anos – idade em quartis), gênero (referência: feminino), hábito tabagista (referência: não fumantes) e hábito etilista (referência: não etilistas) e também para análise das variáveis clínico-histopatológicas. A classificação T foi dividida em tumores com pequena extensão (T1,T2) e com grande extensão (T3, T4). A classificação N foi dicotomizada em comprometimento de linfonodos negativo (N0), e positivo (N1, N2, N3). Os resultados foram apresentados em odds ratio (OR) e intervalo de confiança de 95% (IC – 95%). O nível de significância foi estabelecido em 5% ($p \leq 0,05$). O método de Kaplan-Meier foi aplicado para avaliar a taxa de sobrevida considerando como ponto final da análise (*end point*) o período compreendido entre o diagnóstico da doença e o óbito.

RESULTADOS

Os resultados do teste de regressão logística múltipla mostraram diferenças significantes entre pacientes e controles em relação às variáveis: tabagismo e idade superior a 42 anos ($p<0,05$) e, portanto, foram preditores da doença (Tabela 1).

O teste de Hardy-Weinberg mostrou que a distribuição genotípica estava em equilíbrio na amostra estudada (caso: $X^2=0,7096$; P=0,3996, e controle: $X^2=0,0707$; P=0,7903). O polimorfismo *MTHFD1* G1958A não foi associado ao risco dessa doença. As freqüências genotípicas *MTHFD1* 1958GG, GA e AA foram 35,83, 45,83, 18,34% respectivamente, para os casos, e 35,46, 48,68 e 15,86% respectivamente, para os controles. A freqüência do alelo selvagem 1958G foi 0,59 e 0,6, e do alelo polimórfico 1958A foi 0,41 e 0,4 entre casos e controles, respectivamente.

Os resultados do teste de regressão logística múltiplapara interação entre os fatores de risco e o polimorfismo *MTHFD1* G1958A mostraram que tabagismo (OR: 1,68; IC=95% 1,01-2,78; P=0,46) e etilismo (OR:1,83; IC=95% 1,06-3,15; P=0,03) associados aos genótipos *MTHFD1* 1958GA ou AA aumento o risco para o desenvolvimento de câncer de cabeça e pescoço (Tabela 2).

Em relação aos parâmetros clínico-histopatológicos dos tumores, os resultados do teste de regressão logística múltipla mostraram associação com o estadiamento tumoral, no qual os genótipos *MTHFD1* 1958GA ou AA foram mais freqüentes em indivíduos com estadio 3 e 4 (P=0,044) (Tabela 3).

A média de sobrevida dos pacientes no período do estudo obtida pela estimativa de Kaplan-Meier foi de 82,57 meses para os pacientes com genótipo *MTHFD1* 1958GG e de 59,03 para os pacientes com genótipo *MTHFD1* 1958GA ou AA, conforme Figura1(P=0,031).

DISCUSSÃO

Os resultados mostraram que tabagismo e idade superior a 42 anos aumentam o risco para câncer de cabeça e pescoço, corroborando com dados da literatura, que confirmam que esse tipo de neoplasia é mais freqüente a partir da 4ª década de vida[1,27] e em indivíduos tabagistas[28-32].

O folato age como coenzima em várias reações celulares fundamentais e é necessário na divisão celular devido ao seu papel na biossíntese de purinas e pirimidinas, e, conseqüentemente, na formação do DNA e do RNA[33].

O gene *Metilenotetrahidrofolato desidrogenesase 1* (*MTHFD1*), envolvido no metabolismo do folato, codifica uma proteína trifuncional cistólica que compreende 5,10-metileno-THF dehidrogenase, 5,10-metenil-THF ciclohidrolase, e 10-formil-THF sintase. As enzimas metileno-THF desidrogenase e metenil-THF ciclohidrolase, localizadas no mesmo domínio da proteína, catalisam a oxidação do 5,10-metileno-THF a 5,10-metenil-THF, convertida para 10-formil-THF. Estas três reações seqüenciais estão envolvidas na interconversão de derivados do carbono-1 do THF, substratos para a síntese de metionina, timidilato e purinas[19,34]. O polimorfismo G1958A desse gene pode estar associado ao câncer devido a alterações na síntese de DNA e conseqüentemente, descontrole celular[20].

No presente estudo foi observado que a distribuição genotípica está em equilíbrio, corroborando com a pesquisa de Kruszyna *et al*.[17], que também não encontrou diferenças estatísticas significantes na freqüência genotípica e alélica do polimorfismo *MTHFD1* A1958G.

Em nosso estudo, o polimorfismo *MTHFD1* G1958A não foi associado ao risco de câncer de cabeça e pescoço, assim como os achados de Kruszyna *et al*.[17] em 131 pacientes com câncer de laringe e 250 indivíduos controles, Matakidou *et al*.[18] em 619 pacientes com câncer de pulmão e Chen *et al*.[19] em 274 pacientes com câncer colorretal e 461 indivíduos controles.

Entretanto, Li *et al*.[20] que avaliaram a 227 pacientes, mostraram que o genótipo polimórfico *MTHFD1* 1958AA ocorreu em maior proporção em pacientes com câncer de mama do que o genótipo selvagem *MTHFD1* 1958GG. Também foi encontrado no mesmo estudo associação entre uma maior freqüência de metilação em pacientes com câncer de mama e o genótipo polimórfico *MTHFD1* 1958AA.

Em nosso estudo houve uma interação significante ente os genótipos *MTHFD1* 1958GA ou AA e hábitos tabagista e etilista, sugerindo que indivíduos com esses hábitos e genótipos GA ou AA possuem um risco maior no desenvolvimento do câncer de cabeça e pescoço. Não existem dados na literatura que comprovem essa associação.

A análise dos parâmetros clínico-histopatológicos confirmou que o tamanho do tumor T3 e T4 (avançado) foi mais freqüente em pacientes com genótipos GA ou AA. O estudo de Kruszyna *et al.*[17], em análises de significância genotípica entre características do tumor, mostraram uma fraca associação dos genótipos *MTHFD1* e o tamanho do tumor.

A média de sobrevida dos pacientes durante o período do estudo obtida pela estimativa de Kaplan-Meier mostrou que pacientes com o genótipo selvagem *MTHFD1* 1958GG apresentaram uma média de sobrevida maior em relação aos pacientes com genótipos *MTHFD1* 1958GA ou AA (pelo menos um alelo polimórfico), confirmando uma associação entre a presença do alelo polimórfico e a diminuição do tempo médio de sobrevida. De acordo com levantamento bibliográfico realizado, esse é o primeiro estudo que avaliou a associação entre o tempo de sobrevida e a presença do polimorfismo.

CONCLUSÃO

São preditores para o câncer de cabeça e pescoço, independentemente da variável genética o uso de tabaco e idade superior a 42 anos. A presença do polimorfismo *MTHFD1* G1958A associado aos hábitos tabagista e etilista aumentam o risco para o desenvolvimento do câncer de cabeça e pescoço. O polimorfismo é mais freqüente em tumores com estadios mais avançados da doença e em pacientes com menor prognóstico de vida. É importante corroborar por meio de outros estudos a influência do polimorfismo do gene *MTHFD1* e de outros genes envolvidos no metabolismo do folato na tumorigênese do câncer de cabeça e pescoço, para que seja determinada a etiologia e as correlações significativas com as características clínico-histopatológicas desses tumores.

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CONFLITO DE INTERESSE

Não existem conflitos de interesse declarados em relação a este artigo.

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Tabela 1. Distribuição demográfica, fatores de risco, genótipos e odds ratio (OR) para câncer de cabeça e pescoço.

Variáveis	**Caso (%)**	**Controle (%)**	**OR (95%IC)**	**P *value***
Consumo de Tabaco				
Não-fumantes	41 (17,08)	267 (58,81)	Referência	Referência
Fumantes	199 (82,92)	187 (41,19)	3,90 (2,46-6,20)	**P<0,05**
Consumo de Álcool				
Não-etilistas	67 (27,92)	230 (50,66)	Referência	Referência
Etilistas	173 (72,08)	224 (49,34)	1,56 (0,99-2,48)	P=0,056
Gênero				
Feminino	29 (12,08)	129 (28,41)	Referência	Referência
Masculino	211 (87,92)	325 (71,59)	1,65 (0,95-2,86)	P=0,073
Idade				
<42 anos	8 (3,33)	177 (38,99)	Referência	Referência
42-51 anos	49 (20,42)	170 (37,44)	5,22 (2,53-10,77)	**P<0,05**
52-63 anos	99 (41,25)	51 (11,23)	28,75 (13,51-61,18)	**P<0,05**
>64 anos	84 (35)	56 (12,34)	24,51 (11,57-51,92)	**P<0,05**
Genótipo *MTHFD1* G1958A				
GG	86 (35,83)	161 (35,46)	Referência	Referência
GA	110 (45,84)	221 (48,68)	1,38 (0,91-2,10)	P=0,135
AA	44 (18,33)	72 (15,86)		

Tabela 2. Distribuição dos fatores de risco relacionados ao câncer de cabeça e pescoço e polimorfismo *MTHFD1* G1958A.

Variáveis	GG genótipo casos/controles	OR (95%IC)	GA e AA genótipos casos/controles	OR (95%IC)*	P value
Idade					
<42 anos	4/53	1,00 (ref)	6/123	0,38 (0,09-1,50)	P = 0,166
42-51 anos	19/64	1,00 (ref)	36/105	1.78 (0,83-3,80)	P = 0,136
52-63 anos	31/19	1,00 (ref)	60/32	2,02 (0,86-4,79)	P = 0,108
>64 anos	30/22	1,00 (ref)	54/34	1,31 (0,60-2,83)	P = 0,496
Gênero					
Feminino	10/42	1,00 (ref)	19/87	1,41(0,50-3,96)	P = 0,519
Masculino	76/135	1,00 (ref)	119/206	1,37 (0,85-2,19)	P = 0,192
Tabaco					
Não	23/97	1,00 (ref)	18/170	0,98 (0,45-2,14)	P = 0,964
Sim	63/64	1,00 (ref)	136/123	1,68 (1,01-2,78)	**P = 0,046**
Álcool					
Não	29/77	1,00 (ref)	38/153	0,95 (0,49-1,85)	P = 0,890
Sim	57/84	1,00 (ref)	116/140	1,83 (1,06-3,15)	**P = 0,030**

Tabela 3. Distribuição dos parâmetros clínico-histopatológicos e polimorfismo *MTHFD1* G1958A.

Parâmetros clínicos	GG genótipo casos (%)	OR (95%IC)	GA e AA genótipos casos (%)	OR (95%IC)*	P value
Sítio Primário					
Cavidade oral	35 (14,58)	1,00 (ref)	61 (25,42)	0,88 (0,51-1,53)	P = 0,659
Faringe	15 (6,25)	1,00 (ref)	36 (15)	1,42 (0,72-2,81)	P = 0,312
Laringe	28 (11,67)	1,00 (ref)	45 (18,75)	0,80 (0,45-1,43)	P = 0,454
Tamanho do tumor					
T1/T2	47 (19,58)	1,00 (ref)	107 (44,58)	1,00 (ref)	
T3/T4	37 (15,42)	1,00 (ref)	49 (20,42)	0,57 (0,32-0,98)	**P = 0,044**
Envolvimento de linfonodos					
Não	58 (24,17)	1,00 (ref)	111 (46,25)	1,00 (ref)	
Sim	26 (10,83)	1,00 (ref)	45 (18,75)	0,90 (0,50-1,62)	P = 0,721

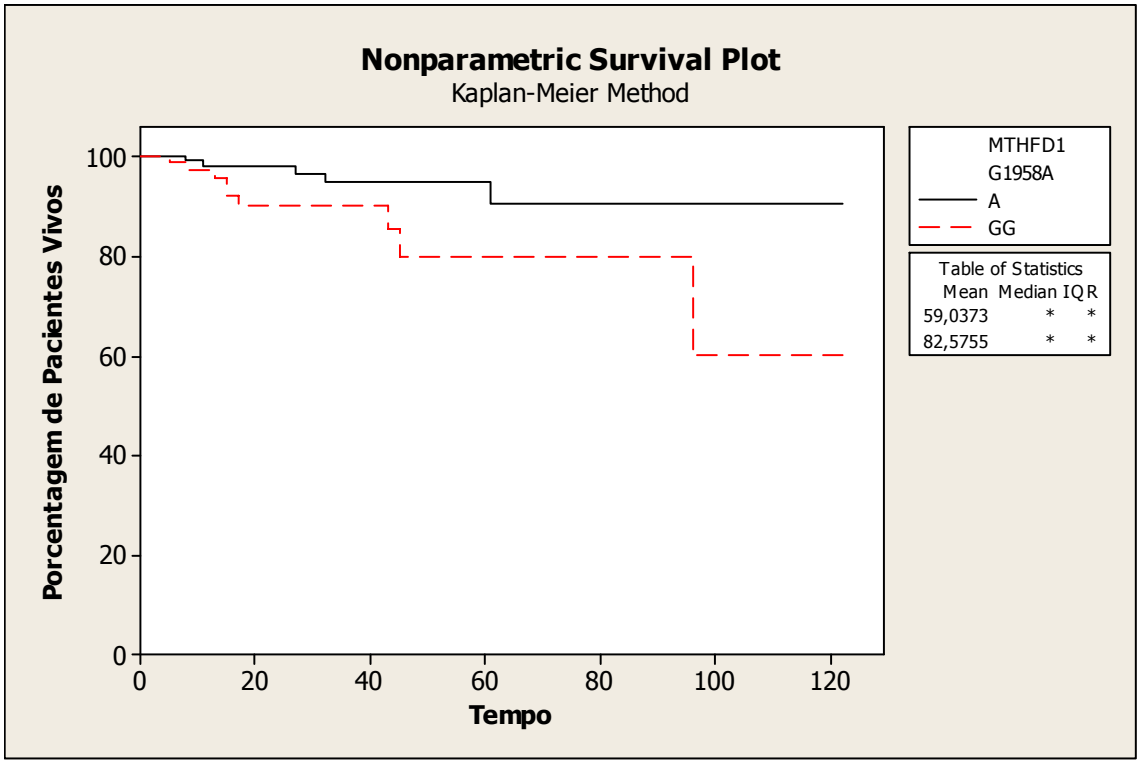

Figura 1. Curva de sobrevida não-paramétrica (Kaplan-Meier) dos pacientes com carcinoma espinocelular de cabeça e pescoço.

ARTIGO CIENTÍFICO 2

Title: *MTHFD1* G1958A, *BHMT* G742A, *TC2* C776G and *TC2* A67G polymorphisms and head and neck squamous cell carcinoma risk

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ABSTRACT

INTRODUCTION: Alterations in folate metabolism may contribute to the process of carcinogenesis by influencing DNA methylation and genomic stability. Polymorphisms in genes encoding enzymes involved in this pathway may alter enzyme activity and consequently interfere in concentrations of homocysteine and S-adenosylmethionine that are important for DNA synthesis and cellular methylation reactions. The objectives were to investigate *MTHFD1* G1958A, *BHMT* G742A, *TC2* C776G and *TC2* A67G polymorphisms involved in folate metabolism on head and neck cancer risk and the association between these polymorphisms with risk factors. **PATIENTS AND METHODS:** Polymorphisms were investigated in 762 individuals (272 patients and 490 controls) by Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP) and Real Time-PCR. Chi-square and Multiple logistic regression were used for the statistical analysis. **RESULTS:** Multiple logistic regression showed that tobacco and male gender were predictors for the disease ($P<0.05$). Hardy-Weinberg equilibrium showed that the genotypic distributions were in equilibrium for both groups in all polymorphisms studied. The *BHMT* 742GA or AA genotypes associated with tobacco consumption ($P=0.016$) increase the risk for head and neck squamous cell carcinoma (HNSCC). **CONCLUSION:** The present study suggests that *BHMT* 742GA associated to tobacco modulate HNSCC risk. However, further investigation of gene-gene interactions in folate metabolism and studies in different populations are needed to investigate polymorphisms and HNSCC risk.

Key words: Genetic polymorphism; Head and neck cancer; *MTHFD1, BHMT* and *TC2 g*enes.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is an aggressive malignant tumour type arising from the epithelial mucosal membranes of the upper-aerodigestive tract (oropharynx, hypopharynx and larynx) and the oral cavity [1,2]. HNSCC is the fifth most common cancer worldwide and is associated with low survival and high morbidity when diagnosed in advanced stage. Tobacco and alcohol consumption have been described as the most important risk factors associated with this carcinoma [3].

Folate is an essential nutrient which plays important roles in DNA synthesis and methylation [4-6]. Three main molecular mechanisms have been proposed: (1) a global decrease in DNA methylation, (2) increased uracil misincorporation during DNA replication, and (3) increased cytosine deamination at sites of DNA methylation. Folate metabolites are required for the conversion of homocysteine to methionine, which in the activated form of S-adenosyl-methionine (SAM) is required for DNA methylation. Folate deficiency can therefore decrease global DNA methylation, which is associated with genetic instability and tumor formation [7].

Low folate intakes have been positively associated with colon [8-11], breast [12-15], lung [12,13,16,17], colorectal [12,13,16,17], cervical [7,16], esophageal [7,16], pancreas [7,16], ovary [12,13,17] and head and neck cancer [18].

A polymorphism in methylenetetrahydrofolate dehydrogenase 1 (*MTHFD1*) gene (1958G>A) results in the substitution of a conserved arginine amino-acid by a glutamine at position 653 [19]. Despite the role of this enzyme in folate pathway, this polymorphism has been little explored in cancer [20,21].

Also involved in folate pathway, betaine-homocysteine methyltransferase (BHMT) is the enzyme, along with methionine synthase, which remethylates homocysteine (Hcy) to methionine [13,17,22,23]. The *BHMT* gene is polymorphic in the nucleotide 742, with a substitution of arginine for glutamine in the protein (G>A) [17,22,24,25]. There are few studies on the influence of this polymorphism on the development of cancer. Koushik *et al* (2006) [26] observed a relation between this polymorphism with colorectal cancer and Xu *et al* (2009) [27] with breast cancer.

A polymorphism in transcobalamin II (*TC2*) gene (776C>G) results in the substitution of a proline amino-acid by an arginine at codon 259 (P259R) [19,28]. Previous studies suggest that the C776G polymorphism in the *TC2* gene may affect transcobalamin binding affinity for Cbl and the ability to transport Cbl into tissues [28,29]. A different polymorphism in the *TC2* gene, the 67A>G transition is located in exon 2 and results in an isoleucine by valine replacement at codon 23 (I23V). There are no studies that have associated *TC2* polymorphisms and cancer; although Biselli *et al* (2008) [30] observed an association between this polymorphisms and maternal risk for Down syndrome, which etiology is related to abnormal folate metabolism. Afman *et al* (2002) [28] did not associate *TC2* C776G and A67G polymorphisms with neural tube defects risk.

Thus, the objectives of this study were to investigate *MTHFD1* G1958A, *BHMT* G742A, *TC2* C776G and *TC2* A67G polymorphisms involved in folate metabolism on head and neck cancer risk and the association between these polymorphisms with risk factors.

PATIENTS AND METHODS

After approval by the National Ethics Committee (CONEP - 5566/2005; SISNEP 0976.0.140.000-05), the individuals who agreed to participate in the study signed an informed consent. A total of 762 individuals (272 patients with head and neck cancer and 490 controls) were included in the study. The diagnosis was made from pathological specimens after biopsy or total excision of the tumor. The inclusion criterion was squamous cell carcinoma tumor cell types and the exclusion criterion was patients previously treated for tumors.

The control group consisted of 490 Brazilian blood donors without a diagnosis of cancer according to government guidelines for donated blood that is tested for 20 related diseases (http://www.hemonline.com.br/portarias/rdc153/indexframe.htm) [31]. The inclusion criterion was age higher than 40 years and the exclusion criterion was a family history of cancer. Each eligible subject was interviewed to obtain data on gender, smoking habit, use of alcohol, and family history of cancer. The variables analyzed were gender and exposure to risk factors (tobacco and alcohol consumption). Individuals who had smoked more than 100 cigarettes in their lifetime were considered to be tobacco consumers and individuals who drank 4 doses of alcohol per week were considered to be alcohol consumers [32,33].

Genomic DNA was obtained from peripheral blood according to Miller *et al*. (1988) [34]. The *MTHFD1* G1958A and *TC2* C776G polymorphisms were investigated by polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP), according to Hol *et al*. (1998) [35] and Pietrzyk *et al*. (2003) [36], with some alterations. The primers and enzyme used were: sense: 5'–CAC TCC AGT GTT TGT CCA TG–3', anti-sense: 5'–GCA TCT TGA GAG CCC TGA C–3' and *MspI* for *MTHFD1* gene; and sense: 5'–CAT CAG AAC AGT GCG AGA GG–3', anti-sense: 5'–GTG CCA GAC AGT CTG GGA AG–3' and *ScrFI* for *TC2* gene. The *BHMT* G742A and *TC2* A67G polymorphisms were investigated by Allelic Discrimination (*Applied Byosistems*, USA) using TaqMan probes (TaqMan SNP Genotyping Assay C_11646606_20 and C_25967461_10, respectively) in Step One Plus™ Real-Time PCR System equipament (*Applied Byosistems*).

STATISTICAL ANALYSIS

Multiple logistic regression was used to determine the interaction effect between the genetic polymorphisms and risk factors related to HNSCC. The model included gender (reference: female), tobacco consumption (reference: non-smokers) and alcohol consumption (reference: non-drinkers) using the Minitab for Windows computer program (Version 14.0). The Chi square test was used for to verify whether the genotypes frequencies were in Hardy-Weinberg equilibrium with BioEstat program. For deleterious alleles, the analysis was made using the criteria: risk 0 for no allele, risk 1 for one deleterious allele, risk 2 for two deleterious alleles, risk 3 for three deleterious alleles and risk 4 for four deleterious alleles or more. $P < 0.05$ was considered statistically significant. Results are shown as odds ratio (OR) and 95% confidence intervals (95%CI). The Kaplan-Meier method was used to evaluate survival rates, considering as *end point* the period between the diagnosis of disease and obit.

RESULTS

The results for comparison between groups showed that tobacco (P<0.05) and male gender (P<0.05) were predictors of the disease. Five hundred and eighty-seven (77.03%) participants were men (239 patients and 348 controls) and 175 (22.97%) were women (33 patients and 142 controls). Of the cases, 72.42% consumed alcohol compared to 50% of the controls. Tobacco also differed greatly between cases (83.09%) and controls (39.80%). None of the polymorphisms were associated to HNSCC risk (Table 1).

Hardy-Weinberg equilibrium showed that the genotypic distributions were in equilibrium for both groups in all polymorphisms studied: *MTHFD1* G1958A (case: X^2=1.0876; P=0.2970, and control: X^2=0.0061; P=0.9378); *BHMT* G742A (case: X^2=0.4320; P=0.5110, and control: X^2=0.7325; P=0.3921); *TC2* C776G (case: X^2=1.8042; P=0.1792, and control: X^2=0.3262; P=0.5679) e *TC2* A67G (case: X^2=1.5657; P=0.2108, and control: X^2=0.8329; P=0.3614).

For the *MTHFD1* G1958A polymorphism, GG, GA, and AA genotype frequencies were 37.87, 44.85 and 17.28%, respectively, for the cases, and 36.53, 47.96 and 15.51%, respectively, for controls. The variant *MTHFD1* 1958G allele frequencies were 0.60 among the cases and 0.61 among the controls, while the *MTHFD1* 1958A allele frequencies were 0.40 and 0.39 among cases and controls, respectively.

For the *BHMT* G742A polymorphism, GG, GA, and AA genotype frequencies were 43.01, 43.75 and 13.24%, for the cases, and 43.26, 46.33 and 10.41%, respectively, for the controls. The variant *BHMT* 742G allele frequencies were 0.65 among the cases and 0.66 among the controls, while the *BHMT* 742A allele frequencies were 0.35 and 0.34 among cases and controls, respectively.

For the *TC2* C776G polymorphism, CC, CG, and GG genotype frequencies were 37.87, 44.12 and 18.01%, for the cases, and 36.53, 47.96 e 15.51%, respectively, for the controls. The variant *TC2* 776C allele frequencies were 0.60 among the cases and 0.61 among the controls, while the *TC2* 776G allele frequencies were 0.40 and 0.39 among cases and controls, respectively.

For the *TC2* A67G polymorphism, AA, AG, and GG genotype frequencies were 72.29, 23.90 and 3.31%, for the cases, and 71.02, 27.14 and 1.84%, respectively, for the controls. The variant *TC2* 67A allele frequencies were 0.85 among the cases and 0.85 among the controls, while the *TC2* 67G allele frequencies were 0.15 and 0.15 among cases and controls, respectively.

Regarding to polymorphisms and risk factors, we found that tobacco (P=0.016) associated to *BHMT* G742A polymorphism modulate head and neck cancer risk (Table 2).

For deleterious alleles, we did not find any relation between allele number and incresead risk for head and neck cancer (one risk: OR:1.02; 95%CI: 0.39-2.66, P=0.975; two risk alleles: OR:1.40; 95%CI: 0.55-3.59, P=0.481; three risk alleles: OR:0.81; 95%CI: 0.32-2.08, P=0.669; four or more risk alleles: OR:1.11; 95%CI: 0.43-2.85, P=0.830).

The overall actuarial survival rate was 87.21% in 5 years (Figure 1). The Kaplan-Meier survival curves by genotype are presented in Figure 2, and did not demonstrate any association between polymorphisms and overall survival (*MTHFD1* 1958A, P = 0.332; *BHMT* G742A, P=0.650; *TC2* C776G, P=0.250; *TC2* A67G, P=0.807).

DISCUSSION

Head and neck carcinogenesis is a multifactor process in which environmental etiologic factors cause alterations in oncogenes or tumor suppressor genes. Almost 85% of patients with head and neck cancer are alcohol or tobacco abusers or both. Other causative agents include snuffing or chewing tobacco, ill-fitting dental appliances, chronic candidiasis, viral infection (mainly involving certain HPV types) and poor oral hygiene [1,37,38]. Factors which seem to promote the head and neck field cancerization include environmental exposures to tobacco and alcohol, viral infections especially with human papilloma virus (HPV) subtypes 16 and 18, and deficiency or imbalances in vitamins and micronutrients such as folic acid, vitamins A, C and E, and selenium and zinc [10].

Our results showed that tobacco and male gender were predictors for HNSCC. Tobacco and alcohol are the most important factors predisposing to development of HNSCC [1,38-42], it leads to cells damage and the genetic code [43]. If these alterations in DNA structures are left un-repaired, genetic changes can accumulate, which may result in cell-cycle dysregulation, autonomous growth and development of invasive mechanisms, leading to carcinoma [44]. Furthermore, studies show that male gender remains the most affected by this type of tumor [39,41,45]. However, while the incidence of HNSCC is much higher in males, more and more females are developing HNSCC as women adopt the male pattern of alcohol and tobacco consumption [1].

The understanding that folate metabolism influences both DNA synthesis and methylation has turned environmental and genetic variants into attractive candidates for cancer susceptibility. Genetic variants in genes that encode enzymes involved in one-carbon metabolism are good candidates for studying the impact of both genetic and environmental effects and their interactions on cancer risk. Our study did not find association between studied polymorphisms and risk for HNSCC.

Consequently, deficiency in *MTHFD1* gene may exacerbate the disruption of folate metabolism [46]. Our study did not find any association between *MTHFD1* G1958A polymorphism and head and neck cancer as Kruzyna *et al* (2010) [47] study, that also did not find association between laryngeal cancer and this polymorphism. In other cancer types, the studies are controversy, Liu *et al* (2008) [48], reported that the *MTHFD1* 1958A allele is less frequency in patients with lung cancer by studying a Chinese population with 500 cases and 517 controls. Matakidou *et al* (2007) [49] did not associate lung cancer with this polymorphism in 619 United Kingdom patients with this disease. Difference among these reports could be explained by the difference in study populations, given that UK population is mostly composed by Caucasian, whereas the Liu *et al* (2008) [48] study was restricted to Chinese population. In colorectal cancer, Chen *et al* (2004) [46] and Koushik *et al* (2006) [26] did not relate the polymorphism with the disease. The only study that showed significance between the frequency of methylation in breast cancer and *MTHFD1* G1958A was the study of Li *et al* (2006) [50].

Although not directly involved in folate metabolism, *BHMT* is involved in the metabolism of homocysteine. *BHMT* may play a critical role in the remethylation of homocysteine when the folate-dependent pathway is compromised by either genetic or dietary factors [14,17]. There are no studies in head and neck cancer related to this polymorphism, however in our study, smoking associated to *BHMT* G742A polymorphism increased the risk for this disease. A case-control study of Koushik *et al* (2006)

[26] in 376 colorectal patients and 849 controls observed that 742A polymorphic allele and the GA heterozygous genotype were associated to higher risk of colorectal cancer than those who have the wild-type genotype. The same polymorphic allele was related to a higher survival for those who had HNSCC by Xu *et al* (2008 and 2009) [17,27]. This could be explained by the difference in alimentation and other habits like smoking and etilism.

Cobalamine (Cbl) is an essential nutrient that plays an important role as coenzyme in two metabolic reactions: 1) the conversion of L-methylmalonyl-CoA to succinyl-CoA; 2) the remethylation of homocysteine to methionine. Methyl-Cbl serves as coenzyme for the enzyme methionine synthase (MTR) acting as a carrier for the methyl group donated by 5-methyltetrahydrofolate [51]. Cbl is bound to transcobalamin II, which is required for cellular uptake of this vitamin [19]. The present study showed that *TC2* C776G and *TC2* A67G polymorphisms were not associated to HNSCC. In other hand, Hazra *et al* (2007) [52] found that *TC2* C776G polymorphism is associated with higher risk of colorectal adenoma. Curtin *et al* (2007) [53] also find that 766G allele is associated with colon tumorigenesis. There are no studies in the literature about this association in HNSCC. In relation to *TC2* A67G polymorphism, there are no studies in the literature in cancer. However, Biselli *et al* (2008) [30] observed an association between this polymorphisms and maternal risk for Down syndrome. Afman *et al* (2002) [28] did not associate *TC2* A67G and neural tube defects risk.

For deleterious alleles, we did not find any relation between allele number and incresead risk for head and neck cancer. There are no studies in the literature relating deleterious alleles and the polymorphisms evaluated in this study.

In conclusion, the present study suggests that *BHMT* G742A associated to tobacco modulate HNSCC risk. There is no association between *MTHFD1* G1958A and *TC2* C776G polymorphisms, risk factors and the disease.

Considering the high prevalence of these polymorphisms in the general population, results from the study will improve the identification of risk factors for disease prevention. However, further investigation of gene-gene interactions in folate metabolism and studies in different populations are needed to investigate polymorphisms and HNSCC risk.

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Table1. Distribution in odds ratio (OR) of the gender, polymorphisms and risk factors between head and neck squamous cell carcinoma patients and controls

Variables	Patients N (%)	Controls N (%)	OR (95%CI)	P *value*
Tobacco consumption				
Non-smokers	41 (17.08)	267 (58.81)	Reference	Referência
Smokers	199 (82.92)	187 (41.19)	3.89 (2.45-6.19)	**P<0.05**
Alcohol consumption				
Alcohol non-consumers	67 (27.92)	230 (50.66)	Reference	Referência
Alcohol consumers	173 (72.08)	224 (49.34)	1.58 (0.99-2.51)	**P<0.05**
Gender				
Female	29 (12,08)	129 (28.41)	Reference	Referência
Male	211 (87.92)	325 (71.59)	1.64 (0.94-2.84)	P=0.08
Age				
<42 years	8 (3.33)	177 (38.99)	Reference	Referência
42-51 years	49 (20.42)	170 (37.44)	5.36 (2.59-11.09)	**P<0.05**
52-63 years	99 (41.25)	51 (11.23)	29.02 (13.61-61.88)	**P<0.05**
>64 years	84 (35)	56 (12.34)	25.26 (11.87-53.75)	**P<0.05**
***MTHFD1* G1958A genotypes**				
GG	86 (35.83)	161 (35.46)	Reference	Referência
GA	110 (45.84)	221 (48.68)	1.39 (0.91-2.12)	P=0.12
AA	44 (18.33)	72 (15.86)		
***BHMT* G742A genotypes**				
GG	101 (42.08)	188 (41.41)	Reference	Referência
GA	109 (24)	215 (47.35)	1.02 (0.68-1.53)	P=0.20
AA	30 (33.92)	51 (11.24)		
***TC2* C776G genotypes**				
CC	85 (35.42)	156 (34.36)	Reference	Referência
CG	109 (45.42)	227 (50)	0.76 (0.50-1.16)	P=0.91
GG	46 (19.16)	71 (15.64)		
***TC2* A67G genotypes**				
AA	178 (74.17)	320 (70.48)	Reference	Referência
AG	55 (22.92)	126 (27.75)	1.21 (0.78-1.87)	P=0.40
GG	7 (2.91)	8 (1.77)		

Table2. Odds ratio of head and neck cancer related to *MTHFD1*, *BHMT* and *TC2* genotypes by age, gender, tobacco and alcohol consumption

	OR (95%CI) – *P* value			
Risk Factors	***MTHFD1* G1958A***	***BHMT* G742A***	***TC2* C776G***	***TC2* A67G***
Tobacco consumption				
Yes	1.69 (1.01-2.80) **P = 0.044**	1.07 (0.66-1.74) P = 0.790	0.68 (0.41-1.14) P = 0.144	1.24 (0.73-2.08) P = 0.427
No	1.00 (0.45-2.20)P = 0.997	0.89 (0.41-1.90) P = 0.756	0.78 (0.35-1.73) P = 0.536	1.40 (0.59-3.34) P = 0,441
Alcohol consumption				
Yes	1.81 (1.05-3.14) **P = 0.033**	1.28 (0.76-2.15) P = 0.353	0.70 (0.40-1.22) P = 0.212	0.99 (0.56-1.74) P = 0.972
No	1.01 (0.51-2.01) P = 0.968	0.66 (0.34-1.29) P = 0.223	0.82 (0.42-1.63) P = 0.576	1.62 (0.79-3.33) P = 0.185
Gender				
Female	1.34 (0.47-3.85) P = 0.581	0.40 (0.15-1.07) P = 0.068	1.01 (0.34-2.97) P = 0.989	0.79 (0.27-2.33) P = 0.676
Male	1.37 (0.86-2.21) P = 0.188	1.19 (0.75-1.88) P = 0.455	0.75 (0.47-1.21) P = 0.239	1.34 (0.82-2.20) P = 0.248
Age				
<42 years	0.37 (0.09-1.52) P = 0.167	0.85 (0.21-3.45) P = 0.825	1.34 (0.30-5.88) P = 0.699	0.93 (0.21-4.12) P = 0.923
42-51 years	1.84 (0.85-3.97) P = 0.120	1.49 (0.74-3.02) P = 0.263	1.29 (0.62-2.68) P = 0.503	2.02 (0.96-4.24) P = 0.063
52-63 years	2.35 (0.95-5.81) P = 0.065	1.72 (0.69-4.32) P = 0.247	0.35 (0.13-0.95) **P = 0.039**	0.37 (0.14-0.98) **P = 0.046**
>64 years	1.47 (0.66-3.28) P = 0.347	0.55 (0.25-1.22) P = 0.140	0.40 (0.17-0.95) **P = 0.039**	1.94 (0.79-4.76) P = 0.148

* *MTHFD1* G1958A Reference: GG wild type genotype; *BHMT* G742A Reference: GG wild type genotype; *TC2* C776G Reference: CC wild type genotype; *TC2* A67G Reference: AA wild type genotype.

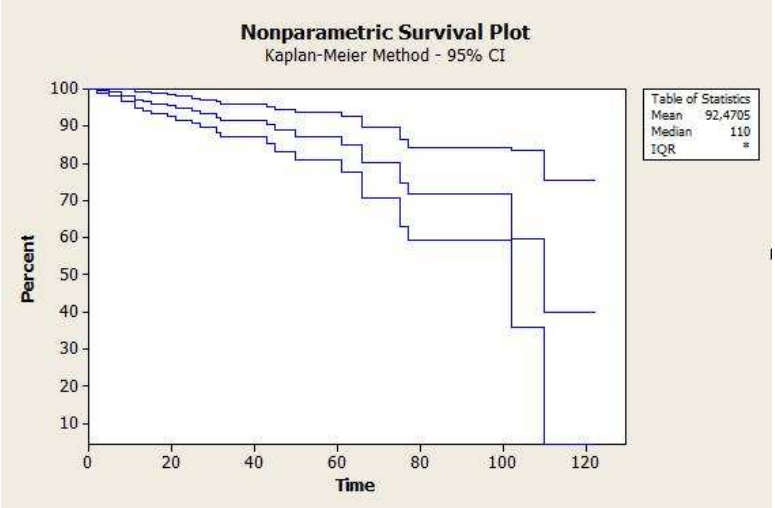

Figure1. Overall survival rates by Kaplan-Meier method.

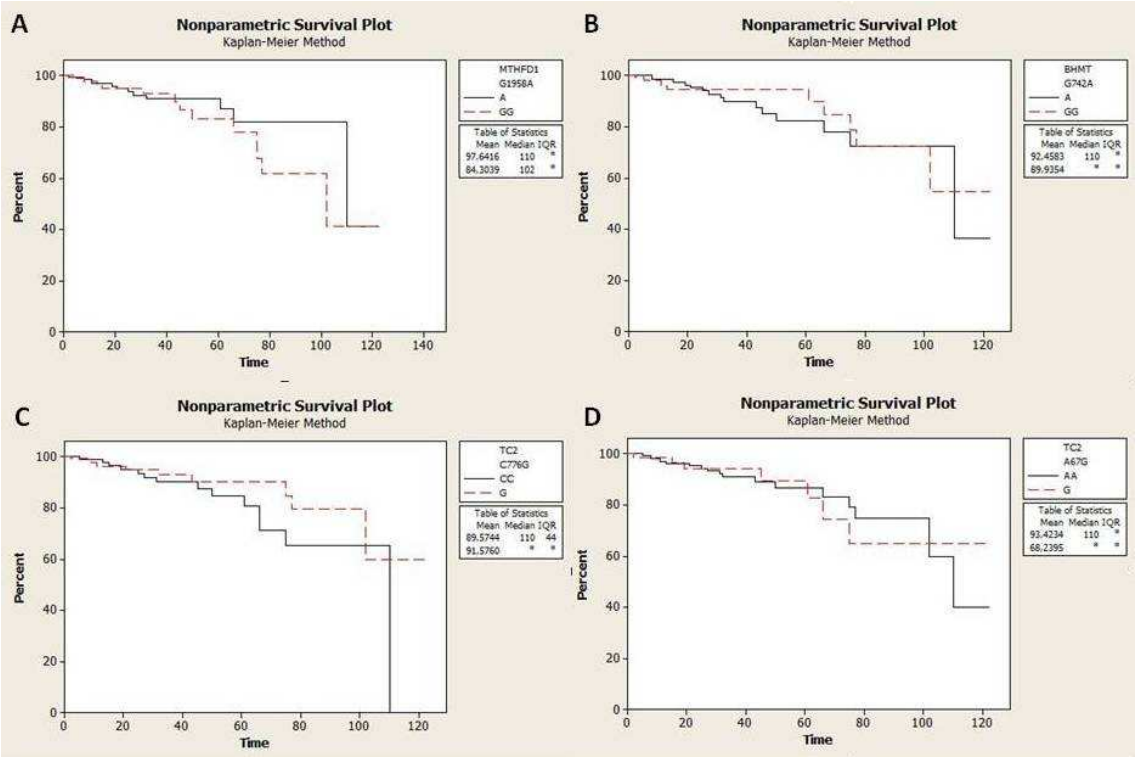

Figure2. Nonparametric survival plot (Kaplan Meier) from patients with head and neck cancer. (A: *MTHFD1* G1958A; B: *BHMT* G742A; C: *TC2* C776G and D: *TC2* A67G).

3 CONCLUSÕES

3. Conclusões

1. Os polimorfismos estudados *MTHFD1* G1958A, *BHMT* G742A, *TC2* C776G e *TC2* A67G não estão associados ao desenvolvimento do câncer de cabeça e pescoço.

2. Os genótipos *MTHFD1* 1958GA ou 1958AA associados ao tabagismo e etilismo e os genótipos BHMT 742GA ou 742AA associados ao tabagismo, modulam o risco de desenvolver carcinoma espinocelular de cabeça e pescoço.

3. Os genótipos *MTHFD1* 1958GA ou 1958AA apresenta-se mais frequentes em indivíduos com estadios tumorais 3 e 4 e estão associados a uma sobrevida menor em relação ao genótipo *MTHFD1* 1958GG.

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5 OUTRAS PRODUÇÕES CIENTÍFICAS

Artigo.

Título: Clinical and epidemiological characteristics of patients of the head and neck surgery department in a university hospital of northwest state of São Paulo

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Title: Clinical and epidemiological characteristics of patients of the head and neck surgery department in a university hospital of northwest state of São Paulo

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ABSTRACT

BACKGROUND: Head and neck cancer is located in upper aerodigestive tract, and accounts for nearly 650,000 new cases worldwide. Squamous cell carcinoma is the most frequent histological type, and the main risk factors are tobacco and alcoholism. PATIENTS AND METHODS: A total of 995 patients from a head and neck surgery department were evaluated. The variables analyzed included: age, gender, skin color, tobacco and alcohol consumption, primary site, histological tumor type, stage, treatment and number of deaths. RESULTS: This disease was more frequent among men (79.70%), smokers (75.15%) and alcohol consumers (58.25%). The most represented results were oral cavity (29.65%) and larynx (24.12%) for primary site, squamous cell carcinoma (84.92%) for histological type, surgery (29.04%) and radiotherapy (14.19%) for patient treatment. CONCLUSION: Tumors in patients treated by the head and neck surgery department occur mainly in males, tabagists and etilists, with the oral cavity and larynx having the highest incidence.

Keywords: Epidemiology, cancer, head and neck surgery department, alcohol and tobacco.

Introduction

Head and neck cancer is not a specific entity, but rather a broad category of diverse tumor types arising from various anatomic structures including the craniofacial bones, soft tissues, salivary glands, skin, and mucosal membranes.[1]

Although the head and neck surgery department treats patients with malignant tumors of the upper aero digestive tract, skin and thyroid, the term "head and neck cancer" is used only for the group of neoplasms located in the upper aero digestive tract, with approximately 40% occurring in the oral cavity, 15% in the pharynx, 25% in larynx and 20% in other anatomic sites.[2,3] Approximately 95% of these tumors have squamous cell carcinoma as the primary histological type.[4]

Head and neck cancer is the fifth most common cancer worldwide among all neoplasias.[4] The overall survival rate for this cancer is variable, depending on the primary site and disease stage. For oral cavity cancer, the overall survival rate is 50% over five years.[5] For other sites (pharynx and larynx), the rate is above 50% for early stage disease (T1-T2, N0) and generally below 50% in advanced stage (T3-T4, N0, T3-T4, N +, or any T, N2-N3). [6] In 2008, 6,214 deaths were observed in Brazil as a result of this disease.[7]

Oral cavity cancer, the most representative site of the disease, has a high frequency in Southeast Asia and India, due to the habit within such regions of chewing tobacco leaf and betel nut, a stimulant commonly used among indians.[8] In the United States, about 21,000 new cases of oral cancer are diagnosed each year and it is estimated that more than 650,000 new cases of head and neck cancer are diagnosed each year worldwide, two-thirds of them in developed countries.[6]

The estimated new cases of oral cavity cancer for 2010 is 14,120 and 4,120 for Brazil and Sao Paulo State, respectively.[7] The preliminary search conducted by our group in a period of five years, in a reference hospital in the northwestern state of Sao Paulo showed 427 patients diagnosed with head and neck cancer.[10]

This tumor type occurs mainly in male subjects, and its occurrence increases with age.[11] In the last decade, there has been a significant increase in this cancer in younger individuals, possibly due to increased infections by human papilloma virus (HPV) .[6,12,13]

The development of head and neck cancer is the result of the interaction of both environmental factors and genetic inheritance, and is therefore, multifactorial. Tobacco use associated with alcohol consumption is a well-established risk factor for the head and neck cancer.[14] Alcohol can act as a solvent for some tobacco carcinogens, increasing cellular uptake of these. According Maruri and Forastiere (2008),[6] the consumption of tobacco associated with alcohol consumption increases head and neck cancer risk by a rate of 40 times.

A significant proportion of head and neck tumors is a result of infection by some HPV types. These virus affect cells and produce viral oncoproteins (E6 and E7) that promote tumor progression by inactivating the product of some tumor suppressor genes such as Tp53 (tumor protein 53) and pRb (retinoblastoma tumor suppressor gene)[13]. Other factors that may contribute to head and neck carcinogenesis include diet, with risk reduced by fruit and vegetable consumption, and increased risk by [15] inadequate oral hygiene, leading to chronic infections by bacteria responsible for the pathogenesis of this tumor type, and[16] body mass, which can modulate toxin and carcinogenic metabolism. [17]

Occupational activity also appears to be associated with development of head and neck cancer. The study by Conway et al. (2010) [18] showed that manual occupational activities, low income, low occupational-social class, low educational attainment and unemployment correlate with increased risk for disease development. The individuals who work in rural activities are in constant exposure to sunlight and in contact with carcinogenic substances that contribute to the development of oral cavity cancer.[19]

Skin cancer is also associated with excessive exposure to solar radiation and occurs more frequently in the portions of the body exposed to the sun (head, neck and limbs). Also influencing the appearance of these lesions are factors such as age, sex, ethnicity, smoking, alcohol abuse, geographical distribution, old scars, persistent physical aggression and exposure to radioactives.[20]

Thyroid gland tumors, with their own unique characteristics, but also treated in the head and neck surgery department, have an origin related to iodine deficiency, external beam radiotherapy in childhood and adolescence, exposure to ionizing radiation and pre-existing thyroid disease.[21]

This study aimed to describe the socio-demographic aspects and clinical-pathology of patients in head and neck surgery department treated at a university hospital in the northwestern state of Sao Paulo, from January 2000 to May 2010.

Patients and methods

We retrospectively evaluated medical records of 1,351 cancer patients treated at the Otolaryngology and Head and Neck department of a university hospital in the northwestern state of Sao Paulo, from January 2000 to May 2010.

The variables analyzed were age, gender, skin color, tobacco and alcohol consumption, primary site, histological type, staging, treatment, death and occupational therapy of patients with the upper aero digestive tract, skin and thyroid cancer. Individuals who smoked more than 100 cigarettes in their lifetime were considered smokers, and individuals who consumed at least four drinks per week were considered alcohol drinkers. [22.23].

Tumors of the upper aero digestive tract were classified according to the anatomical site in the oral cavity, pharynx, larynx, nasal cavity, salivary glands and unknown primary site. Skin tumors and thyroid cases were also included.

Clinical staging of patients was performed according to the International Union Against Cancer based on the classification of malignant tumors (TNM) .[24] According to these standards, for the classification stage, T represents the tumor size, and tumors classified as Tx and T0 indicate primary indefinite tumor and no signs of primary tumor, respectively. N1, N2 and N3 indicate the existence of lymph nodes and N0 the absence of them. Tumors classified as Nx indicate undetermined lymph node status. Metastasis is represented by M1 and M0 for absence. In cases of failure to diagnose the presence or absence of metastasis, tumors were classified as Mx.

The occupations of patients were classified in such sectors as agriculture, construction, domestic service, driver, commercial, administrative, surveillance, metalwork, tapestries and aesthetics.

Data were analyzed using descriptive statistics, using the software Excel (version 2007).

Results

We analyzed records of 1,351 patients, of whom it was possible to obtain the most comprehensive information in hospital records of 995 cases, except for tumor staging and treatment, which were obtained for 785 and 909 cases, respectively.

A substantial majority of the cases were males (79.68%) and the mean age of patients was 60.48 years. Of the total patients, 747 were smokers (75.15%), 579 were etilists (58.25%) and 547 (54.00%) were both. For skin color classification, the subjects were divided into white and nonwhite, according to medical records, and was found a 90.04% frequency of patients with white skin. The oral cavity was the primary site of occurrence (29.65%), followed by the larynx and pharynx (24.12% and 18.29%, respectively). The thyroid gland tumors corresponded to 5.43%, while skin tumors accounted for 6.83% of patients. In 110 patients, it was not possible to identify the primary site (Table 1). The predominant histological type was squamous cell carcinoma, representing 84.92% of cases, followed by basal cell carcinoma (6.03%) and papillary carcinoma (5.22%). Other types of malignant tumors such as adenocarcinoma, melanoma, sarcoma, chondrosarcoma, fibrosarcoma and follicular carcinoma accounted for 3.08% (Table 2).

The tumor stage (TNM) in relation to primary sites and the main methods of treatment made by patients are described in Tables 3 and 4, respectively.

Regarding the occupation of the patients, the main activity was related to agriculture represented by 207 patients (20.82%) (Table 5).

Discussion

In the present study, the mean age of patients, regardless of gender, was 60.48 years, similar to that observed in Brazilian[25] and American[26] populations. Although the

frequency of patients with head and neck cancer is higher in individuals with advanced age, an increasing number of cases, especially for oral cavity and oropharynx cancer in young people has been observed and associated with HPV 16 infection,[13] an etiological agent related to carcinogenesis of this tumor type.[12]

Head and neck cancer affects mostly males [10, 27-29]. Likewise, in the present study, 79.68% of individuals affected by this type of cancer were males. Despite the low incidence of malignancies in women, an increase in number of cases is expected as a result of increased tobacco and alcohol consumption in the female population.[30]

In the present study, a prevalence of white individuals (90.04%) was observed, similar to findings in a study conducted recently in southern states of Brazil[31]and Midwest United States.[32] However, Hayat et al (2007)[33] observed a higher prevalence of head and neck cancer in African-American descendents from different areas of the United States. The ethnic differences in the distribution found in patients with head and neck cancer in different studies are mainly due to population composition where the research was done. Our study was conducted in northwestern São Paulo State, predominantly colonized by Europeans, comprising a high percentage of white-skinned people in the population.

Tobacco consumption was 75.15%; alcohol consumption was 58.25%; and both were 54.00% among the patients studied. The association between alcohol and tobacco with head and neck cancer is well established and has been reported in several studies [14, 27, 34]. An association between experiencing passive smoking for over 15 years and the development of head and neck cancer has been reported, independent of alcohol consumption.[35]

Squamous cell carcinoma was the most common histological type, representing 84.82% of cases, with a frequency close to that observed in the literature, which is approximately 90%[5]. In relation to primary site of tumor, oral cavity was the most representative (29. 65%), followed by larynx (24.12%) and pharynx (18.29%). The incidence of oral cancer worldwide is highest compared to other anatomical sites and is more common in individuals with low income, low occupational social class, low educational attainment.[36] Although it was not possible to obtain all information in our study , most patients treated in the hospital were from public health system, which assists low-income patients.

Surgical procedure and radiotherapy were performed in 29.04% and 14.19% of patients, respectively. The use of surgical practice followed by radiotherapy is a common practice in the treatment of head and neck squamous cell carcinoma, especially in early stages of the disease (I or II) with a high percentage of cure.[37] In this study, both treatment procedures were used in 29.92% of cases. In advanced stages (III or IV), chemotherapy is usually used in conjunction with other forms of treatment, promoting, especially in conjunction with radiotherapy, increased locoregional control in many cases.[37] Among the patients analyzed in this study, 13.86% underwent surgical, radiotherapy and chemotherapy procedures.

Patients with skin tumors in our study underwent surgery, which has cure rates above 95% when treated early and properly.[20]

Regarding thyroid gland tumors, our results indicate the prevalence of surgery associated with iodine therapy as an optional treatment. The use of less conservative surgery and the exploration of lymph nodes, as well as the use of adjuvant iodine

therapy seems to determine a more favorable prognosis for patients with thyroid cancer.[38]

In the analysis of the tumor stage, a high proportion of tumors classified as T3 and T4 were observed. These data reveal a significant number of patients diagnosed in advanced stages of disease and demonstrated the difficulty in obtaining an early diagnosis, since symptoms rarely appear in the early stages. According to the literature, on average, 40% of patients with oral cancer are diagnosed in advanced stages.[39]

In Brazil, some studies reported less than 50% of patients receive an early diagnosis[40,41]. Nearly two-thirds of patients with head and neck cancer have an advanced stage of the disease, usually involving regional lymph nodes. The incidence of distant metastases is relatively small in malignant tumors of head and neck cancer compared with other regions.[42] Approximately one-third of patients of this study had lymph node involvement and only 1% of metastasis.

During the study period, 265 patients died. The high mortality rate for this tumor type remains virtually constant over past decades.[11] Nevertheless Zigon et al (2010)[28] observed an increase in the relative rate of five-year survival for head and neck cancers study in the European population. In another study in the Brazilian population, we observed a significant increase in survival rate over five years for oral and oropharyngeal cancer, ranging from 28.7% in patients treated in the 1950s to 43.2% in the 1990s.[41]

In relation to occupational activities performed by study subjects, the most frequent were those related to agriculture (20.82%) and construction (19.01%), which is consistent with Conway et al.[36] who showed a correlation between higher rates of head

and neck cancer and individuals who practice manual occupational activities and have a low occupational social class.

Sartor et al.[43] also showed an association between laryngeal cancer and exposure to respirable-free crystalline silica. In this study, we found a risk twice as high for exposed individuals, such as those working in construction, compared to unexposed individuals.

Recent research in molecular biology has broadened our understanding of the etiology of these tumors. The combination of prognostic factors and molecular parameters could be beneficial for patients with the application of new therapeutic strategies. Such advances in studies of molecular markers may also improve the diagnosis in early stages of the disease, which would not be possible by traditional clinical methods[27,44].

Conclusion

Malignant tumors that affect patients treated by head and neck surgery department of a university hospital in northwestern São Paulo State are more frequent in subjects from regions with low socioeconomic development, and limited access to education. The incidence of this disease in this specific population affects mostly male patients in their sixties, smokers and etilists, with the oral cavity and larynx being the regions most affected. The high rate of patients with stage III and IV indicates a late demand in treatment centers, which reflects the need for prevention education campaigns for early diagnosis of the disease.

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Table 1. Distribution of cases according to demographic characteristics and sites of tumor.

Variables	Number of patients (%)
Gender	
Male	793 (79.70)
Female	202 (20.30)
Skin color	
White	895 (90.04)
Non-white	99 (9.96)
Tobacco use	
Tobacco users	747 (75.15)
Non tobacco users	247 (24.85)
Alcohol use	
Etilists	579 (58.25)
Non-etilists	415 (41.75)
Tobacco and alcohol use	547 (54.00)
Tumor sites	
Oral cavity	295 (29.65)
Larynx	240 (24.12)
Pharynx	182 (18.29)
Skin	68 (6.83)
Thyroid	54 (5.43)
Nasal cavity	16 (1.61)
Other sites	30 (3.01)
Unknown primary site	110 (11.06)

DP = standard deviation

Table 2. Most frequent histological types in patients attending a head and neck surgery department.

Histological types	Number of patients (%)
Squamous cell carcinoma	845 (84.92)
Basal cell carcinoma	60 (6.03)
Papillary carcinoma	52 (5.22)
Other	38 (3.83)

Table 3. Case distribuition by clinical-histopathological characteristics.

Category	Tumoral Staging					
	T1 e T2 n (%)	T3 e T4 n (%)	N0 n (%)	N1, N2 e N3 n (%)	M0 n (%)	M1 n (%)
Oral Cavity	169 (40.14)	102 (31.19)	202 (37.75)	69 (25.46)	249 (33.73)	2 (28.57)
Larynx	105 (24.94)	114 (34.86)	160 (29.90)	64 (23.62)	208 (28.18)	-----
Pharynx	66 (15.68)	98 (27.97)	89 (16.60)	78 (28.78)	141 (19.10)	2 (28.57)
Skin	39 (9.26)	1 (0.31)	41 (7.66)	-----	41 (5.55)	-----
Thyroid	24 (5.70)	7 (2.14)	23 (4.29)	5 (1.85)	28 (3.79)	-----
Nasal Cavity	5 (1.19)	3 (0.92)	7 (1.13)	1 (0.37)	8 (1.08)	-----
Other Sites	13 (3.09)	2 (0.61)	13 (2.49)	3 (1.11)	15 (2.03)	-----
Unknown Site	0 (0)	0 (0)	0 (0)	51 (18.82)	48 (6.54)	3 (42.86)

Table 4. Treatment forms of patients attending the head and neck surgery department.

Treatment	Number of Patients (%)
Surgery	264 (29.04)
Radiotherapy	129 (14.19)
Chemotherapy	18 (1.98)
Iodotherapy	1 (0.11)
Surgery and Iodotherapy	23 (2.53)
Surgery and Radiotherapy	272 (29.92)
Surgery and Chemotherapy	17 (1.87)
Radiotherapy and Chemotherapy	59 (6.49)
Surgery, Radiotherapy and Chemotherapy	126 (13.86)

Table 5. Occupation of patients attending the head and neck surgery department.

Ocupacional activities	Number of patients (%)
Farming	207 (20.82)
Civil construction	189 (19.01)
Domestic services	175 (17.60)
Driver	89 (8.95)
Commercial	82 (8.25)
Administrative	43 (4.32)
Vigilance	26 (2.61)
Metallurgy	21 (2.11)
Tapestry	9 (0.90)
Aesthetics	5 (0.50)
Other*	149 (14.89)

* Less than 1% in each profession

6 ANEXOS

FACULDADE DE MEDICINA DE SÃO JOSÉ DO RIO PRETO
Autarquia Estadual - Lei n° 8899 de 27/09/94
(Reconhecida pelo Decreto Federal n° 74.179 de 14/06/74)

COMITÊ DE ÉTICA EM PESQUISA

O Comitê de Ética em Pesquisa da Faculdade de Medicina de São José do Rio Preto tomou ciência e aprovou a ampliação da metodologia datada de 11 de novembro de 2005, referente ao protocolo n.° *5566/2005* sob a responsabilidade de Maurício José Cabral Ruback, com o título "Câncer de cabeça e pescoço: um levantamento epidemiológico do Hospital de Base/FAMERP de SãoJosé do Rio Preto".

São José do Rio Preto, 19 de dezembro de 2005.

Prof. Dr. José Paulo Cipullo
Vice- Coordenador do CEP/FAMERP

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FACULDADE DE MEDICINA DE SÃO JOSÉ DO RIO PRETO
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(Reconhecida pelo Decreto Federal n.° 74.179 de 14/06/74)

COMITÊ DE ÉTICA EM PESQUISA

O Comitê de Ética em Pesquisa em Seres Humanos da Faculdade de Medicina de São José do Rio Preto tomou ciência e autorizou a **dispensa do Termo de Consentimento Livre e Esclarecido conforme solicitação datado de 03/07/2008**, referente ao protocolo n.° **5566/2005** sob a responsabilidade de **Maurício José Cabral Ruback**, com o título "Câncer de cabeça e pescoço: um levantamento epidemiológico do Hospital de Base/FAMERP de São José do Rio Preto".

São José do Rio Preto, 08 de julho de 2008.

Prof. Dr. Antonio Carlos Pires
Coordenador do CEP/FAMERP

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