MARIELA DUTRA GONTIJO DE MOURA

TRATAMENTO TÓPICO DA LEUCOPLASIA PILOSA BUCAL E DESENVOLVIMENTO E VALIDAÇÃO DE UM INSTRUMENTO PARA AVALIAÇÃO DO CONHECIMENTO DE CIRURGIÕES DENTISTAS SOBRE HIV/AIDS

Faculdade de Odontologia
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Tese apresentada ao Colegiado do Programa de Pós-Graduação da Faculdade de Odontologia da Universidade Federal de Minas Gerais, como requisito parcial para obtenção do grau de Doutor em Odontologia - área de concentração em Estomatologia

Orientador: Prof. Dr. Ricardo Alves de Mesquita

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Faculdade de Odontologia - UFMG
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PREFÁCIO

Esta tese é resultado de duas pesquisas, sendo a primeira desenvolvida no Centro de Treinamento e Referência em Doenças Infecciosas e Parasitárias (CTR-DIP) conjuntamente com a Faculdade de Odontologia da Universidade Federal de Minas Gerais (UFMG) e a segunda desenvolvida na cidade de Córdoba, na Argentina, conjuntamente com as Faculdades de Odontologia da Universidade Nacional de Córdoba (UNC) e da UFMG. A tese foi estruturada em duas partes, sendo que a primeira compreende as considerações iniciais, descritas como uma breve introdução contendo os dados mais relevantes dos dois estudos, justificativas, objetivos, metodologias e referências bibliográficas exclusivas dessa primeira parte. Na segunda parte são expostos os artigos, sendo dois deles (Artigos 1 e 2) referentes à primeira parte e outros dois (Artigos 3 e 4) referentes à segunda parte, além das considerações finais e anexos. As normas das revistas selecionadas para a submissão do artigo 1 (Anexo 1- página 119) e do artigo 3 (Anexo 2- página 131) encontram-se nos anexos.

ABREVIATURAS, SÍMBOLOS E SIGLAS

AIDS	- Síndrome da Imunodeficiência Adquirida (do inglês *"Acquired Immunodeficiency Syndrome"*)
AZT	- Zidovudina
Candida sp	- Espécies de *Candida*
CD4	- Do inglês *"cluster of differentiation 4"*
CD8	- Do inglês *"cluster of differentiation 8"*
CFA	- Do inglês "confirmatory factorial analysis" ou análise fatorial confirmatória
CFI	- Do inglês *"Comparative Fit Index"*
COEP	- Comitê de Ética em Pesquisa
CTR-DIP	- Centro de Treinamento e Referência em Doenças Infecciosas e Parasitárias
DK-HIV-Q	-*Do ingles "questionnaire on dentists' knowledge of HIV/AIDS"* ou questionário do conhecimento dos dentistas sobre HIV/AIDS
EBV	- Do inglês *"Epstein Barr vírus"*
EFA	- Do inglês "exploratory factorial analysis" ou análise fatorial exploratória
ELISA	- Do inglês *"Enzyme Linked Immunosorpent Assay"*
GFI	- Do inglês *"Goodness of Fit Index"*
HAART	- Do inglês *"Highly active antiretroviral therapy"* ou terapia antirretroviral altamente ativa
HIV	- Do inglês "*Human Immunodeficiency Vírus"* ou "Vírus da Imunodeficiência Humana"
ICC	- Coeficiente de Correlação Intraclasse

Linfócito T	- Linfócito T auxiliar ou linfócito T-*helper*
LPB	- Leucoplasia pilosa bucal
n	- Número da amostra
OMS	- Organização Mundial de Saúde
HR	- *"Hazard ratio":* risco, ameaça, perigo, razão das chances, risco de ocorrência ou fator de risco
P	- Solução alcoólica de podofilina a 25%
PA	- Solução alcoólica de podofilina a 25% associada ao aciclovir a 5%
PCR	- Reação em cadeia da polimerase, do inglês "polymerase chain reaction"
PP	- Solução alcoólica de podofilina a 25% associada ao penciclovir a 1%
PAS	- Do inglês *"Periodic Acid Schift"*
RMR	- Do inglês *"Root Mean Square Residual"*
UFMG	- Universidade Federal de Minas Gerais
UNC	-Universidade Nacional de Córdoba

RESUMO

Tratamento tópico da leucoplasia pilosa bucal e desenvolvimento e validação de um instrumento para avaliação do conhecimento de cirurgiões dentistas sobre HIV/AIDS

A tese apresentada constou de duas pesquisas. A primeira foi um estudo de ensaio clínico randomizado com os objetivos de apresentar um novo tratamento tópico para a leucoplasia pilosa bucal (LPB), utilizando-se solução alcoólica de podofilina a 25% associada ao penciclovir creme a 1% (PP); avaliar variáveis de prognóstico que podem influenciar nos tratamentos tópicos com solução alcoólica de podofilina a 25% (P), com solução alcoólica de podofilina a 25% associada ao aciclovir a 5% (PA) e com PP; e avaliar o desempenho desses três protocolos de tratamento tópico através da cura ou não da LPB, do índice de cura em relação ao tempo e da recidiva doze meses após a cura. As variáveis de prognóstico avaliadas foram: gênero, idade, anos de educação formal, rota de transmissão, CD4, CD8, carga viral, plaquetas, candidíase oral, uso de terapia antiretroviral altamente ativa (HAART), uso prévio de aciclovir, uso prévio de antifúngico, uso de drogas injetáveis, uso de AZT, consumo de cigarro e de álcool. Realizou-se também a pesquisa da presença do vírus Esptein-Barr (EBV) antes e depois do tratamento tópico da LBP. Os 42 pacientes HIV-positivos e portadores de 69 lesões de LPB foram recrutados aleatoriamente do Centro de Treinamento e Referência em Doenças Infecciosas e Parasitárias Orestes Diniz (CTR-DIP), em Belo Horizonte, Minas Gerais. A presença do EBV foi avaliada em 15 destes 42 pacientes, onde a extração do DNA foi realizada por meio de raspado da borda lateral de língua e amplificada pela reação em cadeia de polimerase (PCR) antes e após o tratamento tópico. A LPB foi diagnosticada pela citologia esfoliativa. O modelo proporcional de Cox foi usado para

avaliar as variáveis de prognóstico e o desempenho dos três protocolos de tratamento. Os resultados demonstraram que os três protocolos de tratamento tópico apresentaram a mesma taxa de cura da lesão, mas a partir da sexta semana de tratamento, PA foi mais eficiente na cura da LPB comparado à P e à PP. A recidiva foi observada em três e sete lesões tratadas com P e com PP, respectivamente. O uso prévio de antifúngico diminuiu a taxa de cura da LPB em 59%. Todos os 15 pacientes com LPB antes do tratamento tópico foram positivos para o EBV, mas imediatamente após a cura da LPB, 11 pacientes continuaram positivos para o EBV. O tratamento tópico mais recomendado para a LPB é PA. A elevada prevalência do EBV na borda lateral de língua dos pacientes sem LPB após o tratamento tópico reforça o papel desse vírus na recorrência da LPB.

A segunda pesquisa realizou o desenvolvimento e a validação de um questionário para avaliar o conhecimento dos dentistas sobre HIV/AIDS (DK-HIV-Q). O formato inicial do DK-HIV-Q foi desenvolvido com 33 itens e com quatro domínios específicos: conhecimento declarativo de transmissão de HIV/AIDS, conhecimento declarativo de manifestações orais relacionadas ao HIV/AIDS, conhecimento procedimental de prática odontológica, e conhecimento procedimental de medidas de controle de infecção. O estudo foi desenvolvido em Córdoba, Argentina e como o DK-HIV-Q foi desenvolvido por uma pesquisadora brasileira foi necessário realizar tradução, adaptação trans-cultural dos itens e retro-tradução. Em seguida, avaliações conceituais, de item, semântica, operacional e de equivalência do DK-HIV-Q foram executadas por um grupo de experts. Depois, o DK-HIV-Q foi aplicado em dois estudos pilotos, com uma amostra de conveniência de 20 cirurgiões dentistas argentinos. Posteriormente a essas etapas, a confiabilidade do teste-reteste foi determinada pelo cálculo do Coeficiente de Correlação Intraclasse (ICC)

usando uma amostra randomizada de 25 cirurgiões dentistas argentinos. Dados foram coletados de uma amostra randomizada de 251 cirurgiões dentistas argentinos, onde a consistência interna do instrumento foi acessada pelo Coeficiente Alfa de Cronbach e também foram realizadas a análise fatorial exploratória (EFA) e a análise fatorial confirmatória (CFA). Os resultados mostraram que não houve discrepâncias entre a tradução e a retro-tradução. Os experts concordaram completamente com a relevância conceitual do instrumento com 33 itens. A confiabilidade do teste-reteste revelou estabilidade das respostas em um curto período de tempo com satisfatória reprodutibilidade de 0.95. O DK-HIV-Q apresentou uma estrutura com um fator geral de segunda ordem e quatro fatores específicos. A consistência interna foi 0.68 para o conhecimento dos dentistas sobre HIV/AIDS (fator geral), 0.53 para o conhecimento declarativo sobre transmissão de HIV/AIDS (fator 1), 0.71 para o conhecimento declarativo de manifestações orais de HIV/AIDS (fator 2), 0.59 para conhecimento procedimental de prática odontológica (fator 3) e 0.48 para conhecimento procedimental de medidas de controle de infecção (fator 4). A remoção de oito itens melhorou o funcionamento do instrumento e o DK-HIV-Q com 25 itens provou ser um instrumento útil para avaliar o conhecimento dos dentistas sobre HIV/AIDS e mostrou que o fator 2 está mais correlacionado com o conhecimento dos dentistas sobre HIV/AIDS (fator geral). Novas pesquisas são necessárias para adicionar mais itens nos fatores 1, 3 e 4 e para avaliar as propriedades psicométricas em amostras diversificadas de cirurgiões dentistas.

Palavras chaves: Aciclovir, Penciclovir, Leucoplasia pilosa oral, Podofilina, Infecção HIV, Síndrome da Imunodeficiência Adquirida, Questionários

ABSTRACT

Topical treatment for oral hairy leukoplakia and development and construct validation of a questionnaire for assessment of dentists` knowledge about HIV/AIDS

This study presented was comprised of two researches. To first it was a random clinical trial to present a new topic treatment to oral hairy leukoplakia (OHL) with podophyllin resin (25%) together with penciclovir cream (1%) (PP); evaluate the predictor's variables that could influence in podophyllin resin (25%) (P), podophyllin resin (25%) together with acyclovir cream (5%) (PA) and PP topical treatments and access the performance of these treatments,in accordance to the heal or no of OHL, rate of heal in time and recurrence twelve months after the heal of OHL. The predictor variables evaluated were: gender, age, years of formal education, route of transmission, CD4, CD8, viral load, plaques, oral candidiasis, use of highly active anti-retroviral therapy (HAART), prior use of acyclovir, prior use of antifungal, drug use by injection, use of AZT, smoking and alcohol consumption. Other study was also performed in order to identify and compare the presence or not of Epstein-Barr virus (EBV) before and after of the topical treatment of the OHL. The 42 HIV-infected patients with 69 OHL lesions were recruited randomly in the Orestes Diniz's Treatment Center of Parasitic and Infectious Diseases, (CTR/DIP), in Belo Horizonte, Minas Gerais. The presence of the EBV was evaluated in 15 of these 42 patients, where the DNA was extracted from scrapes of the lateral border of the tongue and amplified by Polymerase Chain Reaction (PCR) before and after the topical treatment. OHL was diagnosed in accordance with exfoliative cytology. A Cox proportional hazards model was used to analyze the predictor's variables and the performance of these treatments. The results showed that the three protocols of topical treatment presented the same hates of heal of the OHL, but after sixth week, PA protocol have a more efficiency to heal OHL comparing

with P and PP. Recurrence was observed in three and seven lesions treated with P and PP, respectively. Prior use of antifungal decrease the hate of heal in 59%. The 15 patients with OHL before of the topical treatment were EBV positive, but immediately after heal of the OHL, 11 patients continued EBV positive. The standard topical treatment for OHL is PA. The elevated prevalence of EBV in the lateral border of the tongue of patients without OHL after of the topical treatment supports a role of this virus in recurrence of the OHL.

A study of survival analysis in a random clinical trial was performed in order to evaluate and compare the topical treatment of oral hairy leukoplakia with podophyllin resin 25% solution, podophyllin resin 25% solution in association with acyclovir 5% and podophyllin resin 25% solution in association with penciclovir 1%. For this purpose, 42 HIV-infected patients were selected, with 69 oral hairy leukoplakia treated at Orestes Diniz's Treatment Center of Parasitic and Infectious Diseases, in Belo Horizonte/Minas Gerais. The performances analysis of these topical treatments protocols were evaluated by Cox proportional hazards model in three phases: (1) healing or no; (2) rate of heal in time and (3) recurrence twelve months after the end of treatment. The results of this study showed that the three protocols of topical treatment presented the same hates of healing of the OHL around five weeks. Podophyllin resin 25% solution in association with acyclovir 5% protocol have a more efficiency to heal OHL in a time interaction comparing with podophyllin resin 25% solution and podophyllin resin 25% solution in association with penciclovir 5%, after sixth week. For some time, the standard topical treatment for OHL is podophyllin resin 25% solution in association with acyclovir 5%.

The second research carried out the development and validation of a questionnaire on dentists' knowledge of HIV/AIDS (DK-HIV-Q). The initial format of

the DK-HIV-Q was developed with 33 items and with four specific domains: declarative knowledge of the transmission of HIV/AIDS, declarative knowledge of oral manifestations of HIV/AIDS, procedural knowledge of proper dental practice, and procedural knowledge of infection control measures. The study was carried out in Córdoba, Argentina and because the DK-HIV-Q was developed by a Brazilian researcher, the questionnaire needed to undergo translation, cross-cultural adaptation of the items and back translation. Right away, the combined assessment of conceptual, item, semantic, operational, and measurement equivalence of the DK-HIV-Q was performed by experts. Afterwards, the DK-HIV-Q was tested in two preliminary pilot studies, with a convenience sample of 20 Argentine dentists. Subsequently, test-retest reliability was determined through the calculation of the Intraclass Correlation Coefficient (ICC) collected from 25 randomly selected Argentine dentists. Data were collected from 251 randomly selected Argentine dentists and the reliability of the instrument was assessed by Cronbach's Alpha Coefficient and the validity was assessed by exploratory factorial analysis (EFA) and confirmatory factorial analysis (CFA). The results showed that there were no significant discrepancies between the translation and the back translation. The experts completely agreed with the conceptual relevance of the instrument with 33 items. Test-retest reliability revealed the stability of the answers over a short period of time with satisfactory reproducibility of 0.95. DK-HIV-Q presented the structure with the general factor of the second order, and with four specific factors. The internal reliability was confirmed as 0.68 for the HIV/AIDS dentists' knowledge (general factor); 0.53 for declarative knowledge toward transmission of HIV/AIDS (Factor 1); 0.71 for declarative knowledge toward oral manifestations of HIV/AIDS (Factor 2); 0.59 for procedural knowledge of dentists' practice (Factor 3); and 0.48 for procedural

knowledge toward infection controls (Factor 4). Deletion of eight items improved the goodness of fit for the instrument and the DK-HIV-Q composed of 25 items proved to be a useful instrument for the assessment of dentists' knowledge of HIV/AIDS, and it showed that factor 2 is most closely with HIV/AIDS dentists' knowledge (general factor). Further research is required to add more items to the factors 1, 3 and 4, and to evaluate the psychometric properties with diversified samples of dentists.

Key words: Acyclovir, Penciclovir, Oral hairy leukoplakia, Podophyllin, HIV infection; Acquired Immunodeficiency Syndrome; Promotion of Health; questionnaires

SUMÁRIO

1. Considerações iniciais

Nas últimas décadas, o mundo tem enfrentado a AIDS (*Acquired Immunodeficiency Syndrome*), que mesmo após várias tentativas para a sua erradicação, ainda permanece como uma ameaça de saúde à população mundial. A OMS (Organização Mundial de Saúde) estima existir entre 33 e 46 milhões de pessoas vivendo com HIV (*Human Immunodeficiency Vírus)*/AIDS em todo o mundo, além de estimar uma média diária de 14 mil novos casos, dos quais dois mil são em menores de 15 anos. Para cada caso de HIV/AIDS diagnosticado, deve ser considerado três a quatro casos a mais, pois cerca de 90% das pessoas que vivem com HIV/AIDS desconhecem seu estado de saúde (Brasil, 2009; Unaids, 2009).

A África Subsaariana é a área mais afetada do mundo, com aproximadamente dois terços do total mundial (22,5 milhões de pessoas) vivendo com HIV/AIDS e concentra 76% de mortes por HIV/AIDS. Na América Latina, estima-se que 1,6 a 1,8 milhões de pessoas vivam com HIV/AIDS. O número estimado de novas infecções nesta região foi de 100 mil; e o de mortes, de 58 mil. Principalmente devido às suas grandes populações, a Argentina, o Brasil e a Colômbia concentram as maiores epidemias na região. Somente o Brasil tem mais de um terço da estimativa de pessoas vivendo com HIV/AIDS na América Latina, com aproximadamente, 506.499 casos desde a identificação do primeiro caso em 1980 até junho de 2008. A Argentina registrou 78.184 casos de pessoas vivendo com HIV/AIDS, no período de 1982 a 2007, sendo 35.431 casos de AIDS e 42.753 casos de infecção pelo HIV (Argentina, 2009; Brasil, 2009; Unaids, 2009).

Em quase todos os países da América Latina, as taxas mais altas de infecção por HIV se encontram entre homens que fazem sexo com homens, com prevalência entre 2% e 28%. As segundas maiores taxas se encontram entre mulheres

trabalhadoras do sexo, com prevalência entre 0% e 6,3%. Estima-se que relações sexuais entre homens são responsáveis por 15 a 35% dos casos notificados de HIV/AIDS em países como Argentina e Brasil. Na Argentina, por exemplo, 18% dos casos notificados de HIV/AIDS estão relacionados à transmissão entre homossexuais (Argentina, 2009; Brasil, 2009; Unaids, 2009).

A infecção pelo HIV/AIDS constitui uma pandemia que, apesar da tendência a estabilizar-se em determinadas zonas geográfica, deve ser considerada uma síndrome infecto-contagiosa emergente importante, não somente pelos índices de morbidade e mortalidade, mas também, pelos aspectos sociais, econômicos e de saúde pública. Estudos de HIV/AIDS são de interesse especial para identificar as populações mais vulneráveis e dirigir estratégias de saúde pública mais adequada. Estratégias para melhorar o acesso das pessoas infectadas pelo HIV aos estudos de diagnóstico e de tratamento e estratégias de integração social e desenvolvimento devem ser prioritários para favorecer uma melhor qualidade de vida a essas pessoas com HIV/AIDS (Souza et al., 2000).

Assim, os cirurgiões dentistas desempenham um papel importante no cuidado desses pacientes com HIV/AIDS. Não obstante, o estigma associado com HIV/AIDS continua sendo uma barreira de preocupação, devido a uma mistura de medo, problemas com implementações de precauções apropriadas de controle de infecção, temor de contágio e de morte. Esse mecanismo dificulta a estruturação de condutas ou ações preventivas. Assim, faz-se necessário desenvolver diferentes estratégias, levando em conta todo o conjunto de elaborações mentais, teóricas e práticas do cotidiano, para agir concretamente influenciando as escolhas e alternativas elaboradas por esses cirurgiões dentistas diante do HIV/AIDS (Bennett et al., 1995; Confort et al., 2004; Reis et al., 2005; Rodrigues et al., 2005; Ross et al., 1991).

Esforços educacionais sobre HIV/AIDS para melhorar o conhecimento, descobrindo maneiras de motivar esses profissionais são necessários, demonstrando a necessidade de programas de saúde pública que visem à elucidação das principais dúvidas (Civaner & Arda, 2008; Mulligan et al., 2006; Sadowsky & Kunzel, 1994). Assim a elaboração de novos instrumentos é necessária para esclarecer e melhorar o conhecimento de cirurgiões dentistas sobre HIV/AIDS. O papel de cirurgiões dentistas nas necessidades de cuidado de saúde aos pacientes HIV/AIDS está bem descrita através de questionários (Gachigo & Naidoo, 2001; Kitaura et al., 1997; Senna et al., 2005). Entretanto, não existe questionário validado para dentistas sobre HIV/AIDS. Há um único estudo com questionário validado sobre HIV/AIDS para pacientes, sobre o que eles pensam do seu dentista (Mussard et al., 2008).

Os cirurgiões dentistas tem papel importante no reconhecimento e no tratamento de manifestações bucais relacionadas a HIV/AIDS (Souza et al., 2000). Isso têm impacto significante na sobrevida e na qualidade de vida dos indivíduos com HIV/AIDS (Patel & Glick, 2003). Dentre as manifestações bucais, a leucoplasia pilosa bucal (LPB), que é uma lesão quase que exclusivamente observada em indivíduos infectados pelo HIV que apresentam uma baixa contagem de linfócitos T CD4 (*"cluster of differentiation 4"*), tem demonstrado ser de grande importância no curso da infecção pelo HIV, por ser considerada um dos marcadores de progressão da AIDS, de grande valor preditivo no diagnóstico precoce e também, um indicador de prognóstico para a infecção pelo HIV (Begg et al., 1997; Glick et al., 1994; Husak et al., 1996; Margiotta et al., 1999; Patton, 2000; Ramirez-Amador et al., 1996; Ravina et al., 1996; Schmidt-Westhausen et al., 2000).

A LPB foi descrita pela primeira vez por Greenspan et al. (1984) e é uma lesão assintomática que aparece como placa branca na borda lateral da língua, de superfície plana, corrugada ou pilosa e não removível quando raspada. A etiolopatogenia da LPB está relacionada ao vírus Epstein-Barr (do inglês “Epstein-Barr Vírus”), que pode ser identificado através de técnicas de microscopia eletrônica, hibridização “in situ”, imunoistoquímica e PCR (reação em cadeia da polimerase, do inglês “polymerase chain reaction”). A citologia esfoliativa é uma boa opção para o diagnóstico da LPB, por ser um método simples, confiável, seguro, de custo reduzido, não invasivo e facilmente realizada pelo cirurgião dentista (Bertazzoli et al., 1997; Kratochvil et al., 1990; Migliorati et al., 1993; Milagres et al., 2007).

Apesar da LPB ser, na maioria das vezes, uma lesão assintomática, o tratamento dessa lesão é recomendado para eliminar as pilosidades, restaurar o conforto do paciente, re-estabelecer as características normais da língua e eliminar nichos de bactérias, vírus e fungos desencadeadores de outras patologias bucais (Gowdey et al., 1995; Moura et al., 2007)

As opções de tratamento propostos na literatura incluem a terapia cirúrgica, o uso de terapia antiviral sistêmica e a terapia tópica (Bhandarkar et al., 2008; Giovani, 2000; Gowdey et al., 1995; Herbst et al., 1989; Lozada-Nur & Costa, 1992; Moura et al., 2007; Sanchez et al., 1992).

O tratamento cirúrgico é um procedimento que apresenta resolução imediata, mas causa desconforto e edema de 24 a 48 horas (Herbst et al., 1989). A terapia com antivirais sistêmicos apresenta custo elevado, efeitos colaterais sistêmicos e pode desenvolver resistência ao vírus (Glick & Pliskin, 1990; Resnick et al., 1988). Além disso, há recorrência após a descontinuação do uso dos antivirais sistêmicos, que varia de sete a 20 dias (Glick & Pliskin, 1990; Resnick et al., 1988).

O tratamento tópico tem sido o mais indicado por ser fácil de aplicar, menos invasivo, de baixo custo e com poucos efeitos colaterais (Giovani, 2000; Gowdey et al., 1995; Greenspan & Greenspan, 1989; Lozada-Nur, 1991; Moura et al., 2007; Sanchez et al., 1992; Triantos et al., 1997). São aceitos na literatura o tratamento tópico com solução alcoólica de podofilina a 25% (P), ácido retinóico a 0,05%, solução alcoólica de podofilina a 25% associado ao aciclovir creme a 5% (PA), violeta genciana a 2% (Bhandarkar et al., 2008; Giovani, 2000; Gowdey et al., 1995; Lozada-Nur & Costa, 1992; Moura et al., 2007; Sanchez et al., 1992). Entretanto, são poucos os estudos que se preocupam em estabelecer uma comparação entre o sinergismo de fármacos de uso tópico no tratamento da LPB (Giovani, 2000; Moura et al., 2007).

Considerando a importância do conhecimento de HIV/AIDS pelos cirurgiões dentistas, assim como o conhecimento das principais manifestações bucais e seus respectivos tratamentos, como a LPB, este trabalho se propôs investigar, primeiramente, um novo tratamento tópico para a LPB com solução alcoólica de podofilina a 25% associada ao penciclovir creme a 1% (PP). Incluiu-se, além desse novo tratamento previamente citado, P e PA. Ao mesmo tempo, foram verificadas variáveis que poderiam influenciar nestes tratamentos tópicos e o desempenho desses três protocolos de tratamento tópico, avaliando a cura ou não da LPB, o índice de cura em relação ao tempo e a recidiva 12 meses após a cura. Em sequência, foi avaliada a presença do vírus Epstein-Barr (EBV- do inglês *"Epstein Barr vírus"*) antes e após o tratamento tópico da LPB.

Paralelamente foi realizado, na cidade de Córdoba, na Argentina, o desenvolvimento de um novo questionário para medir o conhecimento dos cirurgiões dentistas sobre HIV/AIDS, verificando sua validade de construto.

2. Justificativa

A presença de poucos estudos de ensaio clínico randomizado no tratamento tópico da LPB, assim como a ausência de estudos comparativos para avaliar o sinergismo de fármacos de uso tópico no tratamento desta lesão e a ausência de estudos mostrando a ação do penciclovir creme a 1% no EBV, agente etiológico principal desta lesão, favoreceu o desenvolvimento deste trabalho. A partir destes questionamentos, o estabelecimento do melhor tratamento tópico para a cura da LPB, determinar a recidiva dessa lesão após a sua cura, esclarecer os efeitos dos principais agentes antivirais de uso tópico usados em associação com P e estabelecer variáveis de prognóstico que poderiam influenciar nos tratamentos com P, PA e PP. Também a precariedade de estudos que estabeleçam comparação entre a presença do EBV antes e após o tratamento tópico da LPB reforça a importância de estudar o papel desse vírus na LPB, principalmente após a sua cura.

Considerando o aumento do foco em HIV/AIDS, assim como a importância do conhecimento dos cirurgiões dentistas sobre HIV/AIDS e a ausência de questionários validados para cirurgiões dentistas sobre esse tema, o desenvolvimento e a validação deste novo instrumento neste estudo contribuirá nesse sentido.

3. Objetivos

3.1. Objetivo geral

Avaliar um novo tratamento tópico, solução alcoólica de podofilina a 25% associada ao penciclovir a 1% (PP) para LPB, verificando sua eficácia em relação aos outros tratamentos tópicos, solução alcoólica de podofilina a 25% (P) e solução alcoólica de podofilina a 25% associada ao aciclovir a 5% (PA) e desenvolver e validar um questionário sobre o conhecimento dos cirurgiões dentistas sobre HIV/AIDS.

3.2 Objetivos específicos

3.2.1 Pesquisa 1

- Verificar as respostas e atuação das modalidades terapêuticas (P, PA e PP) utilizadas no tratamento tópico da LPB
- Analisar as variáveis que possam influenciar no tratamento: localização da LPB, gênero, idade, anos de educação formal, via de transmissão do HIV, contagem de linfócitos TCD4, contagem de linfócitos TCD8 (“cluster of differentiation 8”), carga viral, plaquetas, candidíase bucal, uso de terapiaantiretroviral altamente ativa (HAART- do inglês *“Highly active antiretroviral therapy”*), uso prévio de aciclovir sistêmico, uso prévio de antifúngico sistêmico, uso de droga injetável, uso de AZT associado a HAART, consumo de cigarro e consumo de alcool.
- Verificar a cura da LPB em relação aos três tratamentos tópicos.
- Verificar o índice de cura da LPB em relação ao tempo de aplicação dos fármacos.
- Verificar a presença de recidiva da LPB após 12 meses de cura.

- Comparar a presença do EBV antes e após o tratamento.

3.2.1 Pesquisa 2

- Criação do questionário para avaliar o conhecimento dos cirurgiões dentistas sobre HIV/AIDS (DK-HIV-Q) na linguagem espanhola argentina.
- Verificar a reprodutibilidade, a validação fatorial e a consistência interna deste instrumento.

4. Metodologia

4.1 Aspectos éticos e legais

Estas duas pesquisas 1 e 2 foram aprovadas, respectivamente, pelo Comitê de Ética em Pesquisa (COEP) obedecendo ao exigido pela legislação brasileira, conforme as resoluções CNS n 196/96 do Conselho Nacional de Saúde, sobre Diretrizes e Normas Regulamentadoras de Pesquisa Envolvendo Seres Humanos com os seguintes números de registros ETIC 367/06 (Anexo 3- página 141) e ETIC 545/07 (Anexo 5- página 146). O projeto não foi aprovado na Argentina, porque segundo informações obtidas, pesquisas relacionadas à aplicação de questionário não necessita de aprovação no Comitê de Ética desse país.

Os pacientes e os dentistas, respectivamente, foram esclarecidos sobre os objetivos das pesquisas e assinaram, respectivamente, os termos de consentimento livre e esclarecido (Anexo 4- página 142 e Anexo 6- página 147). Todas as pessoas envolvidas na manipulação dos dados obtidos na pesquisa: pesquisadora, orientador, co-orientador ou possíveis colaboradores tiveram compromisso com o sigilo, preservando integralmente o anonimato dos pacientes e dos dentistas e seu direito a não identificação.

4.2 Pesquisa 1

Neste estudo de ensaio clínico randomizado realizado no período de janeiro de 2003 a julho de 2007, foram selecionados 42 pacientes com 69 LPB localizadas em borda lateral de língua, sendo 24 lesões tratadas com P (unilateral=quatro; bilateral=dez), 23 com PA (unilateral=cinco; bilateral = nove) e 22 com PP (unilateral=seis; bilateral=oito), com um total de 14 pacientes em cada protocolo de tratamento. O tamanho das 69 lesões variou de 5 a 60 mm (media de 35 mm). Os

pacientes foram recrutados do CTR/DIP (Centro de Treinamento e Referência em Doenças Infecciosas e Parasitárias), em Belo Horizonte, Minas Gerais, Brasil. Todos os pacientes eram soropositivos para o HIV ou tinham AIDS; eram de ambos os gêneros (31 do gênero masculino e 11 do feminino), com idade acima de 13 anos, de qualquer procedência, sem distinção de grupo social. Parte da amostra utilizada nesta pesquisa constituiu a mesma amostra, utilizada no mestrado, de 28 pacientes e 46 LPB do estudo de Moura et al. (2007).

A amostra deste estudo de ensaio clínico foi calculada a partir da comparação entre duas médias, empregando o desvio padrão (s) apresentado no estudo de Giovani (2000), nível de significância (α) de 5% e poder de teste (β) de 20%. A diferença entre os grupos, considerada clinicamente relevante, foi de duas consultas. O cálculo amostral teve um n (número da amostra) de 14 em cada grupo, calculado a partir da fórmula descrita abaixo, sugerida por Kirkwood (1996):

$$n = \frac{\left(z_{1-\frac{\alpha}{2}} + z_{\beta}\right)^2 \left(s_1^2 + s_2^2\right)}{d^2}$$

Todos os pacientes por estarem em tratamento no CTR-DIP, já possuíam o diagnóstico da infecção pelo HIV pelo teste anti-HIV ELISA (do inglês *"Enzyme Linked Immunosorpent Assay"*) e confirmados pelo Western-Blot. O diagnóstico da infecção pelo HIV já tinha sido estabelecido durante o período do exame inicial. Exames extra-oral e intra-oral foram executados em todos pacientes por uma única examinadora, devidamente treinada e calibrada, seguindo os padrões da Organização Mundial de Saúde (OMS) (World Health Organization, 1997). As lesões foram medidas, utilizando-se uma régua milimetrada e diagnosticadas pela examinadora, inicialmente, pelo exame clínico, como lesão branca, não removível quando raspada com gaze, de limites imprecisos e superfície plana, corrugada ou

pilosa. Posteriormente, o diagnóstico da LPB foi confirmado através da citologia esfoliativa. O exame citológico consistiu na raspagem vigorosa das células superficiais da lesão, utilizando-se uma espátula de madeira e confecção de esfregaço sobre uma lâmina de vidro, limpa e seca, previamente demarcada. Foram obtidas duas lâminas de cada paciente, coletadas da mesma região. Em seguida, realizou-se a fixação do esfregaço citológico em álcool absoluto e o material foi encaminhamento ao Laboratório de Patologia da Faculdade de Odontologia da Universidade Federal de Minas Gerais (UFMG), onde foi realizada a coloração pelo método Papanicolaou e PAS (do inglês “Periodic Acid Schift”) e exame microscópico, feito por um patologista bucal, que não tinha conhecimento do grupo ao qual o paciente pertencia. De acordo com Bertazzoli et al. (1997), Dias et al. (2001), Epstein et al. (1995) e Migliorati et al. (1993), os aspectos citomorfológicos para o diagnóstico da LPB são presença de células epiteliais das camadas superficial e intermediária, que se apresentam como células poligonais grandes, de limites bem definidos, citoplasma azulado, núcleos ovais e pequenos, com alterações nucleares representadas por fragmentação da cromatina nuclear (núcleo em colar ou núcleo em contas de rosário), inclusões intranucleares centrais e basofílicas do tipo Cowdry A e condensação marginal de cromatina ou núcleo com aspecto de vidro “despolido”. Os blastosporos e hifas compatíveis com *Candida sp* foram visualizados como estruturas arredondadas e filamentosas, respectivamente, de coloração vermelha. Os pacientes que apresentaram LPB confirmada pela citologia esfoliativa foram convidados participar deste estudo, sem levar em consideração o sexo, raça ou condição sócio-econômica. O critério de inclusão foi o paciente ser HIV-positivo e apresentar LPB. Os pacientes com LPB bilateral receberam o mesmo tratamento tópico em ambas lesões. A seleção do tratamento com P, PA e PP foi aleatória. A

citologia esfoliativa foi executada pela mesma examinadora que realizou a aplicação dos fármacos na língua dos pacientes. Outro examinador, devidamente treinado e calibrado, avaliou os pacientes clinicamente antes e depois de cada tratamento tópico, sem ter conhecimento do tratamento que utilizado. As lesões de LPB foram fotografadas antes e após o tratamento, com o objetivo de comparação, utilizando-se uma máquina Pentax K1000, lente sigma, com macro 105mm. Os pacientes que apresentavam outras lesões de mucosa ou ósseas e necessitavam de exames complementares para o diagnóstico e tratamento, também foram atendidos no setor odontológico do CTR/DIP.

Os fármacos utilizados foram: solução alcoólica de podofilina a 25% (P), creme de aciclovir (ACV) a 5% e creme de penciclovir (PCV) a 1%. A podofilina foi fornecida e manipulada por um único farmacêutico, responsável pela Farmácia de Manipulação da Secretaria Municipal de Saúde de Belo Horizonte, sendo que a composição final da solução teve 25% do fármaco. Utilizou-se um total de nove frascos, de solução alcoólica de podofilina a 25%, de 30 mL cada. A composição por 100 g de produto foi:

- Podofilina pó ---------- 25 g
- Álcool etílico 96% q.s.p.------ 100 mL.

ACV e PCV foram comprados pela pesquisadora, numa farmácia brasileira. ACV e PCV são administrados atualmente como creme a 5% e 1%, respectivamente (Boon et al., 2000, Femiano et al. 2001, Lin et al., 2002; Sarisky et al., 2003; Schmid-Wendtner & Korting 2004; Spruance et al.,1997; Sutton & Boyd, 1993).

Para a aplicação dos fármacos, a língua do paciente foi imobilizada utilizando-se uma gaze, afastando-a em direção lateral contrária à lesão pela mão da examinadora. Em seguida, foi feita a lavagem da LPB com água da seringa tríplice,

secagem da mesma com gaze e aplicação de P com auxílio de uma haste de plástico flexível com algodão, fazendo-se leves toques, durante 30 segundos. O tempo foi marcado em um relógio da marca Astro. Após aplicação do fármaco, a língua ficou imobilizada por dois minutos e, em seguida, a cavidade bucal foi lavada com água da seringa tríplice durante 30 segundos. Observou-se, clinicamente, após a aplicação de P, uma coloração acastanhada na LPB. O procedimento foi repetido nos casos em que não se observou essa coloração.

Para as aplicações de PA e PP, após seguir a mesma seqüência de procedimentos realizados na aplicação de P, afastou-se novamente a língua em direção contrária à lesão de LPB. A lesão foi secada pelo ar da seringa tríplice, com o objetivo de não remover a P previamente colocada e o ACV a 5% ou o PCV a 1%, respectivamente, foi aplicado através de uma haste de plástico flexível com algodão, também durante 30 segundos. Aguardaram-se dois minutos, mantendo a língua imobilizada, porém a cavidade bucal não foi lavada com água, com o objetivo de manter o ACV ou o PCV atuando por um período maior.

Após esses procedimentos, o paciente foi orientado a não ingerir nenhum alimento sólido ou líquido durante um período de 60 minutos. As aplicações foram realizadas em intervalos de sete dias até a cura da LPB ou com o máximo de 25 semanas de aplicação. Quando a lesão não desaparecia clinicamente, repetia-se a aplicação na semana subseqüente e assim, sucessivamente, até sua cura. A cura foi considerada quando a placa branca não era mais vista clinicamente no local primário da LPB. Os pacientes que não apresentaram cura com o tratamento tópico foram submetidos à biópsia incisional e encontram-se em acompanhamento no CTR-DIP.

A análise de desempenhos dos protocolos P, PA e PP foi dividida em três tópicos: (1) cura da LPB ou não; (2) índice de cura em relação ao tempo e (3)

recidiva 12 meses após a cura da LPB. Apesar do tempo de avaliação da recidiva ser de 12 meses, a cada três meses após a cura da LPB, o paciente retornava ao serviço para uma nova avaliação clínica, com a finalidade de verificar a presença ou não de recidiva da referida lesão.

Os tópicos 1 e 2 dessa análise de desempenho dos protocolos foram avaliadas através da análise de sobrevida, utilizando-se o modelo proporcional de Cox. No tópico 1, a curva de sobrevida Kaplan Meier foi usada para avaliar a cura da LPB (Hosmer & Lemeshow, 1999). Para testar o o índice de cura em relação ao tempo no sentido de verificar a eficácia dos três protocolos utilizados no período do tratamento, descrito no tópico 2, utilizou-se um teste chamado “Test of Proportional Hazards Assumption” (Hosmer & Lemeshow, 1999). Uma estatística descritiva da recidiva, descrita no tópico 3, também foi realizada.

As variáveis clínicas e laboratoriais também foram usadas nessa análise de sobrevida, através do modelo proporcional de Cox, primeiramente através do “Unconditional Cox proportional harzards model”, que selecionou variáveis com valor de p menor que 0.20, como critério de inclusão a ser selecionado. Assim, essas variáveis que poderiam interferir na resposta ao tratamento foram automaticamente selecionadas para o modelo múltiplo, cujo valor de p deveria ser menor que 0.05. Assim, as variáveis ajustadas no modelo múltiplo foram utilizadas acessando a Hazard Ratio (HR) baseado na cura da LPB em um dado período de tempo, utilizando-se o mesmo teste anteriormente descrito: “Test of Proportional Hazards Assumption” (Hosmer & Lemeshow, 1999).

Para realização dessas análises estatísticas, utilizou-se o programa STATA (version 10.0).

A localização das lesões na borda lateral de língua, direita e esquerda, foi a única variável ao nível de lesão. As demais variáveis estavam ao nível do paciente, tais como: gênero, idade, anos de educação formal, rota de transmissão do HIV, contagem de linfócitos TCD4, contagem de linfócitos TCD8, carga viral, plaquetas, candidíase bucal, uso de HAART, uso prévio de aciclovir sistêmico, uso prévio de antifúngico sistêmico, uso de droga injetável, uso de AZT associado à HAART, consumo de cigarro e consumo de álcool. As variáveis laboratoriais foram coletadas de registros médicos do CTR-DIP, obtendo-se duas coletas, a primeira coleta foi quando o paciente iniciou o tratamento tópico da LPB; e a segunda, quatro meses após essa primeira coleta. Também foi estabelecida a média das variáveis laboratoriais e a média da idade dos pacientes. O ponto de corte dicotomizado para idade foi 35 anos, para educação formal foi se o paciente tinha 12 anos ou mais de educação formal, para contagem de linfócitos de T CD4, 200 cells/mm^3, para contagem de linfócitos de T CD8, 540 ou 680 cells/mm^3, 3.000 cópias/microL para carga viral, e 150.000/mm^3 para plaquetas. O diagnóstico de candidíase bucal foi estabelecido a partir das características clínicas e do PAS (Costa et al., 2006; Fidel, 2006). Os casos de candidíase bucal foram tratados com fluconazol (50mg/day para sete days) (Costa et al., 2006; Ship et al., 2007). Fumo e consumo de álcool foram considerados presentes, respectivamente, quando o paciente fumava pelo menos 20 cigarros por dia durante 10 anos e quando consumia álcool diariamente (Moura et al., 2006).

Para a identificação do DNA do vírus EBV-1 antes e após o tratamento da LPB foi realizada a reação em cadeia da polimerase (PCR) em uma amostra randomizada de 15 pacientes dos 42 tratados, sendo que quatro deles pertenciam ao gênero feminino e 11 ao gênero masculino, com idade variando de 27 a 47 anos.

Cinco pacientes foram tratados com P, seis com PA e quatro com PP, com um total de 23 lesões (unilateral=sete; bilateral=oito).

A coleta das amostras foi obtida por meio de raspado no local da lesão antes e após o tratamento, utilizando espátulas estéreis. O material coletado foi armazenado em solução de Krebs (20% NaCl, 2% KCl, 2% $CaCl_2$ $2H_2O$, $MgSO_4$, KH_2PO_4, $C_6H_{12}O_6$) e estocado a -20°. O DNA de cada amostra foi extraído usando-se o método de Boom et al. (1990) e estocado a -20°C. A reação da PCR foi realizada em um volume final de 25μl contendo DNA genômico, solução tampão para PCR (10 mM $(NH_4)_2SO_4$, 10 mM KCl, 10 mM Tris-Cl pH 8.4, 3.0 mM $MgCl_2$), 0.1 mM de desoxirribonucleotídeos trifosfatos (Amersham Biosciences, Buckinghamshire, UK), 10 pmol de iniciadores (primers) (F:5' GTCATCATCATCCGGGTCTC 3' e R:5' TTCGGGTTGGAACCTCCTTG 3') e Taq DNA polimerase (1U/reação) (Phoneutria, Belo Horizonte, MG, Brazil). A amplificação do DNA foi realizada em termociclador (Gene Amp. PCR System 2400) (Eppendorf, Westbury, NY, USA) cujo programa consistiu em uma desnaturação inicial a 94° por 5 minutos, seguida por 40 ciclos de amplificação que consistiu em 94° por 1 minuto, 56° por 50 segundos e 72° por 1 minuto. A extensão final foi realizada a 72° por 7 minutos. Em todas as reações foram utilizados controle negativo, componentes da PCR sem amostra de DNA, e positivo, componentes da PCR acrescido de uma amostra sabidamente positiva para o vírus EBV-1. Os produtos amplificados foram visualizados em eletroforese com gel de poliacrilamida 6,5% e coloração pela prata. O estudo de PCR para verificar a presença do EBV-1 antes e após o tratamento tópico da LPB foi avaliado a partir da estatística descritiva. As amostras positivas para o EBV-1 consistiram em uma banda de 269 pares de base, as negativas apresentaram ausência da banda.

4.3 Pesquisa 2

O desenvolvimento e validação do questionário para avaliar o conhecimento dos cirurgiões dentistas sobre HIV/AIDS (DK-HIV-Q) foram executados na área urbana da cidade de Córdoba, a capital do estado de Córdoba, localizada na região central de Argentina. A cidade tem 1,5 milhões de habitantes e 2800 dentistas. Somente dentistas que atendiam pacientes no período do estudo foram incluídos nesta pesquisa e foram selecionados aleatoriamente, a partir de uma lista obtida da Escola de Odontologia do Estado de Córdoba, na Argentina.

A amostra deste estudo foi calculada pelo método de estimativa de proporção, considerando o erro de 1%, nível de confiança de 99% e proporção esperada de 50%, totalizando um número da amostra (n) de 250 cirurgiões-dentistas (Kirkwood, 1996).

O formato inicial do DK-HIV-Q foi desenvolvido pela pesquisadora brasileira, na linguagem portuguesa brasileira, baseado parcialmente no estudo de Oliveira et al. (2002) que avaliou um grupo de estudantes de graduação em odontologia através de um questionário não validado sobre atitudes e conhecimento de HIV/AIDS. Assim, pensou-se em um instrumento com 33 itens e com quatro domínios específicos: (1) conhecimento declarativo de transmissão de HIV/AIDS, (2) conhecimento declarativo de manifestações orais relacionadas ao HIV/AIDS, (3) conhecimento procedimental de prática odontológica, e (4) conhecimento procedimental de medidas de controle de infecção. Os itens do DK-HIV-Q foram agrupados teoricamente em relação a cada domínio.

Pelo fato do DK-HIV-Q ter sido desenvolvido por uma pesquisadora brasileira na linguagem portuguesa brasileira e pela necessidade de aplicação desse instrumento nos dentistas de Córdoba, na Argentina, optou-se por realizar,

previamente a sua aplicação, as seguintes etapas: tradução, adaptação transcultural dos itens entre a tradução e a retro-tradução, e retrotradução. Em seguida, o DK-HIV-Q foi avaliado por experts e, depois, aplicado em dois estudos pilotos. Posteriormente a essas etapas, realizou-se o teste re-teste e sua validação.

Desta forma, a tradução do instrumento original na linguagem portuguesa brasileira para a linguagem espanhola argentina inicialmente foi realizada, independentemente por dois tradutores, um tradutor argentino fluente na linguagem portuguesa brasileira e um brasileiro nativo fluente no espanhol argentino, ambos com experiência em tradução de questionários de saúde.

A adaptação transcultural dos itens entre a tradução e a retro-tradução foi realizada por dois dentistas fluentes nas duas linguagens (portuguesa brasileira e espanhola argentina), de modo que todos os itens desta versão traduzida foram revisados durante uma reunião de consenso, em que decisões finais de tradução e adaptações interculturais foram executadas para a determinação de conceito e de equivalência dos itens. Assim esses dois dentistas avaliaram essa versão e comparou-a a versão original na linguagem portuguesa brasileira. A atenção foi dada ao significado de palavras em cada linguagem para obter efeitos semelhantes dos respondentes de culturas diferentes. As palavras foram ajustadas de acordo com vocabulário típico argentino e, assim, adaptadas à cultura argentina. Um esforço foi feito para identificar possíveis dificuldades no entendimento do questionário. Uma versão síntese foi desenvolvida em conseqüência deste processo.

Para verificar a tradução, esta versão síntese foi retro-traduzida à linguagem portuguesa brasileira por um tradutor brasileiro nativo fluente nas duas linguagens e que previamente não estava envolvido no estudo e não teve acesso ao instrumento original.

Uma subseqüente comparação da versão original com a versão retro-traduzida foi realizada por outro tradutor, não envolvido no estudo, mas que apresentava fluência na linguagem portuguesa brasileira, apesar de sua linguagem nativa ser a espanhola argentina.

As avaliações conceitual, de item, semântica, operacional e de equivalência foram executadas por um grupo de experts, compostos de três cirurgiões dentistas fluentes nas duas linguagens e sem nenhum conhecimento prévio do estudo, sendo um epidemiologista, um clínico geral e um estomatologista com experiência no atendimento de pacientes com HIV/AIDS. O objetivo desta fase foi alcançar um "efeito semelhante" no entendimento para respondentes que falavam as duas linguagens, avaliando o comportamento do instrumento e a possibilidade de comparações a estudos conduzidos em culturas diferentes.

Cada item usado neste DK-HIV-Q teve as seguintes opções de resposta: "Sim" = 1 e "Não" = 0.

A aplicação do DK-HIV-Q foi realizada pela primeira vez em dois estudos pilotos, que foram realizados em duas amostras de conveniência, cada uma com 10 cirurgiões dentistas argentinos, selecionados da Escola de Odontologia do Estado de Córdoba, na Argentina. O DK-HIV-Q foi administrado em forma de entrevista, com o objetivo de reduzir o retorno do mesmo não preenchido. Esses 10 cirurgiões dentistas responderam o questionário e foram orientados a especificar todas as palavras que não estavam claras, no sentido de detectar qualquer dúvida concernente ao significado dos itens ou às instruções de como completar o questionário. Depois de algumas adaptações que foram realizadas para melhorar o entendimento, o DK-HIV-Q foi testado uma segunda vez em outro estudo piloto com outros 10 cirurgiões dentistas argentinos.

Para avaliação da confiabilidade do questionário, o teste-reteste foi aplicado duas vezes em uma amostra randomizada de 25 cirurgiões dentistas, que representavam 10% da amostra total do estudo. Assim, os mesmos 25 cirurgiões dentistas preencheram o DK-HIV-Q uma semana após o primeiro preenchimento. A confiabilidade do teste-reteste foi determinada pelo cálculo do Coeficiente de Correlação Intraclasse (ICC), onde foram realizadas estimativas de intervalos de confiança de 95%. O ICC foi medido de acordo com os seguintes valores: ≤0.40, correlação fraca; 0.41–0.60, correlação moderada; 0.61–0.80, boa correlação e 0.81–1.00, excelente correlação (Bartko, 1996; Cronbach, 1951).

O DK-HIV-Q foi aplicado em uma amostra randomizada de 251 cirurgiões dentistas argentinos de ambos gêneros, com idades variando de 20 a 63 anos.

A análise paralela por permutação foi usada para a extração dos fatores na análise fatorial exploratória (EFA) (Horn, 1965). A concepção da análise paralela consiste em gerar uma amostra aleatória randomizada, ou seja, uma amostra com autovalores atribuídos totalmente ao erro, e comparar os autovalores da amostra empírica obtida pelo pesquisador com os autovalores dessa amostra aleatória randômica. Somente são considerados adequados os fatores que possuem um auto-valor maior do que o auto-valor correspondente da amostra aleatória randômica. Essa estratégia possibilita uma garantia de que os fatores selecionados não podem ser atribuídos apenas a erro (Horn, 1965).

Posteriormente, procedimentos de rotação oblíqua foram utilizados para priorizar uma solução de fatores com cargas fortemente concentradas, com itens carregando fortemente em poucos fatores, sem possuir relações impactantes em outros fatores, gerando, deste modo, uma matriz fatorial bem delineada, com fatores analíticos com um grau de “pureza” e precisão maior. Um fator com menos de três

itens normalmente é visto como mal definido e tende a ser eliminado. Além disso, certos itens devem preferencialmente oferecer uma forte carga em apenas um fator. Um fator que não possui nenhum item que contribua com uma carga considerável também é eliminado. Deve-se reter um fator se ele possuir pelo menos dois itens com cargas salientes. A técnica de rotação oblíqua permite ainda verificar se os fatores extraídos possuem uma correlação, identificando fatores de alta-ordem, chamados de fator geral ou fator de segunda ordem e mostrando que o processo de extração de fatores não pára na identificação de fatores primários (Horn, 1965; Nasser et al., 2002; Reise et al., 2000; Thompson, 1994, Zwick & Velicer, 1986).

Assim, a inspeção das cargas fatoriais dos itens foi realizada para mostrar os itens que estavam contribuindo positivamente aos fatores. Os itens com cargas fatorial abaixo de 0,2 foram eliminados, porque eles não colaboravam com carga fatorial significativa necessária a um fator. Fatores válidos foram considerados aqueles com pelo menos três itens, sendo que esses itens apresentavam carga fatorial igual ou acima de 0,2 (Reise et al., 2000; Thompsom, 1994). Uma nova EFA foi executada com os itens restantes.

Após terminar de rotar obliquamente os fatores, o procedimento de análise fatorial confirmatória (CFA) foi aplicado baseado na solução obtida pela EFA. O critério de adaptação satisfatória do modelo aos dados foi adotado de acordo com os seguintes valores de índice: *Comparative Fit Index* (CFI), *Goodness of Fit Index* (GFI) igual ou maior que 0,90, e *Root Mean Square Residual* (RMR) menor ou igual a 0,08. As cargas fatoriais ideais para o fator geral foi considerado igual ou maior que 0,3 (Bentler, 1990; Maraun, 1996; Marsh et al., 1998; McDonald & Ho, 2002; Tompsom, 1994).

Os valores para a consistência interna do DK-HIV-Q foram calculados usando Coeficiente Alfa de Cronbach para cada fator isolado e para o fator geral. As escalas com confiabilidades de ao menos 0,50, mas preferivelmente 0,70 ou maior, foram considerados confiáveis para ser usado (Bartko, 1966; Cortina, 1993, Cronbach, 1951; Shrout, 1995).

O programa de software de SPSS (versão 16,0. SPSS Inc., Chicago, IL, EUA) foi usado para a realização da análise estatística.

5. Referências bibliográficas

1. Argentina. Disponível em: http://www.indec.gov.ar, 2009.
2. Bartko JJ. The intraclass correlation coefficient as a measure of reliability. *Psychol Report* 1966;19(1): 3-11.
3. Begg MD, Lamster IB, Panageas KS, Mitchell-Lewis D, Phelan JA, Grbic JT. A prospective study of oral lesions and their predictive value for progression of HIV disease. *Oral Dis* 1997;3(3):176-183.
4. Bennett ME, Weyant RJ, Wallisch JM, Green G. Dentists' attitudes toward the treatment of HIV-positive patients. *J Am Dent Assoc* 1995;126(4):509-14.
5. Bentler PM. On the equivalence of factors and components. *Multivariate Behavioral Research* 1990;25(1),67-74.
6. Bertazzoli R, Jaeger MMM, Araújo NS. Método auxiliar no diagnóstico de leucoplasia pilosa em pacientes HIV positivos. *Revista APCD* 1997;51(4):339-42.
7. Bhandarkar SS, MacKelfresh J, Fried L, Arbiser JL. Targeted therapy of oral hairy leukoplakia with gentian violet. *J Am Acad Dermatol* 2008;58(4):711-2.
8. Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, Van der Noordaa J. Rapid and simple method for purification of nucleic acids. J Clin Microbiol 1990;28(3):495-503.
9. Boon R, Goodman JJ, Martinez J, Marks GL, Gamble M, Welch C. Penciclovir cream for the treatment of sunlight-induced herpes simplex labialis: a randomized, double-blind, placebo-controlled trial. *Clinical Therapeutics* 2000;22(1):76-90.
10. Brasil. Ministério da Saúde. AIDS no mundo. Disponível em:

http://www.aids.gov.br/data/Pages/LUMIS63943F78PTBRIE.htm, 2009.

11. Civaner M, Arda B. Can "presumed consent" justify the duty to treat infectious diseases? An analysis. *BMC Infect Dis* 2008;8(29):1-11.
12. Comfort AO, Vandana M, Cuttress T, Tuisuva, J, Morse Z, Maimanuku L. Attitude/practices of oral healthcare provider to management of HIV/AIDS patients in the Pacific. *Pac Health Dialog* 2004;11(1):26-30.
13. Cortina JM. What is coefficient alpha? An examination of theory and applications. *Journal of Applied Psychology* 1993;78:98-104.
14. Costa CR, Lemos JA, Passos XS, Araújo CR, Cohen AJ, Souza LK, et al. Species distribution and antifungal susceptibility profile of oral Candida isolates from HIV-infected patients in the antiretroviral therapy era. *Mycopathologia* 2006;162:45-50.
15. Cronbach LJ. Coefficient alpha and the internal structure of tests. *Psychometrika* 1951;16:297–334.
16. Dias EP, Spyrides KS, Silva-Junior A, Rocha ML, Fonseca EC. Leucoplasia pilosa oral: aspectos histopatológicos da fase subclínica. *Pesqui Odontol Bras* 2001;15(2):104-111.
17. Epstein JB, Fatahzadeh M, Matisic J, Anderson G. Exfoliative cytology and electron microscopy in the diagnosis of hairy leukoplakia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995;79:564-569.
18. Fidel PL. Candida-host interactions in HIV disease: relationships in oropharyngeal candidiasis. *Adv Dent Res* 2006;19:80-4.
19. Femiano F, Gombos F, Scully C. Recurrent herpes labialis: efficacy of topical therapy with penciclovir compared with acyclovir (aciclovir). *Oral Dis* 2001;7:31-3.

20. Gachigo JN, Naidoo S. HIV/AIDS: the knowledge, attitudes and behaviour of dentists in Nairobi, Kenya. *SADJ* 2001;56(12):587-91.

21. Giovani EM. *Estudo comparativo entre o uso de ácido retinóico a 0,05% e o da solução alcoólica de podofilina a 0,25% no tratamento da leucoplasia pilosa oral em pacientes HIV positivos.* 2000. 130f. Dissertação (Mestrado em Patologia Bucal) - Faculdade de Odontologia, Universidade Federal de São Paulo, São Paulo, 2000.

22. Glick M, Muzyka BC, Lurie D, Salkin LM. Oral manifestations associated with HIV-related disease as markers for immune suppression and AIDS. *Oral Surg Oral Med Oral Pathol* 1994;77(4):344-349.

23. Glick M, Pliskin ME. Regression of oral hairy leukoplakia after oral administration of acyclovir. *Gen. Dent* 1990;38(5):374-375.

24. Gowdey G, Lee MRK, Carpenter WM. Treatment of HIV-related hairy leukoplakia with podophyllum resin 25% solution. *Oral Surg Oral Med Oral Pathol* 1995;79(1):64-7.

25. Greenspan JS, Greenspan D. Oral hairy leukoplakia: diagnosis and management. *Oral Surg Oral Med Oral Pathol* 1989;67(4):396-403.

26. Hersbst JS, Morgan J, Raab-Traub N, Resnick L. Comparison of the efficacy of surgery and acyclovir therapy in oral hairy leukoplakia. *J Am Acad Dermatol* 1989;22:753-6.

27. Horn JL. A rationale and test for the number of factors in factor analysis. *Psychometrika* 1965;30:179-185.

28. Hosmer DW, Lemeshow S. Applied survival analysis: regression modeling of time to event data. Canada: Wiley Interscience Publication, 1999.

29. Husak R, Garbe C, Orfanos CE. Oral hairy leukoplakia in 71 HIV-

seropositive patients: clinical symptoms, relation to immunologic status, and prognostic significance. *J Am Acad Dermatol* 1996;35(6):928-934.

30. Kirkwood BR. Essentials of medical statistics. Oxford: Blackwell Science, 1996:27-36.
31. Kitaura H, Adachi N, Kobayashi K, Yamada T . Knowledge and attitudes of Japanese dental health care workers towards HIV related disease. *J Dent* 1997;25(3–4):279-283.
32. Kratochvil FJ, Riordan P, Auclair PL, Huber MA, Kragel PJ. Diagnosis of oral hairy leukoplakia by ultrastructural examination of exfoliative cytologic specimens. *Oral Surg Oral Med Oral Pathol* 1990;70(5):613-8.
33. Lin L, Chen XS, Cui PG, Wang JB, Guo ZP, Lu NZ, et al. Topical application of penciclovir cream for the treatment of herpes simplex facialis/labialis: a randomized, double-blind, multicentre, acyclovir-controlled trial. *J Dermatolog Treat* 2002;13(2):67-72.
34. Louzada-Nur F, Costa C. Retrospective findings of the clinical benefits of podophyllum resin 25% sol on hairy leukoplakia. *Oral Surg Oral Med Oral Pathol* 1992;73(5):555-8.
35. Lozada-Nur F. Podophyllin resin 25% for treatment of oral hairy leukoplakia: an old treatment for a new lesion. *J Acquir Immune Defic Synch* 1991;4(5):543-546.
36. Maraun MD. The claims of factor analysis. *Multivariate Behavioral Research* 1996;31(4):673-689.
37. Margiotta V, Campisi G, Mancuso S, Accurso V, Abbadessa V. HIV infection: oral lesions, CD4+ cell count and viral load in an Italian study population. *J Oral Pathol Med* 1999;28(4):173-177.

38. Marsh HW, Hau KT, Balla JR, Grayson D. Is more ever too much? The number of indicator per factor in confirmatory factor analysis. *Multivariate Behavioral Research* 1998;33(2):181-220.

39. McDonald RP, Ho MH. Principles and practice in reporting structural equation analyses, *Psychol.Methods* 2002;7:64-82.

40. Migliorati CA, Jones AC, Baughman PA. Use of exfoliative cytology in the diagnosis of oral hairy leukoplakia. *Oral Surg Oral Med Oral Pathol* 1993;76(6):704-10.

41. Milagres A, Dias EP, Tavares DS, Cavalcante RM, Dantas VA, Oliveira SP, et al. Prevalence of oral hairy leukoplakia and epithelial infection by Epstein-Barr virus in pregnant women and diabetes mellitus patients:cytopathologic and molecular study. *Mem Inst Oswaldo Cruz* 2007;102(2):159-64.

42. Moura MDG, Guimarães TRM, Fonseca LMS; Pordeus IA, Mesquita RM. A random clinical trial study to assess the efficiency of topical applications of podophyllin resin (25%) versus podophyllin resin (25%) together with acyclovir cream (5%) in the treatment of oral hairy leukoplakia. *Oral Surg Oral Pathol Oral Med Oral Radiol Endod* 2007;103:64-71.

43. Moura MDG, Grossmann SMC, Fonseca LMS, Senna MIB, Mesquita RA. Risk factors for oral hairy leukoplakia in HIV-infected adults of Brazil. *J Oral Pathol Med* 2006;35:321-6.

44. Mulligan R, Seirawan H, Galligan J, Lemme S. The effect of an HIV/AIDS educational program on the knowledge, attitudes, and behaviors of dental professionals. *J Dent Educ* 2006;70(8):857-68.

45. Mussard J, Ashley FA, Newton JT, Kendall N, Crayford TJ. What do you think of your dentist? A dental practice assessment questionnaire. *J Eval Clin*

Pract 2008;14(2):181-4.

46. Nasser F, Benson J, Wisenbaker J. The performance of regression-based variations of the visual scree for determining the number of common factors. *Educational and psychological measurement* 2002;62(3):397-419.
47. Oliveira ER, Narendran S, Falcão A. Brazilian dental student's knowledge and attitudes towards HIV infection. *AIDS Care* 2002;14(4):569-576.
48. Patel ASH, Glick M. Oral manifestations associated with HIV infection: evaluation, assessment, and significance. *Gen Dent* 2003,51(2):153-156.
49. Patton LL. Sensitivity, specificity, and positive predictive value of oral opportunistic infections in adults with HIV/AIDS as markers of immune suppression and viral burden. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000;90(2):182-188.
50. Ramirez-Amador V, Esquivel-Pedraza L, Ponce de León S, Ponce de León S. Prognostic value of oral candidosis and hairy leukoplakia in 111 mexican HIV-infected patients. *J Oral Pathol Med* 1996;25:206-211.
51. Ravina A, Ficarra G, Chiodo M, Mazzetti M, Romagnani S. Relationship of circulating CD4+ T-lymphocytes and p24 antigenemia to the risk of developing AIDS in HIV-infected subjects with oral hairy leukoplakia. *J Oral Pathol Med* 1996;25:108-111.
52. Reis C, Heisler M, Amowitz LL, Moreland RS, Mafeni JO, Anyamele C, et al. Discriminatory attitudes and practices by health workers toward patients with HIV/AIDS in Nigeria. *PLoS Med* 2005;2(8):e246.
53. Reise SP, Waller NG, Comrey AL. Factor analysis and scale revision. *Psychological Assessment* 2000;12:287-297.
54. Resnick L, Herbst JS, Ablashi DV, Atherton S, Frank B, Rosen L, et al.

Regression of oral hairy leukoplakia after orally administered acyclovir therapy. *JAMA* 1988;259(3):384-388.

55. Rodrigues MP, Domingos MS, Silva EM. The dental surgeons and the AIDS social representations. *Cienc. Saude Coletiva* 2005;10(2):463-472.

56. Ross MW, Hunter CE. Dimensions, content and validation of the fear of AIDS schedule in health professionals. *AIDS Care* 1991;3(2):175-80.

57. Sadowsky D, Kunzel C. Measuring dentists' willingness to treat HIV-positive patients. *J Am Dent Assoc* 1994;125:705-10.

58. Sanchez M, Spielman T, Epstein W. Treatment of oral hairy leukoplakia with podophyllin. *Arch Dermatol* 1992;128(12):1659.

59. Sarisky RT, Bacon TH, Boon RJ, Duffy KE, Esser KM, Leary J, et al. Profiling penciclovir susceptibility and prevalence of resistance of herpes simplex virus isolates across eleven clinical trials. *Arch Virol* 2003;148(9):1757-69.

60. Schimid-Wendtner MH, Kortinhg HC. Penciclovir cream improved topical treatment for herpes simplex infections. *Skin Pharmacol Physiol* 2004;17(5):214-8.

61. Schmidt-Westhausen AM, Priepke F, Bergmann FJ, Reichart PA. Decline in the rate of oral opportunistic infections following introduction of highly active antiretroviral therapy. *J Oral Pathol Med* 2000;29:336-341.

62. Senna MI, Guimarães MD, Pordeus IA. Factors associated with dentists' willingness to treat HIV/AIDS patients in the National Health System in Belo Horizonte, Minas Gerais, Brazil. *Cad Saude Publica* 2005;21(1):217-25.

63. Ship JA, Vissink A, Challacombe SJ. Use of prophylactic antifungals in the immunocompromised host. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;103(suppl1):S6.e1-S6.e14.

64. Shrout, P.E. Reliability. In *Textbook in Psychiatry Epidemiology* Edited by: Zahner TTA. New York: Wiley-Liss, 213-227,1995.

65. Souza LB, Pinto LP, Medeiros AMC, Júnior RFA, Mesquita OJX. Manifestações orais em pacientes com AIDS em uma população brasileira. *Pesqui Odontol Bras* 2000;14:79-85.

66. Spruance SL, Rea TL, Thoming C, Tucker R, Saltzman R, Boon R. Penciclovir cream for the treatment of herpes simplex labialis: a randomized, multicenter, double-blind, placebo-controlled trial. *JAMA* 1997;277(17):1374-9.

67. Sutton D, Boyd MR. Comparative activity of penciclovir and acyclovir in mice infected intraperitoneally with herpes simplex virus type 1 SC16. *Antimicrob Agents Chemother* 1993;37(4):642-5.

68. Thompson B. Guidelines for authors: *Educational and Psychological Measurement* 1994; 54(4):837-847.

69. Triantos D, Porter SR, Scully C, Teo CG. Oral Hairy Leukoplakia: clinicopathologic features, pathogenesis, diagnostic, and clinical significance. *Clin Infect Dis* 1997;25:1392-6.

70. Unaids. Disponível em: http://www.unaids.org/en/, 2009.

71. World Health Organization. Oral health survey: basic methods, 4 edn. Who Health Organization, Geneva, 1997.

72. Zwick R, Velicer WF. Comparison of five rules for determining the number of components to retain. *Psychological Bulletin* 1986;*99:*432-442.

6. Artigos

ARTIGO 1

A new topic treatment protocol for oral hairy leukoplakia

(Aceito- Oral Surgery Oral Pathology Oral Medicine and Oral Radiologic Endodontic)

Title: A new topic treatment protocol for oral hairy leukoplakia

Abstract

Objective: To present a new topical treatment protocol for oral hairy leukoplakia (OHL), consisting of a 25% podophyllin resin together with a 1% penciclovir cream (PP), and to compare this topical treatment protocol's efficacy with that of two other topical treatment protocols: a 25% podophyllin resin (P) and a 25% podophyllin resin together with a 5% acyclovir cream (PA).

Study Design: Forty-two HIV-positive patients with 69 OHL lesions were randomly treated using P, PA, or PP (14 patients in each topical treatment protocol). Clinical healing was determined when the white plaque could no longer be seen in the primary location of the lesion. Topical treatment performance was evaluated by clinical healing within each week of topical treatment protocol as well as by the recurrence of the lesion. Statistical survival analysis was performed using a Cox proportional hazards model.

Results: Approximately 55% of the patients presented a clinical healing of OHL within seven to eight weeks of each topical treatment protocol. After the sixth week, the PA treatment protocol presented a faster clinical healing rate of OHL. Recurrence could be observed in three and seven OHL lesions treated with P and PP treatment protocols, respectively.

Conclusions: The PP treatment protocol proved to be effective; however; the PA treatment protocol is more efficient in the clinical healing rate for OHL when compared to P and PP treatment protocols after the sixth week of treatment, and no recurrent OHL could be observed in the PA treatment protocol.

Patients with a prolonged immunodeficiency caused by HIV-infection tend to develop oral lesions, as the oral hairy leukoplakia (OHL)[1-3]. OHL has been well-documented since the onset of the HIV-infection pandemic as an early marker of HIV-infection and may be related to the progression to AIDS, the clinical presentation of other oral lesions, and treatment decisions in HIV-infection[3-6]. Broad access to better HIV treatment with highly active antiretroviral therapy (HAART) has resulted in a 12.8% reduction in the prevalence of OHL[5].

OHL is characterized by an asymptomatic white plaque on the lateral borders of the tongue and a flat, corrugated, or hairy surface that is not removable when scraped[1]. OHL is related to the infection of oral epithelium caused by the Epstein-Barr virus (EBV/ human herpes virus [HHV]-4)[1,2,7]. EBV can be identified through electronic microscopy techniques, “in situ” hybridization, immunohistochemistry, and polymerase chain reaction (PCR)[2,7,8]. Since some of these techniques are complex, expensive, and require a biopsy,[7] exfoliative cytology has proven to be a good option in diagnosing OHL. It is a simple, reliable, and safe method which affords easy access and produces quick results in a non-invasive and non-traumatic manner[9-11].

The clinical indications for OHL treatment included the reestablishment of the normal characteristics of the tongue without developing a resistance to EBV as well as of the patient's desire for either aesthetic improvements or the elimination of OHL[9,12-16]. Topical treatment is most commonly recommended for patients with OHL. It is an inexpensive, safe therapy which is easy to apply, noninvasive, does not produce systemic adverse effects, and is effective over a long period of time[9,12-15].

The topical drugs used to treat OHL include 25% podophyllin resin (P), 2% gentian violet, 5% acyclovir cream (ACV), and the combination of P to ACV (PA)[9,12-17]. P is an effective topical treatment for OHL worldwide, whose resolution time for topical therapy varies from 1 to 25 applications[9,12-14,16]. Moura et al.[9] used P and PA to treat 28 patients with 46 OHL lesions. The applied PA presented a 100% clinical healing. In addition, this was the first study in which the OHL, treated only with P, did not present clinical healing after 25 applications when performed weekly on two patients with bilateral lesions[9]. In contrast, the

application of 2% gentian violet has been approved for human use and has proven to be a potent inhibitor of reactive oxygen. However, only one topical treatment of 2% gentian violet performed on one patient with only one OHL has actually presented clinical healing[15]. Concerning ACV, Ficarra et al.[17] reported a clinical healing of OHL in two patients and a partial regression in another after only one topical treatment with ACV.

Few prior studies have used double blind, controlled randomized clinical trials for the topical treatment of OHL, and only one study described the synergism of drugs, such as ACV with P, in the topical treatment of OHL[9]. Further, there are no studies in the literature that demonstrate the action of a 1% penciclovir cream (PCV) with P in oral lesions related to EBV/HHV-4, such as OHL. Most studies have only made the comparison between PCV and ACV in the topical treatment of herpes simplex labials, and subsequently for HHV-1 and HHV-2, and have shown that PCV was superior in this type of topical treatment[18-24]. PCV possesses the same antiviral spectrum as ACV, but has considerably more bioavailability, up to 77% as compared to the 10–20% of ACV[18]. PCV has a prolonged antiviral activity when compared to ACV, but the superior activity of PCV is brought about by the known stability of the intracellular penciclovir triphosphate[18-23]. The intracellular half-life of PCV and ACV in infected cells is 10 to 20 hours and one hour, respectively[22]. The purpose of the current study, therefore, was to show that the association of PCV and P could enhance the activity of topical treatment, thus improving the effectiveness of the clinical response in the topical treatment of OHL. This study aimed to present a new topical treatment protocol for OHL, consisting of a 25% podophyllin resin together with a 1% penciclovir cream (PP), and to compare this topical treatment protocol's efficacy with two other topical treatment protocols: P and PA.

Subject and methods

Data for this clinical trial were obtained from a random sample of 42 HIV-positive patients (31 males and 11 females), with 14 patients in each topical treatment protocol, and a total of 69 OHL located on the lateral border of the tongue. Twenty-four OHL were treated with P (unilateral=4; bilateral=10), 23 with PA (unilateral=5; bilateral=9), and 22 with PP

(unilateral=6; bilateral=8). The size of 69 OHL varied from 5 to 60mm (mean of 27.7mm and median of 25mm). The patients were recruited from the Orestes Diniz Center for Training and Reference in Infectious and Parasitic Diseases (CTR/DIP) (Belo Horizonte, Minas Gerais, Brazil). The period of study ran from January 2003 to July 2007. In this analysis, the same sample size of 28 patients and 46 OHL lesions from the study of Moura et al.[9] were used. The study protocol was approved by the Committee of Bioethics in Research from the Universidade Federal de Minas Gerais (367/6).

Patient population. The target population for drug therapy included patients with OHL lesions and HIV-infection. All patients were first diagnosed with HIV-infection by means of an enzyme-linked immunosorbant assay (ELISA) as a primary detection test and confirmed by Western blot. The diagnosis for HIV-infection had already been established during the period of the first examination. Extra-oral and intra-oral exams were performed on all patients using a single calibrated, trained medical oral examiner in accordance with World Health Organization standards[25]. The same examiner performed exfoliative cytology and applied the drugs to the tongues of the patients. Analysis of the exfoliative cytology was performed in the Oral Pathology Service at the School of Dentistry, Universidade Federal de Minas Gerais (UFMG). Patients with bilateral OHL received the same topical treatment protocol for both lesions. The selection of patients to receive P, PA and PP treatment protocols was randomized. Another double-blinded oral medical examiner clinically evaluated the patients each week before each topical treatment protocol and each month after clinical healing over a period of one year. Clinical healing was considered when the white plaque could no longer be seen in the primary location of the OHL. The clinical healing rate in time interaction was determined when the clinical healing of the OHL occurred in each week of topical treatment protocol.

Study drug. P solution, ACV, and PCV ointments were the drugs of choice. P was provided by a Brazilian manipulation pharmacy, while ACV and PCV were purchased from a traditional Brazilian pharmacy. ACV or PCV were applied once weekly until clinical healing of the OHL had occurred or up to a maximum of 25 weeks.

Study design. The exfoliative cytology and the topical treatment protocols were in accordance with a previous study performed by Moura et al.[9]. In the present study, the PP treatment protocol of OHL was similar to that using PA treatment protocol, as reported by Moura et al.[9] (Figs 1A, 1B, and 1C). The PP treatment protocol applications were performed until 25 weeks. The patient was advised not to eat or drink anything for a period of 60 minutes after the topical treatment protocol. The applications were performed weekly, considering the unit of time in the analysis as the number of weeks of topical treatment protocols. Clinical reevaluation occurred 12 months after the clinical healing of OHL. Topical treatment performance of P, PA, and PP treatment protocols was evaluated by (1) clinical healing of OHL or not, (2) rate of clinical healing in time interaction, and (3) recurrence of OHL twelve months after clinical healing.

Predictor variables. A medical record review was conducted for each participant to ascertain variables to be included in the analyses as measures of secondary outcomes: route of transmission (heterosexual X others), CD4 T lymphocyte count (two measures and mean of both), viral load (two measures and mean of both), oral candidiasis, use of highly active anti-retroviral therapy (HAART), prior use of acyclovir, and prior use of antifungal drugs. These outcomes were used in a subsequent statistical survival analysis (Table 1). The laboratory variables were collected from medical records in two ways: the first when the patient began the topical treatment protocol and the second four months later (Table 1). The diagnosis of oral candidiasis was established upon determining the clinical features and by using the Periodic Acid Schift stain[26,27]. Oral candidiasis was treated with fluconazole[27,28].

Statistical analysis. The primary dependent measure (drug efficacy in the topical treatment protocol) and secondary outcome measure (predictor variables) were performed using a Cox proportional hazards model. Descriptive statistics to measure secondary outcomes were estimated for each topical treatment protocol (Table 1). The Unconditional Cox Proportional Model was conducted on each predictor variable in an attempt to select those that could be used in a full model and to verify the association of each variable with each topical treatment protocol. A p-value of lower than 0.20 and 0.05 was set as the

inclusion criteria for the Unconditional Cox Proportional Model and for the multiple model, respectively. The measure of the primary outcome was evaluated according to three topics. The first topic of the topical treatment performance used to analyze the clinical healing of OHL was assessed according to the survival analysis using Cox's proportional hazards models and Kaplan-Meier survival[29]. The second topic of topical treatment performance evaluated the impacts on the clinical healing rate in a time interaction and was conducted considering a survival analysis using Cox's proportional hazards model by means of the Test of Proportional Hazards Assumption[29]. The Proportional Hazards Assumption for each predictor variable was also conducted to check if the variable could interact with time. The Cox proportional hazards model was applied with the predictor variable selected in an unconditional analysis, where it was possible to estimate the Hazard Ratio (HR) of each variable together with the control of the real effect[29]. All analyses were conducted using the statistical software package STATA (version 10.0, Texas, USA). The third topic of topical treatment performance, regarding the recurrence of each topical treatment protocol, was estimated by descriptive statistics.

Results

Forty-two HIV-positive adults diagnosed with 69 OHL also had AIDS, of which 30 (71%) were men and 12 (29%) were women. The ages varied from 23 to 56 (mean 37.8 and median 38). The route of transmission included 1 (2.4%) drug user, 3 (7.1%) no information provided, 27 (64.3%) heterosexual, 10 (23.8%) men who have sex with men (MSM), and 1 (2.4%) bisexual. Additional descriptive factors are found in table 1.

The survival characteristics of OHL regarding topical treatment protocols are shown in fig. 2. The P treatment protocol required up to 25 applications. However, PA and PP treatment protocols required only up to 18 and 23 applications, respectively, for the clinically healing of OHL (Fig. 1D). Approximately 55% of the patients presented a clinical healing of OHL within seven to eight weeks of topical treatment protocols; where eight, seven, and eight patients presented an effective clinical healing of OHL, in the P, PA and PP treatment protocols, respectively. All cases of OHL treated with PP and PA topical protocols presented

clinical healing and the median time was of seven to eight weeks. The data regarding the first topic of topical treatment performance of P, PA, and PP treatment protocols in the 42 patients using the survival analysis, via a Kaplan-Meier survival estimates graph and a Cox proportional hazards model, are illustrated in fig 2 and table 2, respectively. Table 2 presents the descriptive data using the time (weeks) and in which week the patients no longer presented clinical healing of OHL. Table 2 shows two patients who died of AIDS, one in the PP treatment protocol and the other in the P treatment protocol, both with bilateral OHL. Also, two patients who had not clinical healing of OHL after 25 applications in the P treatment protocol, both with bilateral OHL. These patients were not included in statistical analysis.

Upon P treatment protocol, patients complained of an altered taste due to the bitter taste of the medication for a period of two-hours after the application. The patients reported no local adverse effects regarding the applications of PCV nor ACV together with P. No systemic adverse effects were observed in either topical treatment protocol.

Results form the Unconditional Cox Proportional Hazards Model showed that heterosexual activity (Hazard Ratio (HR)= 1.630; p = 0.164), oral candidiasis (HR=0.577; p=0.121), and prior use of antifungal drugs (HR=0.495, p=0.044) potentially influenced the topical treatment protocols. These three factors were included in the adjusted multiple variables model. Only prior use of antifungal drugs presents a p-value of lower than 0.05 in the adjusted multiple variables model. As the three topical treatment protocols were the main objective of this study, the variable which represents the topical treatment protocol was automatically selected for the multiple model, together with the variables (potential risk factors or confounding variables) that could interfere in the topical treatment protocol.

The adjusted multiple variables were used in assessing the HR of their relationships, based on the clinical healing rate for OHL at a given point of time for OHL. The final model presented a significant association between the prior use of antifungal drugs (HR=0.410; p=0.016) and the topical treatment protocols with time interaction (HR = 0.030 x $7.967^{\ln(t)}$; Int(t)=interaction with time; overall p = 0.034) (Table 3). Table 3 presents the final model and estimates of HR for PA and PP treatment protocols over time. Prior use of antifungal drugs

proved to decrease the clinical healing rate by 59.0% (HR = 0.410; p = 0.016) over a given time (Table 3). The three topical treatment protocols presented the same clinical healing rates for OHL, with the same speed of clinical healing up to the sixth week. After the sixth week, the PA treatment protocol presented a faster clinical healing rate of OHL as compared to the P and PP treatment protocols (HR = $0.030 \times 7.967^{\ln(t)}$; overall p = 0.034) (Table 3).

Recurrence was observed in three OHL of the three different patients of the P treatment protocol. Two of these patients, with one lesion each, observed a recurrence within 12 months of post-treatment, whereas the third patient with a bilateral OHL presented a recurrence in only one OHL after three months of post-treatment. Also, in seven lesions of four patients treated with PP protocol, recurrence could be observed three months after post-treatment; three of whom presented bilateral OHL and one of whom presented only one OHL. OHL treated with PA protocol presented no recurrence.

Discussion

The current study is the first of its kind to compare three topical treatment protocols for OHL in a blinded randomized clinical trial and to analyze the subsequent results using survival analysis by means of a Cox proportional hazards model. The main findings included: the PA treatment protocol presented a faster clinical healing rate of OHL after the sixth week; approximately 55% of the patients presented an effective clinical healing of OHL within seven to eight weeks of topical treatment protocols; and recurrences could be observed in three and seven OHL lesions treated with P and PP protocols, respectively, whereas no OHL recurrence could be observed in the PA treatment protocol. The long period of the current study, from 2003 to 2007, was related to the following factors: (1) difficulty in obtaining OHL lesions, (2) difficulty in maintaining patient collaboration over the course of topical treatment protocols, and (3) the prevalence of OHL tends to decrease with the beginning of HAART[9,13]. The patients undergoing HAART show a lower prevalence of OHL than do patients who do not undergo HAART[6]. All 42 patients were undergoing HAART, two of whom died during the study.

This current study represents the first depiction of the PP treatment protocol for OHL and shows the clinical application of PCV in the oral lesion caused by EBV/HHV-4. The synergy of drugs has been described in prior literature as being able to improve the effectiveness of the clinical healing in several diseases[30]. Moura et al.[9] reported the most positive effect being PA treatment protocol on OHL. Topical antiviral drugs may well enhance the activity of P treatment protocol, thus improving the effectiveness of clinical healing in the OHL topical treatment protocols[9]. After the dekeratinization of superficial epithelial cells by P, the topical antiviral drug then acts upon exposed cells located immediately beneath the surface and which have been infected by EBV/HHV-4[9]. In this study, PCV together with P, in contrast to ACV together with P, proved inefficient in the clinical healing of the all cases of OHL.

Oral candidiasis and prior use of antifungal drugs may well be able to influence OHL topical treatment protocols by decreasing clinical healing of OHL by 43% and 59%, respectively, over a given time. The high incidence of oral candidiasis in HIV positive patients has resulted in the use of systemic antifungal drugs, especially fluconazole. The literature supports the recommendation that systemically applied antifungal drugs were prescribed as secondary prophylaxis against oral candidiasis or other systemic fungal diseases[26-28]. The fact that patients had presented a prior use of antifungal drugs could mean that they were very ill, presented very low CD4 counts, and had AIDS, which required the use antifungal drugs. Their poor health may represent a key contributing factor, given that their poor immune system most likely allows EBV to replicate, thus causing OHL, which may indirectly be responsible for a decrease in the clinical healing of OHL.

The present study investigated, for the first time in the literature, topical treatment protocols with time interaction and verified that P, PA, and PP treatment protocols do in fact present a clinical healing of OHL with the same clinical healing rate up to the sixth week of topical treatment protocol. Sanchez et al.[16] and Gowdey et al.[13] observed the clinical healing of all OHL lesions after a single application of P in six and ten lesions, respectively. Lozada-Nur and Costa[12] observed the clinical healing of nine OHL lesions, five after a single

application of P and four after two applications. Moura et al.[9] performed an analysis comparing P and PA treatment protocols and verified that only PA showed a 100% clinical healing of OHL after various applications. However, those authors did not evaluate the clinical healing rate for OHL together with time interactions[9,12-13,16]. In the current study, the PA treatment protocol proved to be better after the sixth week, showing that P together with ACV was more efficient than P together with PCV in the clinical healing of OHL. The fact that ACV is more concentrated than PCV, 5% as compared to 1%, may be explain why P together with ACV presented a faster clinical healing rate for OHL after the sixth week. This finding is new, as prior studies had only performed the topical treatment on herpes simplex labialis and had demonstrated the superiority of PCV with a significant decrease in the clinical healing time, the lesion area, and pain[18-24].

Patients had recurrence of OHL in the P and PP treatment protocols. In contrast, patients treated with PA protocol presented no recurrence within 12 months of post-treatment. In previous literature, only Sanchez et al.[16] observed a recurrence rate of 33.3% for OHL treated with P after six and nine months of post-treatment. These findings suggest that the synergism of PA treatment protocol decreases the possibility of OHL recurrence after topical treatment protocol.

The PP treatment protocol proved to be effective; however; the PA treatment protocol is more efficient in the clinical healing rate for OHL when compared to P and PP treatment protocols after the sixth week of treatment. In addition, no recurrent OHL could be observed in the PA treatment protocol. Additional studies employing similar methodologies are needed to obtain further knowledge concerning the fact that, after six weeks, P together with ACV presents an increased (exponential) clinical healing of OHL as well as to explain why PA treatment protocol, as compared to P and PP treatment protocols, is more efficient in the clinical healing rate for OHL.

References

1. Greenspan D, Greenspan JS, Conant MA, Petersen V, Silverman-Junior S, Souza Y. Oral hairy leukoplakia in male homosexuals: evidence of association with both papilloma virus and a herpes-group virus. Lancet 1984;2:831-4.

2. Mendoza N, Diamantis M, Arora A, Bartlett B, Gewirtzman A, Tremaine AM, et al. Mucocutaneous manifestations of Epstein-Barr virus infection. Am J Clin Dermatol 2008;9:295-305.

3. Pedreira EN, Cardoso CL, Barroso-Edo C, Santos JA, Fonseca FP, Taveira LA. Epidemiological and oral manifestations of HIV-positive patients in a specialized service in Brazil. J Appl Oral Sci 2008;16:369-75.

4. Moura MDG, Grossmann SMC, Fonseca LMS, Senna MIB, Mesquita RA. Risk factors for oral hairy leukoplakia in HIV-infected adults of Brazil. J Oral Pathol Med 2006;35:321-6.

5. Chattopadhyay A, Patton LL. Risk indicators for HIV-associated jointly occurring oral candidiasis and oral hairy leukoplakia. AIDS Patient Care STDS 2007;21:825-32.

6. Silva CA, Dourado I, Dahia SR, Harzheim E, Rutherford GW. Oral manifestations of HIV infection in patients receiving highly active antiretroviral therapy (HAART) in Bahia, Brazil. J Public Health Dent 2008;68:178-81.

7. Mabruk MJEMF, Antonio M, Flint SR, Coleman DC, Toner M, Leader M, et al. A simple and rapid technique for the detection of Epstein-Barr virus DNA in HIV-associated oral hairy leukoplakia biopsies. J Oral Pathol Med 2000;29:118-22

8. Robaina TF, Valladares CP, Tavares DS, Napolitano WC, Silva LE, Dias EP, et al. Polymerase chain reaction genotyping of Epstein-Barr virus in scraping samples of the tongue lateral border in HIV-1 seropositive patients. Mem Inst Oswaldo Cruz 2008;103:326-31.

9. Moura MDG, Guimarães TRM, Fonseca LMS; Pordeus IA, Mesquita RA. A random clinical trial study to assess the efficiency of topical applications of podophyllin resin

(25%) versus podophyllin resin (25%) together with acyclovir cream (5%) in the treatment of oral hairy leukoplakia. Oral Surg Oral Pathol Oral Med Oral Radiol Endod 2007;103:64-71.

10. Fraga-Fernandes J, Vicandi-Plaza B. Diagnosis of hairy leukoplakia by cytologic methods. Am J Clinic Pathol 1992;97:262-6.
11. Epstein JB, Fatahzadeh M, Matisic J, Anderson G. Exfoliative cytology and electron microscopy in the diagnosis of hairy leukoplakia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995;79:564-9.
12. Lozada-Nur F, Costa C. Retrospective findings of the clinical benefits of podophyllum resin 25% sol on hairy leukoplakia. Oral Surg Oral Med Oral Pathol 1992;73:555-8.
13. Gowdey G, Lee MRK, Carpenter WM. Treatment of HIV-related hairy leukoplakia with podophyllum resin 25% solution. Oral Surg Oral Med Oral Pathol 1995;79:64-7.
14. Lozada-Nur F. Podophyllin resin 25% for treatment of oral hairy leukoplakia: an old treatment for a new lesion. J Acquir Immune Defic Synch 1991;4:543-6.
15. Bhandarkar SS, MacKelfresh J, Fried L, Arbiser JL. Targeted therapy of oral hairy leukoplakia with gentian violet. J Am Acad Dermatol 2008;58:711-2.
16. Sanchez M, Spielman T, Epstein W. Treatment of oral hairy leukoplakia with podophylin. Arch Dermatol 1992;128:1659.
17. Ficarra G, Barone R, Gaglioti D, Milo D, Riccardi R, Romagnoli P, et al. Oral hairy leukoplakia among HIV-positive intravenous drug abusers: a clinicopathologic and ultrastructural study. Oral Surg Oral Med Oral Pathol 1988;65:421-6.
18. Femiano F, Gombos F, Scully C. Recurrent herpes labialis: efficacy of topical therapy with penciclovir compared with acyclovir (aciclovir). Oral Dis 2001;7:31-3.
19. Sutton D, Boyd MR. Comparative activity of penciclovir and acyclovir in mice infected intraperitoneally with herpes simplex virus type 1 SC16. Antimicrob Agents Chemother 1993;37:642-5.

20. Boon R, Goodman JJ, Martinez J, Marks GL, Gamble M, Welch C. Penciclovir cream for the treatment of sunlight-induced herpes simplex labialis: a randomized, double-blind, placebo-controlled trial. Clinical Therapeutics 2000;22:76-90.

21. Spruance SL, Rea TL, Thoming C, Tucker R, Saltzman R, Boon R. Penciclovir cream for the treatment of herpes simplex labialis: a randomized, multicenter, double-blind, placebo-controlled trial. JAMA 1997;277:1374-9.

22. Lin L, Chen XS, Cui PG, Wang JB, Guo ZP, Lu NZ, et al. Topical application of penciclovir cream for the treatment of herpes simplex facialis/labialis: a randomized, double-blind, multicentre, acyclovir-controlled trial. J Dermatolog Treat 2002;13:67-72.

23. Schimid-Wendtner MH, Kortinhg HC. Penciclovir cream improved topical treatment for herpes simplex infections. Skin Pharmacol Physiol 2004;17:214-8.

24. Sarisky RT, Bacon TH, Boon RJ, Duffy KE, Esser KM, Leary J, et al. Profiling penciclovir susceptibility and prevalence of resistance of herpes simplex virus isolates across eleven clinical trials. Arch Virol 2003;148:1757-69.

25. World Health Organization. Oral health survey: basic methods, 4 edn. Who Health Organization, Geneva, 1997.

26. Fidel PL. Candida-host interactions in HIV disease: relationships in oropharyngeal candidiasis. Adv Dent Res 2006;19:80-4.

27. Costa CR, Lemos JA, Passos XS, Araújo CR, Cohen AJ, Souza LK, et al. Species distribution and antifungal susceptibility profile of oral Candida isolates from HIV-infected patients in the antiretroviral therapy era. Mycopathologia 2006;162:45-50.

28. Ship JA, Vissink A, Challacombe SJ. Use of prophylactic antifungals in the immunocompromised host. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:S6.e1-14.

29. Hosmer DW, Lemeshow S. Applied survival analysis: regression modeling of time to event data. Canada: Wiley Interscience Publication; 1999.

30. Karyadi E, West CE, Schultink W, Nelwan RHH, Gross R, Amin Z, et al. A double-blind, placebo-controlled study of vitamin A and zinc supplementation in persons with tuberculosis in indonesia: effects on clinical response and nutritional status. Am J Clin Nutr 2002;75: 720-7.

Tables

Table 1 – Descriptive statistics of the measure of secondary outcomes in patients for 25% podophyllin resin (P), 25% podophyllin resin together with 5% acyclovir cream (PA), or 25% podophyllin resin together with 1% penciclovir cream (PP) treatment protocols

Variables	P (n=14)		PA (n = 14)		PP (n 14)		Total (n = 42)	
	No	Yes	No	Yes	No	Yes	No	Yes
Heterosexual transmission	6	8	5	9	4	10	15	27
First CD4 lower than 200	10	4	10	4	7	7	27	15
Second CD4 lower than 200	10	4	9	5	9	5	28	14
Mean CD4 lower than 200	10	4	9	5	10	4	29	13
First viral load lower than 3,000	11	3	8	6	10	4	29	13
Second viral load lower than 3,000	11	3	10	4	9	5	30	12
Mean viral load lower than 3,000	11	3	10	4	12	2	33	9
Oral candidiasis	4	10	5	9	4	10	13	29
Use of HAART	5	9	2	12	1	13	8	34
Prior use of acyclovir	13	1	12	2	14	0	39	3
Prior use of antifungal drugs	4	10	6	8	5	9	15	27

Table 2 – Weekly survival data of the 42 patients with oral hairy leukoplakia (OHL) treated with 25% podophyllin resin (P), 25% podophyllin resin together with 5% acyclovir cream (PA), or 25% podophyllin resin together with 1% penciclovir cream (PP) treatment protocols

Interval (weeks)	Total OHL	Clinical healing				Lost	Survival Function	Standard Error
		P	PA	PP	TOTAL			
from 2 to 3	42	0	0	1	1	0	0.9762	0.0235
from 3 to 4	41	1	1	1	3	0	0.9048	0.0453
from 4 to 5	38	3	2	1	6	0	0.7619	0.0657
from 5 to 6	32	3	1	2	6	0	0.6190	0.0749
from 6 to 7	26	0	1	1	2	0	0.5714	0.0764
from 7 to 8	24	1	2	2	5	0	0.4524	0.0768
from 9 to 10	19	0	2	1	3	0	0.3810	0.0749
from 10 to 11	16	0	0	1	1	0	0.3571	0.0739
from 11 to 12	15	2	2	0	4	0	0.2619	0.0678
from 12 to 13	11	0	1	0	1	0	0.2381	0.0657
from 13 to 14	10	0	0	0	0	1	0.2381	0.0657
from 14 to 15	9	0	0	1	1	1	0.2101	0.0637
from 17 to 18	7	0	1	0	1	0	0.1801	0.0612
from 19 to 20	6	0	1	0	1	0	0.1501	0.0579
from 20 to 21	5	0	0	1	1	0	0.1200	0.0536
From 23 to 24	4	1	0	1	2	0	0.0600	0.0402
From 25 to 26	2	0	0	0	0	2	0.0600	0.0402

Table 3 – Cox Proportional Hazards Model with time interaction in the treatment of oral hairy leukoplakia using three topical treatment protocols. Hazard ratio of 25% podophyllin resin together with 5% acyclovir cream (PA) or 25% podophyllin resin together with 1% penciclovir cream (PP) treatment protocols estimates considering the influence of time

Variable	**Hazard ratio (HR)**	**Standard error**	**p-value**
P	1.0000	Baseline – overall p value = 0.034	
PA	0.0304	0.0499	0.033
PP	0.3404	0.4278	0.391
Prior use of antifungal drugs	0.4102	0.1522	0.016
Variables interacted with current log values (_t).			
PA	7.9670	6.6172	0.012
PP	2.1140	1.3333	0.235
Estimation of Hazard Ratio for PA and PP treatment protocols in time			
Time in Ln (weeks)	Time in weeks	Hazard Ratio for PA	Hazard Ratio for PP
0.000	1	0.0304	0.3404
0.693	2	0.1281	0.5719
1.099	3	0.2972	0.7747
1.386	4	0.5399	0.9609
1.609	5	0.8579	1.1356
1.792	*6*	*1.2525*	*1.3017*
1.946	7	1.7247	1.4609
2.079	8	2.2754	1.6145
2.197	9	2.9055	1.7633
2.303	10	3.6156	1.9080
2.708	15	8.3874	2.5846
2.996	20	15.2374	3.2057
3.219	25	24.2119	3.7885

P: 25% podophyllin resin; Ln: natural log

Legends

Fig. 1 – Oral hairy leukoplakia (OHL) treated with 25% podophyllin resin together with a 1% penciclovir cream. A- OHL of 32 mm on the right lateral border of the tongue of an HIV positive patient, 33 year-old man. This shows OHL washed with water and dried, after the immobilization of the tongue. The red arrow demonstrates the beginning and end of the length of OHL. B- After the application of 25% podophyllin resin on the right lateral border of the tongue, this turned a brownish color. C- Application of a 1% penciclovir cream for 30 seconds, covering the entire lesion. D- Clinical aspect of the clinical healing of OHL after 23 applications.

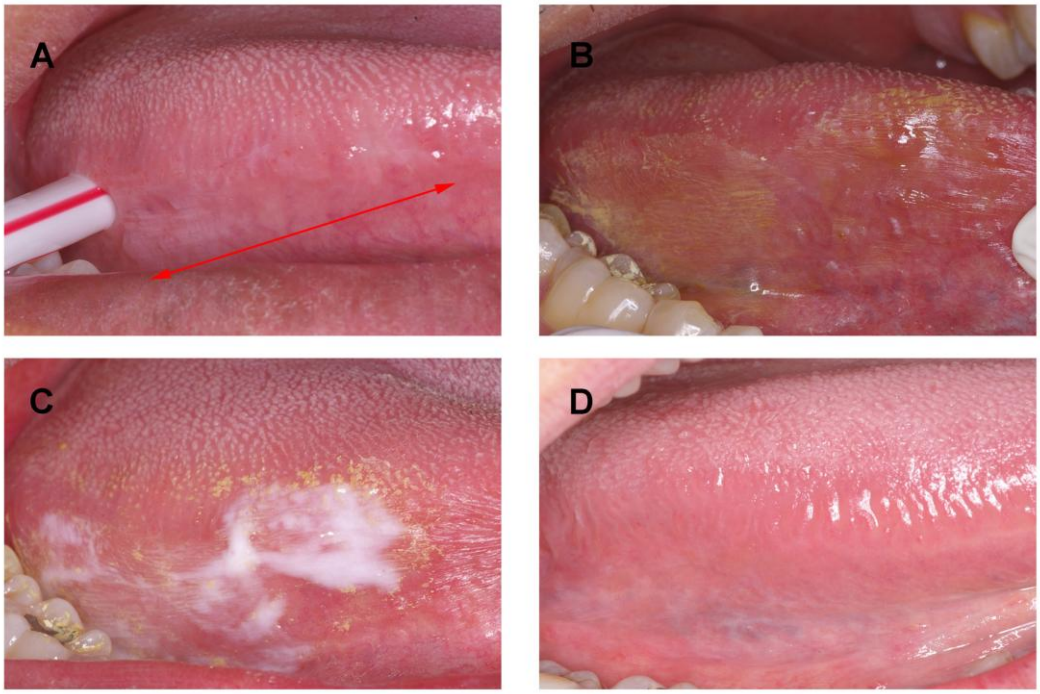
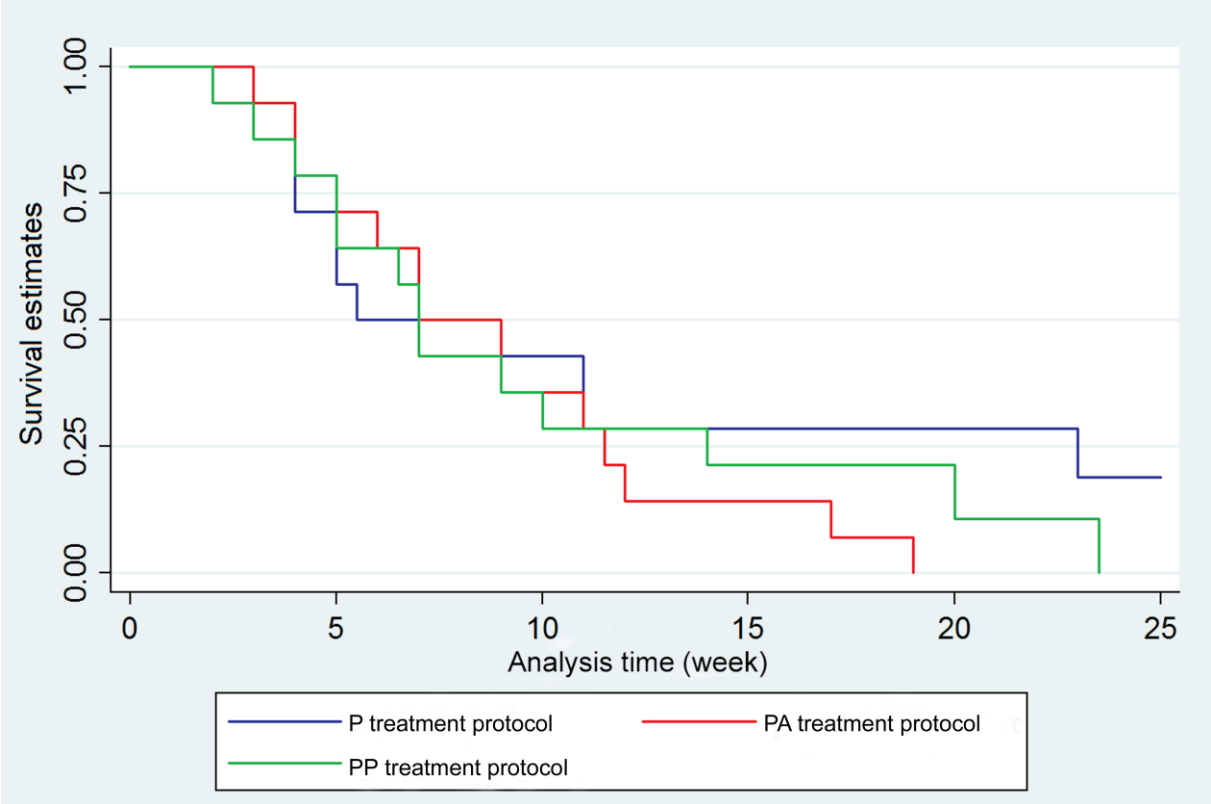


Fig. 2 – Graph of Kaplan-Meier survival estimates of 25% podophyllin resin (P), 25% podophyllin resin together with a 5% acyclovir cream (PA), or 25% podophyllin resin together with a 1% penciclovir cream (PP) treatment protocols for oral hairy leukoplakia.



ARTIGO 2

Epstein-Barr virus: present after the topical treatment of the oral hairy leukoplakia?

(Em elaboração)

Title: Epstein-Barr virus: present after the topical treatment of the oral hairy leukoplakia?

Abstract

Objective: The purpose of the current study was to identify and compare the presence or not of Epstein-Barr virus type 1 (EBV-1) in samples from the lateral border of the tongue of Brazilian HIV-positive adults patients before and after of the topical treatment with different protocols of the oral hairy leukoplakia (OHL).

Study Design: A randomly clinical trial study evaluated 15 HIV-positives patients with OHL. Five OHL were treated with topical applications of podophyllin resin (25%) (P), six with podophyllin resin (25%) together with acyclovir cream (5%) (PA) and four with podophyllin resin (25%) together with pencyclovir cream (1%) (PP) at a clinic for sexually transmitted diseases and HIV. OHL was diagnosed in accordance with exfoliative cytology. DNA was extracted from scrapes of the lateral border of the tongue and amplified by Polymerase Chain Reaction (PCR) using specific primers for EBV-1 before and after the topical treatment. The period of the treatment varied from two to 23 weeks and was suspended after the clinical heal of the OHL. The patients were follow-up for 12 months.

Results: The topical treatment was effective in all patients with 100% of the lesions of OHL healed. All OHL cases (100%) were EBV-1- positive before of the topical treatment. EBV-1-postive in scrapes of the tongue of 11 (73.33%) patients immediately after the clinical heal of the OHL was determined. After of the topical treatment, three patients (20%) treated with PA and one patient (6.66%) with PP was EBV-1-negative. Three patients had recurrence of OHL a year after treatment; all of them were treated with P and EBV-1-positive after treatment.

Conclusions: The acyclovir (5%) or penciclovir (1%) creams together with podophyllin resin (25%) decreased the possibility of recurrence of the OHL. The elevated prevalence of EBV-1 DNA in the lateral border of the tongue of patients without OHL after of the topical treatment supports a role of this virus in recurrence of the OHL.

Key Words: Epstein-Barr virus; HIV infection; oral hairy leukoplakia, polymerase chain reaction.

1. Introduction

Oral hairy leukoplakia (OHL) is related to the infection of oral epithelium by Epstein-Barr virus (EBV). The mechanism by which HIV may cause an increase in the incidence of OHL is poorly understood. The fact that this lesion is primarily and almost exclusively observed in HIV-positive patients with low CD4 T cell counts suggests that it is directly associated with T-cell immunosupression (Greenspan et al., 1984; Greenspan et al., 1985; Husak et al., 1996; Koloktronis et al., 1994; Mabruk et al., 2000; Robaina et al., 2008; Triantos et al., 1997, Walling, 2000). The etiology of OHL has already been established and EBV can be identified through techniques electronic microscopy, in situ hybridization, immunohistochemistry and polymerase chain reaction (PCR) (Cubie et al., 1991; Fraga-Fernández & Vicandi-Plaza 1992; Friedman-Kien, 1986; Greenspan et al., 1984; Greenspan et al., 1985; Gulley 2001; Jaeger et al., 1990; Mabruk et al. 1995, Walling, 2000; Walling et al., 2001; Walling et al., 2003). However, the most of those techniques are very complex, expensive and require a biopsy which may not be readily obtainable, either due to patient refusal or because of coexisting bleeding diatheses due to conditions such as chronic liver disease, haemophilia, or thrombocytopenia, commonly encountered in HIV-positive patients (Longnecker, 2000; Mabruk et al., 2000; Moura et al., 2007). Considering that biopsy is an invasive procedure, the cytopathology should be the best choice to diagnose of the OHL (Epstein et al., 1995; Fraga-Fernandes & Vicandi-Plaza, 1992; Kratochvil et al., 1990; Migliorati et al., 1993; Moura et al., 2007). The PCR is a sensitive technique that can be used on very small tissue samples, but only detects the presence or absence of the target DNA sequence in the sample, and not its location. Therefore, PCR can be useful in the detection of EBV in oral scrapes, and as an adjunct in the diagnosis of OHL (Boom et al., 1990; Cubie et al., 1991; Epstein et al., 1995; Greenspan et al., 1985; Gulley, 2001; Komatsu et al., 2005; Mabruk et al., 1994; Mabruk et al., 1995; Mabruk et al., 2000; Mao & Smith, 1993; Milagres et al., 2007; Scully et al., 1998; Telenti et al., 1990; Walling, 2000; Webster-Cyriaque et al., 2000).

The EBV is a member of the Herpesviridae family and of the Gammaherpesvirinae subfamily. EBV infect lymphocytes and epithelial cells, and were originally isolated from the African form of Burkitt's lymphoma (Epstein et al. 1964). EBV seroprevalence studies demonstrate that EBV antibodies are present in more than 90% of the world's population (Robaina et al., 2008; Webster-Cyriaque et al., 2000; Young; Rickinson, 2004). The EBV is present in a productive, infective state in epithelial cells of the superficial layers of the lingual epithelium and it is present in the epithelial cells of OHL (Cubie et al., 1991; Greenspan et al., 1984; Greenspan et al., 1985; Jaeger et al., 1990; Walling, 2000). The association of herpesviruses with oral disease is not a novel issue. EBV is a herpesvirus group member and is not only associated with OHL, but also with Burkitt's lymphoma, nasopharyngeal carcinoma, infectious mononucleosis and Hodgkin's disease (Komatsu et al., 2005, Longnecker, 2000).

The involvement of EBV in the etiology of OHL is establish, but there is not studies established comparisons among presence the EBV before and after the topical treatment for this condition. Also, the presence or not of EBV after the topical treatment of OHL remain to be elucidated. So, the goal of the current study was to identify the presence of not of genomic sequences of Epstein Barr virus type-1 (EBV-1) in samples from the lateral border of the tongue of Brazilian HIV-positive adults patients before and after of the topical treatment with different protocols of the OHL.

2. Subject and methods

The study protocol was approved by the Committee of Bioethics in Research from the Federal University of Minas Gerais (COEP number 367/6).

A randomly clinical trial study evaluated 15 HIV-positives adults patients, four females and 11 males with age ranged from 27 to 47 years. The patients were recruited in the Orestes Diniz Center for Training and Reference in Infectious and Parasitic Diseases (CTR/DIP; Belo Horizonte, Minas Gerais, Brazil) between January 2003 and July 2007. The patients had a total of 23 OHL lesions, seven patients with unilateral OHL and eight with bilateral OHL. Exfoliative cytology was performed in diagnosis of OHL and all samples of

scrapings from the lateral border of the tongue of the patients presented enough material to cytopathological analysis (Moura et al., 2007). The OHL lesions were treated with topical applications of the applications of podophyllin resin (25%) (P) or podophyllin resin (25%) together with acyclovir cream (5%) (PA) or podophyllin resin (25%) together with penciclovir cream (1%) (PP), in accordance to the Moura et al. (2007). The period of the treatment varied from two to 23 weeks and was suspended after the clinical heal of the OHL. The patients were follow-up for 12 months.

Sample collection preparation

Oral swabs were taken from each patient from the right lateral border of the tongue with OHL before of the topical treatment of OHL and immediately after of the clinical heal of the OHL the same place. The swabs were performed with sterile plastic tips and placed immediately in Eppendorf microtubes contain 500 μL of Krebs buffer (NaCl 20%, KCl 2%, $CaCl_2.2H_2O$ 2%, $MgSO_4$, KH_2PO_4, $C_6H_{12}O_6$). The pellet obtained after 10 min of centrifugation at 17 900 g was stored at -20º C until processing. The DNA extraction was carried out as described by Boom et al. (1990).

Polymerase chain reaction

EBV DNA was identified by PCR and primers were directed to conserved regions of the EBV genome encoding Epstein-Barr nuclear antigen 1 (EBNA-1) (Ammatunal et al., 1998; Telenti et al., 1990). The primers used by PCR were, forward primer 5' GTCATCATCATCCGGGTCTC 3' and reward primer 5' TTCGGGTTGGAACCTCCTTG 3' (Ammatuna et al. 1998). The human β-globin gene was amplified in order to assess the adequacy of each specimen (Gall-Troselj et al., 1990). Positive (one case of OHL known positive for EBV) (Fig. 1; line C2) and negative (PCR reagents without DNA) (Fig. 1; line 3) controls were routinely included. The Primers and PCR conditions is presented.

The amplification was performed in a final volume of 25 μl containing genomic DNA, PCR buffer (10 mM $(NH_4)_2SO_4$, 10 mM KCl, 10 mM Tris-Cl pH 8.4, 3.0 mM $MgCl_2$), 0.1 mM deoxynucleosidetriphosphates (Amersham Biosciences, Buckinghamshire, UK), 10 pmol/reaction primers, and Taq DNA polymerase (1 unit/reaction) (Phoneutria, Belo

Horizonte, MG, Brazil). PCR amplification was performed in a thermal cycler (Gene Amp. PCR System 2400) (Eppendorf, Westbury, NY, USA). Samples were subjected to 5 min at 94°C, followed by 40 cycles of amplification at 94°C for 1 min, 56°C for 50s and 72°C for 1 min. The run was terminated by a 7 min elongation step at 72°C. The product of PCR, 269bp, was analyzed in a 6.5% polyacrylamide gel electrophoresis followed by silver stain.

3. Results

The topical treatment was effective in all patients with 100% of the heal of OHL. In all 23 samples of the clinical OHL it was possible the detection of EBV-1 before of the topical treatment. EBV-1-postive in scrapes of the tongue of 11 (73.33%) patients immediately after the clinical heal of the OHL was determined (Fig. 1; lines C4, C5 and C7). After of the topical treatment, three patients (20%) treated with PA and one patient (6.66%) with PP was EBV-1-negative (Fig. 1; lines C6 and C8). Three patients treated with P and EBV-1-positive after treatment had recurrence of OHL a year after the treatment. The patients that received treatment with PA and with PP did not have recurrence a year after the treatment.

4. Discussion

EBV-1 virus DNA was amplified by PCR in tongue scrapes from HIV-positive adults patients before and after the topical treatment for OHL. The use of PCR in oral scrapes suggests a high sensitivity but low specificity for the diagnosis of OHL. Therefore, PCR can be useful in the detection and identification of EBV-1 in oral scrapes, and as an adjunct in the diagnosis of OHL (Cubie et al., 1991; Komatsu et al., 2005; Mabruk et al., 1994; Mabruk et al., 2000). The identification of EBV-1 by PCR has been used in many studies to investigate questions concerning the biology of infection and the nature of viral persistence (Cubie et al., 1991; Komatsu et al., 2005; Mabruk et al., 1994; Mabruk et al., 1995; Mabruk et al., 2000; Scully et al., 1998; Walling et al., 2001; Walling et al., 2004a; Walling et al., 2004b; Webster-Cyriaque et al., 2000).

Two EBV strains, type-1 (EBV-1) and type-2 (EBV-2), have been identified and the results of many serologic studies have demonstrated that the distribution of EBV-1 versus EBV-2 has characteristically different frequencies in distinct geographic locations (Gratama &

Ernberg, 1995; Khanim et al., 1996). EBV-1 is prevalent in Western countries, whereas EBV-2 is prevalent in Africa, Oceania (New Guinea), and in HIV-1 seropositive patients, even in the Western countries. In addition, EBV-1 is more efficient to transform and immortalize infected B lymphocytes in vitro; whereas, EBV-2 is reported to be a weaker transformer (Cohen 2000; Khanim et al. 1996).

As in the findings of Milagres et al (2007), this study verified that EBV-1 DNA can be found in all of OHL before the topical treatment. However, Komatsu et al. (2005) found the absence of amplification of the two DNA samples to EBV-1 from patients with clinical evidence of OHL and Scully *et al.* (1998) also did not observe EBV-1 amplification in all samples of OHL. Considering the fact that the differential diagnosis of a white patch lesion on the lateral border of the tongue is extensive, atypical forms of OHL may mimic some of these conditions, and may contribute to the underreporting of this lesion (Komatsu et al., 2005). The possibility of inaccurate diagnosis must be considered because of other white oral lesions that can mimic OHL such as lichen planus, white sponge nevus, idiopathic and tobacco-associated leukoplakia, galvanic lesions and frictional keratosis or hyperplastic candidiasis (Komatsu et al., 2005).

Furthermore, the data presented here reveal that the presence of EBV-1 was in the tongue scrapes of 73.33% the patients even after the clinical heal of the OHL with topical treatment. These data suggest that normal oral epithelium supports persistent EBV-1 infection in HIV-positive patients and that productive EBV-1 replication is necessary but not sufficient for the pathogenesis of OHL (Walling et al., 2001). There are not studies established comparisons among presence the EBV-1 after the topical treatment of OHL, but the current study contributes for a possible role of the EBV-1 in recurrence in this lesion or it is questioned if OHL might represent a transient and isolated EBV infection of surface epithelium without serious or profound prognosis implications (Komatsu et al., 2005). According to Scully et al. (1998) the detection of EBV-1 in oral scrapes cannot be regarded as reliable or specific for OHL. It is known that EBV is not only associated with this lesion, but also with nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's disease, mononucleosis

and with oral squamous cell carcinoma. Thus, the detection of EBV could represent other infections associated with this virus previously presented by the individuals. Scully et al. (1998), Walling et al. (2001) and Mabruk et al. (1994) demonstred that EBV-1 DNA can be found not only in tongue scrapes of HIV-positive patients with or without OHL but also in healthy volunteers. EBV-1 appeared to enter the tongue from the blood reservoir of infection and, possibly, from exogenous sources as well, suggesting EBV-1 entry into tongue epithelial tissue as a cell-associated latent infection (Walling et al. 2004a). The persistence and transition of EBV-1 as a dynamic interaction between the blood and epithelial reservoirs of EBV-1 infection and suggest a role for entry, persistence, and reactivation of oral epithelial EBV-1 in the pathogenesis of OHL (Walling et al. 2004a).

The presence of the EBV-1 in the saliva is another important fact to be considered. EBV-1 is localized only in the superficial squamous layers, favoring the theory of lingual infection by saliva rather than by reactivation of latent lingual infection (Brandwein et al., 1996). This can be explained by the fact that PCR has the inconvenient of not determining if the amplified EBV-1 was present in the cell scrapes or if it was present in saliva. According to Wolf et al. (1984) the virus tropism for ephitelial cells of the nasopharynx, and also for salivary glands, suggests that these could be persistant sites of the virus, with viral particles of these places liberated to be shed by the saliva. After the primary EBV-1 infection, the virus is episodically recoverable from the saliva, suggesting that healthy, normal individuals regularly reactivate and shed the virus (Komatsu et al., 2005; Mabruk et al., 1994; Walling et al., 2004a).

It is known that PCR is highly sensitive, amplifying small amounts of the target sequence. The results of the current study showed the presence of EBV-1 in the tongue scrapes of 73.33% the patients even after the clinical heal of the OHL one week after the topical treatment end. The virus expression can precede the appearance of OHL, caused by re-infection of the epithelium by EBV in a quantity detectable by PCR. This re-infection, however, would not necessarily evolve to a clinical manifestation, liable to happen only if the immune response of the host in controlling the viral replication is suppressed (Dias et al.

2000; 2001; Mao & Smith, 1993; Scully et al., 1998). Factors that can contribute to the success of the establishment of EBV in the oral ephitelium are the immune system dysfunction of the host and the absence of Langerhans' cells observed in OHL lesions (Greenspan et al., 1984; Greenspan et al, 1985; Triantos et al, 1997; Walling et al., 2004b). However, it is not clear. More studies need to be done in order to answer this question.

OHL in patients with AIDS might represent a reactivation of latent lingual infection accompanied by a dramatic increase in viral copy number in the more mature, superficial, squamous cells (Brandwein et al., 1996). Webster-Cyriaque et al. (2000) verified that the immunodeficiency-associated OHL is the pathologic manifestation of permissive EBV infection, with concurrent expression of viral proteins characteristic of latent infection within OHL. The EBV expression transforming proteins within the infected OHL tissue create an optimal environment for viral replication, contribute to OHL development, and induce many of the histologic features of OHL, such as acanthosis and hyperproliferation. Interestingly, the expression of these proteins is dependent on viral replication within OHL (Hille et al., 2002; Webster-Cyriaque et al., 2000). This finding may indicate that could help supports possible explain for the presence of EBV in 73.33% tongue scrapes of the patients even after the clinical heal of the OHL with the topical treatment and the OHL recurrence in the patient treated with P a year after the treatment. Absence of the EBV after the clinical heal of OHL with the topical treatment with antiviral (PA and PP) explain that could be possible that the topical antiviral could enhance the activity of P topical treatment, thus improving the effectiveness of the clinical response in the treatment of OHL (Moura et al., 2007). The acyclovir (5%) or penciclovir (1%) creams together with podophyllin resin (25%) decreased the possibility of recurrence of the OHL.

The complete identification of EBV that infects HIV-positive patients is important to elucidate the elements of EBV infection, including virus transmission, compartmentalization, persistence, and the development of pathology, all of which are poorly understood. These analyses reveal that the nature of EBV infection can be very dynamic. The investigation of the EBV infection in the keratinocytes of the tongue scrapes after the topical treatment of the

OHL in Brazilian HIV-infected adults patients, will determine the real contribution of OHL and EBV infection as markers of immunodeficiency and it would be an alternative tool in patients' clinical follow up. The current study demonstrated the persistence of EBV in HIV-seropositive subjects with and without OHL before and after the topical treatment, respectively. The elevated prevalence of EBV-1 DNA in the lateral border of the tongue of patients without OHL after of the topical treatment supports a role of this virus in recurrence of the OHL.

References

1. Ammatuna P, Capone F, Giambelluca D, Pizzo I, D'Alia G, Margiotta V. Detection of Epstein-Barr virus (EBV) DNA and antigens in oral mucosa of renal transplanta patientswithout clinical evidence of oral hairy leucoplakia (OHL). J Oral Med 1998;27:420–427.

2. Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, Van der Noordaa J. Rapid and simple method for purification of nucleic acids. J Clin Microbiol. 1990;28(3):495-503.

3. Brandwein M, Nuovo G, Ramer M, Orlowski W, Miller L. Epstein-Barr virus reactivation in hairy leukoplakia. Mod Pathol 1996;9(3):298-303.

4. Cohen JI. Epstein-Barr virus infection. N Engl J Med 2000;343:481-492.

5. Cubie HA, Felix DH, Southam JC, Wray D. Aplication of molecular techniques in the rapid diagnosis of EBV-associated oral hairy leukoplakia. J Oral Pathol Med 1991;20:271-274.

6. Dias EP, Rocha ML, Silva Júnior A, Spyrides KS, Ferreira SM, Polignano GA, et al. Oral hairy leukoplakia: histopathologic and cytopathologic features of a subclinical phase. Am J Clin Pathol 2000;11:394-400.

7. Dias EP, Spyrides KS, Silva Júnior A, Rocha ML, Fonseca EC. Oral hairy leukoplakia: histopathologic features of subclinical stage. Pesq Odontol Bras 2001;15:104-111.

8. Epstein M, Achong B, Barr Y. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet 1964;15:702-703.

9. Epstein JB, Fatahzadeh M, Matisic J, Anderson G. Exfoliative cytology and electron microscopy in the diagnosis of hairy leukoplakia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995;79:564-569.

10. Fraga-Fernandes J, Vicandi-Plaza B. Diagnosis of hairy leukoplakia by cytologic methods. Am J Clinic Pathol 1992;97:262-266.

11. Friedman-Kien AE. Viral origin of hairy leukoplakia. Lancet 1986;2:694-695.

12. Gall-Troselj K, Mravak-Stipetic M, Jurak I, Ragland WL, Pavellc J. Helicobacter pylori colonization of tongue mucosa – increased incidence in atrophic glossitis and burning mouth syndrome (BMS). J Oral Pathol Med 2001;30:560–563.

13. Gratama JW, Ernberg I. Molecular epidemiology of Epstein-Barr virus infection. Adv Cancer Res 1995;67:197-253.

14. Greenspan D, Greenspan JS, Conant MA, Petersen V, Silverman JS, Souza Y. Oral hairy leukoplakia in male homosexuals: evidence of association with both papilloma virus and a herpes-group virus. Lancet 1984;2:831-834.

15. Greenspan JS, Greenspan D, Lennette ET, Abrams DJ, Conant MA, Petersen V, et al. Replication of Epstein-Barr virus within the epithelial cells of oral hairy leukoplakia, an AIDS associated lesions. N Engl J Med 1985;313:1564-1571.

16. Gulley ML. Molecular diagnosis of Epstein-Barr virus – Related diseases. J Mol Diag 2001;3:1-10.

17. Hille JJ, Webster-Cyriaque J, Palefski JM, Raab-Traub N. Mechanisms of expression of HHV8, EBV and HPV in selected HIV-associated oral lesions. Viral infections – mechanisms of expression. Oral Dis 2002;8(suppl.2):161-168.

18. Husak R, Garbe C, Orfanos CE. Oral hairy leukoplakia in 71 HIV-seropositive patients: clinical symptoms, relation to immunologic status, and prognostic significance. J Am Acad Dermatol 1996;35(6):928-934.

19. International Agency for Research on Cancer. Epstein-Barr virus and Kaposi's sarcoma herpesvirus human herpesvirus-8. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (vol. 70). Lyon, France : WHO, 1997.

20. Jaeger MMM, Jaeger RG, Araújo NS. Leucoplasia pilosa. Estudo clínico, histopatológico, imuno-histoquímico e ultra-estrutural. An Bras Dermatol 1990;65(6):298-302.

21. Khanim F, Yao QY, Niedobitek G, Sihota S, Rickinson AB, Young LS. Analysis of Epstein-Barr virus gene polymorphisms in normal donors and in virus-associated tumors from different geographic locations. Blood 1996;88:3491-3501.

22. Koloktronis A, Kioses A, Antoniades D, Mandraveli K, Doutos I, Papanyoyou P. Immunologic status in individuals infected with HIV oral candidiasis and hairy leukoplakia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1994;78:41-6.

23. Komatsu TL, Rivero ERC, Magalhães MHC, Nunes FD. Vírus Epstein-Barr em esfregaço de leucoplasia pilosa oral: identificação por PCR. Braz Oral Res 2005;19(4):317-321.

24. Kratochvil FJ, Riordan P, Auclair PL, Huber MA, Kragel PJ. Diagnosis of oral hairy leukoplakia by ultrastructural examination of exfoliative cytologic specimens. Oral Surg Oral Med Oral Pathol 1990;70(5):613-618.

25. Longnecker R. Epstein-Barr virus latency: LMP2, a regulator or means for Epstein-Barr virus persistence? Adv Cancer Res 2000;79:175-200.

26. Mabruk MJEMF, Antonio M, Flint SR, Coleman DC, Toner M, Leader M, et al. A simple and rapid technique for the detection of Epstein-Barr virus DNA in HIV-associated oral hairy leukoplakia biopsies. J Oral Pathol Med 2000;29:118-122.

27. Mabruk MJEMF, Antonio M, Flint SR, Coleman DC, Toner M, Kay E, Leader M, et al. Detection of Epstein-Barr virus DNA in tongue tissues from AIDS autopsies without clinical evidence of oral hairy leukoplakia. J Oral Pathol Med 1995;24(3):109-112.

28. Mabruk MJEMF, Flint SR, Toner M, Balluz I, Coleman D, Sullivan D, et al. In situ hybridization and the polymerase chain reaction (PCR) in the analysis of biopsies and exfoliative cytology specimens for definitive diagnosis of oral hairy leukoplakia (OHL). J Oral Pathol Med 1994;23:302-308.

29. Mao EJ, Smith CJ. Detection of Epstein-Barr virus (EBV) DNA by the polymerase chain reaction (PCR) in oral smears from healthy individuals and patients with squamous cell carcinoma. J Oral Pathol Med 1993;22:12-7.

30. Migliorati CA, Jones AC, Baughman PA. Use of exfoliative cytology in the diagnosis of oral hairy leukoplakia. Oral Surg Oral Med Oral Pathol 1993;76:704-710.

31. Milagres A, Dias EP, Tavares DS, Cavalcante RM, Dantas VA, Oliveira SP, Leite JPG. Prevalence of oral hairy leukoplakia and epithelial infection by Epstein- Barr virus in

pregnant women and diabetes mellitus patients: cytopathologic and molecular study. Mem Inst Oswaldo Cruz 2007;102(2):159-164.

32. Moura MDG, Guimarães TRM, Fonseca LMS; Pordeus IA, Mesquita RM. A random clinical trial study to assess the efficiency of topical applications of podophyllin resin (25%) versus podophyllin resin (25%) together with acyclovir cream (5%) in the treatment of oral hairy leukoplakia. Oral Surg Oral Pathol Oral Med Oral Radiol Endod 2007;103:64-71.

33. Robaina TF, Valladares CP, Tavares DS, Napolitano WC, Silva LE, Dias EP, et al. Polymerase chain reaction genotyping of Epstein-Barr virus in scraping samples of the tongue lateral border in HIV-1 seropositive patients. Mem Inst Oswaldo Cruz 2008;103(4):326-331.

34. Scully C, Porter SR, Di Alberti L, Jalal M, Maitland N. Detection of Epstein-Barr virus in oral scrapes in HIV infection, in hairy leukoplakia, and in healthy non-HIV infected people. J Oral Pathol Med 1998;27(10):480-2.

35. Telenti A, Marshall WF, Smith TF. Detection of Epstein-Barr virus by polymerase chain reaction. J Clin Microbiol. 1990;28(10):2187-90.

36. Triantos D, Porter SR, Scully C, Teo CG. Hairy Leukoplakia: clinicopathologic features, pathogenesis, diagnostic, and clinical significance. Clin Infect Dis 1997;25:1392-1396.

37. Walling DM, Etienne W, Ray AJ, Flaitz CM, Nichols CM. Persistence and transition of Epstein-Barr virus genotypes in the pathogenesis of oral hairy leukoplakia. J Infect Dis 2004a;190(2):387-95.

38. Walling DM, Flaitz CM, Hosein FG, Montes-Walters M, Nichols CM. Effect of Epstein-Barr virus replication on Langerhans cells in pathogenesis of oral hairy leukoplakia. J Infect Dis 2004b;189(9):1656-63.

39. Walling DM, Flaitz CM, Nichols CM. Epstein-Barr virus replication in oral hairy leukoplakia: response, persistence, and resistance to treatment with valacyclovir.J Infect Dis 2003;188(6):883-90.

40. Walling DM, Flaitz CM, Nichols CM, Hudnall SD, Adler-Storthz K. Persistent productive Epstein-Barr virus replication in normal epithelial cells in vivo. J Infect Dis 2001;184(12):1499-507.

41. Walling DM. Oral hairy leukoplakia: an Epstein-Barr virus-associated disease of patients with HIV. Res Initiat Treat Action 2000;6(4):10-15.

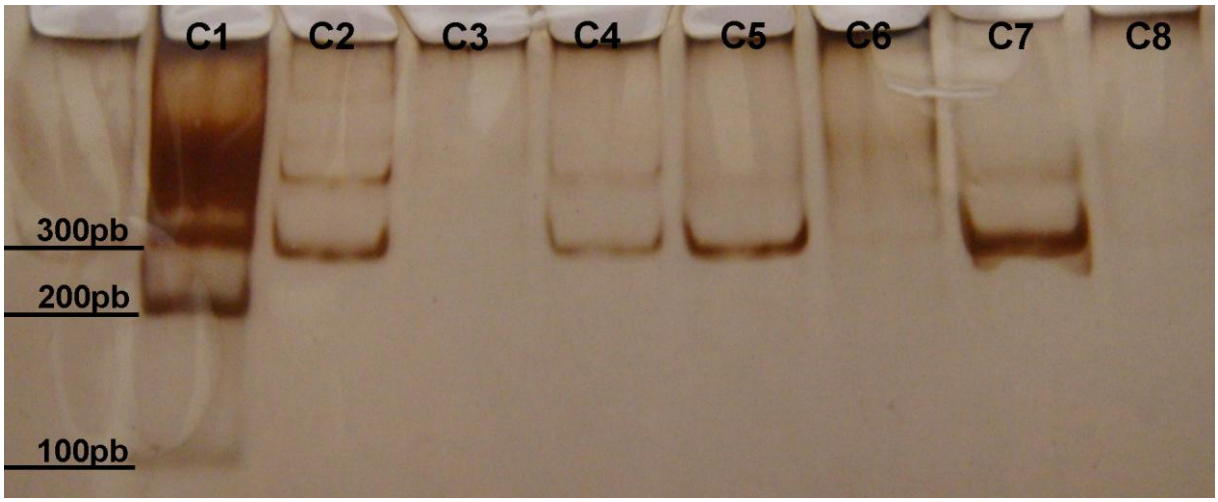
42. Webster-Cyriaque J, Middeldorp J, Raab-Traub N. Hairy Leukoplakia: an unusual combination of transforming and permissive Epstein-Barr virus infections. Journal of Virology 2000;74(16):7610–7618.

43. Wolf H, Haus M, Wilmes E. Persistence of Epstein-Barr virus in parotid gland. J Virol 1984;51:795-8.

44. Young LS, Rickinson A. Epstein-Barr virus: 40 years on. Nat Rev Cancer 2004;4:757-768.

Figure 1: Electrophoretic pattern for EBV-1 run in a 6.5% polyacrylamide gel from representative samples analyzed by PCR-based methods. C1: 100 bp ladder; C2: positive control; C3: negative control. C4, C5 and C7: samples positives to EBV-1 genome (band of 269 bp); C6, C8: sample negative to EBV-1 genome.

bp = bases pares



ARTIGO 3

Development of dentists' knowledge of HIV/AIDS questionnaire

(Submetido- Journal International Epidemiology)

Title: Development of dentists' knowledge of HIV/AIDS questionnaire

Subtitle: Developing a questionnaire for dentists' knowledge of HIV/AIDS in the Argentine Spanish language

Key Words: HIV infection; dental care; acquired immunodeficiency syndrome; promotion of health; questionnaires

Abstract

Background: To present the development of a questionnaire on dentists' knowledge of HIV/AIDS (DK-HIV-Q) in the Argentine Spanish language, in order to present 1) translation and back translation, 2) analysis of content validity, and 3) assessment of test-retest reliability.

Methods: Translation and back translation were performed by four translators. The analysis of content validity consisted of two phases: (1) cross-cultural adaptation of the items between translation and back translation performed by two dentists and (2) evaluation by experts performed by three dentists. The Argentine Spanish version was tested in two pilot studies with a convenience sample of 20 Argentine dentists. The test-retest reliability of the instrument was assessed by Intraclass Correlation Coefficient (ICC) calculation using the data from a stratified random sample of 25 Argentine dentists.

Results: There were no significant discrepancies between the translation and the back translation. The experts completely agreed with the conceptual relevance of the general domains (dentists' knowledge of HIV/AIDS) and with the four specific domains: (1) declarative knowledge of the transmission of HIV/AIDS, (2) declarative knowledge of the oral manifestations of HIV/AIDS, (3) procedural knowledge of dentists' practices, and (4) procedural knowledge of infection control measures. Test-retest reliability revealed the stability of the answers over a short period of time with satisfactory reproducibility (ICC = 0.95).

Conclusions: The concerns identified by the DK-HIV-Q proved to be satisfactorily conceptual and semantic reproducible, adequate reliability and useful as an initial indicator for a subsequent study of construct validity to be carried out.

Introduction

Dentists play an important role in HIV/AIDS care. Nevertheless, the stigma associated with HIV/AIDS continues to be a barrier to care for many HIV-positive patients and for some dentists. HIV-positive patients are reluctant to disclose their status to their dentists because of fear of refusal of treatment. A similar reluctance is found among dentists that include problems with the implementation of appropriate infection control precautions; and a fear of illness, contagion, and death.[1-3]

More education is needed in dentistry schools and postgraduate and continuing education programs to enhance dentists' knowledge.[4-6] Further researches, include the elaboration of news instruments, are required to clarify the role of continuing professional education in improving the knowledge of dentists regarding HIV/AIDS. The role of dentists in meeting the health care needs of patients infected with HIV has been well described through questionnaires.[7-9] Nevertheless, there are no questionnaires with construct validity about HIV/AIDS for dentist. There is only one study with questionnaire with construct validity about HIV/AIDS, but for patients about what do they think of yours dentists.[10]

Considering the importance of developing methods to enhance the relevant knowledge of dentists worldwide regarding HIV/AIDS in the primary health care setting and considering that our searches showed that there are no studies of questionnaires with construct validity about HIV/AIDS for dentists, the aim of this study was to present the development of a new questionnaire to assess dentists' knowledge of HIV/AIDS (DK-HIV-Q) in the Argentine Spanish language, in order to present 1) translation and back translation, 2) analysis of content validity, and 3) assessment of test-retest reliability; to, in future, investigate the construct validity of this instrument.

Methods

Development and description of the DK-HIV-Q

The initial format of the questionnaire was based on the study of Oliveira et al.[11] that evaluated a group of dental students with a questionnaire about attitudes and knowledge of HIV/AIDS.

Initially it was intended as an instrument for identification of the general domain of dentists' knowledge of HIV/AIDS, with 33 items and with four specific domains: (1) declarative knowledge of the transmission of HIV/AIDS, (2) declarative knowledge of oral manifestations of HIV/AIDS, (3) procedural knowledge of proper dental practice, and (4) procedural knowledge of infection control measures. The items were grouped theoretically in relation to each domain and are shown in Figure1.

The score on each item of the test was used in this DK-HIV-Q, with the following options: 'Yes' = 1 and 'No' = 0.

The analysis of content validity consisted of two phases: (1) cross-cultural adaptation of the items between translation and back translation and (2) evaluation of the DK-HIV-Q by experts.

Translation and back translation of the DK-HIV-Q

Because the DK-HIV-Q was developed by a Brazilian researcher, the questionnaire needed to undergo translation, cross-cultural adaptation of the items between translation and back translation and back translation in order to measure dentists' knowledge of HIV/AIDS in the Argentine Spanish language.

The translation from the original Brazilian Portuguese-language instrument into Argentine Spanish was performed independently by two bilingual translators, an Argentine Spanish translator fluent in the Brazilian Portuguese language and a native Brazilian Portuguese-speaker fluent in Argentine Spanish, who both have experience in health questionnaire translation.

The translated version was analyzed by a group of two bilingual dentists. Special attention was given to the meaning of the words in the different languages (Argentine Spanish and Brazilian Portuguese) in order to obtain similar effects from respondents of different cultures. An effort was made to identify possible difficulties in understanding the questionnaire. A synthesis version was developed as a result of this process.

This synthesis version was then translated back into Brazilian Portuguese by bilingual translator whose native language was Brazilian Portuguese. This translator had no access to the original instrument.

A subsequent comparison between the original version and the backtranslated version was performed by other translator who were not previously involved in the study and who was fluent in Brazilian Portuguese and whose native language was Argentine Spanish.

Evaluation of the DK-HIV-Q by experts

The combined assessment of conceptual, item, semantic, operational, and measurement equivalence was performed by a group of specialists composed of three bilingual dentists, an epidemiology dentist, a general clinical dentist, and an oral pathology specialist dentist in the service of patients with HIV/AIDS, and with no prior knowledge of the study. The aim of this step was to achieve a "similar effect" on respondents who speak two languages.

An assessment of the DK-HIV-Q was performed between items in order to assess the perspective of referential meaning of the constituent terms, words and general meaning of each item. This assessment too was carried out with regard to the behavior of the instrument and the possibility of comparisons to studies conducted in different cultures.

Application of the DK-HIV-Q

Two preliminary pilot studies of the DK-HIV-Q were undertaken with two convenience samples, each of 10 Argentine dentists of both gender and a range of ages,

selected from the School of Dentistry of the State of Córdoba, Argentina, who filled out the Argentine Spanish version of the instrument and were instructed to take note of all unclear words. It was decided to administer the instrument as an interview in order to reduce unreturned questionnaires.

First, the resulting draft of the Argentine version of the DK-HIV-Q was pilot-tested on a convenience sample of 10 Argentine dentists to detect any doubts regarding the meaning of the items or the instructions on how to fill out the questionnaire. After some adjustments to improve the understanding, the DK-HIV-Q was tested a second time in another pilot study with 10 other Argentine dentists.

Reliability

Test-retest reliability was applied twice in another sample of 25 dentists that represented a stratified random 10% sample of the study population. A standard error (SE) of 1% and a 99% confidence interval level were calculated for the study (n=250 samples) with an expected prevalence of 50%.[12] The same dentists were interviewed a second time by the same investigator one week following the first interview.

Only subjects who were treating patients at the time of the study were included in the study. A list of all registered dentists was obtained from the School of Dentistry. Written informed consent was also obtained from all participants. Prior to the interviews, the study received approval by the Committee of Bioethics in Research from the Federal University of Minas Gerais (report number 545/07-COEP).

The test-retest reliability was determined through the calculation of the Intraclass Correlation Coefficient (ICC) with a two-way random effects model for the DK-HIV-Q score. Gender was evenly distributed. Estimations of 95% confidence intervals were made. The ICC was measured according to the following values: ≤0.40, weak correlation; 0.41–0.60,

moderate correlation; 0.61–0.80, good correlation; and 0.81–1.00, excellent correlation.[13-14].

Results

Development and description of the DK-HIV-Q

In items 1 to 4, representing the first domain, the objective was to approach the main methods of transmission of HIV. Items 5 to 15, representing the second domain, list the oral manifestations of HIV/AIDS frequently seen in HIV-positive patients, except for lichen planus, which is present to evaluate the level of knowledge of the dentist. Items 16 to 26 were grouped into the third domain, assessing the procedural knowledge of general dental practices for the service of HIV-positive patients. Items 27 to 33 were grouped into the fourth domain, related to the procedural knowledge of basic infection control frequently used by dentists. The knowledge evaluated was mainly related to conduct in the use of equipment for personal protection and of various surfaces.

Translation and back translation of the DK-HIV-Q

With the translated version in hand, the DK-HIV-Q was compared to the original version by two dentists. They were guided by the information that all words should express the choice of terms often used by dentists. In the back translation, synonyms were considered to have equivalent meanings. Those words that were not synonymous were adapted to keep the same meaning of the original version of the DK-HIV-Q. In addition, even if the words in Spanish selected by the researchers did not have exactly the same meaning as the original, they certainly belonged to the same group of synonyms and had some significance according to Argentine culture. These two versions proved nearly identical.

Evaluation of the DK-HIV-Q by experts

During this stage, the experts agreed 100% with the conceptual relevance of the general and specific domains. The experts too stated that the concept of the dentists'

knowledge of HIV/AIDS used for development of the new instrument was pertinent to Argentine culture. Considering the referential meaning, 93.9% of the 33 items exhibited "complete meaning agreement." That is, the general meaning remained unaltered in 93.9% of the pairs of statements. The interviewees reported that they enjoyed answering the questions and considered the research very important. However, they made a few suggestions for replacing words and expressions. The two replacements suggested were made in two different domains. The first adjustment was in the instructions of the second domain, changing what was previously *¿Cuáles son las manifestaciones bucales que se relacionan con los portadores de HIV/SIDA?* to include the following phrase: *Marque con una "X" (puede ser cualquier número de respuestas) aquellos que usted considera.* The final, corrected instruction was: *¿Cuáles son las manifestaciones bucales que se relacionan con los portadores de HIV/SIDA? Marque con una "X" (puede ser cualquier número de respuestas) aquellas que usted considere.* Another adjustment was made to the fourth domain instruction, which previously was: *Cuál es la combinación de medios de control de infección usted adopta en su práctica odontológica.* Interviewees asked for exclusion of the words *Cuál es la combinación de*, and the final, corrected statement read: *Qué medios de control de infección usted adopta en su práctica odontológica* (Fig 1).

Application of the DK-HIV-Q

During the first application of the preliminary pilot of the DK-HIV-Q, six dentists mentioned difficulties in filling out the questionnaire because of the format. However, none of them reported having any trouble understanding the document. Modifications in the format of the questionnaire were made according to the comments made by the dentists, in order to clarify the content of the questionnaire and to facilitate comprehension. Thereafter, a new version, considered the final version, was prepared by the investigators after adjusting the format of the presentation the DK-HIV-Q. After the second application, there were no

difficulties in filling out the questionnaire or understanding the items. All 20 dentists answered the DK-HIV-Q in these two pilot studies, because the researcher was present during the process of filling out the instrument.

Reliability

All of the volunteers who participated in assessing the test-retest reliability of the DK-HIV-Q filled out the questionnaire correctly and did not demonstrate any difficulties in doing so, because they had been instructed previously on how to take the questionnaire. The dentists spent about 10 minutes to fill the questionnaires out. All questionnaires were correctly filled out, and no difficulties were reported. The value obtained during the test-retest reliability analysis was assessed using the ICC, which was 0.95, revealing satisfactory reproducibility.

The final version of the DK-HIV-Q is presented in Figure 1.

Discussion

This article is the first study of development of a questionnaire assessing dentists' knowledge of HIV/AIDS, to in future investigate the construct validity of this instrument. It is important, in the initial construction of the instrument in question, to carry out content validity assessment in order for subsequent studies to demonstrate the validity of constructs. Instruments without proper validation may generate significant errors that interfere with scientific progress.[15]

The use of a self-completed questionnaire is preferable in population studies due to its lower cost and the ability to assess a wider range of dentists.[15] Dentists have shown a need and desire for greater awareness and knowledge about HIV/AIDS.[5,16-17] It is obviously necessary to provide dentists with important information regarding the transmission and oral manifestations of HIV, infection control measures, and proper practice, everything related to the knowledge that dentists must have about HIV/AIDS.[18-20] A division into four domains

was based on the objective of facilitating the construction of the DK-HIV-Q to include these important items related to the knowledge of dentists about HIV/AIDS.

The initial declarative knowledge in the first and second domains consisted of an understanding of transmission and recognition of the oral manifestations of HIV/AIDS, presumably formed in the process of dentistry schooling and postgraduate training. However, with repeated experience, routines for performing specific tasks can be set with the triggering of procedural learning mechanisms, showed in the domains 3 and 4. Declarative knowledge is transformed into qualitatively distinct procedural knowledge, and subsequently a gradual automation process takes place. To that end, the DK-HIV-Q consisting of 33 items was elaborated including items for each domain. These four domains can be related to a general domain.

Rigorous cross-cultural translation and evaluation of the DK-HIV-Q using emerging international guidelines and standardized analytic methods prior to widespread release were integral components of its development. A noteworthy benefit of translation work prior to an instrument's standardization and dissemination is the identification and adaptation of problem items, ambiguous phrases, idiomatic expressions, and conceptual unequivalencies.[21-22]

Regarding assessment of the semantic aspects, it was concluded that the pairs of translation/back translation statements achieved adequate equivalence vis-à-vis the original questionnaire. The involvement of the group of specialists during the stage of content validity assessment should be emphasized, as they contributed with reflection and discussions, thereby promoting suitable adjustments in the developed synthesis version.[21-22]

Test-retest reliability was confirmed by the ICC (0.95). The analysis of test-retest reliability suggests adequate stability for the questionnaire. The seven-day interval between interviews was important in decreasing the probability of alterations in the reports from each dentist. It is recommended that the interval between measurements be long enough to reduce

the effects of memory and short enough to diminish the likelihood of alterations in the reports. Although the definition of this interval is arbitrary, a period of 2 to 14 days is considered adequate.[23]

At present, this self-completed questionnaire is relevant, considering the importance of cross-cultural instruments to assess dentists' knowledge of HIV/AIDS. With the increasing focus on HIV/AIDS, it is important for dentists from different cultures to understand the value of treatment for patients with HIV/AIDS and important to have a common instrument for assessing multicultural labor forces.[24] The concerns identified by the DK-HIV-Q were largely consistent with dentists' knowledge about HIV/AIDS and proved to be satisfactorily conceptual and semantic reproducible, adequate reliability and useful as an initial indicator for a subsequent study of construct validity to be carried out.

References

1. Bennett ME, Weyant RJ, Wallisch JM, et al. Dentists' attitudes toward the treatment of HIV-positive patients. *J Am Dent Assoc* 1995; 126(4): 509-14.
2. Reis C, Heisler M, Amowitz LL, et al. Discriminatory attitudes and practices by health workers toward patients with HIV/AIDS in Nigeria. *PLoS Med* 2005;2(8):e246.
3. Ross MW, Hunter CE. Dimensions, content and validation of the fear of AIDS schedule in health professionals. *AIDS Care* 1991;3(2):175-80.
4. Civaner M, Arda B. Can "presumed consent" justify the duty to treat infectious diseases? An analysis. *BMC Infect Dis* 2008;8(29):1-11.
5. Mulligan R, Seirawan H, Galligan J, et al. The effect of an HIV/AIDS educational program on the knowledge, attitudes, and behaviors of dental professionals. *J Dent Educ* 2006;70(8):857-68.
6. Sadowsky D, Kunzel C. Measuring dentists' willingness to treat HIV-positive patients. *J Am Dent Assoc* 1994;125:705-10.
7. Gachigo JN, Naidoo S. HIV/AIDS: the knowledge, attitudes and behaviour of dentists in Nairobi, Kenya. *SADJ* 2001;56(12):587-91.
8. Kitaura H, Adachi N, Kobayashi K, et al. Knowledge and attitudes of Japanese dental health care workers towards HIV related disease. *J Dent* 1997;25(3–4): 279-283.
9. Senna MI, Guimarães MD, Pordeus IA. Factors associated with dentists' willingness to treat HIV/AIDS patients in the National Health System in Belo Horizonte, Minas Gerais, Brazil. *Cad Saude Publica* 2005;21(1):217-25.
10. Mussard J, Ashley FA, Newton JT, et al. What do you think of your dentist? A dental practice assessment questionnaire. *J Eval Clin Pract* 2008;14(2):181-4.
11. Oliveira ER, Narendran S, Falcão A. Brazilian dental student's knowledge and attitudes towards HIV infection. *AIDS Care* 2002;14(4):569-576.

12. Kirkwood BR. Essentials of medical statistics. Oxford: Blackwell Science, 1996:27-36.
13. Bartko JJ. The intraclass correlation coefficient as a measure of reliability. *Psychol Report* 1966;19(1): 3-11.
14. Cronbach LJ. Coefficient alpha and the internal structure of tests. *Psychometrika* 1951;16: 297–334.
15. Patel S, Weiss E, Chhabra R, et al. The events in care screening questionnaire (ECSQ): a new tool to identify needs and concerns of people with HIV/AIDS. *AIDS Patient Care & STDs* 2008;22(5):381-93.
16. Kunzel C, Sadowsky D. Comparing dentists' attitudes and knowledge concerning AIDS: differences and similarities by locale. *J Am Dent Assoc* 1991;122(3):55-61.
17. McCartan BE, Samaranayake LP. Oral care of HIV infected patients: the knowledge and attitudes of Irish dentists. *J Ir Dent Assoc* 1991;37(2):41-43.
18. Bishop GD, Oh HM, Swee HY. Attitudes and beliefs of Singapore health care professionals concerning HIV/AIDS. *Singapore Med J* 2000;41(2):55-63.
19. Comfort AO, Vandana M, Cuttress T, et al. Attitude/practices of oral healthcare provider to management of HIV/AIDS patients in the Pacific. *Pac Health Dialog* 2004;11(1):26-30.
20. Gerbert B. AIDS and infection control in dental practice: dentists' attitudes, knowledge, and behavior. *J Am Dent Assoc* 1987;114(3):311-4.
21. Brislin RW. Back-translation for cross-cultural research. *J Cross-Cultural Psychol* 1970;1(3):185–216.
22. Bullinger M, Anderson R, Cella D, et al. Developing and evaluating cross-cultural instruments from minimum requirements to optimal models. *Qual Life Res* 1993;2:451–459.
23. Streiner DL, Norman GR. Health measurement scales: a practical guide to their development and use. Oxford: Oxfor University Press, 2003:85-97.

24. Crossley ML. An investigation of dentists knowledge, attitudes and practices towards HIV+ and patients with other bloodborne viruses in South Cheshire, UK. *Br Dent J* 2004;196(12):749-54.

Figure 1: Dentists' knowledge of HIV/AIDS questionnaire (DK-HIV-Q) with 33 items

DK-HIV-Q	Domains
¿Por qué medios puede ser transmitido el HIV?	
1. Sangre	1
2. Saliva	
3. Secreción vaginal	
4. Semen	
¿Cuáles son las manifestaciones bucales que se relacionan con los portadores de HIV/SIDA? Marque con una "X" (puede ser cualquier número de respuestas) aquellas que usted considere.	2
5. Sarcoma de Kaposi	
6. Leucoplasia Vellosa	
7. Candidiasis	
8. Periodontitis Ulcero Necrotizante	
9. Gingivitis Ulcero Necrotizante Aguda (GUNA)	
10. Eritema gingival lineal	
11. Linfoma no Hodgkin	
12. Herpes Zoster	
13. Aumento de parótida	
14. Líquen Plano	
15. Condiloma acuminado	
Lea el siguiente cuadro. En el mismo se realizan afirmaciones. Marque con un X la opción con la que se sienta más identificado. No deje ninguna sin marcar.	3
16. Usted se considera apto para atender a un portador del HIV/SIDA.	
17. Usted ya ha atendido algún paciente con HIV/SIDA.	
18. Usted sabe cómo proceder durante un accidente punzo cortante.	
19. Después de un accidente punzo cortante, usted se haría un análisis para verificar la posibilidad de infección con HIV.	
20. Sus conocimientos y aplicaciones sobre el control de la infección son adecuados para prevenir la infección cruzada.	
21. Usted necesita recibir un entrenamiento especial sobre cuidados y manejos del paciente portador de HIV/SIDA para atender a los mismos.	
22. Las personas portadoras de HIV/SIDA deben ser tratadas en ambiente aislado o en lugares especializados.	
23. Usted conoce la medicación más comúnmente usada por los pacientes portadores de HIV/SIDA.	
24. Usted vas se ha sometido a un análisis para saber si está infectado por el HIV.	
25. En su historia clínica, usted tiene incluido alguna pregunta sobre HIV/SIDA.	
26. Con qué frecuencia usted revée la historia clínica de sus pacientes durante las consultas.	
¿Qué medios de control de infección usted adopta en su práctica odontológica?	4
27. Barbijo	
28. Gafas de protección	
29. Cofia	
30. Guantes (1 par)	
31. Guantes (2 pares)	
32. Guardapolvo o chaquetilla	
33. Protección de superficies con papel film	

ARTIGO 4

Factorial validity of a questionnaire for assessment of dentists' knowledge about HIV/AIDS

(Em elaboração)

Title: Factorial validity of a questionnaire for assessment of HIV/AIDS dentists' knowledge

Short title: Questionnaire for assessment of HIV/AIDS dentists' knowledge

Key Words: HIV infection, questionnaire validation; dentists' knowledge

Abstract

The aim of this paper was to carry out the construct validation of an HIV/AIDS questionnaire, in the Spanish language, to assess dentists' knowledge of HIV/AIDS (DK-HIV-Q). In worldwide literature there are no studies on this subject. The study sample of 251 Argentine dentists included subjects from 20 to 63 years of age, of both genders, and was randomly selected. The reliability of the instrument was assessed by Cronbach's Alpha Coefficient and the validity was assessed by exploratory factorial analysis (EFA) and confirmatory factorial analysis (CFA). DK-HIV-Q presented the structure with the general factor of the second order, and with four specific factors. The internal reliability was confirmed as: 0.68 for the HIV/AIDS dentists' knowledge (general factor); 0.53 for declarative knowledge toward transmission of HIV/AIDS (factor 1); 0.71 for declarative knowledge toward oral manifestations of HIV/AIDS (factor 2); 0.59 for procedural knowledge of dentists' practice (factor 3); and 0.48 for procedural knowledge toward infection controls (factor 4). Deletion of eight items from the original DK-HIV-Q, with 33 items, improved the goodness of fit for the instrument. The Argentine Spanish language version of the DK-HIV-Q proved to be a useful instrument for the assessment of dentists' knowledge of HIV/AIDS, and it showed that factor 2 is most closely with HIV/AIDS dentists' knowledge. Further research is required to add more items to the factors 1, 3 and 4, and to evaluate the psychometric properties with diversified samples of dentists.

Introduction

Factor analysis remains one of the standard and most widely used methods for demonstrating construct validity of new instruments (Clark & Watson, 1995, Fayers & Hand, 1997; Maraun, 1996; Marsh, Hau, Balla, & Grayson, 1998). When questionnaires are being developed, it is common to apply factor analysis as a means of confirming that the instrument possesses an appropriate structure (construct validity), and sometimes to help develop the instrument further by revealing items that may be removed from the questionnaire because they contribute little to the presumed underlying factors (Fayers & Hand, 1997). Factor analysis was developed by methods of Spearman (1904) and can be used for various purposes, three of which are noted here. First, factor analysis can be used to inform evaluations of score validity. Second, factor analysis can be used to develop theories regarding the nature of constructs and to specify construct dimensions. Third, factor analysis can be used to summarize relationships in the form of a more restrained set of factor scores that can then be used in subsequent analyses (Fayers & Hand, 1997; Jensen, 1992; Tompsom, 1994).

There are actually two discrete classes of factor analysis: exploratory factor analysis (EFA) and confirmatory factor analysis (CFA). In EFA, originally proposed by Spearman (1904), the researcher may not have any specific expectations regarding the number or the nature of underlying constructs or factors, he does not declare any expectations, and the analysis is not influenced by these expectations (Jensen, 1992). CFA was developed much more recently by Joreskog (1969). These analyses require the researcher to have specific expectations regarding the number of factors, which variables reflect given factors, and whether the factors are correlated. CFA explicitly and directly tests the fit of factor models and it is more useful in the presence of theory because the theory is directly tested by the analysis, and the degree of model fit can be quantified in various ways. CFA presumes the application of specific expectations, with the attendant possibility that the predetermined theory in fact does not fit, in which case the researcher may be unable to explain the relationships among the variables being analyzed. Both EFA and CFA remain useful, and the authors selection between these two classes of factor analysis generally depends on whether or not they have a specific theory regarding data structure (Fayers & Hand, 1997; Tompsom, 1994).

It is important to present the construct validity of the dentists' knowledge of HIV/AIDS questionnaire (DK-HIV-Q) for the training and education of dentists, mainly because in the worldwide literature there are no studies of questionnaires with construct validity about HIV/AIDS for dentists. To develop, adapt and validate a new instrument, requires more research time and makes more difficult to compare the data generated by this instrument with data from other parts of the world. The lack of instruments of this type in the world limits researchers. It is important to develop or to adapt questionnaires that are reliable and valid in different countries and cultures (Boileau, Rashed, Sylla, & Zunzunegui, 2008; Florindo et al., 2006; Gil-Monte, 2005; Patel et al., 2008) Thus, there are many issues currently stimulating interest in cross-cultural studies, which makes it more important to understand culturally based differences with respect to constructs that evaluate new instruments (Gil-Monte, 2005). For these reasons, there is an advantage in the construct validity of the new DK-HIV-Q based on the fact that it is an instrument of easy comprehension, it is designed for dentists, and because it makes it possible to compare results from future studies designed for dentists from diverse cultures to develop strategies to deal with HIV/AIDS. The goal of this study was to carry out the validation of construct of the DK-HIV-Q in the Argentine Spanish language.

Methods

The present study was carried out in the urban area of Córdoba, the capital city of the state of Córdoba, located in the central region of Argentina. The city has 1.5 million inhabitants and 2800 dentists. A list of all registered dentists was obtained from the School of Dentistry of the State of Córdoba, Argentina.

By assessing the number of Argentine dentists, a standard error (SE) of 1% and a 99% confidence interval level were calculated for the study (n=250 samples) with an expected prevalence of 50% (Kirkwood, 1996). Data were collected from 251 randomly selected dentists of both genders, between 20 and 63 years of age. Only subjects who were treating patients at the time of the study were included in the study.

Written informed consent was also obtained from all participants. Prior to the interviews, the study was approved by the local ethical commission (report number 545/07-COEP).

The DK-HIV-Q is an instrument to assess dentists' knowledge levels about HIV/AIDS. It comprises 33 items (Figure 1). In the previous study, the test-retest reliability revealed stability of answers in a short period of time. Satisfactory reproducibility (ICC = 0.95) has been developed by Moura, Gomes, Blanc, Mesquita, & Ferreira (ready for publication in 2009). The score on each item of the test was used in this DK-HIV-Q, with the following options: 'Yes' = 1 and 'No' = 0.

The parallel analysis for permutation was used to extract the factors in EFA. The factors that were extracted were submitted to the rotation through the Oblique Minimum (oblimin) technique (Horn, 1965; Nasser, Benson, & Wisenbaker, 2002; Reise, Waller, & Comrey, 2000; Thompson, 1994, Zwick & Velicer, 1986). Inspection of the factorial loads of the items was carried out to show which items were contributing positively to the factors. The items with factorial loads lower than 0.2 were eliminated, because they were not collaborating with the necessary significant factorial loads to a factor. Valid factors were only those with at least three variables and with items of factorial load equal to or above 0.2 (Reise et al., 2000; Thompsom, 1994). A new EFA was carried out with the remaining items.

The CFA procedure was applied based on the one solution obtained through EFA. The criteria for satisfactory adjustment of the model to the data were adopted according to the following indices values: *Comparative Fit Index* (CFI) and *Goodness of Fit Index* (GFI) equal to or greater than 0.90, and *Root Mean Square Residual* (RMR) less than or equal to 0.08. (Bentler, 1990; Maraun, 1996; Marsh et al., 1998; McDonald & Ho, 2002; Tompsom, 1994). The ideal factorial loads for the general factor were considered to be equal to or greater than 0.3.

Values for the internal consistency of the DK-HIV-Q were estimated by using Cronbach's Alpha Coefficient both for each isolated factor, and for the general factor. Scales with reliabilities of at least 0.50, but preferably 0.70 or greater, were considered sufficiently reliable to be used (Bartko, 1966; Cortina, 1993, Cronbach, 1951; Shrout, 1995).

The SPSS software program (version 16.0. SPSS Inc., Chicago, IL, USA) was used for the data analysis. Information was codified in a databank.

Results

Initially descriptive analyses were performed for each participant. The length of time of practice in the profession varied from 1 to 40 years. The average years of practice was 11.4 (SD= 10.2).

A researcher made sure that all dentists filled out the questionnaire.

The initial criterion, analyzed by parallel analysis for permutation, used to identify the number of factors of the 33 items of the DK-HIV-Q resulted in the identification of four factors related to the four domains suggested in the DK-HIV-Q: declarative knowledge toward transmission of HIV/AIDS (Factor 1) with three items (2, 3, 4); declarative knowledge toward oral manifestations of HIV/AIDS (Factor 2) with ten items (5, 6, 7, 8, 9, 10, 11, 12, 13, 15); procedural knowledge toward dentists' practice (Factor 3) with six items (16, 17, 18, 20, 21, 23); and procedural knowledge toward infection controls (Factor 4) with seven items (23, 25, 26, 27, 28, 29, 33) (Figure 2).

Item 1 was excluded because all the dentists had answered positively that contaminated blood transmits the HIV/AIDS (Figure 1). Seven more items (items 14, 19, 22, 24, 30, 31 and 32) were excluded because the inspection of their factorial loads was problematic and they presented less than 0.2 factorial load. Thus, a satisfactory factor structure was obtained. Then, a new EFA was carried out and the final results indicate the factor structure of the scale of 25 items the DK-HIV-Q (Figure 2).

In the analysis of first-order factors, correlations between these four factors were identified. These correlations indicated the possibility of the scale of the DK-HIV-Q to show a factorial structure with the presence of a second-order general factor that was confirmed by the CFA model. The elaborated CFA presented the structure with the general factor of second order, and four specific factors. The CFA model presented an adequate degree of adjustment to the data with RMR 0.05, GFI 0.927 and CFI 0.917.

Table 1 presents factorial loads of the items of each identified first-order factor, and of the second-order general factor. Items with a factorial load of zero were omitted because they did not explain the factor by the dentists' answer to that item, and they were not related to the other factors (Table 1). The inspection of Table 1 allows us to verify that all of the factorial loads were adequate: above 0.20 for first-order factors, and above 0.30 for the second-order general factor.

All items of the four first-order factors had shown significant theoretical content about dentists' knowledge of HIV/AIDS. When the model factors were orthogonal by the CFA, the majority of items of factor 2 (6, 7, 8, 10, 11, 12, 13 and 15) lost their specific quality and were more closely related to the general factor, because they presented a factorial load value above 0.30 for the general factor and below 0.20 for the specific factor 2 (Table 1). The first-order factors 1, 3 and 4 are more specific, and they are not correlated to the general factor (Table 1).

Internal consistency reliability was demonstrated by a Cronbach's Alpha coefficient for the first-order factors and for the second-order general factor, and is also described in Table 2. Cronbach's Alpha for factor 2 demonstrated satisfactory internal consistency. However, factors 1, 3 and 4 presented low internal consistency, but the Cronbach's Alpha for the general factor was 0.68.

Discussion

The techniques of factorial analysis identify the most specific abilities by the EFA, and afterwards identify generic abilities through the CFA (Fayers & Hand, 1997; Maraun, 1996; Marsh et al., 1998). In factorial analysis, defining the number of factors to extract is very important because the sub or super-extraction may be altered by any results of subsequent analysis (Fava & Velicer, 1996, Horn, 1965; Nasser et al., 2002; Reise et al., 2000; Thompson, 1994, Zwick & Velicer, 1986). Studies with the criteria based on the parallel analysis of Horn (1965) are adequate in 92% of the cases (Zwick & Velicer, 1986). The parallel analysis for permutation appears to be among the best methods for deciding how many factors to extract or retain. Despite the advantages of the EFA, no procedures of the EFA generate reliable indices with which to evaluate models. For that reason, the CFA was used to verify that the model indicated by the EFA would be adequate for the data of the study (Fava & Velicer, 1996, Horn, 1965; Nasser et al., 2002; Reise et al, 2000; Thompson, 1994, Zwick & Velicer, 1986).

It is important, for knowledge about HIV/AIDS, to identify the first-order factors: the likelihood of declarative knowledge concerning transmission and oral manifestations of HIV/AIDS, the procedural knowledge regarding dentists' practice and infection control. The dentists' performance in training conditions would initially be dependent on declarative knowledge. However, with repeated experience, specific routines for task performance can be set, with the triggering of procedural

learning mechanisms. The declarative knowledge is transformed into qualitatively different procedural knowledge. The contribution of procedural and declarative knowledge about HIV/AIDS is dynamic and dependent on the amount of practice, rather than being a static characteristic of each type of knowledge (Tompsom, 1994).

It has been suggested that the DK-HIV-Q began with 33 items and four domains. The final results of EFA in the current study resulted in a scale of the DK-HIV-Q consisting of 25 items and four factors of first order. Deletion of the eight items that presented less than 0.2 factorial loads improved the goodness of fit for the instrument. The best factorial load would be above 0.4 to indicate the quality of the factorial solution factorial identified. Tabachnick and Fidell (1996) propose the value of factorial load of 0.2, preferably more than 0.3, as the acceptable minimum for an item to be considered legitimately representative of the construct evaluated. The eight items were eliminated by absence of relationship between the content of the item and the theoretical domain of the construct. Nevertheless, if a new application of the DK-HIV-Q with construct validity of 25 items were given to that same sample, possibly the results would be different, maybe even better, and with stronger factorial loads. Smith and McCarthy (1995) emphasize that it is important to investigate the psychometric characteristics of the items, especially if the items are representing the content approached by the factor. The exclusion of the item 14 in the factor 2 was important and pertinent because the oral lichen planus is not a specific oral manifestation of patients with HIV/AIDS, so this does not show specific knowledge of dentists with regard to HIV/AIDS. Also the items 19, 22, 24, 30, 31 and 32 were shown not to be related to the knowledge of dentists about HIV/AIDS, and were excluded.

The current study identified the structure of the scale of the DK-HIV-Q measuring a general factor of second order composed of four specific factors of the first order. The presence of the second order factor contributed to the theoretical development of the field investigated and to the comprehension of the factorial structure of the central and peripheral elements of the instrument. The concerns identified by DK-HIV-Q were largely consistent with the knowledge of dentists about HIV/AIDS, and this evaluated construct is more adequately investigated in the version composed of 25 items.

The analysis also revealed that the majority of the items in the factor 2 present factorial loads greater than 0.30 for the general factor and less than 0.20 for the specific factor 2. Therefore, factor 2 can be considered more representative of the construct of the dentists' knowledge about HIV/AIDS, while the items of the three remaining factors (1, 3 and 4) do not contribute significantly to the dentists' knowledge about HIV/AIDS.

Cronbach's Alpha coefficient was 0.68 for the total scale and 0.71 for factor 2, indicating adequate internal reliability, as reliability of at least 0.50 is acceptable but 0.70 and greater is preferable (Bartko, 1966; Cronbach, 1951; Shrout, 1995). The item internal consistency is demonstrated when each item in a hypothesized scale is substantially linearly related to the underlying concept being measured (Cronbach, 1951). Clark and Watson (1995) describe that the medium correlation between items is a better indicator of the internal consistency than the alpha coefficient that is affected by the number of items. That can justify the attained Cronbach' s Alpha coefficient of 0.53 for factor 1, 0.59 for the factor 3, and 0.48 for factor 4, representing, respectively, items 3, 6 and 7. It will be important in future studies to elaborate more items for each one of those three factors, because the Cronbach's Alpha coefficient was very low. The subsequent addition of items for those factors would improve the results and give a stronger and more consistent result because the instrument would also identify those three areas of knowledge about HIV/AIDS, thus extending the application of the DK-HIV-Q and not only evaluating specific knowledge regarding general information. Low internal consistency coefficients for the first questionnaire would sometimes be found and, in those cases, may be related to the small number of items that composed the factor. This shortcoming is a problem that should be taken into account in the research. Values of the Cronbach's Alpha coefficient are sometimes between alpha = 0.42 and alpha = 0.64 (Gil-Monte, 2005). Some results of internal consistency are similar in Spanish (Gil-Monte, 2005). Cortina (1993) shows that the alpha coefficient loses its usefulness in scales composed of more than 40 items.

The findings presented here demonstrate their value as a starting point for further inquiry. It would be desirable to add more items, and to determine criterion validity in diverse samples of dentists from other countries. This study also makes a contribution to the cross-cultural validation inventory within Latin America. This contribution is relevant when considering the importance of having cross-

cultural instruments to assess dentists' knowledge regarding HIV/AIDS. With the increasing focus on AIDS, it is important for dentists from different cultures to understand the value of having a common instrument for assessing multicultural labor forces when treating patients with AIDS (Civaner & Arda, 2008; Mussard, Ashley, Newton, Kendall, & Crayford, 2008; Patel et al., 2008) . The DK-HIV-Q proved to be a useful instrument for the assessment of dentists' knowledge of HIV/AIDS, but it is required to add more items to the factors 1, 3 and 4. It has satisfactory psychometric properties, it offers a consistent measurement tool, and its use could be extended without difficulty.

References

1. Bartko, J.J. (1966). The intraclass correlation coefficient as a measure of reliability. *Psychological reports*, 19(1), 3-11.
2. Bentler, P. M. (1990). On the equivalence of factors and components. *Multivariate Behavioral Research*, 25(1), 67-74.
3. Boileau C, Rashed S, Sylla M, Zunzunegui MV. Monitoring HIV risk and evaluating interventions among young people in urban West Africa: development and validation of an instrument. AIDS Educ Prev. 2008 Jun;20(3):203-19.
4. Civaner, M., & Arda, B. (2008). Can "presumed consent" justify the duty to treat infectious diseases? An analysis. *BMC Infectious Disease,* 8(29), 1-11.
5. Clark, A. C., & Watson, D. (1995). Constructing validity: basic issues in objective scale development. *Psychological Assessment*, *7*, 309-319.
6. Cortina, J. M. (1993). What is coefficient alpha? An examination of theory and applications. *Journal of Applied Psychology*, *78*, 98-104.
7. Cronbach, L.J. (1951). Coefficient a and the internal structure of tests. Psychometrika, 16, 297–334.
8. Fava, J. L., & Velicer W. F. (1996). The effect of underextraction in factor and component analysis. *Educational and Psychological Measurement,* 56, 907-929.
9. Fayers, P.M. & Hand, D. J. (1997). Factor analysis, causal indicators and quality of life. *Quality of Life Research*, 6, 139–150.
10. Florindo AA, Latorre MR, Santos EC, Negrão CE, Azevedo LF, Segurado AA. Validity and reliability of the Baecke questionnaire for the evaluation of habitual physical activity among people living with HIV/AIDS. Cad Saude Publica. 2006 Mar;22(3):535-41
11. Gil-Monte, P.R. (2005) Factorial validity of the Maslach Burnout Inventory (MBI-HSS) among Spanish professionals. *Revista de Saude Publica*, 39 (1), 1-8.
12. Horn, J. L. (1965). A rationale and test for the number of factors in factor analysis. *Psychometrika*, *30*, 179-185.

13. Jensen, A. R. (1992). Spearman's hypothesis: methodology and evidence. *Multivariate Behavioral Research*, 27(2), 225-233.
14. Jöreskog, K. G. (1969). A general approach to confirmatory maximum likelihood factor analysis. *Psychometrika, 34,* 183–202.
15. Kirkwood, B.R. (1996). *Essentials of medical statistics*. Oxford: Blackwell Science.
16. Maraun, M. D. (1996). The claims of factor analysis. *Multivariate Behavioral Research*, 31(4), 673-689.
17. Marsh, H. W., Hau, K-T., Balla, J. R. & Grayson, D. (1998). Is more ever too much? The number of indicator per factor in confirmatory factor analysis. *Multivariate Behavioral Research*, 33(2), 181-220.
18. MCDonald, R. P. & HO, M. H. (2002) Principles and practice in reporting structural equation analyses, *Psychol.Methods*, 7, 64-82.
19. Mussard, J., Ashley, F.A., Newton, J.T., Kendall, N., & Crayford, T.J. (2008). What do you think of your dentist? A dental practice assessment questionnaire. *Journal of Evaluation in Clinical Practice,* 14(2),181-4.
20. Nasser, F., Benson, J. & Wisenbaker, J. (2002). The performance of regression-based variations of the visual scree for determining the number of common factors. *Educational and psychological measurement , 62 (3)*, 397-419.
21. Patel, S., Weiss, E., Chhabra, R., Ryniker, L., Adsuar, R., Carness, J., Kahalas, W., Delamarter, C., Feldman, I.S., Delorenzo, J.P., Tanner, E., Rapkin, B. (2008). The events in care screening questionnaire (ECSQ): a new tool to identify needs and concerns of people with HIV/AIDS. *AIDS Patient Care & STDs*, 22(5), 381-93.
22. Reise, S. P., Waller, N. G., & Comrey, A. L. (2000). Factor analysis and scale revision. *Psychological Assessment, 12*, 287-297.
23. Shrout, P.E. (1995). Reliability. In *Textbook in psychiatry epidemiology* Edited by: Zahner TTA. New York: Wiley-Liss, 213-227.
24. Smith, G.T., & McCarthy, D.M. (1995). Methodological considerations in the refinement of clinical assessment instruments. *Psychological Assessment, 7,* 300-308.

25. Spearman, C. (1904). General intelligence: objectively determined and measured. *American Journal of Psychology*, 15, 201-293.

26. Tabachnick, B. G., & Fidell, L. S. (1996). *Using multivariate statistics (3a ed.). Nova York: HarperCollins.*

27. Thompson, B. (1994). Guidelines for authors: *Educational and Psychological Measurement,* 54(4), 837-847.

28. Zwick, R., & Velicer, W. F. (1986). Comparison of five rules for determining the number of components to retain. *Psychological Bulletin*, *99*, 432-442.

Figure 1: Dentists' knowledge of HIV/AIDS questionnaire (DK-HIV-Q) with 33 items

DK-HIV-Q	Domains
¿Por qué medios puede ser transmitido el HIV?	
1. Sangre	1
2. Saliva	
3. Secreción vaginal	
4. Semen	
¿Cuáles son las manifestaciones bucales que se relacionan con los portadores de HIV/SIDA? Marque con una "X" (puede ser cualquier número de respuestas) aquellas que usted considere.	2
5. Sarcoma de Kaposi	
6. Leucoplasia Vellosa	
7. Candidiasis	
8. Periodontitis Ulcero Necrotizante	
9. Gingivitis Ulcero Necrotizante Aguda (GUNA)	
10. Eritema gingival lineal	
11. Linfoma no Hodgkin	
12. Herpes Zoster	
13. Aumento de parótida	
14. Líquen Plano	
15. Condiloma acuminado	
Lea el siguiente cuadro. En el mismo se realizan afirmaciones. Marque con un X la opción con la que se sienta más identificado. No deje ninguna sin marcar.	3
16. Usted se considera apto para atender a un portador del HIV/SIDA.	
17. Usted ya ha atendido algún paciente con HIV/SIDA.	
18. Usted sabe cómo proceder durante un accidente punzo cortante.	
19. Después de un accidente punzo cortante, usted se haría un análisis para verificar la posibilidad de infección con HIV.	
20. Sus conocimientos y aplicaciones sobre el control de la infección son adecuados para prevenir la infección cruzada.	
21. Usted necesita recibir un entrenamiento especial sobre cuidados y manejos del paciente portador de HIV/SIDA para atender a los mismos.	
22. Las personas portadoras de HIV/SIDA deben ser tratadas en ambiente aislado o en lugares especializados.	
23. Usted conoce la medicación más comúnmente usada por los pacientes portadores de HIV/SIDA.	
24. Usted vas e ha sometido a un análisis para saber si está infectado por el HIV.	
25. En su historia clínica, usted tiene incluido alguna pregunta sobre HIV/SIDA.	
26. Con qué frecuencia usted revée la historia clínica de sus pacientes durante las consultas.	
¿Qué medios de control de infección usted adopta en su práctica odontológica?	4
27. Barbijo	
28. Gafas de protección	
29. Cofia	
30. Guantes (1 par)	
31. Guantes (2 pares)	
32. Guardapolvo o chaquetilla	
33. Protección de superficies con papel film	

Figure 2: Dentists' knowledge of HIV/AIDS questionnaire (DK-HIV-Q) with 25 items

DK-HIV-Q	Factors
¿Por qué medios puede ser transmitido el HIV?	1
2. Saliva	
3. Secreción vaginal	
4. Semen	
¿Cuáles son las manifestaciones bucales que se relacionan con los portadores de HIV/SIDA? Marque con una "X" (puede ser cualquier número de respuestas) aquellos que usted considere.	2
5. Sarcoma de Kaposi	
6. Leucoplasia Vellosa	
7. Candidiasis	
8. Periodontitis Ulcero Necrotizante	
9. Gingivitis Ulcero Necrotizante Aguda (GUNA)	
10. Eritema gingival lineal	
11. Linfoma no Hodkin	
12. Herpes Zoster	
13. Aumento de parótida	
15. Condiloma acuminado	
Lea el siguiente cuadro. En el mismo se realizan afirmaciones. Marque con un X la opción con la que se sienta más identificado. No deje ninguna sin marcar.	3
16. Usted se considera apto para atender a un portador del HIV/SIDA.	
17. Usted ya ha atendido algún paciente con HIV/SIDA.	
18. Usted sabe cómo proceder durante un accidente punzo cortante.	
20. Sus conocimientos y aplicaciones sobre el control de la infección son adecuados para prevenir la infección cruzada.	
21. Usted necesita recibir un entrenamiento especial sobre cuidados y manejos del paciente portador de HIV/SIDA para atender a los mismos.	
23. Usted conoce la medicación más comúnmente usada por los pacientes portadores de HIV/SIDA.	
23. Usted conoce la medicación más comúnmente usada por los pacientes portadores de HIV/SIDA.	
25. En su historia clínica, usted tiene incluido alguna pregunta sobre HIV/SIDA.	
26. Con qué frecuencia usted revée la historia clínica de sus pacientes durante las consultas.	
¿Qué medios de control de infección que usted adopta en su práctica odontológica?	4
27. Barbijo	
28. Gafas de protección	
29. Cofia	
33. Protección de superficies con papel film	

Table 1: Cronbach`s alpha coefficient and factorial loads of the items in exploratory and confirmatory factors analysis of the dentists' knowledge of HIV/AIDS questionnaire (DK-HIV-Q)

Items	**Factors of the DK-HIV-Q**				
Number items	**Second-order**	**First order (1)**	**First order (2)**	**First order (3)**	**First order (4)**
2	-0.13	**0.25**			
3	0.13	**0.71**			
4	0.08	**0.91**			
5	0.26		0.04		
6	**0.49**		0.05		
7	**0.35**		0.12		
8	**0.43**		**0.27**		
9	**0.42**		**0.46**		
10	**0.55**		0.05		
11	**0.51**		-0.20		
12	**0.61**		0.00		
13	**0.37**		-0.21		
15	**0.47**		-0.38		
16	0.16			**0.67**	
17	0.22			**0.30**	
18	0.24			**0.28**	
20	0.07			**0.46**	
21	0.16			**0.46**	
23	0.15			**0.25**	0.17
25	0.14				**0.41**
26	0.16				**0.47**
27	0.10				**0.26**
28	0.05				**0.30**
29	0.01				**0.40**
33	0.12				**0.30**
Alpha	**0.68**	**0.53**	**0.71**	**0.59**	**0.48**

7. Considerações finais

Com o foco crescente em HIV/AIDS e considerando as transformações desta epidemia ao longo do tempo, principalmente em relação às tendências de instabilidade e vulnerabilidade, é importante ressaltar que estas duas pesquisas realizadas, a primeira demonstrando que o tratamento tópico padrão para a LPB é o PA e, a segunda mostrando a necessidade e a importância de ter um instrumento comum para avaliar o conhecimento dos cirurgiões dentistas sobre HIV/AIDS, fornecem ganhos científicos não só aos pacientes com HIV/AIDS, mas também aos profissionais de saúde, no sentido de garantir um melhor atendimento e uma melhor qualidade de vida aos pacientes com HIV/AIDS.

O fato de existir raros trabalhos de ensaio clínico randomizado sobre tratamento tópico da LPB favorece um grande ganho científico relacionado a essa primeira pesquisa, no sentido de proporcionar o estabelecimento do melhor fármaco para o tratamento tópico da LPB, que apesar ter diminuído sua prevalência com o advento da HAART, ainda continua sendo uma lesão fortemente associada à imunossupressão nos pacientes com HIV/AIDS.

A avaliação do melhor tratamento tópico para a LPB foi baseada não somente na comparação dos três protocolos de tratamento utilizados (P, PA e PP), mas também em relação ao tempo que ocorreu a cura, aos fatores de prognóstico que poderiam influenciar na cura e através das avaliações de recidiva até 12 meses após a cura e da presença do EBV após a cura. Isso torna o estudo mais completo no sentido de enfatizar o melhor protocolo de tratamento para essa lesão e, além disso, pelo fato da LPB ainda ser uma lesão altamente recidivante, estudos como este ajudam também a explicar, mesmo que em parte como um estudo inicial, a alta prevalência de recidiva desta lesão.

Na primeira pesquisa foi verificado que o PA deve ser preferido como tratamento tópico atual para LPB, porque é melhor que o PP para curar esta lesão. No sentido de compreender melhor este resultado, estudos "in vitro" para avaliar a superioridade do ACV comparado ao PCV para o EBV são necessários para prover outros conhecimentos no sentido de ampliar o uso desses protocolos terapêuticos para a LPB.

O aprendizado relacionado à primeira pesquisa foi primeiramente devido aos obstáculos encontrados na realização deste estudo de ensaio clínico, tanto pela dificuldade de encontrar pacientes com LPB, quanto pela dificuldade de mantê-los em tratamento, uma vez que houve tratamentos realizados com até 25 semanas. Mas por outro lado, o convívio com essas pessoas durante esse período, enriqueceu e favoreceu uma experiência de vida inigualável.

Além disso, a oportunidade de trabalhar com o método de análise estatística de sobrevida, o Modelo Proporcional de Cox, também proporcionou um aprendizado importante, além de enriquecer bastante a qualidade da pesquisa, uma vez que vários fatores foram analisados ao mesmo tempo no sentido de favorecer o estabelecimento do melhor protocolo de tratamento.

Assim, o ganho para os pacientes portadores de LPB é um tempo de tratamento menor, o benefício de usar o sinergismo de fármacos (PA) para tratar de forma mais simples esta lesão, com ausência de efeitos colaterais sistêmicos e com baixo custo. ACV junto com P diminui a possibilidade de recorrência da LPB. A elevada prevalência de DNA do EBV-1 na borda lateral de língua de pacientes sem LPB após o tratamento tópico suporta o papel desse vírus na recorrência da LPB.

Quando iniciado a segunda pesquisa, em 2007, não havia nenhum estudo mostrando questionários validados sobre o conhecimento dos cirurgiões dentistas

sobre HIV/AIDS. O conhecimento de cirurgiões dentistas de culturas diferentes sobre esse assunto é importante para eliminar preconceitos no atendimento de pacientes com HIV/AIDS e, ao mesmo tempo, favorecer o direcionamento de medidas educativas e preventivas para solucionar as principais deficiências de conhecimento desses profissionais, proporcionando um melhor atendimento a esses pacientes, priorizando qualidade de vida e saúde bucal.

Estes dois aspectos são fundamentais para o entendimento do principal ganho científico deste estudo, que foi o desenvolvimento e validação de um instrumento comum, auto-aplicável, simples, que apresenta propriedades psicométricas satisfatórias, funciona como uma ferramenta coerente de medida e pode ter seu uso estendido sem dificuldade para cirurgiões dentistas de diferentes culturas. É necessário saber o nível de conhecimento destes profissionais de saúde para que medidas realmente eficientes sejam tomadas, surtindo efeito ao nível de saúde pública.

Os principais aprendizados desta segunda pesquisa foram a dificuldade encontrada na criação de um instrumento pela primeira vez, a sequência de etapas que precisam ser seguidas durante o processo de validação e o fato de ter sido realizado em outra linguagem. Por outro lado, o método utilizado ajudou na ampliação de conhecimentos sobre o assunto, onde os resultados apresentados representam apenas um passo inicial para que outras pesquisas sejam realizadas, uma vez que é necessário adicionar mais itens ao DK-HIV-Q finalizado com 25 itens.

Estudos adicionais em amostras de cirurgiões dentistas de outros países podem ser realizados para a comprovação deste instrumento, mas estes resultados encontrados já revelam um caminho promissor para explorar instrumentos transculturais para avaliar o conhecimento dos cirurgiões dentistas sobre HIV/AIDS.

Anexos

Anexo 1– Normas da revista científica intitulada “Oral Surgery, Oral medicine, Oral Pathology, Oral Radiology and Endodontology” de submissão do Artigo 1

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The Official Publication for the American College of Oral and Maxillofacial Surgery, American Academy of Oral and Maxillofacial Radiology, American Academy of Oral Medicine, American Academy of Oral and Maxillofacial Pathology, and the Organization of Teachers of Oral Diagnosis

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software such as Photoshop and Illustrator (not presentation software such as PowerPoint, CorelDraw, or Harvard Graphics) should be used in the creation of the art. Color images need to be CMYK, at least 300 DPI, and be accompanied by a digital color proof, not a color laser print or color photocopy. Note: This proof will be used at press for color reproduction. Gray scale images should be at least 300 DPI accompanied by a proof. Combinations of gray scale and line art should be at least 1200 DPI accompanied by a proof. Line art (black and white or color) should be at least 1200 DPI with a proof.

For best possible reproduction, avoid using shading or dotted patterns; if unavoidable, submit this type of illustration in the form of a glossy photograph for best results. Use thick, solid lines and bold, solid type. Place lettering on a white background; avoid reverse type (white lettering on a dark background). Typewritten or freehand lettering is unacceptable. All lettering must be done professionally and should be in proportion to the drawing graph, or photograph. Do not send original art work, radiograph films, or electrocardiographic strips. Any special instructions regarding sizing should be clearly noted.

Legends to illustrations. Each illustration must be accompanied by a legend. These should be typed double-spaced on a separate page. If an illustration has been taken from published material, the legend must give full credit to the original source.

Tables. The tables should be typewritten double-spaced, including column heads, data and footnotes, and submitted on separate pages. Tables should be self-explanatory and should supplement, not duplicate, the text. All table reference citations should be repeats of numbers assigned within the text, not initial citations. A concise title should be supplied for each table. All columns should carry concise headings describing the data therein. Type all footnotes immediately below the table and define abbreviations. If a table or any data therein have been previously published, a footnote to the table must give full credit to the original source.

Video and Computer Graphics. Authors are encouraged to submit videos and computer-generated graphics; eg, a slide presentation with or without animation and sound. An author who wishes to supply such material should notify the editors in the cover letter and note this

intention in the Author Comments area of the online submission. Although the publisher will not edit any video or computer graphic, editors and reviewers may suggest changes. All patient-identifying information must be removed or masked.

The maximum length of a video or computer graphic is 8 minutes. Longer submissions may be divided into smaller clips, each of which should be identified at the beginning of the section (eg, Video Clip 1, Graphic 1). A concise legend for each video clip or computer graphic presentation must be included with the manuscript. Videos are to be submitted in MGEG-1 or MPEG-2 (*.mpg) or QuickTime (*.mov) format. More detailed instructions can be found at http://www.elsevier.com/artwork . Videos and computer graphics accompanying a manuscript declined for publication will not be accepted separately. If the manuscript is accepted for publication, the presentation will be archived at www.mosby.com/tripleo.

Permissions. Direct quotations, tables, or illustrations that have appeared in copyrighted material must be accompanied by written permission for their use from the copyright owner and original author along with complete information with respect to source. Photographs of identifiable persons must be accompanied by signed releases showing informed consent. Articles appear in both the print and online versions of the journal, and wording should specify permission in all forms and media. Failure to obtain electronic permission rights may result in the images not appearing in the print or online versions.

NOTE: FOLLOW INSTRUCTIONS FOR ONLINE SUBMISSION AT

HTTP://EES.ELSEVIER.COM/TRIPLEO

Announcements. Announcements must be received by the Editorial Office at least ten weeks before the desired month of publication. Items published at no charge include those received from a sponsoring society of the Journal; courses and conferences sponsored by state, regional, or national dental organizations; and programs for the dental profession sponsored by government agencies. All other announcements selected for publication by the Editor carry a charge of $60 US, and the fee must accompany the request to publish.

Reprints. Because of the extremely high cost of preparing color articles, author reprints for articles containing color illustrations have to be prepared as overprints (overrun pages).

Order forms will be sent to the corresponding author of articles containing color illustrations, so that overprints of those articles can be ordered the month of publication. No complimentary overprints or reprints will be provided.

Checklist for authors

__Signed copyright transfer statement (signed by all authors) (FAXED to Editorial Office)

__Letter of submission

__Title page

__Title of article

__Full names(s), academic degree(s), affiliation(s) and titles of author(s)

__Author to whom correspondence, galleys, and reprint request are to be sent, including address and business and home telephone numbers, fax number, and e-mail address

__Structured abstract (double-spaced)

__Article proper (double-spaced)

(Figures and tables should not be part of the text of the manuscript but added as separate files)

__Statement of IRB review (stated in manuscript)

__References (double-spaced on a separate page)

__Reprint requests line (on a separate page)

__Tables (double-spaced, on separate pages)

__Legends (double-spaced, on a separate page)

__Illustrations, properly formatted (as separate files)

__Video/computer graphics, properly formatted (as separate files)

__Acknowledgments (on a separate page)

__Source of funding for research (on a separate page)

__Signed permission to reproduce previously published material, in all forms and media (scanned in as a file)

__Signed permission to publish photographs of identifiable persons from the individual specifying permission in all forms and media (scanned in as a file)

__Financial interest disclosure, if applicable (on a separate page)

__If this paper was presented at a meeting identification of organization, city, and year (on a separate page)

Anexo 2 – Normas da revista científica intitulada “Journal of Epidemiology and Community Health” de submissão do Artigo 3

Journal of Epidemiology and Community Health

Instructions for Authors

Manuscript format

All manuscripts must be submitted via Bench>Press.

All material submitted is assumed to be submitted exclusively to the journal unless the contrary is stated. Submissions may be returned to the author for amendment if presented in the incorrect format.

If you are submitting a randomised controlled trial, please send with your manuscript the following:

The registration number of the trial and the name of the trial registry - in the last line of the paper's structured abstract. Trials that begin enrolment of patients after 1 July 2005 must register in a public trials registry at or before the onset of enrolment to be considered for publication. Trials that began patient enrolment on or before 1 July 2005 must register before 13 September 2005 to be considered for publication. Please see the Statement from the International Committee of Medical Journal Editors.

Cover letter

Your cover letter should inform the Editor of any special considerations regarding your submission, including but not limited to:

1. Details of related papers published or submitted for publication.

- Copies of related papers should be submitted as supplementary data to help the Editor decide how to handle the matter.

2. Details of previous reviews of the submitted article.

- The previous Editor's and reviewers' comments should be submitted as supplementary data along with your responses to those comments. Editors encourage

authors to submit these previous communications and doing so may expedite the review process.

Whether any of the material could be published as data supplements rather than in the print version of the article.

Title page

The title page must contain the following information:

1. The title.
2. The name, postal address, e-mail, telephone and fax numbers of the corresponding author.
3. The full names, institutions, city and country of all co-authors.
4. Up to five keywords or phrases suitable for use in an index (it is recommended to use MeSH terms).
5. Word count - excluding title page, abstract, references, figures and tables.

Manuscript format

The manuscript format must be presented in the following order:

1. Title page
2. Abstract (or summary for case reports)
3. Main text (tables should be in the same format as your article and embedded into the document where the table should be cited; images must be uploaded as separate files)
4. Acknowledgments, Competing interests, Funding
5. Copyright licence statement
6. References
7. Appendices

Do not use the automatic formatting features of your word processor such as endnotes, footnotes, headers, footers, boxes etc.

Provide appropriate headings and subheadings as in the journal. We use the following hierarchy: BOLD CAPS, bold lower case, Plain Text, *Italics*.

Cite illustrations in numerical order (fig 1, fig 2 etc) as they are first mentioned in the text.

Tables should be in the same format as your article and embedded into the document where the table should be cited.

Images must not be embedded in the text file but submitted as individual files (view further details in File Formats.)

Filenaming convention

Where possible, please name your manuscript and image files as shown below. (Please note: the manuscript ID # appears at the top of each submission page as soon as you start your submission; author refers to the corresponding author's last name.)

1. Your manuscript file should be named as: yr_manuscript id number_author

(for example: 2005_001234_clark)

2. Your image file should be named as: yr_manuscript id number_F#

(for example: 2005_001234_F1)

Statistics

Statistical analyses must explain the methods used.

Guidelines on presenting statistics.

Guidelines on RCTs: CONSORT, QUORUM, MOOSE, STARD, and Economic submissions.

Style

Abbreviations and symbols must be standard and SI units used throughout except for blood pressure values which are reported in mm Hg.

Whenever possible, drugs should be given their approved generic name. Where a proprietary (brand) name is used, it should begin with a capital letter.

Acronyms should be used sparingly and fully explained when first used.

Figures/illustrations

Black and white images should be saved and supplied as GIF, TIFF, EPS or JPEG files, at a minimum resolution of 300 dpi and an image size of 9 cm across for single column format and 18.5 cm for double column format.

Colour images should be saved and supplied as GIF, TIFF, EPS or JPEG files, to a minimum resolution of 600 dpi at an image size of 9 cm across for single column format and 18.5 cm

for double column format.

Images should be mentioned in the text and figure legends should be listed at the end of the manuscript.

During submission, when you upload the figure files please label them as Figure 1, Figure 2, etc. The file label will not appear in the pdf but the order in which the figures uploaded should be sufficient to link them to the correct figure legend for identification.

We can accept multi-page Powerpoint files. Alternatively, Powerpoint files can be saved as JPEG files and submitted as a standard image file.

Histograms should be presented in a simple, two-dimensional format, with no background grid.

Please note: Do not submit colour figures unless you are willing to pay the cost of publishing your figures in colour. If you do not wish to pay the colour charges please submit your figures in black and white.

The journal charges authors for the cost of reproducing colour images on all unsolicited articles. This charge is heavily subsidised by the journal and covers origination costs only. If an image is supplied as a composite figure that contains numerous parts (for example, fig 1A-D), the image will be considered as a single image, provided that all the parts are supplied within a single file that prints out at an overall size no larger that A4 (210 mm x 297 mm). The charge for colour processing will be £100 + VAT for the figure. Multi-part colour images supplied as separate files will be charged at £100 + VAT for each file. The charge only applies to images accepted for print publication and not online only or data supplement files.

Care should be taken in planning composites because combining different images with widely varying colours can lead to contamination or loss of colour and poor quality results.

When submitting your manuscript, please ensure to include a name and address where the invoice should be sent for the colour reproduction costs. If an address is not included, the invoice will be sent to the corresponding author.

Unacceptable file formats

Any file using OLE (Object Linking and Embedding) technology to display information or embed files, Bitmap (.bmp), PICT (.pict), Photoshop (.psd), Canvas (.cnv), CorelDRAW (.cdr); Excel (.xls); and locked or encrypted PDFs are not acceptable.

Tables

Tables should be submitted in the same format as your article and embedded into the document where the table should be cited. Please note: Bench>Press cannot accept Excel files. If your table(s) are in Excel, copy and paste them into the manuscript file. In extreme circumstances, Excel files can be uploaded as supplementary files; however, we advise against this as they will not be acceptable if your article is accepted for publication.

Tables should be self-explanatory and the data they contain must not be duplicated in the text or figures.

References

Authors are responsible for the accuracy of references cited: these should be checked against the original documents before the paper is submitted. It is vital that the references are styled correctly so that they may be hyperlinked.

In the text

References must be numbered sequentially as they appear in the text. References cited in figures or tables (or in their legends and footnotes) should be numbered according to the place in the text where that table or figure is first cited. Reference numbers in the text must be given in square brackets immediately after punctuation (with no word spacing) - for example.[6] not [6].

Where more than one reference is cited, separate by a comma - for example, [1, 4, 39]. For sequences of consecutive numbers, give the first and last number of the sequence separated by a hyphen - for example, [22-25]. References provided in this format are translated during the production process to superscript type, which act as hyperlinks from the text to the quoted references in electronic forms of the article.

In the reference list

References must be double spaced (numbered consecutively in the order in which they are mentioned in the text) in the [slightly modified] Vancouver style. Only papers published or in press should be included in the reference list. (Personal communications or unpublished data must be cited in parentheses in the text with the name(s) of the source(s) and the year. Authors should get permission from the source to cite unpublished data.)

Punctuation of references must follow the [slightly modified] Vancouver style:

12 Surname AB, Surname CD. Article title. Journal abbreviation. Year;Vol:Start page-End page.

Use one space only between words up to the year and then no spaces. The journal title should be in italic and abbreviated according to the style of Medline. If the journal is not listed in Medline then it should be written out in full.

List the names and initials of all authors if there are 3 or fewer; otherwise list the first 3 and add et al.

Example references:

Journal

13 Koziol-Mclain J, Brand D, Morgan D, et al. Measuring injury risk factors: question reliability in a statewide sample. Inj Prev 2000;6:148-50.

Chapter in book

14 Nagin D. General deterrence: a review of the empirical evidence. In: Blumstein A, Cohen J, Nagin D, eds. Deterrence and incapacitation: estimating the effects of criminal sanctions on crime rates. Washington, DC: National Academy of Sciences 1978:95-139.

Book

(personal author or authors) (all book references should have specific page numbers)

15 Howland J. Social norms and drunk driving countermeasures. In Graham JD, ed. Preventing automobile injury: new findings from evaluative research. Dover, MA: Auburn House Publishing Company 1988:163-96.

Abstract/supplement

16 Roxburgh J, Cooke RA, Deverall P, et al. Haemodynamic function of the carbomedics bileaflet prosthesis [abstract]. Br Heart J 1995;73 (suppl 2):P37.

Electronic citations

Basically, websites are referenced with their URL and access date, and as much other information is given as is available. Access date is important as websites can be updated and URLs change. The "date accessed" can be later than the acceptance date of the paper, and it can be just the month accessed. See the 9th edition of the AMA Manual of Style for further examples.

Electronic journal articles:

Morse SS. Factors in the emergency of infectious diseases. Emerg Infect Dis 1995 Jan-Mar;1(1). www.cdc.gov/nciod/EID/vol1no1/morse.htm (accessed 5 Jun 1998).

Use as much information as the author gives. The volume/number information in the URL will take the user to the start of the individual document; ask the author to supply or confirm. Also ask authors to supply the date they accessed the file.

Online First

Each Online First article has a unique Digital Object Identifier (DOI). This should be included in all citations.

BEFORE the article has appeared in an issue

Use the citation format:

Sabin MA, Ford AL, Holly JMP, Hunt LP, Crowne EC, Shield JPH. Characterisation of morbidity in a UK, hospital based, obesity clinic. Arch Dis Child. Published Online First: 24 October 2005. doi:10.1136/adc.2005.083485

AFTER the article has appeared in an issue

Use the citation format:

Sabin MA, Ford AL, Holly JMP, Hunt LP, Crowne EC, Shield JPH. Characterisation of morbidity in a UK, hospital based, obesity clinic. Arch Dis Child 2006; 91:126-130 doi:10.1136/adc.2005.083485 [published Online First: 24 October 2005].

Electronic Letters

Author. Title of letter. Journal name Online [eLetter] Date of publication. url

eg: Krishnamoorthy KM, Dash PK. Novel approach to transseptal puncture. Heart Online [eLetter] 18 September 2001. http://heart.bmj.com/cgi/eletters/86/5/e11#EL1

Check your citation information using PubMed.

Digital Object Identifiers (DOIs)

DOIs are a unique string created to identify a piece of intellectual property in an online environment, particularly useful for articles which have been published online before appearing in print (therefore the article has not yet been assigned the traditional volume, issue and page number reference).

The DOI is a permanent identifier of all versions of an article, whether raw manuscript or edited proof, online or in print. Thus the DOI should ideally be included in the citation even if you want to cite a print version of an article.

How to cite articles before they have appeared in print

To cite an electronic article that has not yet appeared in print please use the following citation format:

1. Alwick K, Vronken M, de Mos T, et al. Cardiac risk factors: prospective cohort study. Ann Rheum Dis. Published Online First: 5 February 2004. doi:10.1136/ard.2003.001234

How to cite articles once they have appeared in print

Once the article has been printed the citation should also include the traditional year, volume and page numbers, as well as the DOI and original date of publication.

1. Vole P, Smith H, Brown N, et al. Treatments for malaria: randomised controlled trial. Ann Rheum Dis 2003;327:765-8 doi:10.1136/ard.2003.001234 [published Online First: 5 February 2004].

More comprehensive guidance about DOI's.

PLEASE NOTE: RESPONSIBILITY FOR THE ACCURACY AND COMPLETENESS OF REFERENCES RESTS ENTIRELY WITH THE AUTHORS.

Supplementary files

You may submit supplementary material which may support the submission and review of your article. This could include papers in press elsewhere, published articles, appendices, video clips, etc.

Online only material

Additional figures and tables, methodology, references, video clips, raw data, etc may be published online only to supplement the printed article. If your paper exceeds the word count you should consider if any of the article could be published online only as a "data supplement". These files will not be copyedited or typeset.

Bench>Press

All supplementary data files should be uploaded to Bench>Press using the supplementary file section. These files are not converted to PDF but will be provided to reviewers and editors in the format in which you supply them.

Anexo 3 – Aprovação do projeto de pesquisa intitulado “Estudo de ensaio clínico para avaliar a eficácia do tratamento tópico da leucoplasia pilosa bucal com solução alcoólica de podofilina a 25% associada ao penciclovir creme a 1%” pelo COEP/UFMG

UFMG

Universidade Federal de Minas Gerais
Comitê de Ética em Pesquisa da UFMG - COEP

Parecer nº. ETIC 367/06

Interessado: Prof. Ricardo Alves de Mesquita
Depto. de Clínica, Patologia e Cirurgia Odontológicas
Faculdade de Odontologia-UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 29 de novembro de 2006, o projeto de pesquisa intitulado **“Estudo de ensaio clínico para avaliar a eficácia do tratamento tópico da leucoplasia pilosa bucal com solução alcoólica de podofilina a 25% associada ao peniclovir creme a 1%”** bem como o Termo de Consentimento Livre e Esclarecido do referido projeto.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Dra. Maria Elena de Lima Perez Garcia
Presidente do COEP/UFMG

Av. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar - Sala 2005 - 31270-901 – BH - MG
(31) 3499-4592 - FAX: (31) 3499-4516 - coep@prpq.ufmg.br

Anexo 4 – Termo de consentimento livre e esclarecido do “Estudo de ensaio clínico para avaliar a eficácia do tratamento tópico da leucoplasia pilosa bucal”

Termo de consentimento livre e esclarecido

UNIVERSIDADE FEDERAL DE MINAS GERAIS

FACULDADE DE ODONTOLOGIA

DEPARTAMENTO DE CLÍNICA, PATOLOGIA E CIRURGIA ODONTOLÓGICAS

Belo Horizonte, ___de _____ de 20____.

Prezado paciente e/ou responsável,

Estamos realizando uma pesquisa, com objetivo de tratar a leucoplasia pilosa bucal, que é uma lesão presente na boca (borda lateral da língua) e freqüente nos pacientes HIV positivos. Utilizaremos para o tratamento os seguintes medicamentos: podofilina e penciclovir. São medicamentos de uso tópico/local e as aplicações desses dois medicamentos serão feitas somente sobre a lesão, no consultório odontológico do Centro de Treinamento e Referência em Doenças Infecciosas e Parasitárias Orestes Diniz (CTR/DIP), em Belo Horizonte/MG. Gostaríamos de poder contar com sua colaboração, esclarecendo que:

1- A pesquisa consiste em preenchimento de prontuário odontológico do CTR/DIP e aplicação tópica dos medicamentos listados acima, somente nos pacientes portadores dessa lesão, realizados por somente uma dentista, uma vez por semana, até o desaparecimento completo da lesão.

2- O tratamento da leucoplasia pilosa bucal e o preenchimento do prontuário odontológico será realizado independente do paciente aceitar em participar desse estudo. Só participarão da pesquisa aqueles que concordarem, o que implica em assinar o termo de consentimento livre e esclarecido.

3- Este tratamento é feito por meio de material descartável, individual (haste de plástico com algodão, nome comercial cotonete^{R}, luvas e gazes), para aplicação do medicamento em borda lateral de língua.

4- A lesão pode voltar a aparecer após o tratamento, por isso realizaremos o seu acompanhamento após seis meses e após um ano do término do tratamento. Se isso acontecer, você terá a opção de realizar novamente o tratamento. Além disso, você pode vir a apresentar, após cada tratamento, uma sensação de gosto desagradável e/ou amargo, que fica na sua boca por um período de tempo de no máximo duas horas após a aplicação. As aplicações serão realizadas uma vez por semana até a lesão desaparecer, ou com um máximo de 25 aplicações, ou seja, 25 semanas de tratamento no máximo.

5- Os benefícios desse tratamento são: desaparecimento da lesão apenas com o tratamento tópico, pois a leucoplasia pilosa é um local favorável para o acúmulo de microorganismos, principalmente fungos; além de favorecer uma melhora na qualidade de vida, no sentido de fazer com que a sua língua volte a apresentar seu aspecto normal, melhorando a estética da língua para aqueles que têm isso como queixa.

6- Todos os examinadores são dentistas e pesquisadores e estão aptos a fazer esse exame e tratamento.

7- Cada paciente receberá o tratamento dentário completo (que consiste não só no tratamento das lesões de leucoplasia pilosa, mas no tratamento de outras lesões bucais, que possivelmente podem estar presentes na cavidade bucal) e poderá contar com atendimento no setor de odontologia do CTR.

8- Todos os seus dados serão confidenciais, sua identidade não será revelada publicamente, em hipótese alguma, e somente os pesquisadores envolvidos neste

projeto terão acesso a estas informações, que serão utilizadas somente para fins de pesquisa e de divulgação de jornais e/ou revistas científicas especializadas no País e no Exterior.

9- Esta pesquisa foi aprovada pelo Comitê de Ética em Pesquisa da UFMG (COEP). Qualquer dúvida entre em contato com a COEP, no telefone (31) 3499-4592.

10- Não será prevista qualquer forma de remuneração para o voluntário, e todas as despesas relacionadas com o estudo são de responsabilidade da Faculdade de Odontologia da UFMG e do Centro de Treinamento Referencial de Doenças Infecto Parasitárias (CTR/DIP).

11- Todos os participantes poderão, a qualquer momento, desistir de fazer parte dessa pesquisa, sem prejudicar o tratamento realizado no ambulatório. E a lesão presente na sua boca será tratada mesmo se você não desejar participar do estudo.

12- Também os dados coletados de cada paciente poderão ser excluídos, a qualquer momento, a critério do sujeito da pesquisa.

Desde já, agradecemos sua colaboração. Os telefones dos pesquisadores para quaisquer esclarecimentos são: Mariela Dutra Gontijo de Moura 9949-0282 e Prof. Dr. Ricardo Alves de Mesquita 3499-2478.

TERMO DE CONSENTIMENTO

Eu, _____

estou ciente de ser portador de leucoplasia pilosa na borda lateral da minha língua. Apresentando este diagnóstico clínico, concordo em participar desse estudo que objetiva avaliar a eficácia do tratamento tópico da LPB, que será realizado com o uso simultâneo de dois medicamentos (podofilina e penciclovir) já consagrados e aceitos pela literatura.

Após entender os objetivos e métodos da pesquisa anteriormente' descritos, voluntariamente autorizo e aceito participar desta pesquisa, que faz parte do Doutorado em Estomatologia do Departamento de Clínica, Patologia e Cirurgia da Faculdade de Odontologia da Universidade Federal de Minas Gerais. Estou esclarecido(a) de que poderei contar com o atendimento do CTR/DIP Orestes Diniz.

Tenho pleno conhecimento de que o principal objetivo é o tratamento desta lesão bucal. Dou pleno direito de uso, para fins de pesquisa, e de divulgação de jornais e/ou revistas científicas especializadas no País e no Exterior.

_____

(Assinatura do paciente ou responsável)

Documento de Identidade: _____

Anexo 5 – Aprovação do projeto de pesquisa intitulado “Estudo do conhecimento e do comportamento dos odontólogos em relação ao atendimento odontológico a pacientes HIV positivos” pelo COEP/UFMG

Universidade Federal de Minas Gerais
Comitê de Ética em Pesquisa da UFMG - COEP

Parecer nº. ETIC 545/07

Interessado(a): Prof. Ricardo Alves de Mesquita
DCPCO
Faculdade de Odontologia-UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 28 de novembro de 2007, o projeto de pesquisa intitulado **"Estudo do conhecimento e do comportamento dos odontólogos em relação ao atendimento odontológico a pacientes HIV positivos"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – Cep31270-901 – BH-MG
Telefone: (031) 3499-4592- FAX: (031)3499-4516 - e-mail: coep@prpq.ufmg.br

Anexo 6 – Termo de consentimento do questionário de conhecimento dos dentistas argentinos sobre HIV/AIDS

Consentimento Informado

UNIVERSIDADE NACIONAL DE CÓRDOBA - FACULTAD DE ODONTOLOGÍA

Córdoba, ___de _____ de 200____.

Estimado Odontólogo,

Estamos realizando una investigación, con el objetivo de analizar el conocimiento y la actitud de los odontólogos de Córdoba, Argentina, a cerca de la infección por HIV y comparar los resultados entre la población argentina y la brasileña.

Para eso, utilizaremos un cuestionario. El siguiente cuestionario está elaborado para saber cuáles son sus conocimientos sobre la infección por HIV. Su participación será muy valiosa y podrán contestar en forma anónima. Los datos que obtendremos nos permitirán definir estrategias que faciliten su formación.

Nos gustaría poder contar con su colaboración, aclarando que:

1- La investigación consiste en un cuestionario en castellano con preguntas de múltiple opción incluida una colecta de datos personales, como edad, sexo y fecha de nacimiento. Las demás preguntas sobre accidentes con materiales punzo cortantes contaminados con sangre, sobre controles de infección utilizados, conocimientos sobre manifestaciones bucales mas comunes en el paciente portador de HIV/SIDA y modos de transmisión del HIV.

2-Todos sus datos y respuestas serán confidenciales y solamente la investigadora interiorizada en este proyecto tendrá acceso a esas informaciones, que serán utilizadas solamente para los fines de la investigación y de la divulgación en diarios y /o en revistas científicas especializadas en el País y en el Exterior.

3-Solamente participarán de la investigación aquellos que aceptarán, lo que implica firmar el consentimiento informado.

4-El consentimiento informado y el cuestionario serán recogidos inmediatamente después de su obtención.

5-Los datos recolectados de cada participante podrán ser excluidos, a cualquier momento, a criterio de la investigadora.

6-Todos los cuestionarios correspondientes a cada persona incluida en la investigación serán mantenidos en archivo por 5 años después de finalizada la misma, respetando la confidencialidad y el sigilo. Después de ese período los cuestionarios serán descartados.

Desde ya agradecemos su colaboración. Los teléfonos para cualquier consulta son:

Mariela Moura 152321356 o 5269000 (Habitación 1002).

CONSENTIMIENTO

Yo,_____

estoy de acuerdo con las informaciones recibidas, concuerdo en participar en el estudio que tiene por objetivo avalar el conocimiento y el comportamiento acerca de la infección por HIV.

Después de entender los objetivos y métodos de la investigación anteriormente descripta, voluntariamente autorizo y acepto participar de esa investigación, que es parte del Doctorado en Estomatología de la Facultad de Odontología de la Universidad Federal de Minas Gerais, Brasil.

Doy pleno derecho de uso para fines de investigación y de divulgación en diarios y /o en revistas científicas especializadas en el País y en el Exterior.

_____

(Firma del participante)

DNI (Documento Nacional de Identidad) _____