Morphometric study of the fibrosis and mast cell count in the circular colon musculature of chronic Chagas patients with and without megacolon

Estudo morfométrico da fibrose e do número de mastócitos na muscular circular do cólon de chagásicos crônicos com e sem megacólon

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Abstract A morphometric study of the circular colon musculature was performed, in which the mast cell count was determined and the connective fibrous tissue in this layer was measured. The objective was to gain better understanding of Chagas megacolon morphology and contribute towards the knowledge of fibrosis pathogenesis in Chagas megas. An evaluation was made of 15 distal sigmoid rings from Chagas patients with megacolon (MCC), 15 without megacolon (CSMC) and 15 non-Chagas patients (NC). The rings were fixed in formal, embedded in paraffin, and 7mm thick sections were cut and stained using Azan-Heidenhain and Giemsa. The mast cell count and fibrosis were greater in the MCC group than in the CSMC and NC groups (p < 0.05; Kruskal-Wallis test) and there was no significant difference between the latter two. The fibrosis and increased mast cell count in the colon musculature of the MCC group possibly indicates that there is a relationship between mastocytosis and fibrosis, as has already been demonstrated in other pathologies.


Resumo Com os objetivos de conhecer melhor a morfologia do megacólon chagásico e contribuir para o conhecimento da patogênese da fibrose dos megas, realizou-se estudo morfométrico na muscular circular do cólon, contando-se o número de mastócitos e medindo o conjuntivo fibroso nessa camada. Foram avaliados anéis do sigmoidóide distal de 15 chagásicos com megacólon (MCC), 15 sem megacólon (CSMC) e 15 não chagásicos (NC). Os anéis foram fixados em formal, incluídos em parafina, cortados com 7mm de espessura e corados por Azan-Heidenhain e Giemsa. O número de mastócitos e a fibrose foram maiores no grupo com MCC em relação ao CSMC e NC (p < 0,05; teste de Kruskal-Wallis); não houve diferença significante entre os dois últimos grupos. Diante destes achados, é possível, que haja relação entre mastocitose e fibrose no megacólon chagásico, como já se demonstrou em outras doenças.


Muscle layer alterations such as myositis and fibrosis that could contribute to the pathogenesis of Chagas’ disease megas are often found both in the esophagus¹³ and in the colon¹²¹⁶. According to Tafuri and Raso¹⁶, fibrosis in Chagas megasoesophagus sometimes is focal, possibly representing a sequel of myositis and thought to be associated with mast cell infiltrate, and sometimes is diffuse and interstitial without showing a topographic relationship with the inflammation. Andrade & Andrade⁸, analyzing myocardial fibrosis, also accepted that focal fibrosis could result from scarring of inflammatory foci related to the presence of mast cells. Nonetheless, Andrade & Andrade⁸ stressed that the pathogenesis of diffuse interstitial fibrosis had not been clarified.

Quantitative studies made on cardiopathic Chagas’ disease patients⁷ and on the circular esophagus musculature of chronic Chagas patients without megasoesophagus¹¹, have reported a marked increase in the mast cell count in these organs. However, no
statistical difference has been detected between the
mast cell counts in the musculature of the esophagus and
colon of chronic Chagas patients with and without megalos, in relation to non-Chagas patients.

With regard to experimental Chagas’ disease, there
has been a report of increased mast cell count in areas
of reinoculation with Trypanosoma cruzi on the skin of mice. Increased mast cell count has also been found
in the submucosa and especially the musculature of the
small and large intestines of mice chronically infected with the ABC strain of T. cruzi. Chapadeiro et al. demonstrated in rats chronically infected
with T. cruzi that there was an increased mast cell
magnitude in the myocardium, although no relationship with fibrosis could be shown.

The data regarding the increase in mast cell count
is controversial and there is a lack of morphometric studies on fibrosis in the musculature of the digestive tract of chronic Chagas patients. In view of this, we
have made a morphometric evaluation of the fibrosis
and mast cell count in the circular colon musculature of
chronic Chagas patients with and without megalos. Our objective was to gain better understanding of Chagas megacolon morphology and contribute towards
the knowledge of fibrosis pathogenesis in megalos.

MATERIAL AND METHODS

It was necessary that two of the three following tests
were positive in the serum or in the pericardial fluid for
the diagnostic of infection with Trypanosoma cruzi: ELISA (Enzyme-Linked-Immunosorbent-Assay), passive
hemagglutination and indirect immunofluorescence to T. cruzi. The individuals of the control groups had the three
tests negative.

Forty-five segments of large intestine were obtained
via surgery and/or necropsy performed in the Pathology
Service of the University Hospital of FMTM, Uberaba,
Minas Gerais. Of these 45 cases, 30 individuals were
chronic Chagas patients and 15 were non-Chagas patients without any intestinal pathology, who served as
controls. The 30 Chagas patients were subdivided into
two groups: with megacolon, consisting of 15 individuals, and without megacolon, also consisting of 15 cases.

In each case, a ring of around 0.5cm in height was
removed from the rectosigmoid transition. The rings were
fixed in 4% formol and, after fixing, dehydrated, diaphanized and embedded in paraffin to form blocks measuring up to
5x5cm for microtomy. Histological sections with 7mm in
thickness were cut and stained using hematoxylin-eosin,
Azan-Heidenhain and Giemsa techniques. The sections
stained using the hematoxylin-eosin were only utilized for
a general analysis of the rings. Following this, a quantitative
analysis of the fibrosis was made on the sections stained
using the Azan-Heidenhain technique and an analysis of the
mast cells on sections stained using the Giemsa

The count of mast cells in the circular musculature
was done using an standard optical microscope
coupled to a video camera linked to a high-resolution
monitor. The image obtained on the monitor was
integrated with a cursor that could move across a
graphical measuring grid connected to an automatic
image analysis system of Leica Q 500MC brand. The
count was done manually, while the area that was being
evaluated was standardized.

The histological sections were previously marked
at eight locations that were approximately equidistant
from each other. The mast cells were counted in 40
consecutive fields of each subdivision, making a total
of 320 microscope fields with the 10x eyepiece and
40x objective lens, corresponding to a total area of
4mm².

For the analysis of the fibrosis, the histological
sections were again previously marked at eight
locations that were approximately equidistant between
each other. The fibrosis was measured in 10 alternate
fields in each subdivision, making a total of 80
microscope fields covering a total area of 1mm². This
analysis was also done with the 10x eyepiece and
40x objective lens. The same morphometry apparatus
was utilized as described for the mast cell analysis
(Figures 1A, 1B and 1C).

The variables analyzed were submitted to
statistical analysis via the Kruskal-Wallis and Dunn
tests. The significance level considered for the tests
was 5% (p < 0.05). To evaluate whether there was a
relationship between the fibrosis percentage and the
mast cell count, the Spearman test was utilized.

This research was approved by the Research
Ethics Committee of Faculdade de Medicina do
Triângulo Mineiro (FMTM), Uberaba, Minas Gerais.

RESULTS

In the non-Chagas group, no myositis was
identified. In the Chagas group without megacolon,
discrete myositis was found in four cases (30.1%) (n=17, 20, 22 and 23). In the megacolon group there
was myositis in 14 of the 15 cases (93.3%), which
was discrete in one case (n=39), moderate in 6 cases
Figure 1 - Image from the morphometry apparatus monitor, captured for quantitative evaluation of the fibrosis. In A, the original image, the muscle tunic of a Chagas case with megacolon is stained using the Azan-Heidenhain technique (objective 40x): the muscle appears in red and the fibrous connective tissue in blue. In B, the marking of the fibrosis for automatic measurement can be seen, now in yellow. Next, as shown in C, the muscle is marked, excluding the empty areas that appear in white.

Figure 2 - In A, circular musculature of the rectosigmoid transition of a non-Chagas individual, in which a mast cell is observed (arrow). In B, musculature of a Chagas case without megacolon, in which a mast cell can also be seen (arrow). In C, muscle tunic of a Chagas case with megacolon, in which three dispersed mast cells can be seen (arrows). In D, myositis focus in a Chagas case with megacolon, in which two mast cells can be seen (arrows). Giemsa staining – objective 100x.
(No 31, 38, 42, 43, 44 and 45) and severe in 7 cases (No 32, 33, 34, 35, 36, 37 and 40).

As indicated in Table 1, the mast cell count found in the circular colon musculature was significantly greater in the Chagas group with megacolon than in the other groups. When pairs of groups were analyzed, it was noted that the mast cell count was greater in the Chagas group with megacolon in comparison with the Chagas group without megacolon (p < 0.01) and the non-Chagas group (p < 0.01). However, there was no significant difference between the non-Chagas group and the Chagas group without megacolon (p > 0.05).

Table 1 - Average values for mast cell counts in the circular colon musculature, according to the study group.

<table>
<thead>
<tr>
<th>Mast cells</th>
<th>Chagas cases</th>
<th>non</th>
<th>with megacolon</th>
<th>with megacolon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.3</td>
<td>6.4</td>
<td>32.7</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>5.9</td>
<td>10.7</td>
<td>32.1</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1.0</td>
<td>1.0</td>
<td>26.0</td>
<td></td>
</tr>
<tr>
<td>Maximum value</td>
<td>19</td>
<td>39</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>Minimum value</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

Kruskal-Wallis test: p = 0.0016. Dunn test: non-Chagas vs. Chagas without megacolon p > 0.05; non-Chagas vs Chagas with megacolon p < 0.01; Chagas without megacolon vs. Chagas with megacolon p < 0.01.

With regard to fibrosis, fibrous connective tissue in the muscle tunica was rare in the non-Chagas group and was represented by thin bands between the myocells (endomysial connective tissue) and between the muscle bundles (perimysial connective tissue) (Figure 3A).

In the Chagas group without megacolon, the appearance of the connective tissue in the muscle tunica was similar to that described for the non-Chagas individuals (Figure 3B). In the Chagas group with megacolon, there was an evident diffuse increase in endomysial and perimysial connective tissue and frequent substitution fibrosis foci (Figures 3C and 3D).

Figure 3 - In A, circular musculature of the rectosigmoid transition of a non-Chagas individual, in which rare fibrous connective tissue can be seen (stained blue). In B, musculature of a Chagas case without megacolon with an appearance that is similar to the non-Chagas group. In C, Circular colon musculature of a Chagas case with megacolon, showing diffuse increased endomysial connective tissue (stained blue). In D, focal fibrosis interrupting and substituting muscle fibers, and also showing increased endomysial connective tissue in the colon musculature of a Chagas case with megacolon. Azan-Heidenhain staining – objective 40x.
As can be seen in Table 2, there was a statistically significant difference between the groups. The percentage of fibrous connective tissue was greater in the megacolon group, in comparison with the Chagas group without megacolon (p < 0.001) and the non-Chagas group (p < 0.001). However, there was no statistically significant difference between the non-Chagas group and the Chagas group without megacolon (p > 0.05).

Table 2 - Average values for percentages of fibrous connective tissue in the circular colon musculature, according to the study group.

<table>
<thead>
<tr>
<th>Fibrosis</th>
<th>Chagas cases</th>
<th>Non-Chagas cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.19</td>
<td>0.35</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.15</td>
<td>0.20</td>
</tr>
<tr>
<td>Median</td>
<td>0.16</td>
<td>0.35</td>
</tr>
<tr>
<td>Maximum value</td>
<td>0.53</td>
<td>0.67</td>
</tr>
<tr>
<td>Minimum value</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>N° of cases</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test: p < 0.0001. Dunn test: non-Chagas vs Chagas without megacolon p > 0.05; non-Chagas vs Chagas with megacolon p < 0.001; Chagas without megacolon vs Chagas with megacolon p < 0.001.

Tables 3, 4 and 5 show the mast cells counting percentage of fibrous conjunctive tissue and myositis in the three groups studied.

The value for the Spearman correlation coefficient between the mast cell count and the percentage of fibrous connective tissue for the Chagas group with megacolon was -0.2, which was not statistically significant (p > 0.05). However, when we analyzed the Chagas groups with and without megacolon together, the value for the correlation coefficient between the mast cell count and the percentage of fibrous connective tissue was 0.215, which was statistically significant (p < 0.05), (Table 3, 4 and 5).

DISCUSSION

Our data are in agreement with quantitative studies of mast cells in the human myocardium and in the myocardium of rats with chronic Chagas’ disease via experimental infection. The data also agree with the description of increased mast cell counts in the circular musculature of the small and large intestines in mice with chronic Trypanosoma cruzi infection.

Our data disagree with the quantitative studies made by Adad et al on the circular musculature of the esophagus and colon of chronic Chagas cases with or without megas. It is possible that these authors did not find significant differences between the groups because they analyzed a lesser number of microscope fields and cases.
With regard to the results from counting mast cells in the muscularature of the esophagus of chronic Chagas cases obtained by Pereira et al.\(^1\), they appear at first to agree with those obtained in our study. However, those authors only evaluated the esophagus of Chagas cases without megas. In our material, we only observed a statistically significant difference in the group with megacolon, while the Chagas cases without megacolon did not present any significant difference when compared with the non-Chagas patients. We must stress that those authors worked with a greater number of cases and, in addition to this, there may be differences between how the esophagus and colon muscleatures are compromised. It has already been demonstrated that myositis occurs more frequently and severely in the esophagus than in the colon of chronic Chagas cases\(^6\).

With regard to the morphometric analysis of fibrosis that was done in this study, we demonstrated that in the colon muscleature there was a greater percentage of fibrous connective tissue in the Chagas group with megacolon, in comparison with the Chagas group without megacolon and the non-Chagas group. We did not find in the literature any morphometric studies of fibrosis in the colon of Chagas cases. Nonetheless, our findings are in agreement with the observations of Adad\(^2\), who described fibrosis in the muscle tunica of Chagas megacolon that was often moderate to severe, and also with the ultrastructural megaeosophagus data of Tafuri et al.\(^15\) and Tafuri\(^12\).

Our results appear to support the hypothesis that interstitial fibrosis is related to the mastocytopsis present in experimental and human Chagas’ disease, since we found greater mast cell counts in the muscle tunica of the group with megacolon, in relation to the others. Nevertheless, when we analyzed each group of Chagas cases separately, it was not possible to demonstrate a relationship between mast cells and fibrosis, perhaps because of the small number of cases. However, when we analyzed the Chagas groups with and without megacolon together, we observed a significant relationship between the mast cell count and the percentage of fibrous connective tissue.

In conclusion, the analysis of the findings of this study and the data in the literature demonstrate that there is a greater mast cell count and more fibrosis in Chagas cases with megacolon. However, it was not possible to demonstrate whether there is a relationship between the fibrosis and the mast cell count, as suggested by some data in the literature, perhaps because of the sample size.

REFERENCES

13. Tafuri WL. Alterações ultra-estruturais dos componentes muscular, intersticial e nervoso do coração e intestinos na doença de Chagas experimental e humana. Tese de Professor Titular. Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 1974.