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Feasibility of using saliva for Cytomegalovirus detection and genotyping in pediatric hematopoietic stem cell transplant recipients

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ABSTRACT

Human Cytomegalovirus (HCMV) is a major viral pathogen that causes severe complications in immunosuppressed individuals, particularly hematopoietic stem cell transplant recipients. In these patients, Cytomegalovirus has been associated with gastroenteritis, pneumonia, hepatitis, and even graft-versus-host disease, and a possible relationship has been identified between Cytomegalovirus genotypes and clinical course, complications and outcome. Early detection of Cytomegalovirus infection or reactivation is important, and previous findings show that it could potentially be evaluated in saliva, where HCMV causes asymptomatic viral shedding. Since saliva can be collected easily and safely, it is important to evaluate its potential for HCMV detection and genotyping, especially in pediatric patients who are receiving hematopoietic stem cell transplantation. ***Aim:*** *The purpose of this study was to evaluate the feasibility of using saliva to detect and genotype HCMV in a cohort of pediatric hematopoietic stem cell transplant recipients (HSCTR).* ***Materials and Method:*** *This study was conducted at Fundación Hospital Pediátrico la Misericordia, in Bogota, Colombia. Stimulated saliva samples were collected once a week and subjected to HCMV detection by qualitative PCR and genotyping by nested PCR followed by sequencing. Finally, a phylogenetic tree was constructed.* ***Results:*** *Twenty patients were enrolled, and 105 saliva samples were collected, of which 29 were positive for HCMV. Twelve patients had at least one positive sample. The gB1 genotype was identified with no coinfection with any other genotype. Phylogenetic analysis showed that some saliva samples were closer to the sequence reported for the Towne laboratory strain, while others were closer to the Merlin strain, with slight differences between them.* ***Conclusions:*** *It was demonstrated that saliva can be used to detect and genotype Cytomegalovirus in pediatric transplant recipients, and that sample collection is easy, with no risk of bleeding or discomfort in the pediatric patients evaluated.*

Keywords: *cytomegalovirus - saliva - genotype - sequencing - stem cell transplant*

Viabilidad de la saliva para la detección y la genotipificación de citomegalovirus en pacientes pediátricos receptores de trasplante de precursores hematopoyéticos

RESUMEN

Citomegalovirus humano (HCMV) es el principal patógeno viral causante de complicaciones graves en individuos inmunodeprimidos, sobre todo en receptores de trasplantes de precursores hematopoyéticos. En estos pacientes, el citomegalovirus se ha asociado con gastroenteritis, neumonía, hepatitis e incluso enfermedad injerto-hospedero, y se ha identificado una posible relación entre los genotipos de citomegalovirus y el curso clínico, las complicaciones y el desenlace en estos pacientes. La detección precoz de la infección o reactivación por citomegalovirus es importante y, según hallazgos previos, la saliva puede ser una herramienta para evaluar esta infección, especialmente en pacientes pediátricos. Dado que el HCMV causa descarga viral asintomática en la saliva y que este fluido se obtiene de forma fácil y segura, es importante evaluar la posibilidad de utilizar la saliva para la detección y la genotipificación de HCMV en pacientes pediátricos que reciben un trasplante de precursores hematopoyéticos. ***Objetivo:*** *El propósito de este estudio fue evaluar la viabilidad de la saliva para detectar y genotipar HCMV en una cohorte de receptores de trasplantes de células madre hematopoyéticas pediátricas (HSCTR).* ***Materiales y Método:*** *Este estudio fue llevado a cabo en la Fundación Hospital Pediátrico la Misericordia, Bogotá-Colombia. Se recolectaron muestras de saliva estimulada una vez por semana, posteriormente se hizo la detección mediante PCR cualitativa y la genotipificación se logró con una PCR anidada seguida de secuenciación y finalmente se construyó un árbol filogenético.* ***Resultados:*** *Se incluyeron 20 pacientes de los que se obtuvieron 105 muestras de saliva; 29 muestras fueron positivas para HCMV y 12 pacientes tuvieron por lo menos una muestra positiva. El genotipo gB1 fue identificado en todos los casos, sin coinfecciones con otro genotipo; el análisis filogenético mostró que algunas muestras de saliva se ubicaron con mayor proximidad a la secuencia reportada para la cepa de laboratorio Towne y otras estuvieron más próximas a la cepa Merlin, con leves diferencias entre ellas.* ***Conclusiones:*** *Se pudo demostrar que la saliva puede emplearse para detectar y genotipificar citomegalovirus en pacientes pediátricos receptores de trasplante, resaltando que la toma de muestras es fácil, sin riesgo de producir sangrado o molestias en los pacientes pediátricos evaluados.*

Palabras clave: *citomegalovirus - saliva, genotipo - secuenciación - trasplante de células madre hematopoyéticas*

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INTRODUCTION

Human Cytomegalovirus (HCMV), a member of the Herpesvirus family, infects 50–100% of the world's population, and is acquired during pregnancy, childbirth or the early years of life[1,2]. The gB envelope glycoprotein encoded in the *UL55* gene is involved in host cell entry, cell-to-cell virus transmission, and fusion between membranes of infected and uninfected cells, leading to the formation of syncytia[3,4]. The variability and polymorphic sequences in this gene have enabled the classification of five main genotypes (gB1 to gB5)[5,6], and they can influence viral tropism due to the interaction with described viral receptors such as EGFR, PDGFR or integrins, to enter different cell types[7–10]. Moreover, gB participates in the activation of Pattern Recognition Receptors such as TLR2, triggering the innate immune response[11].

After primary infection, HCMV establishes lifelong latency[12], and viral reactivation in different tissues or anatomical compartments results in local asymptomatic viral spread, leading to the transient presence of viral particles in bodily fluids such as saliva and urine[13–15].

Viral shedding in saliva is essential because of the ability of the virus to persist and replicate in salivary acinar cells, resulting in salivary aerosols and droplets being effective horizontal transmission mechanisms during speaking, coughing or spitting[13-16]. Thus, saliva is also valuable for monitoring HCMV infection/reactivation in both symptomatic and asymptomatic individuals[17–19].

Genotyping is based on the detection of differences in the sequence of the UL55 gene. Five main genotypes have been recognized: gB1 to gB5. Several genotyping techniques have been proposed based on a nested or semi-nested PCR, followed by restriction fragment length polymorphism. The use of PCR and phylogenetic analysis through a maximum likelihood tree has also been reported[20-23]. Genotyping by sequencing also relies on a variable fragment of the UL55 gene encoding for gB glycoprotein.

It has been suggested that HCMV genetic variability may be associated with the clinical outcome of infection and demonstrated that there is a relationship between genotypes (mainly gB3) and the development of complications such as myelosuppression, gastrointestinal disease, graft-versus-host disease (GVHD) or patient death[20-23].

HCMV genotyping is not a standard diagnostic procedure in transplant recipients. However, it might be advisable to establish the HCMV genotype(s) and whether there is coinfection/mixed infection with more than one Cytomegalovirus genotype in the same patient, given the possible association with complications or higher viral load in such patients[22]. To date, no HCMV genotyping study has been performed in Colombia or in pediatric transplant recipients. Given that collecting saliva samples is safe and easy, the objective of this study was to evaluate the feasibility of using saliva to detect and genotype HCMV in a cohort of pediatric hematopoietic stem cell transplant recipients.

MATERIALS AND METHOD:

Patients

This research was conducted in the Hematopoietic Stem Cell Transplantation Unit of HOMI – Fundación Hospital Pediátrico la Misericordia (Bogota, Colombia), from April to December 2016. The study was approved by the Ethics Committee of the School of Dentistry (certificate No.15 of 2015, National University of Colombia, Bogota) and classified as minimal risk according to Ministry of Health regulations.

The study was explained to the patients and their relatives who were admitted to the transplant unit, and assent and informed consent forms were signed upon their agreement. For each patient, data including diagnosis, transplant type, complications and outcomes were obtained from the medical records. Patients were excluded if their oral or systemic condition prevented more than two samples from being collected.

Saliva samples were collected weekly during hospitalization. For sample collection, each patient was given a block of dental wax to chew for 30 seconds, after which the patient spat out the block of wax and saliva collection began. Patients provided 1 to 1.5 mL of saliva, which was collected in sterile 15 mL tubes containing 450 µL of Dulbecco's Modified Eagle´s Medium (DMEM) supplemented with 100 IU/mL of penicillin and refrigerated until taken to the laboratory. Then, an equal volume of PBS was added, and the tube was centrifuged for 10 minutes at 4 °C (4000 × g), and the supernatant collected and stored at -80 °C until use.

DNA extraction

A 50 µL aliquot of sample was mixed with 150 µL of Chelex 100® (Sigma-Aldrich C7901) resin prepared at 20% in 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, and 0.1% sodium azide. The solution was mixed for 10 s and incubated at 56 °C for 20 min, followed by a second incubation at 100 °C for 10 min. The suspension was allowed to cool, and the resin was decanted to obtain the upper aqueous phase, which contained the DNA free of protein contaminants and was used to amplify viral DNA by PCR.

HCMV detection and genotyping

The objective was to detect and genotype Cytomegalovirus in saliva. First, a qualitative PCR was conducted to evaluate the presence of viral DNA, followed by a nested PCR to amplify the gene segment where the UL55 gene is found, and finally, sequencing to determine the genotype.

Viral DNA was detected by PCR amplification using the 5′-GTCAGCGTTCGTTCCCA-3′ and 5′-GGGACACAACCGTAAAGC-3′ primers, amplifying a 283 bp fragment of the *UL83* structural protein gene[24]. The PCR program was as follows: 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 60 °C for 1 min, 72 °C for 30 s, followed by final extension at 72 °C for 5 min[24]. Amplification products were visualized on 2% agarose gels stained with ethidium bromide. The Towne strain of HCMV DNA harvested from the MRC-5 cell line was used as positive control. The multiplex nested PCR protocol previously reported by Tarragó et al.[25] was adapted to start the genotyping of HCMV in saliva samples, based on the amplification of the *UL55* gene. The primers used and the expected products are listed in Table 1. For the first round, 100 µg/µL of DNA was amplified using the following PCR program: 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 60 °C for 1 min, 72 °C for 30 s, and 72 °C for 5 min. For the second round, 2 µL of the product from the first PCR was used, and the first amplification program was used[25]. This protocol was applied to samples that tested positive for HCMV by conventional PCR.

To complete the genotyping, the second-round amplification products were subjected to standard sequencing using an ABI 3730XL sequencer (Macrogen Company, Seoul, Korea). DNA sequence similarity analysis was performed using BLAST in the NCBI database. To determine and verify the genotypes of HCMV in saliva samples, a multiple sequence alignment was performed on MEGA X[26] using Clustal W software and the representative nucleotide sequences for the gB HCMV genotypes. The accession numbers for each genotype were used as follows: Towne (M22343), C327A (M60929), Merlin (NC 006273.2) for gB1; AD169 (X04606), C336A (M60931) for gB2; C076A (M85228), Toledo-p7 (MF783090) for gB3, and C128A (M60924) and C194A (M60926) for gB4[27].

These sequences were compared to those published for each genotype, including those reported for the Merlin and Towne Cytomegalovirus laboratory strains. With these sequences, a Neighbor-Joining tree[28] was constructed using evolutionary distances calculated by the Maximum Composite Likelihood[29]. There were 476 nucleotide positions in the final dataset, after removing all ambiguous positions for each sequence pair (pairwise deletion option).

Table 1. Primers used to obtain the amplification products of HCMV genotypes from the positive saliva samples through nested PCR.

First Round				
PCR	**Polarity**	**Position (nucleotides)**	**Sequence 5' → 3'**	**Product bp**
CMVQ1+	HS5GLYBG+	868-885	TTTGGAGAAAACGCCGAC	
CMVQ1-	HS5GLYBG-	1619-1597	CGCGCGGCAATCGGTTTGTTGTA	751
Second round				
Nested PCR				
CMVGT1+	HS5GLYBG+	1111-1130	ATGACCGCCACTTTCTTATC	420
CMVGT2+	HEHCMVGB+	1074- 1096	TTCCGACTTTGGAAGACCCAACG	613
CMVGT3+	HS5GLYBM+	1341–1359	TAGCTCCGGTGTGAACTCC	190
CMVGT4+	HS5GLYBD+	1057–1082	ACCATTCGTTCCGAAGCCGAGGAGTCA	465
CMVGT5+	AF043721+	307–325	TACCCTATCGCTGGAGAAC	139
CMVQ2-	HS5GLYBG-	1531–1513	GTTGATCCACRCACCAGGC	

Statistical analysis

Data collected from medical records and laboratory results were stored in an Excel 2013 database. Descriptive methods were used to present the general characteristics of all participating subjects, including variables such as HCMV positivity, viral genotype and clinical aspects of the evolution of each patient. STATA 13.0 (College Station, Texas, USA) was used for statistical analysis. Univariate and bivariate analyses were performed on the evaluated clinical features and the relationship between the presence of HCMV in saliva, viral genotype, and the complications that the patient developed during the hospital stay and their outcomes. Fisher's exact test and Spearman's correlation were used to determine the differences between variables, and the p-value was obtained for each analysis.

RESULTS

Patient description: demographics, diagnoses, HCMV serostatus, type of transplant, and complications (Table 2)

Twenty pediatric HSCTR were included. No patient was withdrawn from the study. There were 12 boys and eight girls, mean age 10.3 years (range 4 to 16). The most common pre-transplant diagnoses were acute lymphoblastic leukemia (ALL; 40%) (8 patients), followed by congenital and acquired aplastic anemia (CAA), Hodgkin lymphoma (HL), and Fanconi anemia (FA) (2 patients each). In addition, there was one patient with an initial diagnosis of Fanconi's anemia who developed acute myeloid leukemia (AML), one patient with sickle cell anemia, one patient with a chronic granulomatous disease, and one patient with hemophagocytic lymphohistiocytosis (Table 2).

Regarding transplant type, eight patients (40%) received allogeneic umbilical cord blood transplant (UCB), seven (35%) received matched related donor transplant (MRD), and two (10%) received autologous transplant. Of the remaining three patients, one received a syngeneic transplant, another received an allogeneic haplo-cord transplant, and the third received an initial UCB followed by an MRD transplant (Table 2).

Table 2 shows the complications reported in clinical records. Nineteen out of 20 patients (95%) presented some complication. Four patients had one complication, six patients had two complications, and nine patients had three to six complications. The most common complication was febrile neutropenia (17 patients, 85%). Of the three patients without febrile neutropenia, one had received an autologous transplant, and the other two had received an allogeneic transplant. Skin graft versus host diseases (GVHD) was the second most frequent complication, affecting five patients (25%). Hemorrhagic cystitis occurred in 25% of the patients, and pneumonia was diagnosed in 15% of the patients, two of them reported as multilobar pneumonia. Oral mucositis was noted in two patients, but the duration or severity of the mucositis did not preclude weekly sampling. Three patients died during hospitalization.

HCMV detection by PCR

Twenty patients were enrolled during the study period, and saliva samples from 12 patients were positive for HCMV DNA by PCR in at least one sample (60%). A total 105 saliva samples were collected, of which 29 (27.6%) were positive (median saliva sample 5 IQR 4-6). Nine saliva samples were collected from patient 6, and the virus was detected in all samples. Eight saliva samples were collected from patients 5 and 13, and the first two samples were positive for HCMV. In some cases, such as patient 2, the low number of samples is explained by difficulties in obtaining saliva, usually due to the general condition of the patient; however, this patient was not excluded from the study because only two saliva samples were not taken. Table 2 shows the salivary viral shedding data for the enrolled patients.

HCMV genotypes

Sequencing identified the HCMV genotype in the saliva samples of the 12 positive patients. Sequence analysis revealed infection by the gB1 genotype in all positive patients, and other genotypes were not identified (Table 2). Analysis of the UL55 gene fragment showed that all samples were most closely related to gB1 genotype, but samples from patients 5, 6 and 11 were more closely related to Merlin strain and samples from patients 9, 17, 18 y 20 were more closely related to Towne strain. The sequences were deposited in the GenBank database (accession numbers OP781314 - OP781325) (Fig. 1).

The median age of HCMV-positive patients was 10.9 ± 3.6 years. No association was found between genotype and sex (p=0.16), age (p=0.36), or the number of complications (p=0.87).

Table 2. Patient description: demographics, diagnoses, serology for HCMV, transplant type, complications, HCMV detection and genotyping.

Patient number	Gender	Age/ years	Prior diagnosis	Type of transplant	Complications during hospitalization	Outcome	# saliva samples/ HCMV positive	HCMV Genotype
1	F	7	Fanconi anemia	MRD	Febrile neutropenia	Discharged	5/0	ND
2	M	7	ALL	MRD	Febrile neutropenia, GVHD, Amebic colitis	Discharged	2/0	ND
3	M	13	ALL	UCB	Febrile neutropenia, pulmonary aspergillosis, pulmonary tuberculosis, septic shock	Deceased	4/0	ND
4	F	5	Hemophagocytic lymphohistiocytosis	UCB	Febrile neutropenia, mucositis, GVHD	Discharged	6/0	ND
5	M	16	Sickle cell anemia	allogeneic haplo-cord	Convulsive syndrome, Gastrointestinal GVHD	Discharged	8/1	**gB1**
6	M	9	ALL	UCB	Febrile neutropenia, bacteriemia, hemorrhagic cystitis	Discharged	9/9	**gB1**
7	M	8	ALL	UCB	Febrile neutropenia, GVHD, amebic colitis, sepsis, pneumonia, multiorgan failure	Deceased	6/4	**gB1**
8	F	6	ALL	MRD	Febrile neutropenia, leukemia relapse	Discharged	4/0	ND
9	M	12	ALL	UCB	Febrile neutropenia, hemorrhagic cystitis, skin GVHD	Discharged	5/3	**gB1**
10	F	7	AML	UCB	Febrile neutropenia, hemorrhagic cystitis	Discharged	4/0	ND
11	M	4	Chronic granulomatous disease	MRD	Febrile neutropenia	Discharged	6/3	**gB1**
12	M	16	Hodgkin's lymphoma	Autologous	Febrile neutropenia, gastrointestinal mucositis	Discharged	5/0	ND
13	F	14	Aplastic anemia	MRD	Hypertension, jaundice, hemorrhagic cystitis, sinusitis	Discharged	8/2	**gB1**
14	M	12	ALL	UCB + MRD	Febrile neutropenia, primary graft failure, hemorrhagic cystitis, unspecified fungal infection, sepsis, pneumonia	Deceased	7/1	**gB1**
15	F	14	AML	UCB	Febrile neutropenia, skin GVHD	Discharged	5/0	ND
16	F	10	Hodgkin's lymphoma	Autologous	Febrile neutropenia	Discharged	6/1	**gB1**
17	F	7	Fanconi anemia and AML	UCB	Febrile neutropenia	Discharged	6/2	**gB1**
18	M	16	ALL	MRD	Febrile neutropenia, sinusitis, oral mucositis	Discharged	2/1	**gB1**
19	M	11	Fanconi anemia	MRD	None	Discharged	4/1	**gB1**
20	M	12	Acquired aplastic anemia	Syngeneic	Febrile neutropenia, bacteriemia	Discharged	3/1	**gB1**

F=Female; M=Male; ALL=Acute Lymphoid Leukemia; AML=Acute Myeloid Leukemia; MRD= matched related donor transplant; UCB=Umbilical cord blood transplant; GVHD=Graft Versus Host Diseases; ND=Not Detected; gB1=Cytomegalovirus Genotype B1

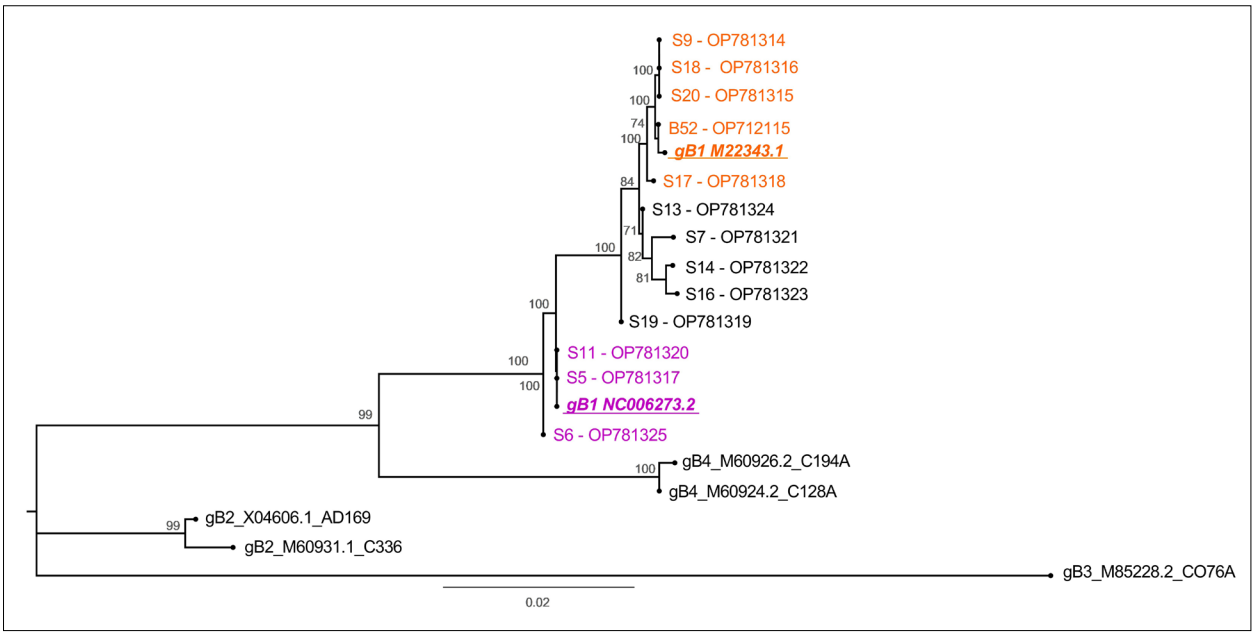

Fig. 1: GenBank database

Among the 12 patients with a positive PCR for HCMV in saliva samples, 50% had three or more complications (7), and no significant difference was observed in the Fisher test (p=0.6). Detailed analysis showed that 75% of HCMV-positive patients presented with febrile neutropenia, with no statistically significant difference (p=0.242).

The patient with primary graft failure and other complications who died was positive for HCMV in saliva. Two other patients died during hospitalization, one of whom was positive for HCMV in saliva.

DISCUSSION

This is the first study of salivary HCMV detection and genotyping in children with HSCT in Colombia. Saliva is an exocrine secretion with essential functions in the oral cavity, and an excellent vehicle for the transmission of HCMV, which can occur by the dispersion of droplets or aerosols in asymptomatic dissemination. Cytomegalovirus infects the acinar cells of the salivary glands, where it persists, so stimulation of salivary secretion may improve viral DNA levels in oral cavity fluid[13,15]. Saliva is currently being used for diagnostic purposes such as tumor marker evaluation, glucose level measurement, virus detection, and many others. The sampling technique depends on the objective or what is to be determined. For virus detection, one of the most important aspects is whether the salivary secretion is stimulated, which may depend on whether the infectious agent is present in the secretion and therefore stimulated saliva increases the possibility of detection, as is the case with Cytomegalovirus. In a previous study of pediatric transplant recipients, we used this technique to collect saliva, and detected Herpes Simplex Virus 2, Epstein Barr Virus and Cytomegalovirus[18].

Saliva is easy to collect with minimal risk to the patient and operator. This is particularly important in pediatric patients, especially as an alternative to blood sampling when the patient does not have venoclysis, or when daily or high frequency sampling is needed. Saliva is easy and safe to transport and does not require highly trained personnel, thus reducing costs. The composition of the medium in which the saliva is transported to the laboratory is intended to maintain the viability of the viral DNA, and the antibiotic inhibits bacterial growth, thus reducing the microbial load in the sample, and facilitating the detection of the viral genetic material. For this reason, it is often used in transplant recipients to diagnose and monitor viruses of the Herpesviridae family, which are one of the main causes of complications[17-19,30].

In this study, 29 of 105 samples collected (27.6%) were positive for HCMV. In some patients, only one sample was positive, while in others, several or all samples were positive. In all cases, the

viral shedding was asymptomatic (no oral lesions of viral etiology were observed) and patients diagnosed with oral mucositis were negative for the virus. Intermittent detection of the virus in saliva may be related to the individual immune status or ganciclovir administration, as suggested by Sarmento et al. upon finding that only four out of 20 kidney recipients had a single positive saliva sample[31]. In addition, because the virus undergoes localized, independent replication in the different anatomical compartments[19], it can sometimes be detected in one type of sample (saliva) but not in the blood, as reported by Correa-Sierra et al. in their evaluation of asymptomatic viral shedding in 27 pediatric solid organ transplant recipients at 32 weeks post-transplant[32]. They also observed that 70.4% of the patients were positive for HCMV in saliva. In another study, the virus was only detected in 10 of the 27 blood samples evaluated[33]. These and other studies have shown that the behavior of salivary virus shedding in HSCTR is variable, dynamic, and may depend on several factors such as immunosuppression status, bone marrow reconstitution and antiviral therapy[18,19]. The above reasons may explain why HCMV was not detected in all the specimens from the patients who were positive for HCMV in the current study.

The gB glycoprotein (encoded by the *UL55* gene) is important for HCMV fusion and entry into cells, and influences viral tropism by interacting with different receptor molecules[7–10]. We genotyped the HCMV using the *UL55* gene from saliva samples and determined the gB1 genotype in the 12 patients. This is the most frequent genotype reported in studies worldwide, although several studies on HSCT recipients have reported coinfections with gB2, gB3 or gB4 genotypes[20-23]. The detection of more than one genotype can be explained by viral infection and/or reinfection[22], but this was not the case in the 12 patients studied here. This is probably due to the size of the sample, which included only 20 patients who were admitted during the proposed period. Other genotypes might have been detected in a larger sample, but in Colombia there is no report suggesting that any HCMV genotypes other than gB1 are in circulation.

Regarding the genotyping technique, DNA sequencing is also a complementary technique for HCMV genotypic variants based on gB cleavage site (CLS) genotypes (gBCLS 1 to 4), since gB exhibits variability at both the N-terminus (gBN 1 to 4) and the C-terminus (gBCLS 1 to 2), which is highly conserved in each gBCLS genotype. Therefore, this approach is based on sequencing a variable fragment of *UL55*, after PCR amplification. In addition, sequencing is very useful for detecting unknown variants. Although the most common genotypic variants are gB1 to 4, there is strong evidence for homologous recombination among gBCLS. In fact, different gBN sequences have been described but not assigned to a genotype numbering system[27]. In these cases, the use of conventional methods, such as specific multiplex PCR, restriction length polymorphism analysis or microarrays are not useful, and sequencing it is recommended.

Genotype confirmation by sequencing provides more robust data, as genotype detection by size can be inaccurate in cases where nested PCR products are similar in size. Currently, the cost of sequencing is low, so the use of both approaches is highly recommended.

HSCT recipient clinical features

More than 100 saliva samples from this cohort of 20 pediatric patients diagnosed with neoplastic and non-neoplastic conditions were analyzed to perform an outcome analysis during hospitalization. ALL was the most frequent pre-transplant diagnosis in the cohort, and the second most common were aplastic anemia (congenital or acquired), HL, and only one case of AML, in contrast to information published by the European Society for Blood and Marrow Transplantation (EBMT), where one third of the cases were AML[34]. The current study also included patients with non-neoplastic diseases such as aplastic anemia, chronic granulomatous disease, hemophagocytic lymphohistiocytosis, and pathologies for which HSCT is recommended by the EBMT, among others[35].

In this cohort, 17 patients (85%) had febrile neutropenia, which is one of the most common complications in patients receiving myeloablative therapy. Empirical antimicrobial therapy is initiated early in patients, with the assumption that the causative agent is bacterial or fungal. However, in some cases, these agents cannot be identified, and viral etiology may be suspected, but HCMV is not frequently reported[35-38]. The second most frequent complication was GVHD (n = 5). The risk of HCMV infection or reactivation after transplantation is

increased by the immunosuppression associated with GVHD prophylaxis and/or treatment. The use of corticosteroids also interferes with immune reconstitution and contributes to increased risk of reactivation. The relationship between HCMV and GVHD is bidirectional, as immunosuppression and the consequent possibility of viral reactivation and dissemination also contribute to the development of GVHD and other serious complications associated with Cytomegalovirus, such as pneumonia, gastroenteritis, and even transplant failure or leukemia relapse[37,38].

HCMV infection or reactivation is frequently associated with morbidity and mortality in HSCT patients, although sensitive methods of detection and early therapy have significantly improved patient prognosis. Viremia detection is followed by ganciclovir or valganciclovir therapy, although these antivirals induce or exacerbate myelosuppression, and contribute to the occurrence of infectious complications[38-40].

Two of the three fatal cases tested positive for HCMV in saliva. Since all the HCMV patients had the gB1 genotype, it was not possible to establish associations with the observed outcomes. Eleven of 12 patients who were HCMV-gB1 positive in saliva had complications that were likely related to HCMV infection/reactivation or other factors such as pre-transplant diagnoses and type of transplant. Although all individuals in this cohort were gB1, genotyping of infectious HCMV to search for coinfections may help to understand the outcome of patients or infection/reactivation episodes[22].

The present study demonstrated the feasibility and usefulness of saliva as a specimen in which to detect HCMV in hematopoietic stem cell pediatric patients. The collection of saliva samples to detect Cytomegalovirus infection or reactivation can be recommended, given the ease of sample collection, patient convenience and reliable results using qualitative PCR. The study also shows that nested PCR followed by sequencing is a suitable approach for determining HCMV genotype. These techniques are accessible and inexpensive at present.

There is no information on the genotypes of Cytomegalovirus in Colombia. It can therefore be assumed that the predominant genotype is gB1, as in other countries. In this cohort, all the patients were gB1 positive. The study demonstrated the feasibility of using saliva to detect HCMV reactivation and genotyping with highly sensitive techniques such as sequencing. In this cohort, patients found it easy to cooperate with the saliva collection, and were not uncomfortable with the procedure.

Several studies have attempted to determine the relationship between HCMV genotypes, complications and outcomes in recipients of hematopoietic precursor and solid organ transplants[20–23], with variable results. Moreover, the correlation between complications and outcomes has not been observed in all groups studied. It is crucial to identify mixed infections with more than one genotype. These coinfections are associated with higher viral loads, which persist longer and are difficult to control[22,23]. It has also been reported that coinfections may be associated with the development of the Cytomegalovirus associated syndrome and the earlier onset of clinical manifestations of reactivation[40].

Although the current study included a small number of patients and did not allow inference of these associations, it reinforces the importance of evaluating this association and determining Cytomegalovirus genotype(s).

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CONFLICT INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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