UNIVERSIDADE DE UBERABA
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AVALIAÇÃO DA FUNÇÃO DA LÍNGUA, DO FLUXO SALIVAR E DOS NÍVEIS DE IgA, IgM E IgG NA SALIVA DE PACIENTES CHAGÁSICOS CRÔNICOS

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Dissertação apresentada ao curso de Mestrado acadêmico em Odontologia, área de concentração Biopatologia da Universidade de Uberaba, como requisito parcial para a obtenção do título de Mestre em Odontologia.

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(Arthur Schopenhauer)

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RESUMO

INTRODUÇÃO: Alterações morfológicas e imunológicas na cavidade oral de chagásicos têm sido descritas. No entanto não encontramos nenhum estudo que avaliasse a função da língua e os níveis de IgA, IgG e IgM totais na saliva de chagásicos comparados aos não chagásicos. OBJETIVO: Avaliar a função da língua, o fluxo salivar e os níveis de IgA, IgG e IgM totais na saliva de pacientes chagásicos comparados aos não chagásicos. METODOLOGIA: Foram selecionados 37 pacientes sendo 17 pacientes chagásicos: 6 na forma cardíaca, 11 na forma mista com megaesôfago e 20 pacientes não chagásicos. Realizou-se o exame da função da língua por uma técnica fonoaudiológica. Foi coletada saliva dos pacientes de maneira não estimulada e o fluxo salivar medido por sialometria. Os níveis de IgA, de IgG e de IgM na saliva dos pacientes foram avaliados pela técnica do ELISA sanduiche. RESULTADOS: Não foram encontradas diferenças significativas entre os grupos quanto à função da língua. Observou-se maior fluxo salivar nos pacientes chagásicos na forma mista com megaesôfago quando comparados aos demais grupos ($p = 0.0302$). Não foram encontradas diferenças significativas quando comparou-se os níveis de IgG, de IgM e de IgA totais entre os grupos. CONCLUSÃO: Apesar de não termos encontrado diferenças significativas quanto à função da língua e quanto aos níveis de imunoglobulinas salivares totais, o maior fluxo salivar nos pacientes chagásicos, com forma mista, provavelmente estaria ocorrendo por alterações esofágicas e/ou por alterações de glândulas salivares provocadas pela DC crônica.

PALAVRAS CHAVE: Doença de Chagas. Immunoglobulinas. Língua. Saliva.

ABSTRACT

INTRODUCTION: morphological and immunological changes in the oral cavity of chagasics have been described. However we found no studies that avaluate the function of tongue and total salivary levels of the IgA, IgG and IgM in total chagasics compared to non chagasics patients.OBJECTIVE: To evaluate tongue function, salivary flow rate, and levels of total immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM) in the saliva of chronic patients (CP) in comparison to non-chagasic patients (NCP). METHODOLOGY: Thirty seven patients were selected: NCP (n=20) and CP (n=17). In the group CP 6 had the cardiac form and 11 had the mixed form with megaesophagus. Examination of tongue function was performed through phonoaudiological evaluation. Saliva was collected from the patients, and the salivary flow rate was measured by sialometry. The levels of total IgA, IgM and IgG in the saliva of patients were evaluated by sandwich ELISA assay. RESULTS: There were no significant differences in the function of the tongue among the groups. Patients with the mixed form of Chagas disease with megaesophagus had a higher salivary flow rate than patients in the other groups ($p = 0.0302$). There were no significant differences in the levels of total IgA, IgM and IgG among the groups. CONCLUSION: Although there were no significant differences in tongue function and in the levels of total salivary immunoglobulins, the higher salivary flow in chagasic patients with the mixed form probably occurred due to esophageal abnormalities and/or changes in the salivary glands caused by chronic Chagas disease.

KEYWORDS: Chagas disease. Immunoglobulin. Saliva. Tongue.

LISTA DE ABREVIATURAS

DC - Doença de Chagas

ELISA - Enzyme-Linked Immunosorbent Assay – Ensaio Imunoenzimático

IFN-γ - Interferon - gama

IgA – Imunoglobulina A

IgM - Imunoglobulina M

IgG – Imunoglobulina G

T. cruzi - *Trypanossoma cruzi*

TGF-β - Tumor necrosis factors – beta

TNF-α - Tumor necrosis factors – alfa

UNIUBE - Universidade de Uberaba

SUMÁRIO

1 INTRODUÇÃO

A doença de Chagas (DC), descrita por Carlos Chagas (1909), é uma zoonose potencialmente letal (DUBNER et al., 2008) que afeta milhões de pessoas na América latina (PÉREZ-AYALA et al., 2011). A DC é causada por infecção pelo protozoário parasita, *Trypanossoma cruzi* (*T. cruzi*) (RAMOS et al., 2011) sendo a principal causa de miocardiopatia e morte por doença cardiovascular em pacientes com idades entre 30-50 anos (PÉREZ-AYALA et al., 2011). Apesar da doença ser um sério problema de saúde pública (PRIOTTO et al., 2009) ainda não são esclarecidas completamente todas as alterações morfológicas provocadas pelo *T. cruzi* (CAROD-ARTAL et al., 2007).

A DC é uma doença endêmica em 21 países (RAMOS et al., 2011), acometendo grande variedade de mamíferos silvestres e domésticos além de seres humanos (KIRCHHOFF, 2011). Historicamente, os triatomíneos, mais conhecidos como barbeiros, são os vetores de transmissão de *T. cruzi*. A transmissão ocorre por transfusão de sangue, transplante de órgãos, via transplacentária (KIRCHHOFF, 2011) e via oral (TOSO; VIAL; GALANTI, 2011). A primeira evidência de transmissão oral foi relatada no Brasil em 1965. A contaminação por via oral ocorre pela ingestão de triatomíneos infectados ou de alimentos contaminados com a urina ou secreção anal de marsupiais infectados (TOSO; VIAL; GALANTI, 2011).

Nos mamíferos infectados, incluindo humanos, a DC apresenta três fases características: aguda, indeterminada e crônica. A fase crônica pode apresentar-se nas formas cardíaca, digestiva ou mista (PRADO et al., 2011). Na fase aguda o parasita encontra-se livre na corrente sanguínea. Posteriormente os parasitas invadem os tecidos dos hospedeiros, com predileção pelo tecido muscular cardíaco e sistema digestório, convertendo-se em formas intracelulares que proliferam podendo atingir qualquer tecido humano (LOPES et al., 2000).

Na forma digestiva da DC existe destruição de gânglios intramurais e desnervação parassimpática em todo o trato digestório, afetando especialmente o esôfago e o retossigmóide (CREMA et al., 2011). Estudo realizado em pacientes chagásicos já demonstrou mudanças no padrão normal de deglutição através da análise do trânsito oral e faríngeo pelo método de cintilografia. Nos indivíduos chagásicos, na forma digestiva, existe maior tempo de trânsito oral e faríngeo quando comparados aos indivíduos saudáveis (dos SANTOS; CASSIANI; DANTAS, 2011).

No coração o processo inflamatório na DC está associado com fibrose intersticial, sendo que nas fases mais precoces da doença existe predomínio de fibronectina, laminina e

colágeno tipo I e tipo III (MAGALHAES-SANTOS; LIMA; ANDRADE, 2002). Sabe-se que o acometimento cardíaco na fase crônica da DC é caracterizado por apresentar destruição de células musculares cardíacas com infiltrado inflamatório e neoformação de tecido conjuntivo fibroso (ELIZARI, 1999; LOPES et al., 2000). Existe destruição progressiva do miocárdio em aproximadamente 30% dos indivíduos infectados cronicamente pelo *T. cruzi (*SOARES et al., 2004). Em um estudo realizado através de autópsias, foram observados focos de necrose e degeneração no miocárdio juntamente com células como fibroblastos, linfócitos T e macrófagos (ROSSI, 1998). Assim como no intestino e coração, os músculos esqueléticos, também foram descritos como locais infectados por *T. cruzi*. Parasitismo em diferentes grupos musculares foi descrito em camundongos (BIJOVSKY et al., 1983). Dores musculares e fraqueza já foram descritas em pacientes chagásicos (KÖBERLE, 1968), assim como o músculo deltóide (CENGET; ROJAS, 1959). Estudo realizado em ratos infectados pelo *T. cruzi* analisou os músculos reto abdominal e músculos plantares, através de estimulação elétrica direta. As propriedades contráteis dos músculos foram significativamente reduzidas durante a infecção (RAMIREZ-ARCHILA et al., 2011). Além disso, em indivíduos infectados cronicamente pelo *T. cruzi* já foram observadas alterações estruturais em miofibrilas através de biópsias musculares (LAGUENS et al., 1975). Há também evidências de que existem danos na junção neuromuscular, fazendo com que haja perda de sensibilidade e flacidez da estrutura contrátil (MIRKIN et al., 1994). No final do período de infecção a inflamação nos tecidos diminui, porém, o processo de reparo do tecido muscular ocorre por fibrose, o que provoca mais complicações na contratilidade muscular (ARAÚJO-JORGE et al., 2008).

Não encontramos, entretanto, nenhum estudo na literatura que descrevesse alterações clínicas na língua de pacientes com DC, ou que avaliasse sua espessura e contratibilidade (FELTON et al., 2007). No entanto já foram descritas alterações morfológicas microscópicas na língua de pacientes chagásicos. Nesses estudos observamos alterações vasculares na língua dos chagásicos como maior diâmetro vascular, aumento da área da parede vascular, maior densidade dos vasos sanguíneos e aumento da espessura da membrana basal capilar (de LIMA PEREIRA et al., 2009), maior densidade de tecido conjuntivo fibrose e maior densidade de mastócitos na língua de chagásicos quando comparados aos não chagásicos (de LIMA PEREIRA et al., 2007). Desta maneira, a fibrose da língua poderia estar contribuindo para a disfagia na doença de Chagas/forma digestiva. Portanto, acreditamos que a língua e saliva desses pacientes possam estar alterados.

Além de alteração vasculares e da matriz extracelular, já foi descrito, na língua de pacientes com DC, inflamação, perineurite e aumento do calibre de ductos de glândulas salivares de Von Ebner (de LIMA PEREIRA et al., 2006). Já foi descrita hipertrofia da glândula submandibular em ratos infectados pelo *T. cruzi*, apresentando proteínas idênticas àquelas produzidas pela estimulação adrenérgica beta (ALVES; ALVES; NAITO, 1994). Porém não encontramos estudos que comparasse a intensidade de fluxo salivar em pacientes chagásicos crônicos, nem que correlacionasse esses achados com as alterações morfológicas.

A produção e secreção de anticorpos frente a infecção por *T. cruzi* é particularmente complexa devido ao alto grau de polimorfismo entre as diferentes espécies e clones, ocasionando as mais diferentes manifestações clínicas, sendo que muitas ainda são pouco esclarecidas (PINHO et al., 1999). Porém não encontramos na literatura nenhum dado que comparasse os níveis de anticorpos na saliva entre chagásicos e não chagásicos, nem entre as formas da doença.

Anticorpos IgG são produzidos ainda na fase aguda da DC (LANA et al., 1991; CARNEIRO et al., 2007) e reagem principalmente com as moléculas de superfície das formas tripomastigotas (BRODSKYN et al., 1989; CORDEIRO et al., 2001). Os principais isotipos de anticorpos envolvidos na eliminação de formas sanguíneas do parasita e na diminuição das taxas de mortalidade são IgG1 e IgG2 (BRODSKYN et al., 1989; CORDEIRO et al., 2001).

Estudos mostram que indivíduos chagásicos produzem no plasma níveis significativamente mais elevados de IgG do que indivíduos não chagásicos (PISSETTI et al., 2009; MORGAN et al., 1996) e que indivíduos com a forma digestiva da DC apresentam níveis mais elevados de IgG-4. Além disso, já foram observados maiores níveis de IL-10 nos indivíduos com a forma digestiva, que poderiam contribuir com os níveis elevados de IgG4 específicos, inibindo, indiretamente, os efeitos de IFN-γ, favorecendo um desvio Th2 o que ajudaria na manutenção do parasita e consequentemente desenvolvimento das lesões de fase crônica (PISSETTI et al., 2009).

Além de IgG, já foram descritos, no sangue de pacientes com a forma digestiva da DC, níveis elevados de anticorpos da isoforma IgA (SÁ FERREIRA et al., 1983; PRIMAVERA et al., 1988). Estudo demonstrou aumento dos níveis de IgA na forma digestiva da DC de acordo com a gravidade do comprometimento esofágico. Os níveis aumentados de IgA poderia ser provocado por danos na mucosa em consequência à irritação provocada pela permanência do bolo alimentar no esôfago, o que favoreceria a passagem de antígenos para o conjuntivo devido à perda de integridade epitelial (WALKER; ISSELBACHER; BLOCH, 1974). Estes antígenos estimulariam precursores de células produtoras de IgA plasmático localizados na

lâmina própria do no trato gastrointestinal (WALKER; ISSELBACHER; BLOCH, 1974). Além disso, sabe-se que reduções transitórias nos níveis de IgA salivar estão associados com aumento da susceptibilidade a infecções do trato gastrointestinal (NOGUEIRA et al., 2005).

Como já demonstramos em outros estudos que existem alterações morfológicas microscópicas das línguas dos chagásicos crônicos, e ainda, como até o presente momento, não foram esclarecidas todas as alterações clínicas e imunológicas da cavidade oral dos pacientes com DC, surgiu a necessidade de avaliar clínica e macroscopicamente as línguas dos chagásicos. Esse estudo, somado aos anteriores, poderá contribuir para posterior elaboração de estratégicas terapêuticas que minimizem as dificuldades de deglutição e de digestão desses pacientes. O conhecimento dos níveis de anticorpos produzidos na fase crônica da DC também ajudaria a explicar a patogênese da doença e seus possíveis mecanismos lesivos na cavidade oral.

2 HIPÓTESE

Os pacientes chagásicos crônicos apresentam menor contratilidade da língua, maiores níveis de IgA, de IgM e de IgG e maior fluxo salivar quando comparados aos não-chagásicos.

3 OBJETIVOS

1. Realizar avaliação funcional da língua de pacientes chagásicos e não chagásicos crônicos;
2. Avaliar o fluxo salivar dos pacientes chagásicos e não chagásicos crônicos, sem estimulação;
3. Determinar os níveis de IgA, IgM e IgG em salivas de pacientes chagásicos e não chagásicos crônicos.

TONGUE FUNCTION, SALIVARY FLOW RATE AND SALIVARY LEVELS OF TOTAL IgA, IgM AND IgG IN PATIENTS WITH CHAGAS DISEASE

Running title: Evaluation of tongue and saliva of chagasics

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Abstract

Objective: To evaluate tongue function, salivary flow rate, and levels of total immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM) in the

saliva of chronic patients (CP) in comparison to non-chagasic patients (NCP). Methodology: Thirty seven patients were selected: NCP (n=20) and CP (n=17). In the group CP 6 had the cardiac form and 11 had the mixed form with megaesophagus. Examination of tongue function was performed through phonoaudiological evaluation. Saliva was collected from the patients, and the salivary flow rate was measured by sialometry. The levels of total IgA, IgM and IgG in the saliva of patients were evaluated by sandwich ELISA assay. Results: There were no significant differences in the function of the tongue among the groups. Patients with the mixed form of Chagas disease with megaesophagus had a higher salivary flow rate than patients in the other groups ($p = 0.0302$). There were no significant differences in the levels of total IgA, IgM and IgG among the groups. Conclusion: Although there were no significant differences in tongue function and in the levels of total salivary immunoglobulins, the higher salivary flow in chagasic patients with the mixed form probably occurred due to esophageal abnormalities and/or changes in the salivary glands caused by chronic Chagas disease.

KEYWORDS: Chagas disease; Tongue; Immunoglobulin; Saliva

1. Introduction

Chagas disease, or American trypanosomiasis, is a systemic parasitic infection caused by the flagellated protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) (Briceño, 2009). In infected mammals, including humans, Chagas disease has three characteristic phases: acute, indeterminate and chronic, and the latter can occur in the cardiac, digestive or mixed forms (Prado et al., 2011). Dysphagia, which is commonly found in chronic chagasic patients with the digestive form of the disease,

occurs due to esophageal involvement. During swallowing, oral and pharyngeal transit of the bolus of these patients is slower than in healthy subjects (dos Santos, Cassiani & Dantas, 2011).

In addition to esophageal involvement, microscopic morphological changes have also been described in the tongue in chronic chagasic patients, such as: increased vascular diameter, increased vascular wall area, increased blood vessel density, thickened capillary basement membrane (de Lima Pereira et al., 2009), and increased collagen density and higher density of mast cells (de Lima Pereira et al., 2007). Although the tongue plays an essential role in swallowing and in early digestion of food (Felton, Gaige, Reese, Wedeen, & Gilbert, 2007), there are no studies describing clinical changes in the tongue of Chagas disease patients.

The salivary glands are also often affected in Chagas disease. Several changes have already been described in the salivary glands in Chagas disease, as increased diameter of the ducts of Von Ebner's glands in humans (de Lima Pereira et al., 2006), and hypertrophy of the submandibular gland in rats (Alves, Alves & Naito, 1994). A study showed dilation of the body of the parotid, submandibular and sublingual glands in 13-33% of patients with chagasic megaesophagus (Vieira, 1961), as well as increased salivary flow rate in chronical Chagas disease (Earlam, 1972; de Oliveira, Bloise & Lopes, 1952; Vieira, 1961).

Even though functional alterations of the tongues of Chagas disease patients have not yet been described, it is known that the parasitism of skeletal muscles of mice infected with *T. cruzi* (Bijovsky, Elizari, Muller, Katzin, & González Cappa, 1983) causes damage to the neuromuscular junction, as well as subsequent loss of sensitivity and contractile structure stretching (Mirkin et al., 1994). Muscle repair with replacement of muscle tissue by collagen was observed at the end of the infection

period, with consequent complications in muscle contractility (Araújo-Jorge et al., 2008).

The production and secretion of antibodies against *T. cruzi* infection leads to several clinical manifestations, many of which are still poorly elucidated (Pinho, Pedrosa, Costa-Martins, Castello-Branco, 1999). Antibodies of different classes specific for *T. cruzi* have been found in the saliva of chagasic patients (Pinho, Pedrosa, Costa-Martins, Castello-Branco, 1999). Although total antibody levels are associated with individual immunity, there is no study in the literature comparing total antibody levels in the saliva of chagasic and non-chagasic patients, or total antibody levels between the forms of the disease.

Even though clinical changes in the oral cavity of patients with Chagas disease are still unclear, we believe that there could be changes in tongue muscle contraction, as well as increased salivary flow rate and changes in total salivary IgA, IgM and IgG levels in patients with Chagas disease.

2. Materials and Methods

Upon approval by the Research Ethics Committee of the Federal University of Triangulo Mineiro (UFTM) under protocol number 2256, a cross-sectional study and analysis of medical records of patients was conducted at the University Hospital of University of Uberaba (UNIUBE), in Uberaba, in the state of Minas Gerais, Brazil.

2.1. Patient selection

A total of 37 adult patients older than 24 were included in the study. All participants were aware of the study and signed an informed consent form. They were homogenized regarding gender, age and ethnicity, and were then subdivided

into three groups according to the clinical criteria: chronic Chagas disease patients with the cardiac form (n = 6), chronic Chagas disease patients with the mixed form with the presence of megaesophagus (n = 11), and non-chagasic patients (n = 20). As a selection criteria, chagasic patients had to have at least one positive reaction to *T. cruzi* and a morphological finding suggestive of Chagas disease, such as cardiomegaly and/or megaesophagus demonstrated by imaging exams.

Approximately 100 medical records of patients with or without Chagas disease were analyzed. Patients with other systemic infectious diseases, patients with a history of craniofacial malformations, with temporomandibular dysfunction, with malignant neoplasms, patients on medications such as antihistamines or decongestants, as well as alcoholic or smoker individuals were excluded from the study. After a review of dental records, patients with caries, gingivitis, periodontitis, disorders that cause stress or chronic pain in the head and neck, mouth breathers, patients with allergic rhinitis, patients who suffered cerebrovascular accident (CVA) or any type of facial paralysis were also excluded.

2.2. Tongue function examination

After anamnesis, clinical examination of the tongue of patients was conducted by a speech therapist at the Speech Therapy Clinic of University of Uberaba (UNIUBE). The following were evaluated: muscle tone, mobility, muscle strength with counter-resistance, muscle strength without counter-resistance, and contraction of the lingual apex. Sterile gloves and spatulas were used so as to perform this evaluation (Fig. 1).

For muscle tone evaluation, the patients were asked to open their mouths and put their tongues up in order to slightly touch the palate. Hence, their ability to

maintain the tongue vertically tensioned was evaluated, and the tongue was considered normal or flaccid (Fig. 1, A). In order to evaluate the mobility, the patients were asked to move their tongues to the left and to the right with their mouths closed. After that, the ability of the patients to lateralize their tongues and touch the sides of the oral cavity was evaluated when they were asked to clearly project their cheeks for the examiner to observe. Mobility of the tongue was, thus, considered to be normal or altered (Fig. 1, B and C). Muscle strength against no resistance was analyzed by protruding the tongue out, followed by free up-and-down movements from one side to the other for as long as the patients managed to. In this case, we tested whether the patients had enough strength to perform the procedure (Fig. 1, D, E and F). Muscle strength against resistance was evaluated by asking the patients to protrude their tongues out and freely move them up and down from side to side for as long as they could, while the examiner applied force against the movement of the tongue, and evaluated whether the patients had strength or not (Fig. 1, G and H). In order to assess the contraction of the lingual apex, the patients were asked to protrude their tongues out of the oral cavity with subsequent elevation of the lingual apex. Thus, the dorsum of the tongue curved upwards and the lingual apex was tapered (Fig. 1, J).

2.3. Analysis of saliva

2.3.1 Collection of saliva

After formation of the groups, saliva collection was performed in a calm environment between 1 p.m. and 3 p.m. Each patient salivated, in an unstimulated way, for five minutes in a sterile container two hours after lunch. The patients themselves held the container in the lower area of the lower lip with maximal mouth opening, so that the saliva would collect passively into the container, without

induction (Fig. 2). Later, saliva was withdrawn from the container with the aid of a 5mL graduated disposable vacuum syringe in order to determine the exact volume of saliva.

2.3.2. Evaluation of salivary flow rate by sialometry

The salivary flow rate was measured by unstimulated whole sialometry technique. Sialometry was performed in accordance with a method previously described and used by other authors (Chao et al., 2001; Navazesh & Kumar, 2008). In order to determine the salivary flow rate, the amount of saliva in mL was divided by five, and the result was expressed in mL/min. The foam that eventually appeared during the collection was not discarded because it became fluid after a few minutes.

2.3.3 Analysis of total salivary levels of immunoglobulins IgA, IgM and IgG

The total levels of IgA, IgM and IgG in the saliva were determined by Enzyme-Linked Immuno Sorbent Assay (ELISA) 96-well polystyrene plates with high affinity for proteins (Costar 3590, USA). Plates were sensitized with 100 µl of affinity purified goat anti-human IgA or IgM or IgG antibody solution (Zymed, USA), diluted in buffer I (sodium bicarbonate, pH 9.6) to a concentration of 2 µg/mL per well. The plates were sealed and incubated at 37^{o}C for two hours, and then kept at 4^{o}C for 16 hours. After sensitization, the plates were washed twice with buffer II containing NaCl (0.9%), Tween 20 (0.05%), and sodium azide (0.02%). Blocking of non-specific binding was performed using 100 µl of bovine serum albumin solution (BSA) at 0.02% buffer III (phosphate-buffered saline – PBS-BSA, pH 7.5) under shaking at room temperature for one hour. The plates were then subjected to further washes with buffer II. One hundred microliters of saliva samples diluted 1:200 in buffer III were added per well in

duplicate, and the plates were then incubated for two hours under shaking at room temperature. Serial dilutions of purified human IgA, IgM and IgG (Sigma and Zymed) were performed at concentrations of 2.0, 1.0, 0.5, 0.25 and 0.125 μg/mL in order to determine a concentration curve for human IgA, IgM or IgG, and for assay reproducibility control. Moreover, 100 μl of adult control saliva sample were applied in a 1:200 dilution in duplicates for control purposes.

After incubation with saliva and purified IgA, IgM and IgG, the plates were washed twice with buffer II. Then, 100 μl/well of a mouse anti-human IgA or IgM or IgG antibody solution was added and diluted in buffer III at a concentration of 1:500. After application, the plate was covered and incubated for one hour under stirring at room temperature. The plates were then washed twice with buffer II and incubated with 1:10.000 goat anti-mouse IgG biotinylated antibody (Sigma, USA) for one hour under shaking at room temperature. The plates were subsequently washed as previously described, and incubated overnight at room temperature with streptavidin-alkaline phosphatase conjugate (Sigma, USA) diluted 1:500 in buffer III. The following day, after further washings, ELISA reactions were developed by incubation with p-nitrophenyl phosphate substrate (Sigma, USA) at a concentration of 1mg/mL diluted in buffer IV (0.2M Na2CO3, 0.2 M NaHCO3 and 0.02% MgCl) pH 9.8 (Sigma, USA). The reaction with the substrate was developed for 30 minutes at room temperature, and the intensity of the reactions was measured at a wavelength of 405nm using ELISA reader (VersaMax, Molecular Devices). Non-sensitized wells of the same plates and sensitized wells which had not been incubated with saliva were used as negative controls. The mean absorbance values of the negative controls were discounted from the absorbance values of the samples tested, and the latter was expressed as ELISA units. A concentration curve for IgA, IgM and IgG by ELISA

unit was determined from reaction samples of purified IgA, IgM and IgG so as to check the linearity of ELISA. This curve was used to determine immunoglobulin levels in saliva samples. After plotting of final results, immunoglobulin levels in the saliva were expressed in mg/dl.

2.3.4 Quantification of total protein using the Bradford method

Determination of total protein concentration was performed using the Bradford method (Bradford, 1976) with bovine albumin in serial dilutions as the standard for plotting the sample analysis results. Saliva samples were diluted in distilled water at a ratio of 1:200. After dilution, 1 mL of Bradford reagent was added, and the samples were incubated for 15 minutes at room temperature, and were then read in a spectrophotometer (Synergy HT, BioTek, USA). The spectrophotometer used to measure the reaction was previously reset by a sample containing only water and reagent in a ratio of 1:1, which acted as the negative and blank control. The absorbance was read at 595 nm and the results were expressed in mg/dl.

All data were recorded in an electronic spreadsheet, and the amount of IgA, IgM and IgG was divided by the amount of total proteins.

2.4 Statistical analysis

GraphPad Prism 4.0 statistical software was used for statistical analyses. The Shapiro-Wilk normality test was performed in all groups. The Fisher’s exact test was used to compare genders and ethnic groups, and the ANOVA test was used to compare ages. All qualitative variables of tongue function analysis were transformed into quantitative variables, and then the Kruskal-Wallis test with Dunn’s post-test was used. The ANOVA and Tukey’s post-test were used to compare the salivary flow rate

between groups. In order to evaluate tongue function and the levels of IgA, IgM and IgG, the Kruskal-Wallis test with Dunn's post-test was used. The differences were considered significant when the probability of rejecting the null hypothesis was lower than 0.05 (5%).

3. Results

The number of chagasic patients with the cardiac form of the disease, patients with the mixed form of Chagas with presence of megaesophagus, and non-chagasic patients was, respectively, as follows: Caucasian (5 / 11 / 19), non-Caucasian (1 / 0 / 1), male (4 / 7 / 8), and female (2 / 4/ 12). There was no significant difference regarding gender, ethnicity and age between the groups, hence showing a homogeneous distribution between the three groups (Table 1).

There were no statistical differences between the groups concerning the following parameters: muscle tone, mobility, muscle strength with resistance, muscle strength without resistance, and contraction of the lingual apex (Table 2).

Chagasic patients with the mixed form of the disease with the presence of megaesophagus had a higher salivary flow rate than non-chagasic patients ($p = 0.0302$) (Fig. 3).

There was no statistical difference between the groups when total IgA ($p = 0.3881$), IgM ($p = 0.1558$) and IgG levels ($p = 0.6492$) were compared to total salivary proteins (Table 3).

4. Discussion

It has been reported that during the chronic phase of Chagas disease, *T. cruzi* can invade and parasitize different cell types, including muscle cells in the tongue (Barbosa Jr & Andrade, 1984). The role of *T. cruzi* in the destruction of muscle cells in the tongue likely results in local fibrosis by a repair mechanism similar to that described in the heart of chagasic individuals (Chapadeiro, 1967). Cell destruction could be due to the adsorption of *T. cruzi* antigens (Ribeiro Dos Santos & Hudson, 1980) and/or cross-reactivity between the parasite and the muscle cells in the tongue (Santos-Buch & Teixeira, 1974), as described in other organs of chronic chagasic patients (de Lima Pereira et al., 2007).

A study on rats infected with *T. cruzi* showed decreased muscle contractility during infection (Ramirez-Archila et al., 2011). Damaged myofibrils were observed in biceps brachial muscle biopsies of humans chronically infected with *T. cruzi* (Laguens et al., 1975), thus compromising skeletal muscle contractile function. This is due to damage to the neuromuscular junction of skeletal muscle cells, which causes loss of sensitivity and contractile structure stretching (Mirkin et al., 1994).

There is less tissue inflammation over the course of *T. cruzi* infection; even so, muscle repair and replacement of muscle tissue by collagen are triggered, leading to more complications associated with muscle contractility (Araújo-Jorge et al., 2008). However, there are no studies on Chagas disease describing functional changes in the tongue. In this study, changes in muscle tone, mobility, muscle strength with resistance, muscle strength without resistance, and contraction of the lingual apex were not observed in the tongues of chagasic patients. Perhaps the microscopic changes in the tongues of chagasic individuals, previously described before (de Lima Pereira et al., 2006; 2007; 2009), were not sufficient to cause functional changes.

In the present study, a higher salivary flow rate was observed in chagasic patients with megaesophagus compared to other groups, which corroborates other studies that observed a higher salivary flow rate in Chagas patients (de Oliveira, Bloise & Lopes, 1952; Earlam, 1972; Vieira, 1961). We believe that individuals with megaesophagus undergo changes in the salivary glands, such as duct dilatation, which is likely caused by the typical neuronal destruction of Chagas disease (de Lima Pereira et al., 2006; Vieira, 1961). This would be an inductive stimulus that would lead to increased saliva secretion in the oral cavity (de Lima Pereira et al., 2006). Moreover, increased capillary filtration due to the increase in the number and diameter of blood vessels (de Lima Pereira et al., 2006) may also lead to an increase in salivary flow rate in patients with chronic Chagas disease. In Chagas disease, persistent inflammation may cause progressive vasodilatation, increasing blood demand and, hence, increased production of saliva.

Furthermore, it is known that pharyngeal transit time is longer in patients with Chagas disease than in normal individuals, especially with a bolus of pasty consistency (dos Santos, Cassiani & Dantas, 2011). Therefore, the permanence of bolus in the pharynx may also contribute to higher salivary flow rate in chagasic patients with megaesophagus (Earlam, 1972).

There were also no significant differences regarding salivary IgA levels between the groups. IgA is an immunoglobulin present mostly in mucosal surfaces that represents the first line of defense of the adaptive immune system against infectious diseases. Reduced levels of IgA have been associated with more episodes of gastrointestinal tract infection (Nogueira, Alves, Napimoga, Smith, & Mattos-Graner, 2005). The identification of antigens with a high level of virulence in the oral cavity may help to investigate the mechanisms of antigenic stimulation and to better

understand the immune response of the oral mucosa (Borges et al., 2015). Increased levels of total IgA isoform antibodies in the blood of chagasic patients have been related to digestive forms of the disease (Sá Ferreira, Galvão-Castro, Macedo, & Castro, 1983). A study showed higher levels of IgA anti-amastigotes in the blood in the chronic digestive form of Chagas disease, especially in patients with severe esophageal involvement (Primavera et al., 1988), hence suggesting that IgA levels could be used as markers for the early diagnosis of esophageal lesions in Chagas disease. This is believed to occur due to local mucosal damage caused by esophageal content retained in the area with altered motility, which would allow for food and bacterial antigens that do not usually cross the epithelial barrier to eventually enter the circulation (Walker, Isselbacher, & Bloch 1974). These antigens would stimulate the precursors of IgA-producing plasma cells located in the lamina propria of the gastrointestinal tract (Walker, Isselbache & Bloch, 1974). Nonetheless, as there were no differences in IgA levels between chagasic and non-chagasic patients in the present study, we believe that the saliva was not responsible for the blood disorders found in other studies, perhaps due to the fact that IgA is more diluted in saliva than in blood.

There was not a significant difference in salivary IgM and IgG levels between the groups, which is in consonance with previous studies on blood (Freitas, Costa, Pereira, Quintão, & Souza, 1976; Marsden, Seah, Mott, Prata, & Platt, 1970). However, other authors have reported increases in blood IgG levels in chronic chagasic patients (Lelchuk, Dalmasso, Inglesini, Alvarez, & Cerizola, 1970; Vattuone, Szarfman, & Gonzklez-Cappa, 1973). These contradictory results may be attributed to methodological differences associated with samples from control patients used in the experiments (Brener, 1980; Sá Ferreira, Galvão-Castro, Macedo, & Castro,

1983), as many of these studies had not been performed in endemic areas of Chagas disease (Sá Ferreira, Galvão-Castro, Macedo, & Castro, 1983).

It is known an experiment with dogs, there was increased production of blood IgM in the acute phase of *T. cruzi* infection only until day 28 (Coura-Vital et al., 2008). When Chagas disease becomes chronic, specific IgM immunity is terminated and induction of *T. cruzi*-specific IgG production is triggered (Coura-Vital et al., 2008). High levels of specific IgGs are thought to be important for the elimination of blood forms of the parasite and for the reduction of mortality rates (Brodskyn, Silva, Takehara, & Mota, 1989; Cordeiro et al., 2001). Even though there are no studies in the literature assessing the levels of total salivary immunoglobulin in Chagas disease, the results of this study indicate that the levels of salivary immunoglobulin do not play an important role in the pathogenesis of Chagas disease.

Although there were no significant differences regarding tongue function and total salivary immunoglobulin levels, the highest salivary flow rate in patients with the mixed form of Chagas disease with presence of megaesophagus may likely occur due to esophageal abnormalities and/or changes in salivary glands caused by chronic Chagas disease.

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Conflict of interest: None.

References

1. Briceño-Leon, R. (2009). Chagas disease in the Americas: an ecohealth perspective. Cadernos de Saúde Pública do Rio de Janeiro, 25(1), S71–82.
2. Prado, C. M., Celes, M. R., Malvestio, L. M., Campos, E. C., Silva, J. S., Jelicks, L. A., et al. (2011). Early dystrophin disruption in the pathogenesis of experimental chronic Chagas cardiomyopathy. Microbes and Infection, 14(1), 59-68.
3. dos Santos, C. M, Cassiani, R. A., & Dantas, R. O. (2011). Videofluoroscopic evaluation of swallowing in Chagas' disease. Dysphagia, 26(4),361-365.
4. de Lima Pereira, S. A., Severino, V. O., Kohl, N. L., Rodrigues, D. B., Alves, P. M., Clemente-Napimoga, J. T., et al. (2009). Expression of cytokines and chemokines and microvasculature alterations of the tongue from patients with chronic Chagas' disease. Parasitology Research, 105(4), 1031-1039.
5. de Lima Pereira, S. A., dos Santos, V. M., Rodrigues, D. B., da Cunha Castro, E. C., dos Reis, M. A., de Paula Antunes Teixeira, V. (2007). Quantitative analysis of fibrosis and mast cells in the tongue of chronic chagasic patients: autopsy study. Médecine et Maladies Infectieuses, 37(4),229-233.
6. Felton, S. M., Gaige, T. A., Reese, T. G., Wedeen, V. J., Gilbert, R. J. (2007). Mechanical basis for lingual deformation during the propulsive phase of swallowing as determined by phase-contrast magnetic resonance imaging. Journal of Applied Physiology, 103(1),255-265.
7. de Lima Pereira, S. A., Rodrigues, D. B. R., Ferraz, L. M. F., Castro, E. C. C., Reis, M. A., Teixeira, V. P. A. (2006). Inflammation and glandular duct dilatation of the tongue from patients with chronic Chagas disease, Parasitology Research, 98, 153-156.
8. Alves, J. B., Alves, M. S., & Naito, Y. (1994). Induction of synthesis of the rat cystatin S protein by the submandibular gland during the acute phase of experimental Chagas disease. Memórias do Instituto Oswaldo Cruz, 89(1), 81-85.
9. Vieira, C. B. (1961). Hyperamylasemia and hyperactivity of salivary glands associated with megaesophagus. American Journal of Digestive Diseases, 6(8),727-741.

10. Earlam, R. J. (1972). Gastrointestinal Aspects of Chagas' Disease. The American Journal of Digestive Diseases, 17(6), 559-571.
11. de Oliveira, H. L., Bloise, W., & Lopes, A. G. Hyperactivity of the salivary glands as a cause of hyperamylasemia verified in a case of megaesophagus; possible semeiological value of the amylasemia-lipasemia relation. (1952). Revista Paulista de Medicina, 41(4), 238-245.
12. Bijovsky, A. T., Elizari, M. V., Muller, L. A., Katzin, V. J., González Cappa, S. M. (1983). Chronic infection in mice with Trypanosoma cruzi. Revista do Instituto de Medicina Tropical de São Paulo, 25(5), 207-214.
13. Mirkin, G. A., Jones, M., Sanz, O. P., Rey, R., Sica, R. E, González Cappa, S. M. (1994). Experimental Chagas' disease: electrophysiology and cell composition of the neuromyopathic inflammatory lesions in mice infected with a myotropic and a pantropic strain of Trypanosoma cruzi. Clinical Immunology and Immunopathology, 73(1), 69-79.
14. Araújo-Jorge, T. C., Waghabi, M. C., Soeiro, Mde. N., Keramidas, M., Bailly, S., Feige, J. J. (2008). Pivotal role for TGF-beta in infectious heart disease: The case of Trypanosoma cruzi infection and consequent Chagasic myocardiopathy. Cytokine and Growth Factor Reviews,19(5-6), 405-413.
15. Pinho, R. T., Pedrosa, R. C., Costa-Martins, P., Castello-Branco, L. R. (1999). Saliva ELISA: a method for the diagnosis of chronic Chagas disease in endemic areas. Acta Tropica, 72(1), 31-38.
16. Chao, K. S., Deasy, J. O., Markman, J., Haynie, J., Perez, C. A., Purdy, J. A., et al. (2001). A prospective study of salivary function sparing in patients with head-and-neck cancers receiving intensity-modulated or three-dimensional radiation therapy: initial results. International Journal of Radiation Oncology, 49(4),907-916.
17. Navazesh, M., Kumar, S. K. (2008). Measuring salivary flow: challenges and opportunities. The Journal of the American Dental Association, 139, S35-40.
18. Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry, 72:248-254.
19. Barbosa, Jr. A. A., Andrade, Z. A. (1984). Identificação do Trypanosoma cruzi nos tecidos extracardíacos de portadores de miocardite crônica chagásica. Revista da Sociedade Brasileira de Medicina Tropical, 17(3), 123-126.

20. Chapadeiro, E. (1967). Peso do coração e intensidade do processo inflamatório na cardiopatia chagásica crônica. Boletín de la Oficina Sanitaria Panamericana, 63:236-239.
21. Ribeiro dos Santos, R., & Hudson, L. (1980). Trypanosoma cruzi: binding of parasite antigens to mammalian cell membrane. Parasite Immunology. 1980, 2(1), 1-10.
22. Santos-Buch, C. A., & Teixeira, A. R. (1974). The immunology of experimental Chagas' disease. 3. Rejection of allogeneic heart cells in vitro. The Journal of Experimental Medicine, 140(1), 38-53.
23. Ramirez-Archila, M. V., Muñiz, J., Virgen-Ortiz, A., Newton-Sánchez, O., Melnikov, V. G., Dobrovinskaya, O. R. (2011). Trypanosoma cruzi: correlation of muscle lesions with contractile properties in the acute phase of experimental infection in mice (Mus musculus). Experimental Parasitology, 128(4), 301-308.
24. Laguens, R. P., Cossio, P. M., Diez, C., Segal, A., Vasquez, C., Kreutzer, E., et al. (1975). Immunopathologic and morphologic studies of skeletal muscle in Chagas' disease, American Journal of Pathology, 80(1),153-162.
25. Nogueira, R. D., Alves, A. C., Napimoga, M. H., Smith, D. J., Mattos-Graner, R. O. (2005). Characterization of salivary immunoglobulin A responses in children heavily exposed to the oral bacterium Streptococcus mutans: influence of specific antigen recognition in infection. Infection and Immunity, 73(9), 5675-5684.
26. Borges, M. C., Sesso, M. L., Roberti, L. R., de Menezes Oliveira, M. A., Nogueira, R. D., Geraldo-Martins, V. R., et al. (2015). Salivary antibody response to streptococci in preterm and fullterm children: A prospective study. Archives of Oral Biology,60(1),116-125.
27. Sá Ferreira, J. A., Galvão-Castro, B., Macedo, W., Castro, C. Immunoglobulins and other serological parameters in Chagas' disease: evidence for increased IgA levels in the chronic digestive form. (1983). Clinical and Experimental Immunology, 52(2):266–270.
28. Primavera, K. S., Hoshino-Shimizu, S., Umezawa, E. S., Peres, B. A., Manigot, D. A., Camargo, M. E. (1988). Immunoglobulin A antibodies to Trypanosoma cruzi antigens in digestive forms of Chagas' disease. Journal of Clinical Microbiology, 26(10), 2101-2104.

29. Walker, W. A., Isselbacher, K. J., & Bloch, K. J. (1974). The role of immunization in controlling antigen uptake from the small intestine. Advances in Experimental Medicine and Biology. 45(0), 295-303.
30. Mardsen, P. D., Seah, S. K. K., Mott, K. E., Prata, A., Platt, H. (1970). Immunoglobulins in Chagas disease. Journal of Tropical Medicine. 73(7), 157-161.
31. Freitas, G., Costa, S. C. G., Pereira, N. M., Quintão, L. G., Souza, J. G. (1976). Imunoglobulinas na fase crônica da doença de Chagas. Memórias do Instituto Oswaldo Cruz, 74(2), 183-190.
32. Lelchuc, R., Dalmasso, A. P., Inglesini, C. L., Alvarez, M., Cerizola, J. A. (1970). Immunoglobulin studies in serumof patients with american trypanossomiasis (Chagas'Disease). Clinical & Experimental Immunology. 6, 547-555.
33. Vattuone, N. H., Szarfman, A., Gonzklez-Cappa, S. M. (1973). Antibody response and immunoglobulin levels in human with acute or chronic Trypanosoma cruzi infections (Chagas' disease). Journal of American Tropical Medicine and Hygiene, 76(2), 45-47.
34. Brener, Z. (1980). Immunity to Trypanosoma cruzi. Advances in Parasitology, 18:247-292.
35. Coura-Vital, W., Carneiro, C. M., Martins, H. R., de Lana M., Veloso, V. M., Teixeira-Carvalho, A., et al. (2008). Trypanosoma cruzi: immunoglobulin isotype profiles during the acute phase of canine experimental infection with metacyclic or blood trypomastigotes. Experimental Parasitology, 120(3), 269-274.
36. Brodskyn, C. I., Silva, A. M., Takehara, H. A, Mota I. (1989). IgG subclasses responsible for immune clearance in mice infected with Trypanosoma cruzi. Immunology and Cell Biology, 67(6), 343–348.
37. Cordeiro, F. D., Martins-Filho, O. A., da Costa Rocha, M. O., Adad, S. J., Correa-Oliveira, R., Romanha, A. J. (2001). Anti-Trypanosoma cruzi immunoglobulin G1 can be a useful tool for diagnosis and prognosis of human Chagas' disease. Clinical and Diagnostic Laboratory Immunology, 8(1), 112–118.

Figure Legends

Fig. 1 - Analysis of tongue function through speech therapy evaluation.

Fig. 2 - Collection of saliva.

Fig. 3 - Comparison of salivary flow rate between cardiac chagasic individuals, chagasic individuals with the mixed form of the disease with the presence of megaesophagus, and non-chagasic individuals. *ANOVA, post-test (Tukey's test),* p = 0.0302.

Table 1
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Table 1 - Data demographics of groups: chagasics cardiac form, chagasics mixed form with megaesophagus and non-chagasics.

	Chagasics cardiac form (n =6)	Chagasics mixed form with megaesophagus (n=11)	Non-chagasics (n = 20)
Ethnicity * (C/NC)	5:1	11:0	19:1
Gender** (M/F)	4:2	7:4	8:12
Age***	69.2±4.4	65.7± 10.7	66.4± 10.7

C= Caucasians; NC=Non-Caucasians; M= Male; F= Female; **Exact of Fisher Test*, p=0.6419; ** *Exact of Fisher Test*, p=0.4256; *** *ANOVA Test*, p=0.7837 groups values about age expressed in mean ± standard deviation.

Table 2
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Table 2 - Analyses of tongue function.

	Chagasics cardiac form (n =6)	Chagasics mixed form with megaesophagus (n=11)	Non-chagasics (n = 20)
Muscletone (normal/flaccid)*	1:5	4:7	8:12
Mobility(normal/amended)**	3:3	7:4	13:7
Muscle strength with counter-resistance (yes/no)***	2:4	3:8	7:13
Muscle strength without counter-resistance (yes/no)****	5:1	11:0	19:1
Apex lingual tapered (with/without)*****	5:1	10:1	16:4

**Kruskal Wallis test* p=0.5819; ***Kruskal Wallis test* p=0.4436; ****Kruskal Wallis test* p=0.9090; *****Kruskal Wallis test p*=0.3560; ******Kruskal Wallis test* p=0.7386.

Table 3
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Table 3 - Total salivary levels of IgA, IgM, and IgG for total salivary protein of groups: chagasics cardiac form, chagasics mixed form with megaesophagus and non-chagasics.

	Chagasics cardiac form (n =6)	Chagasics mixed form with megaesophagus (n=11)	Non-chagasics (n = 20)
IgA/total protein (mg/dl)*	1.9	3.6	2.4
IgM/total protein (mg/dl)**	1.4	2.1	1.4
IgG/total protein (mg/dl)***	0.8	1	1

**Kruskal Wallis test* p=0.3881; ***Kruskal Wallis test* p=0.1558; ****Kruskal Wallis test* p=0.6492.

Figure 1
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Figure 2
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Figure 3
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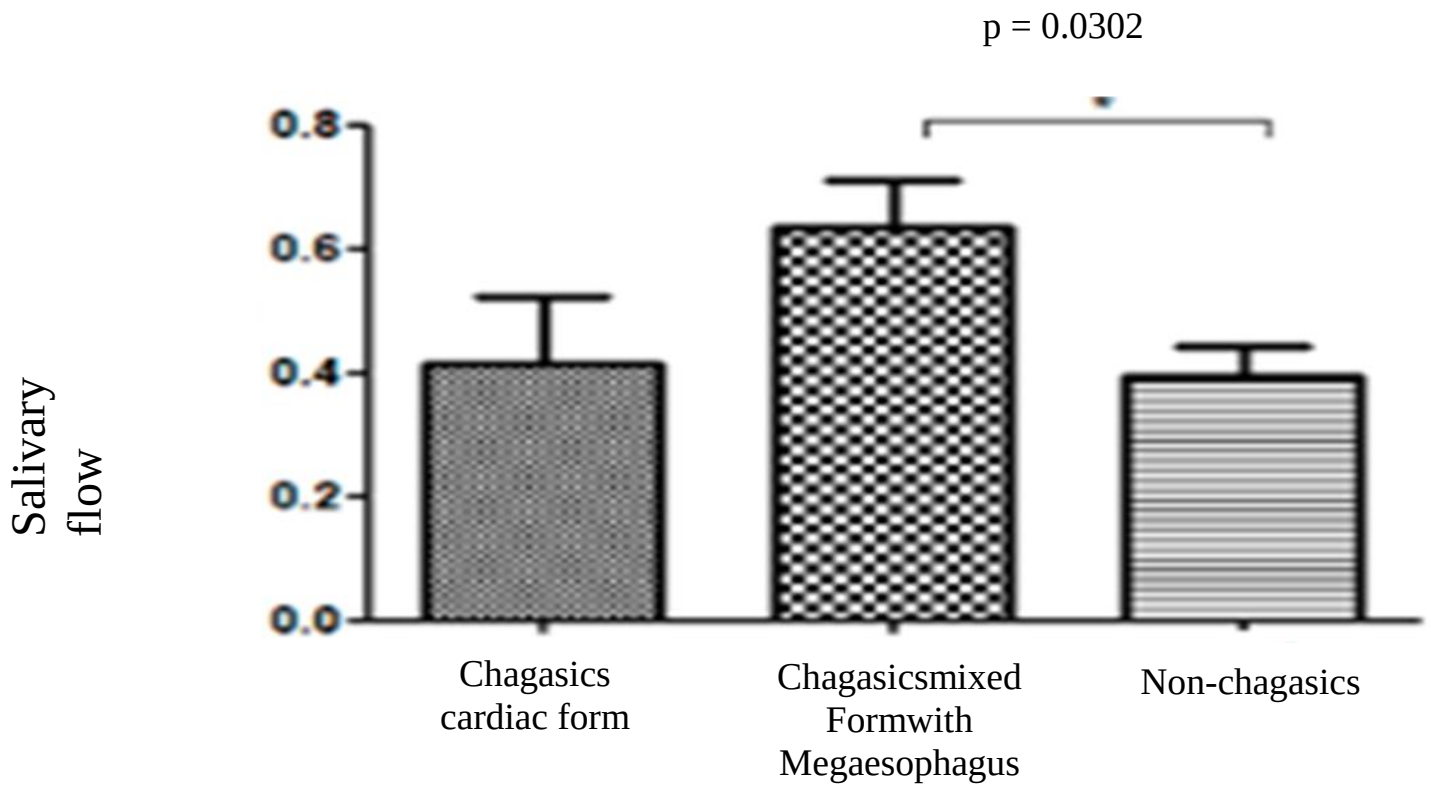

REFERÊNCIAS

ALVES, J.B; ALVES, M.S; NAITO, Y. Induction of synthesis of the rat cystatin S protein by the submandibular gland during the acute phase of experimental Chagas disease. **Memórias do Instituto Oswaldo Cruz**, v. 89, n. 1, p. 81-85, 1994.

ARAÚJO-JORGE, T.C. et al. Role for TGF-beta in infectious heart disease: The case of *Trypanosoma cruzi* infection and consequent Chagasic myocardiopathy. **Cytokine & Growth Factor Reviews**, v. 19, n. 5-6, p. 405-413, 2008.

BIJOVSKY, A.T. et al. Chronic infection in mice with *Trypanosoma cruzi*. **Revista do Instituto de Medicina Tropical de São Paulo**, v. 5, n. 25, p. 207-14, 1983.

BRODSKYN, C. I. et al. IgG subclasses responsible for immune clearance in mice infected with *Trypanosoma cruzi*. **Immunology and Cell Biology**, v. 67, p. 343–348, 1989.

CARNEIRO, C.M. et al. Differential impact of metacyclic and blood trypomastigotes on parasitological, serological and phenotypic features triggered during acute *Trypanosoma cruzi* infection in dogs. **Acta Tropica**, v. 101, n.2, p. 120-129, 2007.

CAROD-ARTAL, F.J. et al. Cardioembolic stroke and ischemic small bowel infarction in a Chagas' disease patient. **European Journal of Neurology**, v. 14, n. 5, 2007.

CENGET, D.D; ROJAS, R. La biopsia de músculodeltoidesen la enfermedad de Chagas. **Revista da Faculdad de Medicina de Tucuman**, v.1, n. 2, p. 27-37, 1959.

CORDEIRO, F.D. et al. Anti-*Trypanosoma cruzi* immunoglobulin G1 can be a useful tool for diagnosis and prognosis of human Chagas' disease. **Clinical and Diagnostic Laboratory Immunology**, v. 8, n. 1, p. 112-118, 2001.

CREMA, E. et al. Prevalence of cholelithiasis in patients with chagasic megaesophagus. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 44, n. 3, p. 324-326, 2011.

de LIMA PEREIRA, S.A. et al. Expression of cytokines and chemokines and microvasculature alterations of the tongue from patients with chronic Chagas' disease. **Parasitology Research**, v. 105, n. 4, p.105-1031, 2009.

de LIMA PEREIRA, S.A. et al. Quantitative analysis of fibrosis and mast cells in the tongue of chronic chagasic patients: autopsy study. **Médecine et MaladiesInfectieuses**, v. 37, n. 4, p. 229-33, 2007.

de LIMA PEREIRA, S.A. et al. Inflammation and glandular duct dilatation of the tongue from patients with chronic Chagas disease. **Parasitology Research**, v. 98, n. 2, p. 153-156, 2006.

dos SANTOS, C.M; CASSIANI, R.A; DANTAS, R.O. Videofluoroscopic evaluation of swallowing in Chagas' disease. **Dysphagia**, v. 26, n. 4, p. 361-365, 2011.

DUBNER, S. et al. Chagas disease: state-of-the-art of diagnosis and management. **Cardiology Journal**, v. 15, n.6, p. 493-504, 2008.

ELIZARI, M.V. Chagasicmyocardiopathy: historical perspective. **Medicina (B Aires)**, v. 59, p. 25-40, 1999.

FELTON, S.M. et al. Mechanical basis for lingual deformation during the propulsive phase of swallowing as determined by phase-contrast magnetic resonance imaging. **Journal of Applied Physiology**, v. 103, n. 1, p. 255-265, 2007.

KIRCHHOFF, L.V. Epidemiology of American trypanosomiasis (Chagas disease). **Advances in Parasitology**, v. 75, p. 1-18, 2011.

KÖBERLE, F. Chagas' disease and Chagas' syndromes: the pathology of American trypanosomiasis. **Advances in Parasitology**, v. 6, p. 63-116, 1968.

LAGUENS, R.P. et al. Immunopathologic and morphologic studies of skeletal muscle in Chagas' disease. **The American Journal of Pathology**, v. 80, n. 1, p. 153-162, 1975.

LANA, M. et al. Humoral immune response in dogs experimentally infected with *Trypanosoma cruzi*. **Memórias do Instituto Oswaldo Cruz**, v. 86, n. 4, p. 471–473, 1991.

LOPES, E.R. et al. In: BOGLIOLO, Luigi; BRASILEIRO FILHO, Geraldo. **Bogliolo Patologia**. 8 ed. Rio de Janeiro: Guanabara Koogan, 2000, 1328 p, p. 1165-1275.

MAGALHAES-SANTOS, I.F; LIMA, E.S; ANDRADE, S.G. Fibrogenesis and collagen resorption in the heart and skeletal muscle of Calomyscallosus experimentally infected with *Trypanosoma cruzi*: immunohistochemical identification of extracellular matrix components, **Memórias do Instituto Oswaldo Cruz**, v. 97, n. 5, p. 703-710, 2002.

MIRKIN, G.A. et al. Experimental Chagas' disease: electrophysiology and cell composition of the neuromyopathic inflammatory lesions in mice infected with a myotropic and a pantropic strain of *Trypanosoma cruzi*. **Clinical Immunology and Immunopathology**, v. 73, n. 1, p. 69-79, 1994.

MORGAN, J. et al. Anti-*Trypanosoma cruzi* antibody isotype profiles in patients with different clinical manifestations of Chagas' disease. **The American Journal of Tropical Medicine and Hygiene**, v. 55, n. 4, p. 355-359, 1996.

NOGUEIRA, R.D. et al. Characterization of salivary immunoglobulin A responses in children heavily exposed to the oral bacterium *Streptococcus mutans*: influence of specific antigen recognition in infection. **Infection and Immunity**, v. 73, n. 9, p. 5675-5684, 2005.

PEREZ-AYALA, A. et al. Chagas disease in Latin American migrants: a Spanish challenge. **Clinical Microbiology and Infection**, v. 17, n. 7, p. 1108-1113, 2011.

PINHO, R.T. et al. Saliva ELISA: a method for the diagnosis of chronic Chagas disease in endemic areas. **Acta Tropica**, v. 72, n. 1, p. 31-38, 1999.

PISSETTI, C.W. et al. Association between the plasma levels of TNF-alpha, IFN-gamma, IL-10, nitric oxide and specificIgG isotypes in the clinical forms of chronic Chagas disease. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 42, n. 4, p. 425-430, 2009.

PRADO, C.M. et al. Early dystrophin disruption in the pathogenesis of experimental chronic Chagas cardiomyopathy. **Microbes and Infection**, v. 14, n. 1, p. 59-68, 2011.

PRIMAVERA, K.S. et al. Immunoglobulin A antibodies to *Trypanosoma cruzi* antigens in digestive forms of Chagas' disease. **Journal of Clinical Microbiology**, v. 26, n. 10, p. 2101-2104, 1988.

PRIOTTO, S. et al. *Trypanosoma cruzi*: participation of cholesterol and placental alkaline phosphatase in the host cell invasion. **Experimental Parasitology**, v.122, n.1, p. 70-73, 2009.

RAMIREZ-ARCHILA, M.V. et al. *Trypanosoma cruzi*: correlation of muscle lesions with contractile properties in the acute phase of experimental infection in mice (Mus musculus). **Experimental Parasitology**, v. 128, n. 4, p. 301-308, 2011.

RAMOS, J.M. et al. Chagas disease in Latin American pregnant immigrants: experience in a non-endemic country. **Archives of Gynecology and Obstetrics**, v. 285, n. 4, p. 919-923, 2011.

ROSSI, M.A. Fibrosis and inflammatory cells in human chronic chagasic myocarditis: scanning electron microscopy and immunohistochemical observations. **International Journal of Cardiology**, v. 66, n. 2, p. 183-194, 1998.

SÁ FERREIRA, J.A. et al. Immunoglobulins and other serological parameters in Chagas' disease: evidence for increased IgA levels in the chronic digestive form. **Clinical of Experimental Immunology**, v. 52, n. 2, p. 266–270, 1983.

SOARES, M.B. et al. Transplanted bone marrow cells repair heart tissue and reduce myocarditis in chronic chagasic mice. **The American Journal of Pathology**, v. 164, n. 2, p. 441-447, 2004.

TOSO, M.A.; VIAL, U.F.; GALANTI, N. Oral transmission of Chagas' disease. **Revista Medica de Chile**, v. 139, n. 2, p. 258-266, 2011.

WALKER, W.A.; ISSELBACHER, K.J; BLOCH, K.J. The role of immunization in controlling antigen uptake from the small intestine. **Advances in Experimental Medicine and Biology**, v. 45, n. 0, p. 295-303, 1974.

ANEXO A

TERMOS DE ESCLARECIMENTO E DE CONSENTIMENTO

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO TRIÂNGULO MINEIRO - Uberaba-MG
Comitê de Ética em Pesquisa- CEP

TERMO DE ESCLARECIMENTO

A doença de Chagas tem como um dos principais órgãos afetados, os órgãos do sistema digestório. Sendo a língua parte desse sistema, e antes nunca pesquisada em humanos com a doença, o presente estudo "avaliação da função da língua, do fluxo salivar e dos níveis de IgA, IgM e de IgG na saliva de pacientes chagásicos crônicos" tem como objetivo avaliar a função da língua, utilizando método clínico e verificar componentes da saliva de pacientes com a doença. Como os avanços na área da saúde ocorrem através de estudos como este, sua participação é importante. Caso você participe, será necessário fazermos coletas de saliva, exames clínico, perguntas sobre sua saúde geral e odontológica. Este estudo permitirá identificar se você tem redução da contratilidade da musculatura lingual bem como alterações na saliva. Não será feito nenhum procedimento que traga risco a sua vida ou maior desconforto, além da coleta da saliva e exame clínico.

Você poderá ter todas as informações que quiser e poderá não participar da pesquisa ou retirar seu consentimento a qualquer momento, sem prejuízo no seu atendimento. Por sua participação no estudo, você não receberá qualquer valor em dinheiro, mas terá a garantia de que todas as despesas necessárias para a realização da pesquisa não serão de sua responsabilidade. Seu nome não aparecerá em qualquer momento do estudo, pois você será identificado com um número.

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO TRIÂNGULO MINEIRO - Uberaba-MG
Comitê de Ética em Pesquisa- CEP

TERMO DE CONSENTIMENTO LIVRE, APÓS ESCLARECIMENTO

Eu,_____, li e/ou ouvi o esclarecimento acima e compreendi para que serve o estudo e qual procedimento a que serei submetido.A explicação que recebi esclarece os riscos e benefícios do estudo. Eu entendi que sou livre para interromper minha participação a qualquer momento, sem justificar minha decisão e que isso não afetará meu tratamento. Sei que meu nome não será divulgado, que não terei despesas e não receberei dinheiro por participar do estudo. Eu concordo em participar do estudo.

Uberaba,___/____/_____

_____ _____
Assinatura do voluntário ou seu responsável legal Documento de identidade

_____ _____
Assinatura do pesquisador responsável Assinatura do pesquisador orientador

Telefone de contato da pesquisadora: Sanívia: 9113-8830

Em caso de dúvida em relação a esse documento, você pode entrar em contato com o Comitê Ética em Pesquisa da Universidade Federal do Triângulo Mineiro, pelo telefone 3318-5854.

ANEXO B

APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO TRIÂNGULO MINEIRO – Uberaba (MG)
COMITÊ DE ÉTICA EM PESQUISA – CEP
Av. Frei Paulino, 30 (Centro Educacional e Administrativo da UFTM) – 2º andar – Bairro Nossa Senhora da Abadia
38025-180 - Uberaba-MG - TELEFAX: 34-3318-5854
E-mail: cep@pesqpg.uftm.edu.br

IDENTIFICAÇÃO

TÍTULO DO PROJETO: AVALIAÇÃO DA FUNÇAÕ DA LINGUA, DO FLUXO SALIVAR E DOS NÍVEIS DE IGA, IGG E DE TGF-B NA SALIVA DE PACIENTES CHAGÁSICOS CRONICOS
PESQUISADOR (A) RESPONSÁVEL: SANIVIA APARECIDA L. PEREIRA
INSTITUIÇÃO ONDE SE REALIZARÁ A PESQUISA: UFTM
DATA DE ENTRADA NO CEP/UFTM: 07/03/2012
PROTOCOLO CEP/UFTM: 2256

PARECER

De acordo com as disposições da Resolução CNS 196/96, o Comitê de Ética em Pesquisa da UFTM considera o protocolo de pesquisa **aprovado**, na forma (redação e metodologia) como foi apresentado ao Comitê.

Conforme a Resolução 196/96, o pesquisador responsável pelo protocolo deverá manter sob sua guarda, pelo prazo de no mínimo cinco anos, toda a documentação referente ao protocolo (formulário do CEP, anexos, relatórios e/ou Termos de Consentimento Livre e Esclarecidos – TCLE assinados, quando for o caso) para atendimento ao CEP e/ou à Comissão Nacional de Ética em Pesquisa – CONEP.

Toda e qualquer alteração a ser realizada no protocolo deverá ser encaminhada ao CEP, para análise e aprovação.

O relatório anual ou final deverá ser encaminhado um ano após o início da realização do projeto.

Uberaba, 18 de maio de 2012.

Profª. Ana Palmira Soares dos Santos
Coordenadora do CEP/UFTM

ANEXO C

ANAMNESE

- Dados de Identificação:
Nome: _____
Data de nascimento: ___/___/___ Idade: _____ Sexo : _____
Telefone: _____
Data da anamnese: ____/____/____

2- Queixas a respeito de cavidade oral
_____
_____
_____

3- Histórico do caso (Tratamentos)
_____
_____
_____

4- Aspectos respiratórios :
( ) Rinite alérgica ( ) Asma ( ) Bronquite
( ) Hipertrofia de Adenóide ( ) Hipertrofia de amígadalas
( ) Resfriados constantes ( ) Ronco
( ) Baba no travesseiro ( ) Respiração oral diurna
( ) Respiração oral noturna ( ) Outros: _____

Tratamentos realizados: ( ) Homeopatia ( ) Alopatia ( ) Vacina
( ) Cirurgia de amígdala ( ) Cirurgia de adenóide
( ) Outros: _____

5- Saúde:
( ) Fumante ( ) Não-fumante
( ) Faz uso de bebidas alcoólicas? Frequência:_____
( ) Paralisia facial ( ) Acidente Vascular Cerebral
( ) Hipertensão _____ ( ) Diabetes_____
( ) Está em tratamento médico? _____
( ) Faz uso de medicamento de uso contínuo?_____
_____
_____
( ) Doenças psiquiátricas_____
( ) Problema neurológico: _____
( ) Histórico de malformações craniofaciais_____
( ) Doenças infecciosas _____
( ) Alergia Qual (s)_____
( ) Outros: _____

6- Hábitos viciosos e posturais:

( ) Bruxismo ( ) Onicofagia ( ) Morder a língua
( ) Apoio de braços ( ) Apoio de mãos
Frequência: _____

Quando ocorre? _____
( ) Outros _____

7- Aspectos alimentares :

Alimentação atual :
_____
_____

Apresenta dificuldade na mastigação? _____

Engasgos na deglutição? _____

Sensação de boca seca?_____

( ) Mastigação com lábios fechados ( ) Mastigação com lábios abertos

Mastigação ( ) lenta ( ) rápida ( ) ruidosa

Preferência de lado para mastigar ( ) direito ( ) esquerdo ( ) não tem

Ingere líquidos durante as refeições ? _____
Outros : _____

8- Dores orofaciais
Qual região: _____
Frequência: _____
O que provoca o aumento da dor: _____
O que faz para melhorar: _____
Como é a dor: _____

9- Tratamento Odontológico :
Faz ou já fez uso de algum tipo de aparelho ortopédico ou ortodôntico ? _____
Qual ? _____
Quando iniciou o tratamento? _____
Quando acabou o tratamento? _____
Apresenta Disfunção Temporomandibular? _____
Dentista: _____
Outras informações : _____

10- Problemas posturais :
_____
_____

11- Informações adicionais :
_____
_____

ANEXO D

AVALIAÇÃO ESTRUTURAL E FUNCIONAL (MIOFUNCIONAL) DA LÍNGUA

1) Postura e morfologia

( )posição (dorso elevado; região alveolar inferior; assoalho bucal; entre os dentes)
( ) marcas
( ) alargada
( ) normal
( )espessura (grossa, fina, normal):
( ) simetria

2) Tônus (Estado de tensão)

( ) normal
( ) flácida

3) Mobilidade:

Movimentos básicos: protrusão, retração, elevação, abaixamento, estalo, vibração, rotação e lateralização

( ) normal
( ) alterada

4) Força:

- força com contra-resistência: ( ) sim ( ) não
- força sem contra-resistência: ( ) sim ( ) não

5) Ápice lingual afilado:

( ) com

( ) sem