Immunohistological insight into the correlation between cardiac and lingual musculature in chronic chagas disease

Percepção imunohistológica sobre a correlação entre a musculatura cardíaca e lingual na doença chagásica crônica

Visión inmunohistológica de la correlación entre la musculatura cardíaca y lingual en la enfermedad de chagas crónica

Abstract

Aim: To assess and compare the inflammatory infiltrate, area of muscle cells, microvessel density (MVD) and expression of cytokines in the myocardium (MM) and lingual (LM) musculature between chagasic and non chagasic individuals.

Methods: Fragments of MM and LM from autopsied chronic chagasic (CC) (n=18) and non chagasic individuals (n=24) were histologically processed for histochemical and immunohistochemical evaluation. Was assessed the MVD by CD31 and quantification of immune cells infiltrated in MM and LM, and also expression of IL-1, IL-10 and TGF-β1.

Results: There were higher intensity of inflammation and larger area of muscle cells in the myocardium and tongue of chagasic compared to non-chagasic individuals (p<0.0001). In the chagasic individuals, there was a positive correlation: regarding to the MVD between the LM and MM (p=0.049); between area of MM and density of leukocytes expressing TGF-β1 (p=0.049); and IL-10 correlate with the MM and LM (p=0.029).

Conclusions: The correlation between the density of leukocytes immunostained by anti-TGF-β1 and hypertrophy in the myocardium suggests the participation of this cytokine in cardiac muscle hypertrophy. Moreover, the hypertrophy in the myocardium and lingual muscles, and the concomitant expression of CD31 and IL-10 between the myocardium and lingual musculature in chagasic individuals suggests that evaluation of the tongue musculature presents certain similarities changes as a myocardium muscle in CC. However, it needs more studies to verify in other phases of the...
disease, if the parasite is installed in the tongue or if the mechanisms are reflex of systemic changes in level of immune response.

**Keywords:** Chagas disease; Cytokines; Tongue; T. cruzi.

**Resumo**
Objetivo: Avaliar e comparar o infiltrado inflamatório, área de células musculares, densidade de microvasos (MVD) e expressão de citoquinas no miocardio (MM) e na musculatura lingüística (LM) entre indivíduos chagásicos e não chagásicos. Métodos: Fragmentos de MM e LM de indivíduos chagásicos crônicos autopsiados (CC) (n=18) e não chagásicos (n=24) foram processados histologicamente para avaliação histoquímica e imunohistoquímica. Foi avaliado o MVD pelo CD31 e quantificação das células imunes infiltradas em MM e LM, e também expressão de IL-1, IL-10 e TGF-β1. Resultados: Houve maior intensidade de inflamação e maior área de células musculares no miocardio e línha chagásica em comparação com indivíduos não chagásicos (p<0,0001). Nos indivíduos chagásicos, houve uma correlação positiva: em relação à MVD entre a LM e MM (p=0,049); entre área de MM e densidade de leucócitos expressando TGF-β1 (p=0,049); e IL-10 correlacionada com MM e LM (p=0,029). Conclusões: A correlação entre a densidade de leucócitos imunizados por anti-TGF-β1 e a hipertrofia no miocardio sugere a participação desta citoquina na hipertrofia do músculo cardíaco. Além disso, a hipertrofia nos músculos do miocardio e lingual, e a expressão concomitante de CD31 e IL-10 entre o miocardio e a musculatura lingual em indivíduos chagásicos sugere que a avaliação da musculatura da língua apresenta certas similaridades como um músculo do miocardio em CC. Entretanto, são necessários mais estudos para verificar em outras fases da doença, se o parasita está instalado na língua ou se os mecanismos são reflexos de mudanças sistêmicas no nível de resposta imunológica.

**Palavras-chave:** Doença de Chagas; Citocinas; Língua; T. cruzi.

**Resumen**
Objetivo: Evaluar y comparar el infiltrado inflamatorio, el área de células musculares, la densidad de microvasos (MVD) y la expresión de citoquinas en el miocardio (MM) y la musculatura lingual (LM) entre individuos chagásicos y no chagásicos. Métodos: Se procesaron histológicamente fragmentos de MM y LM de individuos chagásicos crónicos (n=18) y no chagásicos (n=24) autopsiados para su evaluación histoquímica e inmunohistoquímica. Se evaluó la MVD por CD31 y la cuantificación de las células inmunos infiltradas en el MM y LM, así como la expresión de IL-1, IL-10 y TGF-β1. Resultados: Hubo mayor intensidad de inflamación y mayor área de células musculares en el miocardio y la lengua de los individuos chagásicos en comparación con los no chagásicos (p<0,0001). En los individuos chagásicos, hubo una correlación positiva: respecto a la MVD entre el LM y el MM (p=0,049); entre el área del MM y la densidad de leucocitos que expresan TGF-β1 (p=0,049); y la IL-10 se correlaciona con el MM y el LM (p=0,029). Conclusiones: La correlación entre la densidad de leucocitos inmunotransparentes por anti-TGF-β1 y la hipertrofia en el miocardio sugiere la participación de esta citoquina en la hipertrofia del músculo cardíaco. Además, la hipertrofia en el miocardio y en la musculatura lingual, y la expresión concomitante de CD31 e IL-10 entre el miocardio y la musculatura lingual en los individuos chagásicos sugiere que la evaluación de la musculatura lingual presenta ciertas similitudes cambios como músculo del miocardio en CC. Sin embargo, se necesitan más estudios para verificar en otras fases de la enfermedad, si el parásito se instala en la lengua o si los mecanismos son reflejo de cambios sistémicos a nivel de respuesta inmune.

**Palabras clave:** Enfermedad de Chagas; Citoquinas; Lengua; T. cruzi.

1. Introduction

Chagas disease, or American trypanosomiasis, described by Carlos Chagas, is a potentially lethal zoonosis, caused by the protozoan hemoflagellate Trypanosoma cruzi (T. cruzi) first found in the Americas (Chagas, 1909) and can be characterized by an inflammatory response in the affected tissues. Chagas disease is a serious public health problem, the estimates indicate that there are 2.9 - 7.2 million people with Chagas disease in Brazil, which accounts for approximately six thousand deaths per year (Martins-Melo et al., 2014; Mizzaci et al., 2017). Although of this disease being one of the main causes of heart failure (Wang et al., 2011), morbidity and mortality in Latin America (Regueiro et al., 2013) all the morphologic alterations caused by T. cruzi have not yet been completely explained.

The etiology of chagasic cardiopathy is multifactorial, involving persistence of the parasite, inflammation, autoimmunity, vascular alterations and hypertrophic response of the cardiomyocytes (Petersen & Burleigh, 2003). Vascular alterations in Chagas disease include infection associated with microvascular spasm, increase in platelet aggregation7, decapillarization and reduction in arterial density (Ferreira et al., 1980). In addition to the vascular alterations in the myocardium, such alterations including reduction in the number of capillaries, thickening and reduplication of the basement
membrane and proliferation of endothelial cells have also been demonstrated in the vastus lateralis muscle in humans with Chagas disease (Torres et al., 2004). In a preview study of our group we demonstrated vascular alterations in the lingual muscle of autopsied chronic chagasic individuals, such as increase in the density of blood vessels, increase in vascular wall and diameter (de Lima Pereira et al., 2009).

However, another study demonstrated microvascular spasm in chagasic myocardiopathy, probably associated with interleukin-1 (IL-1) expression, which promotes alterations in endothelial cell function, in addition to modulating the expression of adhesion cells that participate in the inflammatory process in Chagas disease (Petersen & Burleigh, 2003). IL-1 also causes myocardocyte hypertrophy in the beginning of the infectious process, thus contributing to the maintenance of cardiac function during the infection (Petersen & Burleigh, 2003). In Chagas disease, in addition to cardiac-muscle hypertrophy, intestinal smooth muscle (Borges et al., 2008) and gastrocnemius muscle hypertrophy have also been demonstrated in chronically infected rats (Lugo de Yarbuh et al., 2006).

Moreover, interleukin 10 (IL-10) and the transforming growth factor β1 (TGF-β1) are capable of reducing the intracellular infection by T. cruzi (Cardoni et al., 1999) controlling inflammation and tissue destruction secondary to the anti-parasite immune response in the organs, especially in the myocardium. On the other hand, IL-10 plays an important role in controlling progression of the disease (de Araújo et al., 2011). Therefore, understanding the role of cytokines in the pathogenesis of Chagas disease is fundamental for tracking strategies with the aim of controlling inflammation and preventing or minimizing cardiac dysfunction (Cardoni et al., 1999; de Araújo et al., 2011).

In the present study, the hypothesis was raised that simultaneous morphologic alterations could be occurring in the lingual and cardiac musculature of chronic chagasic individuals, such as muscle hypertrophy and increase in the density of cells immunostained for IL-1, IL-10 and TGF-β1. Moreover, as chronic Chagas disease is an inflammatory disease, in addition to larger density of inflammation, and expects to find higher microvessel density (MVD) by anti-CD31 in the lingual musculature and myocardium of chagasic individuals.

The aim of the present study was to compare the intensity of inflammation, area of muscle cells, density of vessels immunostained by anti-CD31 and density of cells immunostained by anti-IL-1, anti-TGF-β1 and anti-IL-10 in the myocardium and lingual musculature between autopsied chronic chagasic and non chagasic individuals.

2. Methodology

2.1 Selection of Individuals

After approval by the Human Research Ethics Committee of the University of Uberaba, Protocol No. CEP-078/06, a cross sectional study was conducted with the analysis of protocols of complete autopsies performed at the General Pathology Division, Triangulo Mineiro Federal University (UFTM), Uberaba, MG, Brazil, in the period of January/2008 to December/2010. Protocols of 42 adult individuals were selected, whose tongues were removed during autopsy. The individuals were paired with regard to gender, ethnicity (Caucasian and non Caucasian) and age, being divided into two groups: chronic chagasic individuals (n = 18) and non chagasic individuals (n = 24) (Table 01). This classification was based on the autopsy protocols and on the indirect immunofluorescence, hemagglutination and Enzyme-Linked Immunosorbent Assay (ELISA) reactions for Chagas disease, performed in pericardial fluid collected during the autopsies. Individuals with the form cardiac of Chagas disease in the chronic phase were selected. For this selection individuals had to present at least one positive reaction and one morphologic finding suggestive of Chagas disease, such as cardiomegaly and left ventricle lesion. Patients with other forms of cardiomyopathies were excluded from both groups.
2.2 Collection of fragments and histochemical and immunohistochemical processing:

Once the groups have been formed, fragments were collected from the lingual musculature and myocardium for histochemical, morphometric and immunohistochemical evaluation. The fragments from the lingual musculature were removed by longitudinal section made with a scalpel blade in the region that accompanies the median lingual sulcus, from the base to the apex of the tongues. A longitudinal segment approximately 0.5 cm thick was removed. This segment was then subdivided into five fragments in the transverse direction. The central fragment, which corresponded to the third fragment, was used for analyses. The heart fragments were removed in the myocardial region, in the median portion of the left ventricle measuring approximately 2.0 x 1.0 x 0.5 cm. These fragments were embedded in paraffin and processed for histopathologic analysis. Sagittal sections of 6 µm were mounted on glass slides and stained with hematoxylin and eosin (HE) for semiquantitative evaluation of inflammation. Additional sections of the myocardium and lingual musculature of 21 individuals were mounted on glass slides pre-treated with 3-aminopropyl triethoxysilane (Sigma Chemical, St. Louis, USA) and used for immunohistochemical analysis. For each case, one tongue tissue slide and one myocardial tissue slide were examined.

After deparaffinization, antigen retrieval was performed in pan steam at 97°C, for 20 min with citrate buffer, pH 6. The histological sections were then washed with Phosphate Buffer Saline (PBS) 0.01 M, pH 7.2 and treated with 3% hydrogen peroxide. The sections were incubated for 40 min to block endogenous peroxidase in the tissues. Next, the sections were incubated in a solution containing the primary antibody (Sigma Chemical, St. Louis, USA), diluted in 2% Bovine Serum Albumin (BSA) (Sigma Chemical, St. Louis, USA), for 1h and 30 min at ambient temperature in the following concentrations: anti-TGFβ1 (1:50), anti-IL1 (1:15), anti-IL10 (1:25) and anti-CD31 (1:40). Afterwards the sections were washed with PBS as previously described. For antibody detection the Universal LSAB™ Kit+ HRP Kit (DAKO, Glostrup, Denmark) were used, with an incubation time of 30 min. This system was revealed for 5 to 30 min, using 3,3′ diaminobenzidine (DAKO, Glostrup, Denmark) at ambient temperature sheltered from light. Right after this the sections were washed in distilled water, counterstained with Harris Hematoxylin and mounted with Entelan (MERCK, Darmstadt, Germany). As negative control, sections that were not incubated with primary antibody were used, but had been through all the stages of the technique.

2.3 Semiquantitative analysis of the inflammation:

For semiquantitative analysis of the inflammation was used a light microscope (Zeiss, Berlin, Germany) and 40x objective that allowed greated amplification (de Lima Pereira et al., 2009). This analysis was carried out as follows: 0 = no inflammation; 1 = discrete inflammation (until 10 cells per field); 2 = moderate inflammation (between 11 and 20 cells per field); and 3 = severe inflammation (more than 21 cells per field) (de Lima Pereira et al., 2009). All the fields of the tongue musculature and myocardial fragments were evaluated.

2.4 Morphometric analysis of myocardial muscle and lingual muscle cells:

Morphometric evaluation of the myocardial and tongue muscle cells was performed on HE stained slides using a light microscope (Zeiss, Berlin, Germany) and 40x objective being performed in all fields in which it was possible to observe muscle cells transversely sectioned. For this evaluation, the slides were placed under the microscope, coupled to a color video camera plugged into a video capture card in a computer, in which Image J software was installed (Bethesda, Maryland, USA). The images were captured and the muscle cells were outlined with a cursor to determine the area of each cell, which was expressed in µm². The accumulated mean test was performed to determine the number of cells to be measured in the lingual and myocardial musculature. In each case the measurements of 100 lingual muscle cells and 150 myocardial muscle cells were taken.
2.5 Immunohistochemical evaluation:

The cells immunostained for anti-IL-1, anti-IL-10 and anti-TGF-β1 were quantified in all the fields of myocardial and lingual muscle fragments. For quantification of capillaries, venules and arterioles, both in the myocardial and lingual musculature, the slides in which the immunohistochemical technique with anti-CD31 antibodies was performed, were used. Immunostained cells were quantified in all the fields of the fragment, using a light microscope (Zeiss, Berlin, Germany) and 40x objective. To obtain the area evaluated, the area of the field was calculated with the use of a blade micrometer being 0.14 mm². Next, this area was multiplied by the number of fields evaluated to obtain the total area. Once the total area evaluated and the number of immunostained cells and blood vessels were known, the density was calculated, and was expressed as number of vessels/cm² and number of cells/cm² respectively. All analyzes were formed blind by a single, previously trained examiner.

2.6 Statistical Analysis

Statistical analysis were performed with the use of GraphPad Prism 4.0 software (GraphPad, San Diego, USA). For normality tests, the Shapiro-Wilks test was used. The Mann-Whitney test was used to compare to two groups when the quantitative variables were shown to be not normally distributed. The values were expressed as median with minimum and maximum values. For the ratios female: male and Caucasian: non Caucasian the exact Fisher test was used. For correlations, BioEstat 5.0 software (Instituto de Desenvolvimento Sustentável Mamirauá, Tefé, Manaus, Brazil) and Spearman’s correlation test were used. Student’s-t test was used to compare the age between groups and for comparing the intensity of inflammation. The level of significance considered was 5%.

3. Results

The demographic data of the chagasic and non chagasic groups was: Caucasians (16 vs. 17) and non Caucasian (2 vs. 7), male (12 vs. 15) and female (6 vs. 9), respectively. Mean age ± standard deviation was 64.06 ± 3.12 in the chagasic group and 55.29 ± 3.31 in the non chagasic group. There was no significant difference as regards gender, ethnicity and age between chagasic and non chagasic individual, demonstrating homocedastic distribution between the two groups (Table 1).

| Table 1. Demographic data of autopsied chronic chagasic and non chagasic individuals |
|---------------------------------------------------------------|
| Chronic chagasics (n = 18) | Non chagasics (n = 24) |
|-----------------------------|------------------------|
| Ethnicity \( ^a \) (C/NC)  | 16/2                   | 17/7                   |
| Gender \( ^b \) (M/F)       | 12/6                   | 15/9                   |
| Age (years, mean±SD) \( ^c \) | 64.06 ± 3.12           | 55.29 ± 3.31           |

\( ^a \) Exact Fisher Test, \( p>0.05 \), \( ^b \) Exact Fisher Test, \( p>0.05 \), \( ^c \) Student’s-\( t \) Test, \( p>0.05 \)

Source: Authors.

Intensity of inflammation in the myocardium (Fig.1A; 1B), was significantly greater in chagasic when compared to non chagasic individuals \( (p<0.0001) \) (Fig.2A). In the lingual musculature (Fig. 1C; 1D) there was no significant difference in the intensity of inflammation between chagasic and non chagasic individuals. The areas of myocardial muscle (Fig. 1E) and
tongue muscle (Fig. 1F) cells were significantly larger in chagasic when compared to non chagasic individuals (p<0.0001) (Fig. 3C, Fig. 3D).

Figure 1 – Histological sections of lingual musculature and myocardium of chagasic and non chagasic patients. (A). Histological section of the myocardium with accentuated inflammation (arrow) in chagasic group (HE, x1600); (B). Histological section of the myocardium with moderate inflammation (arrow) in non chagasic group (HE, x1600); (C). Histological section of the lingual musculature with moderate inflammation (arrow) in chagasic group (HE, x1600); (D). Histological section of the lingual musculature with moderate inflammation (arrow) in non chagasic group (HE, x1600); (E). Histological section of the myocardium with large cells in chagasic group (HE, x800); (F). Histological section of the myocardium with small cells in non chagasic group (HE, x800); (G). Histological section of the tongue with large cells in chagasic group (HE, x1600); (H). Histological section of the tongue with small cells in non chagasic group (HE, x1600).

Source: Authors.

There was no significant difference in the MVD between chagasic and non chagasic individuals, both in the myocardial and lingual musculature (Fig. 2A and Fig. 2B). However, in chagasic individuals there was positive and significant correlation of the MVD between the myocardial and lingual musculature (Fig. 3B).
There was no significant difference in the density of leukocytes and fibroblasts immunostained for anti-IL-1 (Fig. 2C; 2D) and for anti-TGF-β1 (Fig. 2E; 2F) between chagasic and non chagasic individuals, both in the myocardial and lingual musculature.

There was positive and significant (p=0.049) correlation between the density of leukocytes immunostained with anti-TGF-β1 and the area of myocardial muscle leukocytes and fibroblasts in chagasic individuals (Fig. 3E). However, there was not significative correlation between the area of muscle cells, both in the myocardial and lingual musculature, with the density of leukocytes and fibroblasts immunostained for anti IL-1, anti IL-10 and anti-CD31 (data not shown).

In chagasic individuals there was positive and significant correlation of the density of leukocytes and fibroblasts immunostained for anti-IL-10 between the myocardial (Fig. 2G) and lingual musculature (Fig. 2H) (p=0.029) (Fig. 3F). However, there was no significant difference in the density of leukocytes and fibroblasts immunostained for anti-IL-10 between chagasic and non chagasic individuals, both in the myocardial and lingual musculature.

Both in chagasic and non chagasics, there was not significative correlation between the myocardial and lingual musculature for the following parameters: Intensity of inflammation, muscle cell area, density of leukocytes and fibroblasts immunostained for anti-IL-1 and density of cells immunostained for anti-TGF-β1 (data not shown).
**Figure 2** – Histological sections of lingual musculature and myocardium of chagasic patients. (A). Histological section of the myocardium demonstrating vessels immunostained for anti-CD31 (arrow) (immunohistochemistry; x1600); (B). Histological section of the lingual musculature demonstrating vessels immunostained for anti-CD31 (arrow) (immunohistochemistry; x1600); (C). Histological section of the myocardium demonstrating vessels immunostained for anti IL-1 in the myocardium (arrow) (x1600); (D). Histological section of the lingual musculature demonstrating cell immunostained for anti-IL-1 (arrow) (x1600); (E). Histological section of the myocardium demonstrating cells immunostained for anti- TGF-β1 (arrow) (x 1600); (F). Histological section of the lingual musculature demonstrating cell immunostained for anti- TGF- β (arrow) (x1600); (G). Histological section of the myocardium demonstrating cell immunostained for anti- IL-10 (arrow) (x1600); (H). Histological section of the lingual musculature demonstrating cell immunostained for anti- IL-10 (arrow) (x1600).

Source: Authors.
Figure 3 - Comparisons of lingual muscles and myocardium between chagasic and non-chagasic patients. A. Intensity of inflammation in the myocardium of chagasic compared with non chagasic individuals. * Indicates statistically significant difference between the groups. Student’s-t test, p<0.0001. The values are presented as mean ± standard deviation. B. Correlation between Microvessel density immunostaining by CD31 in the myocardial and lingual musculature of chagasic individuals (number of capillaries/cm²). *Correlation is significant at p=0.049 level (Spearman’s correlation coefficient). C. Area of myocardial muscle cells in chagasic and non chagasic individuals; Mann Whitney test, p<0.0001. * Indicates statistically significant difference between the groups. D. Area of tongue muscle cells in chagasic and non chagasic individuals; Mann Whitney test, p<0.0001. * Indicates statistically significant difference between the groups. E. Correlation between density of leukocytes and area of muscle cells in the myocardium immunostained for anti-TGF-β1 in chagasic individuals; Spearman’s correlation test, p<0.049. F. Correlation between the density of leukocytes and fibroblasts immunostained for anti IL-10 in the myocardial and lingual musculature of chagasic individuals; Spearman’s correlation test, p<0.0298.

Source: Authors.
4. Discussion

It is known that the cardiac form of Chagas disease is characterized by intense myocarditis (Gutierrez et al., 2011), with generalized focus of inflammation and fibrosis17 and that T. cruzi modulates the expression of co-receptors in lymphocytes after infection, capable of causing unregulated inflammation due to unbalanced expression of inflammatory molecules (Vilas-Boas et al., 2011). Thus, as higher intensity of inflammation in the myocardium of chagasic when compared with non chagasic individuals was demonstrated in the present study, although without significant difference, these findings corroborate those described in the literature, which demonstrated greater intensity of inflammation in tissue infected by T. cruzi (Gutierrez et al., 2011). However, a previous study using the same methodology observed a higher intensity of inflammation in the lingual musculature of individuals with chronic Chagas disease (de Lima Pereira et al., 2006). Therefore, we believe that the tongues used in the present study would not be presenting destruction so intense that it could cause exacerbation of the inflammatory response as observed in the tongues used in the previous study (de Lima Pereira et al., 2006).

Moreover, in the cases analyzed there was perhaps greater tropism of T. cruzi for the myocardium, as has been demonstrated in other studies (Andrade et al., 2010), which would justify our finding of greater intensity of inflammation in the myocardium of chagasic individuals.

Some vascular endothelial adhesion molecules, such as the platelet adhesion molecule (PECAM-1/CD31), play an important role in angiogenesis and inflammation (Park et al., 2010). Although the increase in CD49 (Laucella et al., 2001) and CD44 (Reis et al., 1993) has been described in Chagas disease, we not found articles that studied the MVD by CD31 in chagasic individuals. In the present study, we found no significant difference between chagasic and non chagasic individuals with regard to the MVD, both in the myocardial and lingual musculature. Although it is expected to find significant increase in CD31 expression in the lingual and myocardial musculature of chronic chagasic individuals, in the present study compression of the microvasculature by hypertrophic muscle cells may have occurred, inducing vascular scarcity as has been demonstrated in some studies (Jorge, 1973; Ferreira et al., 1980). Therefore, we believe that in the present study, compression and rupture of some vessels, caused by the pathogenesis of these lesions, maybe have made it difficult to quantify the blood vessels.

There is a positive and significant correlation between the density of blood vessels immunostained for anti-CD31 in the myocardium, and in the lingual musculature of chagasic individuals. In a previous study we described an increase in the density and caliber of blood vessels, in addition to an increase in vascular wall thickness in the lingual musculature of chronic chagasic individuals that could be caused by inflammatory response due to persistence of T. cruzi antigens at this site (de Lima Pereira et al., 2009). In the present study, although no positive and significant correlation was found between the intensity of inflammation in the lingual musculature and the intensity of inflammation in the cardiac musculature of chagasic individuals, the concomitant increase MVD by CD31 in the tongues and hearts of chagasic individuals demonstrates a behavior similar to the expression of this molecule in blood vessels of these two organs in Chagas disease.

As regards muscle cells in heart and tongue, in this study we showed hypertrophy of lingual and cardiac musculature in chagasic individuals. Although the cardiac hypertrophy has been shown in other studies (Dhiman & Garg, 2011), this is the first report of lingual musculature hipetrophy in Chagas disease. Therefore, the other pathologic processes in the tongues of chagasic patients, previously described by us (de Lima Pereira et al., 2009; Vilas-Boas et al., 2011) associated with the musculature hypertrophy showed in present study could contribute to alterations in swallowing demonstrated in studies conducted with chronic chagasic patient (Santos et al., 2011).

As concerning as about immune response in Chagas disease, it has been demonstrated that IL-1 promotes hypertrophy of the cardiomyocytes in the beginning of the infectious process, and could contribute to the maintenance of cardiomyocyte function during establishment of myocardial infection by T. cruzi (Petersen & Burleigh, 2003). However, in the present study no significant correlation was found between the density of cells immunostained for anti-IL-1 and the area of myocardial and...
lingual muscle cells. Therefore, we believe that IL-1 alone did not play a significant role in the hypertrophy of the lingual and cardiac musculature, although Sousa and Cols (2017) (Sousa et al., 2017), related that IL-17A induce the IL-1 expression and IL-17A was correlated with better left ventricular function in Chagas disease patients.

The increase in the production of IL-1 in the myocardium of animals6 and humans chronically infected by T. cruzi (Cardoni et al., 1999) has been demonstrated. However, in present study there was no significant difference between chagasic and non chagasic individuals when comparing the density of leukocytes and fibroblasts immunostained for anti-IL-1 in the myocardial and lingual musculature.

In this context of immune response, another cytokine assessed was TGF-β1, a peptide growth factor that may play a role in the myocardial response to hypertrophic stimulus (Cardillo et al., 2015). Previous study performed by Rosenkranz (2004) (Rosenkranz, 2004), demonstrated that TGF-β1 induced cardiac hypertrophy and β-adrenergic signaling in vivo. Morphological alterations in the myocardium are induced by the direct effects of TGF-β1 or may result from increased β-adrenergic signaling, which may contribute to excessive catecholamine stimulation during the transition from compensatory hypertrophy to the condition of heart failure (Rosenkranz, 2004). In the present study, although no significant difference was found between chagasic and non chagasic individuals with regard to the density of cells immunostained for anti-TGF-β, there was a positive and significant correlation between muscle hypertrophy and the density of leukocytes immunostained for anti-TGF β in the myocardium of chagasic individuals. Therefore, these findings suggest that TGF-β1 would collaborate in the process of myocardial hypertrophy in chagasic individuals.

The influx of leukocytes into the tissues is regulated by cytokines and the extracellular matrix, which are components that may represent possible therapeutic targets in individuals infected by T. cruzi (Savino, 2007). IL-10 is an anti-inflammatory cytokine that inhibits the release and diminishes the activity of the subgroup of Th1 cells, and is capable of reducing intracellular infection by T. cruzi (Laucella et al., 2001) and regulate the response to the parasite in individuals with the cardiac form of Chagas disease (Medeiros et al., 2009). In a literature review performed by Dutra-Walderez and Cols (2013) (Dutra-Walderez, Hojo-Souza, Gollob, 2013), these authors reported that some studies analysing clinical parameters that measure cardiac function has observed a direct and positive correlation of better cardiac function and IL-10 expression. Thus, it is possible that IL-10 displays an important role in maintaining this protective phenotype. In the present study there was a positive and significant correlation between the myocardial and lingual musculature with regard to the density of cells immunostained by anti-IL-10 in chagasic individuals. Although no studies were found in the literature correlating the density of fibroblasts and leukocytes immunostained for anti-IL-10 between the lingual musculature and the myocardium of chagasic individuals, we believe that in the present study the IL-10 could play a similar role both in the myocardium (Laucella et al., 2001) and the lingual musculature of chagasic individuals.

5. Conclusion

Therefore, in the present study chagasic individuals presented greater intensity of inflammation in the myocardium, larger area of cardiac muscle cells and larger area of tongue muscle cells when compared with non chagasic individuals. In addition, in chagasic individuals there was positive and significant correlation between the density of leukocytes immunostained by anti-TGF-β1 and hypertrophy in the myocardium suggesting the participation of this cytokine in cardiac muscle hypertrophy. In chagasic individuals a positive and significant correlation was also demonstrated between both the cardiac and lingual musculature, as regards CD31 and IL-10 expression.

Taken together, we suggest that changes in the skeletal striated musculature of the tongue present certain similarities with myocardial changes in chronic Chagas disease. However, it needs more studies to verify in other phases of the disease, if the parasite is installed in the tongue or if the mechanisms are reflex of systemic changes in level of immune response.
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