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Chagas disease / COVID-19 comorbidity. An advantage to chagasic patients?

Néstor Añez[1*], Gladys Crisante[1], Siham Salmen[2], Cesar Paredes[3], Henry Parada[4]

[1]Investigaciones Parasitológicas "J.F. Torrealba", Facultad de Ciencias, Universidad de Los Andes, Mérida, 5101, Venezuela; [2] Instituto de Inmunología, Facultad de Medicina, Universidad de Los Andes, Mérida, 5101, Venezuela; [3] Hospital Central de Sabaneta, Ministerio de Salud, Barinas, Venezuela; [4] Unidad de Cardiología, Hospital "Luis Razetti", Ministerio de Salud, Barinas, Venezuela.

RESUMEN

Comorbilidad enfermedad de Chagas-COVID-19. ¿Ventaja para pacientes chagásicos?

Introducción. La enfermedad de Chagas es una dolencia desatendida y debilitante, causada por la infección con *Trypanosoma cruzi* que afecta millones de pobladores humanos en los países de América latina. En la misma región han sido registrados, durante los últimos años, frecuentes y severos casos de infección viral causados por *SARS-CoV-2*, agente etiológico de COVID-19, considerada la peor pandemia global del siglo XXI.

Objetivo. Investigar la relación enfermedad de Chagas-COVID-19, y el efecto de la comorbilidad en pacientes chagásicos en comunidades donde *SARS-CoV-2* ha circulado durante los dos últimos años, causando casos severos y muertes en la población.

Materiales y métodos. Un grupo de 50 pacientes chagásicos provenientes de localidades rurales del occidente de Venezuela, afectadas por la pandemia de COVID-19, seleccionados al azar, fueron evaluados con el propósito de saber si habían sufrido la infección por *SARS-Cov-2*. Los pacientes chagásicos fueron comparados clínicamente con individuos no-chagásicos (N=22) quienes habían sufrido COVID-19 en las mismas comunidades.

Resultados. La infección por *SARS-CoV-2* en pacientes chagásicos provocó efectos significativamente menos agresivos que en pacientes no-chagásicos ($p<0.05$). Mientras que en pacientes chagásicos el COVID-19 cursó con atenuados perfiles clínicos de escasos y leves síntomas de corta duración, sin complicaciones posteriores, en controles no-chagásicos infectados con COVID-19 fueron detectados 45% y 13% de casos severos y fatales, respectivamente.

Conclusión. En pacientes chagásicos con comorbilidad por COVID-19, pareciera expresarse una robusta respuesta inmune (Th1/Th2/Th17), la cual en combinación con la acción de anticuerpos circulantes anti-glicoproteinas

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*Autor para correspondencia: Néstor Añez, Investigaciones Parasitológicas "J.F. Torrealba"; Departamento de Biología, Facultad de Ciencias, Universidad de Los Andes, Mérida, 5101, Venezuela
Tel.: +58 414 717 4627
ORCID: 0000-0003-4758-2573
E-mail: noar1510@gmail.com
https://revistabiomedica.mx.

de *T. cruzi* dirigidas contra ciertas glicoproteínas de membrana y/o de espículas de *SARS-CoV-2,* podría inducir perfiles clínicos de leve sintomatología y corta duración. Se recomiendan más investigaciones en poblaciones donde la enfermedad de Chagas es endémica.

ABSTRACT

Introduction. Chagas's disease a neglected and debilitating tropical illness, caused by *Trypanosoma cruzi*-infection, afflicts millions of people in most Latin-American countries. In the same region, frequent and severe viral infection cases have been reported due to *SARS-CoV-2*, the causative agent of COVID-19, deemed as the worst global pandemic in the 21st century.

Objective. To investigate the Chagas disease-COVID-19-relationship, and the comorbidity effect on chagasic patients living in communities where *SARS-CoV-2* have circulated during the last two years causing severe cases and deaths.

Material and methods. Randomly selected chagasic patients (N=50) from rural localities of western Venezuela, where COVID-19 has occurred, were evaluated in order to know whether they had suffered *SARS-CoV-2*- infection. COVID-infected chagasic patients were clinically compared with non-chagasic individuals (N=22) who had suffered the viruses in the same localities.

Results. *SARS-CoV-2*-infection caused significantly less aggressive effects in chagasic patients than in non-chagasic patients, evidenced by the short-lasting scarce mild symptoms and the attenuated clinical profile ($p<0.05$), without further complications. Comparison revealed COVID-19 detection in 10% of chagasic patients, showing all of them mild clinical pattern and few symptoms, while non-chagasic COVID-infected control individuals, showed 45% and 13% severe and fatal cases, respectively.

Conclusion. Chagasic patients suffering from COVID-19 comorbidity seem to express a robust immune response (Th1/Th2/Th17), which associated with anti-*T. cruzi*-glycoproteins circulating antibodies, directed to certain *SARS-CoV-2* glycoproteins (membrane/spike), may induce a short lasting and milder clinical profile. More investigations in populations where Chagas disease is endemic, is recommended.

INTRODUCTION

Chagas's disease, a neglected and debilitating tropical illness, caused by *Trypanosoma cruzi*-infection, afflicts millions of people in most Latin-American countries. This vector-borne parasitic-infection represents a complex public health problem due to the lack of vaccines and effective treatments. Additionally, *T. cruzi* is a paninfective parasite able to colonize several organs and tissues, affecting the cardiovascular, respiratory, nervous, and digestive systems (1).

Coronavirus Disease 2019 (COVID-19) generates a complex clinical profile, which can be asymptomatic or, it may cause symptoms similar to those of the common cold, such as fever, cough, myalgia, fatigue, headache, and diarrhea, which can progress to dyspnea, severe respiratory distress syndrome, acute cardiac injury, and even death (2-8). Additionally, olfactory and taste disorder (i.e., anosmia and ageusia) seem to be two particular symptoms associated with COVID-19 (9, 10).

Previous reports suggest that effects on the human host caused by *SARS CoV-2* infection is due, among other, to the virus binding to angiotensin-converting enzyme (ACE2)-receptors. This molecular binding has also been detected in Chagas disease pathology and cardiopathies correlated with the level of ACE2-receptors found in perivascular pericytes, pneumocytes, macrophages, cardiomyocytes, capillary endothelial, and capillaries, where they play an essential role in microcirculation causing inflammatory response, and consequently, inducing endothelial dysfunction and frequent myocardial damage (2, 11, 12).

Herein, we report observations carried out in individuals, randomly selected from a cohort of patients who had suffered different Chagas disease (acute, chronic and subclinical) phases. Both, chagasic patients and non-chagasic-COVID-infected control individuals, were recruited from localities where *SARS-CoV-2* had circulated during the last two years, causing symptoms of various

severity and deaths. The aim of the present study was to investigate the Chagas disease-COVID-19-relationship, and the comorbidity's effect on chagasic patients.

MATERIALS AND METHODS

Fifty *Trypanosoma cruzi*-infected individuals were selected, by simple random sampling, from a cohort made up of 240 chagasic patients (20%) previously diagnosed by clinical, serological, parasitological, and molecular methods during the period 1989-2017. The patients were diagnosed based on acute (N=25), chronic (N=6), or subclinical (N=19) Chagas disease phases, using criteria previously established (13-15).

Additionally, to compare with the above indicated selected chagasic patients, 22 non-chagasic individuals, previously diagnosed as suffering COVID-19, were included as control after recording their symptomatic profile and clinical conditions. Both, chagasic and control individuals came from same rural localities of Barinas, Merida and Portuguesa states in western Venezuela, where Chagas disease is endemic and COVID-19 occurred.

The selected chagasic patients were evaluated in order to know whether they had suffered infection by *SARS-CoV-2*. After selection, the patient's serological status was confirmed considering the last recorded evaluation. This consisted of detecting the specific *T. cruzi*-antibody (Ab) titers by using the Direct Agglutination Test (DAT) and Immunofluorescent Antibody Test (IFAT), both by polyvalent and specific IFAT-IgM/IgG levels, respectively. Details on protocols for the detection of circulating Abs have been previously reported (14,16).

Once corroborated the chagasic patients' serological status, they were grouped by locality/state, and then evaluated to find out: **i.** whether they were aware of the presence of people positive to COVID-19 in the community. **ii.** if they were living (family situation) or had been in contact (community situation) with people positive to SARS-CoV-2 infection. **iii.** if they had suffered similar symptoms. **iv.** if they admitted to having shown symptoms, they were asked to define if they caused mild, moderate, or severe conditions, and **v.** they were asked to estimate the symptoms' lasting time.

All patients, chagasic and symptomatic COVID-control, were then classified for COVID-19 affliction according to Yuki *et al*. (17) as follows: **i.** Mild, with upper respiratory tract symptoms (fever, fatigue, myalgia, cough, sore throat, runny nose, sneezing or digestive symptoms). **ii.** Moderate respiratory, showing fever, cough, hypoxemia and positive chest imaging and **iii.** Patients with severe respiratory, hypoxemia, and systemic complications, including those with severe pneumonia, heart failure, cardiovascular shock, encephalopathy and acute kidney injury.

During the study, the chagasic patients were evaluated twice (two months apart) using the same methodology as detailed above.

Statistical analysis to compare number of symptoms, lasting time and clinical conditions between chagasic and non-chagasic patients afflicted by COVID-19, was carried out using the U-Mann-Whitney test (18). In addition, to establish differences between affliction by gender, a Chi-square test was used. To compare the elapsed time chagasic patients had suffered *T. cruzi*-infection before getting SARS-CoV-2-infection, the Fisher exact test was used.

ETHICAL CONSIDERATION

This study was approved by the ethical committees of "Luis Razetti" General Hospital, Barinas, "J A Camacho" Hospital, Sabaneta, Barinas, and Hospital Universitario de Los Andes, Mérida, Ministry of Health, Venezuela, and by the Research Council of Universidad de Los Andes, Mérida, Venezuela. A written consent was obtained from each of the patients, and their representative in case of children, in order to comply with the criteria established by the Biomedical Committee of the National Research Council of Venezuela.

RESULTS

From the total selected chagasic individuals, 27 (54%) were females and 23 (46%) males, with a ratio of 1♀:0.85♂. The current average age for the time the study was carried out showed 44±19 years (range 8-82 years). The females showed an average of 45±18 years (range 11-82 years) and males 43±19 years (range 8-78 years). The time of primary diagnosis for *T. cruzi*-infection ranged from 32 to 5 years, for patients diagnosed in1989 and 2017, respectively.

All the chagasic patients included in the study showed specific anti-*T. cruzi* Abs. This was demonstrated by serologic titers detected by DAT and IFAT including both polyvalent and IgM/IgG specific for *T. cruzi*. The 50 selected chagasic patients, 31 symptomatics and 19 asymptomatics, were diagnosed when suffered the acute phase (50%), the chronic phase (12%) and the subclinical inapparent infection (38%) recognized for the chagasic infection (14). Fifty percent of them received treatment either with benznidazole (42%) or nifurtimox (8%) and the remaining 50% did not receive treatment at all.

From the 50 chagasic patients included in the study, the presence of SARS-CoV-2 infection was detected in 5 (10%) of them (2♂ and 3♀; 4 [8%] in Barinas and 1[2%] in Merida state) who had been treated with benznidazole-recommended dose (5mg/kg/day for 60 days).

The SARS-Cov-2 infection among the selected chagasic patients was detected at local health centers. They were diagnosed: **i.** clinically, considering specific symptoms (i.e., fever, myalgia, anosmia, ageusia) recognized for COVID-19, thoracic X rays for those referring cough or throat pain, c-reactive protein level and, in some cases, specific PCR assay. **ii.** Epidemiologically, taking into consideration the presence of symptomatic members in the same family and/or the contact with infected people in the same community.

Clinical patterns were recorded as mild showing, in general, symptoms as fever-anosmia, in all the five infected individuals and, fever-anosmia-myalgia or fever-anosmia-ageusia in four of them, lasting 1 week in two patients and 2 weeks in the other three. All the 5 cases were described as attenuated when compared with control people who had not suffered Chagas disease and got SARS-CoV-2 infection in the same communities. The mild condition allowed them to fulfill their respective activities after a short period without any physical or mental inconvenience. Details on the compared clinical patterns between chagasic and non-chagasic patients afflicted by COVID-19 are summarized in Table 1.

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Table 1. Clinical comparison between chagasic and non-chagasic patients infected with COVID-19

	Variables	Chagasic patients	Non-chagasic patients	*p* value
Baseline characteristics	Patient N°	50	22	
	N° (%) infected	5 (10)	22 (100)	
	X±SD Age	44±19	45±17	0.553
	Gender	3♀-2♂	12♀-10♂	0.825
	Diagnostic	Clinical-PCR	Clinical 5 PCR 12 Clinical-PCR 5	
COVID-19 general detected Symptoms N° (%)	Fever	5 (100)	17 (77)	
	Headache		15 (68)	
	Cough		17 (77)	
	Thoracic pain		12 (54)	
	Myalgia	4 (80)	15 (68)	
	Malaise		22 (100)	
	Arthralgia		16 (72)	
	Anosmia	5 (100)	19 (86)	
	Ageusia	4 (80)	19 (86)	
	Dyspnea		12 (54)	
	Pneumonia		8 (36)	
	Confusion		1 (45)	
	Heart failure		4 (18)	
	O2 saturation	95	80	
Average symptoms		3.60	7.40	0.000*
Average Lasting time (Weeks)		1.6	3.4	0.004*
Patients clinical condition N° (%)		Mild: 5 (100)	Mild: 2 (9.1) Moderate: 7 (31.8) Severe: 10 (45.5) Fatal: 3 (13.6)	

***: Significant differences between chagasic and non-chagasic SARS-CoV-2 infected patients**

Regarding the elapse time before chagasic patients were afflicted by COVID-19, two of them got SARS-CoV-2 infection, 7 and 5 years after diagnosed with *T. cruzi*-infection, one from Barinas detected in 2014 and another from Merida state in 2016, both suffering the acute phase of Chagas disease. The remaining three patients were diagnosed in 1989, 1994 and 1997 in the Barinas state, also suffering the acute phase of Chagas disease. Additionally, the 5 chagasic patients who suffered COVID-19, showed in previous clinical diagnostic heart failures (abnormal EKG/ECO, frequent arrhythmia or pericardial effusion) as well as common symptoms of Chagas disease like fever, myalgia, headache, and Romaña's sign or facial edema in those patients infected by vectorial and oral routes respectively (19, 20).

Statistical analysis using the Fisher exact test, revealed no significant differences (p-value=0.6092; 95% CI) between the elapsed time chagasic patients had suffered *T. cruzi*-infection before getting SARS-

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CoV-2-infection. However, the comparison of the number of symptoms, and lasting time for recovering of SARS-CoV-2-infection, between chagasic and non-chagasic control, showed significant differences when U-Mann-Whitney test was applied (p-value=0.000; 95% CI; p-value=0.004; 95% CI, respectively). Details on the statistical analysis and its significance are summarized in the boxplot showed in Figure 1.

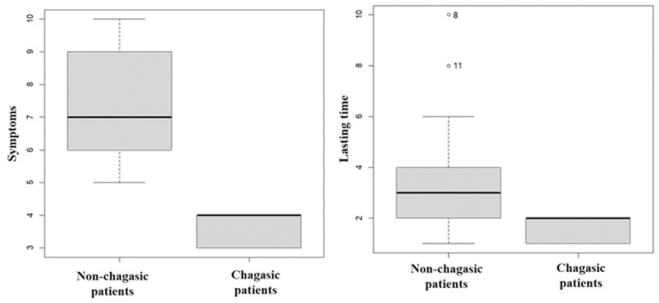

Figure. 1. Boxplot showing statistical comparison of symptoms and lasting time between chagasic and non-chagasic patients afflicted by COVID-19.

DISCUSSION

We report the detection of SARS-CoV-2 infection in patients who had suffered Chagas disease 5 to 32 years previous to the spread of COVID-19 pandemic. From the 50 chagasic patients selected for the study, 5 (10%) were mildly afflicted by the viral illness.

The diagnosis of COVID-19 in chagasic patients was established on clinical presentation of the scarce symptoms, including fever and anosmia in all of them; fever, anosmia, ageusia and myalgia in three, and fever, anosmia and ageusia in one, lasting 1-2 weeks without further clinical complications.

The few mild symptoms detected in the studied chagasic patients, resulted contrary to the expected severe clinical condition referred to SARS-CoV-2 infection, which has been hypothesized as cause of devastating distress in infected people. Such a hypothesis has been explained as a possible consequence of an immunocompromised condition suggested as a synergistic action of COVID-19 on chagasic patients (11, 21). However, although this explanation sounds reasonable from the theoretical point of view, it was only a hypothesis and no previous study has tested it using patient data (22). In addition, the here obtained results did not show evidence to support the hypothesis, neither in the number nor in the severity of COVID-19 in Chagas disease-infected individuals.

In relation to the scarce number of symptoms detected in the few chagasic patients infected with *SARS-CoV2*, we have not a clear explanation yet. However, it should be possible that the robust immunity generated in the human host by *T. cruzi*-infection, although it does not prevent *SARS-CoV2*-infection, it may limit virus density and severity of symptoms.

In this respect, 90% (45/50) of the here studied proven chagasic patients did not show infection at all, and/or if so, they were undetected, asymptomatic or subclinical cases. This fact seems to be supported by previous reports showing no significant differences and/or trends of worsening clinical profiles in chagasic patients due to *SARS-CoV-2*-infection (22).

On the other hand, more elements must be considered to explain the *SARS-CoV-2 / T. cruzi*-coinfection relationship, regarding present outcomes of COVID-19 in a group of chagasic patients compared with non-chagasic control from the same localities.

At risk to be speculative to answer the many questions surged from the Chagas disease-COVID-19 comorbidity, whose relationships are not fully understood yet, the following comments are proposed to explain the present results:

i. *T. cruzi* fate in the human host depends upon a balanced immune response (Th1/Th2), which allows to control parasite spread without compromising host tissue integrity. This robust immunity against *T. cruzi* is provoked by the concerted action of macrophages, neutrophils, dendritic cells, CD4+ and CD8+T cells and B cells.

ii. The above response may be reinforced for a similar function attributable to Th17 cells (23).

Consequently, a high frequency of IL-producing cells should be found in patients with Chagas disease who show milder disease correlated with a high expression of IL-17.

iii. From the observed behavior in chronic chagasic patients infected with COVID-19, it is possible to speculate the preexisting of a microenvironment favoring the Th17 response in chagasic patients, modulating effects against *SARS-CoV2* virus. In addition to the immune response, other factors such as cardiac remodeling or even the medication the patients receive should have a favorable effect on *T. cruzi*-infected individuals exposed to the virus.

iv. One more characteristic of *T. cruzi* that might help to support present results is its great surface antigenic variation, allowing the host to produce Abs with different specificities. Such an activity should be attributed, in part, to the presence of *T. cruzi*-membrane glycoproteins, a potential antigenic component able to mediate in the expression of surface proteins with the capability to induce immunogenicity, an activity also demonstrated in *Trypanosoma rangeli* and *Leishmania braziliensis* (24-30).

In this respect, we have previously demonstrated that individuals who have suffered Chagas disease, have circulating antibodies (IgG/IgM) against specific *T. cruzi* antigens. These Abs are directed to Glycosyl-phosphatidyl-inositol (GPI), the anchored molecule of some membrane proteins of *T. cruzi*, expressed in individuals with acute, chronic and inapparent infections (31).

GPI is a phospholipid which activates leukocytes and the release of proinflammatory cytokines inducing the expression of adhesion molecules via Toll like receptors (TLRs). Activation of TLR pathways leads to secretion of pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α), as well as type 1 interferon. Different TLRs, like TLR2 and TLR4, are potentially important in COVID-19 infection. The modulation of TLRs expressed on T and B cells has a great potential to develop adaptive immune responses to target various microbial infections with antiviral response, including current COVID-19 pandemic (32).

Regarding the latter, *SARS-CoV-2* has various glycoproteins (GPs), including membrane- GPs, spike-GPs and GPs that have acetyl esterase and hemagglutination features. These viral GPs could be identified by the anti-GPI antibodies developed in the human host during *T. cruzi*-infection, which may result in protection against virus infection or in the induction of a milder disease pattern (31).

CONCLUSION

The results obtained during the present study led us to reach the following conclusions: **i.** individuals previously infected with *T. cruzi* were comparatively less affected *by SARS-CoV-2* infection than non-chagasic control patients living in the same localities ($P<0.05$). **ii.** infection in COVID-afflicted chagasic patients showed attenuate clinical profiles consisting of short-lasting and scarce-mild symptoms, without further complications. **iii.** chagasic patients suffering COVID-19 comorbidity appear to express a long lasting-robust immune response (Th1/Th2/Th17), which in association with anti *T. cruzi*-glycoproteins circulating antibodies, directed to certain *SARS-COV-2*-GPs (membrane/spike) may induce a short lasting and milder clinical profile.

CONFLICT OF INTEREST

The authors declare they have not conflict of interest.

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