ORIGINAL ARTICLE

Association between inflammatory interleukins and intraventricular conduction disturbances in patients with positive serology for Chagas disease and preserved ventricular function

Asociación entre interleuquinas inflamatorias y la presencia de trastornos intraventriculares de la conducción en pacientes con serología positiva para enfermedad de Chagas y función ventricular conservada

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ABSTRACT

Background: Intraventricular conduction disturbances are common in patients with Chagas disease and preserved left ventricular ejection fraction, but their association with higher inflammatory activity is unknown.
Objectives: The aim of this study was to determine the presence of an association between interleukin levels and intraventricular conduction disturbances in patients with positive serology for Chagas disease and preserved left ventricular function.
Methods: Twenty-two patients between 22 and 80 years of age with positive serology test for Chagas disease of more than 20 years progression and left ventricular ejection fraction ≥50% were included in the study and compared with a control group of 14 healthy individuals. Plasma levels of IFN-γ, IL-1β, IL-6, IL-10, IL-12 (p70), IL-15, IL-17A, MCP-1/CCL2, MIP-1 α/CCL3, TNF α and IL-2 were measured in patients and controls. Right bundle branch block, left anterior hemiblock or left bundle branch block were considered intraventricular conduction disturbances.
Results: Among the 22 patients with positive serology for Chagas disease, 10 presented intraventricular conduction disturbances (45.4%). This group had elevated levels of interleukins with high inflammatory effect, such as INF-γ, IL-15, IL-2, IL-12, MIP-1 α, compared with the control group, and high levels of IL-10 as a regulatory mechanism of an excessive immune response.
Conclusions: The association between elevated levels of inflammatory interleukins and intraventricular conduction disturbances suggests that chronic inflammation may play a role in the development of these abnormalities in patients with positive serology for Chagas disease and preserved left ventricular ejection function.

Key Words: Chagas Cardiomyopathy – Interleukins - Cardiac Conduction System Disease

RESUMEN

Introducción: Los trastornos de la intraventriculares de la conducción constituyen una manifestación habitual en los pacientes con enfermedad de Chagas con función ventricular izquierda conservada. Se desconoce si su presencia puede estar asociada a una mayor actividad inflamatoria.
Objetivos: Determinar si existe una correlación entre los niveles de interleuquinas y la presencia de trastornos intraventriculares de la conducción en pacientes con serología positiva para enfermedad de Chagas y fracción de eyección ventricular izquierda conservada.
Material y métodos: Se evaluó a 22 pacientes con edades comprendidas entre 21 y 80 años, seropositivos para enfermedad de Chagas, de más de 20 años de evolución y fracción de eyección ventricular izquierda mayor del 50%. Se analizó, además, un grupo control de 14 individuos sanos. Se determinaron las concentraciones en plasma de IFN-γ, IL-1β, IL-6, IL-10, IL-12 (p70), IL-15, IL-17A, MCP-1/CCL2, MIP-1 alfa/CCL3, TNF alfa e IL-2. Se consideró trastornos intraventriculares de la conducción a la presencia de bloqueo de rama derecha, hemibloqueo anterior izquierdo o bloqueo de rama izquierda.
Resultados: De los 22 pacientes con serología positiva para enfermedad de Chagas, 10 presentaron trastornos intraventriculares de la conducción (45,4%). En el grupo con trastornos intraventriculares de la conducción, se observaron niveles elevados de interleuquinas de alto efecto inflamatorio como INF-γ, IL-15, IL2, IL-12, MP1α, en comparación al grupo control, además de presentar altos valores de IL 10 como mecanismo modulador de una respuesta inmunitaria excesiva.
Conclusiones: La asociación entre niveles elevados de interleuquinas y la presencia de trastornos intraventriculares de la conducción plantea un posible proceso inflamatorio crónico para su desarrollo en pacientes chagásicos con fracción de eyección ventricular izquierda conservada.

Palabras claves: Cardiomiopatía chagásica - Interleuquinas - Trastorno del sistema de conducción cardíaco

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Abbreviations

RBBB	Right bundle branch block	**IL**	Interleukin
LBBB	Left bundle-branch block	***T. cruzi***	*Trypanosoma cruzi*
CD	Chagas disease	**IVCD**	Intraventricular conduction disturbances
LVEF	Left ventricular ejection fraction	**TNF-α**	Tumor necrosis factor-α
LAH	Left anterior hemiblock		

INTRODUCTION

Chagas disease (CD), also called American trypanosomiasis, is a potentially fatal disease caused by the protozoan parasite Trypanosoma cruzi (T. cruzi). (1)

Between six and seven million people are estimated to have CD worldwide, particularly in endemic areas of 21 Latin American countries, where it is mostly transmitted to humans by contact with feces or urine of triatomine bugs (vector-borne) which have different names depending on the geographical area. (2)

About 15%-30% of people affected with CD develop Chagas cardiomyopathy. Other manifestations include conduction disturbances, gastrointestinal disorders, and abnormalities of the peripheral, central and autonomic nervous systems. (3, 4)

There are several theories to explain why CD has so many manifestations, many of which are complementary to each other and are still under constant investigation. However, how the disease will progress is still unpredictable.

Immune activity in CD is one of the most extensively studied branches. Circulating immunoglobulin G (IgG) antibodies against muscarinic M2 receptors have been associated with early dysautonomia in patients with chronic CD and symptoms as sinus bradycardia or atrioventricular conduction disturbances. (5-7)

Another line of research is represented by the analysis of autoantibodies against β1 and β2 adrenergic receptors that can be produced by patients with Chagas cardiomyopathy. (8) These autoantibodies, originally targeted against the parasite, would recognize similar antigens in the host, a phenomenon known as mimicry. The relationship between their concentration and the extent of cardiac involvement (complex arrhythmias, ventricular dysfunction, or conduction disturbances) remains controversial. (9)

The action and concentration of cytokines is another immunological factor that has been evaluated, particularly in advanced stages of the disease but not in early or asymptomatic stages. Some studies have shown a significant correlation between plasma levels of some inflammatory cytokines, as IFN-γ, tumor necrosis factor-alpha (TNF-α) and IL-6, and ventricular dysfunction. In contrast, low levels of IL-10 have been observed in patients with reduced ejection fraction. (10)

It is unknown whether there is a relationship between cytokine profile and concentration and the presence of conduction disturbances in the early stages of the disease.

OBJECTIVES

The aim of this study was to determine the concentration of proinflammatory and regulatory cytokines in asymptomatic patients with chronic CD and preserved ejection fraction and evaluate their association with intraventricular conduction disturbances (IVCD).

METHODS

The study included 44 subjects between 21 and 80 years of age with positive serology for CD, known by the patient for more than 20 years and certified by the treating physician or by serology tests, and left ventricular ejection fraction (LVEF) ≥50% estimated by color-Doppler echocardiography within 12 months before inclusion. In patients with definite pacemaker, ventricular paced beats should be <50%. These patients were compared with a control group of 14 healthy individuals with similar age and male-to-female ratio, and negative serology for CD.

Patients who refused to sign the informed consent form, or with known severe conditions (excluding cardiovascular disease) with a life expectancy <1 year, or who were participating in research protocols within 30 days before blood sampling, or who would not be able to be contacted in person or by telephone were excluded from the study. Other exclusion criteria were alcohol or drug abuse within the past 6 months, symptoms of liver failure with abnormal laboratory tests (AST and ALT levels three times higher than the normal range and bilirubin >2 mg/dL), LVEF <50% in subjects who received medication with known action on the cardiovascular parameters considered effects (immunosuppressants, nitrates, estrogens, statins, non-steroidal anti-inflammatory drugs, other anti-inflammatory drugs or corticosteroids), history of acute or chronic coronary artery disease, or indication of percutaneous coronary intervention or myocardial revascularization surgery within the previous 6 months. Patients with kidney dysfunction (creatinine levels >3 g/dL), severe obstructive pulmonary disease, cardiomyopathies, diabetes, hypertension, significant valvular heart diseases (except for those secondary to dilation of the mitral annulus or tricuspid annulus), pacemaker with ventricular pacing >50% or with autoimmune diseases (rheumatoid arthritis, hepatitis B or C, among others) were also excluded.

After signing the informed consent form, a complete medical record was taken and the corresponding diagnostic and prognostic procedures were performed. All the patients underwent 12-lead ECG and color Doppler-echocardiography with estimation of LVEF.

Fasting blood sampling was performed, and the total blood obtained (6 mL) was placed in Vacutainer tubes with

EDTA (Becton, Dickinson, USA) and immediately centrifuged at 1200×g for 10 min. A freshly prepared protease inhibitor cocktail (P8340, Sigma-Aldrich) (1% V/V) was immediately added to each plasma sample. The frozen plasma samples were then sent to the IATIMET laboratory for aliquoting and storage at -20 °C until analysis.

Plasma levels of IFN-γ, IL-1β, IL-6, IL-10, IL-12 (p70), IL-15, IL-17A, MCP-1/CCL2, MIP-1 α/CCL3, TNF α and IL-2 were measured in patients and controls using HCYTOMAG-60K, a bead-based multiplex human cytokine/chemokine panel (Merck Millipore, MO, USA) and a Magpix® device. All the samples were analyzed in duplicate. The concentrations were determined from standard curves using xPONENT software 4.2 and expressed in pg/mL. All patients received standard treatment according to national and international treatment guidelines and were free to withdraw from the project if they so decided. Right bundle branch block (RBBB), left anterior hemiblock (LAH) or left bundle branch block were considered IVCD.

Statistical analysis

Non-parametric tests were used for statistical analysis and categorical variables were analyzed with the chi-square and Cramer's V test. The Mann-Whitney U test and the Kolmogorov-Smirnov test were used to establish the relationship between a categorical variable and a quantitative variable. The critical value selected for hypothesis testing was 0.05.

Ethical considerations

The research was conducted in compliance with the Ministry of Health Resolution No. 1490/07, the words and spirit of the Declaration of Nuremberg, the Declaration of Helsinki and its amendments, and the Law 3301 "Protection of the Rights of Subjects under Research" of the city of Buenos Aires. Medical confidentiality was ensured and all information was protected according to the Argentine personal data protection law 25 326.

The study protocol, the informed consent form and the patient information sheet were approved by the Research Ethics Committee of Hospital J. M. Ramos Mejía. After the consent form was signed, an original copy was handed to the patient.

RESULTS

A total of 44 patients were evaluated, 22 of which had reduced LVEF and 22 had preserved LVEF. The latter group was used for the analysis and was subdivided into two groups: with and without IVCD. Population characteristics are detailed in Table 1.

Among the 22 patients with positive serology test for CD and preserved LVEF, 10 presented IVCD (45.4%). In the group without IVCD, mean age was 50.92±7.1 years, LVEF was 62±7.07% and 41.7% were men, while in the group with IVCD, age was 61.3±13.36 years, LVEF was 59.60± 6.28% and 60% were men.

Cytokine levels in patients without IVCD showed no significant differences compared with those of the control group (Table 2). According to these observations, the profile of the group without IVCD is similar to that of the control population.

Patients with IVCD had significantly higher levels of interleukin 10, 2, 12 p70, 15, INFγ and MIP-1 α than those without IVCD (Table 3).

DISCUSSION

The underlying immune-mediated and inflammatory mechanisms resulting from the contact of the immune system with the parasite, together with disturbances of the autonomic nervous system, seem to be involved in the transition of CD from the asymptomatic form to the cardiac form.

There are two main subpopulations of T lymphocytes based on their markers and functionality, CD4+, or helper T cells, and CD8+, or cytotoxic T cells, both involved in the control of T. cruzi infection. In a model of acute infection in mice, deletion of CD8+ T lymphocytes increased parasitemia, while in humans a reduction in CD4+ lymphocytes, as in HIV infection, leads to parasite reactivation, indicating that T lymphocytes are crucial in controlling the infection. (11)

Different studies have shown that CD4+ and CD8+ T cells play a major role in the control of parasite multiplication during the acute and chronic phases of the infection through their effector cytokines, but paradoxically, they would also have an important role in the pathogenesis of chronic CD. In turn, CD4+ T cells differentiate into Th1 and Th17 which are important in the control of the infection in the acute phase but have a negative effect during the chronic phase. (12)

On the other hand, the cytotoxic effect of CD8+ T cells is both direct and through the production of cytokines, as TNF-α. Elevated circulating levels of TNF-α have been observed in patients with more severe cardiac involvement. (13)

Patients with chronic Chagas cardiomyopathy may exhibit myocytolysis, cellular infiltrate, interstitial fibrosis, and myocyte basement membrane thinning. (14) This cellular infiltrate is predominantly made up of lymphocytes, especially CD8+ and CD4+ T cells,

Table 1. Characteristics of the population included in the study

	With conduction disturbances	Without conduction disturbances	*p*=
N (patients)	10	12	
Age (mean ± SD, years)	61.30±13.36	50.92±7.10	0.107
Sex (M, %)	6 (60)	5 (41.7)	0.392
LVEF (mean ± SD)	59.60±6.28	62.00±7.07	0.539

M: Men. LVEF: Left ventricular ejection fraction.

Interleukins	Controls	Without conduction disturbances	p =
TNF-α (X, min.-max.)	15.19 (9.8-23.48)	14.53 (10.78-34.76)	1.00
IL-6 (pg/mL) (X, min.-max.)	1.79 (0.0-7.01)	0.79 (0.00-3.81)	0.12
MCP-1 pg/mL (X, min.-max.)	181.57 (94.20-228.34)	186.94 (89.00-392.81)	0.57
IL-10 (pg/mL) (X, min.-max.)	18.48 (5.45-44.40)	9.83 (0.00-27.71)	0.09
MIP-1 α (pg/mL) (X, min.-max.)	10.61(0.0-21.50)	3.35 (0.00-9.20)	0.06
IL-12 p70 (pg/mL) (X, min.-max.)	14.20 (5.43-28.60)	9.02 (2.93-15.09)	0.11
IL-2 (pg/mL) (X, min.-max.)	3.32 (1.13-5.60)	2.07 (1.14-3.18)	0.04
IL-1β (pg/mL) (X, min.-max.)	2.64 (0.84-4.43)	1.87 (0.67-2.89)	0.14
IL-15 (pg/mL) (X, min.-max.)	6.25 (1.32-12.30)	3.36 (1.37-7.74)	0.08
IL-17A (pg/mL) (X, min.-max.)	4.64 (1.82-9.58)	3.69 (0.83-6.87)	0.09
INF-γ (pg/mL) (X, min.-max.)	9.33 (4.62-33.46)	7.25 (2.43-10.55)	0.07

TNF-α: Tumor necrosis factor-α, IL-6: Interleukin-6, MCP-1: Monocyte chemotactic protein-1, IL-10: Interleukin-10, MIP-1 α: Macrophage inflammatory protein-1 α, IL-12 p70: Interleukin-12 p70 subunit, IL-2: Interleukin-2, IL-1β: Interleukin-1 beta, IL-15: Interleukin-15, IL-17A: Interleukin-17A, INF-γ: Interferon gamma.

Table 2. Comparison of interleukin levels between Chagas disease patients without conduction disturbances and controls

Interleukins	With conduction disturbances	Without conduction disturbances	p =
TNF-α (X, min.-max.)	22.16 (11.89-39.21)	14.53 (10.78-34.76)	0.093
IL-6 (pg/mL) (X, min.-max.)	3.31 (80.00-8.15)	0.79 (0.00-3.81)	0.059
MCP-1 pg/mL (X, min.-max.)	194.78 (74.76-402.08)	186.94 (89.00-392.81)	1.000
IL-10 (pg/mL) (X, min.-max.)	24.49 (7.10-62.31)	9.83 (0.00-27.71)	0.007
MIP-1 α (pg/mL) (X, min.-max.)	10.33 (0.00-17.63)	3.35 (0.00-9.20)	0.013
IL-12 p70 (pg/mL) (X, min.-max.)	13.20 (4.67-17.62)	9.02 (2.93-15.09)	0.034
IL-2 (pg/mL) (X, min.-max.)	2.70 (2.12-5.43)	2.07 (1.14-3.18)	0.020
IL-1β (pg/mL) (X, min.-max.)	2.62 (0.39-3.77)	1.87 (0.67-2.89)	0.295
IL-15 (pg/mL) (X, min.-max.)	7.09 (2.11-10.33)	3.36 (1.37-7.74)	0.020
IL-17A(pg/mL) (X, min.-max.)	4.94 (1.73-9.71)	3.69 (0.83-6.87)	0.107
INF-γ (pg/mL) (X, min.-max.)	10.83 (5.85-24.13)	7.25 (2.43-10.55)	0.004

TNF-α: Tumor necrosis factor-α, IL-6: Interleukin-6, MCP-1: Monocyte chemotactic protein-1, IL-10: Interleukin-10, MIP-1 α: Macrophage inflammatory protein-1 α, IL-12 p70: Interleukin-12 p70 subunit, IL-2: Interleukin-2, IL-1β: Interleukin-1 beta, IL-15: Interleukin-15, IL-17A: Interleukin-17A, INF-γ: Interferon gamma.

Table 3. Comparison of interleukin levels between Chagas disease patients with and without conduction disturbances

with a patchy distribution. (15)

Therefore, natural and acquired immunity contribute to parasite control and thus, to cardiovascular risk. The mechanisms produced by natural immunity in the presence of the pathogen -in this case, the parasite- induce inflammatory reactions. The inflammatory response is associated with the expression of many cytokines, such as interferon γ, interferon α, TNF-α, interleukin 1 (IL-1) and interleukin 3 (IL-3). (12)

Some proinflammatory cytokines, such as TNF-α, produced by monocytes and lymphocytes, have shown a positive correlation with the degree of cardiac damage. (16)

There is evidence that patients with Chagas cardiomyopathy exhibit a strong immune response, expressed by higher levels of IFN-γ, TNF-α and IL-6, compared with patients infected with the parasite without cardiac involvement and with healthy controls. In turn, patients without cardiac involvement have higher levels of interleukin 10 (IL-10) expression. (17)

Asymptomatic chronic CD patients appear to have higher levels of interleukin-10 (IL-10) production and secretion by regulatory T cells compared with patients with cardiomyopathy. The higher expression of IL-10 also correlates with better left ventricular function. (16)

Absence of IL-17RA and IL-17A/F during T. cruzi infection could be due to increased tissue parasitism and reduced response of parasite-specific CD8+ T cells. Thus, IL-17RA and IL-17A are considered critical factors for sustaining CD8+ T cell immunity to T. cruzi infection. (18)

In our study, we did not find differences in IL-17 A levels between patients with and without IVCD. In contrast, high levels of IL-10 were observed in patients with IVCD and preserved LVEF compared with those without IVCD. In addition, patients with IVCD had higher concentrations of inflammatory cytokines such as MIP-1 α, interleukins 2, 12, 15 and INF-γ.

CONCLUSIONS

The results of our study would suggest that CD patients with ICVD and preserved LVEF (stage B1), have

increased levels of proinflammatory cytokines such as MIP-1 α, IL-2, 12, IL-15 and INF-γ, and elevated IL-10 levels, which would act as a regulatory factor of the inflammatory process. High levels of cytokines with higher inflammatory activity, as IL-6 and TNF-α, were observed without reaching statistical significance. So far, this is the first report about this association between inflammatory interleukins and ICVD in patients with positive serology for Chagas disease and preserved LVEF.

Conflicts of interest

None declared.

(See authors' conflicts of interest forms on the website/ Supplementary material)

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REFERENCES

1. La enfermedad de Chagas (tripanosomiasis americana) Organización mundial de la salud https://www.who.int/es/news-room/fact-sheets/detail/chagas-disease-(american trypanosomiasis)

2. Rodrigo Sousa G, Assis Silva Gomes J, Gomes Fares R, de Souza Damasio M, Chaves A, Silvestre Ferreira K, et al. Plasma Cytokine Expression Is Associated with Cardiac Morbidity in Chagas Disease. PLoS One 2014;9:e87082.

3. WHO. Weekly Epidemiological Record 2015;90:33-44.

4. Giménez L, Mitelman JE. Situación epidemiológica de la enfermedad de Chagas Mazza en Iberoamérica. 2010. https:// www.intramed.net/sitios/libro_chagas.

5. Kölliker-Frers RA, Otero-Losada M, Razzitte G, Calvo M, Carbajales J and Capani F. Chagas cardiomyopathy: role of sustained host-parasite interaction in systemic inflammatory burden. In: Veeranoot Nissapatorn, Helieh S. Oz (Eds.) - Chagas disease - Basic investigations and challenges, September 2018. https://doi.org/10.5772/intechopen.77980.

6. Thiers C, Barbosa J, Pereira B, Nascimento E, Nascimento J, Medei E, Pedrosa R. Autonomic dysfunction and anti-M2 and anti--1 receptor antibodies in Chagas disease patients. Arq Bras Cardiol 2012; 99:732-9.

7. Marin Neto JA, Rassi Jr A. Update on Chagas Heart Disease on the first centenary of Its discovery. Rev Esp Cardiol 2009;62:1211-6. https://doi.org/10.1016/s1885-5857(09)73346-8.

8. Rosenbaum M B, Chiale P A, Schejtman D, Levin M, Elizari M V. Antibodies to Beta-Adrenergic Receptors Disclosing Agonist-Like Properties in Idiopathic Dilated Cardiomyopathy and Chagas' Heart Disease. J Cardiovasc Electrophysiol 1994;5:367-75. https://doi.org/10.1111/j.1540-8167.1994.tb01174.x.

9. Chiale P A, Rosenbaum M B, Elizari M V, Hjalmarson A, Magnusson Y, Wallukat G, et al. High Prevalence of Antibodies against Beta 1- and Beta-Adrenoceptors in Patients with Primary Electrical Cardiac Abnormalities. J Am Coll Cardiol 1995; 26:864-9. https://doi.org/10.1016/0735-1097(95)00262-2.

10. Rodrigo Sousa G. Plasma Cytokine Expression Is Associated with Cardiac Morbidity in Chagas Disease. PLoS One 2014; 9:e87082. https://doi.org/10.1371/journal.pone.0087082. eCollection 2014.

11. Teixeira de Freitas V, Teixeira de Freitas VL, Vicente da Silva SC, Sartori AM, Bezerra RC, Visone Nunes Westphalen E, et al. Real-time PCR in HIV/Trypanosoma cruzi coinfection with and without Chagas disease reactivation: association with HIV viral load and CD4 level. PLoS Negl Trop Dis 2011;5: e1277. https://doi.org/10.1371/journal.pntd.0001277

12. Padilla A, Xu D, Martin D, Tarleton D. Limited role for CD4+ T-cell help in the initial priming of Trypanosoma cruzi-specific CD8+ T cells. Infect. Immun 2007;75:231-5. https://doi.org/10.1128/IAI.01245-06.

13. Talvani A, Rocha M, Barcelos L, Gomes Y, Ribeiro A. Teixeira M. Elevated concentrations of CCL2 and tumor necrosis factor-alpha in chagasic cardiomyopathy. Clin Infect Dis 2004;38:943-50. https://doi.org/10.1086/381892

14. Ferrans V, Milei J, Tomita Y, Storino R. Basement membrane thickening in cardiac myocytes and capillaries in chronic Chagas' disease. Am J Cardiol 1988;61:1137-40. https://doi.org/10.1016/0002-9149(88)90148-8

15. Benvenuti LA, Higuchi ML, Reis MM. Upregulation of adhesion molecules and class I HLA in the myocardium of chronic chagasic cardiomyopathy and heart allograft rejection, but not in dilated cardiomyopathy. Cardiovasc Pathol 2000;9:111-7. https://doi.org/10.1016/s1054-8807(00)00027-2

16. Vasconcelos RH, Azevedo E de AN, Diniz GT , Cavalcanti M da GA de M , Oliveira de W Jr , Morais C N L, et al. Interleukin-10 and tumour necrosis factor-alpha serum levels in chronic Chagas disease patients. Parasite Immunol 2015;37:376-9. https://doi.org/10.1111/pim.12183

17. Sousa GR, Gomes JA, Fares RC, Damásio MP, Chaves AT, Ferreira KS, et al. Plasma Cytokine Expression Is Associated with Cardiac Morbidity in Chagas Disease. PLoS One. 2014; 9: e87082. https://doi.org/10.1371/journal.pone.0087082

18. Tosello Boari J, Araujo Furlan CL, Fiocca Vernengo F, Rodríguez C, Ramello MC, Amezcua Vesely MC, et al. IL-17RA-Signaling Modulates CD8+ T Cell Survival and Exhaustion During Trypanosoma cruzi Infection. Front Immunol 2018; 9:2347. https://doi.org/10.3389/fimmu.2018.02347. eCollection 2018