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CLAUDIO MARANHÃO PEREIRA

INVESTIGAÇÃO DO HHV-6 EM SANGUE PERIFÉRICO E FLUIDOS BUCAIS DE PACIENTES PORTADORES DE MANIFESTAÇÕES BUCAIS DA DOENÇA DO ENXERTO CONTRA O HOSPEDEIRO CRÔNICA

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção de título de Doutor em Estomatopatologia, Área de Patologia

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Sumário

Lista de Abreviaturas

AIDS – síndrome da imunodeficiência adquirida

CD – cluster de diferenciação

CMV – citomegalovirus

DECHc – doença do enxerto contra o hospedeiro crônica

DNA – ácido desoxirribonucléico

HBLV – vírus humano linfotrópico B

HHV – vírus herpes humano

HHV-1 – vírus herpes humano tipo 1

HHV-6 – vírus herpes humano tipo 6

HIV – vírus da imunodeficiência humana

IgG – imunoglobulina G

IgM – imunoglobulina M

NK – *natural killer*

OMS – organização mundial de saúde

PCR – reação em cadeia da polimerase

RT – transcriptase reversa

SNC – sistema nervoso central

TMO – transplante de medula ósseo

Resumo

Doença do enxerto contra o hospedeiro crônica (DECHc) é a mais freqüente complicação tardia do transplante de medula ósseo (TMO) alogênico. Em sua forma crônica, acomete cerca de 50% dos indivíduos submetidos ao TMO alogênico, sendo que mais de 80% dos pacientes apresentam envolvimento bucal. O vírus herpes humano tipo 6 (HHV-6) é o agente etiológico do exantema súbito. Após a infecção primária geralmente o vírus fica em latência nas glândulas salivares e a saliva torna-se seu principal modo de transmissão. HHV-6 em latência pode ser reativado em pacientes imunocomprometidos podendo causar graves complicações.

O objetivo deste estudo foi padronizar uma técnica para coleta e extração de DNA viral de fluidos bucais (saliva total, saliva de glândula parótida e fluido gengival); Assim como avaliar o quadro clínico e investigar a presença do vírus HHV-6 nas manifestações bucais da DECHc.

O vírus foi pesquisado no sangue periférico, saliva total, saliva de glândula parótida, fluido gengival e amostras de tecido de mucosa labial. Os fluidos bucais e sangue periférico foram coletados de 19 pacientes portadores de manifestações bucais da DECHc e de 28 indivíduos saudáveis doadores de sangue. Fluido gengival e saliva da glândula parótida foram coletados utilizando cones endodônticos de papel para evitar a contaminação destes fluidos com a saliva total. Amostras de tecido bucal foram obtidas de 12 pacientes portadores de manifestações bucais da DECHc e de 12 indivíduos saudáveis. Reação em cadeia da polimerase (PCR) foi realizada com o objetivo de identificar a presença do HHV-6.

Seis (31.6%) pacientes apresentaram a forma liquenóide clássica da DECHc em cavidade bucal, 6 (31.6%) a forma atrófica-ulcerativa, 2 (10.5%)

hiperqueratótica e 3 (15.8%) lesão bucal mista. Dois pacientes não apresentaram lesões bucais no momento do exame clínico. O vírus foi identificado na saliva total de 13 (68%) pacientes portadores da DECHc e de 19 (67.8%) doadores de sangue. HHV-6 não foi identificado em nenhuma amostra de fluido gengival e de saliva de glândula parótida de pacientes portadores da DECHc. No grupo controle, 4 amostras de fluido gengival e 4 amostras de saliva de glândula parótida (14.3%) apresentaram resultados positivos. Duas amostras de tecido bucal foram positivas para o HHV-6 nos pacientes portadores da DECHc.

Os resultados demonstraram predominância e similar incidência das formas clínicas liquenóide e ulcerativa-atrófica. Os dados sugerem que os pacientes portadores de DECHc e indivíduos saudáveis apresentam alta e similar incidência do HHV-6 nos fluidos bucais e não mostram influência do vírus na severidade das lesões bucais da DECHc.

Abstract

Chronic graft versus host disease (cGVHD) is the most late complication of allogeneic bone marrow transplantation. Chronic GVHD affects approximately 50% of the surviving adults and oral manifestations can be found in around 80% or more, of patients. Human herpesvirus 6 (HHV-6) is the etiologic agent of exanthem subitum. The virus is latent in salivary glands and saliva is the main form of viral transmission. Latent HHV-6 may be reactivated in immunocompromised patients causing severe complications. The aims of this study were to standardize a technique for collecting and extracting viral DNA from oral fluids (gingival crevicular fluid, whole saliva, and parotid gland saliva), evaluate the oral manifestation and clinical features of the cGVHD and investigate the HHV6 in patients with oral manifestation of cGVHD. Peripheral blood and oral fluids (whole saliva, gingival crevicular fluid and parotid gland saliva) from 19 cGVHD patients and 28 blood donors were searched for viruses. Gingival crevicular fluid and parotid gland saliva were collected using endodontic paper cones in order to not contaminate these fluids with whole saliva. Buccal's mucosa samples were obtained from 12 cGVHD patients and 12 healthy individuals. Nested-polymerase chain reaction was employed to identify the HHV6.

The results showed that six (31.6%) patients presented the lichenoid form of cGVHD in buccal mucosa, 6 (31.6%) atrophic-ulcerative, 2 (10.5%) hyperceratotic and 3 (15.8%) the mixing form. Two patients did not present oral lesions at the moment of the clinic exams. The virus was detected in whole saliva in thirteen (68%) cGVHD patients and in ninetheen (67.8%) blood donors. HHV6 was not identified in any samples of gingival crevicular fluid and parotid gland saliva in the cGVHD patients. In the control group, four gingival crevicular fluid and four (14.3%)

parotid gland saliva samples were positive. Two buccal's mucosa samples of cGVHD patients presented positives results.

The results demonstrated predominance of lichenoid and ulcerative-atrophic forms with similar incidence of these lesions in patients with GVHDc. The data also showed that, cGVHD patients and healthy individuals present high and similar incidence of HHV-6 in oral fluids and did not support the virus influence in the severity of the cGVHD oral lesions.

Introdução

O vírus herpes humano 6 (HHV-6) é um dos oito tipos de vírus da família *Herpesviridae* que tem como hospedeiro primário o homem (Pellett *et al*., 1992). Foi primeiramente isolado pôr Salahuddin *et al*., (1986) em cultura de linfócitos do sangue periférico de pacientes portadores de distúrbios linfoproliferativos e síndrome da imunodeficiência adquirida (AIDS). O vírus foi denominado de HBLV (vírus humano linfotrópico B) pelo fato de acreditarem que o mesmo apresentava tropismo pelos linfócitos B. Trabalhos posteriores comprovaram que esse tropismo se dava para linfócitos T (Ablashi *et al*., 1987), mas especificamente para linfócitos T CD4+, além de poder infectar uma variedade de outras células da linhagem T e B (Becker *et a*l., 1989). Devido a sua semelhança genética com o citomegalovírus humano (CMV), foi incluído na subfamília beta-HHV (Roizman *et al*., 1992). Duas variantes do HHV-6 são consideradas, A e B, sendo suas diferenças baseadas principalmente em suas características de crescimento "*in vitro*", imunorreatividade e sequência de DNA (Pellett *et al*., 1992). A variante B é considerada a principal causa do exantema súbito*,* enquanto a variante A apresenta-se mais citolítica além de ser mais virulenta (Schiemer *et al*., 1991; Dewhurst *et al*., 1993).

O HHV-6 assemelha-se a outros vírus herpéticos da subfamília beta-herpesvirinae devido a sua permanência, quando em latência, nas glândulas salivares e células mononucleares do sangue (Fox *et al*., 1990; Roizman *et al*., 1992; Luppi *et al*., 1993). Esta característica pode explicar a alta incidência do vírus na saliva de adultos saudáveis, bem como a alta taxa de infecção em crianças (Jarret *et al*., 1990; Harnett *et al.,* 1990; Levy *et al*., 1990; Cone *et al*., 1993; Di Luca *et al*., 1995; Aberle *et al*., 1996).

A prevalência do vírus HHV-6 na população adulta saudável pode variar de 50 a 90% (Pellett *et al*., 1992), sendo que infecção primária se dá geralmente nos dois primeiros anos de vida. A saliva tem sido considerada um meio de transmissão viral importante (Piertroboni *et al*., 1988). No Japão a freqüência de exantema súbito em crianças infectadas pelo HHV-6 é de cerca de 60%. Nos Estados Unidos, 70% dos casos de infecção primária não apresentam manifestações clínicas ou resultam apenas em febre ou "*rash* cutâneo" (Niederman *et al*., 1988; Sobue *et al*., 1991; Akashi *et al*., 1993; Drago *et al*., 1999).

Após a infecção primária, o HHV-6 geralmente permanece em latência e pode ser isolado em saliva de indivíduos infectados que não apresentem quaisquer alterações clínicas (Cone *et al*., 1993). Ainda não foi totalmente esclarecido os locais de latência viral, porém pode ser identificado em células mononucleares de sangue periférico de 17 a 90% dos adultos saudáveis, além das glândulas salivares e no sistema nervoso central (Gopal *et al*., 1990; Cuende *et al*., 1994, Yoshikawa, 2004). Isto leva crer que o retículo linforeticular é o local de permanência do vírus, enquanto as glândulas salivares podem representar os sítios de replicação e o reservatório viral (Fox *et al*., 1990).

Ao exame histológico em culturas de linfócitos, pode-se notar o efeito citopático do HHV-6, caracterizado pela presença de células baloniformes e sincícia, indicando a porcentagem de células infectadas (Sallahuddin *et al*., 1986; Lusso *et al*., 1989). Culturas mistas de HHV-6 e HHV-1 têm demonstrado co-infecção de linfócitos T CD4+, resultando na aceleração da expressão viral do HHV-1 e da apoptose celular. Além, disso, o HHV-6 pode infectar os linfócitos T CD8+, células "*natural Killer*" (NK), fagócitos mononucleares além de células do sistema nervoso central (Lusso *et al*., 1989; Yoshikawa, 2004).

Em indivíduos imunocompetentes a infecção pelo HHV-6 está relacionada a doenças inflamatórias como o exantema súbito, febre infantil, hepatite, síndrome da fadiga crônica, doenças auto-imunes, histiocitose, síndrome das "luvas e meias", mononucleose infecciosa "*like*" e ainda estar relacionado a casos de

esclerose múltipla. Também são encontrados indícios dessa infecção herpética em pacientes com distúrbios linfoproliferativos malignos como linfomas e leucemias linfoblásticas (Niederman *et al*., 1988; Sobue *et al*., 1991; Akashi *et al*., 1993; *Drago & Rebora, 1999*). Ghodratnama *et al*., em 1997, identificaram anticorpos contra o HHV-6 em pacientes com estomatites aftosas recorrentes. Yadav *et al*., em 1997, também identificaram o DNA do HHV-6 em lesões ulcerativas em cavidade bucal.

Em pacientes imunossuprimidos, o HHV-6 pode tornar-se ativo ou infectá-los de forma primária. Pacientes portadores sintomáticos do vírus HIV podem manifestar infecções disseminadas nos pulmões, fígado, rins, baço e linfonodos além de doenças no Sistema Nervoso Central (SNC) como distúrbios de desmielinização. O uso de medicações imunossupressoras mieloablativas em pacientes submetidos ao tratamento antineoplásico, propicia a infecção secundária pelo HHV-6. As manifestações comuns nesses pacientes são os "*rash*" de pele, pneumonite interticial e encefalites. Formas mais graves da doença do enxerto contra hospedeiro (DECH), podem estar relacionados à presença do HHV-6 (Drago & Rebora, 1999).

Em pacientes receptores de medula óssea, devido a períodos extensos de imunossupressão, a infecção pelo HHV-6 tem papel importante na perda do enxerto e no aumento da morbidade dos pacientes (Yoshikawa *et al*., 1991).

Transplante de Medula Óssea (TMO) tem sido rotineiramente usado para o tratamento de uma variedade de doenças incluindo neoplasias hematológicas, imunodeficiências e síndromes com deficiência medular. Infecções virais, especialmente com herpes vírus, são causas de morbidade e mortalidade depois do transplante de medula óssea (Maeda *et al*., 1999).

Infecção pelo HHV-6 logo após o transplante de medula óssea é comum com predominância, mas não exclusividade, da variante B. Há uma associação do HHV-6 com uma variedade de doenças clínicas incluindo encefalites, pneumonias, linfoma T pos-transplante, microangiopatia trombótica, perda do enxerto e supressão da medula óssea. Estudos têm demonstrado atividade supressiva do

HHV-6 na maturação e crescimento dos precursores da medula óssea normal, incluindo granulócitos / macrófagos, eritrócitos e linhagens megacariocíticas (Clark *et al*., 1990).

A quimioterapia e radioterapia, assim como o transplante de medula óssea, têm fundamental importância no tratamento contra o câncer. Estas terapias causam de forma primária ou secundária, com alta freqüência na cavidade bucal, um efeito adverso denominado mucosite. A mucosite é uma alteração extremamente dolorosa produzida por citotoxicidade, que restringe a função mastigatória e de deglutição (Sonis, 1998). Clinicamente varia de edema a úlceras graves podendo agir como sítio de infecção secundária e porta de entrada para microbiota bucal endógena, tornando os pacientes vulneráveis a infecções locais ou sistêmicas (Sonis, 1998).

A mielossupressão em decorrência da quimioterapia resulta em neutropenia, o que faz com que a mucosite seja um fator de risco significante para infecção local e sistêmica. Pacientes com mucosite e neutropenia estão quatro vezes mais expostos ao risco de septicemia quando comparados aos pacientes sem mucosite (Sonis, 1998).

Ainda não há consenso quanto à classificação das mucosites. A Organização Mundial de Saúde (OMS), em 1990, classificou a mucosite da seguinte forma (Sonis *et al*., 1999):

- Grau 0: ausência de estomatite;
- Grau I: eritema clinicamente visível e/ou paciente menciona sensação de ardência na cavidade oral;
- Grau II: eritema e ulceração ou mancha branca presente ao exame clínico. Paciente queixa-se de dor intra-oral, mas está hábil para comer;
- Grau III: eritema e ulceração ou mancha branca presente ao exame clínico. Paciente queixa-se de severa dor intra-oral e está impossibilitado de ingerir alimentos sólidos.

- Grau IV: eritema e ulceração ou mancha branca presente ao exame clínico. Paciente queixa-se de severa dor intra-oral e está impossibilitado de ingerir alimentos sólidos e líquidos.

Para se propor um tratamento adequado a essas lesões, faz-se necessário o diagnóstico diferencial das lesões resultantes da citotoxicidade medicamentosa daquelas fúngicas ou virais que se sobrepõem a essas alterações epiteliais (Sonis, 1998).

Diversos são os exames utilizados para o diagnóstico da infecção pelo HHV-6. A presença do anticorpo IgM anti-HHV-6 pode indicar infecção recente ou reativação viral, enquanto a infecção crônica é geralmente caracterizada pela presença do anticorpo IgG anti-HHV-6, presente em 80 a 90% da população adulta (Briggs *et al*., 1988; Levy *et al*., 1990; Suga *et al*, 1992). A investigação do genoma viral pode ser feita em fluidos corporais como a saliva e sangue periférico, em amostras de tecidos (biópsias) ou citologias esfoliativas através da técnica de PCR (reação em cadeia da polimerase) (Niederman *et al.,* 1988; Briggs *et al*., 1988; Pietroboni *et al*., 1998; Gopal *et al*., 1990; Kido *et al*., 1990; Jarret *et al*., 1990; Aubin *et al*., 1991; Yamomoto *et al.,* 1994; Cuende *et al*., 1994; Wilborn *et al*., 1994; Aberle *et al*., 1996).

O PCR é um método para amplificação específica de seqüência de ácidos nucléicos através de repetidos ciclos de síntese de DNA sendo, portanto, de grande valia para a detecção de agentes infecciosos (Peter, 1991). Este exame apresenta alta sensibilidade em relação a outros métodos de quantificação molecular, tornando o método de escolha para a quantificação de ácidos nucleicos virais presentes em pequenas amostras de material biológico (Clementi *et al.,* 1995).

A determinação de anticorpos IgG anti-HHV-6 sorológicos tem apenas valor limitado devido à alta prevalência da infecção pelo HHV-6 na população mundial e pela reatividade cruzada antigênica com outros beta-herpesvirus, como CMV e HHV-7. Similar para a determinação da titulação de anticorpos IgG, ensaios qualitativos de PCR para detecção de DNA viral em leucócitos sanguíneos

periféricos não podem distinguir entre infecção latente, presente e a vasta maioria dos adultos saudáveis (Locatelli *et al*., 2000).

Apenas PCR quantitativo baseado em transcriptase reversa (RT-PCR) com células sanguíneas ou técnicas de PCR quantitativos aplicadas para a detecção de DNA viral livre em fluidos corporais, podem determinar infecção ativa pelo HHV-6. Entretanto esses ensaios desenvolvidos até o presente momento apresentam duas grandes limitações: primeiro não são extremamente sensíveis; segundo, eles não podem estabelecer se o DNA viral é derivado de vírions circulantes ou de infecções latentes onde células são danificadas durante a manipulação para a técnica (Locatelli *et al*., 2000).

Objetivos

Foi proposto identificar o HHV-6 no sangue periférico, na saliva total, no fluido gengival e saliva de glândula parótida, de pacientes portadores de manifestações bucais da doença do enxerto contra o hospedeiro crônica e em indivíduos sadios, por meio de PCR, correlacionando com a presença e estadiamento das mucosites bucais. Visamos com isso atingir os seguintes objetivos:

1. Avaliar o HHV-6 no sangue periférico (lise de leucócitos), saliva total, saliva de glândula parótida e fluido gengival de indivíduos sadios por meio de PCR;

2. Avaliar o HHV-6 na saliva total, sangue (lise de leucócitos), saliva de glândula parótida e fluido gengival de pacientes submetidos ao transplante de medula óssea alogênico portadores da doença do enxerto contra o hospedeiro crônica;

3. Investigação do HHV-6 na mucosa bucal de pacientes portadores da doença do enxerto contra o hospedeiro crônica e de indivíduos saudáveis;

4. Avaliação da incidência e aspectos clínicos das manifestações bucais da doença do enxerto contra o hospedeiro crônica.

Capítulo 1

HUMAN HERPESVIRUS 6 IN ORAL FLUIDS FROM HEALTHY INDIVIDUALS

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Summary

Background: Human herpesvirus 6 (HHV6) is the etiologic agent of exanthem subitum. The virus is latent in salivary glands and saliva is the main form of viral transmission. The objective of this study was to assess HHV6 incidence in the fluids from healthy individuals using a standardized technique for collecting and extracting viral DNA from gingival crevicular fluid, whole saliva and parotid gland saliva.

Design: Samples of oral fluids and peripheral blood were collected from 28 blood donors and HHV6 was detected using PCR assay. Parotid gland saliva and gingival crevicular fluid were collected by endodontic paper cones in order to not contaminate these fluids with whole saliva.

Results: Of the 28 donors, 20(71.4%) presented positive results in at least 1 of the 3 oral fluids researched. Whole saliva was positive in 19 (67.8%) volunteers, while only 4 (14.2%) samples of gingival crevicular fluid and 4 of parotid gland saliva proved to be positive.

Conclusions: The results suggest that HHV6 is present in the saliva of a large proportion of the healthy adult population. The use of endodontic paper cones for oral fluid collection and viral extraction was efficient, simple, cheap and painless. In spite of, the small number of cases studied it was possible to demonstrate that neither gingival crevicular fluid nor parotid gland saliva were the principal source of HHV 6 in whole saliva.

Key Words: HHV 6, exanthema subitum, saliva, parotid gland, viral shedding
Running title: HHV6 in oral fluids

Introduction

Human herpes virus 6 (HHV6) is one of eight types of viruses from the *Herpesviridae* family. Humans are considered the primary host[1]. HHV6 isolates can be separated into two closely related and distinct groups designated variant A and B. Primary infection with HHV6 variant B is known to cause exanthema subitum in infants[2], while the role of variant A in disease has yet to be defined[3]. HHV6 remains latent after primary infection and persists in selected anatomical sites. Reactivation or reinfection may occur, particularly in patients with immune deficiency[4]. The latent virus site is believed to be in B-lymphocytes, and salivary glands[5, 6].

HHV-6 infection can be detected using serological methods. The presence of IgM indicates recent infection or viral reactivation and detection of IgG indicates chronic or latent infection[7, 8]. Latent infection has been found in 80% to 90% of the healthy population over 2 years of age[6, 7, 9]. HHV-6 can also be detected by the isolation of HHV6 DNA from saliva using PCR.[10-13] . However, the presence of possible PCR inhibitors in saliva may reduce the sensitivity of this method[13-15]. Studies using this approach report infection in 0 - 100% of the population [6, 10, 12, 16, 17].

The aims of this study were to standardize a technique for collecting and extracting viral DNA in 3 different oral fluids (whole saliva, parotid gland saliva and gingival crevicular fluid), and to assess the HHV6 incidence in these fluids in healthy individuals.

Material and methods

Samples of whole saliva, parotid gland saliva and gingival crevicular fluid, as well as peripheral blood were collected from 28 blood donors. All individuals were submitted to a physical examination in which the absence of systemic diseases and oral lesions was established. The donors were instructed regarding the nature and non-compulsory character of the research, and signed informed consent was obtained before sample collection. The protocol was approved by the Ethical Committee of this Hospital.

Peripheral blood (10 mL) was collected in a tube with anticoagulant (EDTA) and was submitted to DNA extraction from peripheral blood mononuclear cells (PBMCs) as described by Wilborn *et al* (1994)[18]. DNA solution (5 uL) was included in each PCR reaction. Unstimulated whole saliva was collected in sterile plastic tubes and 400μL samples were treated overnight with proteinase K solution (0.5% sodium dodecyl sulfate, 0.5M NaCl, 25mM EDTA, 0.01M Tris – pH8.0 and 0.05mg of proteinase K per mL). DNA was extracted using phenol-chloroform purifications as described by Cone *et al* (1993)[10]. The extracted DNA was resuspended in 20 μL of TE buffer (10mM Tris – HCl, pH 7.5, 0.1mM EDTA). 5 uL of DNA solution was included in each PCR reaction. Parotid gland saliva and gingival crevicular fluid were collected using sterile paper endodontic cones. Five cones were placed at the outlet of the parotid duct after relative isolation and prior to drying of the region.

No salivary stimulation technique was used. The individual's face was massaged during collection with the intention of milking the gland. Five cones, corresponding to approximately 75μL of parotid gland saliva, were collected in a sterile microtube and stored in a freezer at -20º C. Gingival crevicular fluid was collected placing five endodontic cones at the gingival fold of previously selected clean teeth.

Cones were treated overnight with proteinase K solution and DNA was extracted from 75μL of each fluid using phenol-chloroform purifications[10]. The extracted DNA was resuspended in 20 μL of TE buffer (10mM Tris – HCL, pH 7.5, 0.1mM EDTA). DNA solution (5 uL) was included in each PCR reaction.

HHV6 PCR was carried out using four specific primer pairs as previously described [10]. In order to confirm the presence of intact DNA in the samples a beta globin primer pair was used as control[10]. Particular care was taken to avoid contamination of PCR samples. Positive and negative DNA control samples, as well as DNA-free samples were included in each set of reactions.

Results

Beta globin DNA gene was clearly amplified in all samples analyzed. The results showed 8 (28,5%) positive reactions in both whole saliva and in blood; 6 (21,4%) positive results only in whole saliva; 1 (3,5%) positive reaction occurred in whole saliva and gingival crevicular fluid; 1 (3,5%) positive reaction occurred in both whole saliva and parotid gland saliva; 1 (3,5%) positive reaction occurred in whole saliva, gingival crevicular fluid and in parotid gland saliva; 1 (3,5%) positive result in whole saliva, gingival crevicular fluid, parotid gland saliva and blood; 1 (3,5%) positive in whole saliva, parotid gland saliva and blood and 1 (3,5%) positive in gingival crevicular fluid and blood. Eight (28,5%) samples presented negative results in all 4 fluids analyzed. Of the 28 donors, 20 (71,4%) presented positive result in at least 1 of the 3 oral fluids researched and whole saliva was positive in 19 (67,8%) of them (Table 1).

Discussion

HHV-6 may persist in saliva, although its reported isolation from this fluid has ranged from 0 to 100%[6, 10, 12, 16, 17]. The reasons for this variation are unclear. Some primers used may provide cross-reaction between HHV6 and HHV7[5]. This study used primers derived from a highly conserved sequence of the HHV6 genome, which would eliminate this possibility. The highest number of positive results (90% to 100%) was found in expectored saliva[10, 12] . Zerr *et al.*[14] compared two different methods for whole saliva collection and HHV6 detection using a real time quantitative fluorescent probe PCR assay. The results from expectorated saliva (400μl) and saliva collected with paper strips (60μl) were similar. Increasing the volume of saliva did not necessarily increase the sensitivity of the test but could increase the action of inhibitors found in this fluid[14]. In our study approximately 75μL of saliva were analyzed corresponding to 5 cones with 15μl of fluid in each strip. For whole saliva, 400μl were analyzed.

Whole saliva is a complex mixture of glandular secretions, microorganisms, epithelial cells, leucocytes, erythrocytes and food remains[13, 19]. Gingival crevicular fluid is the main source of leucocytes for saliva. Pure saliva direct from salivary glands does not contain leucocytes[18,19]. Our results show that 19 samples (67.8%) of whole saliva gave positive results for HHV6 while only 4 samples (14%) of gingival crevicular fluid and 4 of parotid gland saliva (14%) did. Although, we found a small number of HHV6 positive results in gingival crevicular fluid and parotid gland saliva all samples presented positive results for the β-globin gene

amplification. These data suggest that gingival crevicular fluid and parotid gland saliva were not the principal source of the HHV6 found in whole saliva.

These data suggest that HHV6 is present in the saliva in a large proportion of the healthy adult population. The use of endodontic paper cones for oral fluid collection and viral DNA extraction was efficient, simple, cheap and painless.

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Tables

Blood donor (n=28)	**HHV 6** PCR reactions
20 (71.4%)	Positive
8 (28,5%)	Whole saliva, blood
6 (21,4%)	Whole saliva
1 (3,5%)	Whole saliva, gingival crevicular fluid
1 (3,5%)	Whole saliva, parotid gland saliva
1 (3,5%)	Whole saliva, gingival crevicular fluid, parotid gland saliva
1 (3,5%)	Whole saliva, gingival crevicular fluid, parotid gland saliva, blood
1 (3,5%)	Whole saliva, parotid gland saliva, blood
1 (3,5%)	Gingival crevicular fluid, blood
8 (28,5%)	Negative

Table 1 – HHV 6 detected by PCR reaction in blood, whole saliva, parotid gland saliva and gingival crevicular fluid from 28 healthy individuals.

Capítulo 2

Human herpesvirus 6 (HHV 6) in patients with oral chronic graft-versus-host disease (cGVHD) following allogeneic hematopoietic progenitor cell transplantation

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Running title: HHV6 in oral cGVHD

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Abstract

Chronic graft-versus-host disease (cGVHD) is a major cause of morbidity in long-term survivors of allogeneic hematopoietic progenitor cell transplantation. Herpesviruses are involved in the occurrence and progression of various oral diseases. The aim of this study was to investigate the human herpesvirus 6 (HHV6) in patients with oral manifestation of cGVHD. Peripheral blood and oral fluids (whole saliva, gingival crevicular fluid and parotid gland saliva) from 19 cGVHD patients and 28 blood donors were searched for HHV6. Buccal mucosa samples were collected from 12 cGVHD patients and 12 healthy individuals. Nested-polymerase chain reaction was employed to identify the HHV6. The virus was detected in whole saliva in 13 cGVHD patients (68%) and in 19 blood donors (67%). HHV6 was not identified in any of the gingival crevicular fluid and parotid gland saliva samples in the cGVHD patients. In the control group 14.3% of either, 4 gingival crevicular fluid and 4 parotid gland saliva samples were positive. Two buccal mucosa samples of cGVHD patients were positive for HHV 6. These results indicate that patients with oral manifestations of cGVHD and healthy individuals

present high and similar incidence of HHV6 in blood and oral fluids. These data do not support the participation of HHV6 in oral lesions of cGVHD.

Key Words: Human herpesvirus 6, graft-versus- host disease, hematopoietic progenitor cell transplantation, saliva, polymerase chain reaction, mounth

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Introduction

Allogeneic bone marrow transplantation (BMT) has been used as a therapeutic modality for patients with malignant neoplasms, immunodeficiency diseases and syndromes of marrow failure (1, 2). Graft versus host disease (GVHD) is a common complication in hematopoietic progenitor cell transplant patients (3). Acute GVHD usually appears within the first three months following transplantation; it is diagnosed in 30% to 50% of hematopoietic cell transplant recipients and has a mortality rate of 20% to 30% (3, 4, 5). Chronic GVHD (cGVHD) develops after three or more months following hematopoietic cell transplantation affecting 60-80% of the long-term survivors (1, 3, 6).

Oral manifestations have been described in approximately 80% of the patients with cGVHD. Oral lesions closely resemble those seen in a variety of autoimmune connective tissue diseases, including reticular and ulcerative lichen planus, systemic sclerosis, lupus erythematosus and Sjögren's syndrome (7-12). It can cause severe pain, dysphagia and lead to nutritional deficits, impacting negatively on quality of life. Lichenoid reactions typically seen as white striae are the most distinctive oral lesions and occur in 80-100% of patients with cGVHD. Salivary glands can also be affected (8, 9, 12, 13).

Human herpes virus 6 (HHV6) is one of the eight types of viruses from the *Herpesviridae* family, of which humans are considered the primary host (14). HHV6 isolates can be separated into two closely related and distinct groups, designated variant A and B (15). Primary infection with HHV6 variant B causes exanthem

subitum in infants, while the clinical features of variant A infection remain poorly defined (16). HHV6 remains latent after primary infection in selected anatomical sites, as salivary glands (17-19), mononuclear cells (20) and the central nervous system (21-23). Reactivation or reinfection may occur, particularly in patients with immune deficiency. (24). In hematopoietic cell recipient patients, the virus might enhance immune system reactivity or alter the antigenicity of the host tissue, thereby leading to an increased incidence of GVHD (24-25).

Saliva has been considered an important means for HHV6 transmission, but the main source of the virus found in the oral cavity is not yet clear (26-29). HHV6 can be detected in periodontal diseases, recurrent aphthous ulceration, leukoplakia, epithelial tumors and oral lichen planus (30-31). The participation of the virus, on these diseases however, has yet to be fully delineated (30-32).

The aim of this study was to investigate the presence of HHV6 in blood, oral fluids and buccal mucosa samples of patients with oral manifestations of cGVHD.

Patients, controls and methods

Nineteen patients with cGVHD were submitted to a medical and dentistry evaluations. Data regarding the graft type (bone marrow or peripheral blood cell source), organs involved by cGVHD and drugs received at the moment of sample collection were obtained by reviewing the medical records. Oral manifestations of cGVHD were qualified in lichenoid (white striae), atrophic-ulcerative, hyperceratosis and mixing form (7, 33, 34). cGVHD was graded as limited, when affecting the skin or oral cavity locally, or extensive, when affecting two or more organs with a generalized skin and/or oral involvement (35).

Samples of whole saliva, parotid gland saliva and gingival crevicular fluid were collected from 17 patients with cGVHD. It was not possible to collect oral fluids in two patients due to severe hyposalivation

Biopsies of minor salivary glands of the lower lip from 12 patients, used to diagnose and stage cGVHD, were used to detect HHV6. The material was fixed in 10% formalin and embedded in paraffin (37).

Control groups

Samples of whole saliva, parotid gland saliva and gingival crevicular fluid, as well as peripheral blood were collected from 28 healthy blood donors.

Normal oral mucosa fixed in 10% formalin and embedded in paraffin, was obtained from twelve individuals with mucoceles of the lower lip, who were submitted to

surgical treatment. All individuals of the control group were healthy, without any oral lesions.

Patients with cGVHD and controls were instructed regarding the nature and non-compulsory character of the research, and signed informed consent was obtained before sample collection.

Peripheral blood, oral fluids and buccal mucosa analysis:

Peripheral blood (10mL) was collected in a tube with anticoagulant (EDTA) and was submitted to leukocyte and neutrophil counting (Cell-Dyn 3500 – Abbott, Amstelveen - The Netherlands). DNA extraction from peripheral blood mononuclear cells (PBMCs) was performed as described by WILBORN *et al,* 1994 (24).

Unstimulated whole saliva (2.0mL) was collected in sterile plastic tubes and 75μL of parotid gland saliva and gingival crevicular fluid were collected using sterile paper endodontic cones (29). The samples were submitted to DNA extraction as described by Cone et al 1993 (18).

Genomic DNA was extracted from 5μm thick sections of formalin-fixed paraffin–embedded tissue as described by SHIBATA, 1994 (38). Briefly, two sections were submitted to paraffin extraction with xylene, washed twice with ethanol (100%), and vacuum dried. This was followed by digestion for 4 hours at 55C°, with 400 μg/mL

proteinase K, 100mM Tris-HCl, 4mM EDTA (pH 8.0), and boiled for 10 minutes to inactivate the enzyme. The samples were stored at -20C° until analysis.

HHV6 nested - PCR was carried out using two sets of nested primers previously described (39). In order to confirm the presence of intact DNA in the samples a beta globin primer pair was used as control (18). Particular care was taken to avoid contamination of PCR samples. Positive and negative DNA control samples, as well as DNA-free samples were included in each set of reactions.

Results

Fourteen (73.7%) patients of the cGVHD group were male and 5 (26.3%) female, with age range of 19 to 63 years (mean 39.0). Twelve (63.1%) patients had chronic myeloid leukemia (CML), 3 (15.8%) severe aplastic anemia (AA), 2 (10.5%) acute myeloid leukemia (AML), 1 (5.3%) acute lymphocytic leukemia (ALL) and 1 (5.3%) multiple myeloma (MM). Thirteen (68.4%) patients received bone marrow cells (BM) and 6 (31.6%) peripheral hematopoietic cells (PHC).

Thirteen (68.4%) presented extensive and 6 (31.6%) limited cGVHD. Among 6 limited cGVHD, 1 envolved the skin and 5 buccal mucosa.

Three patients (15.8%) from 19 had previous history of acute cGVHDA involving the buccal mucosa. Nine (47.3%) patients had Xerostomia, in four it was severe and in 5, mild (Table 1).

At the moment of the sample collection, 17 patients were receiving cyclosporine-A (10mg/Kg/day) and 13 prednisone (1mg/Kg/day). Four patients were taking acyclovir (800mg/day), 3 for *herpes simplex* infection and one for *varicela-zoster* .

Six patients presented the lichenoid form of cGVHD in buccal mucosa, 6 the atrophic-ulcerative form, 2 the hyperceratotic form and 3 mixing form. Two patients did not present oral lesions at the moment of the clinic exams but did present a histopatological diagnosis of cGVHD in buccal mucosa and minor salivary gland (Table 1).

Leukocytes above $11.0X10^3$/µl was observed in 2 patients and below $4.5x10^3$ /µl in 7 patients out of 19 with cGVHD (Table1).

HHV 6 PCR analysis

Beta globin DNA gene was clearly amplified in all samples analyzed in both groups, control and cGVHD patients. Tests for HHV 6 in oral fluids of cGVHD patients were performed in 17 out of 19 patients. Two individuals presented severe hiposalivation and collection of saliva was not possible (Table 1).

Table 2 shows the results. Briefly, 8 patients were positive for HHV 6 in both whole saliva and in blood; 5 only in whole saliva, 2 only in blood and 2 were negative in all 4 fluids analyzed

Twenty-eight blood donors composed the control group, 23 (83.1%) were male and 8 (17.8%) of female, with age range of 20-60 years, mean of 39.6 years. Eight blood donors were positive for HHV 6 in whole saliva and blood; 6 only in whole saliva; one in blood and in gingival crevicular fluid; one positive in blood, whole saliva and in parotid gland saliva; one in blood, whole saliva, gingival crevicular fluid and in parotid gland saliva; one in whole saliva and in gingival crevicular fluid; one in whole saliva and in parotid gland saliva; one positive in whole saliva, gingival crevicular fluid and in parotid gland saliva and 8 donors were negative for all 4 fluids analyzed. Twenty controls presented positive result for HHV 6 in at least 1 of 3 oral fluids analized and whole saliva was positive in 19 of them (Table 2).

Two (16.6%) patients with cGVHD presented positive results for HHV6 in buccal mucosa samples, and all individuals from control group were negative. Both positive patients presented oral atrophic ulcerative widespread lesions.

Discussion

Patients undergoing chemotherapy and hematopoietic cell transplantation are susceptible to infections with several opportunistic pathogens (40). Reactivation of HHV6 following hematopoietic cell transplantation was first reported in 1991 by Yoshikawa *et al* (41), and clinical manifestations of this reactivation have been reported since then, including pneumonitis, encephalitis and GVHD (42-44). On the other hand, the relationship between increased HHV6 replication in blood and clinically recognizable disease of these patients has generally been difficult to establish (21).

We studied patients with cGVHD and our results demonstrated that 13 (68.4%) out of 19 patients presented positive results for HHV6 in oral fluids. However, these data were similar to those of the control group.

HHV6 is detected and may persist in human saliva, but the available data is variable (17, 18, 45, 46). The reasons for this variation are yet unclear. Some primers used for HHV 6 may provide cross-reaction with HHV7 (19), however we used primers derived from a highly conserved sequence of the HHV6 genome.

Gingival crevicular fluid is the main source of leukocytes found in saliva (47). Thirteen (68.4%) samples of whole saliva from patients with cGVHD were positive for HHV6 while all the samples of gingival crevicular fluid and of parotid gland saliva were negative. Nevertheless, all samples of gingival crevicular fluid and parotid saliva were positive for the β-globin gene amplification. These data suggest

that gingival crevicular fluid and parotid gland saliva were not the principal source of the HHV6 found in whole saliva.

Wright et al. (1986) demonstrated that patients who received allogeneic bone marrow cells following high-dose chemotherapy, neutrophils levels recover first in the mounth than in peripheral blood (48). Although we had two patients with neutrophils count above the normal maximum and 9 below the normal minimum, we found no association between the neutrophil counts and presence of HHV6 or severity of cGVHD oral lesions.

Epithelium of the salivary glands has been shown to be affected early in the course of cGVHD, with an incidence of 80-100%. Additionally, a direct correlation has been observed between the degree of hypo salivation and the severity of GVHD (9, 12, 13). Nine of our patients (47.3%) complained of xerostomia, four of them were classified as severe and five mild form, nevertheless there was no association between the xerostomia and the severity of oral lesions.

The influence of the graft type in HHV6 infection is still controversy. Maeda et al., (1999) (49) detected higher rates of HHV6 DNA in patients who had undergone allogeneic transplantation using bone marrow cells, comparing to peripheral hematopoietic cells. However, LJUNGMAN *et al.* (2000) (50) described that the graft type did not influence the HHV6 viral load. In our study, 10 of 13 BMC transplantation presented positive results for HHV6 and 5 of 6 peripheral hematopoietic cell transplantation presented positive results for HHV 6. In spite of, the small number of studied cases, our results agree with those of LJUNGMAN *et al*. (50) in which cell source did not influence in the HHV6 infection incidence.

Four patients were receiving acyclovir during sample collection. These patients presented oral manifestations of cGVHD and they were positive for HHV 6 in oral fluids. These results support others reported in the literature, where HHV6 is reported to be less sensitive to acyclovir, than ganciclovir and foscarnet (42, 44).

Lichenoid lesions with predominant reticular and papular forms are the most frequent cGVHD oral manifestations and occur in 80-100% of these patients. Ulcerative, atrophic and hyperkeratotics lesions are less common (8-12). In our study, the incidence of lichenoid lesions was similar to the other forms of oral lesions, particularly ulcerative-atrophic. The data also indicate that the type of lichenoid lesionis not associated with the detection of virus.

Although PCR is extremely effective with pure and fresh biological specimens, its success with complex biological samples like paraffin-embedded tissues is variable (51). The efficiency of PCR amplification decreases in accordance with an increase in fixation time and the acidification of formalin with formic acid (52). The main obstacle in preparing DNA suitable for PCR amplification is the removal of paraffin wax and purification (53). Nevertheless, this methodology has been used successfully, and it is important to improve the technique since many valuable samples are routinely embedded in paraffin for diagnosis.

In our study, only two patients with cGVHD presented positive results for HHV6 in the oral mucosa tissues. Both patients presented oral atrophic-ulcerative widespread lesions. In spite of the detection of HHV6 in these two patients, it was difficult to associate with the clinical alterations. It was not possible to discard that

the small number of positive cases were due to the presence of PCR inhibitor in the samples.

In conclusion, the results of this work showed a high and similar presence of HHV6 in the oral fluids of cGVHD patients and healthy individuals. We could not confirm the participation of HHV 6 in the development of oral lesions associated to cGVHD. Further studies are necessary, to verify the role of HHV6 in oral manifestations of cGVHD.

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Tables

Table 1- Clinical evaluation, cell source, leukocyte and neutrophil counts and HHV 6 investigation in peripheral blood and oral fluids of 19 patients with cGVHD.

N	Organ affected	Cell Source	xerostomy	Oral lesion cGVHD	leuk	Neut	HHV6	
							Oral fluid	blood
1	Mouth, eyes, skin, lung	BMC	Severe	Atrophic-ulcerative widespread	N	N	+	-
2	Mouth, skin, liver	BMC	Absent	Atrophic-ulcerative buccal mucosa	N	N	+	+
3	Mouth, liver	PHC	Mild	Atrophic-ulcerative widespread	<	<	+	-
4	Mouth	BMC	Severe	Lichenoid widespread	<	<	+	+
5	Mouth	BMC	Mild	Atrophic-ulcerative widespread	<	<	+	+
6	Mouth, eyes	BMC	Absent	Hyperkeratosis tongue	N	N	-	-
7	Mouth, skin	BMC	Severe	Lichenoid widespread	N	N	-	+
8	Mouth	BMC	Absent	Mixing form	<	<	+	+
9	Mouth, skin	PHC	Absent	Atrophic-ulcerative widespread	N	>	-	-
10	Mouth, lung	BMC	Severe	Mixing form	N	N	-	-
11	Mouth, skin, liver, kidney	PHC	Absent	Hyperkeratosis buccal mucosa	N	N	+	+
12	Mouth, skin, eyes	PHC	Mild	Lichenoid widespread	>	>	+	+
13	Mouth	BMC	Absent	Without lesion	N	<	+	-
14	Mouth, skin	BMC	Mild	Lichenoid widespread	<	<	-	-
15	Skin	BMC	Absent	Without lesion	N	<	+	+
16	Mouth, skin	PHC	Absent	Lichenoid widespread	<	<	+	+
17	Mouth, skin, liver	BMC	Absent	Mixing form	N	N	+	-

18	Mouth	BMC	Absent	Atrophic-ulcerative widespread	<	<	+	-
19	Mouth, skin, eyes, liver	PHC	Mild	Lichenoid widespread	>	N	-	+

Leuk = leukocytes, neut = neutrophils, BMC = bone marrow cells, PHC = peripheral hematopoietic cells, N* = normal, < = above of normal maximum and > = below of normal minimum.
*Normal leukocytes count (4.5-11.0 x10^3/µl) and normal neutrophils count (3.0-5.8x10^3/µl) (54)

Table 2 - HHV 6 detected by PCR reaction in peripheral blood, whole saliva, parotid gland saliva and gingival crevicular fluid from 19 cGVHD patients and 28 healthy individuals.

HHV6 investigation	Control group (n=28)	cGVHD (n = 19)
HHV6 positive		
Peripheral blood	0	2 (10.5%)
Peripheral blood, whole saliva	8 (28.6%)	8 (42.1%)
Peripheral blood, gingival crevicular fluid	1 (3.6%)	0
Peripheral blood, whole saliva, parotid gland saliva	1 (3.6%)	0
Peripheral blood, whole saliva, gingival crevicular fluid, parotid gland saliva	1 (3.6%)	0
Whole saliva	6 (21.4%)	5 (26.3%)
Whole saliva, gingival crevicular fluid	1 (3.6%)	0
Whole saliva, parotid gland saliva	1 (3.6%)	0
Whole saliva, gingival crevicular fluid, parotid gland saliva	1 (3.6%)	0
HHV 6 negative		
Peripheral blood, oral fluids	8 (28.5%)	2 (10.5%)
Peripheral blood*		2(10.5%)
Total	**28 (100%)**	**19 (100%)**

* It was not possible to perform the research of HHV 6 in oral fluid of two patients due to severe hiposalivation

Conclusões

1. O uso de cones de papéis endodônticos para a coleta de fluidos bucais e extração de DNA viral mostrou ser eficaz, simples, barata e indolor.

2. Forma liquenóide e atrófica-ulcerativa foram as formas clínicas mais comum da doença do enxerto contra o hospeiro crônica em cavidade bucal.

3. As principais queixas dos pacientes portadores da doença contra o hospedeiro crônica com envolvimento bucal foram xerostomia e disfagia.

4. O HHV-6 foi encontrado nos fluidos orais em similar porcentagem nos indivíduos portadores da doença do enxerto contra o hospedeiro crônica e indivíduos saudáveis

5. Nossos dados indicaram que o fluido gengival e a saliva de glândula parótida não são a principal fonte de origem do HHV-6 identificado na cavidade bucal.

6. Não foi encontrado evidencias da participação do HHV-6 na gravidade das lesões bucais dos pacientes portadores da doença do enxerto contra o hopedeiro crônica.

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Anexos

TERMO DE CONSENTIMENTO PARA PESQUISA DE HHV-6 EM INVÍDUOS SADÍOS DOADORES DE SANGUE DO BANCO DE SANGUE DO HEMOCENTRO/UNICAMP

Pesquisa: Análise da influência do vírus HHV-6 em pacientes submetidos a Transplante de Medula Óssea.
Pesquisador: Claudio Maranhão Pereira.

MAIOR DE IDADE

Eu , anos de idade, , **R.G** **, HC** , residente em autorizo ao cirurgião-dentista Claudio Maranhão Pereira, membro da equipe de odontologia do Hemocentro/ UNICAMP, sob a coordenação da Dra. Maria Elvira Pizzigatti Corrêa, a realizar em mim a coleta de saliva e de fluido gengival para investigação da presença de HHV-6. Também autorizo a coleta de sangue por profissionais do banco de sangue do HEMOCENTRO/UNICAMP.

Foram discutidos comigo os detalhes dos procedimentos. Fica ciente também, que o mesmo procedimento descrito acima, não acarretara nenhum dano a minha saúde bucal e geral. Esses procedimentos serão realizados sem quaisquer ônus para mim e, mesmo no caso de desistência por minha parte, não terei nenhum prejuízo no procedimento o qual estou sendo submetido. O material coletado será utilizado posteriormente para a realização de testes para quantificação da carga viral do HHV-6 no Laboratório de Biologia Molecular do HEMOCENTRO/UNICAMP.

Todas as minhas perguntas relativa a esses procedimentos foram adequadamente respondidas, e minhas dúvidas esclarecidas.

Será mantido sigilo e caráter confidencial sobre minha identidade quando do momento da exposição das conclusões e da publicação do projeto e de documentações.

Hemocentro/ Departamento de Odontologia: tel.: (19) 37888729.

Dentista responsável: Drª Maria Elvira P. Corrêa

Pesquisador: Claudio M. Pereira: tel.: (19) 34125315
Comitê de Ética para Pesquisa: Tel.: (19) 788-8936

TERMO DE CONSENTIMENTO PARA PESQUISA DE HHV-6 EM PACIENTES PORTADORES DE GVHD CRÔNICO EM BOCA

Pesquisa: Análise da influência do vírus HHV-6 em pacientes portadores de GVHD crônico
Pesquisador: Claudio Maranhão Pereira.

MAIOR DE IDADE

Eu , anos de idade, , **R.G** **, HC** , residente em autorizo
ao cirurgião-dentista Claudio Maranhão Pereira, membro da equipe de odontologia do Hemocentro/ UNICAMP, sob a coordenação da Dra. Maria Elvira Pizzigatti Corrêa, a realizar em mim a coleta de saliva e de fluido gengival para investigação da presença de HHV-6. Também autorizo a coleta de sangue por profissionais da equipe de Enfermagem do Ambulatório de Hematologia do HEMOCENTRO/UNICAMP.
Estou ciente que a saliva e fluido gengival serão coletados com auxílio de 10 cones de papel absorventes e o sangue será coletado em quantidade de 5 ml de uma veia do braço por meio de uma agulha estéril e descartável. Fica ciente também, que o mesmo procedimento descrito acima, não acarretara nenhum dano a minha saúde bucal e geral. Esses procedimentos serão realizados sem quaisquer ônus para mim e, mesmo no caso de desistência por minha parte, não terei nenhum prejuízo no tratamento o qual estou sendo submetido. O material coletado será utilizado posteriormente para a realização de testes para quantificação da carga viral do HHV-6 no Laboratório de Biologia Molecular do HEMOCENTRO/UNICAMP.
Todas as minhas perguntas relativa a esses procedimentos foram adequadamente respondidas, e minhas dúvidas esclarecidas.
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FICHA CLÍNICA PARA PESQUISA DE ANÁLISE DA INFLUÊNCIA DO VÍRUS HHV-6 EM PACIENTES PORTADORES DE GVHD CRÔNICO

Nome: _____

HC:_____ R.G.: _____

Data de nascimento: _____ Local: _____

Sexo: _____ Raça:_____

Doença de base: _____

Data de diagnóstico e história da doença atual: _____

_____

_____

_____

História pregressa de saúde:_____

_____

Medicações:_____

_____

_____

Terapêutica proposta: _____

_____

Tipo de TMO:_____

Complicações: _____

_____

Data do TMO: _____

Estado de saúde atual: _____

_____

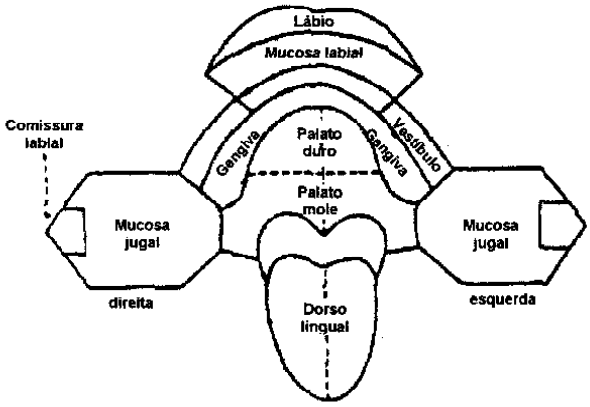
_____

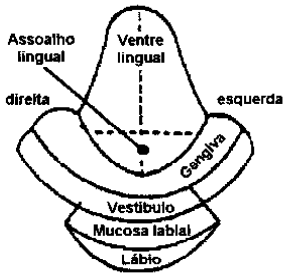
Estado de saúde bucal: _____

_____

_____

_____





Data de coleta do material: _____

Carga viral:_____

Observações _____