UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

ASSOCIAÇÃO ENTRE POLIMORFISMOS EM GENES ENVOLVIDOS NO METABOLISMO DO METOTREXATO E A OCORRÊNCIA DE MUCOSITE ORAL EM PACIENTES PEDIÁTRICOS COM LEUCEMIA

José Maria Chagas Viana Filho

2019

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, da Universidade Federal da Paraíba, como parte dos requisitos para obtenção do título de Mestre em Odontologia – Linha de Pesquisa em Fisiopatologia e morfologia dos tecidos buco-maxilo-faciais.

Orientadora: Prof. Dr.ª Naila Francis Paulo de Oliveira
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João Pessoa
2019

Catalogação na publicação
Seção de Catalogação e Classificação

V614a Viana Filho, José Maria Chagas.
Associação entre polimorfismos em genes envolvidos no metabolismo do metotrexato e a ocorrência de mucosite oral em pacientes pediátricos com leucemia / José Maria Chagas Viana Filho. - João Pessoa, 2019.
82 f.

Orientação: Naila Francis Paulo de Oliveira.
Coorientação: Ana Maria Gondim Valença.
Dissertação (Mestrado) - UFPB/CCS.

1. Mucosite oral. 2. Polimorfismos genéticos. 3. Crianças. I. Oliveira, Naila Francis Paulo de. II. Valença, Ana Maria Gondim. III. Título.

UFPB/BC

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DEDICATÓRIA

Dedico este trabalho a todos os pacientes do Hospital Napoleão Laureano que se dispuseram a participar da pesquisa em meio a tanto sofrimento físico e psicológico. Que todo o mérito desta pesquisa seja destinado a eles e aos familiares deles.

AGRADECIMENTOS

Agradeço a **Deus**, em primeiro lugar, por ser tão presente em minha vida e por ter segurado na minha mão durante estes dois anos de mestrado. Nos momentos em que fraquejei, Ele me reergueu e me impulsionou a continuar, e nos momentos de glória, Ele estava lá para me mostrar que eu era capaz. Hoje finalizo com a certeza de que sem Ele eu não teria conseguido. "Obrigado, Deus, porque você é muito bom!".

À minha madrinha/mãe, **Fátima**, que é a pessoa mais importante que eu tenho aqui na Terra, por acreditar em mim e por correr comigo em busca dos meus sonhos. Ela é o tipo de mãe que esquece dos seus sonhos para realizar os sonhos dos seus filhos. Obrigado por ter aceitado o chamado de Deus para ser minha mãe!

À intercessão da minha mãe, **Zélia** (*in memoriam*), que está junto a Deus olhando por mim. Sei que do céu ela está sempre olhando por mim e se alegrando a cada conquista minha.

Aos meus irmãos, **Maria José**, **Sâmara** e **Denys**, por todo o amor e companheirismo desde que me entendo por gente. Eles são os melhores! Em especial, gostaria de agradecer a Maria José e ao seu esposo Jefferson, que desde o meu primeiro dia de aula da graduação me acolheram em sua casa e nunca deixaram faltar nada para mim. Obrigado por tanto!

Ao meu pai, **José Maria**, e aos meus irmãos, **Charles e Daniel**, que mesmo distantes fisicamente, se fazem presentes com palavras de apoio e se alegram junto comigo a cada vitória.

A **Nikácio**, por ter me incentivado a não desistir da carreira docente, por dividir comigo momentos diários de alegria e tristeza, pela paciência e persistência ao longo desses três anos de convivência, por se alegrar a cada vitória minha como se fosse sua e por me impulsionar a sonhar sempre alto. Muito obrigado!

A minha orientadora, **Prof.ª Naila**, pela paciência, dedicação, ensinamentos, companheirismo e oportunidades durantes estes dois anos. Naila é um exemplo a ser seguido, seja como professora, seja como pessoa! Agradeço por ter abraçado a ideia de pesquisar numa área que fugia um pouco da sua vivência e por ter mergulhado profundamente nessa temática. O pouco que sei hoje de genética e biologia molecular, devo a você. Muito obrigado!

A minha coorientadora, **Prof.ª Ana Maria**, por ter aceitado o meu pedido de pesquisar em pacientes oncopediátricos e por ter me apresentado essa área tão

encantadora. Ana Maria foi a idealizadora desse estudo junto com a **Prof.ª Isabella**, a qual sou grato pela ajuda com as análises estatísticas. Muito obrigado, Ana, pela minha inserção nessa área, no seu grupo de pesquisa (GPOCI) e por ter sido tão presente na realização desse estudo.

Às minhas companheiras e amigas de laboratório, **Marina** e **Ingrid**, pelo companheirismo e amizade durante todo esse tempo. Por terem me recebido tão bem e por terem passado de forma tão magnifica todos os conhecimentos a respeito de cada técnica que utilizamos nesse estudo. Muito obrigado por tudo!

Aos meus amigos do mestrado: **Hugo**, **Renata**, **Arella**, **Isis**, **Raíres**, **Larissa**, **Juliana**, **Danielle**, por terem dividido comigo os melhores e mais angustiantes momentos acadêmicos desse mestrado.

Aos meus amigos de longas datas, aos amigos feitos a pouco tempo, aos amigos do EJC e ECRI Auxiliadora, que passaram por minha vida e deixaram sua marca. Em especial a **Aninha**, **Eduardo**, **Blenda (Joaquim)**, **Cecília**, **Bianca** (responsável pelas aulas de inglês às vésperas da prova de seleção).

Aos professores do PPGO-UFPB, pelos ensinamentos durante o curso e por sempre buscarem pela excelência em suas pesquisas realizadas com tanta responsabilidade e esmero. Em especial, gostaria de agradecer a ajuda e o apoio dos professores: **Prof. Yuri**, **Prof.ª Leopoldina**, **Prof.ª Bianca**, **Prof. Ricardo** e **Prof. Frederico**.

Aos funcionários do Hospital Napoleão Laureano, por dividirem conosco seu espaço de trabalho, sempre pensando na saúde dos pacientes que passam por ali. Em especial a **Carol**, **Ana**, **Zu** e **Nubia**. Aos pais das crianças, por autorizarem a participação de cada um e a todas as crianças que tive a oportunidade de conhecer.

“Basta-te minha graça, porque é na fraqueza que se revela totalmente a minha força”

2Co 12,9

RESUMO

A mucosite oral quimioinduzida é uma resposta inflamatória da mucosa à ação dos quimioterápicos. Polimorfismos em genes envolvidos no metabolismo do metotrexato (MTX) têm sido referidos como indícios associativos na ocorrência da mucosite oral. O objetivo da presente dissertação foi investigar a relação dos polimorfismos genéticos no desenvolvimento da mucosite oral em crianças e adolescentes submetidos à quimioterapia envolvendo o MTX. Realizou-se, para tanto, uma revisão sistemática com meta-análise e uma pesquisa longitudinal. Para a revisão, buscas bibliográficas foram realizadas nas bases de dados: PubMed, Scopus, Lilacs, Cochrane, Web of Science e Open Grey, até maio de 2019. Estudos observacionais com crianças e/ou adolescentes submetidos à quimioterapia com MTX, portadores de algum polimorfismo e que apresentaram a mucosite oral como desfecho foram selecionados por meio da estratégia PECO. Estudos que continham o número indivíduos com e sem polimorfismos genéticos, com e sem mucosite oral, e que realizaram análises estatísticas associativas foram selecionados. Avaliou-se a qualidade metodológica pelo instrumento de Fowkes e Fulton (1991). Duas meta-análises foram conduzidas no Review Manager v. 5.2 (com todos os polimorfismos encontrados e com os polimorfismos que se repetiram em mais de um estudo). A qualidade da evidência foi avaliada pelo GRADE. Para o estudo longitudinal, 64 pacientes foram avaliados pelo OAG (*Oral Assessment Guide*) modificado para diagnóstico da mucosite oral. O material biológico utilizado foi a saliva, de onde foram isoladas células epiteliais orais e extraído o DNA. Os polimorfismos: *MTHFR* C677T (rs1801133), *DNMT3B* C-149T (rs2424913), *ABCC2* C-24T (rs717620), *ABCG2* G34A (rs2231137) e *ABCG2* C421A (rs2231142), foram analisados por PCR-RFLP (*Polymerase Chain Reaction - Restriction Fragment Length Polymorphism*) e os resultados observados em gel de poliacrilamida 6%, corado com nitrato de prata ou GelRed®. Os dados demográficos e hematológicos foram coletados a partir dos prontuários. Foi realizada estatística descritiva e inferencial, utilizando os testes: Qui-quadrado, exato de Fisher e U de Man-Whitney ($p \leq 0,05$). A busca da revisão sistemática resultou em 61 artigos, dos quais 9 eram elegíveis. A amostra total foi de 1775 indivíduos, com idades entre 1 e 19 anos, portando leucemia ou linfoma. Investigaram-se 29 polimorfismos estudados e suas associações com a mucosite oral, sendo o MTHFR C677T o mais investigado. Sete estudos apresentaram vieses na seleção da amostra. Não foi observada associação entre os polimorfismos e a ocorrência de mucosite oral, tanto na meta-análise com todos os polimorfismos (RR= 1,14; I^2=18%; p=0,22) quanto na realizada com os polimorfismos que se repetiram nos estudos (RR=0,96; I^2=20%; p=0,29), com nível de evidência científica muito baixo. No estudo longitudinal, houve predomínio do sexo masculino (56,2%) e idade média de 10,8 anos (±4,9). Dentre os pacientes que apresentaram mucosite oral, 65,3% foram acometidos pela forma grave (MOG). Houve associação entre o polimorfismo C421A (rs2231142) e a mucosite oral (RR=8,33; IC=0,09 – 0,47; p=0,02;), bem como entre MOG e contagem de leucócitos (p=0,03) e MOG e idade (p=0,02). A revisão sistemática concluiu que a mucosite oral e polimorfismos genéticos não estão associados, no entanto, o risco de viés e a baixa qualidade da evidência científica sugerem a realização de novos estudos com maior rigor metodológico, o que impulsionou a realização do estudo longitudinal. Já neste estudo, conclui-se que a presença de polimorfismo C421A *ABCG2* (rs2231142) aumenta a probabilidade de ocorrência mucosite oral. As crianças e adolescentes mais jovens e com menor quantidade de leucócitos apresentam maior probabilidade de desenvolverem a mucosite oral grave.

Palavras-chave: Mucosite oral, Polimorfismos genéticos, Crianças

ABSTRACT

Chemoinduced oral mucositis is an inflammatory mucosal response to chemotherapeutic action. Polymorphisms in the genes involved in methotrexate metabolism (MTX) have been caused as evidence associated with the occurrence of oral mucositis. The aim of this dissertation was to investigate a relationship of genetic polymorphisms in the development of oral mucositis in children and adolescents who used chemotherapy using MTX. For this, a systematic review with meta-analysis and a longitudinal survey were performed. For review, bibliographic searches were performed in the databases: PubMed, Scopus, Lilacs, Cochrane, Web of Science and Open Gray, until May 2019. Observational studies with children and / or adolescents with MTX chemotherapy, with some polymorphism and who described an oral mucositis as unfocused were selected using the CEEC strategy. Studies containing the number of individuals with and without genetic polymorphisms, with and without oral mucositis, and who performed statistical statistics associated with selected items. Prohibit methodological quality by the instrument of Fowkes and Fulton (1991). Two meta-analyzes were conducted in Review Manager v. 5.2 (with all polymorphisms found and with polymorphisms that were repeated in more than one study). The quality of the evidence was assessed by GRADE. For the longitudinal study, 64 patients were evaluated by the modified oral assessment guide (OAG) for diagnosis of oral mucositis. The biological material used was saliva, where oral epithelial cells were extracted from DNA. Polymorphisms: MTHFR C677T (rs1801133), DNMT3B C-149T (rs2424913), ABCC2 C-24T (rs717620), ABCG2 G34A (rs2231137) and ABCG2 C421A (rs2231137) and were analyzed by PCR-RFLP polymorphism and length the results observed on 6% polyacrylamide gel stained with silver nitrate or GelRed®. Demographic and hematological data were collected from medical records. Descriptive and inferential statistics were performed using the chi-square, Fisher's exact test and Man-Whitney U test ($p \leq 0.05$). The search for systematic review resulted in 61 articles, 9 of which were eligible. A total sample was 1775 individuals, aged 1 to 19 years, with leukemia or lymphoma. Twenty-nine polymorphisms studied and their associations with oral mucositis were investigated, being MTHFR C677T or more investigated. Seven studies published in the sample selection. No association was observed between polymorphisms and occurrence of oral mucositis, either meta-analysis with all polymorphisms (RR = 1.14; $I^2 = 18\%$; p = 0.22) or performed with the polymorphisms that were repeated in the studies (RR = 0.96; $I^2 = 20\%$; p = 0.29), with very low level of scientific evidence. In the longitudinal study, there was a predominance of males (56.2%) and average age of 10.8 years (± 4.9). Among the patients with oral mucositis, 65.3% were severely affected (MOG). There was an association between C421A polymorphism (rs2231142) and oral mucositis (RR = 8.33; CI = 0.09 - 0.47; p = 0.02;), as well as between MOG and leukocyte count (p = 0 .03) and MOG and age (p = 0.02). The systematic review concluded that oral mucositis and genetic polymorphisms are not associated; however, the risk of disease and the poor quality of scientific research suggest further studies with greater methodological rigor, or what drives longitudinal studies. In this study, we concluded that the presence of C421A ABCG2 polymorphism (rs2231142) increases the likelihood of oral mucositis. Younger children and adolescents with fewer leukocytes are more likely to develop an oral mucositis severe.

Keywords: Oral mucositis, Genetic polymorphisms, Child

LISTA DE ABREVIATURAS E SIGLAS

ABC – ATP Binding Cassete

ABCC2 – ATP Binding Cassete subfamília C membro 2

ABCG2 – ATP Binding Cassete subfamília G membro 2

CCG – *Children's Cancer Group*

CTCAE – *Common Terminology Criteria for Adverse Eventes*

DANT – Doenças e Agravos Não Transmissíveis

DNA – Ácido Desoxirribunucleico

DNMT – DNA metiltransferase

GBTLI – Grupo Brasileiro de Tratamento da Leucemia na Infância

IL-6 – Interleucina 6

INCA – Instituto Nacional do Câncer

LLA – Leucemia Linfoblástica Aguda

LMA – Leucemia Mieloide Aguda

MOG – Mucosite Oral Grave

MTHFR – Metilenotetrahidrofolato redutase

MTX – Metotrexato

NCI – *National Cancer Institute*

OAG – *Oral Assessment Guide*

RCBP – Registros de Câncer de Base Populacional

SAM – S–adenosilmetionina

SNP – *Single Nucleotide Polymorphism*

TNFα – Fator de Necrose Tumoral

WHO – *World Health Organization*

SUMÁRIO

1 INTRODUÇÃO

1.1 Câncer pediátrico

O câncer é uma síndrome classificada como uma Doença e Agravo não Transmissível (DANT), responsável por altos índices de morbimortalidade no mundo e no Brasil. Apresenta alta incidência e estimativa crescente ao passar dos anos. Calcula-se, para cada ano do biênio 2018-2019, o surgimento de 417.010 novos casos no Brasil, com exceção do câncer de pele não melanoma (1,2).

Nos Registros de Câncer de Base Populacional (RCBP), 3% (percentual mediano) dos tumores malignos são encontrados no público infanto-juvenil (0 – 19 anos), estimando-se a ocorrência de 12.500 novos casos de câncer em crianças e adolescentes, com maior incidência nas regiões sudeste e nordeste brasileiro (2).

Nesta população, as leucemias correspondem a 26% das neoplasias, sendo a Leucemia Linfoblástica Aguda (LLA) a mais prevalente em crianças, embora haja um predomínio da Leucemia Mieloide Aguda (LMA) no primeiro ano de vida (3,4). As leucemias são resultado da não-maturação de células sanguíneas da série branca, produzidas na medula óssea, que se transformam em células neoplásicas. Elas não realizam suas funções, não sofrem morte celular, mas substituem as células sanguíneas saudáveis e proliferam-se (2,5).

Esta neoplasia é classificada de acordo com o tipo de célula branca afetada e com a velocidade de evolução, sendo assim denominadas: leucemia mieloide ou mieloblástica, quando a estirpe mieloide é atingida; leucemia linfoide, linfocítica ou linfoblástica, quando a estirpe linfoide é atingida; apresentando evolução aguda (rápida) ou crônica (mais lenta) (2,5).

No ano de 2016, o Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA) publicou um levantamento a respeito da morbimortalidade e incidência de câncer em pacientes hospitalizados. As análises de tendência das taxas de incidência para as leucemias no público infanto-juvenil, na cidade de João Pessoa, demonstraram um aumento significativo. Isso pode ser reflexo da melhoria do serviço oncopediátrico, no que diz respeito ao diagnóstico e tratamento das leucemias, bem como na melhoria das informações destas neoplasias para a população (6).

Mais da metade dos pacientes pediátricos acometidos pelo câncer é tratada com quimioterapia, sendo este o recurso terapêutico empregado no tratamento das leucemias.

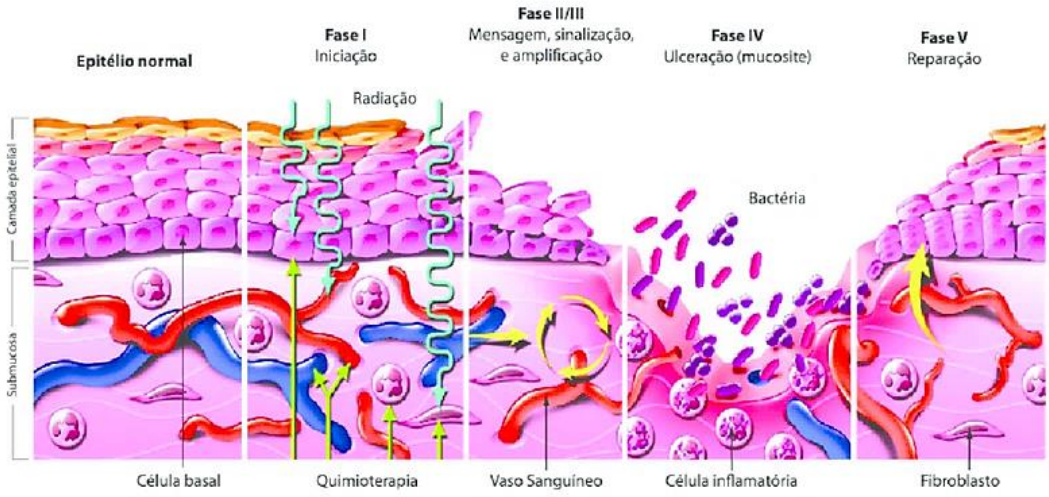
No entanto, esse tratamento apresenta diversos efeitos colaterais, como: alopecia, náusea, vômito, diarreia, imunossupressão, hipossalivação e mucosite oral (7–9).

1.2 Mucosite oral quimioinduzida

A mucosite oral quimioinduzida consiste em uma resposta inflamatória da mucosa à ação bioquímica de medicamentos antineoplásicos. Esta condição é mais frequente e mais severa em crianças, por apresentarem alto teor mitótico. Clinicamente, inicia-se com a formação de lesões eritematosas atróficas, podendo evoluir para lesões edemaciadas e/ou ulceradas, atingindo o tecido submucoso, sendo frequente a presença de sangramento e dor intensa (9–13).

De acordo com o modelo patobiológico da doença, a mucosite oral pode perpassar por cinco fases: (1) iniciação, (2) geração da mensagem, (3) amplificação do sinal, (4) ulceração e (5) cicatrização (11,14). O início da inflamação ocorre com um dano irreversível no DNA, causado pelos quimioterápicos, resultando numa injúria na camada basal da mucosa. Este dano tecidual pode desencadear a liberação de espécies reativas de oxigênio e ativar a sinalização de eventos submucoso, como a quimiotaxia de células de defesa (macrófagos) e sinalização de citocinas inflamatórias (TNF-a e IL-6). A partir desses eventos, as células da camada basal da mucosa oral começam a sofrer apoptose e não proliferam-se para promover o reparo tecidual, formando uma ulceração, que pode ser colonizada por patógenos oportunistas e gerar sepse nos pacientes. A recuperação das lesões ocorre espontaneamente, variando o período de cicatrização, mas normalmente a mucosite regride em até 15 dias (11,13,14).

Figura 1. Fisiopatologia da Mucosite Oral



Adaptado de: Sonis ST. ***A Biological Approach to Mucositis.*** J Support Oncol 2004;2:31-36.

Esta injúria acontece em cerca de 46% a 70% dos pacientes infanto-juvenis tratados com quimioterapia, iniciando entre o 3º e 15º dia após administração do medicamento. No entanto, observa-se resolutividade, em 90% dos casos, entre a 2ª e 3ª semanas subsequentes ao término do ciclo quimioterápico (4,14).

Nos casos mais avançados, classificados como Mucosite Oral Grave (MOG), a presença de ulcerações confluentes, dor e sangramento impedem os pacientes de se alimentarem por via oral (11,13). Esta condição causa debilidade nutricional, aumento do tempo de hospitalização e de custos, e aumento do risco de bacteremia e sepse, sendo necessária a interrupção do tratamento quimioterápico (15,16).

Para mensurar a evolução clínica da mucosite oral são utilizadas algumas escalas que avaliam a integridade dos tecidos bucais, dentre elas estão: *National Cancer Institute* (NCI), *Common Terminology Criteria for Adverse Eventes* (CTCAE), *World Health Organization* (WHO) e *Children's Cancer Group* (CCG), no entanto não são direcionadas apenas para os efeitos adversos na cavidade bucal. A escala *Oral Assessment Guide* (OAG) modificado, por sua vez, é um instrumento destinado apenas para a avaliação oral, sendo de fácil aplicação. Consiste numa escala com escores variando de 1 a 3, onde o valor 1 indica normalidade da mucosa, o 2 indica alterações leves e/ou moderadas, com relação à integridade epitelial ou função, e o 3 é o resultado de complicações severas. São avaliados 8 itens e, ao final, é feito um somatório, totalizando em valores compreendidos entre 8 e 24, não havendo um ponto de corte para a estimação da mucosite (17).

Com relação à etiologia da doença, alguns fatores já foram relacionados ao aparecimento desta inflamação, dentre os quais uns são inerentes ao indivíduo, como: mielossupressão, neoplasia de base, idade, sexo, fatores genéticos, condições sistêmicas e orais; outros são resultados dos quimioterápicos empregados nos protocolos terapêuticos: citoxicidade terapêutica direta (posologia), poder cumulativo das drogas, aumento dos ciclos quimioterápicos e associação de drogas (4,14).

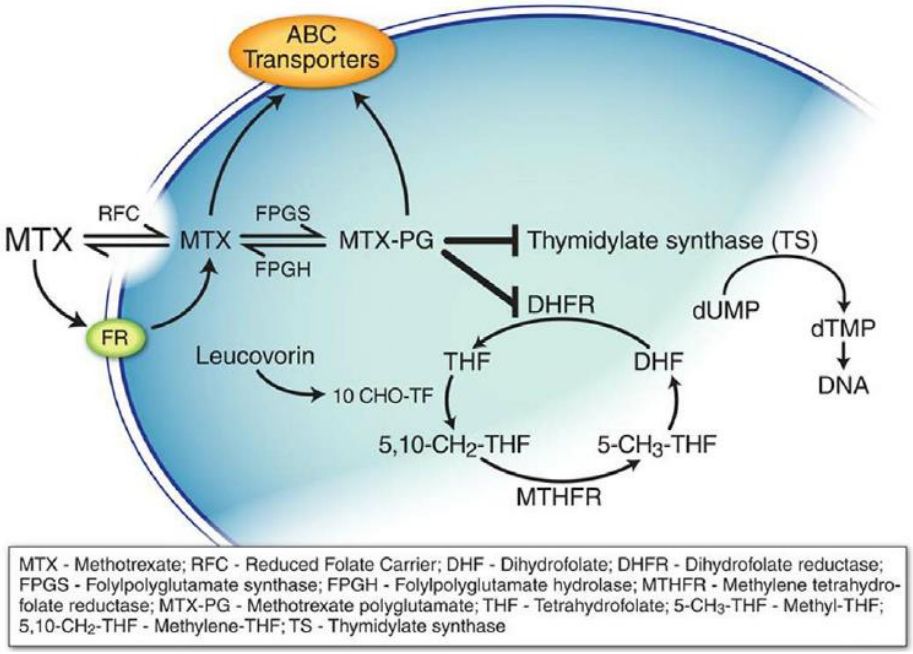
Dentro dos fatores genéticos, os polimorfismos de nucleotídeo único (SNP – *Single Nucleotide Polymorphism*) também têm sido referidos como um indício associativo no aparecimento desta complicação, sobretudo em genes que regulam o metabolismo do Metotrexato (MTX). Este quimioterápico é empregado no tratamento de diferentes tipos de câncer infanto-juvenil, inclusive nas leucemias, que são as neoplasias mais prevalentes em pacientes pediátricos (3,4,18–24).

1.3 Metotrexato

O MTX é um metabólito antagonista do folato, que atua na fase S do ciclo celular, impedindo a síntese do DNA e interrompendo a proliferação celular exponencial de células malignas e de tecidos sadios com esta característica. Dessa forma, ocorre uma redução no reparo epitelial da mucosa, contribuindo para o desenvolvimento da mucosite oral (25,26).

Essa droga atua em uma variedade de proteínas, começando por sua entrada na célula e metabolização e finalizando com sua saída (27,28). A ação desta medicação no ciclo do folato interfere no metabolismo de aminoácidos e síntese de nucleotídeos resultando em um bloqueio na formação do DNA e, consequentemente, morte celular (27,29). Em adição, é possível que o MTX tenha influência sobre o perfil de metilação do DNA, uma vez que ele pode diminuir os níveis de doadores de radical metil (SAM) gerados no ciclo do folato. Como visto no plasma sanguíneo de crianças com leucemia linfoblástica aguda tratadas com MTX (30).

Figura 2. Metabolização intracelular do MTX



McBride, A. et al. (2012). Suspected Methotrexate Toxicity From Omeprazole. Journal of Pharmacy Practice, 25(4), 477–485.

Uma vez que a toxicidade do MTX esteja ligada a sua capacidade de inibir a enzima metionina-adenosina transferase e, consequetemente, a produção do doador de radical metil (SAM), alterações no pefil de metilação de DNA derivada de seu tratamento não é exatamente um evento surpresa (31). De fato, alguns estudos mostraram que o MTX

está associado a alterações no perfil de metilação do DNA. Em um modelo de defeito do tubo neural de camundongos, o MTX causou hipometilação de DNA (32). Em contraste, baixas doses de metotrexato causaram hipermetilação global em pacientes com artrite reumatóide (33,34). Outro estudo sugeriu que neurotoxicidade derivada do tratamento com MTX em leucemias pode estar relacionada a alterações no perfil de metilação de DNA tanto global quanto sítio-específica (35).

O mecanismo pelo qual o MTX causa citotoxicidade está associado à intervenção causada na produção de purinas e pirimidinas, bloqueando a síntese do DNA (28,36). Sua metabolização e armazenamento ocorre nas células hepáticas, onde há um maior estresse oxidativo, implicando em um retardo na depuração da droga. Esse retardo também pode acontecer nos túbulos renais, causando obstrução e, consequentemente, maior pernamência do MTX no plasma sanguíneo, aumentando os riscos dos efeitos adversos (37,38)

Alguns genes codificam proteínas que atuam diretamente na farmacodinâmica do MTX, como as proteínas de efluxo de drogas, a exemplo das transmembrana da família ABC, e enzimas que participam do ciclo do folato (38–40).

1.4 Metilenotetrahidrofolto redutase (*MTHFR*)

O gene *MTHFR* codifica a enzima metilenotetrahidrofolato redutase que participa da síntese do folato, substância essencial na formação do DNA e reprodução celular (41,42). Essa enzima promove a conversão da 5,10–metilenotetrahidrofolato em 5–metiltetrahidrofolato, que resultará nos aminoácidos metionina e S–adenosilmetionina (SAM), utilizados para as reações de metilação do DNA (41,42).

O gene *MTHFR* está localizado no cromossomo 1p36.3, estando o polimorfismo C677T localizado no éxon 04 deste gene. Indivíduos que apresentam essa substituição de nucleotídeos, resultando nos genótipos CT e TT, codificam uma enzima alterada portando um aminoácido diferente do esperado. Nesta substituição pode-se observar a troca do códon que codifica o aminoácido alanina para o códon que codifica o aminoácido valina (43).

O polimorfismo C677T leva a uma diminuição na atividade enzimática em cerca de 70% nos indivíduos com genótipo TT e 35% nos indivíduos com genótipo CT, o que configura um aumento moderado das concentrações plasmáticas de homocisteína, levando a um quadro de hiper-homocisteinemia, quadro que está associado a doenças vasculares, pela toxicidade da homocisteína no endotélio vascular (44,45).

Vários polimorfismos já foram descritos para esse gene, em especial o polimorfismo C677T, que é um dos mais estudados na literatura e mostra associação com diversas doenças inflamatórias e tumorais, tais como: artrite reumatoide (46), arterosclerose e doenças cardiovasculares (47,48), psoríase (49), câncer de tireoide (50) e mucosite oral (51).

Uma coorte realizada no Egito com 40 pacientes oncopediátricos diagnosticados com leucemia estudou a relação entre o polimorfismo C677T e a ocorrência de mucosite oral quimioinduzida por MTX. Os autores concluíram que os indivíduos com genótipo TT, comparados aos ídivíduos com genótipo CC, apresentavam associação com a ocorrência da mucosite oral (51).

1.5 DNA-metiltransferase (*DNMT3B*)

As DNA-metiltransferases são enzimas que utilizam o substrato SAM, gerado no ciclo do folato, para transferir radicais metil (CH_3) ao DNA. Essas enzimas são classificadas em: metilases de manutenção, responsável pela metilação das fitas de DNA em processo de replicação e que já apresentavam metilação prévia, como a DNMT1, e as metilases *de novo* (DNMT2 e DNMT3), responsáveis pela maioria dos processos de metilação naqueles sítios sem metilação prévia (52). Essas enzimas promovem a metilação do DNA resultando comumente na diminuição da transcrição ou silenciamento gênico e, em alguns casos, na ativação da expressão gênica (53,54).

A enxima DNA metiltransferase 3B é codificada pelo gene *DNMT3B* localizado no cromossomo 20q11.2, estando o polimorfismo C-149T localizado na região promotora deste gene, sendo um dos mais estudados dentre tantos outros já descritos na literatura. Esse polimorfismo leva a um aumento da transcrição deste gene, que pode levar a uma hipermetilação do DNA e, consequentemente, diminuição ou silenciamento da expressão gênica (52)

Diversas doenças inflamatórias, neurológicas e tumorais já foram associadas à presença do polimorfismo C-149T, tais como: Alzheimer (55), Parkinson (55), câncer de pumão (56), gliomas (57), câcer colorretal (58), câncer de cabeça e pescoço (19). No entanto, ainda não foi realizado nenhum estudo associando a presença deste polimorfismo com a ocorrência de mucosite oral.

1.6 Proteínas da família ABC (ATP Binding Cassete)

Os genes da família *ABC* codificam proteínas de transporte transmembrana, envolvidas no processo de efluxo de drogas, que utilizam a energia do trifosfato de adenosina (ATP) de ligação e de hidrólise para realização desta função. Proteínas da subfamília *C* e *G* transportam o MTX para fora das células (59,60).

A proteína ABC membro 2 da subfamília C é codificada pelo gene *ABCC2* localizado na posição 10q24.2. Um dos polimorfismos mais estudados desse gene é o C-24T, localizado na região promotora do gene *ABCC2*. Essa alteração leva a uma diminuição na expressão desse gene e já foi associada a várias doenças, dentre elas estão: câncer de pâncreas (62), câncer de mama (63), sepse (64), disfunção tubular renal (65) e mucosite oral (40).

O estudo que associou a ocorrência da mucosite oral com o polimorfismo C-24T foi realizado na China com 112 crianças com leucemia tratadas com MTX. Foi observado, nessa coorte, que este polimorfismo não somente estava associado com a ocorrência da doença, como também com a sua forma mais grave (MOG) (40).

Outra proteína transmembrânica da família ABC envolvida no efluxo de MTX é a membro 2 da subfamília G, que está localizada na posição cromossômica 4q22.1. Alguns polimorfismos já foram estudados no gene *ABCG2*, sobretudo dois que estão associados a uma maior permanência de drogas no interior da célula: o G34A e o C421A (20,21,66,67).

O polimorfismo G34A resulta em uma troca do códon do aminoácido valina pelo códon do aminoácido metionina, consequentemente ocorre a formação de uma proteína estruturalmente e funcionalmente diferente. O mesmo acontece com o polimorfismo C421A, que ocorre a troca do códon do aminoácido glicina pelo códon do aminoácido lisina. Estudos já observaram que há um retardo na atividade dessas proteínas quando elas apresentam esses polimorfismos. Como consequência, as drogas permanecem por mais tempo no interior da célula e podem aumentar o risco citotóxico no indivíduo (20,21,66,67).

Alguns efeitos biológicos podem ser observados em decorrência desses polimorfismos, como por exemplo: aumento de ácido úrico em indivíduos que portam o G34A (68) e associação com a incidência de câncer colorretal (69).

1.7 Polimorfismos genéticos e medicina personalizada

Essa identificação de associações contribuiria para a melhoria do cuidado preventivo de pacientes que, porventura, apresentassem riscos ao desenvolvimento da doença, contribuindo com a redução da morbidade e severidade da mucosite oral em crianças e adolescentes. Uma vez que seria possível lançar mão da medicina personalizada, recurso ideal para minimizar reações adversas.

O estudo de polimorfismos genéticos consegue identificar biomarcadores que poderiam ser utilizados como ferramentas para uma terapia personalizada. Essa, por sua vez, tem sido considerada como uma importante ferramenta para um tratamento mais eficiente, mais barato e com menos eventos adversos, na qual a dose e o tempo de tratamento se ajustariam de acordo com a resposta do paciente (70). O efeito de polimorfismos genéticos na suscetibilidade à mucosite oral induzida pelo MTX ainda é pouco explorado, enquanto o apelo para a prática da medicina personalizada vem crescendo significativamente (70,71).

Embora alguns estudos apontem a associação entre polimorfismos genéticos e a incidência da mucosite oral (40,51), ainda são poucos os estudos que investigam essa associação na população infanto-juvenil tratada com MTX e os resultados encontrados não são convergentes (4,72). Ainda não foram realizados estudos desta natureza na população brasileira, dado observado pela revisão sistemática apresentada no capítulo 2 dessa dissertação, e não há na literatura a associação do polimorfismo *DNMT3B* C-149T (rs2424913) com a ocorrência da mucosite oral, justificando a originalidade do presente estudo.

Diante disto, objetiva-se investigar a associação dos polimorfismos genéticos: *MTHFR* C677T (rs1801133), *DNMT3B* C-149T (rs2424913), *ABCC2* C-24T (rs717620), *ABCG2* G34A (rs2231137) e *ABCG2* C421A (rs2231142) no desenvolvimento da mucosite oral em crianças submetidas a protocolos quimioterápicos envolvendo o MTX.

2 CAPÍTULO 1

O manuscrito a seguir foi submetido para publicação no periódico *Clinical Oral Investigations*.

THE *ABCG2* POLYMORPHISM (RS2231142) IN PEDIATRIC PATIENTS WITH LEUKEMIA TREATED WITH METHOTREXATE MAY CONTRIBUTE TO ORAL MUCOSITIS, AND AGE AND LEUKOCYTE COUNT MAY CONTRIBUTE TO SEVERITY

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ABSTRACT

Objective The objective of this study was to investigate the relationship between the genetic polymorphisms *MTHFR* C677T (rs1801133), *DNMT3B* C-149T (rs2424913), *ABCC2* C-24T (rs717620), *ABCG2* G34A (rs2231137) and *ABCG2* C421A (rs2231142) and the development of oral mucositis in pediatric patients undergoing chemotherapy involving methotrexate.

Materials and Methods A retrospective and prospective longitudinal study was conducted with 64 patients, and oral mucositis was evaluated by the modified Oral Assessment Guide (OAG). The biological material used was saliva, from which oral epithelial cells were isolated and DNA was extracted. The polymorphisms were analyzed by PCR-RFLP method. Demographic, hematological and biochemical data were collected from medical records. Statistical analysis was performed using the SPSS software adopting a p-value of 0.05.

Results Male sex predominated (56.2%), and the mean age was 10.8 years (± 4.9). Among the patients with oral mucositis, 65.3% were affected by the severe form. There was an association between the rs2231142 polymorphism and oral mucositis (RR = 8.33; CI = 0.09 - 0.47; p = 0.02) as well as between severe mucositis and leukocyte count (p = 0.03) and between severe mucositis and age (p = 0.02).

Conclusions It is concluded that the presence of the *ABCG2* polymorphism (rs2231142) increases the likelihood of oral mucositis. Younger patients with fewer leukocytes are more likely to develop severe mucositis. Female sex and every 10,000-platelet increase are protective factors against the onset of oral mucositis.

Clinical Relevance This polymorphism may be a promising biomarker for predicting the occurrence of oral mucositis.

Keywords: Oral mucositis. Genetic polymorphisms. Children. Chemotherapy.

INTRODUCTION

Chemo-induced oral mucositis consists of an inflammatory mucosal response to the biochemical action of antineoplastic drugs, being more frequent and more severe in children [1, 2]. This inflammation is observed in patients undergoing therapeutic protocols involving methotrexate (MTX), the main chemotherapeutic agent used in the treatment of leukemias [3, 4]. This cancer type is more common in children and adolescents (26%), and acute lymphoblastic leukemia (ALL) is the most prevalent in children (40%) [5, 6].

Clinically, oral mucositis begins with the formation of atrophic erythematous lesions, which can progress to edematous and/or ulcerated lesions that reach the submucosal tissue. The occurrence of bleeding and intense pain is frequently observed in the most advanced stages [1, 7]. For the more severe inflammatory responses, it necessary to pause chemotherapy because patients are unable to eat orally, causing nutritional impairment and, consequently, affecting them systemically [8–10].

Some factors have already been related to the onset of this inflammation, including myelosuppression, direct therapeutic cytotoxicity, increased chemotherapy cycles, cumulative power of drugs, underlying neoplasia, oral and systemic patient conditions and age [2, 11]. Genetic factors, such as polymorphisms in specific genes, have also been reported as an associated factor of the onset of this complication, especially in genes involved in MTX metabolism [7, 12–16].

MTX reaches the cytosol through transmembrane proteins, interferes with cell metabolism and can be eliminated by efflux proteins [17, 18]. It is an antagonist of folic acid and acts by inhibiting enzymes that participate in the folate cycle. The folate cycle is important for amino acid metabolism, nucleotide synthesis and DNA methylation [17, 19].

The mechanism by which MTX causes cytotoxicity is associated with its ability to intervene in the folate cycle, leading to a deficiency in the synthesis of nucleotides and thus blocking DNA synthesis and promoting cell death [17, 19]. Because the toxicity of MTX is linked to its ability to inhibit methionine-adenosine transferase and, consequently, the production of the methyl radical donor (S-adenosylmethionine, SAM), changes in the DNA methylation profile derived from MTX treatment are not unexpected [20]. In fact, some studies have shown that MTX is associated with changes in the DNA methylation profile, both global and site-specific [21–23].

Thus, the interest in studying specific genes that encode proteins that act on MTX pharmacodynamics is justified. The *MTHFR* gene, for example, encodes the enzyme methylenetetrahydrofolate reductase, which participates in the folate cycle, converting 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is used for the production of the amino acid methionine and SAM and for DNA methylation reactions [24, 25]. MTX inhibits the first and other enzymes in the chain, leading to a decrease in tetrahydrofolate and, consequently, a decrease in *MTHFR* substrate, which may lead to reduced levels of methionine and SAM [4, 26–28].

The *DNMT3B* gene encodes DNA methyltransferase, which transfers a methyl radical (CH_3) to DNA from the SAM radical generated in the folate cycle. This enzyme promotes DNA methylation, which may lead to a decrease in transcript levels or complete silencing of gene expression [29, 30]. Polymorphisms in the *MTHFR* gene that reduce enzymatic activity (rs1801133) and/or in the *DNMT3B* gene that increase gene expression (rs2424913) have been associated with inflammatory diseases that affect the oral cavity, including mucositis in the case of *MTHFR* [29, 31].

Among the genes encoding transmembrane proteins, genes from the *ABC* family (ATP-binding cassette), such as *ABCC2* and *ABCG2,* encode transport proteins involved in the drug efflux process, including MTX [32, 33]. Polymorphisms in the genes of subfamilies *C* and *G* member 2 are associated with loss of function of transport proteins and have also been associated with inflammatory diseases of the oral cavity [34–37].

Although some studies have noted an association between genetic polymorphisms and the incidence of oral mucositis [12, 13, 15, 34, 38], there are few studies that have investigated this association, and the results are not necessarily convergent [11, 39].

Therefore, the objective of the present study was to investigate the relationship between the genetic polymorphisms *MTHFR* C677T (rs1801133), *DNMT3B* C-149T (rs2424913), *ABCC2* C-24T (rs717620), *ABCG2* G34A (rs2231137) and *ABCG2* C421A (rs2231142) and the development of oral mucositis in children and adolescents undergoing MTX chemotherapy protocols. This is the first study of this nature conducted in Brazil and the first to test the association of C-149T (rs2424913), in the *DNMT3B* gene, with oral mucositis.

MATERIALS AND METHODS

Ethical considerations

The procedures for conducting this study complied with the guidelines and norms that regulate research involving human subjects and was approved by the Research Ethics Committee of the Center for Health Sciences of Federal University of Paraíba (CAAE: 64249317.3.0000.5188). These procedures were also in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Study design

A retrospective observational analytical study was conducted. Data were obtained at the pediatrics department of Hospital Napoleão Laureano (HNL), a reference center for cancer treatment in the state of Paraíba, located in the Northeast region of Brazil, between July 2015 and March 2019. Laboratory data were processed in the Laboratory of Human Molecular Genetics of the Department of Molecular Biology, Federal University of Paraíba (DBM/CCEN/UFPB, for its acronym in Portuguese).

Patients who underwent oncological treatment and those in the maintenance phase were investigated by means of direct observation. Information on the oral health of patients was collected from dental records.

At the beginning of the study, the main oral complications were observed via weekly assessments of each included patient, over four weeks following the beginning of oncological treatment, considering that this is the period when the main oral disorders resulting from therapy are reported [11].

Sample selection

The sample consisted of patients aged between 3 and 19 years who were diagnosed with leukemia (acute lymphoblastic, acute myeloid, chronic myeloid and acute promyelocytic) by the HNL and who underwent chemotherapy involving MTX. These therapeutic protocols were proposed by the Brazilian Childhood Leukemia Treatment Group (Grupo Brasileiro de Tratamento da Leukemia in Infância - GBTLI) [40].

The sample size was calculated based on the prevalence of leukemia patients treated at HNL between 2015 and 2018. The mean number of patients was 45.5 per year, or 29% of all patients. However, the largest number of patients with leukemia treated at

the hospital over these four years was adopted to include a larger number of patients. In 2016, 52 patients were recorded, or 31% of all patients.

The sample size was calculated using the program OpenEpi version 3.01 (Bill and Melinda Gates Foundation - Emory University, Atlanta, USA), adopting the prevalence of patients with leukemia diagnosed in 2016. A type I error (α) of 5% (two-tailed) and a type II (β) error of 20% were adopted, as was a statistical power of 80%, and the sample size was estimated as 46 patients. However, the sample size included 64 individuals, obtained by census sampling.

Eligibility criteria

Inclusion criteria: Individuals aged between 3 and 19 years; primary diagnosis of leukemia; patients who underwent chemotherapy with MTX or containing this substance in the chemotherapeutic compound, who were monitored weekly until the 4th week of treatment; patients in the maintenance phase (who completed chemotherapy); and patients who had dental follow-up during treatment.

Exclusion criteria: Patients with cognitive or motor skills impairment hindering the collection procedures; and patients with a compromised health status or in isolation from respiratory contact, with restricted care, whose condition would preclude the performance of the data and biological sample collection procedures.

Data collection and genetic polymorphism analysis

Oral mucosal condition: Mucositis was evaluated using the modified Oral Assessment Guide (OAG), which is an easy-to-apply instrument that measures changes in the oral mucosa resulting from chemotherapy in pediatric patients. It consists of a scale with scores ranging from 1 to 3, where 1 indicates normality of the mucosa, 2 indicates mild and/or moderate changes in epithelial integrity or function, and 3 indicates severe complications. Eight items are evaluated, and the item scores are summed, with a final score ranging from 8 to 24, with no cutoff point for the estimation of mucositis [41]. Previously calibrated individuals (Kappa = 0.87) were responsible for evaluating the oral mucosa. Patients were classified according to mucositis severity: severe oral mucositis, grade 3, and mild/moderate, grades 1 or 2.

Collection of data for the study sample: General data of the patients were recorded on a specific form to describe the study sample. Data from patients who were in maintenance and were followed up by the dental team during treatment were searched in

the dental and/or medical records as well as in databases of previous studies conducted at HNL. Hematological and biochemical data were obtained from HNL software that stores the laboratory tests of patients throughout cancer treatment. The hematological and biochemical data of patients who presented with oral mucositis were recorded according to disease severity. The hematological and biochemical data of patients categorized as having severe mucositis with a score of 3 on any of the parameters evaluated by the OAG, were collected from this time point. However, the hematological and biochemical data of patients who did not develop severe mucositis corresponded to the time of occurrence of maximum changes in the parameters evaluated by the OAG. If only one parameter was altered, the hematological and biochemical data corresponding to this time point were recorded. The collected hematological data of patients who did not develop oral mucositis corresponded to the last chemotherapy session.

Collection of oral epithelial cells: Oral mucosa cells were obtained from a 1-minute mouth rinse with 6 mL of autoclaved 3% dextrose. Next, 3 mL of TNE solution was added, and the sample was taken to the laboratory, where it was centrifuged at 11,000 rpm for 10 minutes, after which the supernatant discarded [42]. Lysing solution was added to the oral epithelial cell pellet, and the samples were frozen at -20 °C until DNA extraction.

DNA extraction and quantification: Genomic DNA was purified using 8 M ammonium acetate [42]. The amount of purified DNA and its purity were measured in a spectrophotometer at an OD ratio of. DNA with a 260/280 ratio above 1.8 was considered pure.

Analysis of single nucleotide polymorphisms (SNPs) in the MTHFR, DNMT3B, ABCC2 and ABCG2 genes: Genetic polymorphisms were analyzed using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP), in which DNA fragments were amplified by PCR, followed by enzymatic digestion by restriction enzymes (REs). Polymorphisms were identified by the actions of REs; the presence or absence of polymorphisms is determined from the products generated during digestion. The genotypes were analyzed by electrophoresis in 6% polyacrylamide gel stained with 0.5% silver nitrate or GelRed® (Biotium) [35, 43–47]. The PCR primers, their references, the optimal conditions for PCR, the REs, digestion time, SNP localization and size of the original and cleaved fragments are provided in Table 1.

Statistical Analysis

The data were categorized and organized into a database to allow analysis. Data normality was assessed using the Kolmogorov-Smirnov test. Relationships between variables were measured using the chi-square test, Fisher's exact test, Mann-Whitney U test and Student's t-test, adopting a p-value of 0.05, in SPSS version 20.0 (IBM, New York, USA). For Hardy-Weinberg equilibrium analysis, the Court Lab-HW calculator (Seattle Pacific University, Washington, USA) was used. Binary logistic regression analyses (univariate and multiple) were also performed, with variable selection performed with the stepwise-backward technique, in R version 3.4.2 (The R Foundation, St. Louis, Missouri, USA), for the outcome "oral mucositis." Patients were divided into two groups: "1" with oral mucositis and "0" without oral mucositis. All independent variables evaluated in the present study were analyzed sequentially by univariate logistic regression, and those with p-values at the 0.30 level were included together in a multiple logistic regression model. The multiple logistic regression model was fit to the 5% significance level by the stepwise-backward method. Subsequently, the fit of the multiple logistic regression model was determined by obtaining the receiver operating characteristic (ROC) curve.

RESULTS

The demographic and clinical data of the study sample are provided in Table 2. Of a total of 64 patients, 56.25% (n = 36) were male, with a median age of 9 years (minimum 3 and maximum 19 years). Among the diagnosed cases of leukemia, ALL was the most common (82.82%). Regarding the condition of the oral mucosa after chemotherapy involving MTX, 65.63% (n = 42) of patients developed mucositis, and 61.91% (n = 26) presented with severe mucositis.

The hematological and biochemical data are provided in Table 3. The groups with and without mucositis were compared; there were no differences in platelet (mm^3) ($p = 0.074$), hemoglobin (g/dl) ($p = 0.520$), urea (mg/dl) ($p = 0.871$), and creatinine (mg/dl) ($p = 0.420$) levels or the urea/creatinine ratio (mg/dl) ($p = 0.577$); however, there was a difference in white blood cell count (mm^3) ($p = 0.013$).

The mucositis severity data, compared with other parameters, are presented in Figure 1. Differences were observed between the groups in regard to the variables age and leukocyte count. There was a higher frequency of severe mucositis in children

between 5 and 10 years of age, and the predominant age of the patients in the mild/moderate group was 15 to 19 years old (p = 0.029). That is, when comparing the age range of both groups, severe mucositis occurred in younger patients. In addition, the number of leukocytes in individuals who developed the most severe form of the disease was lower than that in individuals who presented a mild or moderate form (p = 0.038).

The allelic frequencies of four genes were balanced according to Hardy-Weinberg equilibrium (HWE): C677T (rs1801133), p = 0.47; C-24T (rs717620) p = 0.07; G34A (rs2231137) p = 0.59; and C421A (rs2231142) p = 0.54. However, C-149T (rs242424913), in the *DNMT3B* gene, showed an equilibrium lower than 5% (p = 0.0023) due to the prevalence of individuals with the CT genotype (n = 32, 76.2%) in the group of patients with mucositis, which generated an HWE of p = 0.0006 in this group. The polymorphism data are provided in Table 4.

The non-rare C allele was the most frequent in the entire population for the *MTHFR*, *DNMT3B* and *ABCC2* genes, and no differences were detected between the groups. For the *ABCG2* gene (rs2231137), the non-rare G allele was also the most frequent in the entire population. For the *ABCG2* gene (rs2231142), the rare A allele was more frequent in individuals with mucositis (10.7%) than in individuals without mucositis (0%). Likewise, the CA genotype was more frequent in individuals with mucositis (22.5%) than in individuals without mucositis (0%). Thus, an association was identified between the C421A polymorphism (rs2231142), in the *ABCG2* gene, and the incidence of oral mucositis (p = 0.022), with the presence of the A allele being indicative of this association (RR = 8.33; CI = 0.09 - 0.47; p = 0.024).

According to the generated logistic regression model (Table 5), it is inferred that both female sex and higher platelet count constitute protective factors against oral mucositis and that being female reduces the odds of a patient having oral mucositis by 1.61-fold compared to being male. In addition, at each increase of 10,000 platelets, the odds of a patient having oral mucositis are reduced by 1.05-fold.

A ROC curve was generated for the fitted model presented in Table 5. The accuracy of patient classification regarding oral mucositis, based on the variables sex and platelet count, was 72.40%, and the positive and negative predictive values were 44.40% and 18.90%, respectively.

DISCUSSION

Oral mucositis is one of the most common adverse reactions (46% to 70%) in pediatric patients undergoing chemotherapy protocols involving MTX due to the high proliferative capacity of the mucosa [2, 6]. An increase in the severity of this inflammation may compromise the course of anticancer treatment, requiring its pause, and negatively affect the quality of life of these children and adolescents [8–10].

This adverse reaction is commonly observed at HNL [11], drawing the attention of researchers seeking to better understand the genetic aspects involved in the development of chemo-induced oral mucositis in children and adolescents. The genes chosen have some relationship with the metabolism of MTX, the main therapeutic resource in the treatment of leukemias, which are the most frequent cancers observed in the juvenile population [35, 43–46].

MTX inhibits the exponential proliferation of malignant cells; however, it does not restrict its action to only those cells and acts on healthy tissue with a high proliferative capacity. As the oral mucosa exhibits high proliferation, it is affected by the use of this chemotherapeutic agent, and the tissue response to this stimulus allows the development of mucositis [9, 48, 49].

Clinical data from the present study showed that the majority of children and adolescents with leukemia treated with MTX had oral mucositis and that severe mucositis was the most incident form. These data suggest that the GBTLI protocols used in these patients have high cytotoxic levels, causing direct therapeutic toxicity to oral mucosa cells. Because this is the first Brazilian study of this nature, there are differences compared to previous studies conducted in different countries. In these other studies, therapeutic protocols different from those developed by the GBTLI are administered for leukemias, and lower incidences of oral mucositis are observed [12–15, 38, 50–53].

In addition, the use of the modified OAG scale allows a more thorough assessment of oral parameters, specifically measuring changes in the oral mucosa resulting from chemotherapy [41]. It is suggested, therefore, that the modified OAG may overestimate the diagnosis of oral mucositis compared to other scales that evaluate this inflammation as just another adverse effect of chemotherapy protocols.

It was also observed that the severity of oral mucositis was associated with age and leukocyte count. Severe mucositis was more frequently identified in younger individuals. Prior studies have reported that younger individuals have a higher risk of

developing severe mucositis, suggesting that a high mitotic index in the mucosal cells of these individuals is a likely factor contributing to the severity of mucositis [1, 39, 54].

Regarding the leukocyte count, patients with severe mucositis had lower counts than those who did not develop the severe form of the disease. In the pathobiological model of oral mucositis, severe mucositis is the third stage of disease progression, termed ulcerative or bacterial. At this stage, there is exposure of connective tissue and contact of the lesion with opportunistic bacteria, which attempt to invade and spread in the body, causing systemic infections [1, 2]. Individuals undergoing chemotherapy are already immunosuppressed and susceptible to opportunistic infections [55]. Therefore, severe mucositis promotes a reduction in the number of leukocytes by contributing to the entry of opportunistic microorganisms, which recruit the few defense cells still present in immunosuppressed individuals [56].

Genetic polymorphism data from the present study showed an association between the C421A polymorphism (rs2231142) in the *ABCG2* gene and the incidence of oral mucositis. None of the patients without oral mucositis had the rare A allele, suggesting that the presence of this allele increases the risk of developing oral mucositis by 8-fold. In addition, the CA genotype was more frequent in individuals with mucositis and responsible for the association with this inflammation. C421A (rs2231142) results in the loss of function of the protein encoded by the *ABCG2* gene [36, 57]; the polymorphism causes an amino acid substitution (Gln141Lys), resulting in a structurally and functionally defective protein [35–37, 44]. Another study showed that the presence of the rare A allele results in a reduction in protein efflux capacity and consequently in a greater accumulation of MTX inside cells [18, 58].

There are no data in the literature on the C421A polymorphism (rs2231142) associated with adverse effects of MTX in children with hematologic tumors; however, two studies previously performed in adults [3, 59] concluded that there was no association of this polymorphism with any toxic effect of MTX, especially with regard to oral mucositis, in opposition to the findings of the present study. In contrast, the polymorphisms evaluated herein, C677T *MTHFR* (rs1801133) and C-24T *ABCC2* (rs717620), which were not associated with oral mucositis, were analyzed in previous studies with children and adolescents and showed an association with this inflammation [14, 15].

The study of genetic polymorphisms is able to identify biomarkers that could be used as tools for personalized therapy. This, in turn, has been considered an important

strategy for developing more efficient, inexpensive treatments with fewer adverse events, in which the treatment dose and duration would be adjusted according to the patient's response [60]. The effect of genetic polymorphisms on MTX-induced susceptibility to oral mucositis is still little explored, while the appeal for precision medicine has been growing significantly [60, 61]. Thus, it is necessary to explore genetic polymorphisms in the context of oral mucositis in different populations to better understand the molecular mechanisms involved, contributing to a therapy for which adverse events, such as oral mucositis, are minimal.

Regarding protective factors against oral mucositis, the logistic regression model fitted in the present study showed that a higher platelet count is a protective factor against oral mucositis, i.e., individuals with platelet reduction are at a higher risk of developing the disease. It is known that the indirect effect of chemotherapy results in bone marrow suppression, which then leads to a decrease in platelet and leukocyte levels, which in turn are correlated with the occurrence of oral mucositis [62, 63].

Platelets are anucleate structures, derived from megakaryocytes, produced in the bone marrow and responsible for hemostasis [64]. Currently, other functions of these elements have been investigated, such as their role in inflammation [65, 66]. In the presence of tissue damage, platelets activate and trigger fibrinogen bonds between each other and with endothelial cells to promote a decrease in tissue damage and tissue repair [67, 68]. In addition, when activated, platelets trigger a signaling pathway in the immune system and promote binding with leukocytes in order to quell antigen microorganisms invasion in the body [69]. It is suggested, therefore, that platelet reduction hinders tissue repair and exposes the patient to opportunistic infections, thus increasing the inflammatory response.

Additionally, the logistic regression model generated showed that female sex was a protective factor against the occurrence of oral mucositis. This indicates that there was a higher prevalence of oral mucositis among males, which can be explained by a higher number of boys in the sample. Data from Brazilian epidemiological surveys show a higher incidence of childhood cancer in males [70]; therefore, the data in the present study corroborate this finding.

The limitations of this study include the lack of assessing plasma MTX levels, as we preferred to collect noninvasive saliva samples rather than peripheral blood samples. It is important to mention that the present study was the first of its kind in the country,

and our data show an association of genetic, demographic and clinical factors with oral mucositis resulting from chemotherapy involving MTX for leukemias in children.

CONCLUSIONS

The presence of the C421A *ABCG2* polymorphism (rs2231142) increases the likelihood of oral mucositis. Younger children and adolescents with lower leukocyte counts are more likely to develop severe oral mucositis. Female patients and each 10,000-platelet increase are protective factors against the onset of oral mucositis.

CONFLICT OF INTEREST - There is no conflict of interest.

ACKNOWLEDGEMENTS - This work was financial supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) (407394/2016-8) and Programa de Pós Graduação em Odontologia - Universidade Federal da Paraíba, Brazil. José MC Viana Filho was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil). Marina C Coêlho and Ingrid C Queiroz were supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil).

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Table 01. PCR-RFLP Characterization.

SNP	*PRIMERS*	*REF.*	*PCR*	*RE*	*GENOTYPE*
MTHFR *rs1801133*	*F: 5'-TGA AGG AGA AGG TGT CTG CGG GA–3'* *R: 5'-AGG ACG GTG CGG TGA GAG TG–3'*	[43]	35x 61ºC 30''	*Hinf* I	198bp (CC) 198pb/175pb/23bp (CT) 175pb/23bp (TT)
DNMT3B *rs2424913*	*F: 5'-TGG CTA CCA GGT CTC CTT GGC C-3'* *R: 5'-GGT AGC CGG GAA CTC CAC GG-3'*	[45]	35x 68,4ºC 1'	*XmaJ* I	230bp (CC) 230pb/172pb/58bp (CT) 172pb/58bp (TT)
ABCC2 *rs717620*	*F: 5'-TAA ATG GTT GGG ATG AAA GG-3'* *R:5'-GCT TTA GAC CAA TTG CAC ATC-3'*	[46]	35x 54ºC 30''	*Bbs* I	301bp (TT) 301pb/188bp/113pb (CT) 188pb/113bp (CC)
ABCG2 *rs2231142*	*F: 5'-ATG TTG TGA TGG GCA CTC TG-3'* *R: 5'-TGC TGA TCA TGA TGC TTT CAG-3'*	[35]	35x 50ºC 30''	*Tru1*1	100pb/84bp (CC) 100pb/84pb/64pb/36bp (CA) 84pb/64pb/36bp (AA)
ABCG2 *rs2231137*	*F: 5'-AAA TGT TCA TAG CCA GTT TCT TGG A-3'* *R:5'-ACA GTA ATG TCG AAG TTT TTA TCG CA-3'*	[44]	35x 58ºC 30''	*BseM* I	291bp (GG) 291pb/261pb/30bp (GA) 261pb/30bp (AA)

F = forward, R = reverse; REF = reference articles; PCR: number of cycles, temperature and annealing time; RE = restriction enzyme; bp = base pairs

Table 02. Descriptive analysis of demographic and clinical data of the studied population.

Sex		**n (%)**
Male		36 (56.25%)
Female		28 (43.75%)
	Overall	64 (100%)
Age		
Mean age (± standard deviation)		10.81 (±4.98)
Median age (max - min)		09 (3 – 19)
Basic disease		**n (%)**
Acute Lymphoblastic Leukemia (ALL)		53 (82.82%)
Acute Myeloid Leukemia (AML)		07 (10.93%)
Acute Promyelocytic Leukemia (ALI)		03 (4.68%)
Chronic Myeloid Leukemia (CML)		01 (1.57%)
	Overall	64 (100%)
Oral mucosa condition		**n (%)**
With mucositis		42 (65.63%)
Without mucositis		22 (34.37%)
	Overall	64 (100%)
Severity of oral mucositis		**n (%)**
With severe oral mucositis		26 (61.91%)
No severe oral mucositis		16 (38.09%)
	Overall	42 (100%)

Table 03. Descriptive analysis of hematological and biochemical parameters of the studied population (mean ± standard deviation).

Hematological and biochemical parameters	**With mucositis**	**Without mucositis**	***p-valor**
Hemoglobin (g/dl)	9.1 (±1.91)	10.06 (±2.32)	0.520
Leukocytes (mm^3)	4.564.28 (±4.802.02)	13.359.09 (±36.883.54)	**0.013**
Platelets (mm^3)	17.1738.09 (±13.0640.27)	23.7818.18 (±15.1183.47)	0.074
Urea (mg/dl)	19.09 (±10.27)	16.82 (±6.68)	0.871
Creatinine (mg/dl)	0.46 (±0.18)	0.45 (±0.12)	0.420
Urea/Creatinine Ratio (mg/dl)	44.66 (±26.38)	39.34 (±19.95**)**	0.577

***** Mann-Whitney U test

Table 04. Association between allelic and genotypic frequency with presence or absence of mucositis of the studied population.

Gene and SNP	Frequencies					
	Allelic / Genotypes	**With mucositis n (%)**	**Without mucositis n (%)**	**p-value**	**RR**	**HWE**
***MTHFR* C677T (rs1801133)** (n=64)	C	56 (66.7%)	32 (73.0%)			
	T	28 (33.3%)	12 (37.0%)	p=0.482		
	CC	18 (43.9%)	11 (50.0%)		–	p=0.47
	CT	20 (46.3%)	10 (45.5%)			
	TT	04 (9.8%)	01 (20.0%)	p=0.738		
***DNMT3b* C46359T (rs2424913)** (n=64)	C	44 (52.0%)	24 (55.0%)			
	T	40 (48.0%)	20 (45.0%)	p=0.815		
	CC	06 (14.3%)	06 (27.0%)		–	p=0.0023
	CT	32 (76.2%)	12 (55.0%)			
	TT	04 (9.5%)	04 (18.0%)	p=0.389		
***ABCC2* C-24T (rs717620)** (n=63)	C	72 (88.0%)	36 (82.0%)			
	T	10 (12.0%)	08 (18.0%)	p=0.359		
	CC	33 (79.2%)	15 (68.2%)		–	p=0.07
	CT	06 (15.3%)	06 (27.3%)			
	TT	02 (5.5%)	01 (4.5%)	p=0.475		
***ABCG2* G34A (rs2231137)** (n=64)	G	79 (94.0%)	41 (93.0%)			
	A	05 (6.0%)	03 (7.0%)	p=0.847		
	GG	37 (88.0%)	19 (86.0%)		–	p=0.59
	GA	05 (12.0%)	03 (14.0%)			
	AA	0	0	p=0.870		
***ABCG2* C421A (rs2231142)** (n=64)	C	75 (89.3%)	44 (100%)			
	A	09 (10.7%)	0	**p=0.024**		
	CC	33 (78.5%)	22 (100%)		8.33	p=0.54
	CA	09 (21.5%)	0			
	AA	0	0	**p=0.022***		

*Chi-square and Fisher Exact; HWE = Hardy-Weinberg Equilibruim

Table 05. Logistic regression model adjusted for the outcome “oral mucositis”

Variable	p-value	OR	Error	CI95%	
				Upper Limit	Lower Limit
Female*	0.021	0.240	0.620	1.455	-0.975
Platelets **	0.021	0.951	0.022	0.994	0.907

* Reference Category = male. ** With each increase of 10,000 platelets.

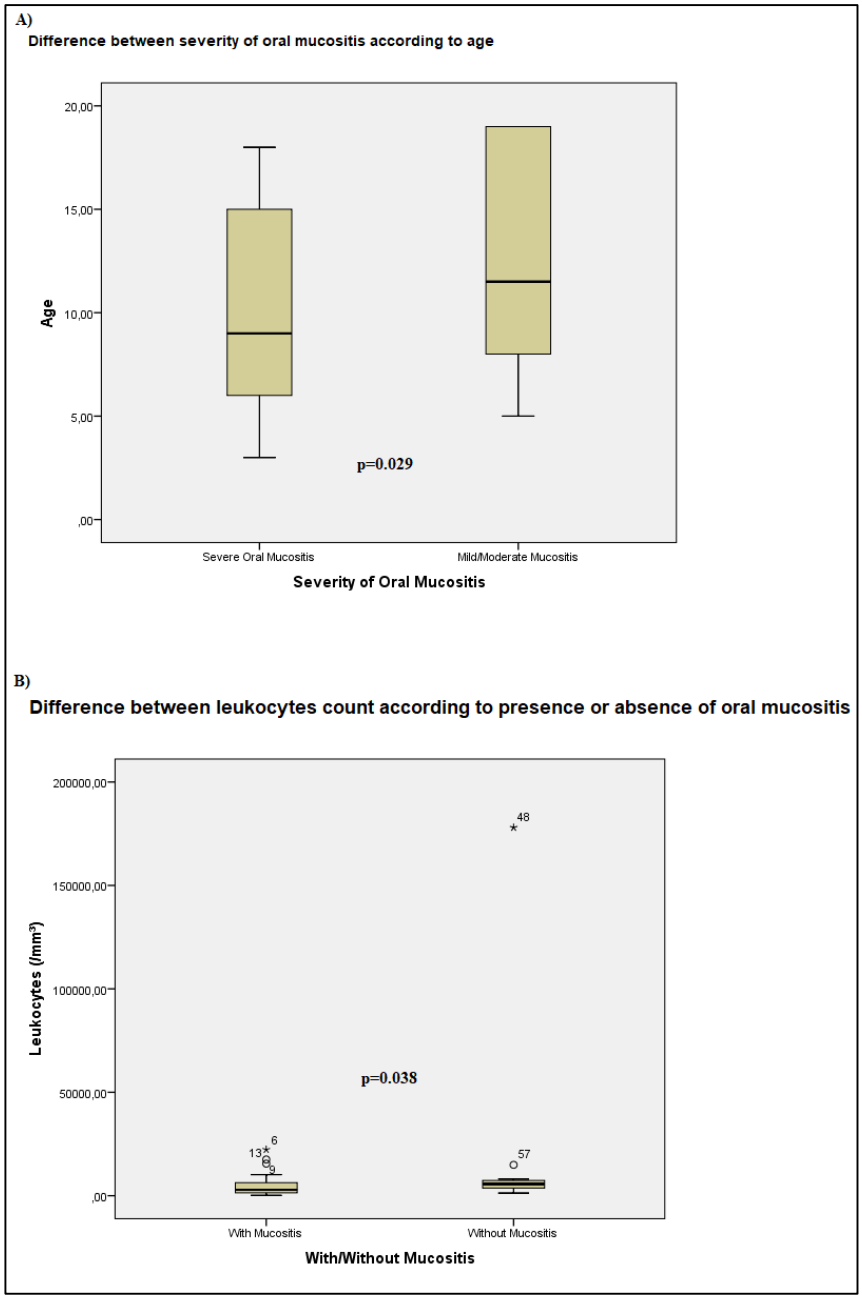

Fig. 1 Differences between variables according to presence or absence of mucositis and severity of mucositis. **a)** Age and leukocyte count in patients with mild/moderate oral mucositis and with severe oral mucositis **b)** Age and leukocyte count in patients with oral mucositis and without oral mucositis.

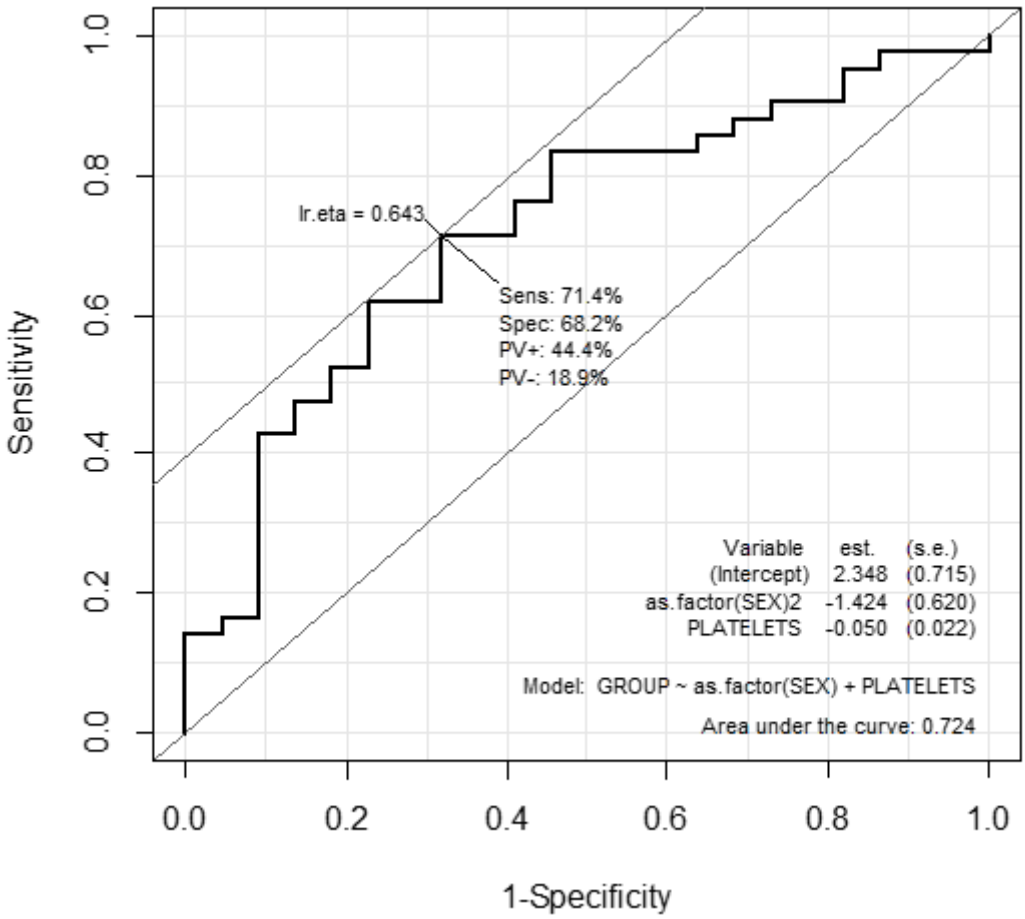

Fig. 2 Receiver Operating Characteristic curve (ROC curve) for the outcome "oral mucositis" adjusted for the variables gender and number of platelets.

3 CAPÍTULO 2

O manuscrito a seguir será submetido para publicação no periódico *Oral Diseases*.

Genetic changes and methotrexate chemoinduced oral mucositis in pediatric patients: A systematic review and meta-analysis

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Genetic changes and methotrexate chemoinduced oral mucositis in pediatric patients: A systematic review and meta-analysis

ABSTRACT

Objective: To evaluate the association between genetic alterations and the occurrence of methotrexate chemoinduced oral mucositis in pediatric patients.
Methods: Literature search was performed on five databases, until may/2019. Observational studies with pediatric patients submitted to chemotherapy with methotrexate, bearers of any polymorphisms and that presented oral mucositis as the outcome were selected through the PECO strategy. Quality of methodological approach was assessed using the Fowkes & Fulton tool (1991).
Results: Two meta-analysis were conducted and the quality of evidence was assessed with GRADE. Sixty-one studies were found, out of which 9 were considered eligible. Total sample size was 1775 individuals ranging from 1 to 19 years old with leukemia or lymphoma. The *MTHFR* C677T was the most investigated regarding its association to oral mucositis occurrence. Seven studies presented bias during sample selection. No associations were observed between polymorphisms and oral mucositis occurrence, neither in the meta-analysis that included all polymorphisms (RR=1.14; I^2=18%; p=0.22) nor in the one that included the repeated ones (RR=0.96; I^2=20%; p=0.29), which had a low level of scientific evidence.
Conclusions: There was no association, however, the risk of bias and low quality of scientific evidence demonstrate the necessity of new studies with greater methodological rigor.

Key words: Child; Polymorphism, Genetic; Stomatitis

INTRODUCTION

Chemotherapy-induced oral mucositis is an inflammatory response of the oral mucosa to the biochemical action of anticancer medications. This condition is more frequent and severe in children due to the high mitotic activity in this population. Clinically, it starts with the formation of atrophic erythematous lesions that may evolve to a swollen and/or ulcerative state, penetrate into the submucosal tissue and frequently result in bleeding and severe pain (Ribeiro, 2015; Sandoval, Koga, Buloto, Suzuki, & Lauria, 2003; Santos, Coradini, Ribeiro, & Caldo-Teixeira, 2010; Sonis, 2004; Sonis & Yuan, 2017).

Single-nucleotide polymorphisms (SNPs) have been referred to as associative indications of this inflammation, especially in genes related to the metabolism of methotrexate (MTX). This drug belongs to the class of antifolate metabolites administered in treatment protocols for leukemias (Bachour & Sonis, 2018; Farias et al., 2010; Haenisch et al., 2007; Ribeiro et al., 2018; Salimizand, Amini, Abdi, Ghaderi, & Azadi, 2016; Wu et al., 2015). MTX hinders the production of folate and inhibits DNA replication, thus reducing epithelial healing and contributing to the development of oral mucositis (Park & Shin, 2016).

Some genes encode proteins that act directly in the pharmacodynamics of MTX. *MTHFR*, for example, encodes the enzyme methylenetetrahydrofolate reductase, which takes part in the metabolism of folate molecules (Costea, Moghrabi, Laverdiere, Graziani, & Krajinovic, 2006; Pakakasama et al., 2007). A meta-analysis published in 2012 studied the association between polymorphisms rs1801133 and rs1801131, both in in *MTHFR*, and direct therapeutic toxicity in children and adults with leukemia. This study concluded that the presence of the T allele was associated to a threefold increase in the chance of developing oral mucositis (Yang, Hu, & Xu, 2012). Other genes also participate in the metabolism of MTX and bring about the interest of clinicians and researchers towards investigating additional polymorphisms that may be associated to the occurrence of this disease during anticancer therapy.

Another example is the *TYMS* gene, which encodes thymidylate synthase, an enzyme involved in DNA replication and repair. A tandem repeat polymorphism (rs34743033) in *TYMS* has been proportionally correlated to oral mucositis reduction (Radtke et al., 2013). However, a recent meta-analysis revoked the association of this polymorphism with MTX-induced oral mucositis in adults (Oosterom et al., 2018).

The *ABCG2*, *ABCC2*, *ABCB1/MDR1* genetic sequences encode membrane transporters involved in cellular drug efflux (Borst & Elferink, 2002; Gottesman, Fojo, & Bates, 2002); while *SLC19A1* encodes membrane proteins related to folate transport (Zhao, Diop-Bove, Visentin, & Goldman, 2011). Studies have demonstrated that polymorphisms in these genes are also associated to the occurrence of chemotherapy-induced oral mucositis (Aplenc et al., 2005; Bektas-Kayhan et al., 2013; Gutierrez-Camino, Oosterom, Den Hoed, et al., 2017; S.-G. Liu et al., 2017; Y. Liu et al., 2014a; Radtke et al., 2013; Shimasaki et al., 2006; Tantawy et al., 2010; Fausto Zaruma-Torres et al., 2015).

Additional factors such as direct therapeutic toxicity, physical and clinical conditions, cancer type and demographic profile have been studied and associated to mucositis occurrence. However, few studies investigated the genetic alterations associated to the disease in children and adolescents, and results are not necessarily convergent (Damascena et al., 2018; Ribeiro, Limeira, de Castro, Bonan, & Valença, 2017). Also, systematic reviews about genetic polymorphisms and oral mucositis in patients submitted to chemotherapy treatment usually focus on a single gene in adult populations. Therefore, an evidence review about the effect of genetic polymorphisms on oral mucositis occurrence in children who experience chemotherapy is necessary.

To that end, this study aimed to investigate the relation between genetic polymorphisms and the occurrence of oral mucositis in pediatric patients submitted to chemotherapy protocols involving MTX.

MATERIALS AND METHODS

A literature systematic review previously registered in the PROSPERO database (CRD42018110235) was conducted following the PECO strategy, which aimed to respond the question: “Children with genetic polymorphisms have higher probability of developing oral mucositis after chemotherapy with MTX?”. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were respected and directed this systematic review with meta-analysis.

Bibliographic searches were performed in six databases: PubMed, Scopus, Lilacs, Cochrane, Web of Science and Open Grey. MeSH terms and their synonyms were applied for the construction of search strategies, according to the syntax of each bibliographic base (Table 1). Searches were carried out until may of 2019, without language or publication date restrictions. A reference manager software (Mendeley Desktop) was

used for duplicate removal as well as title and abstract screening. Studies with animals, case reports, observational studies with adults and clinical studies that did not have oral mucositis as the outcome were excluded.

The search followed the PECO strategy, collecting observational studies that included children and/or adolescents submitted to chemotherapy with MTX (P), bearing any genetic polymorphisms (E), compared to individuals without polymorphisms (C) in order to determine association with oral mucositis (O). To identify such association, the included studies should present statistical analysis and measures of association such as odds ratio (OR) or relative risk (RR).

Two reviewers (JMCVF and HVD) independently performed bibliographic searches as well as title and abstract screening, in order to identify potentially eligible studies. Studies that fit the inclusion criteria underwent full-text screening, as well as the ones in which title/abstract were not sufficient to confirm the eligibility criteria. Full-text screening also occurred independently. Queries concerning study eligibility were settled through consensus or discussion with a third reviser (BMS).

Two reviewers (JMCVF and HVD) extracted and tabulated the studies' data concerning authors, year of publication, country, study design, participants details (age, number of participants in case and control groups, and sample source), study methods (evaluation criteria and follow-up period) and main results (measures of association and p values). Authors were contacted through e-mail whenever any information was not clear. When there was no response, the study would not be included in the meta-analysis.

Quality of methodological approach and risk of bias of the included studies were evaluated according to the recommendations described by Fowkes and Fulton (1991) (Fowkes F & Fulton P, 1991), considering the following aspects: study design, sample representativeness, validity, reproducibility, distorting influences and participant loss. The reviewers assessed the studies independently according to the guidelines of the tool's original reference (Fowkes F & Fulton P, 1991). Sample size and selection as well as study instruments were considered fundamental aspects for quality assessment of the included studies. Control group definition and participant blindness were not considered key factors to determine each study's methodological quality. A qualitative summary was made taking into account all items from the checklist, according to the following categories: major problem (++), minor problem (+), no problem (0) and not applicable (NA). In cases of divergence between the examiners, a third researcher performed the same evaluation and proposed a consensus (BMS). Finally, study quality was summarized

considering possible impairment due to bias, confounding variables and/or likelihood that results occurred by chance. When the studies did not present necessary information or were incomplete, an attempt of contact with the authors was made through e-mail and response was awaited for a 5-week period.

Data were assessed quantitatively using the Review Manager software (Review Manager v. 5.2, The Cochrane Collaboration; Copenhagen, Denmark) aiming to evaluate the association between the presence of genetic polymorphisms and oral mucositis occurrence. Two meta-analysis were carried out: one with all polymorphisms that were found and another with the polymorphisms that were repeated amongst the studies. Only bias-free studies were included in both meta-analysis.

Heterogeneity was measured with the I^2 index and sensitivity analysis were performed to estimate and verify each studies' influence, one by one, in the subgroups' and grouped results when heterogeneity was substantial or considerable (50 to 100%) (Ryan & Group, 2013). The random effect model was applied since the studies were not functionally equivalent.

Certainty of evidence was assessed according to the Grading of Recommendations Assessment, Development and Evaluation system (GRADE), which ranks the quality of evidence and strength of recommendations as foundations for decision-making. This classification is carried out in four levels representing result consistency and precision: high, moderate, low and very low, (Guyatt, Oxman, Kunz, Vist, & Falck-Ytter, Y Schunemann, 2008). Observational studies begin this evaluation with low quality and certainty of evidence. If problems concerning risk of bias, inconsistency, indirect evidence, imprecision, publication bias and possible confounding factors are identified, the certainty of evidence for these studies is considered very low.

RESULTS

Initially, 61 potentially eligible registers were found, followed by removal of 8 duplicates. After title and abstract screening, 35 studies were excluded for not presenting the desired outcome. Eighteen studies were analyzed in full-text screening, out of which 9 were excluded: two were systematic reviews, four did not use MTX and three did not present only children and/or adolescents in their samples (Figure 1). The nine remaining studies (Aplenc et al., 2005; Bektas-Kayhan et al., 2013; Gutierrez-Camino, Oosterom, Den Hoed, et al., 2017; S.-G. Liu et al., 2017; Y. Liu et al., 2014a; Radtke et al., 2013; Shimasaki et al., 2006; Tantawy et al., 2010; Fausto Zaruma-Torres et al., 2015) were

submitted to data extraction and quality assessment (Table 2), according to Fowkes & Fulton (1991) (Fowkes F & Fulton P, 1991) for methodological rigor and risk of bias evaluations (Table 2).

Studies were from China, EUA, Germany, Turkey, the Netherlands, Mexico, Japan and Egypt. Considering all 9 studies, the total number of patients was 1775 children aged from 1 to 19 years old, with Acute Lymphoblastic Leukemia (LLA) (Aplenc et al., 2005; Bektas-Kayhan et al., 2013; Gutierrez-Camino, Oosterom, Den Hoed, et al., 2017; S.-G. Liu et al., 2017; Y. Liu et al., 2014a; Radtke et al., 2013; Shimasaki et al., 2006; Tantawy et al., 2010; Fausto Zaruma-Torres et al., 2015) or lymphoma (Shimasaki et al., 2006). In total, 8 studies were cohorts and one was a case-control study (Aplenc et al., 2005).

During the assessment of methodological quality for the selected studies, seven of them (Bektas-Kayhan et al., 2013; Gutierrez-Camino, Oosterom, Den Hoed, et al., 2017; S.-G. Liu et al., 2017; Y. Liu et al., 2014a; Shimasaki et al., 2006; Tantawy et al., 2010; Fausto Zaruma-Torres et al., 2015) indicated risk of bias in their sample selection for groups with and without mucositis. Although the studies present risks in their selection process, their results were trustworthy and did not compromise their initial proposal. Therefore, all studies were deemed methodologically admissible according to the used qualifier.

Twenty-nine polymorphisms were found in different genes related to proteins that take part in the pharmacodynamics of MTX and *MTHFR* C677T was the most investigated one. The genotyping techniques consisted of real-time PCR, PCR-RFLP, sequencing and Sequenom MassARRAY. Scales from the *National Cancer Institute* (NCI), *Common Terminology Criteria for Adverse Events* (CTCAE), *World Health Organization* (WHO) and *Children's Cancer Group* (CCG) were applied for mucositis diagnosis (Table 3). Associations were found between polymorphisms *miR*-1206 rs2114358, *ABCC2* rs717620, *TYMS* rs34743033, *MDR1* rs1045642, *MTHFR* rs1801133 and occurrence of oral mucositis (Table 3).

From all of the evaluated children, 1276 were classified for oral mucositis occurrence without considering disease severity. Also, among the studied individuals, a frequency of 16,5% (n=210) was observed for mucositis.

All studies (Aplenc et al., 2005; Bektas-Kayhan et al., 2013; Gutierrez-Camino, Oosterom, Den Hoed, et al., 2017; S.-G. Liu et al., 2017; Y. Liu et al., 2014a; Radtke et al., 2013; Shimasaki et al., 2006; Tantawy et al., 2010; Fausto Zaruma-Torres et al., 2015)

that contained information on the polymorphisms' genotypic frequency associated to the occurrence of oral mucositis were elected for the meta-analysis, aiming to verify if these genetic variations are associated to the development of the outcome (Figure 2).

Six studies (Bektas-Kayhan et al., 2013; Gutierrez-Camino, Oosterom, Den Hoed, et al., 2017; S.-G. Liu et al., 2017; Y. Liu et al., 2014a; Tantawy et al., 2010; Fausto Zaruma-Torres et al., 2015) were included in the meta-analysis (Figure 2A), presenting investigations on 25 polymorphisms and their relation to mucositis appearance. Therefore, the analysis considered the number of children bearing or not the polymorphisms while presenting or not the outcome.

In general, polymorphisms are not related to the occurrence of oral mucositis (RR=1.14; IC=0.97-1.35; p=0,22; I^2=18%). Only one study demonstrated an association between a polymorphism (*rs717620*) and the disease (Y. Liu et al., 2014a). This SNP encodes a mutated ABCC2 membrane transporter, causing a twofold increase in the risk of stomatits occurrence among children and adolescents who bear the genetic variant when compared to those without the polymorphism (RR=2.23; IC=1.36-3.64).

Aiming to evaluate the isolated relative risk of these genetic alterations in the appearance of the outcome, studies that investigated repeated polymorphisms (Figure 2B) were grouped for a second meta-analysis. Once more, no associations between presence of polymorphisms and oral mucositis occurrence were observed (RR=0.96; IC=0.68-1.36; p=0.29) (Figure 2A).

GRADE results (Figure 3) showed inconsistency problems in the meta-analysis involving all studies as well as imprecision problems in the meta-analysis with repeated polymorphisms, culminating in a very low level of scientific evidence for the primary studies.

DISCUSSION

This study investigated the relation between genetic polymorphisms of sequences involved in MTX metabolism and oral mucositis occurrence in pediatric patients with leukemia or lymphoma.

From 2005 to 2017, studies evaluating the association of different polymorphisms to the occurrence of MTX-induced oral mucositis in pediatric patients were published. The genes analyzed in such studies encode proteins that metabolize MTX, including *ABCB1, ABCG2, ABCC5, ABCC2, ABCC4, CNOT4, miR-1206, miR-2053, MTHFR, TYMS, RFC1, SLC19A1, SLCO1B1* and *XO* (Aplenc et al., 2005; Bektas-Kayhan et al.,

2013; Gutierrez-Camino, Oosterom, den Hoed, et al., 2017; S.-G. Liu et al., 2017; Y. Liu et al., 2014b; Radtke et al., 2013; Shimasaki et al., 2006; Tantawy et al., 2010; F Zaruma-Torres et al., 2015).

Although some primary studies found an association between the investigated polymorphism (*miR-1206* rs2114358, *ABCC2* rs717620, *TYMS* rs34743033, *MDR1* rs1045642, *MTHFR* rs1801133) and disease occurrence, no associations were observed in the meta-analysis that included all polymorphisms nor in the one including only the repeated genetic variants (*ABCB1* rs1128503 and rs1045642) (Bektas-Kayhan et al., 2013; S.-G. Liu et al., 2017; F Zaruma-Torres et al., 2015).

These results demonstrate that the investigated polymorphisms did not have a clinically significant effect on oral mucositis occurrence. However, these genetic alterations might explain risk prediction for the disease's development (Bachour & Sonis, 2018; Sonis & Yuan, 2017); thus, new studies are encouraged, focusing on the investigation of other genomic *loci*.

Different mucositis grading scales were used (NCI, CTCAE, WHO and CCG) for ranking of disease progression. There was no convergence concerning these scales amongst the studies that correlated polymorphism presence to disease occurrence. Some studies (Bektas-Kayhan et al., 2013; Y. Liu et al., 2014a; Radtke et al., 2013; Shimasaki et al., 2006; Fausto Zaruma-Torres et al., 2015) consider presence of oral mucositis since its lightest forms, which corresponds to erythematous lesions with low levels of pain (2, 38) (scores $\geq$ 1). On the other hand, other studies (Aplenc et al., 2005; Gutierrez-Camino, Oosterom, Den Hoed, et al., 2017; S.-G. Liu et al., 2017; Tantawy et al., 2010) only classify the more severe states of mucosal inflammation as oral mucositis, with presence of confluent ulcerative lesions, pain and bleeding (Sonis, 2004; Villa & Sonis, 2015) (scores $\geq$ 3).

This may have influenced the meta-analysis' results since 4 studies (Aplenc et al., 2005; Gutierrez-Camino, Oosterom, Den Hoed, et al., 2017; S.-G. Liu et al., 2017; Tantawy et al., 2010) evaluated the association of polymorphisms with only the severe form of the disease, underestimating the fact that early stages of mucositis development also correspond to cytotoxic effects of chemotherapy treatment (Bachour & Sonis, 2018) and should be accounted for in associative analysis.

For future studies, it is suggested that associative analysis with polymorphisms also include the initial states of oral mucositis since these may be indicate a decrease in

enzymatic activity due to polymorphic alteration. Therefore, oral mucositis classifications ranging from the lightest to the most severe stages should be accounted for.

Methodological issues also might explain the findings in this study. The absence of some results in primary studies, such as the genotyping of polymorphisms non-related to oral mucositis or even the subgrouped genotypic frequencies in relation to the outcome (Aplenc et al., 2005; Y. Liu et al., 2014a; Radtke et al., 2013; Shimasaki et al., 2006; Tantawy et al., 2010) interfered with the execution of both meta-analysis.

It is suggested for primary studies to include a description of the observed genotypic frequencies in each group (with and without oral mucositis) so that it is possible to evaluate the effect of each genotype in the association with the outcome and in inflammation severity.

The GRADE evaluation began with a low level of certainty of evidence since observational studies were included (Villa & Sonis, 2015). This certainty was reduced even more when inconsistency problems were detected in the meta-analysis involving all the polymorphisms due to the overlapping of confidence intervals in the effect estimate. Thus, it was observed a statistically equivalent distribution of patients, healthy ones and those presenting the disease, in both groups with and without the polymorphism.

In relation to imprecision, the certainty of evidence of the subgrouped meta-analysis (*ABCB1* gene) was lowered since the effect size was based in a relatively small number of events. The ideal number, in this case, would be at least 300 individuals per subgroup, using the effect size's absolute value (relative risk – RR) and the 95% confidence interval obtained in the subgrouped meta-analysis for *ABCB1*.

Therefore, the quality assessments of scientific evidence were considered very low since the observational studies presented limited effect estimate of their own results and the observed effect size for a sample size was far from the needed amount. Nevertheless, the absence of association demonstrated in this study should be carefully evaluated. Better-designed primary studies are necessary to increase the certainty of evidence and corroborate the findings of this meta-analysis.

In summary, it is important to emphasize that primary studies presenting distinct populations, each one submitted to evaluation with different clinical instruments and methodological approaches, were grouped in this study in order to answer one research question. Hence, future studies should be conducted in a more homogeneous and rigorous way when it comes to methodological execution in the interest of presenting more

consistent and strict results concerning the association of genetic polymorphisms to chemotherapy-induced oral mucositis occurrence in children and adolescents.

CONCLUSIONS

There is no association between genetic polymorphisms of genes that encode proteins involved in methotrexate pharmacodynamics and occurrence of oral mucositis in children and/or adolescents. However, this result reveals the need for primary studies of higher methodological quality when it comes to both sample selection and effect estimation of their findings.

CONFLICT OF INTEREST

The authors declare no conflict of interest. Master's degree students performed this study with their mentors and teachers from the module "Topics in Systematic Reviews", part of the Graduate Program in Odontology of the Federal University of Paraíba (João Pessoa, PB - Brazil).

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Table 1. Search Keys

PUBMED	(((((((((child[MeSH Terms]) OR child, preschool[MeSH Terms]) OR infant[MeSH Terms]) OR child[Title/Abstract]) OR children[Title/Abstract]) OR preschool child[Title/Abstract]) OR Preschool Children[Title/Abstract]) OR infant[Title/Abstract]) OR infants[Title/Abstract]) AND (((Polymorphism, Genetic[MeSH Terms]) OR genetic polymorphism[Title/Abstract]) OR Genetic Polymorphisms[Title/Abstract])) AND (((((((((Stomatitis[MeSH Terms]) OR Stomatitis[Title/Abstract]) OR Stomatitides[Title/Abstract]) OR Oral Mucositis[Title/Abstract]) OR Oral Mucositides[Title/Abstract]) OR Oromucositis) OR Oromucositides))
SCOPUS	TITLE-ABS-KEY("child" OR "child, preschool" OR "infant" OR "children" OR "preschool child" OR "Preschool Children" OR "infants") AND TITLE-ABS-KEY("Polymorphism, Genetic" OR "genetic polymorphism" OR "Genetic Polymorphisms") AND TITLE-ABS-KEY("Stomatitis" OR "Stomatitides" OR "Oral Mucositis" OR "Oral Mucositides" OR "Oromucositis" OR "Oromucositides")
WEB OF SCIENCE	TS=("child" OR "child, preschool" OR "infant" OR "children" OR "preschool child" OR "Preschool Children" OR "infants") AND TS=("Polymorphism, Genetic" OR "genetic polymorphism" OR "Genetic Polymorphisms") AND TS=("Stomatitis" OR "Stomatitides" OR "Oral Mucositis" OR "Oral Mucositides" OR "Oromucositis" OR "Oromucositides")
COCHRANE	ID Search Hits #1 MeSH descriptor: [Child] explode all trees 1409 #2 child* 133052 #3 MeSH descriptor: [Child, Preschool] explode all trees 491 #4 preschool child* 33905 #5 MeSH descriptor: [Infant] explode all trees 15022 #6 infant* 51357 #7 #1 or #2 or #3 or #4 or #5 or #6 149277 #8 MeSH descriptor: [] explode all trees 0 #9 Drug Therpy* 3 #10 MeSH descriptor: [Drug Therapy] explode all trees 132117 #11 Chemotherap* 58435 #12 Pharmacotherap* 9389 #13 #8 OR #9 OR #10 OR #11 OR #12 179098 #14 MeSH descriptor: [Polymorphism, Genetic] explode all trees 3000 #15 Genetic Polymorphism* 5361 #16 #14 or #15 5861 #17 MeSH descriptor: [Stomatitis] explode all trees 943 #18 Stomatiti* 3105 #19 Oral mucositi* 1905 #20 Oromucositi* 1 #21 #17 or #18 or #19 or #20 4486 #22 #7 and #13 and #16 and #21 0
LILACS	(tw:((mh:(child)) OR (mh:("child, preschool")) OR (mh:(infant)) OR (tw:(child)) OR (tw:(children)) OR (tw:(preschool child)) OR (tw:(preschool children)) OR (tw:(infant)) OR (tw:(infants)))) AND (tw:((mh:(drug terapy)) OR (tw:(Drug Therp*)) OR (tw:(Chemotherap*)) OR (tw:(Pharmacotherap*)))) AND (tw:((mh:(Polymorphism, Genetic)) OR (tw:(genetic polymorphism*)))) AND (tw:((mh:(Stomatitis)) OR (tw:(Stomatiti*)) OR (tw:(Oral Mucositi*)) OR (tw:(Oromucositi*))))

OPEN GREY	(child OR "child, preschool" OR infant OR child OR children OR preschool child OR preschool children OR infant OR infants) AND (drug terapy OR Drug Therp* OR Chemotherap* OR Pharmacotherap*) AND (Polymorphism, Genetic OR genetic polymorphism*) AND (Stomatitis OR Stomatiti* OR Oral Mucositi* OR Oromucositi*)

Table 2. Quality assessment according to Folkes & Fulton (1991)

Guideline	Checklist	Gutierrez-Camino et al, 2017	Liu el al, 2017	Torres et al, 2015	Liu Yan et al, 2014	Radtke et al, 2013	Bektas et al, 2011	Tantawy et al, 2010	Shimasaki et al, 2006	Aplenc et al, 2005
Study design appropriate?	**Cross-sectional (prevalence)**	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Cohort (prognosis)	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Controlled Trial (treatment)	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Cohort, case-control, cross-sectional (cause)	0	0	0	0	0	0	0	0	0
Study sample representative?	**Source of sample**	0	0	0	0	0	0	0	0	0
	Sampling method	0	0	0	0	0	0	0	0	0
	Sample size	+	+	+	+	0	+	+	+	0
	Entry criteria and exclusions	0	+	0	0	0	0	0	+	0
Control group acceptable?	**Definition of control**	0	0	0	+	+	0	+	+	+
	Source of control	+	+	+	+	+	0	+	+	0
	Matching/randomization	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Comparable characteristics	+	+	+	+	+	0	+	+	+
Quality of measurements and outcomes?	**Validity**	0	0	0	0	0	0	0	0	0
	Reproducibility	0	0	0	0	0	0	0	0	0
	Blindness	NA	NA	NA	NA	NA	NA	NA	NA	NA

	Quality control	0	0	0	0	0	0	0	0	0
Completeness?	**Compliance**	0	0	0	+	+	0	+	+	+
	Drop out	0	0	0	0	0	0	0	+	0
	Death	0	0	0	0	0	0	0	0	0
	Missing data	0	0	0	+	+	0	0	0	0
Distorting influence?	**Extraneous treatments**	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Contamination	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Changes over time	0	0	0	0	0	0	0	0	0
	Confounding factors	0	0	0	0	0	0	0	0	0
	Distortion reduced by analysis	0	0	0	0	0	0	0	0	0
Summary questions	**Bias – Are the results erroneously biased in a certain direction?**	NO	NO	NO	NO	NO	NO	NO	NO	NO
	Confounding – Are there any serious confounding or other distorting influences?	NO	NO	NO	NO	NO	NO	NO	NO	NO
	Chance – Is it likely that the results occurred by chance?	NO	NO	NO	NO	NO	NO	NO	NO	NO

Table 3. Descriptive Data Extraction

AUTHOR	GEOGRAPHIC LOCATION	TYPE OF STUDY	OVERALL SAMPLE	POLYMORPHISMS	ANALYSIS METHOD (Polymorphism / Mucositis)	RESULTS (p/OR/IC)	CONCLUSION
Gutierrez-Camino et al, 2017	Netherlands	Coorte	117	1. *CNOT4 rs3812265;* 2. *miR-1206* rs2114358; 3. *miR-2053* rs10505168	PCR em tempo real / NCI≥3	1. *CNOT4:* 0.45/0.69/0.27-1.80 2. *miR-1206*: 0.02/3.58/1.12-11.46 3. *miR-2053*: 0.12/2.50/0.76-8.24	Association between occurrence of oral mucositis and miR-1206 polymorphism
Liu et al, 2017	China	Coorte	322	4. *SLCO1B1* rs11045879; 5. *SLCO1B1* rs4149056; 6. *SLCO1B1* rs2306283; 7. *SLCO1B1* rs10841753; 8. *SLC19A1* rs1051266; 9. *SLC19A1* rs3788200; 10. *SLC19A1* rs1131596; 11. *SLC19A1* rs2838958; 12. *ABCB1* rs1128503; 13. *ABCB1* rs1045642; 14. *ABCG2* rs2231137	Sequenom MassARRAY / NCI≥3	4. *SLCO1B1*: 0.86/0.92/0.36-2.33 5. *SLCO1B1*: 0.99/0/0 6. *SLCO1B1*: 0.48/1.28/0.64-3.00 7. *SLCO1B1*: 0.99/0.99/0.33-3.00 8. *SLC19A1*: 0.43/0.70/0.29-1.67 9. *SLC19A1*: 0.45/0.71/0.30-1.70 10. *SLC19A1*: 0.67/0.85/0.40-1.79 11. *SLC19A1*: 0.75/0.88/0.41-1.90 12. *ABCB1*: 0.054/2.01/0.99-4.10 13. *ABCB1*: 0.93/0.97/.048-1.96 14. *ABCG2*: 0.43/1.51/0.54-4.21	There was no association between oral mucositis and the polymorphisms studied.
Torres et al, 2015	Mexico	Coorte	35	15. *ABCB1 rs1128503;* 16. *ABCC5 rs3792585;* 17. *ABCC5 rs9838667;* 18. *XO rs1701368;* 19. *XO rs17323235*	PCR em tempo real / NCI≥1	15. *ABCB1*: 0.22/2.25/0.39-12.97 16. *ABCC5*: 0.13/0.31/0.05-1.76 17. *ABCC5*: 0.049/0.24/0.05-1.06 18. *XO*: 0.16/4.12/0.44/38-52 19. *XO*: 0.14/2.67/0.57-12.56	There was no association between oral mucositis and the polymorphisms studied.
Liu Yan et al, 2014	China	Coorte	112	20. *ABCC2 rs717620;* 21. *ABCC2 rs3740065;* 22. *ABCC4 rs9516519;* 23. *ABCC4 rs868853;* 25. *ABCC4 rs2274407;* 26. *ABCG2 rs2231137*	PCR + Sequenciamento / CTCAE≥1	20. *ABCC2*: <0.01/9.00;2.11-38.35	Polymorphism is associated with the onset of oral mucositis and its severe form
Radtke et al, 2013	Germany	Coorte	499	27. *SLCO1B1 rs4149056;* 28. *SLCO1B1 rs11045879* 29. *SLCO1B1 rs2306283;* 30. *ABCC2 rs717620;* 31. *MTHFR rs1801131;* 32. *MTHFR rs1801133;*	PCR + Sequenciamento / NCI≥1	33. *TYMS:* 0.009/-0084-0.12 B= -0.48; R^2= 0.018	Polymorphism (3 * / * 3) correlates with reduction of oral mucositis in the studied population.

				33. *TYMS rs34743033*			
Bektas et al, 2011	Turkey	Coorte	115	34. *MDR1 rs1045642*	PCR-RFLP / WHO≥1	34. *MDR1*: 0.042/0.17/0.03-0.94	Individuals with CT genotype are more susceptible to the development of oral mucositis
Tantawy et al, 2010	Egypt	Coorte	40	35. *MTHFR rs1801133;* 36. *MTHFR rs1801131*	PCR-RFLP / NCI≥3	35. MTHFR: 0.0001	TT genotype is associated with the appearance of oral mucositis in relation to CC
Shimasaki et al, 2006	Japan	Coorte	15	37. *MTHFR rs1801133;* 38. *RFC1 r*s1051266	PCR + Sequenciamento / NCI≥2	37. MTHFR: 0.432/2.052 38. RFC1: 0.171/0.301	There was no association between oral mucositis and the polymorphisms studied.
Aplenc et al, 2005	USA	Case control	520	39. *MTHFR rs1801133;* 40. *MTHFR rs1801131*	PCR-RFLP / CCG≥3	39. MTHFR: 0.55/1.32	There was no association between oral mucositis and the polymorphisms studied.

Some polymorphisms are studied by more than one study: rs11045879 (4 and 28), rs4149056 (5 and 27), rs1128503 (12 and 15), rs1045642 (13 and 34), rs2231137 (14 and 26), rs717620 (20 and 30), rs1801133 (32, 35, 37 and 39) and rs1801131 (31, 36, and 40).

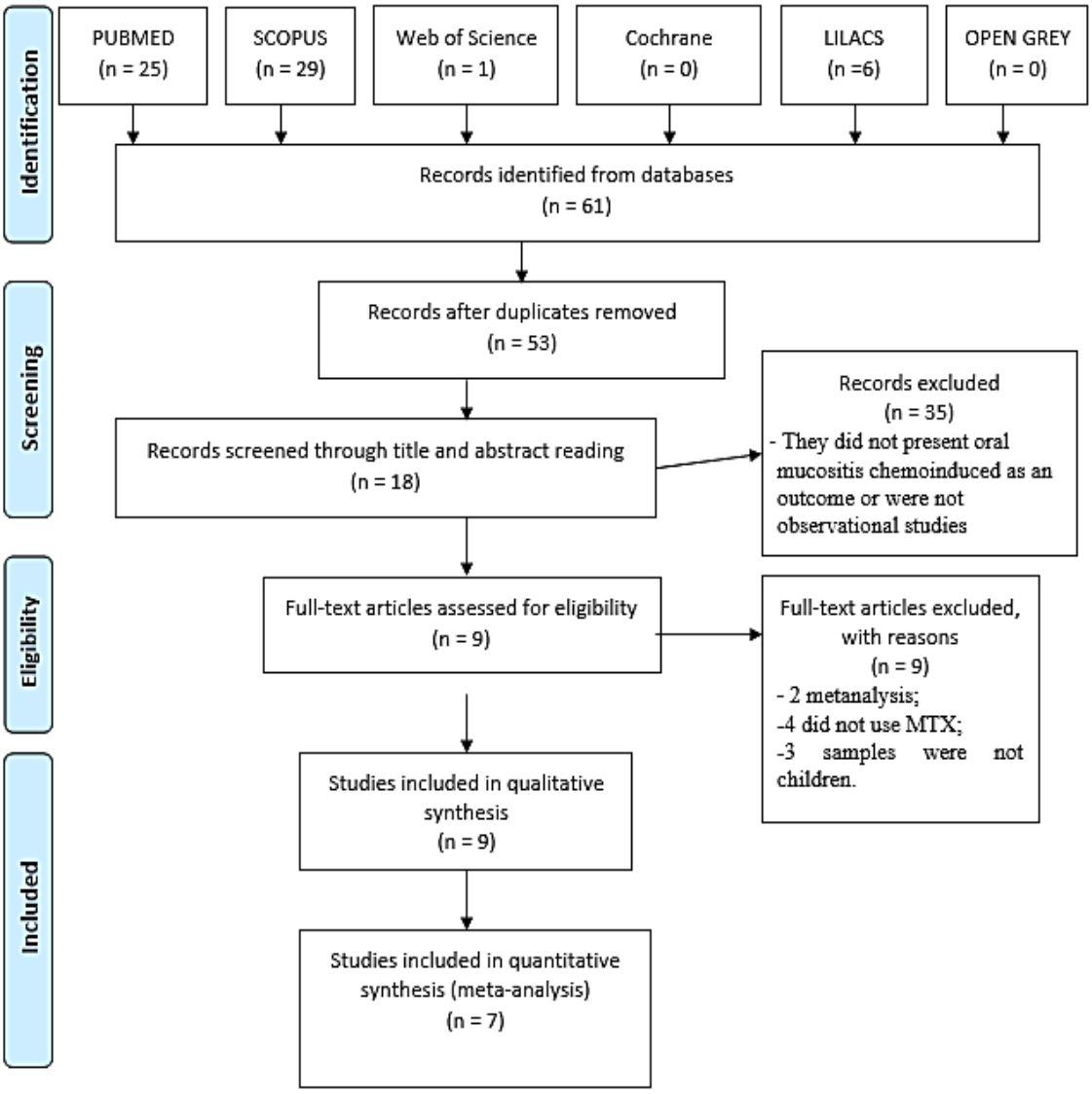
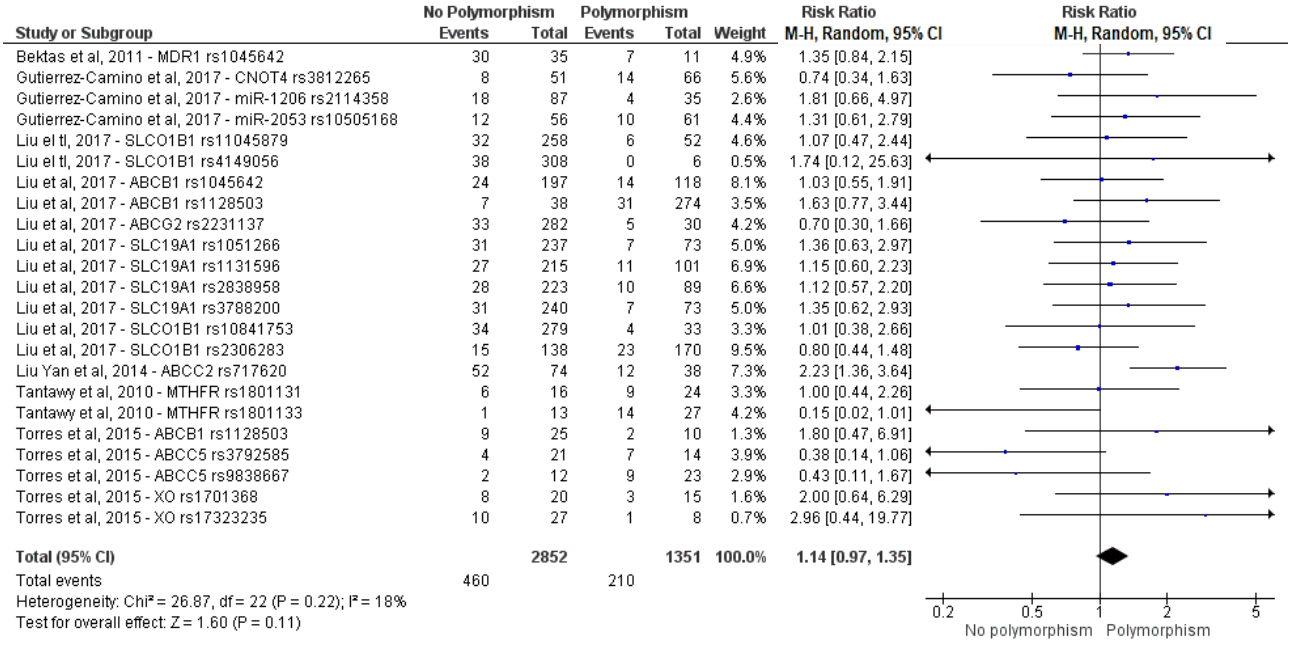
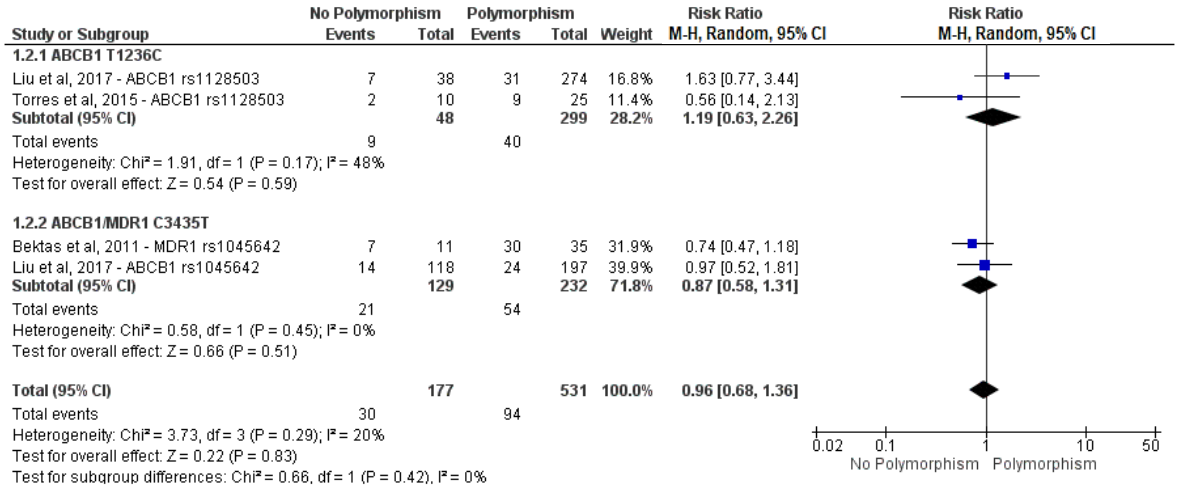


Figure 1. Prism diagram containing the article selection flowchart

A - Florest plot with the association between genetic polymorphisms and oral mucositis



B - Florest plot with the association between repeated genetic polymorphisms in the studies and oral mucositis



SNP in the same gene (ABCB1, also called MDR1)

Figure 2: Meta-analyzes. Relative risk (RR) and 95% confidence interval (CI) were presented alongside each study; center line of graph corresponds to null hypothesis; the vertical diagonal of the diamond corresponds to RR; the horizontal diagonal of the diamond corresponds to the CI; the size of the blue square corresponds to the weight of the study; the position of the square corresponds to RR; The presence of arrows on some horizontal lines means that the CI goes beyond the values shown on the X axis.

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Presence	absence of genetic polymorphisms	Relative (95% CI)	Absolute (95% CI)		
Associação entre a ocorrência de mucosite oral e polimorfismos encontrados												
25	observational studies	not serious	serious [a]	not serious	not serious	none	0 cases 0 controls 482/2882 exposed 238/1385 unexposed		**RR 1.12** (0.96 to 1.30)	-	⨁◯◯◯ VERY LOW	
							-	0.0%		**0 fewer per 1.000** (from 0 fewer to 0 fewer)		
Mucosite oral e ABCB1 T1236C												
2	observational studies	not serious	not serious	not serious	serious [b]	none	40/299 (13.4%)	9/48 (18.8%)	**RR 0.84** (0.44 to 1.59)	**30 fewer per 1.000** (from 105 fewer to 111 more)	⨁◯◯◯ VERY LOW	
Mucosite oral e ABCB1/MDR1 C3435T												
2	observational studies	not serious	not serious	not serious	serious [b]	none	21/129 (16.3%)	54/232 (23.3%)	**RR 0.87** (0.58 to 1.31)	**30 fewer per 1.000** (from 98 fewer to 72 more)	⨁◯◯◯ VERY LOW	

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. There is a large overlap of confidence intervals associated with effect estimation. It has been downgraded by one point.
b. Studies include a relatively small number of individuals, resulting in a sample size of less than 300 participants per group.

Figure 3: GRADE – *Grading of Recommendations Assessment, Development and Evaluation*

4 CONSIDERAÇÕES GERAIS

O capítulo 2 do presente trabalho foi realizado no componente curricular de Tópicos em Revisão Sistemática, em dupla com outro mestrando Programa de Pós-Graduação em Odontologia, sob orientações dos docentes desta disciplina em unidade com os orientadores dos estudantes.

Serviu como subsídio para a construção das hipóteses de pesquisa levantadas no início da coleta de dados para realização do artigo do capítulo 1. Este contém os resultados do trabalho realizado durante o mestrado, a fim de ser apresentado como requisito obrigatório para obtenção do título de Mestre em Odontologia.

Embora a revisão sistemática tenha concluído não haver associação entre os polimorfismos genéticos encontrados e a ocorrência da mucosite oral, ela revelou a necessidade da elaboração de estudos primários com uma melhor qualidade metodológica, no que diz respeito à seleção da amostra e estimativa do efeito dos seus achados.

Na realização do estudo apresentado no capítulo 1, observamos algumas limitações com respeito a seleção amostral. Por apresentar critérios de elegibilidade bem específicos, a seleção da amostra, embora tenha extrapolado o cálculo amostral e ter coletado de maneira censitária, passou por dificuldades. Alguns pacientes que estavam em acompanhamento desde 2015 já tinham vindo a óbito ou mudado de cidade, inviabilizando a coleta do material biológico. Além disso, houve dificuldade na alocação de pacientes no grupo controle, por motivos da alta incidência de mucosite na população do presente estudo.

Diante disto, observa-se a dificuldade em realizar estudos dessa natureza com evidência alta, por se tratar de estudo observacional, que já inicia com uma evidência baixa, e por enfrentar dificuldades na seleção amostral. No entanto, essas limitações não configuram vieses no resultado das hipóteses que foram testadas.

Além disso, vale ressaltar a importância desta pesquisa como impacto positivo para os pacientes, para o serviço oncológico e para a linha de pesquisa de avaliações de alterações genéticas e ocorrência de mucosite oral. Tendo em vista que uma das alterações genéticas observadas configura um fator de risco para o desenvolvimento da mucosite oral, a utilização de uma terapia personalizada poderia reduzir a probabilidade de efeitos adversos.

CONCLUSÃO

Conclui-se que embora não se tenha observado associação nos achados da meta-análise, a presença de polimorfismo C421A *ABCG2* (rs2231142) aumenta a probabilidade de ocorrência mucosite oral em pacientes pediátricos com leucemia tratados com metotrexato.

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APÊNDICE I

INFORMAÇÕES GERAIS DA AMOSTRA

Nome –

Sexo – (1) – Masculino (2) Feminino

Idade –

Cor da pele – (1) Branca (2) Negra (3) Amarela (4) Parda (5) Indígena

Cidade de residência – (1) João Pessoa (2) Cidade do interior da Paraíba

(3) Demais Estados

Tratamento – (1) Q (Quimioterapia) (2) QC (Quimioterapia/Cirurgia)

(3) QR (Quimioterapia/Radioterapia) (4) QRC (Quimioterapia/Radioterapia/Cirurgia)

Recidiva – (1) Sim (2) Não

APÊNDICE II

Termo de Consentimento Livre e Esclarecido

PESQUISA:

Associação entre polimorfismos genéticos e ocorrência de mucosite oral

João Pessoa, ___/___ de 20__

Prezado(a) Senhor(a),

Estamos realizando uma pesquisa para verificar se fatores genéticos podem influenciar na ocorrência de inflação da mucosa da boca (mucosite) em crianças e adolescentes atendidos no Hospital Napoleão Laureano que fazem uso de quimioterapia para tratamento da Leucemia.

Faremos um exame da boca das crianças e adolescentes no consultório dentário do setor de Pediatria do hospital ou no leito, caso o paciente esteja internado. No exame iremos verificar a situação dos dentes, da gengiva, da bochecha, da língua, do palato e dos lábios. Este exame não representa riscos para quem será examinado, havendo apenas a necessidade de abrir a boca por cerca de 5 minutos.

Também coletaremos a saliva das crianças e adolescentes e na saliva veremos a quantidade de alguns microrganismos. Nesta saliva será verificado, ainda, se existem alterações em alguns genes, pois algumas destas alterações poderiam fazer com que pessoas que fazem quimioterapia venham a ter maior chance de apresentar inflamação na boca.

A saliva será utilizada apenas para fazer os testes que já lhe explicamos e, após os testes serem feitos, o material será descartado.

Obteremos informações do prontuário da criança/adolescente sobre a: idade dela, histórico médico e o tratamento que está sendo realizado.

Os dados individuais não serão divulgados em nenhuma hipótese, mas os resultados da pesquisa ajudarão a compreender se alterações genéticas influenciam no aparecimento da inflamação de gengiva e, com isto, identificar quem são as crianças e adolescentes com maior possibilidade de ter inflamação na boca. Por isso, sua colaboração, autorizando no quadro abaixo a realização do exame da pessoa pela qual é responsável, é muito importante.

Esclarecemos que a sua participação nesta pesquisa é decorrente de sua livre decisão após receber todas as informações que julgar necessárias.

Nem você nem a criança pela qual é responsável serão prejudicadas de qualquer forma caso sua vontade seja de não colaborar.

A qualquer momento você pode desistir de continuar colaborando na nossa pesquisa e sua decisão não trará quaisquer prejuízos para o paciente pelo qual você é responsável.

Os procedimentos adotados nesta pesquisa obedecem aos critérios da Ética em Pesquisa com Seres Humanos, conforme Resolução no. 466/12 do Conselho Nacional de Saúde.

Se quiser mais informações sobre o nosso trabalho, por favor ligue para Profa. Ana Maria Gondim Valença – Telefones (83) 32167796 / (83) 999864397.

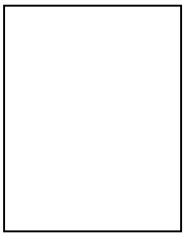
Profa. Ana Maria Gondim Valença

Coordenadora da Pesquisa

AUTORIZAÇÃO

Após ter sido informado sobre as características da pesquisa **Associação entre polimorfismos genéticos e ocorrência de mucosite oral**

AUTORIZO a realização do exame em:



_____

Em _____ de _____ de 20____

_____ _____

Nome do responsável Assinatura do responsável

Testemunha _____

Telefone do Comitê de Ética em Pesquisa do Centro de Ciências da Saúde – (83) 32167791

Centro de Ciências da Saúde - 1º andar

Campus I - Cidade Universitária CEP: 58.051-900 - João Pessoa-PB

eticaccsufpb@hotmail.com

APÊNDICE III

Termo de Assentimento

Associação entre polimorfismos genéticos e ocorrência de mucosite oral

João Pessoa, ___/___ de 201__

Você está sendo convidado/a para participar da pesquisa: **Associação entre polimorfismos genéticos e ocorrência de mucosite oral.**

Seus pais permitiram que você participasse.

Com esta pesquisa queremos que colabore conosco permitindo que examinemos sua boca (lábios, dentes, gengiva, língua, palato e bochechas). Também pedimos que você permita que seja feita a coleta de sua saliva.

As crianças que irão participar desta pesquisa têm de 0 a 19 anos de idade. Caso não queira participar da pesquisa, é um direito seu e não terá nenhum problema em recusar ou desistir.

A qualquer momento você pode nos procurar pelo telefone (83) 999864397 ou pelo e-mail anamvalenca@gmail.com da pesquisadora Ana Maria Gondim Valença. Ao persistirem as dúvidas sobre os seus direitos como participante desta pesquisa, você também poderá fazer contato com Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal da Paraíba pelo telefone (83) 3216-7791.

Ninguém saberá que você está participando da pesquisa; não falaremos a outras pessoas, nem daremos a estranhos as informações que você nos der.

Os resultados da pesquisa vão ser divulgados, mas sem identificar as crianças que participaram.

Se você tiver alguma dúvida, você pode me perguntar. Eu escrevi os telefones na parte de cima deste texto.

CONSENTIMENTO PÓS INFORMADO

Eu _____ aceito participar da pesquisa **associação entre polimorfismos genéticos e ocorrência de mucosite oral.** Entendi que posso dizer "sim" e participar, mas que, a qualquer momento, posso dizer "não" e desistir e que ninguém vai ficar com raiva se eu desistir. Os pesquisadores tiraram minhas dúvidas e conversaram com os meus responsáveis. Recebi uma cópia deste termo de assentimento e li e concordo em participar da pesquisa.

João Pessoa, ____de _____de _____.

_____ _____

Assinatura do menor ou impressão dactiloscópica Assinatura do(a) pesquisador(a)

ANEXO I

APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA DO CENTRO DE CIÊNCIAS DA SAÚDE (CCS) – UFPB

UFPB - CENTRO DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DA PARAÍBA

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: CONDIÇÃO DE SAÚDE BUCAL E FATORES ASSOCIADOS EM PACIENTES PEDIÁTRICOS ONCOLÓGICOS ASSISTIDOS EM UM HOSPITAL DE REFERÊNCIA NA CIDADE DE JOÃO PESSOA/PB ¿ ESTUDO LONGITUDINAL

Pesquisador: Ana Maria Gondim Valença

Área Temática:

Versão: 1

CAAE: 64249317.3.0000.5188

Instituição Proponente: Universidade Federal da Paraíba

Patrocinador Principal: Financiamento Próprio
CONS NAC DE DESENVOLVIMENTO CIENTIFICO E TECNOLOGICO

DADOS DO PARECER

Número do Parecer: 1.915.531

Apresentação do Projeto:

Pesquisadora Responsável: Ana Maria Gondim Valença

Grupo de Pesquisa Cadastrado no CNPq: Grupo de Pesquisa em Odontopediatria e Clínica Integrada

Centro: Centro de Ciências da Saúde

Departamento: Departamento de Clínica e Odontologia Social

Objetivo da Pesquisa:

Objetivo Geral

- Identificar os fatores associados às condições de saúde bucal e analisar o cuidado ofertado aos pacientes oncológicos na faixa etária de 0 a 18 anos, assistidos no Hospital Napoleão Laureano, na cidade de João Pessoa, Paraíba.

Objetivos Específicos

- Caracterizar o perfil clínico-epidemiológico de pacientes pediátricos oncológicos assistidos no Hospital Napoleão Laureano, João Pessoa-PB;
- Classificar os pacientes quanto aos aspectos socioeconômicos, de acesso e da auto percepção em saúde bucal;

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Bairro: CASTELO BRANCO **CEP:** 58.051-900
UF: PB **Município:** JOAO PESSOA
Telefone: (83)3216-7791 **Fax:** (83)3216-7791 **E-mail:** eticaccs@ccs.ufpb.br

- Identificar a ocorrência de cárie e doença periodontal nestas crianças e adolescentes e os fatores associados ao aparecimento destes agravos;
- Identificar a ocorrência de mucosite oral nestes pacientes e os fatores associados ao seu aparecimento;
- Analisar os aspectos microbiológicos, mediante a contagem de Streptococcus totais e Streptococcus mutans (UFC/mL), os aspectos físicos como viscosidade e fluxo salivar não estimulado, de forma longitudinal, dos pacientes pediátricos oncológicos;
- Conhecer o nível de satisfação dos pacientes e/ou seus cuidadores sobre atenção prestada em saúde bucal;
- Analisar a organização dos serviços, fluxos e rotinas de funcionamento voltadas para estes pacientes;
- Propor estratégias e ferramentas que permitam estruturar um modelo de tomada de decisão que possibilite o sentido universal, integral, resolutivo, equânime e democrático ao acesso ao cuidado em saúde bucal para pacientes oncológicos no estado da Paraíba.

Avaliação dos Riscos e Benefícios:

Riscos:

- Possível desconforto em razão do tempo que o paciente permanecerá de boca aberta para avaliação das condições de saúde bucal (3 a 5 minutos)
- Desconforto devido ao leve ruído produzido pelo sialômetro (2 minutos - tempo de coleta).

Benefícios:

No Brasil, em especial no estado da Paraíba, são escassas as informações sobre a condição de saúde bucal de pacientes oncológicos, bem como quanto ao acesso e à utilização dos serviços odontológicos. Desta forma, devem ser avaliadas as necessidades odontológicas destes indivíduos e, face às complicações decorrentes do tratamento a que são expostos, torna-se fundamental a instituição de estratégias educativas, preventivas e curativas em saúde bucal com vistas a resolução e/ou minimização dos desconfortos apresentados pelos pacientes e a melhoria da qualidade de vida destas crianças e de seus familiares. O desenvolvimento do presente projeto proporcionará a produção de conhecimentos inéditos sobre a relação entre a terapia antineoplásica administrada a pacientes pediátricos oncológicos e as condições de saúde bucal apresentadas por esses pacientes. A identificação de associação entre os eventos aqui estudados será importante para a prevenção de intercorrências que prejudicam o andamento dos protocolos estabelecidos no tratamento das diferentes patologias, bem como auxiliará o cirurgião-dentista a planejar melhor a assistência antes e durante o tratamento antineoplásico.

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Comentários e Considerações sobre a Pesquisa:

A população a ser estudada será composta por todos os pacientes de 0 a 18 anos, em atendimento no Hospital Napoleão Laureano. Estima-se que a amostra seja composta por 250 pacientes, levando-se em consideração os dados atuais fornecidos pelo Hospital Napoleão Laureano.

Considerações sobre os Termos de apresentação obrigatória:

Anexados.

Recomendações:

Todos os resultados de uma pesquisa deverão ser divulgados junto aos participantes da mesma, assim como na(s) instituição(ões) onde os dados foram obtidos. ACONSELHAMOS A TODOS OS PESQUISADORES (RESPONSÁVEL/ASSOCIADO/ASSISTENTE) QUE ANTES DO ENVIO DE QUALQUER PROTOCOLO DE PESQUISA, VIA PLATAFORMA BRASIL, SEJA FEITA UMA LEITURA DA RESOLUÇÃO N.466/12, ASSIM COMO DA NORMA OPERACIONAL N.001/13, AMBAS DO CONSELHO NACIONAL DE SAÚDE.

Conclusões ou Pendências e Lista de Inadequações:

Considero este projeto sem pendências ou inadequações.

Este é meu parecer, salvo melhor juízo.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_858234.pdf	27/01/2017 16:00:07		Aceito
Outros	AnuenciaLaureanoProjetoCondicoesSaudeBucal.pdf	27/01/2017 15:59:37	Ana Maria Gondim Valença	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TermoAssentimentoProjetoCondicaoSaudeBucal.pdf	27/01/2017 10:16:00	Ana Maria Gondim Valença	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEProjetoCondicaoSaudeBucal.pdf	27/01/2017 10:15:47	Ana Maria Gondim Valença	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoAnaValencaPaicentesOncologicos2017.pdf	27/01/2017 10:12:14	Ana Maria Gondim Valença	Aceito
Folha de Rosto	FolhaDeRostoProjetoCondicaoSaudeB	27/01/2017	Ana Maria Gondim	Aceito

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Folha de Rosto	ucal.pdf	10:10:49	Valença	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

JOAO PESSOA, 10 de Fevereiro de 2017

Assinado por:
Eliane Marques Duarte de Sousa
(Coordenador)

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