Brazilian Dental Journal (2021) 32(2): 14-26
http://dx.doi.org/10.1590/0103-6440202103768

ISSN 0103-6440

ABCG2 polymorphism, age and leukocyte count may contribute to oral mucositis in oncopediatric patients

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The study investigated the relationship between genetic polymorphisms and the development of oral mucositis in pediatric patients undergoing chemotherapy involving methotrexate. A longitudinal study was conducted with 64 patients, and oral mucositis was evaluated by the modified Oral Assessment Guide, which aims to diagnose and classify oral mucositis. Epithelial cells were obtained by mouthwash and DNA was extracted. The polymorphisms MTHFR (rs1801133), DNMT3B (rs2424913), ABCC2 (rs717620), ABCG2 (rs2231137) and ABCG2 (rs2231142) 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. Male sex predominated (56.2%), and the mean age was 10.8 years (± 4.9). Oral mucositis affected 65.6% of the patients, of which 61.9% developed the severe form of the disease. For the ABCG2 gene (rs2231142), the rare A allele and CA genotype were more frequent in individuals with mucositis (p= 0.02; RR = 0.60; CI = 0.387 - 0.813). The severity of the disease was mainly observed in younger patients (median = 9 years; p=0.02). Patients with severe oral mucositis presented lower leukocytes count (median = 2.150 mm3) compared to patients with the mild/moderate form (median = 4.200 mm3; p=0.03). Female patients and each 10,000-platelet increase were protective factors against the onset of oral mucositis (p=0.02). It is concluded that rs2231142 polymorphism increases the likelihood of oral mucositis and younger patients and patients with low leukocytes counts are more likely to develop severe form.

Key Words: Oral mucosa. Mucositis. Polymorphism. 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). This inflammation is observed in patients undergoing therapeutic protocols involving methotrexate (MTX), the main chemotherapeutic agent used in the treatment of leukemias (2), cancer type more common in children and adolescents (26%) (3).

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 (4). 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 (5,6).

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 (1,7). 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 (8).

MTX reaches the cytosol through transmembrane proteins, interferes with cell metabolism and can be eliminated by efflux proteins (9,10). 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 (9).

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 (11). 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 (2).

The DNMT3B gene encodes DNA methyltransferase, which transfers a methyl radical (CH3) 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 (12). 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 (12,13).

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 (14). 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 (15,16).

Although some studies have noted an association between genetic polymorphisms and the incidence of oral mucositis (15,17,18), there are few studies that have investigated this association, and the results are not necessarily convergent (8).

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 (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 longitudinal study was conducted. Data were obtained at the pediatrics department of Hospital Napoleão Laureano (HNL), João Pessoa-PB, 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).

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 (7).

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 The patients included in the study could be in any phase of treatment (initial phase: intensification or remission or final phase: maintenance), however, the data regarding the occurrence of oral mucositis were always obtained at the time of diagnosis of the disease, which occurred in the initial phase of treatment, since it is the period of higher incidence of oral mucositis. The therapeutic protocols were proposed by the Brazilian Childhood Leukemia Treatment Group (GBTLI) (19).

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, and were included in this study a total of 64 individuals.

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, at any stage of chemotherapy 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. Brothers and sisters and syndromic patients were also excluded.

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 (20). Eight items are evaluated for the estimation of mucositis according to OAG: voice, swallowing, lips, tongue, saliva, labial/palate mucosa, labial mucosa, and gingiva. Previously calibrated individuals (Kappa = 0.87) were responsible for evaluating the oral mucosa.

Collection of data for the study sample: General data of the patients were recorded on a specific form to describe the study sample. The patients were followed up weekly for four weeks, which may be longer, according to the hospitalization period, and the signs of oral mucositis were expected to fit them in the sick group. If they did not develop mucositis during this follow-up period, they were allocated to the control group. The hematological and biochemical data of patients who presented with oral mucositis were recorded at the time of diagnosis of the disease. If the patient developed severe oral mucositis while being monitored, exams and registration were selected from this moment. The collected hematological and biochemical 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 mouthwash 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. Lysing solution was added to the oral epithelial cell pellet, and the samples were frozen at -20 °C until DNA extraction (21).

DNA extraction and quantification: Genomic DNA was purified using 8 M ammonium acetate (21). 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 (NanoDrop® 2000).

Analysis of single nucleotide polymorphisms (SNPs) in the MTHFR, DNMT3B, ABCC2 and ABCG2 genes:

The studied genes encode enzymes involved in folate metabolism (MTHFR and DNMT) or transport proteins involved with drug efflux (ABCC2 and ABCG2). The MTHFR gene is located on chromosome 1 and the SNP C677T (G>A; rs1801133) in exon 4 comprises Ala263Val substitution, which implies a decrease in enzyme activity (12). The DNMT3B gene is located on chromosome 20 and the SNP C-149T (C>A; rs2424913) in the promoter implies an increase in gene expression. (13). The ABCC2 gene is located on chromosome 10 and the SNP C-24T (C>T; rs717620) in the promoter implies a decrease in gene expression. (14). The ABCG2 gene is located on chromosome 4 and the SNPs G34A (C>T; rs2231137) in exon 2 comprises Val12Met substitution and C421A (G>C,T; rs2231142) in exon 5 comprises Glu141Lys substitution, which implies a reduction in transport activity (15,16).

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) as previously described (16, 22–26).

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, adopting α=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 (non-adjusted) and multiple (adjusted)) 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.

Results

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, acute lymphoblastic leukemia 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 severe mucositis (Table 1).

Table 1. Descriptive results for demographic and clinical data of the studied population. João Pessoa, Brazil.

Variable	
Sex	**n (%)**
Male	36 (56.25%)
Female	28 (43.75%)
Overall	64 (100%)
Age	
Mean age (± standard deviation)	10.81 (±4.98)
Base disease	**n (%)**
Acute Lymphoblastic Leukemia (ALL)	53 (82.82%)
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%)

The oral mucositis were presented by 27 (75.00%) of the male patients and by 15 (53.60%) of the female patients (p=0.073; Chi-Square test). In the group of patients with mucositis, 64.3% (n = 27) were male and 35.7% (n = 15) female. In control group, 59.1% (n = 13) were female and 40.9% (n = 9) male (p = 0.073; Chis-Square test).

The median of age of patients was significantly different between groups with (median=9.50, p25=6.75; p75=17.00) and without (median=8.00, p25=7.00; p75=14.00), (p=0.041; Mann-Whitney U test) oral mucositis. In addition, the median of age of patients with severe oral mucositis (median = 9.00, p25 = 5.75; p75 = 15.25) was less than the median of age of patients that developed the mild and moderate forms (median = 11.50, p25 = 8.00; p75 = 19.00), (p=0.029; Mann-Whitney U test).

The hematological and biochemical data are provided in Table 2. 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). When the severity levels were separated into mild/ moderate and severe, it was observed that the leukocyte count was lower in individuals with the severe form (median = 2.150, p25 = 1.100; p75 = 3.900) compared to the other patients (median = 4.200, p25 = 2.700; p75 = 6.950) (p = 0.038; Mann-Whitney U test).

Table 2. Hematological and biochemical parameters of the studied population (median (percentil 25; percentil 75)). João Pessoa, Brazil. n=64.

Hematological and biochemical parameters	Group		p-value
	With mucositis (n= 42)	Without mucositis (n= 22)	
Hemoglobin (g/dl)	9.40 (7.77; 10.72)	9.75 (8.67; 11.87)	0.520
Leukocytes (mm^3)	2,850.00 (1,375.00; 6,425.00)	5,650.00 (3,625.00; 7,500.00)	**0.013**
Platelets (mm^3)	151,000.00 (54,000.00; 261,250.00)	229,000.00 (99,000.00; 342,250.00)	0.074
Urea (mg/dl)	16.00 (11.00; 27.75)	15.50 (10.75; 22.00)	0.871
Creatinine (mg/dl)	0.42 (0.35; 0.52)	0.45 (0.39; 0.52)	0.420
Urea/Creatinine Ratio (mg/dl)	33.59 (24.41; 66.14)	36.62 (23.83; 46.69)	0.577

Mann-Whitney U test. Significance level=5%.

The allelic frequencies of four genes were balanced according to Hardy-Weinberg equilibrium (HWE): (rs1801133) p = 0.47; (rs717620) p = 0.07; (rs2231137) p = 0.59; and (rs2231142) p = 0.54. However, (rs2424913), 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 3.

Table 3. Allelic and genotypic frequency of the studied population

SNP	Frequencies					
	Allelic / Genotypes	With mucositis n (%)	Without mucositis n (%)	p-value	RR	HWE
MTHFR C677T	C	56 (66.7%)	32 (73.0%)			
(rs1801133)	T	28 (33.3%)	12 (37.0%)	p=0.482		
(n=64)	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	C	44 (52.0%)	24 (55.0%)			
(rs2424913)	T	40 (48.0%)	20 (45.0%)	p=0.815		
(n=64)	CC	06 (14.3%)	06 (27.0%)		–	p=0.00
	CT	32 (76.2%)	12 (55.0%)			
	TT	04 (9.5%)	04 (18.0%)	p=0.389		
ABCC2 C-24T	C	72 (88.0%)	36 (82.0%)			
(rs717620)	T	10 (12.0%)	08 (18.0%)	p=0.359		
(n=63)	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	G	79 (94.0%)	41 (93.0%)			
(rs2231137)	A	05 (6.0%)	03 (7.0%)	p=0.847		
(n=64)	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	C	75 (89.3%)	44 (100%)			
(rs2231142)	A	09 (10.7%)	0	**p=0.024**		
(n=64)	CC	33 (78.5%)	22 (100%)		0.60 (0.387 – 0.813)	p=0.54
	CA	09 (21.5%)	0			
	AA	0	0	**p=0.022***		

P-values calculated by the tests: Chi-square and Fisher Exact *; HWE = Hardy-Weinberg Equilibruim

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 = 0.60; CI = 0.387 - 0.813; p = 0.024).

According to the generated logistic regression model (Table 4), 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.

Table 4. Logistic regression model adjusted for the outcome "Oral Mucositis". João Pessoa, Brazil. n=64.

Variable	Categories	Univariate (Non adjusted)		Multiple (Adjusted)	
		p-value	OR (CI95%)	p-value	OR (CI95%)
Sex	Male	-	1	-	1
	Female	0.077	0.385 (0.133 - 1.109)	0.021	0.240 (0.072 – 0.811)
Age [I]		0.462	1.041 (0.936 - 1.157)		
Disease	ALL	-	1		
	AML	0.572	0.630 (0.127 – 3.132)		
	APL	0.252	0.236 (0.020 – 2.788)		
	CML	0.985	0.760 (0.980 – 2.552)		
MTHFR C677T (rs1801133)	CC	-	1		
	CT	0.712	1.222 (0.420 – 3.553)		
	TT	0.449	2.444 (0.241 – 24.778)		
DNMT3b C46359T (rs2424913)	CC	-	1		
	CT	0.172	2.500 (0.671 – 9.310)		
	TT	0.640	1.500 (0.275 – 8.189)		
ABCG2 G34A (rs2231137)	GG	-	1		
	GA	0.842	0.856 (0.184 – 3.970)		
ABCG2 C421A (rs2231142)	CC	-	1		
	CA	0.990	0.986 (0.055 – 3.931)		
ABCC2 C-24T (rs717620)	CC	-	1		
	CT	0.229	0.455 (0.126 – 1.644)		
	TT	0.940	0.909 (0.076 – 10.821)		
MTHFR C677T (rs1801133) + *DNMT3b* C46359T (rs2424913)	CC+TT/CC	-	1		
	CC/CT+TT	0.600	1.511 (0.323 – 7.071)		
	CT+TT/CT+TT	0.334	2.171 (0.450 – 10.486)		
	CC/CC	0.512	0.400 (0.026 – 6.176)		
ABCC2 C-24T (rs717620) + *ABCG2* G34A (rs2231137) + *ABCG2* C421A (rs2231142)	CC/GG/CC	-	1		
	CC/GG/CA/+AA	0.991	0.953 (0.011 – 2.315)		
	CC/GA+AA/CC	0.863	0.870 (0.177 – 4.275)		
	CT+TT/GG/CC	0.223	0.447 (0.123 – 1.632)		
	CT+TT/GG/CA+AA	0.995	0.991 (0.036 – 3.198)		
Hemoglobine [II]		0.086	0.791 (0.605 – 1.034)		
Leucocytes [III]		0.396	1.000 (0.880 – 1.120)		
Platelets [IV]		0.080	1.000 (0.970 – 1.030)	0.021	0.951 (0.907 - 0.994)
Urea [V]		0.349	1.029 (0.969 – 1.093)		
Creatinine [VI]		0.753	1.688 (0.065 – 43.856)		

ALL (Acute lymphoid leukemia); AML (Acute Myeloid Leukemia); APL (Acute Promyelocytic Leukemia); CML (Chronic myeloid leukemia); [I]= For each increase of 1 year; [II]=For each increase of 1g in the blood concentration; [III]=For each increase of 1.000 leucocytes; [IV]=For each increase of 10.000 platelets; [V]= For each increase of 1 unit in the blood concentration; [VI]= For each increase of 0.1 unit in the blood concentration

Discussion

The oral mucositis is commonly observed at HNL (7), 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 leukemia, which are the most frequent cancers observed in the juvenile population (16,22–24). This medication is responsible for the appearance of some adverse effects, including oral mucositis (2).

Clinical data showed that most children and adolescents (> 60%) with leukemia treated with MTX had oral mucositis and that severe mucositis was the most incident form. Another recent study conducted at Hospital Napoleão Laureano showed that more than 50% of oncopediatric patients developed mucositis. These data suggest that the GBTLI protocols used in these patients have high cytotoxic levels, causing direct therapeutic toxicity to oral mucosa cells. Other studies have shown rates between 40-46%, including a study conducted in Brazil more than a decade ago. In these studies, therapeutic protocols different from those developed by the GBTLI are administered for leukemias, and lower incidences of oral mucositis are observed (2,27-29).

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 (20). 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 (28,30,31). Regarding the leukocyte count, patients with severe mucositis had lower counts than those who did not develop the severe form of the disease. This association has also been shown recently in adult cancer patients receiving radiotherapy (32). In addition, a study of oncopediatric patients receiving chemotherapy showed an association between severity and low neutrophil counts (33). Individuals immunosuppressed are susceptible to opportunistic infections, since this deficiency impairs the patient's defenses. Leukopenic patients are at increased risk of bacterial colonization of the damaged epithelium, which leads to an increase in pro-inflammatory cytokines that aggravate oral mucositis (33).

Genetic polymorphism data from the present study showed an association between the 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. 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 (34); the polymorphism causes an amino acid substitution (Gln141Lys), resulting in a structurally and functionally defective protein (16,35). 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 (10), which could lead to greater adverse effects of MTX, including the development of oral mucositis.

There are no data in the literature on the polymorphism (rs2231142) associated with adverse effects of MTX in children with hematologic tumors; however, two studies previously performed in adults (36,37) 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, MTHFR (rs1801133) and 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 (15,17).

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 (38). 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 (39). 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. As a perspective, the data shown in the present study (genetic, demographic and hematological) can be markers to predict

the occurrence of oral mucositis and, thus, contribute to a personalized therapy, where the occurrence of oral mucositis is minimal and milder.

Regarding protective factors against oral mucositis, the logistic regression model adjusted 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 (40).

Platelets are anucleate structures, derived from megakaryocytes, produced in the bone marrow and responsible for hemostasis (41). Currently, other functions of these elements have been investigated, such as their role in inflammation (42). 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 (43). 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 (44). 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 (45); 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. Another important point is that the patients' general oral health condition was not evaluated, which could also imply the incidence of mucositis.

Another limitation is the sample size for the polymorphism study that can be considered small. This aspect should be taken into consideration when interpreting our findings since it could influence the association between the presence of the rs2231142 polymorphism and the occurrence of oral mucositis.

However, it is common knowledge that most childhood cancer cohorts are relatively small in size and one reason is that these diseases are relatively rare (42). In addition, our sample was obtained in a single cancer center. Thus, it is difficult to control all variables related to chemotherapy treatment and patient-related variables, and there is a practical difficulty in obtaining a sufficient number of participants due to the low incidence of childhood cancer.

Unfortunately, the loss to follow-up, which may compromise internal validity and the conclusion of a study, is an occurrence frequently observed in research with cancer patients. Yet, the lack of power is a frequently encountered problem in cancer studies due to small sample size (43), as previously mentioned in this section. Even so, we highlight that the power of this study was able to reach 80% even with a small sample.

However, it is also 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. So, we conclude the presence of the ABCG2 polymorphism (rs2231142) increases the likelihood of oral mucositis. Severe oral mucositis is associated with younger patients and patients with low leukocytes counts.

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. JMCVF was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil). MCC was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil).

Resumo

O presente estudo investigou a relação entre cinco polimorfismos genéticos e o desenvolvimento de mucosite oral em pacientes pediátricos recebendo quimioterapia com metrotexato. O estudo longitudinal foi conduzido com 64 pacientes e a mucosite oral avaliada pelo Oral Assessment Guide modificado, que tem como objetivo diagnosticar e classificar a mucosite oral. Células epiteliais bucais

foram obtidas por bochecho e o DNA foi extraído. Os polimorfismos MTHFR (rs1801133), DNMT3B (rs2424913), ABCC2 (rs717620), ABCG2 (rs2231137) e ABCG2 (rs2231142), foram analisados pela técnica de PCR-RFLP. Dados demográficos, hematológicos e bioquímicos foram coletados a partir de registros médicos. Análise estatística foi realizada utilizando o software SPSS adotando um valor de p=0,05. Observou-se que, o sexo masculino foi predominante (56,2%), e a idade média foi de 10,8 anos (± 4.9). A mucosite oral acometeu 65,6% dos pacientes, dos quais, 61,9% desenvolveram a forma grave da doença. Para o gene ABCG2 (rs2231142), o alelo raro A e o genótipo CA foram mais frequentes em indivíduos com mucosite (p= 0.02; RR = 0.60; CI = 0.387 - 0.813). A gravidade da doença foi observada principalmente em pacientes mais jovens (mediana = 9 anos; p=0.02). Além disso, os pacientes com mucosite oral grave apresentaram menor contagem de leucócitos (mediana = 2150 mm3) em comparação aos pacientes com a forma leve/moderada (mediana = 4200 mm3; p=0.03). Pacientes do sexo feminino e aumento a cada 10.000 plaquetas foram fatores de proteção contra o aparecimento de mucosite oral (p=0.02). Concluiu-se que a presença do polimorfismo rs2231142 aumenta o risco de o paciente desenvolver a mucosite oral, bem como pacientes mais jovens e menor contagem de leucócitos contribui com a severidade.

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Received: 18/06/2020
Accepted: 15/02/2021