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LIDIA YILENG TAY CHU JON

EFEITO CLÍNICO DO USO TÓPICO DE *UNCARIA TOMENTOSA*WILLD D.C.EM PACIENTES COM ESTOMATITE PROTÉTICA

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Tese de Doutorado apresentada como requisito à obtenção do título de Doutor na Universidade Estadual de Ponta Grossa, no Curso de Doutorado em Odontologia – Área de concentração em Clínica Integrada

Orientador: Prof. Dr. Fabio André dos Santos

Co-orientadora: Profa. Dra. Brenda Paula Gomes

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DADOS CURRICULARES

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RESUMO

Tay LY. Efeito clínico do uso tópico de *Uncaria tomentosa*Willd D.C*.* em pacientes com estomatite protética. [Tese de Doutorado em Clínica Integrada – Faculdade de Odontologia]. Ponta Grossa: Universidade Estadual de Ponta Grossa; 2013.

A Estomatite protética (EP) é uma doença inflamatória crônica que afeta usuários de prótese removível, sendo a *Candida spp.* o principal agente etiológico. *Uncaria tomentosa*, conhecida como unha de gato, é utilizada usualmente na medicina alternativa para o tratamento de diversas doenças. O propósito deste estudo clínico randomizado duplo cego foi determinar a eficácia de um gel de *Uncaria tomentosa Willd D.C.* contra a EP. Cinquenta pacientes portadores de EP foram distribuídos aleatoriamente em três grupos para receber tratamento contra a doença: grupo M: gel de miconazol 2% como controle positivo; grupo P: gel de hydroxietilcelulose (natrosol) como placebo; e grupo UT: gel de *Uncaria tomentosa* 2% como o grupo experimental. Os pacientes receberam uma bisnaga contendo o medicamento de acordo com o grupo e foram instruídos para aplicar o gel na base da prótese três vezes ao dia durante uma semana. Além disso, instruções sobre a higiene bucal e das próteses foram prescritas a todos os pacientes. Os pacientes foram avaliados inicialmente (dia 0), após uma semana de tratamento (dia 7) e uma semana após terminado o tratamento (dia 14). A efetividade do tratamento foi avaliada clinicamente pela utilização dos critérios de Newton para grau de EP. Além disso, exame micológico foi realizado contando as unidades formadoras de colônias por mililitro (UFC/mL) de amostras tomadas da mucosa do palato e da superfície interna da prótese. As*Candida spp.*foram identificadas com o HiCrome Candida Agar e submetidas ao teste bioquímico Api 20 C AUX. Não houve diferença significativa entre os três grupos ($p>0,05$) segundo o teste estatístico Kruskal-Wallis e o teste de comparação múltipla de Dunn. Pôde-se observar clinicamente uma diminuição no grau de EP e na análise microbiológica redução significativa da contagem de UFC/mL, após uma semana de tratamento ($p<0,05$) a qual permaneceu semelhante após 14 dias ($p>0,05$) nos três grupos avaliados. Foi possível reconhecer *C. albicans, C. tropicalis, C. glabrata e C. krusei* encontradas nas amostras dos pacientes com EP. Pode-se concluirque o gel de *Uncaria tomentosa* teve o mesmo efeito que o agente antifúngico miconazol 2%.

Palavras-chave: Estomatite protética, Candida spp, *Uncaria tomentosa.*

ABSTRACT

Tay LY.Clinical effect of topical Uncaria tomentosa Willd DC in patients with denture stomatitis.[Tese de Doutorado em Clínica Integrada – Faculdade de Odontologia]. Ponta Grossa: Universidade Estadual de Ponta Grossa; 2013.

Denture stomatitis is a chronic oral disease that affects denture wearers. It presents as an inflammatory reaction in denture-bearing patients, under maxillary prosthesis. *Candida spp.* has been reported as the principal etiological agent. Despite the existence of a great number of antifungal agents, treatment failure is observed frequently. *Uncaria tomentosa*Willd D.C., known as cat's claw, is habitually used in alternative medicine for the treatment of several diseases. The aim of this clinical study was to determine the efficacy of *Uncaria tomentosa* gel against denture stomatitis (DS). Fifty patients with DS were randomly assigned into three groups to receive either 2% miconazole gel as a positive control (Group M) or 1.5% hydroxyethylcellulose (natrosol) as a placebo group (Group P) or 2% *Uncaria tomentosa* gel as the experimental group (Group UT). All patients received their treatment on admission and were told to apply the gel on the base of the denture three times a day for a period of 1 week. In addition, instructions for oral and denture hygiene were prescribed to all patients. DS level was registered initial (day 0), after 1 one week of treatment (day 7) and 7 days after treatment (day 14). The effectiveness of treatment was assessed clinically using Newton's criteria for severity degree of DS; mycological examination was performed counting colony-forming units per milliliter (CFU/mL) from samples of the palatal mucosa and from the tissue surface or the upper denture. *Candida spp.* were identified with HiCrome Candida and submitted to the biochemical test API 20 C AUX. Results were statistically evaluated using Kruskal-Wallis test and Dunn's multiple comparsion test. Clinically it was observed decrease in the severity of DS in all groups ($p<0.05$). At the mycological examination, significant reduction in number of CFU/ml after one week of treatment ($p<0.05$) which remained after 14 days ($p>0.05$) were found in the three groups with no significant difference among the three groups ($p < 0.05$). It was possible to identify*C. albicans, C. tropicalis, C. glabrata* and *C. krusei* found on the samples of DS patients. Furthermore, it was observed that *Uncaria tomentosa* gel has the same effect as the antifungal agent.

Keywords: Denture stomatitis, Candida spp, *Uncaria tomentosa.*

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1 INTRODUÇÃO

Há aumento da população idosa em vários países desenvolvidos ou em desenvolvimento, o qual pode indicar problemas de saúde potenciais em certas populações (Evren et al.[1] 2011). Na odontologia, existe maior ênfase na manutenção da saúde bucal nos idosos, já que as alterações nos tecidos bucais estão associadas com outras condições (Ferreira et al.[2] 2010). A candidose bucal é a infecção fúngica mais comum entre os humanos, afetando principalmente idosos, usuários de próteses e pessoas imunocomprometidas. Pode acometer entre 15 a 70% das pessoas idosas que utilizam prótese dental, principalmente total superior, podendo também aparecer em menor frequência na mucosa de suporte das próteses parcial removível(Budtz-Jörgensenet al.[3]1975,Jorge et al.[4] 1991, Rabiei et al.[5] 2010). Quando esta infecção está relacionada com o uso de próteses é chamada de Estomatite Protética (EP), também encontrada na literatura com outras nomenclaturas como estomatite por dentadura, estomatite por Candida e candidíase atrófica crônica. Dependendo da população estudada e/ou métodos diagnósticos, a epidemiologia da EP tem uma grande variabilidade (Gendreau, Loewy[6] 2011).

A EP é uma reação inflamatória dos tecidos de suporte das próteses totais e parciais removíveis que se caracteriza por hiperemia e edema, acompanhados algumas vezes por petéquias hemorrágicas, podendo a inflamação ser moderada ou intensa, e raramente o processo é sintomático. A etiologia mostra-se extremamente variável, sendo considerada de causa multifatorial (Glass, Belobraydic[7] 1990, Webb et al.[8,9] 1998). Presença de biofilme, infecção por *Candida spp.*, trauma, uso contínuo da prótese e hiposalivação constituem fatores predominantes nesta alteração. Quando a doença apresenta sintomas clínicos, estes podem ser: dor e irritação, entretanto, muitos pacientes portadores desta doençanão apresentam sintomatologia. A *Candida albicans* é a espécie mais comumente encontrada neste tipo de infecção. Outras espécies de Candida, como *C. tropicalis, C. krusei* e *C. glabrata* também foram identificadas, porém em menores porcentagens (Dorko et al.[10] 2001, Cross et al.[11] 2004, Coco et al.[12] 2008, Dagistan et al.[13] 2009, Rautemaa, Ramage[14] 2011, Sanitá et al.[15] 2011).

Diversos são os tratamentos propostos para a EP. A utilização de antifúngicos de aplicação tópica ou sistêmica, orientação do paciente quanto à higienização da prótese e substituição das próteses antigas são parte do protocolo de tratamento mais usado (Aldana et al.[16] 1994). Além disso, é consenso na literatura que a suspensão do uso da prótese favorece a diminuição do componente inflamatório. As principais classes de agentes antifúngicos usados constituem-se de: polienos (nistatina e anfotericina B) e azóis. Este último grupo se divide em imidazóis (clotrimazol e miconazol) e triazóis (fluconazol, cetoconazol e itraconazol). Atualmente, o tratamento da EP tem se tornado cada vez mais difícil e oneroso, em decorrência de fatores como resistência fúngica e toxicidade dos medicamentos (Chandraet al.[17] 2001,Tobudicet al.[18] 2012). Dessa forma, métodos alternativos de tratamentos devem ser considerados (Amanlou et al.[19] 2006, Reding et al.[20] 2009).

Existe crescente interesse dos países desenvolvidos procurarem alternativas complementares para melhorar as condições de saúde da população (Marshal[21] 2000), sendo que a fitoterapia, a ciência que usa as plantas medicinais, é uma alternativa viável para atingir esse objetivo. A literatura hoje nos mostra uma diversidade de fitoterápicos para diversos tratamentos de doenças principalmente as de origem inflamatória. O uso das plantas medicinais tem custo baixo em comparação com os medicamentos convencionais, podem chegar a ser 70% mais econômico,o que despertou grande interesse em pesquisas nesta área.

Das plantas amazônicas, a mais promissora é a *Uncaria tomentosa* (Willd.) D.C., conhecida como unha de gato. Esta é uma planta que pertence à família *Rubiaceae*, às quais se atribuem muitos usos em medicina tradicional. A tribo Ashaninka, da Amazônia peruana, tem usado a *U. tomentosa* por séculos para cicatrização de feridas e úlceras, febre, dor de cabeça, dores estomacais e infecções por bactérias e fungos (Williams[22] 2001, Mammone et al.[23] 2006). Estudos científicos têm permitido aos pesquisadores colaborarem com o conhecimento das propriedades farmacológicas da *U. tomentosa*. Diversos artigos (Wurm et al.[24] 1998, Lemaire et al.[25] 1999, Hardin[26] 2007), foram encontrados a respeito da utilização da *U. tomentosa* como tratamento farmacológico e terapêutico, incluindo estudos pré-clinicos em animais e estudos em humanos, demonstrando a importância dessa planta medicinal. Têm sido reportados estudos que apresentam a efetividade da *U. tomentosa* na artrite, pela sua atividade antiinflamatória; no

tratamento do câncer, pelas suas propriedades antimutagénicas e imunoestimulantes; como antiviral antibacteriano e antifúngico. A resistência dos fungos às drogas disponíveis no mercado tem sido reportada como uma preocupação, sendo de interesse a descoberta de alternativas no tratamento antifúngico (Walker[27] 2000).

As propriedades da *U.tomentosa* podem ser atribuídas à atividade combinada dos seus diferentes compostos e não pelas propriedades de qualquer produto isolado (Ribeiro et al.[28] 2013). Além disso, *U.tomentosa* é fonte importante de substâncias bioativas, como os alcaloides óxidos monoterpenoides, os quais têm propriedades imunomodulatórias e antitumorais (Heitzman et al.[29] 2005). Diversos estudos (Lemaire et al.[25] 1999, Keplinger et al.[30] 1999, Sandoval et al.[31] 2002, Herrera et al.[32] 2010, Santos Araújo et al.[33] 2012)demonstram que o uso de fitoterápicos no tratamento contra candidose podem ser tão efetivos quanto os antifúngicos convencionais, tornando a utilização de fitoterapia uma opção viável, efetiva e econômica. Partindo do extrato liofilizado de *U. tomentosa* e utilizando o natrosol como base de gel inerte, é possível obter um gel de *U. tomentosa* emconcentrações de 2%, o qual tem mostrado efeito antifúngico sobre colônias de *Candida albicans* (Herrera et al.[32] 2010). Diante do exposto anteriormente, pode se supor que tal gel possa ser efetivo no tratamento da EP.

2 PROPOSIÇÃO

2.1 Objetivo geral:

Avaliar a efetividade do uso tópico do gel*Uncaria tomentosa*a 2% sobre o tratamento da Estomatite Protética (EP) em pacientes usuários de prótese total superior.

2.2 Objetivos específicos:

- Determinar a atividade dos produtos testados no tratamento da EP e no controle de *Candida spp*.
- Determinar a prevalência da EP em pacientes usuários de prótese total e relacionar a doença com a idade, gênero e tempo de uso da prótese.
- Identificar as espécies de *Candida* presentes em pacientes com EP.

3 MATERIAIS E MÉTODOS

3.1 Desenho do estudo

Este estudo clínico randomizado duplo cego foi aprovado pela Comissão de Ética em Pesquisa da Universidade Estadual de Ponta Grossa (UEPG), Paraná, Brasil (Parecer 14/2011; Protocolo 17572/10) (Anexo 1).

Para o estudo, foram avaliados 204pacientes portadores de prótese total removível da cidade de Ponta Grossa – Paraná – Brasil, que foram atendidos na clínica de prótese da UEPG.Os critérios de inclusão do estudo foram pacientes entre 45 e 85 anos de idade, com boa saúde geral, usuários de prótese total superior por pelo menos um ano, portadores EP, selecionados segundo os critérios de Newton (Newton[34], 1962) em EP tipo I, II e III. Foram excluídos indivíduos imunossuprimidos ou sistemicamente comprometidos, pacientes que estavam sob algum protocolo de medicamentos imunossupressores, antibióticos e/ou antimicóticos. Não foram considerados para este estudoos pacientes com necessidades especiais, deficiências físicas, ou com destreza manual comprometida.

3.3 Seleção dos pacientes

Dos 204 pacientes que participaram do estudo, 50 foram selecionados para participar do estudo clínico da eficacia da Uncaria tomentosa no tratamento da EP. Este estudo foi realizado de acordo com os critérios mencionados por meio de questionário de saúde geral e exame clínico inicial. Foram selecionados 50 pacientes, os quais assinaram o Termo de Consentimento Livre e Esclarecido, autorizando a realização da pesquisa (Anexo 2).

Baseado em dados de estudos clínicos prévios (Amanlou et al.[19] 2006, Neppelenbroek et al.[35] 2008, Santos et al.[36], 2008) foi realizado um cálculo amostral resultando em uma amostra mínima de 15 pacientes por grupo. Os pacientes selecionados foram randomizados por sorteio e distribuídos

aleatoriamente por estratos em três grupos. Todos os pacientes avaliados receberam instruções de higiene bucal e da prótese.Os pacientes receberam diferentes tipos de tratamentos tópicos de acordo com cada grupo. O grupo M(n=16) recebeu uma bisnaga contendo 20 g de miconazol em gel (20 mg/gr, Daktarin gel, Janssen-Cilag, São Paulo, Brasil) como o controle positivo; o grupo P(n=17) recebeu uma bisnaga contendo 20 g de natrosol gel (2%, Fleming Manipulações, Ponta Grossa, Brasil) como placebo; e finalmente, o grupo UT(n=17) recebeu uma bisnaga contendo20 g de *U. tomentosa* em gel 2% (Fleming Manipulações, Ponta Grossa, Brasil) como o grupo experimental. A utilização de um hidrogel (Natrosol) na formulação do gel de *U. tomentosa* neste estudo justifica-se pela necessidade de controlar o escoamento do produto final nas próteses.

3.4 Avaliação dos grupos

O delineamento do estudo foi duplo cego, no qual nem o paciente, nem o avaliador tinham conhecimento do grupo para o qual o voluntario foi designado. Para isso, todos os géis foram entregues num mesmo tipo de embalagem (Figura 1) e os mesmos foram distribuídos para os grupos pelo pesquisador responsável pela pesquisaque não participou das análises.

Os pacientes de todos os grupos foram instruídos a aplicarem os géis três vezes ao dia após a limpeza da prótese e higienização bucal, durante sete dias. Os géis foramaplicados na superfície interna da prótese e imediatamente colocados na boca e mantida em contato com a mucosa. Todos os pacientes, independentemente dos grupos a que pertenciam, receberam, de forma escrita e falada, instruções de higiene bucal e das próteses, incluindo: escovar as próteses e a mucosa palatal com escova macia e dentifrício e retirar as próteses para dormir deixando-as imersas em água filtrada (Neppelenbroek et al.[35] 2008; Ribeiro et al.[37] 2012).

Figura 1: Bisnaga (20g)usada para armazenar o gel dos três produtos.

A condição clínica de cada voluntário do estudo foi monitorada pelo pesquisador durante os seguintes períodos: tempo zero, após 7 dias de tratamento e 7 dias após o término do tratamento. Todos os sinais e sintomas que ocorreram foram analisados durante as visitas, sendo questionados com perguntas diretas. Todos os efeitos adversos, que por ventura fossem observados pelo pesquisador ou relatados voluntariamente pelo sujeito da pesquisa, seriam registrados na ficha clínica.

A efetividade dos tratamentos foi avaliada clinicamente com base nos critérios de Newton[34] (1962) para os graus de severidade de EP antes, após 7 dias de tratamento e 7 dias após finalizado o tratamento, sendo atribuídos os seguintes escores: 0 = palato saudável; 1= hiperemia puntiforme (EP Tipo I de Newton); 2 = hiperemia difusa (EP Tipo II de Newton); e 3 = hiperemia granular (EP Tipo III de Newton) (Figura 2). Nestes períodos, as mucosas palatinas de todos os pacientes foram fotografadas. Todas as fotografias foram tomadas com a mesma câmera digital (EOS Rebel T2i; Canon, Tokyo, Japão), pelo mesmo operador sob as mesmas condições (lugar, luz, ângulo e posição do paciente) para facilitar a reprodutibilidade. Dois observadores cegos calibrados, com experiência na área de estomatite,analisaram as fotografias tomadas de cada paciente.

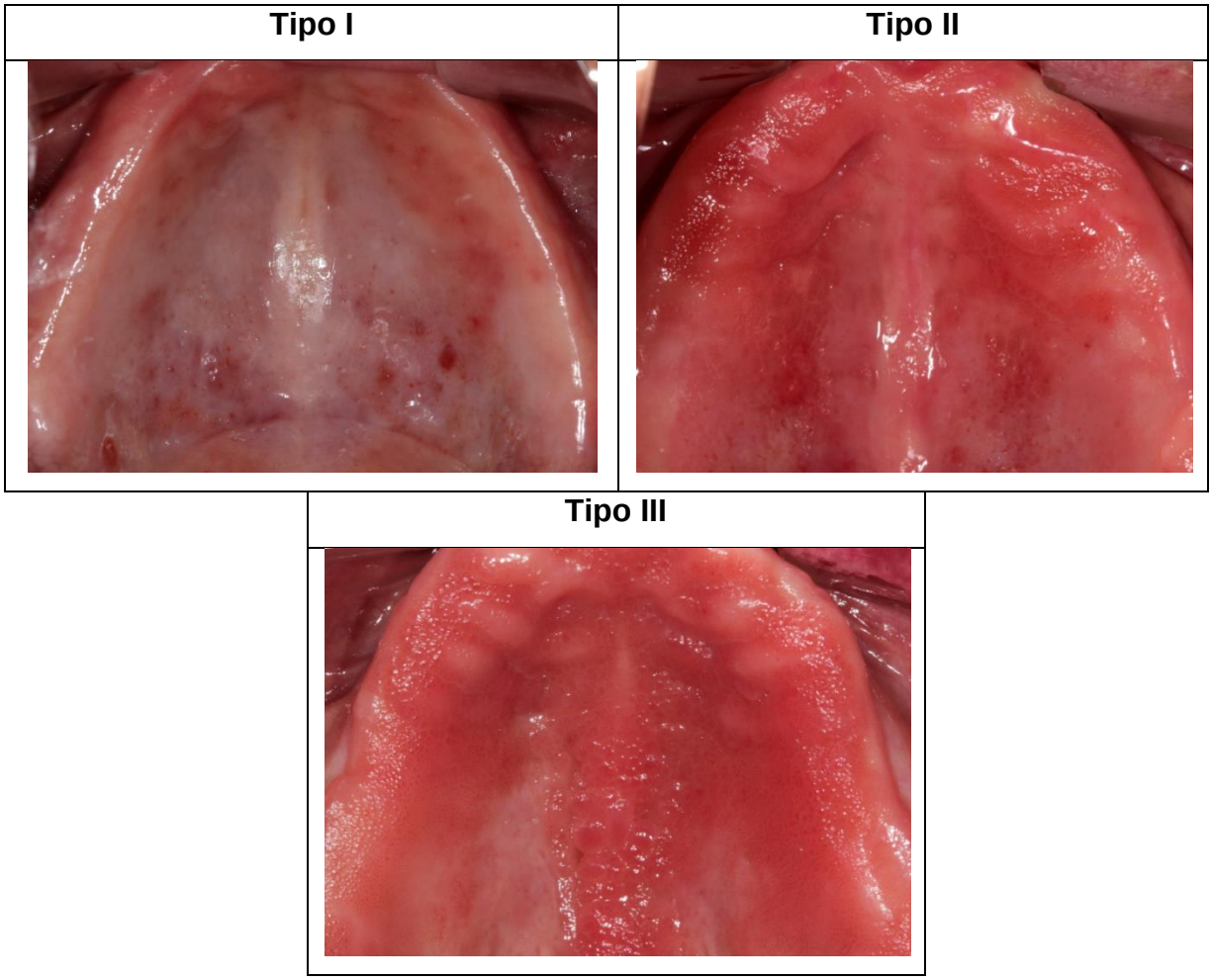

Figura 2: Aspectos clínicos da estomatite protética, segundo a classificação de Newton[34].

Além do exame clínico, para avaliação da efetividade dos tratamentos propostos, o método de contagem de colônias viáveis foi utilizado. Para isso, amostras de material da mucosa palatina de cada paciente e da superfície palatina de suporte de cada prótese foram coletadas por meio de swab oral (Neppelenbroeket al.[35] 2008; Ribeiro et al.[37] 2012). Foram feitas coletas antes do tratamento, após os 7 dias de tratamento e 7 dias após finalizado o tratamento. Após cada coleta, os swabs foram imersos em tubo de ensaio contendo 5 mL de meio de cultura BHI (HiMedia Laboratories Pvt. Ltd., Mumbai, Índia). Os tubos de ensaio foram agitados no vortex durante 30 s para promover a suspensão dos microrganismos dos swabs (CB Products Ind. e Com. Ltda, Brasil). 50 µL da

suspensão foram semeadas em duplicatas nas placas de Petri contendo Ágar Sabouraud com gentamicina (HiMedia Laboratories Pvt. Ltd., Mumbai, Índia). Outros 50 µL da suspensão foram semeadas em duplicatas em placas de Petri contendo HiCrome Candida Agar (Himedia Laboratories – Mumbai – India) para identificação das espécies de Candida segundo as cores das colônias (Quadro 1).As placas de Petri foram incubadas a 37 °C por 48 h e assim as colônias viáveis foram contadas em um contador de colônia digital (CP 600 Plus; Phoenix Ind. Com. Equipamentos Científicos, Araraquara, Brazil) e o número de unidades formadoras de colônias por mililitro (UFC/mL) foi determinado. As UFC/mL da mucosa do palato foi correlacionada com as UFC/mL da superfície interna da prótese superior.

Quadro1: Tipificação das espécies de *Candida spp*. de acordo com a cor da cultura no HiCrome Candida Agar.

Organismo	**Cor da cultura**
Candida albicans	Verde
Candida glabrata	Creme ou branco
Candida krusei	Roxo, penugem
Candida tropicalis	Azul

As colônias no HiCrome Candida foram identificadas pela cor das colônias (Figura 3) e submetidas ao teste bioquímica para confirmação das identificações. Uma colônia de cada cor no HiCrome Candidafoi transferida ao Agar Sabouraud para obter colônias de uma mesma espécie de cor branca. Após as 48 h a 37 °C, os fungos isolados foram identificados segundo o teste bioquímico Api 20 C AUX (BioMérieux SA, Marcy-l'Etoile, França)o qual é um sistema de identificação preciso para a maioria dos fungos encontrados da boca; para isso, foi retirada parte de uma colônia e colocada em 2mL de NaCl até chegar ao grau 2 de turbidez McFarland, desse tubo foi retirado 100µL e colocados num tubo com 7mL de C Medium (Api 20 C AUX), esse tubo foi homogenizado com o vortex e foram levados 200µL dessa amostra para cada microtubo da placa do Api 20 C AUX.

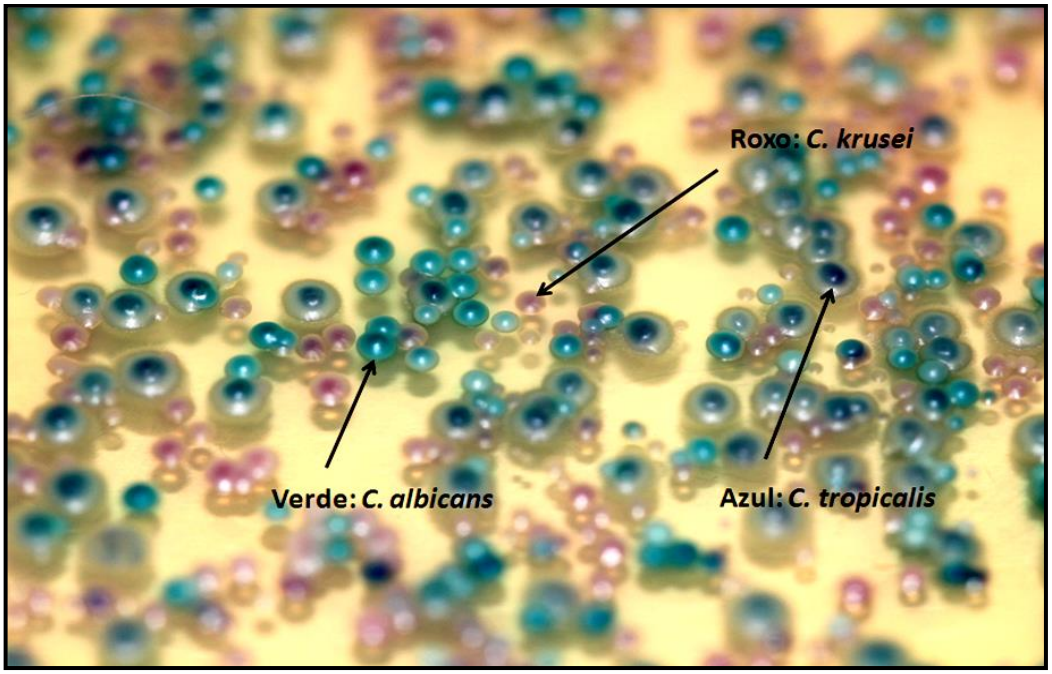

Figura 3: Colônias de *Candida spp*. identificadas por cor no HiCrome Candida.

O Api 20 C AUX consiste em 20 microtubos contendo substratos desidratados para demonstração da atividade enzimática ou fermentação de açúcares (Figura 4). Os microtubos são inoculados com um meio semi-sólido e os fungos crescem apenas se forem capazesde utilizar cada substrato. Para leitura, as placas com as amostras foram colocadas em estufa a 37 °C e após as 48 h as reações foram lidas anotando o código se o teste para cada microtubo deu positivo (turbidez) ou negativo (transparente), isso da um código de identificação o qual é analizado pelo programa computadorizado de identificação Apiweb software (BioMérieux SA, Marcy-l'Etoile, França) e da como resultado a possibilidade de espécie de Candida analizada.

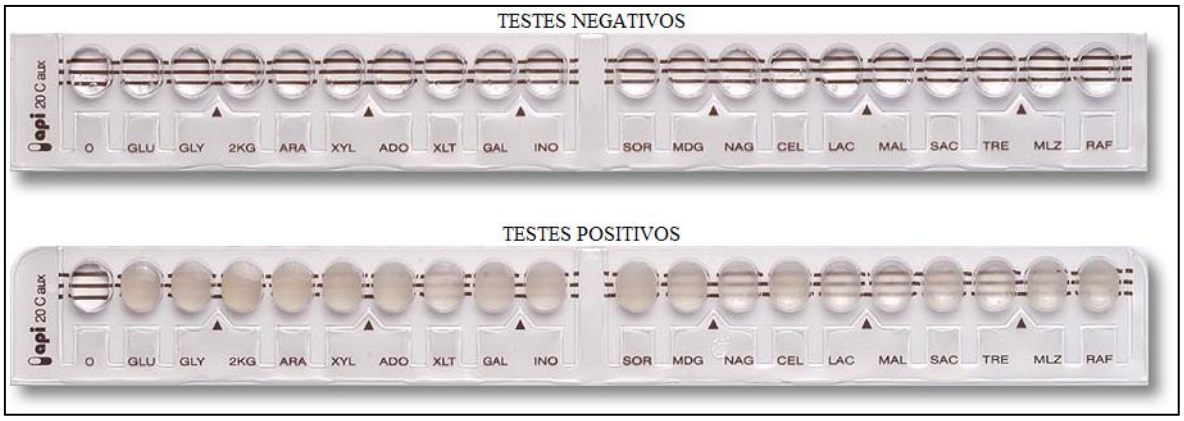

Figura 4: Placa com 20 microtubos Api 20 C AUX, cada uma contendo substratos para identificação de atividade enzimática.

3. 2 Teste de PCR simples para identificação de *Candida spp.*

Foi realizado o teste de PCR (Polimerase Chain Reaction) para identificação de *Candida spp.*somente em pacientes com sinais clínicos de EP, amostras de material da mucosa palatina de cada paciente e da superfície palatina de suporte de cada prótese foram coletadas por meio de swab oral. Este foi colocado num tubo de ensaio com 5 mL de BHI (Brain-Heart Infusion) e agitados no vortex durante 30 s para promover a suspensão dos microrganismos dos swabs. Essas amostras foram levadas para identificacao de *C. albicans, C. tropicalis, C. krusei, C. glabrata, C. guillermondi and C. dubliniensis*mediante o teste de PCR simples.

De cada amostra foi extraidoo DNA utilizando o QIA amp DNA kit (QIAGEN, Chatsworth, CA, USA) segundo as indicações do fabricante. Obtendo 20 µL de DNA de cada amostra. Além dessas amostras foi utilizado um DNA puro de cada microrganismo como controle positivo e água estéril Mili Q como controle negativo.

Candidaspp. foi identificado usando *primers* específicos de*C. albicans, C. tropicalis, C. krusei, C. glabrata, C. guillermondi and C. dubliniensis.* As sequencias dos *primers* e os ciclos são mostrados na Tabela 1. As reações foram realizadas em microtubos de 200µL num volume total de 20 µL por cada amostra, contendo2 µL de DNA extraído de cada amostra; 2 µL de 10X PCR tampão; 0,6 µL

de 50 mmol/L $MgCl_2$; 1,6 µL da mistura de trifosfato deoxinucleotido dNTP (2.5 mmol/L); 1 µL de cada primer da espécie avaliada (10 µmol); 0,1 µL de 5U/mL Platinum Taq DNA Polymerase e 11,7µL de água estéril. Todos os microtubos foram levados para termocicladore os tempos foram determinados para cada espécie (Tabela 1).

Os resultados do PCR de cada amostra foram levados para leitura em gel de agarose 1% contendo EDTA tampão com brometo de etidio (Invitrogen® - Life Technology do Brasil) e eletroforese durante 45 min para posterior visualização em luz ultravioleta. Reações positivas foram determinadas segundo a presença de bandas com o mesmo peso molecular que o controle positivo. Foi utilizado a 1kb DNA ladder (Invitrogen) como marcador de longitud universal de PCR.

Tabela 1: Detecção de espécies de Candida pela reação em cadena polimerase (PCR) usando primers específicos.

Candida	**Primers (5' – 3')**	Amplificação	**Ciclos**
Candida albicans	F: TTTATCAACTTGTCACACCAGA R: ATCCCGCCTTACCACTACCG	273 bp	Desnaturação inicial: 96°C por 5' e 40 ciclos de: 94°C por 30s, 58°C por 30s, 72°C por 30s eetapa final: 72°C por 15'.
Candida tropicalis	F: CAATCCTACCGCCAGAGGTTAT R: TGGCCACTAGCAAAATAAGCGT	357 bp	Desnaturação inicial: 96°C por 5' e 40 ciclos de: 94°C por 30s, 58°C por 30s, 72°C por 30s eetapa final: 72°C por 15'.
Candida krusei	F: GAGCCACGGTAAAGAATACACA R: TTTAAAGTGACCCGGATACC	227 bp	Desnaturação inicial: 96°C por 2' e 30 ciclos de: 96°C por 30s, 57°C por 30s, 94°C por 60s etapa final 72°C por 15'.
Candida glabrata	F: CCCAAAAATGGCCGTAAGTATG R: ATAGTCGCTACTAATATCACACC	674 bp	Desnaturação inicial: 96°C por 2' e 30 ciclos de: 96°C por 30s, 57°C por 30s, 94°C por 60s etapa final 72°C por 15'.
Candida guillermondi	F: CCCAAAATCACAAAGCTCAAGT R: TACGACTTGAAGTTGCGAATTG	205 bp	Desnaturação inicial: 96°C por 2' e 30 ciclos de: 96°C por 30s, 57°C por 30s, 94°C por 60s

			etapa final 72°C por 15’.
Candida dubliniensis	F: AAATGGGTTTGGTGCCAAATTA R: GTTGGCATTGGCAATAGCTCTA	816 bp	Desnaturação inicial: 96°C por 2’ e 30 ciclos de: 96°C por 30s, 57°C por 30s, 94°C por 60s etapa final 72°C por 15’.

3.5 Análise estatística

. Os dados foram analisados com o software GraphPad Prism 6. As análises de prevalência foram realizadas com o teste de Qui-Quadrado (α=0,05). Idade, gênero dos pacientes e tempo de uso da prótese de cada grupo estudado foi comparado utilizando ANOVA one-way e o teste de comparação múltiplade Tukey. A efetividade da cada tratamento foi analisada com o teste Friedman e o teste de comparação múltipla de Dunn (α=0,05). Os resultados clínicos e microbiológicos dos três grupos foram comparados com o teste de Kruskal-Wallis e o teste de comparação múltipla de Dunn (α=0,05). A correlação entre as UFC da mucosa do palato e as UFC da prótese foi analisada com o teste Spearman r (α=0,05).

4 RESULTADOS

Dos 204 pacientes, a estomatite protética foi evidenciada em 54% da amostra (Tabela 2). Segundo o gênero 41% dos homens e 58% das mulheres apresentarama doença sendo a diferença estatisticamente significante (p=0,032).Dos pacientes que apresentaram EP, foi encontrada: hiperemia puntiforme foi encontrada em 36% da amostra, hiperemia difusa em 41% e hiperemia granular em 23%. Apesar dos três tipos de estomatite protética ter sidomais prevalentes em mulheres, essa diferença não foi estatisticamente significante (p=0,75) (Tabela 3).

Tabela 2: Prevalência da estomatite protética segundo gênero, idade e tempo de utilização da prótese.

		Estomatite Protética			
		Ausente	**Presente**	**Total**	**p**
Genero	Masc	27 (58,7%)	19 (41,3%)	46 (100%)	0,032
	Fem	66 (41,8%)	92 (58,2%)	158 (100%)	
Idade (anhos)	<= 60	14 (34,1%)	27 (65,9%)	41 (100%)	0,070
	> 60	79 (48,5%)	84 (51,5%)	163 (100%)	
Tempo de uso da prótese (anhos)	1-3	40 (67,8%)	19 (32,2%)	59 (100%)	0,0001
	3-10	31 (43,7%)	40 (56,3%)	71 (100%)	
	> 10	22 (29,7%)	52 (70,3%)	74 (100%)	

Teste Qui-quadrado

Tabela 3: Prevalência da estomatite protéticasegundo Newton[34]

Estomatite Protética

		I	II	III	total	p
Genero	Masc	7 (15,2%)	8 (17,4%)	4 (8,7%)	19 (100%)	0,754
	Fem	32 (20,3%)	37 (23,4%)	23 (14,6%)	92 (100%)	
Idade (anhos)	<= 60	7 (17,1%)	13 (31,7%)	7 (17,1%)	27 (100%)	0,397
	>60	32 (19,6%)	32 (19,6%)	20 (12,3%)	84 (100%)	
Tempo de uso da prótese (anhos)	1-3	9 (15,3%)	4 (6,8%)	6 (10,2%)	19 (100%)	0,021
	3-10	19 (26,8%)	16 (22,5%)	5 (7,0%)	40 (100%)	
	>10	11 (14,9%)	25 (33,8%)	16 (21,6%)	52 (100%)	

Teste Qui-quadrado

A identificação de *Candida spp.*por meio do teste PCR simples foi realizada em 56 pacientes com sinais clínicos de EP. A tabela 4 mostra as espécies de Candida encontradas.

Tabela 4: Distribuicao (%) de espécies de Candida mais frequentes encontradas em pacientes com estomatite protética.

Candida spp.	**Número de pacientes (%)**
Candida albicans	54 (96,4%)
Candida tropicalis	7 (10,7%)
Candida glabrata	4 (7,1%)
Candida krusei	2 (3,5%)
Candida dubliniensis	1 (1,7%)
Candida guillermondi	0

Quarenta e oito pacientes com idade média de 63,8± 8,9 anos participaram do estudo, sendo 90% mulheres e 10% homens.Foram excluídos dois

pacientes do estudo por eles não ter voltado ao período de acompanhamento clínico do uso da medicação. O grupo miconazol (Grupo M) teve 13 mulheres e 2 homens com idade media de 65,8 anos e o tempo de uso da prótese médio de 11,4 anos. O grupo placebo (Grupo P) teve 16 mulheres com idade media de 63,2 anos e o tempo de uso da prótese foi de 19,5 anos. O grupo experimental da *U. tomentosa* (Grupo UT) teve 14 mulheres e 3 homens com idade media de 62,7 anos e o tempo de uso da prótese de 14,8 anos. Não houve diferença significante em gênero entre os grupos (p=0,59) nem no tempo de uso da prótese (p=0,21).Nenhum voluntário relatou efeito adverso após aplicação tópica dos géis.

O grau de severidade da EP está apresentado na Tabela 5. Pode-se observarantes do tratamento (dia 0) escore 2 em relação ao grau da EP nos três grupos, o qual foi reduzido nos outros períodos de avaliação (dia 7 e 14), sem diferenças significantes entre os tratamentos (p>0,05) (Figura 5).

Tabela 5: Média e desvio padrãodo grau de severidade de estomatite protética em funcao do tempo no tratamento dos grupos.

	Dia 0	**Dia 7**	**Dia 14**
GrupoM	2,25 ± 0,57 ^{aA}	1,18 ± 0,65 ^{bB}	0,81 ± 0,65 ^{cB}
Grupo P	2,18 ± 0,75 ^{aA}	1,31 ± 0,94 ^{bB}	1,18 ± 1,04 ^{cB}
Grupo UT	2,11 ± 0,60 ^{aA}	1,47 ± 0,62 ^{bB}	0,82 ± 0,72 ^{cB}

Letras minúsculas iguais indicam resultados similares estatisticamente significantes entre as linhas. Letras maiúsculas iguais indicam resultados similares estatisticamente significantes nas colunas (p>0,05).

Grupo	**Inicial (Dia 0)**	**Controle (Dia 14)**

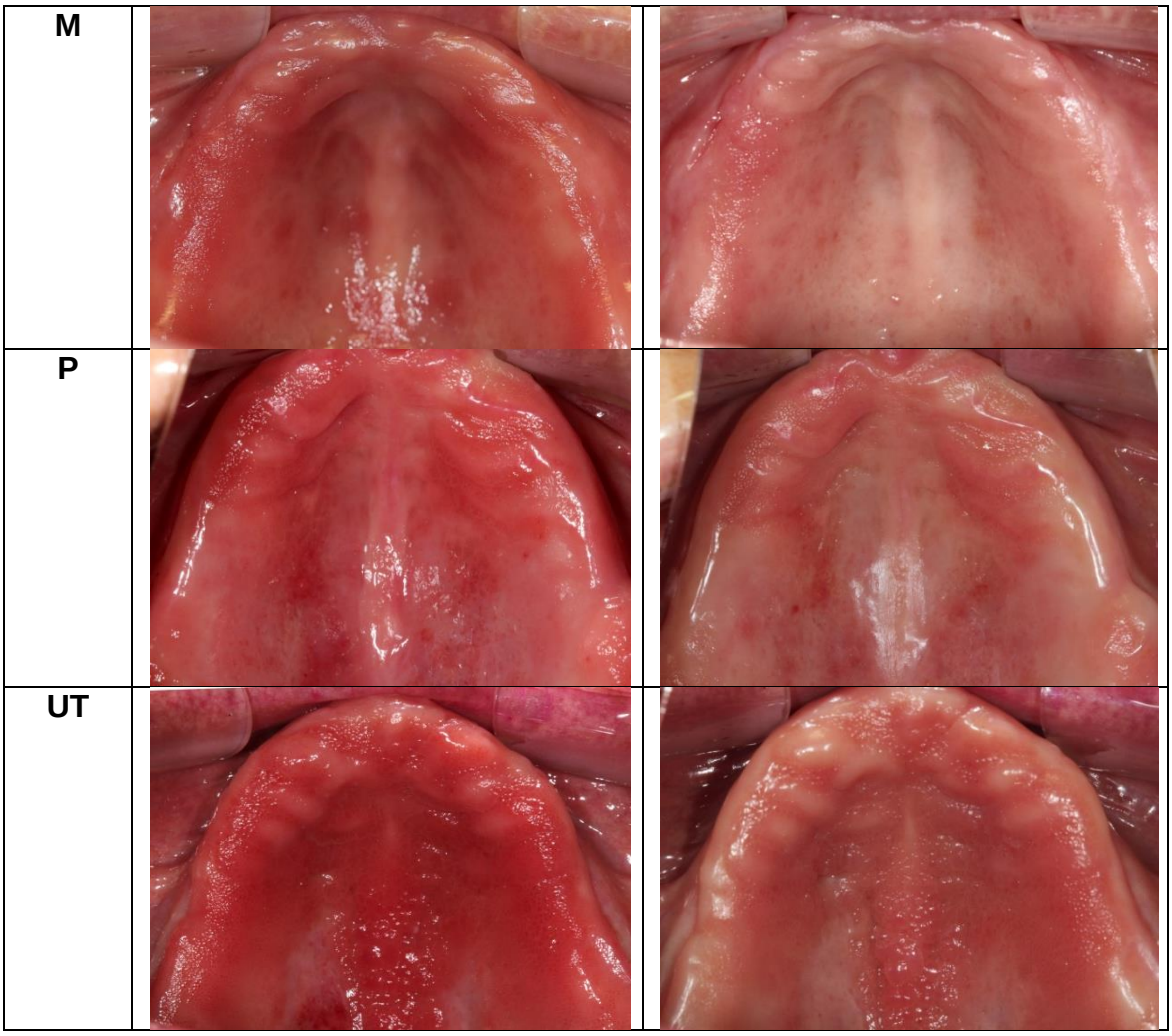

Figura 5: Avaliação clínica do grau de estomatite protética antes e após o tratamento. M: miconazol 2%, P: placebo, UT: Uncaria tomentosa 2%.

De cada paciente foram tomadas amostras da mucosa de palato e superfície interna da prótese, para fazer a análise estatística dos dados e o número das UFC/mL foi transformado a logaritmo.Foi encontrada uma diferença significante no número de UFC/mL das amostras tomadas da mucosa do palato comparadas com aquelas tomadas da prótese, sendo o número de UFC/mL da prótese maior do que da mucosa do palato. Ao fazer a correlação entre os resultados, foi encontrada uma correlação direta entre o número de UFC/mL do palato e da prótese ($r=0,452$) ($p<0,0001$). A Tabela 6 mostra a media de UFC/mL em log das amostras tomadas da prótese e a Tabela 7 mostra os resultados das amostras tomadas da mucosa. Resultados similares aos clínicos foram encontrados na análise das UFC/mL. Houve número maior de UFC/mL no dia 0, o qual foi reduzido nos outros períodos de observação (dia 7 e 14) sem diferença significante

entre os grupos (p>0,05). Além disso, pôde se observar que no dia 7 houve menor quantidade de UFC/mL no grupo M comparado com o grupo P e UT.

Tabela 6: Media e desvio padrãodas UFC/mL (Log) na prótese em função do tratamento dos grupos.M: miconazol 2%, P: placebo, UT: Uncaria tomentosa 2%.

	Dia 0	**Dia 7**	**Dia 14**
GrupoM	2,04± 0,79 ^{aA}	0,60± 0,81 ^{aB}	0,86± 0,97 ^{cB}
Grupo P	2,09± 0,88 ^{aA}	1,41± 1,08 ^{bB}	1,20± 1,16 ^{cB}
Grupo UT	2,20± 1,04 ^{aA}	1,32± 1,03 ^{bB}	1,03± 0,77 ^{cB}

Letras minúsculas iguais indicam resultados similares estatisticamente significantes entre as linhas. Letras maiúsculas iguais indicam resultados similares estatisticamente significantes nas colunas (p>0,05).

Tabela 7: Media e desvio padrãodas UFC/mL (Log) na mucosa do palato em função do tratamento dos grupos.M: miconazol 2%, P: placebo, UT: Uncaria tomentosa 2%.

	Dia 0	**Dia 7**	**Dia 14**
GrupoM	0,90± 0,74^{aA}	0,23± 0,50^{aB}	0,39± 0,69 ^{cB}
Grupo P	0,67± 0,57^{aA}	0,52± 0,82^{bA}	0,30± 0,62^{cB}
Grupo UT	1,11± 0,99^{aA}	0,49± 0,65^{bB}	0,33± 0,49 ^{cB}

Letras minúsculas iguais indicam resultados similares estatisticamente significantes entre as linhas. Letras maiúsculas iguais indicam resultados similares estatisticamente significantes nas colunas (p>0,05).

A Tabela 8 mostra os resultados da identificação de espécies de Candida nos pacientes com o HiCrome Candida Agar e o teste bioquímico Api 20 C AUX. Pôde se observar que *Candida albicans* foi o microorganismo mais

prevalente, seguido pela *C. tropicalis, C. glabrata* e *C. krusei*, independente do grupo avaliado.Para os períodos avaliados, houve redução no número de microorganismos do dia 0 para o dia 7 e do dia 7 para o dia 14, com exceção da *C. tropicalis* no grupo UT e *C. glabrata* no grupo M, onde houve maior porcentagem de microrganismos no dia 14 em comparação com o dia 7.

Tabela 8: Frequência absoluta e relativa (%) de *Candida spp.*encontrados nos pacientes de acordo com os grupos experimentais.M: miconazol 2%, P: placebo, UT: Uncaria tomentosa 2%.

	Dia	**Grupo M (n=15)**	**Grupo P (n=16)**	**Grupo UT (n=17)**
	0	14 (93,3)	15 (93,7)	15 (88,2)
C. albicans	7	7 (46,6)	7 (43,7)	8 (47,1)
	14	3 (26,6)	6 (37,5)	3 (17,6)
	0	3 (20)	5 (31,2)	5 (29,4)
C. tropicalis	7	2 (13,3)	4 (25)	3 (17,6)
	14	1 (6,6)	1 (6,2)	5 (29,4)
	0	2 (13,3)	3 (18,7)	1 (5,8)
C. glabrata	7	0	1 (6,2)	1 (5,8)
	14	2 (13,3)	2 (12,5)	1 (5,8)
	0	2 (13,3)	1 (6,2)	3 (17,6)
C. krusei	7	0	0	0
	14	0	0	1 (5,8)

5 ARTIGOS

5.1 Artigo 1:

Influence of gender, age, time of use of the prosthesis and presence of Candida spp. in patients with denture stomatitis.

16-May-2013

Dear Mrs. Tay:

Your manuscript entitled "Prevalence of denture stomatitis and presence of Candida spp. in complete denture wearers" .by Tay, Lidia Yileng; Herrera, Daniel Rodrigo; Jorge, Janaina; Gomes, Brenda Paula; dos Santos, Fabio Andre, has been successfully submitted online and is presently being given full consideration for publication in Gerodontology.

Co-authors: Please contact the Editorial Office as soon as possible if you disagree with being listed as a co-author for this manuscript.

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Sincerely,

Gerodontology Editorial Office

Influence of gender, age, time of use of the prosthesis and presence of Candida spp. in patients with denture stomatitis.

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Running title: Prevalence of denture stomatitis

Abstract:

Objective: To evaluate the prevalence of Denture Stomatitis (DS) in full denture users in relation to gender, patient age and time of use of the prosthesis and to identify Candida *spp.* in DS.

Background: In dentistry, there isan increasing emphasis on maintaining oral health into the old age, whenalterations in oral tissues are associated with various local conditions.DS is considered as a multifactorial disease. It has been strongly correlated with denture hygiene, reaction to oral biofilms, dental materials used in the fabrication and denture trauma.

Materials and Methods:204 denture patients (46 males and 158 females) were selected. DS was classified according to Newton's classification and it was related to gender, age and time of use of the denture. Samples from the palatal mucosa and the surface of the upper denture of patients with DS were evaluated using PCR test for identification of *Candida* species. T-test, chi-square and Fisher's exact tests were used for statistical analysis.

Results: DS was evidenced in 54.4% of the sample. Although all types of DS were more prevalent in females, this difference was not statistically significant ($p>0.05$). DS and the type of DS are directly influenced by the time of use of the prosthesis ($p<0.001$), but it was not significantly related to the age of the participants ($p>0.05$). Presence of C*. albicans, C. tropicalis, C. glabrata, C. krusei* and *C. dubliniensis* were identified by PCR.

Conclusion: DS was more prevalent in female. There were no differences according to the age of the patients and DS. The prevalence of DS was influenced by the the time of denture use (years). *Candida albicans* was identified as the most frequent specie in patients with DS.

Key words: denture stomatitis, Candida spp., epidemiology

Introduction:

The different colonization by microorganisms and subsequent biofilm formation are relevant factors in the development of pathogens, for example: denture stomatitis (DS), which may be considered a public health problem. This disease is the most frequent oral mucosal lesions in elderly people.[1] The DS is an erythematous inflammatory condition found more frequently in partial or full dentures users.[2,3,4] This disease is characterized by diffuse erythema, which may have homogeneous appearance or reddish focal points or areas, besides showing various changes in texture and surface of the palatine mucosa. It is most often asymptomatic. Only few patients have experienced pain or burning sensation.[5]

DS is considered as a multifactorial disease. It has been strongly correlated with denture hygiene, reaction to oral biofilms, dental materials used in the fabrication or denture trauma.[6,7]The results of Santos et al.,[8] indicate key role for the denture biofilm accumulation in DS. In addition, in the elderly population, it can expect reduce in tissue regeneration and higher susceptibility to diseases, which may favor the appearance of the DS. Systemic complexity, aging process, metabolic changes, nutritional factors, medications, prosthetic use, psychobiological habits and alcohol or tobacco use could explain the high susceptibility to diseases in the elderly population.[9]

Although *Candida spp.* are often present as benign commensal organisms in healthy individuals, these kind of microorganisms produces a broad range of serious illnesses in compromised hosts.[10]Different *Candida* species have been frequently isolated from the oral cavities of patients with DS. The most commonly recovered specie is *Candida albicans* with a prevalence of up to 80%. This oral fungal pathogen is the most virulent of the *Candida* species and is able to grow as biofilm. Other *Candida* species such as *C. tropicalis, C. krusei* and *C. glabrata* were also identified, but in smaller percentages.[11-16]

Elderly population is increasing in manydeveloped and developing countries, and this increase can result in potential health problemsin certain populations.[17]In dentistry, there isan increasing emphasis on maintaining oral health into the old age,

whenalterations in oral tissues are associated with various conditions.[1]Epidemiological researches are important to understand the distribution and etiology of oral diseases.The epidemiology of DS has great clinical variability depending on the population studied and diagnostic methods.[18]Rabiei et al.[19] found a prevalence of 45.6%, and Evren et al.[17] the rate of DS was found to be 44.8%. Oral mucosal alterations, including stomatitis, were observed in 60% of the elderly population in Brazil.[20] On the other hand, Cueto et al.[21] observed that denture-induced stomatitis corresponded to 37.1% of the population. In spite of these studies, the researches that show the relationship between patient age, denture status and denture stomatitis are scarce. So, the aim of this study was to evaluate the prevalence of denture stomatitis classified according Newton[22] in full denture users in relation to gender, patient age and time of use of the prosthesis, and to identify presence of *Candida* spp. in patients with denture stomatitis.

Material and Methods:

This study was carried out after the approval by the Committee of Ethics in Research of the State University of Ponta Grossa (Protocol: 17572/10). 204 denture patients (46 males and 158 females) were selected according to the inclusion criteria for evaluation. The inclusion criteria were male or female adults who frequently go to the long-term care institutions of Ponta Grossa city and maxillarycomplete denture wearers for at least one year.

In the first, the patients were interviewed using a structured questionnaire. Demographic data (name, age, gender, educational level, etc.) and medical records were recorded using the attendant's files in the Prosthodontic Clinic of the State University of Ponta Grossa, as well as the duration of maxillary denture experience, the age of the last denture, their wearing frequency and denture hygiene habits (after each meal, once per day, less often than once per day).

The subjects were examined using a portable high-intensity light, a dental mirror and a tongue blade. All parts of the oral cavity were examined. A clinical examination was performed by two trained examiners after interexaminer calibration (k=0.9). The informationobtained was registered on a clinical recordspecially designed for this purpose.The clinical examination included the assessment of the oral mucosa covered by the maxillary denture.The presence of denture stomatitis according to Newton's classification was observed as follow: pinpoint hyperaemia (type I), diffuse erythema confined to the mucosa under the denture base (type II) and inflammatory papillary hyperplasia of the palate (type III).

Presence and type of DS was correlated with gender (male and female), age (60 years old or younger and older than 60 years old) and time of use of the last denture (1-3 years old, 3-10 years old and older than 10 years). Statistical analysis was performed using SPSS 13.5 (SPSS Inc, Chicago, IL, USA). T-test, chi-square and Fisher's exact tests were used for comparative analysis. A p-value below 0.05 was considered to indicate statistical significance.

Only for patients with clinical symptoms of denture stomatitis, recovery of *Candida spp*. was performed by rubbing oral swabs along the palatal mucosa and

the tissue surface of the upper denture. Each swab was placed into a test tube containing 5 mL of Brain-Heart Infusion (BHI) and vortexed for 1 minute to suspend the organisms from the swab. These samples were taken for recognition of *C. albicans, C. tropicalis, C. krusei, C. glabrata, C. guillermondi and C. dubliniensis* presents in the sample with simple PCR test (Polymerase Chain Reaction).These Candida specieswere correlated with the patient's DS type.

DNA extraction was performed using the QIA amp DNA kit(QIAGEN, Chatsworth, CA, USA) according to the manufacturer's instructions from each patient sample. Besides the samples, purified genomic DNA of each microorganism was used as positive control, and Mili Q sterile water was used as the negative control.

*Candida*spp. were identified by using a nested amplification with species-specific primers for*C. albicans, C. tropicalis, C. krusei, C. glabrata, C. guillermondi and C. dubliniensis.* Primer sequence and cycles are shown inTable 1. The reactions were performed in a total volume of 20 µL containing 2 µL of DNA sample; 2 µL of 10X PCR buffer; 0.6 µL of 50 mmol/L $MgCl_2$; 1.6 µL of a mixture of each deoxynucleoside triphosphate (2.5 mmol/L); 1 µL of each species-specific primer (10 µmol); 0.1µL of 5U/mL Platinum Taq DNA Polymerase and 11.6 µL of sterile water.

The PCR products were electrophoresed on 1% agarose gel and tri-acetate-EDTA buffer stained with 0.5uL/ml ethidium bromide (Invitrogen® - Life Technology do Brasil) and visualized under ultraviolet light. Positive reactions were determined by the presence of bands of the appropriate size. A 1kb DNA ladder (Invitrogen) was used as size marker for universal PCR.

Results:

Denture stomatitis was evidenced in 54.4% of the sample (Table 2). According to gender41.3% of the males and58.3% females had the disease and the differences were statistically significant (p=0.032). Pinpoint hyperaemia was recorded in 19.1% of the sample (males: 3.43%, females: 15.68%), diffuse erythema in 22.1% (males: 3.93%, females: 18.13%) and papillary hyperplasia in 13.2% (males: 1.96%, females: 11.27%). Although all three types of denture stomatitis were more prevalent in females, this difference was not statistically significant (p=0.75) (Table 3).

The mean age of the 204 patients was 65.9 ± 8.6 years. No differences were found comparing the patients 60 years old or younger (65.9%) and patients older than 60 years (51.5%) (Table 2). Neither the types of denture stomatitis were related to the age of the patients (p=0.39) (Table 3).

Participants had been edentulous in the maxilla for 17.4 ± 12.2 years. The statistically significant associations using Chi-square test are presented in Table 2. The mean age of the last denture was 12.21 ± 12.1 years (range: 1–45 years). The prevalence of denture stomatitis significantly increased with the increasing age of the denture (p<0.001). DS was observed in 32,2% of the participants dentures users of 1 to 3 years, increasing to 56,3% subjects dentures users of 3 to 10 years, but increased to over 70,3% of the participants dentures users more than 10 years. When the three Newton types were separately investigated, the age of the last denture was significantly related (p<0.05). 15.3% of the subjects wearing dentures less than 3 years had dentures stomatitis type I, 6.8% type II and 10.2% type III. In subjects wearing denture more than 10 years, it was found DS type I in 14.9% patients, type II in 33.8% and in type III 21.6% (Table 3).

PCR test was randomly performed in 56 patients with DS for identification of different *Candidaspp.* Table 4 shows the number and the frequency distribution of the patients who had presence of *Candidaspp*. Only two patients with DS type I did not present *Candida spp*. *Candida albicans* was present in the three types of DS, the others species of *Candida* were present along with *Candida albicans*, none of them had an isolated growth.

Discussion:

Prevalence studies of denture stomatitis have been conducted over the years. Contradictory results are found, mainly due the population studied and the diagnostic method used. In addition, the differences may be due to the origin of the examined patients and training of examiners in the diagnosis of oral mucosal lesions.[21]Because of this, the comparisonof the findings of this study with those ofother epidemiological studies is difficult. For the clinical diagnosis of DS, should consider some clinical signs, such as changes in color and texture of the mucosa. In addition to clinical examination, other diagnostic methods available are cell culture and histopathological examination. These methods are used for diagnosis of chronic hyperplastic candidiasis.[23]In this study, denture stomatitis was assessed clinically and the patients were classified according to Newton, based on clinical lesions: class I, class II and class III.[22]

The prevalence of DS in the study population was 54.4%, in compliance with other studies asRabiei et al.[19], who found a prevalence of 45.6%. In the study of Evren et al.[17], the rate of denture stomatitis was found to be 44.8%. Oral mucosalalterations, including stomatitis, were observed in 60% of the elderly population in the Brazil.[20] On the other hand, Cueto et al.[21] observed that denture-inducedstomatitis corresponded to 37.1% of the population. The factors that can increase the risk of DS are poor denture fit, poor denture hygiene, and colonization of the denture surface and oral mucosa.[18]These factors were not assessed in this study. Thus, future studies should be performed to associate DS with these factors.

The survey showed statistically significant difference in theprevalence of DS between males (41.3%) and females(58.3%). These results are in agreement with previous studies.[6,7] In contrast, the results of Evren et al.[17]showed that there were no differences between males and females incidence of DS. Furthermore, for classification of Newton, in this study was observed prevalence similar for all kinds regardless of gender.

Age may influence the prevalence of DS. Older patients have less manual hability to hygiene the denture and cleaning oral mucosa.[17,24] Other problems associated with aging may favor the onset of disease such as oral epithelium thins, decrease synthesis of collagen in connective tissues, decrease in tissue regeneration and lower resistance to diseases.[21] Contrarily, in relation to age, the results of this

study showed no difference between patients younger than 60 years (65.9%) or older than 60 years (51.5%). This shows that other factors not related to age, may favor the appearance of DS, such as systemic diseases, inadequate hygiene habits and very old prostheses.[1,17] However, as previously reported, further studies are needed to correlate the etiological factors with the prevalence of DS.

In this study, the factor that most influenced the prevalence of denture stomatitis was the time of use of the denture. The quality of the dentures was notevaluated in this study, but the results point to more prevalence of denture stomatitis with the increasing time of the denture use. The prevalence of the disease was 70% for users who used the same prosthesis for more than 10 years. More than 50% of the patients wearing their prosthesis from 3 to 10 years had DS, which represents a high prevalence of the disease in these cases. Over time, changes occur in the surface acrylic base, such as increased surface roughness,[25]favoring the accumulation of biofilm and thus the appearance of DS.

The abilityof adherence of *Candidaspp.* to saliva-coatedacrylic resin may play a role in establishing itself onthe denture surface.[26]The majority of the denturewearersin our study was low-income individualsor no income elderly. Nearly half of the maxillarydentures worn by these patients wereacrylic-based and were defective. Also, the acrylic-based dentures are less stable than the cobaltchrome based dentures causing accumulation of plaque or mucosal lesions.[27]

Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of *Candidaspp*., the commonest being *C. albicans*.[28] When it is associated to the use of dental prosthesis, it is called denture stomatitis. Not surprisingly 96.8% (54 patients) harbored C. *albicans*, which was by far the predominant yeast isolated. This finding is in agreement with earlier reports. Coco et al.[13] demonstrated that *C. albicans* was isolated from 75% of patients without denture stomatitis and from 81% of those with clinical signs of the infection. In addition, *C. albicans* has the ability to adhere to mucosal and denture surfaces, which is considered to be the first step in the pathogenesis of DS.

Although *C. albicans* is still the most frequently isolated species from patients with *Candida* infections, the growing prevalence of non-albicans species is clearly a concern. In the present study, *C. tropicalis* and *C. glabrata* were isolated from 12.5%

and 7.14% of patients with denture stomatitis. *C. krusei* and *C. dubliniensis* were isolated from 3.5% and 1.7% each. In terms of frequency distribution, some studies have shown that *C. tropicalis* was the second most prevalent species identified.[29] However, contrasting results have been found in other studies, in which *C. glabrata* was the most common yeast after *C. albicans.*[30]

Despite maxillary and mandibular denture were evaluated, DS lesions were found only in the maxillary mucosa. Maxillarydentures cover a greater area of oral mucosathan mandibular dentures; therefore a greaterchance of plaque and yeast retention as well asmechanical injury would be expected.[26]This study confirmed that high prevalence of *Candida albicans* and the subsequent infection in the palatal mucosa is due to poor hygiene of full denture and the time of use of it,careful instruction should be provide to elderly people with more emphasis in women.In addition, the high prevalence of DS pointed in this study is one of themotives why oral examination of patients using total denture is of significant importance, so it is recommended to reevaluate the prosthesis every three years to assess hygiene and the need of relining or replacement of it.

Conclusion:

According to the findings in this study can conclude that:

1. There is a high prevalence of dentures stomatitis in the patients evaluated in this study; being DS type II the most prevalent;
2. DS is more prevalent in women;
3. There are no differences according to the age of the patientson the prevalence of DS;
4. DS is more prevalent in patients that wear their dentures for more than 10 years.
5. *Candida albicans* was identified as the most frequent specie in patients with denture stomatitis.

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Table 1: PCR species-specific primer pairs used for detection of six*Candida*species

Candida	Primer pairs (5' – 3')	Amplification	Cycles
Candida albicans	F: TTTATCAACTTGTCACACCAGA R: ATCCCGCCTTACCACTACCG	273 bp	Initial denaturation at 96°C for 5 min and 40 cycles of: 94°C for 30s, 58°C for 30s, 72°C for 30s and a final step 72°C for 15min.
Candida tropicalis	F: CAATCCTACCGCCAGAGGTTAT R: TGGCCACTAGCAAAATAAGCGT	357 bp	Initial denaturation at 96°C for 5 min and 40 cycles of: 94°C for 30s, 58°C for 30s, 72°C for 30s and a final step 72°C for 15min.
Candida krusei	F: GAGCCACGGTAAAGAATACACA R: TTTAAAGTGACCCGGATACC	227 bp	Initial denaturation at 96°C for 2 min and 30 cycles of: 96°C for 30s, 57°C for 30s, 94°C for 60s and a final step 72°C for 15min.
Candida glabrata	F: CCCAAAAATGGCCGTAAGTATG R: ATAGTCGCTACTAATATCACACC	674 bp	Initial denaturation at 96°C for 2 min and 30 cycles of: 96°C for 30s, 57°C for 30s, 94°C for 60s and a final step 72°C for 15min.
Candida guillermondi	F: CCCAAAATCACAAAGCTCAAGT R: TACGACTTGAAGTTGCGAATTG	205 bp	Initial denaturation at 96°C for 2 min and 30 cycles of: 96°C for 30s, 57°C for 30s, 74°C for 60s and a final step 72°C for 15min.
Candida dubliniensis	F: AAATGGGTTTGGTGCCAAATTA R: GTTGGCATTGGCAATAGCTCTA	816 bp	Initial denaturation at 96°C for 2 min and 30 cycles of: 96°C for 30s, 57°C for 30s, 74°C for 60s and a final step 72°C for 15min.

Table 2: Denture stomatitis prevalence according the distribution of some personal characteristics.

		Denture Stomatitis			
		Not prevalent	**Prevalent**	**total**	**p**
Gender	Masc	27 (58.7%)	19 (41.3%)	46 (100%)	0.032
	Fem	66 (41.8%)	92 (58.2%)	158 (100%)	
Age (years)	<= 60	14 (34.1%)	27 (65.9%)	41 (100%)	0.070
	>60	79 (48.5%)	84 (51.5%)	163 (100%)	
Time of denture use (years)	1-3	40 (67.8%)	19 (32.2%)	59 (100%)	0.0001
	3-10	31 (43.7%)	40 (56.3%)	71 (100%)	
	>10	22 (29.7%)	52 (70.3%)	74 (100%)	

Chi-square test

Table 3: Prevalence of denture stomatitis types (Newton).

		Denture Stomatitis				
		I	**II**	**III**	**total**	**p**
Gender	Masc	7 (15.2%)	8 (17.4%)	4 (8.7%)	19 (100%)	0.754
	Fem	32 (20.3%)	37 (23.4%)	23 (14.6%)	92 (100%)	
Age (years)	<= 60	7 (17.1%)	13 (31.7%)	7 (17.1%)	27 (100%)	0.397
	>60	32 (19.6%)	32 (19,6%)	20 (12.3%)	84 (100%)	
Time of denture use (years)	1-3	9 (15.3%)	4 (6.8%)	6 (10.2%)	19 (100%)	0.021
	3-10	19 (26.8%)	16 (22.5%)	5 (7.0%)	40 (100%)	
	>10	11 (14.9%)	25 (33.8%)	16 (21.6%)	52 (100%)	

Chi-square test

Table 4: Presence of *Candida spp.* in patients with denture stomatitis and frequency distribution.

Candida spp.	Number of patients (%)
Candida albicans	54 (96.4%)
Candida tropicalis	7 (12.5%)
Candida glabrata	4 (7.14%)
Candida krusei	2 (3.5%)
Candida dubliniensis	1 (1.7%)
Candida guillermondi	0

Table 5: *Candida spp.* distribution in the three types of DS.

Candida spp.	DS type I	DS type II	DS type III
Candida albicans	37	45	27
Candida tropicalis	1	3	2
Candida glabrata	0	3	1
Candida krusei	0	1	1
Candida dubliniensis	0	0	1
Candida guillermondi	0	0	0

5.2Artigo 2:

Uncaria tomentosa gel against Denture Stomatitis: Case report

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Uncaria tomentosa gel against Denture Stomatitis: Case report

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Uncaria tomentosa gel against Denture Stomatitis: Case report

Abstract

Patient: A 65-year-old woman, denture-wering patient. At the clinical examination, her palate showed hyperplasic and erythematous mucosa indicating Denture Stomatitis type III according to the criteria proposed by Newton. The alternative treatment included topical application of a gel of Uncaria tomentosa 2% and hygiene of the prosthesis three times a day for one week. After treatment, the patient reduced the signs of the disease.

Discussion: Denture stomatitis is a chronic oral disease that affects denture wearers. It presents as an inflammatory reaction in denture-bearing patients, under maxillary prosthesis. *Candida albicans* have been reported as the principal etiological agent. Despite the existence of a great number of antifungal agents, treatment failure is observed frequently. Phytotherapy is becoming more popular all over the world. Currently, the most promising medicinal Amazonian herb is *Uncaria tomentosa* (Willd.) DC, which is known as cat's claw. Studies of the chemical and pharmacological properties of this medicinal plant have allowed researchers to develop indications for its use.

Conclusion: this case report demonstrated the effectiveness of Uncaria tomentosa against denture stomatitis.

Key words:*Uncaria tomentosa*, Denture stomatitis, Candida

Introduction

Oral candidiasis is the most common fungal infection in humans, which primarily affects elderly people, denture users and immunocompromised patients. It can affect up to 60% of elderly people who use full upperdenture.[1,2] When this infection is related to the use of dental prosthesis, it is called denture stomatitis. It's also found in the literature with other nomenclatures as oral candidiasis and chronic atrophic candidiasis.

Denture Stomatitis is an inflammatory reaction of the supporting tissues of the full and partial removable prosthesis, which is characterized by hyperemia and edema sometimes accompanied by hemorrhagic petechiae. Clinical symptoms of the disease may include: pain, irritation and disturbance of salivation. However, many patients with this disease show no symptoms. The etiology shows extremely variable, being considered of multifactorial cause[3-8] and *Candida albicans* is the principalcausative agent. Other Candida species such as *C. glabrata* arecommonly identified especially in medically compromisedpatients.[9-10]

Different treatments have been proposed against denture stomatitis. Antifungal medication is the most commonly indicated which can be applied topically or systemically; nystatin, amphotericin B, clotrimazole and miconazole are some antifungal agents used against oral candidiasis[11,12]; prosthesis hygienic orientation and the verification of the need for change the denture are included in the treatment.[13] In addition, suspension of the use of the prosthesis can reduce the inflammatory component. However, some factors such as fungal resistance to the medicine and drug toxicity lead us to search for alternative treatments.[14]

The literature shows a variety of herbal treatments for various diseases, especially those of inflammatory origin. Medicinal plants have been used by many generations and its low cost represents an advantage.[15] Research on these plants has increased.[14] Among the several phytotherapeutic reported in the literature we can find chamomile[16], Brazilian pepper (*Schinus terebonthifolius Raddi*), pomegranate (*Punica granatum*), and propolis.[14,16,17,18] One of the most promising medicinal Amazonian herb is *Uncaria tomentosa* (Willd.) DC, which is known as cat's claw. This is a plant that belongs to the *Rubiaceae* family and many uses in traditional medicine are described. The Ashaninka tribe from the Peruvian Amazon

has used the *U. tomentosa* for centuries for healing wounds and ulcers, fever, headache, stomach pain and infections by bacteria and fungi.[19]In the last years, many scientific papers have described the use of the *U. tomentosa* as pharmacological and therapeutic treatment. Studies including in vitro, animal and human clinical studies, have demonstrated the importance of this medicinal plant. Studies have also showed the effectiveness for treatment arthritis for its anti-inflammatory activity, in the treatment of cancer cause its immune antimutagenic properties, such as antiviral, antibacterial and antifungal activities.[19-23]

Considering the aspects mentioned, this case report shows the clinical use of the *U. tomentosa* gel 2% against denture stomatitis as an alternative treatment.

Case Report

A 64-year-old woman, denture-wearing patient, was referred to the Dental Clinic of the State University of Ponta Grossa (UEPG). She had worn her denture for 7 years continuously, without removing it for sleeping, only removing it for hygiene after meals. Initial anamnesis containing information about age, gender, race, medications, diseases and habits were asked to the patient. At the clinical examination, her palate showed hyperplasic and erythematous mucosa indicating DS type II (Figure 1). DS was classified according to the criteria proposed by Newton.

After consultation with the patient, a sample was taken from the palatal mucosa and complete dentures with sterile oral swabs to analyze the presence of colonies of Candida spp. Each swab was placed into a test tube containing 5mL of BHI solution and vortexed for 1 minute to suspend the organisms from the swab. An aliquot of 50 µL from this suspension was spread plated on Hi-crome agar Candida (HiMedia Laboratories Pvt Ltd., Mumbai, India) and incubated at 37°C for 48 hours. Colonies were counted and identified by colony color. The results showed the presence of large amount of colonies of *Candida albicans*(96 UFC/mL) and *Candidatropicalis*(5 UFC/mL) (Figure 3).

After acceptance by the Human Research Ethics Committee of the Department of Dentistry of the Ponta Grossa State University (Protocol: 17572/10), the treatment was starting. Treatment includes the use of *U. tomentosa* gel

2%(Fleming Manipulações, Ponta Grossa, Brasil), three times daily for one week, brushing with soft bristle brush was perfomed before treatment. The gel covered the entire surface of the prosthesis and then placed into the mouth; instructions were given to the patient of the correct hygiene of the prosthesis and storing the denture in water overnight was indicated.

After one week of treatment, it was observed decline of palatine mucosa hyperemia and reduction of the degree of DS (Figure 2). Mycological analysis found a significant reduction of viable colonies of *Candidaalbicans* and *Candidatropicalis*, after one week of treatment there were no UFC growth. Control was also performed after one week of the end treatment and found the palatal mucosa without any degree of hyperemia, reducing the DS degree of the patient. After treatment, the patient was referred to the prosthetic clinic for replacing of the old prosthesis. In addition, the old denture was relined for use by the patient as a provisory.

Discussion

Fungi resistance to medicines available in the market has been reported as a concern, arousing interest in discovering new alternative antifungal treatment. Studies show that the use of herbal medicine in treating candidiasis can be as effective as conventional antifungal therapy.[11,18,23,24] When properly used, medicinal plants extracts have the advantage of low toxicity.[25]

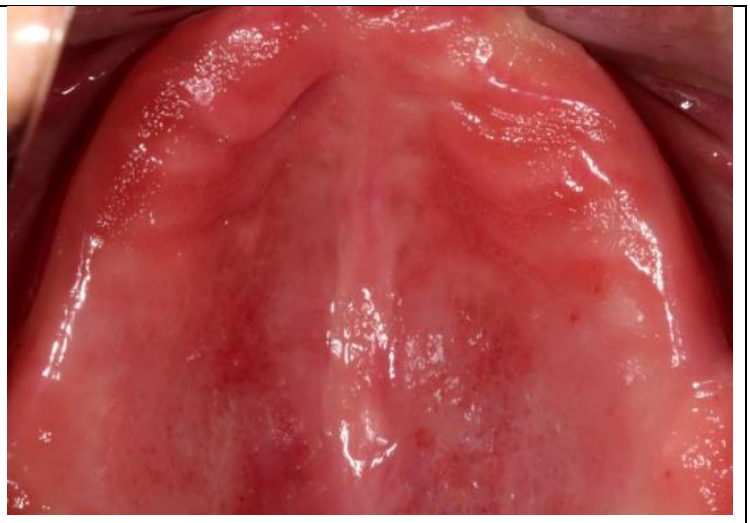
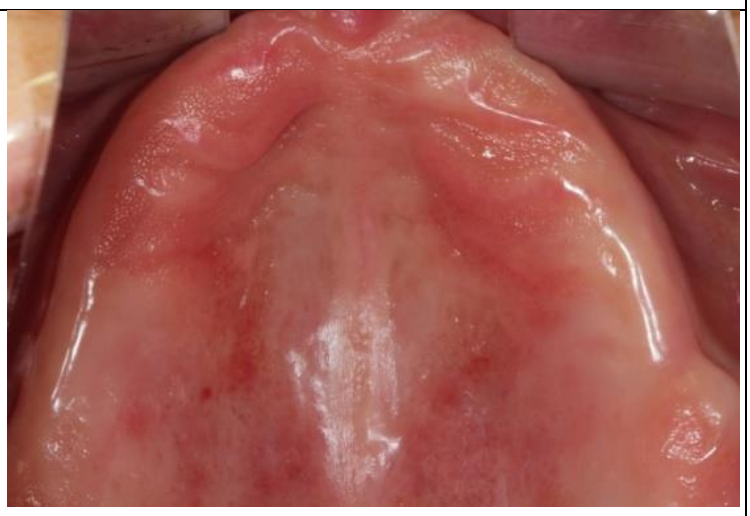
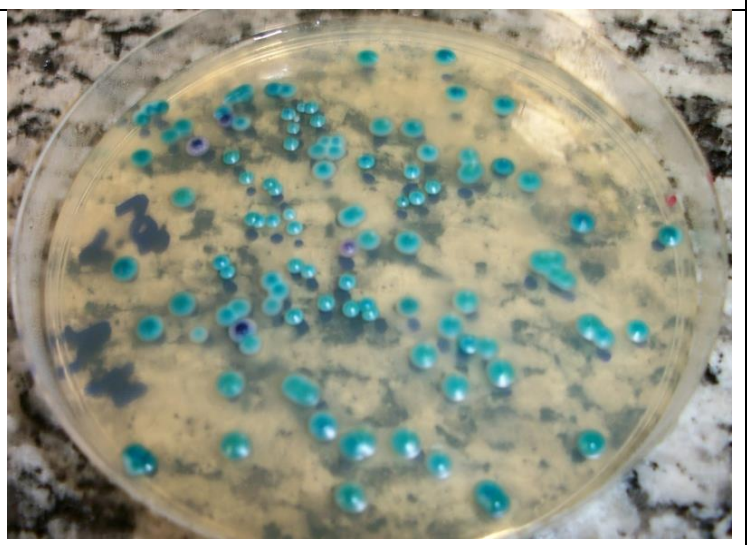
Candida albicans is the most commonly specie found in denture stomatis. Other Candida species such as *C. tropicalis, C. krusei* and *C. glabrata* have also been identified but in a lesser percentage.[26,27] From the lyophilized extract of *Uncaria tomentosa* and using the Natrosol gel as inert base, it is possible to obtain a gel of *U. tomentosa* at concentrations of 2% which have demonstrated *in vitro* an antifungal effect on colonies of *Candida spp*.[23] In this context, Paiva et al.[24] conducted a study to evaluate clinical effectiveness of the *U. tomentosa* on oral candidiasis. After mycological confirmatory analysis, 20 patients were selected and divided into two groups: 10 patients received *Uncaria tomentosa* to perform three applications per day for two weeks, and the other 10 patients formed the control group and must

perform the same treatment with Miconazole gel 4%. The clinical results were similar in both groups, showing the *Uncaria tomentosa* as effective as Miconazole.

Based on the clinical observations, it can observe a significant improvement in DS after using the *U. tomentosa* gel 2%. This can be explained by the effect of anti-inflammatory and antifungal medicinal extract.[23] Silva et al.[28] 1998, registered 66% of inhibition of *C. albicans* isolates with the *U. tomentosa*. In the other hand, an in vitro study conducted by Ccahuana-Vasquez et al.[29] showed no activity of *Uncaria tomentosa* on *C. albicans* growth. This difference may be associated to the concentration employed in the studies. In addition, the presentation in gel form of the treatment has shown success in their application, to the detriment of other antifungals such as nystatin, found in oral solution that is in contact with the lesion for a shorter period than the gel. The product is packaged directly to the prosthesis, previously cleaned, which acts as a "tray", which gives the product a longer contact with the lesion, reflecting a better response and more rapid regression of symptoms.

Other factors may have contributed to the improvement of the case, such as brushing and storing dentures in water overnight. According to Ramage et al.[30]the presence of mature biofilm may result in high levels of inflammation, but this can be controlled through denture cleansing.

It is important to remark that the patient didn’t have symptoms. The awareness about the disease, the information and guidance offered to the patient may have favored the results of this case, since the patient was encouraged to take the correct hygiene of the mucosa and their prostheses, and remove them during sleep. Therefore, further studies will be needed to demonstrate the effectiveness of *Uncaria tomentosa* gel in treating denture stomatitis. Furthermore, tests that assess the biocompatibility of the product are also needed.

Figure 1: DS type II (before treatment)	
Figure 2: After one week of treatment with *Uncaria tomentosa* gel 2%	
Figure 3: Presence ofUFC of *Candida albicans* (green) and *Candida tropicalis* (blue).	

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5.3Artigo 3:

Evaluation of an *Uncaria tomentosa Willd D.C.* gel against denture stomatitis: a randomized clinical study.

Dear Dr. Tay,

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Evaluation of *Uncaria tomentosa* gel against denture stomatitis: a randomized clinical study

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Abstract

The aim of this clinical study was to determine the efficacy of*Uncaria tomentosa* against Denture Stomatitis. 50 patients with DS were randomly assigned into three groups to receive either 2% miconazole, placebo or 2%*Uncaria tomentosa* gel. DS level was registered initial, after one week of treatment and 7 days after treatment. The effectiveness was assessed clinically using Newton's criteria; mycological examination was performed counting CFU/mL. Candida species were identified with HiCrome Candida and biochemical test API20CAUX. It was observed decrease in the DSseverity in all groups ($p<0.05$). Significant reduction in number of CFU/mL after one week ($p<0.05$) which remainedafter 14 days ($p>0.05$) were found in the three groups. *C.albicans* was the most prevalent before treatment, followed by *C.tropicallis, C.glabrata* and *C.krusei*, regardless of the group and the time evaluated. It was observed that *Uncaria tomentosa* gel has the same effect that the 2% miconazole gel.

Keywords: Denture stomatitis, Candida*spp*., *Uncaria tomentosa.*

Introduction

Although they are often present as benign commensal organismsin healthy individuals, Candidaspp. produce a broad range of serious illnesses in compromisedhosts.[1]Therefore, the treatment has become increasingly difficult and expensive. Oral candidiasis is the most common fungal infection in humans, primarily affects elderly people, denture users and immunocompromised patients. It can affect up to 65% of elderly people who use total denture.[2,3] When this infection is related to the use of dental prosthesis, it is called Denture Stomatitis (DS).Species of Candida are the principalcausative agent of DS, an inflammatory reaction of the supporting tissues of the total and partial removable prosthesis. DS ischaracterized by hyperemia and edema sometimes accompanied by hemorrhagic petechiae. Clinical symptoms of the disease may include: pain, irritation and disturbance of salivation, however, many patients with this disease show no symptoms.[4]

Different treatments have been proposed against Denture Stomatitis. Antifungal medication is the most commonly indicated which can be applied topically or systemically; nystatin, amphotericin B, clotrimazole and miconazole are some antifungal agents used against oral candidiasis[5,6]; prosthesis hygienic orientation and the verification of the need for change the denture are included in the treatment.[7] In addition, suspension of the use of the prosthesis can reduce the inflammatory component. However, some factors such as fungal resistance to the medicine and drug toxicity lead us to search for alternative treatments.[8]

Phytotherapics are prepared exclusively with plants or parts of medicinal plants.Recently, a lot of research activities have been shown onthe utilization of medicinal plants to control of several diseases. The use of medicinal plant has a lower cost when compared to synthetic products. Additionally, phytotherapics possess effectively biological action with low toxicity without side effects.[9]

Uncaria tomentosa(Rubiaceae), known as cat’s claw, is usually used in peruvian medicine for the treatment of several diseases.[10]Studies including *in vitro*, animal and human clinical studies, have demonstrated the importance of this medicinal plant. These studies showed the effectiveness for arthritis treatment for its anti-inflammatory activity.[11-15] The properties of *U.tomentosa* have been mainly attributed to thecombined activity of its different componentsrather than the

properties of any one isolated compound.[16]In addition, *U. tomentosa*isan important source of bioactive substances, as monoterpenoidoxindole alkaloids,which have immunomodulatory and antitumoral properties.[17]

Few studies, with contradictory results were found in the literature regarding the effect of *U. tomentosa* on opportunistic fungi.Ccahuana-Vasquez et al.[18]observed, in vitro, that the tested concentrations of *U. tomentosa* did not present inhibitory effect on *Candida albicans*. On the other hand, the results of in vitro study of Herrera et al.[15] indicated that 2% cat's claw gel had antimicrobial activity against microorganisms frequently found in the oral cavity.Even though de studies including *in vitro*, animal and human clinical studies, have demonstrated the importance of this medicinal plant, no clinical studies of the effect of *U. tomentosa* on DS was found. Thus, the purpose of this clinical study was to determine the efficacy of*U. tomentosa* gel against DS. The hypothesis of this study was that *U. tomentosa* gel could reduce the infection of the supporting tissues of the dentures.

Material and methods

Subjects

Using a sample size calculator for an equivalence trial (significance level: 5%, power: 80%) and from previous clinical studies published[4,19], calculation resulted in a minimal sample size of 15 patients in each group. Fifty individuals, ranging between 45 and 85 years of age with good general health, denture wearers for at least one year, patients with denture stomatitis type I, II and III according to the criteria of Newton[20], were selected from Department of Dentistry, State University of Ponta Grossa. The selection of patients according to the criteria mentioned was performed using general health questionnaire and initial clinical examination. The procedures carried out in the study were in compliance with the criteria of Resolution 196/96 of the Brazilian Health Ministry and the protocol of the entire project was approved by the Ethics Committee of the Ponta Grossa State University (Protocol: 17572/10). All subjects voluntarily entered the study and signed an informed consent form before their enrolment.

The exclusion criteria were based on the medical history of each individual. Females of childbearing age, patients with impaired hepatic or renal function, diabetes, xerosthemia, hypoparathyroidism, immune alterations, chemotherapy and radiation therapy and patients who had received any recent treatment with antibiotics, antifungal or steroids agents within 4 weeks before the study and poorly fitting dentures were excluded.

All selected subjects were examined for oral lesions, oral hygiene and denture conditions. DS was classified according to the guidelines given by Newton: type I (bleeding spots); II (diffuse erythematous areas) and III (granular inflammation). Diagnosed candidiasis confirmed by microbiologic cultures from the palatal mucosa and from the denture surface. One colony or more from the palatal mucosa and more than 100 colonies from the denture were considered as positive results.[5]

Study design

The study was carried out as a double-blind study. According to the stratified randomization list, the 50 patients were randomly assigned to one of the 3

experimental groups to receive either 2% miconazole gel (20 mg/gr, Daktarin gel, Janssen-Cilag, São Paulo, Brasil) as a positive control (Group M) or hydroxyethylcellulose (1.5% Natrosol, Fleming Manipulações, Ponta Grossa, Brasil) as a placebo group (Group P) or 2% *Uncaria tomentosa* gel (manipulated by Fleming Manipulações, Ponta Grossa, Brasil; according to the authors instructions) as the experimental group (Group UT). The patients didn't know which group they belong. The identities of the treatment codes were not known during the clinical or mycologic examination.

All patients (from all groups)received their treatment on admission (day 0) and were told to apply the gel on the base of the denture three times a day (after breakfast, after lunch and after dinner) for a period of 1 week. At each application, 2.5 mL (one teaspoonful) of gel was applied on the denture, which was immediately placed in and surplus gel was kept in the mouth until the next application. The patients were asked to clean their denture before each application with water and a toothbrush and to suspend the use of the denture during night leaving it in a glass of regular water. At each visit the palatal mucosa was observed for local adverse reactions. The patients were questioned as to whether they experienced any adverse reactions such as irritation, burning, nausea, vomiting and diarrhea.

The effectiveness of treatment was assessed clinically using the criteria of Newton[20] (1962) for severity degree of DS. Denture stomatitis was registered initial (day 0), after 1 one week of treatment (day 7) and 7 days after treatment (day 14), being assigned the following scores: 0 = healthy palate; 1 Type I; 2 = Type II; and 3 = Type III.At review visits, in addition to the visual observations, color photographs were taken of the mucosa as visual documentation of the treatment efficacy.All of the photographswere taken with the same digital camera (EOS Rebel T2i; Canon, Tokyo, Japan), by the same operator andunder the same conditions (place, light, angle, and patientposition) to facilitate their reproducibility. Two independent calibrated observers were engagedto blindly analyze of the photographs taken of eachpatient.[21]

Mycologic examination

For each patient, recovery of *Candida spp*. was performed by rubbing oral swabs along the palatal mucosa and the tissue surface of the upper denture.[22]Swabs were collected before treatment (day 0), at the end of the treatment (day 7), and at

the follow-up time intervals (day 14). Each swab was placed into a test tube containing 5 mL of Brain-Heart Infusion (BHI) (Himedia Laboratories – Mumbai – India) and vortexed for 1 minute to suspend the organisms from the swab. An aliquot of 50 µL from this suspension was spread plated on Sabouraud's dextrose agar (SDA) plates (Himedia Laboratories – Mumbai – India) with 5 µg/mL chloramphenicol and another 50 µL of the sample was inoculated onHiCrome Candida Agar (Himedia Laboratories – Mumbai – India) and both samples were incubated at 37 °C for 48 hours. Colonies on SDA were quantified using a digital colony counter (CP 600 Plus; Phoenix Ind. Com. EquipamentosCientíficos, Araraquara, Brazil) and the number ofcolony-forming units per milliliter (CFU/mL) determined. CFU/mL from samples of the palatal mucosa were correlated with the CFU/mL from the samples of the tissue surface of the upper denture.

Colonies on HiCrome Candida were presumptively identified by colony color and submitted to biochemical tests to confirm all identifications. For biochemical identification, one colony of each color type on HiCrome Candida was transferred onto fresh SDA for purity. After 48 hours at 37°C, yeast isolates were identified using the following biochemical tests: API 20 C AUX(BioMérieux SA, Marcy-l'Etoile, France) and the results were analized by the Apiweb software (BioMérieux SA, Marcy-l'Etoile, France).

Data were analyzed by GraphPad Prism 6 software. Age, gender and time of use of the prosthesis of each group's participant were compared with one-way ANOVA and Tukey multiple comparison test. The effectiveness of each treatment were analyzed with Friedman test and Dunn's multiple comparision test (α=0.05). Clinical and microbiological results of the three groups were compared also with Kruskal-Wallis test and Dunn's multiple comparision test (α=0.05). Correlation between CFU/mL of the palatal mucosa and CFU/mL of the prosthesis were analyzed with Spearman r test (α=0.05).The CFU/mL values were logarithm transformed to achieve anormal distribution.

Results

Of the 50 patients, two were withdrawn from the study for not returning to the controls. Forty-eight patients with a mean age of 63.83 ± 8.9 years entered the trial. The 89.58% were female and 10.41% were male. Themiconazole group (group M) consisted of 13 females and 2 males with a mean age of 65.8 years, the mean time of use of the prosthesis was 11.4 years. The placebo group (group P) contained 16 females with a mean age of 63.18years,the mean time of use of the prosthesis was 19.5 years. The *Uncaria tomentosa* group (group UT) consisted of 13 females and 3 males with a mean age of 62.7 years, the mean time of use of the prosthesis was 14.76 years. There was nostatistically significant difference in gender between the groups (p = 0.59) neither in the time of use of the prosthesis (p = 0.21).

The severity degree of DSis presented in Figure 1. The examination scores assigned by the blind observers were in high degree of correlation and concordance. It can be observed that before treatment (day 0), themean of severity of DS was type II in all groups, which was reduced over the evaluation periods (7 and 14 days) with no significant differences between the treatments (p>0.05) (Figure 2).

For each patient, recovery of *Candida spp*. was performed by rubbing oral swabs along the palatal mucosa and the tissue surface of the upper denture. The number of CFU/mL was transformed into Log for statistical analyses. Significant difference between the number of CFU/mL of samples taken from the palate mucosa and samples taken from the prosthesis were found,CFU/mLfound in samples taken from the prosthesis was greater than samples taken from the palate mucosa. When performing the correlation between the results, it was found that there is a direct correlation between the number of CFU/mL on the palate and the prosthesis(r=0.452) (p<0.0001).The Figure 3 shows the mean of CFU/mL in log. Similar to the results observed clinically, there was a higher number of CFU/mL on day 0, which was reduced in the other evaluation periods (7 and 14 days) with no significant differences between the groups (p>0.05). One can also observe that, on the 7^{th}, there was a lower number of CFU for group A than in groups B and C.

The Table 1 shows the results of Candida spp. identification found in the patients by HiCrome Candida agar and biochemical test API 20 C AUX.It can be seen that the *C.albicans* was the most prevalent Candida spp., followed by *C.*

tropicallis, *C. glabrata* and *C. krusei*, regardless of the group evaluated. For the period of analysis, there was a reduction in the number of microorganisms from day 0 to day 7 and from day 7 to day 14, with the exception of *C. tropicallis* in group C and *C. glabrata* in group A, in which there was higher percentage of these species on the 14^{th} than on day 7.

Discussion

Oral candidiasis is a common opportunistic infection ofthe oral cavity caused by an overgrowth of Candida spp., the commonest species being *C. albicans*.[23]When it is associated to the use of dental prosthesis, it is called denture stomatitis (DS).There are several treatments for this disease, however, treatment with medicinal plants have been widely used and its low cost represents an advantage.[24]Thus, the purpose of this clinical study was to determine the efficacy of*U. tomentosa* gel against DS. The hypothesis of this study was that *U. tomentosa* gel could eliminate the infection of the supporting tissues of the dentures.

Clinically, the hypothesis could be considered accepted, since there was a reduction of theDS' severity after 7 days of treatment, from grade 2 to grade 1, and the condition remained after 14 days (Figure 1). However, it was also observed that there were no differences among the three experimental groups. Thus, we cannot affirm that the*U. tomentosa*gel was actually responsible for this reduction. This findingmay be attributed to the reduction of fungal load fromdentures and palates after the treatments associatedwith the patient compliance with the recommendationsabout oral and denture hygiene, including removingdentures during sleeping. According to Könsberg and Axéll[25], treatment should start with meticulous denture hygiene and removing of other local factors. In addition, although not sufficient to treat DS[22,26] denture and oral hygiene are essential to maintain a low level of microorganisms on dentures, as well as oral health. According to Ramage et al.[27] the presence of mature biofilm may result in high levels of inflammation, but this can be controlled through denture cleansing. Also Kanli et al.[28]found that there was a significant correlation between poor denture hygiene and prevalence of oral candidosis. Kulak-Ozkan et al.[29] determined oral hygiene habits, denture cleanliness, presence of yeasts and DS in elderly people, and no statistical relationship was foundbetween DS and frequency ofdenture brushing and denture cleaning methods.However, there was a statistically significant relationship between DS, yeasts' presence and denture cleanliness. For the mentioned aspects, studies that compare methods of cleaning dentures with the*U. tomentosa* gel treatment should be performed.

Based on the clinical results of DS severity, significant improvement after using the *U. tomentosa* gel 2% was observed. This can be explained by the anti-inflammatory and antifungal effect of the medicinal extract,[15] although an in vitro study conducted by Ccahuana-Vasquez et al.[18] showed no activity of *U. tomentosa* on *C. albicans* growth; Paiva et al.[30]conducted a study to evaluate clinical effectiveness of the *U. tomentosa* on oral candidiasis. After mycological confirmatory analysis, 20 patients were selected and divided into two groups: 10 patients received *U. tomentosa* to perform three applications per day for two weeks, and the other 10 patients formed the control group and must perform the same treatment with miconazole gel 4%. The clinical results were similar in both groups, showing the *U. tomentosa* as effective as miconazole. These results agree with the results of the present study concluding that *U. tomentosa* could be an alternative treatment for denture stomatitis. The positive results could be explained because *U. tomentosa* has two chemotypes with a different pattern of tetracyclic or pentacyclic oxindole alkaloids. The pentacyclic oxindole alkaloids have immune modulatory effects and these effects are antagonistically inhibited by tetracyclic oxindole alkaloids.[11,31,32]However, it is important to perform new research to identify the potentially therapeutic agents of the plant. The positive results relative to placebo group could be explained by fact that patients know about their disease turned them more concerned about the recommendations about oral and denture hygiene. Some studies have found that simply monitoring a particular outcome can change study participant behavior if they know the investigators are interested in that outcome.[33] In addition, the application of a placebo gel can have prevented retention of yeasts on the denture surface.[25] The presentation in gel form of the products has shown success in their application, when comparing with other antifungals such as nystatin, oral solution that is in contact to the lesion for a shorter period than the gel. The product is packaged directly to the prosthesis, previously cleaned, which acts as a "tray", which gives the product a longer contact with the lesion, reflecting a better response and more rapid regression of symptoms.

From the analysis of Figure 3 it can be seen that, similar to the clinical outcome, a greater number of CFU/mL found in the prosthesis on day zero, which was reduced in the remaining periods of observation (days 7 and 14). The maintenance of the results after 14 days could be explained by the improvement and

maintenance of oral hygiene and denture retained by patients. We can also observe that, on the 7th day, there was a lower number of CFU/mL for group A than in groups B and C. Thus, it is considered that miconazole was more effective in reducing the number of colonies than *U. tomentosa* and Natrosol (placebo group). This result could be explained by the action of the antifungal miconazole scientifically proven.[5] Azoles wield a cytostatic or cytotoxic effect via inhibition of synthesis reactions in the metabolism of essential fungal cell membrane components.[34,35] In addition, it has provided that azole induces intracellular reactive oxygen species, that are inducers of apoptosis in yeasts.[35,36] Despite the proven antifungal action of miconazole, it is important to remember that some clinical isolates have showed resistance. A decrease in susceptibility to miconazole has been observed,[36] which confirmed previous findings concerning the protective effect of antioxidants during miconazole treatment.[35] Thus, the use of alternative treatments like phytotherapic can be considered promising.

Currently, there are a variety of methods for identifying yeasts from clinical samples. These include traditional methods, such as the germ-tube test, morphology studies, and carbohydrate utilization; rapid methods, such as enzymatic and fluorogenic tests; commercially available methods; automated systems; and recently developed molecular typing techniques.[37]In this study, HiCrome Candida agar and biochemical test API 20 C AUX were used. Through the analysis of Table 4 *C. albicans* was the most prevalent species, followed by *C. tropicallis, C. krusei* and *C. glabrata*, regardless of the group evaluated. These results are in agreement with several studies.[37-41]As Sanitá et al.[38] who compared the prevalence of Candida spp. in diabetics and nondiabetics with and without DS, they found that *C.albicans* was the predominant species isolated (100%), with *C. tropicalis* and *C. glabrata* demonstrating similar prevalence (20% and 10%, respectively). These results were significantly higher than in the group without DS. Cross et al.[41] also found that *C. albicans* was isolated at baseline from all patients with DS, either alone or in combination with another species as *C. glabrata, C tropicalis, C guilliermondii, C krusei, C parapsilosis* and *C kefyr*, all of them less frequently.

In terms of frequency, a large variation in species frequency has been reported in different regions of the world. Although several investigators reported that C. tropicalis is one of the most common non-albicans species isolated in Brazil and

South America, the literature contains substantial data indicating that C. glabrata is found much more frequently in North America.[42,43]

Moreover, for the period of analysis, there was a reduction in the number of microorganisms from day 0 to day 7 and from day 7 to day 14, with the exception of *C. tropicalis* in group C and *C. glabrata* in group A, in which there was higher percentage of microorganisms on the 14th than on day 7, suggesting that the treatments were not effective for this species. Literature shows that C. glabrata exhibits considerable clinically significant cross-resistance between older azole drugs (fluconazole and itraconazole) and voriconazole.[44] On the other hand, it has been demonstrated that C. tropicalis obtained from the oral cavity of denture wearers with denture stomatitis were more adherent to buccal ephitelial cells than those obtained from patients without signs of disease.[45]The ability of C. tropicalis strains to form biofilm on different surfaces is another potential virulence trait, which may increase their resistance to antifungal treatment.[46]Although these specific virulence factors related to C. glabrata and C. tropicalis may help explain the results obtained in the present study, further investigations will be required to examine and better understand the mechanism of pathogenicity of this Candida species.

Literature shows several alternative treatments and different times of treatment against DS with optimal results, time treatment ranging between 1 to 4 weeks showing effectiveness depending on the medical therapy or the prosthesis treatment.[21,22] In this study one week of treatment was enough in the effectiveness of DS treatment. However, further studies increasing the time of treatment with *U. tomentosa* gel will be needed to compare the effectiveness of treatment in terms of time.

Conclusion

Based on the present findings, it was possible to identify different Candida *spp*. found on the prosthesis of denture stomatitis patients. Furthermore, it was observed that *Uncaria tomentosa* gel has the same effect as the 2% miconazole gel; however, the influence of the prescribed hygiene methods should be evaluated.

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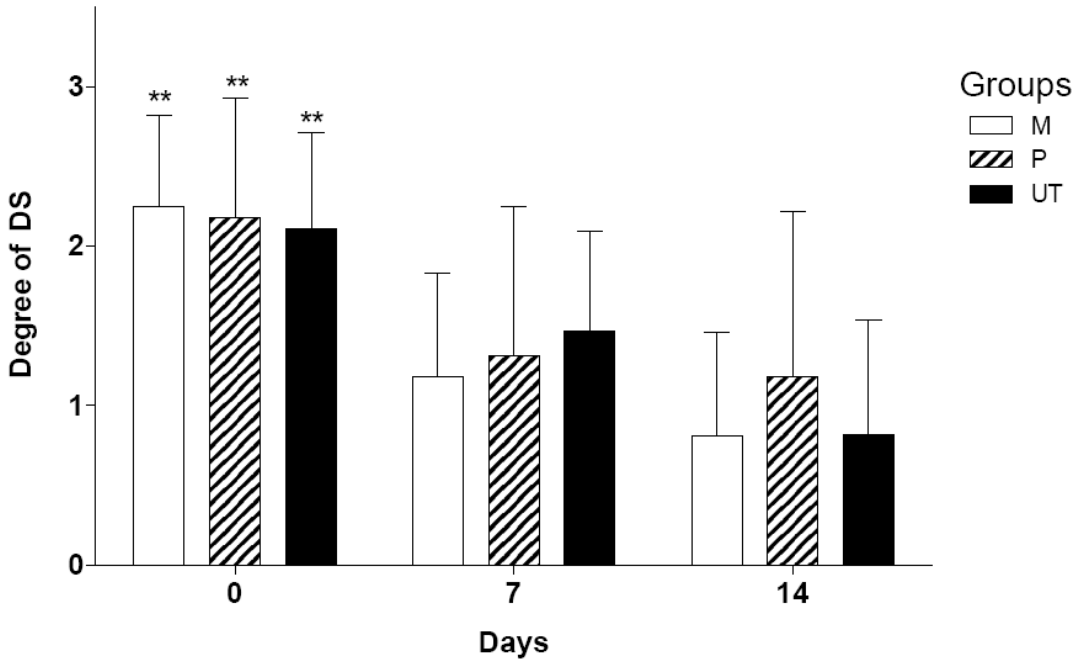


Figure 1: Mean and SD of severity degree of denture stomatitis (scores) for each group (M. Miconazol; P. Placebo; UT. *Uncaria tomentosa*) in different timepoints. Considering groups in different timepoints: (**)p<0.05 with 7 and 14 days. Kruskal-Wallis and Dunn post hoc tests.

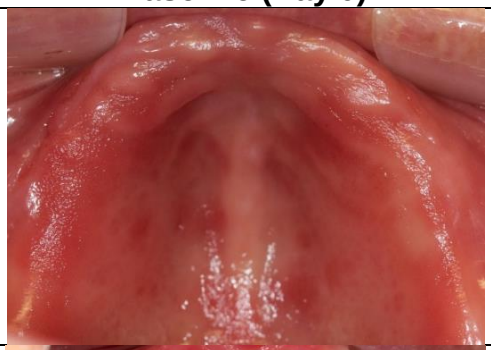
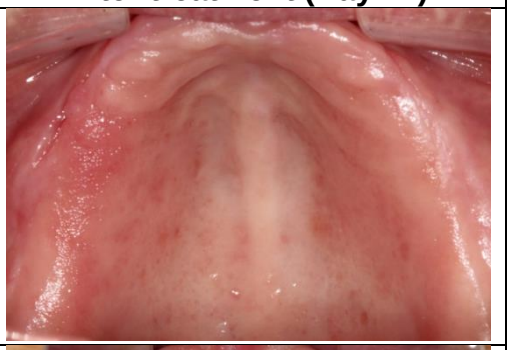
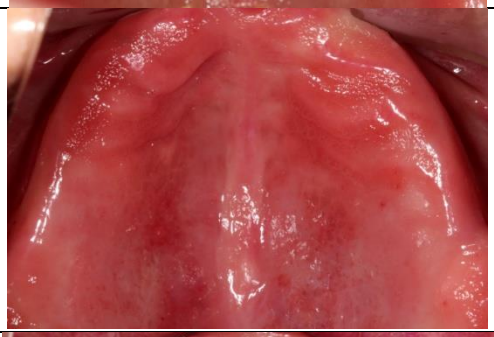
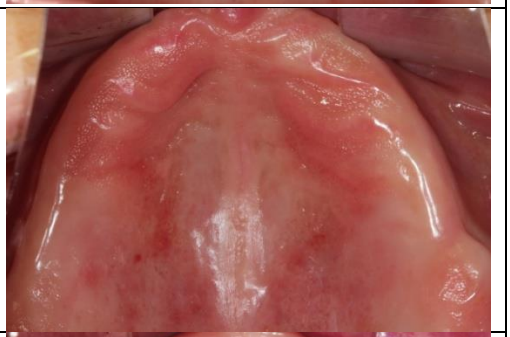
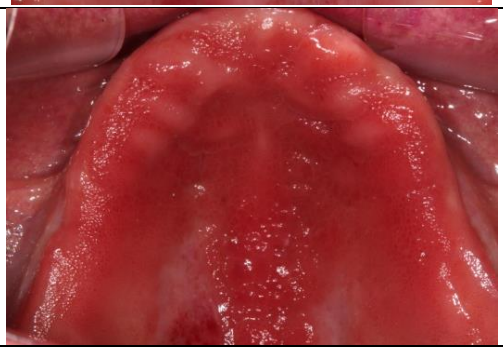
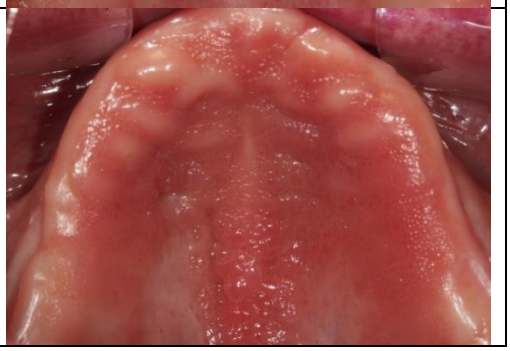
Group	Baseline (Day 0)	After treatment (Day 14)
M: 2% miconazole		
P: 1.5% Natrosol		
UT: 2% *Uncaria tomentosa*		

Figure 2: Palate of patients from 3 experimental groups

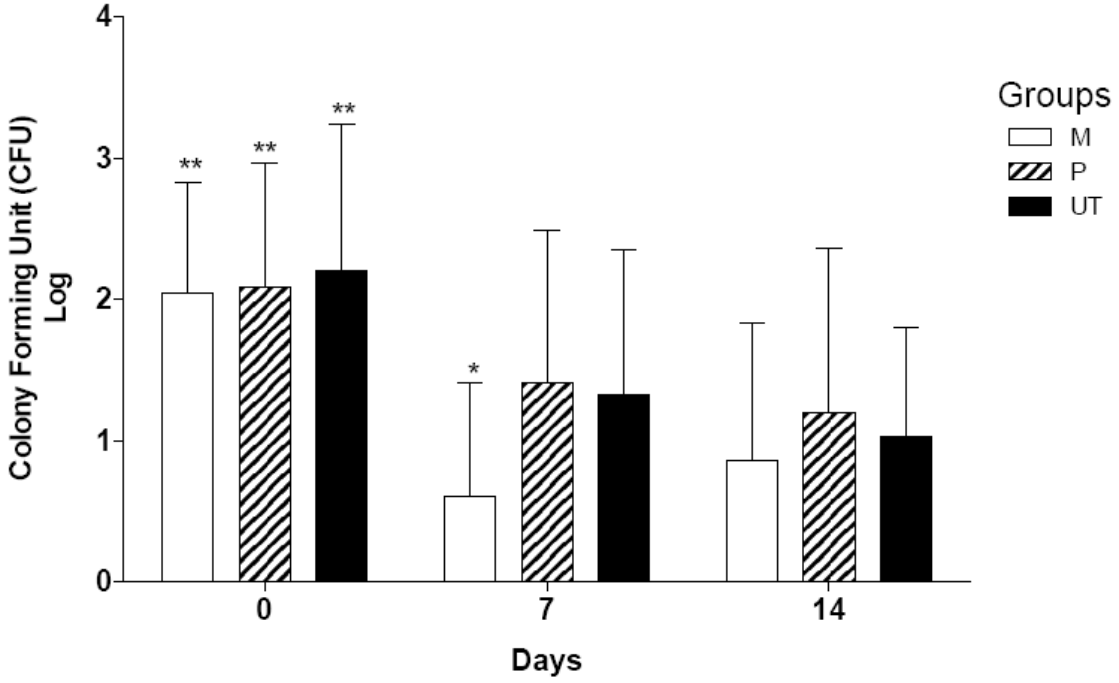


Figure 3. Mean and SD of CFU for each group (M. Miconazol; P. Placebo; UT. *Uncaria tomentosa*) in different timepoints. Considering timepoints: (*)p<0.05 with P and UT groups only 7 days. Considering groups in different timepoints: (**)p<0.05 with 7 and 14 days. Kruskal-Wallis and Dunn post hoc tests.

Table 1: Absolute and relative (%) frequency of Candida species found in the patients.

	Day	**Group M (n=15)**	**Group P (n=16)**	**Group UT (n=17)**
	0	14 (93,3)	15 (93,7)	15 (88,2)
C. albicans	7	7 (46,6)	7 (43,7)	8 (47,1)
	14	3 (26,6)	6 (37,5)	3 (17,6)
	0	3 (20)	5 (31,2)	5 (29,4)
C. tropicalis	7	2 (13,3)	4 (25)	3 (17,6)
	14	1 (6,6)	1 (6,2)	5 (29,4)
	0	2 (13,3)	3 (18,7)	1 (5,8)
C. glabrata	7	0	1 (6,2)	1 (5,8)
	14	2 (13,3)	2 (12,5)	1 (5,8)
	0	2 (13,3)	1 (6,2)	3 (17,6)
C. krusei	7	0	0	0
	14	0	0	1 (5,8)

6 DISCUSSÃO

Neste estudo a prevalência de estomatite protética (EP) foi de 54%, resultado que pode ser comparado com o de Rabiei et al.[5]2010que encontraram uma prevalência de 46%. No estudo de Evren et al.[1] 2011, relataram 45% de casos de EP. Por outro lado, Cueto et al.[38] 2012 observaram que a EP ocorre em 37% da população. De acordo com Gendreau e Loewy[6] 2011, os fatores que podem aumentar o risco de EP são a má adaptação das dentaduras, má higiene, e colonização de microrganismos na superfície da dentadura e da mucosa bucal. No presente estudo foi demonstrada uma diferença significativa na prevalência de EP entre homens (41%) e mulheres (58%). Estes resultados concordam com estudos prévios (Budtz-Jørgensen[39] 1981, Kulak-Ozkan et al.[40] 2002), porém resultados de Evren et al.[1] 2011 mostraram que não existe tal diferença. Além disso, quando foi avaliada a prevalência segundo a classificação de Newton, foi observado resultados similares para todos os tipos, indiferente do gênero.

A idade do paciente pode influenciar na prevalência de EP devido a dificuldade de higiene da dentadura ou limpeza da mucosa bucal em pacientes com menor destreza manual (Evren et al.[1] 2011, Akpan, Morgan[41] 2002). Outros problemas associados com a idade que podem favorecer a doença são o epitélio bucal delgado, diminuição da síntese do colágeno nos tecidos conjuntivo, diminuição na regeneração tissular e uma resistência menor às doenças (Cueto et al.[38] 2012). Porém, os resultados deste estudo não mostraram diferença nos pacientes com menos de 60 anos de idade (66%) ou com mais de 60 anos (52%). Isso demonstra que outros fatores que não estão relacionados a idade, podem favorecer a aparição de EP, como doenças sistêmicas, hábitos de higiene bucal e dentaduras com muito tempo de uso (Evren et al.[1] 2011, Ferreira et al.[2] 2010).

Neste estudo, o fator que mais influenciou a prevalência da EP foi o tempo de uso da prótese. A qualidade das próteses não foi avaliada neste estudo, mas os resultados mostram maior prevalência de EP ao aumentar o tempo de uso da prótese. A prevalência desta doença foi mais de 50% em pacientes que usaram a mesma prótese entre 3 e 10 anos; e 70% dos pacientes que usaram a

prótese por mais 10 anos tiveram EP, o que representa alta prevalência da doença nestes casos. Com o tempo, ocorrem mudanças na superfície da resina acrílica da prótese, como o aumento na rugosidade desta (Oliveira et al.[42] 2010), favorecendo o acúmulo de biofilme e a aparição de EP. Além disso, a habilidade de aderência da*Candida spp*. à mucosa bucal e à resina acrílica pode ter um papel importante na patogênese da EP (Budtz-Jorgensen[43] 2000). Por isso, indica-se a reavaliação das próteses a cada três anos com o objetivo de avaliar a higiene bucal e da prótese feita pelo paciente e a necessidade de reembasamento ou troca da mesma.

A candidose é uma infecção oportunista dos tecidos bucais causada por um aumento de *Candida spp*., sendo a *C. albicans* a espécie mais prevalente (Petersen, Yamamoto[44] 2005). Não foi surpresa encontrar que dos pacientes avaliados com EP, 97% apresentaram *C. albicans,*a qual foi a espécie mais isolada. Este achado esta de acordo com estudos anteriores como o de Coco et al.[12] 2008 que demonstrou presença de *C. albicans* em 75% de pacientes sem EP e em 81% dos pacientes com sinais clínicos da doença. Apesar de que a *C. albicans* é espécie frequentemente isolada, o crescimento de outras espécies ainda não foi adequadamente estabelecido. No presente estudo, *C. tropicalis* e *C. glabrata* foram as duas espécies isoladas com maior frequência depois da *C. albicans*. Alguns estudos mostram a *C. tropicalis* como a segunda mais prevalente (de Resende et al.[45] 2006) e outros a *C. glabrata* como a segunda mais comum (Webb et al.[46] 2005). Esta diferença pode ser atribuída aos diferentes sítios de coletas das amostras, enquanto *C. tropicalis* é mais frequente no Brasil e outros países da América do Sul. A literatura também mostra que *C. glabrata* se encontra com maior frequência na América do Norte (Colombo et al.[47] 2003. Pfaller et al.[48] 2011).

Existem vários tratamentos para EP, porém, o tratamento usando plantas medicinais está sendo cada vez mais frequente pela vantagem do menor custo (Williams[22] 2001), neste estudo encontramos que o uso do gel de *U.tomentosa* foi 70% mais econômico que o gel de miconazol. Por isso, o objetivo deste estudo clínico foi determinar a efetividade do gel de *Uncaria tomentosa* em pacientes com EP. A hipótese foi que o gel de *U. tomentosa* reduz ou elimina a reação inflamatória dos tecidos de suporte das próteses removíveis. Clinicamente a hipótese pode ser considerada aceita, pela redução da severidade de EP após os 7 dias de tratamento, sendo mantida até 14 dias. Porém, também foi observado que

não houve diferenças entre os três grupos experimentais. Isso pode indicar que a *U.tomentosa* gel não foi a única responsável por esta redução. Estes achados podem ser atribuídos à redução de fungos das próteses e palatos pelas indicações dadas aos pacientes acerca de higienização da dentadura e a remoção destas durante o sono. Além disso, somente o fato do paciente ter consciência de que apresenta uma doença e que é parte de um estudo aonde ele é avaliado, faz com que o paciente fique mais atento nas recomendações. Segundo Ramage et al.[49] 2012, a presença de biofilme estruturado pode resultar em altos níveis de inflamação, mas este pode ser controlado por meio da higienização da dentadura. Kanli et al.[50] 2005, também acharam que existe uma correlação entre pobre higiene da dentadura e a prevalência de candidose bucal. Kulak-Ozkan et al.[40] 2002, determinaram os hábitos de higiene bucal, limpeza das dentaduras, presença de fungos e EP em idosos e não encontraram relação significante entre EP com frequência de escovação das dentaduras ou métodos de higienização, porém, houve relação significante entre EP, presença de fungos e limpeza da dentadura. Pelos aspectos acima mencionados, estudos que comparem métodos de limpeza juntamente com o tratamento de *U.tomentosa* devem ser realizados.

Baseados nos resultados das avaliações clínicas de EP, pode-se observar uma melhora significativa após o uso de *U. tomentosa* gel 2%. Isso pode ser explicado pela atividade anti-inflamatória e anti-fúngica do extrato (Herrera et al.[32] 2010). Apesar de que o estudo de Ccahuana-Vasquez et al.[51] 2007 não ter demonstrado atividade anti-fúngica da *U.tomentosa* contra o crescimento de *Candida albicans*; estudos como o de Paiva et al.[52] 2009, avaliaram clinicamente a efetividade da *U. tomentosa* para candidose bucal, os resultados foram similares ao miconazol. Os resultados foram similares com os dopresente estudo.

Além disso, a apresentação no formato gel para o tratamento mostra sucesso na aplicação, em comparação com outros anti-fúngicos como a nistatina em solução oral que tem um contato com a lesão por um tempo menor quando comparado com o gel. O produto é colocado diretamente na prótese, previamente higienizada, a qual atua como uma “férula” a qual permite que o produto fique em maior contato com a lesão, mostrando melhor resposta e uma regressão mais rápida dos sintomas.

A contagem de UFC/mL foi maior no dia 0, a qual foi reduzida nos demais períodos de observação (7 e 14dias). Podemos observar também, que no dia 7, houve uma diminuição de UFC/mL para o grupo M, diferente do grupo P e UT. Por isso, o miconazol foi considerado mais efetivo na redução imediata de colônias quando comparado com a *U. tomentosa* e o placebo. Os resultados demonstram a efetividade do antifúngico miconazol, a qual é comprovada em outros estudos (Amanlou et al.[19] 2006). Porém no dia 14 os três grupos tiveram uma redução significativa das UFC/mL, sendo que o grupo que recebeu *U.tomentosa* teve uma resposta muito similar ao grupo que recebeu miconazol, indicando efetividade anti-fúngica da *U.tomentosa*.

Quando foi avaliada a efetividade dos tratamentos contra as espécies de Candida, foi encontrada redução no número de pacientes com *C. albicans* do dia 0 para o dia 7 e do dia 7 para o dia 14, mas no grupo M não houve redução de *C. glabrata* e no grupo UT não houve redução de *C. Tropicalis*, sugerindo que estes tratamentos não são efetivos contra estas espécies de Candida. A literatura demonstra que a *C. glabrata* mostra uma resistencia fúngica contra os azóis antigos (fluconazol e itraconozal) e o voriconazol (Panackal et al.[53] 2006). Por outro lado, tem se demonstrado que a *C. tropicalis* obtida da cavidade bucal de pacientes com EP é mais aderente à células epiteliais da boca quando comparado com as *C. tropicalis* obtidas de pacientes portadores de prótese sem a EP (Lyon. Resende[54] 2007). A habilidade da *C. tropicalis* de formar biofilme em diferentes superfícies é outro potencial de virulência, que pode incrementar a resistência aos tratamentos anti-fúngicos (Ramage et al.[55] 2004). Apesar de que estes fatores de virulência relacionados à *C. glabrata* e *C. tropicalis* podem explicar os resultados obtidos neste estudo, futuras pesquisas devem ser realizadas para examinar e entender melhor o mecanismo e patogenia destas espécies de Candida.

A literatura mostra diversos tratamentos alternativos com diferentes tempos de aplicação para a estomatite protética com bons resultados, o tempo varia de 1 a 4 semanas, mostrando efetividade dependendo da terapia medicamentosa ou do tratamento da prótese (Sanitá et al.[15] 2011. Neppelenbroek et al.[35] 2008). Neste estudo, foi utilizada uma semana de tratamento, suficiente para se obter resultados efetivos para EP. Cabe ressaltar que nenhum dos pacientes apresentou queixa pelo uso dos produtos testados, e também não apresentaram

qualquer efeito adverso durante o tratamento ou após nas avaliações. Porém, futuros estudos devem ser realizados para comparar a efetividade do tratamento em diferentes períodos de tempo.

7 CONCLUSÕES

Considerando os dados do presente estudo clínico, pôde-se concluir que:

1. O gel de *Uncaria tomentosa (Willd.) D. C.* a 2% foi tão efetivo no tratamento da estomatite protética (EP) quanto miconazol a 2%;

2. Todos os tratamentos propostos nos três grupos avaliados foram efetivos no tratamento da EP;

3. A EP é mais prevalente no gênero feminino e o tempo de uso da prótese tem uma relação direta na prevalência desta;

4. *Candida albicans* foia espécie de Candida mais isolada em pacientes com estomatite protética.

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* De acordo com a norma do Programa de Pós-Graduação em Odontologia da UEPG, baseado no modelo Vancouver. Abreviaturas dos periódicos em comformidade com o Medline.

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ANEXOS

UEPG
UNIVERSIDADE ESTADUAL DE PONTA GROSSA

PROPESP
Pró-Reitoria de Pesquisa e Pós-Graduação

COEP
COMISSÃO DE ÉTICA EM PESQUISA

PARECER Nº 14/2011
Protocolo: 17572/10

No dia 24 de fevereiro de 2011, a Comissão de Ética em Pesquisa, **APROVOU** o protocolo de pesquisa intitulado **"Estudo clínico do uso tópico de Uncaria tomentosa em pacientes usuários de prótese total, portadores de candidose bucal"** de responsabilidade da pesquisadora Janaina Habib Jorge.

Conforme Resolução CNS 196/96, solicitamos que sejam apresentados a esta Comissão, relatórios sobre andamento da pesquisa, conforme modelo (http://www.uepg.br/coep/).

Data para entrega do relatório parcial:24 de fevereiro de 2012
Data para entrega do relatório Final: 24 de fevereiro de 2013.

Ponta Grossa, 25 de fevereiro de 2011.

UNIVERSIDADE ESTADUAL DE PONTA GROSSA
COMISSÃO DE ÉTICA EM PESQUISA - COEP

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TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

1. Título da Pesquisa: Estudo clínico do uso tópico de *Uncaria tomentosa* em pacientes usuários de prótese total, portadores de candidose oral.

2. Pesquisadores: Profª Dra. Janaina Habib Jorge, CD. Daniel Rodrigo Herrera Morante, CD. Lidia Yileng Tay Chu Jon.

3. Proposição: O objetivo deste estudo é avaliar o efeito da utilização de um gel a base do fitoterápico *Uncaria tomentosa*, conhecido como unha de gato, no tratamento da candidose oral em pacientes portadores de prótese total superior.

4. Procedimentos do Experimento: Após exame clinico, os pacientes que manifestem seu desejo de participar, e após assinatura deste termo, serão distribuídos aleatoriamente em três grupos. Cada grupo receberá as mesmas instruções e indicações para a limpeza da prótese e higiene bucal para o qual receberam uma escova para limpeza de prótese. Cada grupo também recebera uma bisgana contendo 20 g de gel sendo instruídos na aplicação do mesmo no palato após as refeições e a higienização oral e limpeza da prótese. Será feito o registro fotográfico do palato e das próteses antes do tratamento e após 7 dias do uso dos medicamentos. Esfregaços serão obtidos de ambos dos sítios, corados para analise microscópico para verificação da presença ou não de leveduras e/ou hifas de *Candida spp.* (fungos)

5. Local da pesquisa: O tratamento e exame clínico serão realizados na Clínica Odontológica do Curso de Odontologia da Universidade Estadual de Ponta Grossa. Durante este período os voluntários serão acompanhados pelos pesquisadores para a verificação de qualquer efeito adverso.

6. Resultados esperados: **Espera-se com esse estudo verificar a eficácia de *Uncaria tomentosa* em gel ao 2% no tratamento da candidíase oral.**

7. Análise crítica dos riscos e benefícios: As técnicas que serão utilizadas neste estudo são descritas na literatura, na determinação, tratamento e controle da candidíase oral. Os produtos utilizados na pesquisa têm mostrado atividade antifúngica, sendo que a concentração utilizada tem se mostrado segura. Assim, todos os pacientes receberam tratamento efetivo para candidíase oral. Os indivíduos participantes da pesquisa receberão gratuitamente os geis, assim como uma escova para a limpeza da prótese.

8. Forma de acompanhamento, assistência e garantia de esclarecimentos: Os indivíduos terão a garantia de que receberão esclarecimento a qualquer dúvida, acerca dos procedimentos, riscos, benefícios e outros assuntos relacionados com a pesquisa. Os pesquisadores responsáveis assumem o compromisso de proporcionar informação atualizada obtida durante o estudo, ainda que esta possa afetar a vontade do indivíduo em continuar participando dele.

9. Retirada do consentimento: Os sujeitos têm a liberdade de se recusar a participar da pesquisa ou de retirar seu consentimento a qualquer momento, sem sofrer qualquer tipo de prejuízo, ou represálias de qualquer natureza.

10. Garantia de sigilo: Os pesquisadores se comprometem a resguardar todas as informações

individuais. tratando-as com impessoalidade e não revelando a identidade do sujeito que as originou.

11. Formas de ressarcimento de despesas e de indenização: Os indivíduos não deverão ter efetivamente qualquer despesa, pois o estudo será realizado nos períodos normais durante o tratamento odontológico (candidíase oral). Para o tratamento de efeitos adversos os custos estão previstos no orçamento do projeto.

12. Consentimento pós-informação:

Eu, _____, certifico que tendo lido as informações acima e suficientemente esclarecido de todos os itens, pelos pesquisadores clínicos responsáveis: Profª Dra. Janaina Habib Jorge, CD. Daniel Rodrigo Herrera Morante, CD. Lidia Yileng Tay Chu Jon; estou plenamente de acordo com a realização do experimento. Assim, eu concordo em participar como voluntário do trabalho de pesquisa, exposto acima.

Certifico também ter recebido uma cópia deste Termo de Consentimento Livre e Esclarecido.

Ponta Grossa, ____ de _____ de ____.

Nome: _____

Assinatura: _____

Para entrar em contato com os pesquisadores:
Profª Dra. Janaina Habib Jorge (42) 3220-3741
CD. Daniel Rodrigo Herrera Morante (42) 99027233
CD. Lidia Yileng Tay Chu Jon (42) 99027233

ATENÇÃO: A sua participação em qualquer tipo de pesquisa é voluntária. Em caso de dúvida quanto aos seus direitos, entre em contato com a Comissão de Ética em Pesquisa da UEPG. Endereço – Av. Carlos Cavalcanti, n.4748, Bloco M, Sala 12, CEP- 84030-900 – Ponta Grossa – PR. Fone: (42) 3220-3108.
e-mail: seccoep@uepg.br