Gallbladder neuron count in cholelithiasis patients with and without Chagas disease

Contagem de neurônios da vesícula biliar de pacientes chagásicos e não chagásicos portadores de colelitíase

Eduardo Crema[1], Lara Beatriz Prata Ribeiro[2], Sheila Jorge Adad[3], Renata Margarida Ectchebehere[3], Aiodair Martins Júnior[1] and Alex Augusto Silva[1]

ABSTRACT

Various investigators agree that the incidence of cholelithiasis is greater in patients with Chagas disease. The most plausible explanation for this is based on the parasympathetic denervation that occurs over the whole digestive tract due to Chagas disease. In order to analyze the occurrence of this alteration, gallbladder neuron counts were performed on cholelithiasis patients with and without Chagas disease who were being treated at the Department of Digestive Surgery, Universidade Federal do Triângulo Mineiro, Uberaba, Brazil. In the present study, a notable reduction in the number of neurons in the gallbladder wall was observed in Chagas patients, in comparison with non-Chagas subjects.

Key-words: *Chagas disease. Gallbladder. Cholelithiasis. Neuron count.*

RESUMO

Vários autores concordam que a incidência de colelitíase encontra-se elevada nos pacientes portadores de doença de Chagas. A explicação mais plausível para este fato baseia-se na desnervação parassimpática existente em todo o tubo digestivo na doença de Chagas. No intuito de analisar a ocorrência desta alteração, foi realizada contagem neuronal da vesícula biliar de pacientes chagásicos e não chagásicos, portadores de colelitíase, tratados na Disciplina de Cirurgia do Aparelho Digestivo, Universidade Federal do Triângulo Mineiro, Uberaba, Brasil. No presente estudo, observou-se uma redução expressiva do número de neurônios na parede da vesícula biliar dos pacientes chagásicos quando comparado com os não chagásicos.

Palavras-chaves: *Doença de Chagas. Vesícula biliar. Colelitíase. Contagem neuronal.*

Chagas disease is characterized by variable clinical manifestations including cardiac, digestive, cardiodigestive (mixed), nervous and indeterminate forms, and a form showing acute exacerbations[1,2]. The histopathological basis for digestive alterations in Chagas disease consists of the non-uniform destruction of neuron cells of the enteric nervous system of the digestive tract[1,2,6,7,8,12], especially in the esophagus[2,14] and at the rectosigmoid transition[1], which lead to the formation of Chagas megaesophagus and megacolon. The esophagus is the organ that shows the highest degree of denervation even in the absence of ectasia, with almost total denervation present in most cases of megaesophagus[2,7].

From an anatomopathological point of view, frequent qualitative and quantitative lesions in the intramural ganglia and changes in muscle layers of the gastrointestinal tract have been observed both in man and in experimentally infected animals[1,2].

Parasympathetic denervation of the digestive tract and the consequent morphological and functional alterations have been extensively described[1,2,6,7,8]. However, involvement of the glands and attached organs of the digestive tract has been little studied. Particularly, the gallbladder in Chagas disease has been the subject of very few studies[3,9,15,16].

The incidence of cholelithiasis is greater in cases of parasympathetic nerve injury to the bile ducts, which may be

1. Disciplina de Cirurgia do Aparelho Digestivo, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brasil. 2. Acadêmica de Medicina, Universidade Federal do Triângulo Mineiro, Uberaba, MG. 3. Disciplina de Patologia Especial, Universidade Federal do Triângulo Mineiro, Uberaba, MG.
Financial Support: CNPq
Address to: Dr. Eduardo Crema. Disciplina de Cirurgia do Aparelho Digestivo/UFTM. Rua Getúlio Guaritá s/n, 38025-440 Uberaba, MG, Brasil.
Tel: 55 34 3318 5228.
e-mail: cremauftm@mednet.com.br
Recebido para publicação em 5/12/2005
Aceito em 12/1/2007

caused either by surgery (vagotomy)[5] or by neuron destruction as observed in the chronic digestive form of Chagas disease[1 2 7 8]. In the latter case, vesicular lithiasis has been studied by some investigators[3 9 10 13 15 16], but the results have been controversial and most studies were retrospective.

In view of the scarcity of data in the literature and the conflicting results obtained, the aim of the present study was to microscopically analyze gallbladder intramural plexuses in Chagas patients and to compare these data with data obtained from non-Chagas patients.

MATERIAL AND METHODS

Thirty-two patients with symptomatic cholelithiasis treated at the Department of Digestive Surgery, Universidade Federal do Triângulo Mineiro, Uberaba, Brazil, underwent gallbladder neuron counts. All the patients received detailed information regarding the procedures to be performed and regarding the study, which was approved by the Research Ethics Committee of this institution. The patients were divided into two groups - Chagas and non-Chagas - based on positive or negative *Trypanosoma cruzi* serology, respectively. The diagnosis of Chagas infection was made on the basis of a positive reaction in at least two of the following three blood tests: ELISA, passive hemagglutination and indirect immunofluorescence for *T. cruzi*. All controls showed three negative reactions. The Chagas group consisted of 17 patients: 12 women (70.5%) and 5 men (29.5%) with a mean age of 57.6 years (range: 41-68). The non-Chagas group consisted of 15 patients: 12 women (80%) and 3 men (20%) with a mean age of 51.8 years (range: 25-71).

Microscopic analysis of the gallbladder was performed on surgical specimens obtained from patients with symptomatic cholelithiasis at the Special Pathology service of the Federal School of Medicine, Uberaba. Immediately after their removal, the gallbladders were opened by means of an incision made along their major axis and their borders were fixed with pins in such a way that the specimen remained extended and attached to a plane polystyrene surface.

The neck of the gallbladder was chosen as the location for the neuron counts, on the basis of the study by Conte[4], who observed a larger number of nerve cells in this region in healthy subjects and in patients with cholelithiasis.

For each gallbladder, 160 serial sections (7µm thick) were obtained and every fourth section in the sequence was stained with hematoxylin-eosin for neuron counting, thus giving a total of 40 stained slides for counting. All neurons in these 40 sections obtained from the 32 patients were counted under an optical microscope equipped with a 10x eyepiece and a 40x objective lens.

The one-in-four staggered sequence was used in order to prevent counting of the same neuron more than once. This was established on the basis of the diameter of the neurons (± 30µm), which was determined by measuring the diameters of the neurons of two normal gallbladders.

RESULTS

Statistical analysis of age and gender showed no significant difference between the two groups.

The mean neuron count obtained from the 40 stained slides was 177.1 ± 149.7 in the non-Chagas group, compared with 60.2 ± 69.5 in the Chagas group, thus showing a significant difference ($p = 0.003$) (Figure 1).

In addition to the significant reduction in the number of gallbladder neurons in Chagas patients, histopathological analysis revealed that these neurons were more frequently in the process of degeneration. It also showed the presence of sparse ganglionitis, periganglionitis, neuritis and perineuritis (Figure 2). No ganglionitis and/or neuritis was observed in the non-Chagas group (Figure 3).

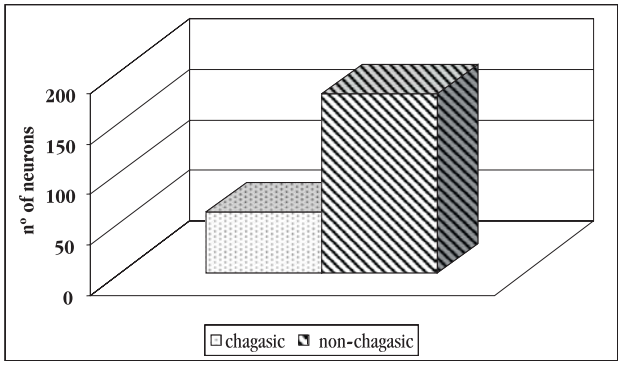

Figure 1 - Graphical representation of the number of gallbladder neurons in the Chagas and non-Chagas groups.

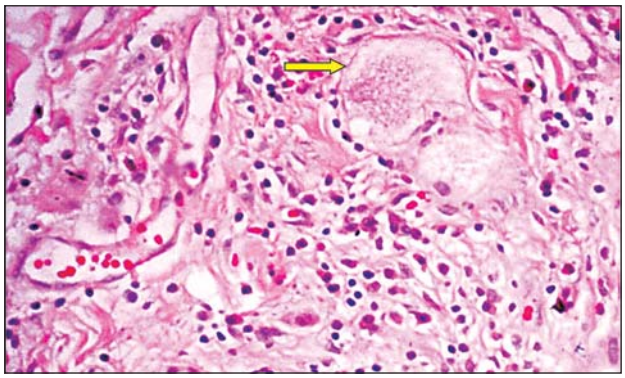

Figure 2 - Gallbladder neuron from a Chagas patient, in the process of degeneration (HE 400X).

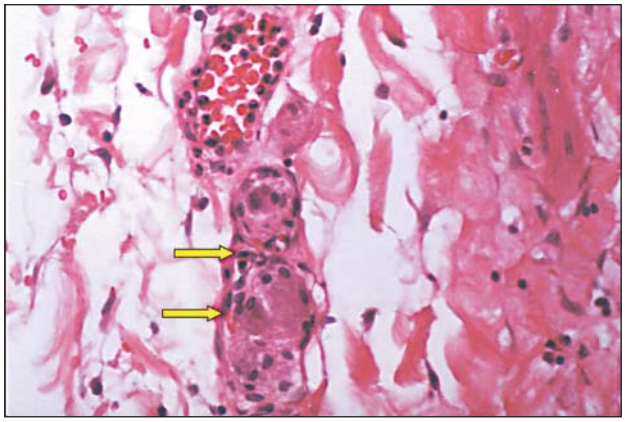

Figure 3 - Gallbladder neurons from a non-Chagas patient (HE 400X).

DISCUSSION

In the presence of parasympathetic nerve injury to the bile ducts caused either by surgery (vagotomy)[5] or by neuron destruction as observed in the chronic digestive form of Chagas disease, the incidence of cholelithiasis is higher than in the general population[9 10 13]. Pinotti et al[11] reported the presence of cholelithiasis in 8.4% of their patients with Chagas megaesophagus. Most investigators agree with the above finding, although the difference is not always significant. In contrast, Rocha et al[13] demonstrated that biliary lithiasis shows a significant association with Chagas megaesophagus and megacolon but not with Chagas disease when considered overall in terms of all of its anatomoclinical forms.

The explanations for this increase are: 1) denervation of the gallbladder leading to changes in tonus and contractility, with a reduction in bile flow and greater stasis in patients with megaesophagus[9]; and 2) hypostimulation of the gallbladder by cholecystokinin due to a) reduced food supply to the stomach and duodenum as a result of poor deglutition in cases of megaesophagus and of anorexia in cases of megacolon; b) vagal denervation of the stomach, thereby reducing its motility and the acidity of the gastric juices; c) increased salivary and mucus secretion in cases of megaesophagus, which neutralizes the gastric juices and reduces the stimulation of cholecystokinin secretion[6]; and d) in patients with megaesophagus and megacolon it is possible that the denervation of the small bowel reduces the absorption of bile salts, an event triggering the formation of lithogenic bile[9].

In the present study, a significant reduction in the number of gallbladder neurons was observed in Chagas patients, plus degenerating neurons, periganglionitis, perineuritis, ganglionitis and neuritis. Such findings were not detected in the gallbladders of non-Chagas patients. All of these neuronal and ganglionic changes have also been described in esophagopathy and in Chagas colopathy[1 2].

We found only one study in the literature investigating neuron counts in the gallbladders of Chagas patients, which reported the absence of neuron cells throughout the segments of the gallbladder in lithiasic or non-lithiasic Chagas patients[3].

The present results allow us to conclude that a significant reduction in the number of neurons in the gallbladder wall was observed in Chagas patients, in comparison with non-Chagas controls.

REFERENCES

1. Adad SJ. Contribuição ao estudo da anatomia patológica e patogênese do megacólon chagásico. Tese de Doutorado, Universidade Federal do Triângulo Mineiro, Uberaba, MG, 1996.
2. Adad SJ, Resende AV, Jorge BH. Estudo sistematizado do plexo mioentérico nos diferentes terços do esôfago de chagásicos crônicos com e sem megaesôfago. Revista da Sociedade Brasileira de Medicina Tropical 25(supl III):101, 1992.
3. Conte VP. Aspectos anátomo-funcionais da vesícula biliar em pacientes com megaesôfago chagásico. Revista do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo 36: 69-77, 1981.
4. Conte VP. Normal neuronal features of the human gallbladder and structural changes in cholelithiasic patients. Revista do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo 44: 211-213, 1989.
5. Diaconescu MR, Simon I, Costea I, Glod M, Terinte R. Litiaza biliar a dup a chirurgia gástrica. Chirurgia 92: 343-3470, 1997.
6. Köberle F. Patogenia da moléstia de Chagas. Revista Goiana de Medicina 3: 155-180, 1957.
7. Köberle F. Patogenia do megaesôfago brasileiro e europeu. Revista Goiana de Medicina 9: 79-116, 1963.
8. Köberle F. Chagas disease and Chagas syndromes: The pathology of American trypanosomiasis. Advances in Parasitology 6: 63-116, 1968.
9. Oliveira LCM, Nascimento RS, Rocha A, Gonçalves EG, da Silva JM, de Oliveira VA, Ferreira RM, Buso AG. Colelitíase em chagásicos crônicos. Arquivos de Gastroenterologia 34: 222-226, 1997.
10. Palmero HA, Caero TF, Iosa DJ, Bas J. Increased prevalence of cholelithiasis in chronic Chagas disease. Medicina (Buenos Aires) 42: 47-50, 1982.
11. Pinotti HW, Raia A, Bettarello A, Conte VP. Ocorrência de colelitíase em portadores de megaesôfago chagásico. Estudo comparativo com não chagásicos. Revista do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo 35: 21-24, 1980.
12. Prata A. Classificação da infecção chagásica no homem. Revista da Sociedade Brasileira de Medicina Tropical 23: 109-113, 1990.
13. Rocha A, Almeida H, Teixeira VPA, Silva AM. Prevalência da colelitíase em chagásicos crônicos necropsiados no Triângulo Mineiro – correlação com o megaesôfago, o megacólon e a insuficiência cardíaca. Arquivos de Gastroenterologia 22: 3-6, 1985.
14. Tafuri WL. Alterações das células musculares e dos componentes do interstício no megaesôfago da tripanosomíase cruzi humana. Estudo ao microscópio eletrônico. Revista da Associação Médica de Minas Gerais 21: 1-21, 1973.
15. Troncon LEA, Rezende Filho J, Iazigi N. Estudo cintilográfico do esvaziamento da vesícula biliar na doença de Chagas crônica. Arquivos de Gastroenterologia 24: 157-163, 1987.
16. Villanova MG, Meneghelli UG, Dantas RO. Gallbladder motor function in chagasic patients with megacolon and/or megaesophagus. Digestion 36: 189-194, 1987.