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PROGRESSIVE CHAGAS' CARDIOMYOPATHY IS ASSOCIATED WITH LOW SELENIUM LEVELS

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Abstract. Selenium (Se) deficiency is linked with some cardiomyopathies. Its status was determined in 170 patients with chronic Chagas' disease from 2 Brazilian regions (Rio de Janeiro and Belo Horizonte), clinically stratified into groups as follows: indeterminate or asymptomatic (IND); cardiac asymptomatic (CARDa); cardiac symptomatic with moderate to severe heart dysfunction (CARDb); and healthy adults (HA), used for comparison. In most HA, Se levels were normal, excluding an overall Se deficiency. Se was significantly lower in CARDb than in HA, IND, or CARDa patients. This was not associated with a concomitant decrease in activity of glutathione peroxidase. Thyrotropin was normal, excluding iodine deficiency. Se correlated positive and significantly with ventricular ejection fraction (assessed via echocardiography). Asymptomatic children with acute Chagas' disease had normal Se as well as 5 noninfectious cases of cardiomyopathy. Low Se was found in 6 of 10 chagasic patients with digestive megasyndromes. Thus, the decrease in Se in chagasic patients seems to be a biological marker for *Trypanosoma cruzi* infection and related to the progression of pathology.

INTRODUCTION

Chagas' disease (American trypanosomiasis) is caused by infection with the flagellated parasite *Trypanosoma cruzi* and continues to be a major cause of morbidity and mortality, particularly among poor population in rural and urban areas of Central and South America.[1,2] Most of the seropositive infected persons evolve to an asymptomatic form. However, 20–30% of patients develop chronic chagasic cardiomyopathy, a pathology not yet well understood.[3–5] Clinical groups can be clearly distinguished: indeterminate or asymptomatic patients, with normal or with only slight alterations in electrocardiogram and normal radiological chest appearance; and patients with cardiopathy, with different and progressive levels of ventricular dysfunction and arrhythmias. Some patients show only electrical alterations, seen as abnormal electrocardiogram, with disturbances in the conduction system or ventricular arrhythmias, but without evidence of ventricular dysfunction, congestive heart failure, or cardiomegaly. Moderate or severe cardiopathy cases demonstrate abnormal electrocardiogram and congestive heart insufficiency associated with cardiomegaly, seen as abnormal via radiograph or echocardiography.[6] Histopathological findings in people with ongoing cardiomyopathy revealed that a mononuclear infiltration is associated with parasite antigens, characterizing myocarditis, which is associated with microvascular alterations and ventricular remodeling with fibrosis.[3]

In endemic areas, the population is also affected by malnutrition.[7] Few studies have been performed that specifically point to the effect of nutrition on the course of human infection with *T. cruzi.*[7] However, information about the role of dietary factors in disease prevention has been the focus of a growing number of studies.[8,9] Nutrition has a profound influence on the immune system, and nutritional deficiencies can impair immune responsiveness and lead to a decrease in the development of resistance to infection, in both animals and humans.[10–12]

Selenium (Se), a trace element, is an essential micronutrient for organisms ranging from bacteria to humans. Its main role is to function as an antioxidant at the cellular level, providing protection against free radical damage and oxidative stress.[13–15] One of the best-characterized roles of Se in mammalian systems is its incorporation into the active site of the different isoforms of the enzyme glutathione peroxidase (GPx).[16] Several studies have shown that plasma or erythrocyte GPx activity may serve as a useful index of Se nutritional status.[12] However, other metabolic functions for Se exist, and other Se-containing enzymes have been well characterized, such as the iodothyronine deiodinases converting the prohormone thyroxine to the active metabolite triiodothyronine in peripheral tissues, selenoprotein P, and thioredoxin reductase.[17–19]

Selenium deficiency has been implicated as a contributing factor in some cases of congestive cardiomyopathy and increased cardiovascular complications, including myocardial infarction.[20] In China, such deficiency has been involved in the pathogenesis of Keshan disease, a well-described cardiomyopathy.[21,22] In many regions, Se deficiency is associated with iodine (I) deficiency, indicated by a increase in serum thyrotropin levels that has been used as a biological marker.[23] Combined Se and I deficiencies have been implicated in the etiology of Kashin-Beck osteoarthropathy, endemic in rural parts of the People's Republic of China, including the Tibet Autonomous Region.[12]

The purpose of this study was to investigate whether Se status could be involved as a risk factor for the clinical severity of Chagas' cardiomyopathy as a result of nutritional deficiency of Se or to altered Se metabolism. Se status was measured in chronic chagasic patients, in healthy reference adults, and in children during the acute phase of *T. cruzi* infection, and confirmed the hypothesis that chronic Chagas' cardiomyopathy is associated with a decrease in Se.

MATERIALS AND METHODS

Study design and subjects. *Chronic chagasic patients.* Blood samples from 170 *T. cruzi*–infected people were obtained from patients during clinical survey at two different Brazilian

regional centers of the Oswaldo Cruz Foundation (Fiocruz) in two important urban areas: in the city of Rio de Janeiro (Rio) at the Centro de Pesquisas Hospital Evandro Chagas, and in the city of Belo Horizonte (BH) at the University Hospital of the Federal University of Minas Gerais. These patients originated from diverse endemic areas of Brazil but had been living in Rio or BH (nonendemic areas for Chagas' disease) for at least 10 years, and all had confirmed positive serology for this disease. Blood sample collection was voluntary, and the patients provided written informed consent for their participation, according to the official Fiocruz ethics committee rules (Brazilian Ministry of Health); the Brazilian Ministry of Health had also previously approved the study.

The study subjects in the Rio group were composed of 122 chronic chagasic patients, 67 men and 65 women, with ages ranging 23–80 years (values expressed as mean ± standard deviation; 49 ± 12 years). In BH, the group was composed of 48 chronic patients (31 men, 17 women, aged 24–68 years; 43 ± 10 years). The samples of chronic chagasic patients coming from the two cities were analyzed separately because regional differences in Se levels were reported for animal feed and in animals consumed by humans.[24] To our knowledge, no previous studies have been conducted on the normal range of Se reference serum levels in the Brazilian population in different regions.

The individuals were stratified into three groups according to the severity of Chagas' disease, as assessed by clinical examination, electrocardiography (ECG), and echocardiography: a group comprising members with an indeterminate or asymptomatic form of the disease (IND; Rio: n = 32, mean ventricular ejection fraction [VEF] = 68 ± 5%; BH: n = 23, mean VEF = 63 ± 8%), corresponding to functional class I indicated by the New York Heart Association (NYHA); cardiac patients with ECG alterations and slight or no heart dysfunction, corresponding to NYHA functional class II (CARDa; Rio: n = 50, mean VEF = 68 ± 7%, BH: n = 7, mean VEF = 61 ± 8%); cardiac patients with ECG alterations, echocardiography alterations, or both and moderate or severe heart dysfunction, corresponding to NYHA functional classes III–IV (CARDb; Rio: n = 40, mean VEF = 39 ± 12%, BH: n = 18, mean = 37 ± 12%).

Reference healthy adults. For noninfected persons, the samples were taken from healthy adult (HA) members of the staff at Fiocruz in Rio (n = 16, 9 men, 7 women, aged 33 ± 8 years) and in BH (n = 16, 7 men, 9 women, aged 39 ± 12 years).

Other comparative groups. A group composed of chagasic Brazilian patients without cardiomyopathy but displaying the digestive form of Chagas' disease (megasyndromes, or MS; n = 10, 6 men, 4 women, aged 57 ± 10 years) was also studied. An additional group composed of patients with noninfectious cardiomyopathy (NIC) (n = 5, all men, aged 53 ± 13 years), obtained both from Belgium and Brazilian patients, was also included.

Acute cases. Plasma samples from *T. cruzi*–infected (n = 22) acute asymptomatic children and noninfected (n = 14) children aged 2 to 15 years were studied to ascertain acute Chagas' disease cases (18 girls and 18 boys). A study that used a larger panel of those samples has been published, showing that patients were all asymptomatic, with normal electrocardiogram.[25]

Laboratory measurements. Serum or plasma samples were obtained for measurement of Se, thyrotropin, and GPx activity. The samples were kept frozen until analysis. Se levels were determined in all the samples. As a result of marked decrease of enzymatic activity with increasing temperature of storage, we took the methodological precaution for measurement of serum GPx only in samples submitted to rapid freezing and storage at temperatures lower than –20°C.[26] Samples subjected to a first defreezing for Se measurement were not used for measurement of GPx activity. Serum Se was measured by atomic absorption spectrometry with the Zeeman background correction (model Z3030, Perkin-Elmer, Uberlingen, Germany) with a limit of detection of 5 ng/mL (64 nmol/L). GPx activity was measured spectrophotometrically (λ = 340 nm) by the decrease in nicotinamide-adenine dinucleotide phosphate (0.28 mmol/L) at 37°C on a biochemical analyzer (Hitachi 717, Boehringer Mannheim, Mannheim, Germany), with aromatic organic peroxide (isopropylbenzene [cumene] hydroperoxide; final concentration, 018 nmol/L) and glutathione (final concentration, 4 mmol/L) as substrates in 0.05 mol/L of phosphate buffer (pH, 7.2) and 4.3 mmol/L of ethylene diamine tetraacetic acid in the presence of excess glutathione reductase (≥ 0.05 U/L). The limit of detection of serum GPx was 50 U/L. Serum thyrotropin was measured with the use of an automated immunoassay with chemiluminescence detection (ACS 180; Corning, Los Angeles, CA) and commercial reagents. The limit of detection of serum thyrotropin was 0.01 mU/L.

Anti-galactosyl immunoglobulin G immunoreactivity. Levels of immunoglobulin (Ig) G anti-galactosyl (Gal) α1-3 Gal epitopes were measured by enzyme-linked immunoassay by using mouse laminin as a capture antigen and by using sera diluted 1:10, 100, 500, and 1,000, as described in detail elsewhere.[25]

Statistical analysis. The results for serum Se, thyrotropin, and GPx were analyzed by Kruskall-Wallis test. Frequency distribution differences were assessed by Kolmogorov-Smirnov test. Correlation studies were performed by the Pearson product moment test. Statistical significance was defined as $P < 0.05$.

RESULTS

Selenium status was determined by measuring serum Se concentration and serum GPx activity in adult Brazilian subjects affected by Chagas' disease and in adult Brazilian reference samples in the same age range (Table 1). Patients from Rio (Table 1, Rio) in Groups IND and CARDa had a Se status identical to the one of healthy reference patients. In patients with chronic disease (CARDb), the Se concentration was significantly lower than in HA (65 ng/mL versus 72 ng/mL; $P < 0.01$) and than in patients from IND and CARDa (Figure 1A). This significantly lower Se level of CARDb patients was not related to an effect of age because the mean ages of groups CARDa and CARDb were similar. However, even these low levels were considered within the normal range (50–120 ng/mL) and were not followed by a parallel decrease of serum GPx activity (Table 1, Rio), excluding endemic Se deficiency, at least in Rio. No case of iodine deficiency or associated hypothyroidism (serum thyrotropin would be > 10 mU/L) was observed in the 39 chagasic patients studied for serum thyrotropin (Table 1, Rio).

The finding of statistical significance of low Se levels in the

TABLE 1
Levels of serum selenium concentration (Se), glutathione peroxidase activity (GPx), and thyroid-stimulating hormone concentration (TSH) during the progression of chagasic cardiomyopathy*

Origin	Group	Age (y)	Se (ng/mL)	GPx (U/L)	TSH (mU/L)
Rio	HA	33 ± 8, 30 (15)	72 ± 10, 70 (16)	870 ± 140, 884 (16)	2.9 ± 2.9, 1.9 (10)
Rio	CHA	48 ± 12, 48 (118)	70 ± 16, 68 (122)	958 ± 244, 908 (35)	2 ± 1, 1.9 (39)
Rio	IND	44 ± 13, 42 (32)	70 ± 13, 71 (32)	847 ± 144, 827 (3)	1.9 ± 1.0, 1.8 (19)
Rio	CARDa	48 ± 13, 51 (48)	73 ± 15, 73 (50)	970 ± 285, 900 (20)	2.1 ± 1.1, 1.2 (12)
Rio	CARDb	52 ± 10, 52 (38)	65 ± 19, 62 (40)†,‡	964 ± 190, 950 (12)	2.1 ± 1.1, 2.4 (8)
BH	HA	39 ± 12 (10), 24–60	55 ± 10 (16), 39–72	ND	ND
BH	CHA	43 ± 10 (48), 24–68	43 ± 11 (48), 23–65†	ND	ND
BH	IND	41 ± 8 (23), 24–52	46 ± 9 (23), 24–63†	ND	ND
BH	CARDa	40 ± 10 (7), 24–51	42 ± 15 (7), 23–65†	ND	ND
BH	CARDb	46 ± 12 (18), 24–68	39 ± 10 (18), 25–62†	ND	ND

* The normal ranges of the values are as follows: Se, 50–120 ng/mL; GPx, 550–1,100 U/L; TSH, 0.3–5.0 mU/L. Data for Rio de Janeiro are presented as mean ± standard deviation, median (n); data for Belo Horizonte are presented as mean ± standard deviation (n), minimum-maximum. For origin, BH = Belo Horizonte; Rio = Rio de Janeiro. For group, CARDa = cardiac asymptomatic; CARDb = cardiac symptomatic with moderate to severe heart dysfunction; CHA = all chagasic patients; HA = healthy adults; IND = indeterminate or symptomatic. ND = not done.
† $P < 0.05$ as compared with healthy adults or to IND.
‡ $P < 0.01$ versus CARDa.

CARDb group, but remaining within the normal range, led us to search for confirmation of these results in samples from another geographic region. Chronic patients followed up clinically at BH were thus also studied (Table 1, BH). Selenium levels in the control healthy population (HA) for this sample were significantly lower (mean, 55 ng/mL) than those observed in the HA group at Rio (mean, 72 ng/mL). Four (40%) of the 10 healthy adults had Se levels below the minimum of the reference normal range (50 ng/mL), thus indicating that this group of samples could not be analyzed altogether with the group of samples from Rio (Table 1, Rio).

In the group of patients from BH, comparison of Se levels in chagasic patients and healthy controls indicated a significant reduction in the former groups, with the mean value dropping from 55 to 43 ng/mL (Table 1). All clinical groups displayed mean Se values that were significantly lower than those of healthy controls (Figure 1F). As already observed in the patients from Rio, the group of chagasic patients with moderate to severe disease showed a statistically significant more pronounced reduction in Se levels than patients in the indeterminate or asymptomatic stage (Table 1, BH, and Figure 1F), dropping from a mean of 46 to 39 ng/mL.

Because in both geographical regions we observed a significant decrease of Se in patients from CARDb as compared with HA or IND (Table 1 and Figure 1A,F), but with a relative heterogeneity observed among individuals, we carried out a study of frequency distribution of patients with different levels of Se. In patients from Rio (Figure 1B–E), there was a shift toward lower Se concentrations in the distribution curve of the chronic chagasic CARDb patients (Figure 1C), whereas in the control HA group (Figure 1B) and in the chronic IND (Figure 1E) and CARDa (Figure 1D) groups, we found few or no patients with Se concentrations ranging 40–60 ng/mL. Kolmogorov-Smirnov statistical test indicated that a significant frequency of cases in CARDb (Figure 1C) had low levels of Se. Se concentrations lower than 50 ng/mL were not observed in the healthy adults and were observed in 3% (1 of 32) of IND patients, in 4% (2 of 50) of CARDa patients, and in 12.5% (5 of 40) of CARDb patients ($P < 0.05$ versus HA or IND or CARDa).

The study of frequency distribution of patients from BH (Figure 1G–J), despite their different reference Se levels (Figures 1F, 2G), also clearly showed the progressive shift toward the lower Se levels in the curves obtained from the group of patients with the more severe forms of chronic Chagas' disease. The percentage of cases below the normal reference range (< 50 ng/mL) in the samples from BH increased from 40% (4 of 10) in HA patients (Figure 1G) to 56.5% (13 of 23) in the IND group (Figure 1J), 71% (5 of 7) in the CARDa group (Figure 1I), and 89% (16 of 18) in the CARDb group (Figure 1H). Kolmogorov-Smirnov test confirmed the statistically significant differences in frequency of patients with low Se levels in the CARDb group, as compared with HA and IND groups.

Selenium decrease in a significant proportion of patients undergoing active cardiomyopathy in two different Brazilian regions led us to investigate if this feature was associated more specifically with *T. cruzi* infection or with the cardiomyopathy. When sera samples from NIC patients were analyzed, a significant decrease in Se levels was not observed (Table 2). On the other hand, chagasic patients with the digestive form of Chagas' disease (with clinically assessed MS) but without cardiomyopathy also presented a decrease in Se levels (Table 2), indicating a possible association of Se decrease with *T. cruzi* long-standing infection and the consequent cardiac or digestive pathology. The result obtained with MS patients was also significantly different from HA and from patients in CARDa, but not from CARDb. In the MS group, this decrease of serum Se was accompanied by a parallel decrease of serum GPx activity.

The finding of low Se levels in symptomatic chagasic chronic patients (in both CARDb and in MS groups) interested us, so we tested whether acute infected patients would also display a decrease in Se. Because acute cases are no longer observed in Brazil as a result of vector control, the analysis was carried out in Bolivian children, where the acute form of the disease is still frequent.[27] Acutely infected children were studied and compared with their own age- and region-matched noninfected reference cases (Table 2). They were all clinically asymptomatic, and the etiological diagnostic was made by serology, as described previously.[25] Both had a mean serum Se concentration within the normal range (50–120 ng/mL), and no significant differences were observed. Serum GPx activity was similar and at the normal levels in both groups.

To determine whether the decrease in Se was associated to

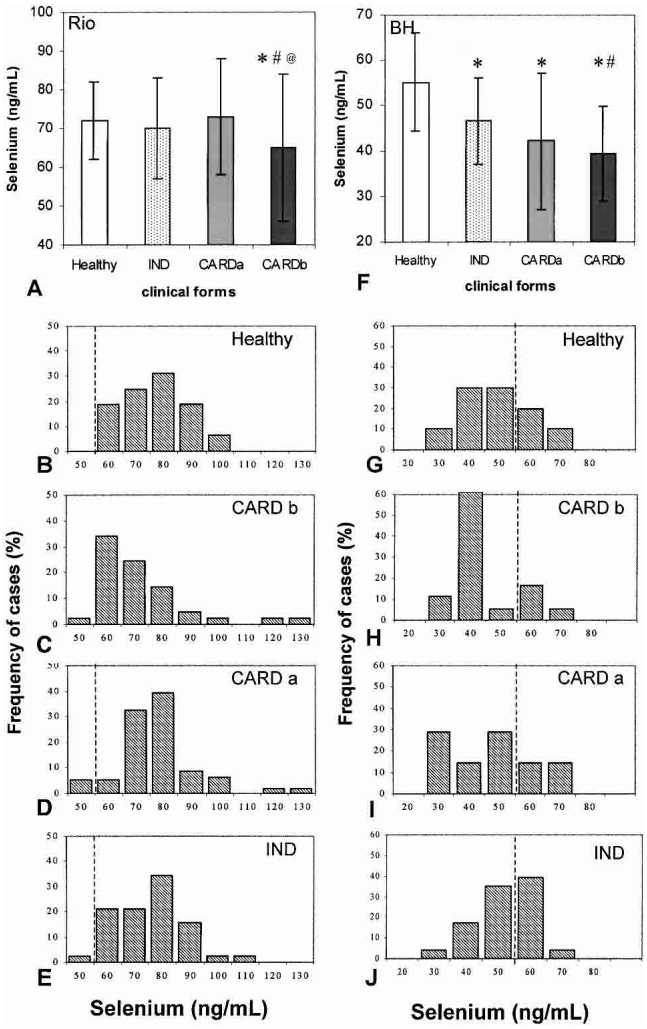

FIGURE 1. Selenium levels **(A, F)** and frequency distribution **(B–E, G–J)** in chronic chagasic patients from different clinical groups living in the urban area of Rio de Janeiro **(A–E)** and Belo Horizonte **(F–J).** IND = indeterminate or asymptomatic, CARDa = cardiac asymptomatic patients, with electrocardiographic alterations and absence or only slight heart dysfunction; CARDb = patients with cardiac symptomatic with moderate or severe heart dysfunction. *$P <$ 0.05 compared with healthy adults; #$P < 0.05$ compared with IND patients; @$P < 0.05$ compared with CARDa. The frequency distribution curve in **(C)** is significantly different ($P < 0.05$) from that of **(B).** Distribution curve in **(H)** is significantly different ($P < 0.05$) from curves in **(G)** and **(J).** The minimum selenium level in the normal international range is indicated **(vertical dotted line).**

other immunological or clinical parameters, we studied the correlation between its levels and IgG anti-Gal reactivity (n = 34), which indicates the intensity of humoral response against living parasites (that express the epitope Gal α1-3 Gal), as well as the VEF (n = 116), which indicates the contractility power of the myocardium.[28,29] Anti-Gal reactivity was high in patients in the CARDa and CARDb groups as compared with patients in the IND group (Figure 2A). Although no significant differences between the CARDa and CARDb groups were observed, a slight trend for higher anti-Gal levels in the CARDb group corresponded to a decrease in both Se levels and VEF (Figure 2A). Se correlated directly and significantly to the VEF ($r = 0.31$, $P = 0.0005$, Figure 2B), but no significant correlation was observed with anti-Gal levels ($r = 0.13$, $P = 0.447$, Figure 2C).

TABLE 2

Levels of serum, selenium concentration (Se), and glutathione peroxidase activity (GPx) in chagasic children of Cochabamba, Bolivia and in reference groups*

Group	Age (y)	Se (ng/mL)	GPx (U/L)
MS	57 ± 10, 54 (10)	60 ± 12, 57 (10)†	799 ± 380, 812 (4)
NIC	53.2 ± 12.9, 59 (5)	66 ± 7, 67 (5)	833 ± 151, 887 (5)
HC	8.9 ± 3.7, 8.5 (14)	55 ± 17, 49 (6)	800 ± 190, 779 (14)
AAC	9.1 ± 3.2, 9 (19)	69 ± 12, 66 (5)	776 ± 97, 772 (19)

* The normal range of Se is 50–120 ng/mL; the normal range for GPx is 550–1,100 U/L. AAC = acute asymptomatic children; HC = healthy children; MS = megasyndromes; NIC = noninfectious cardiomyopathy. Data are presented as mean ± standard deviation, median (n).

† $P < 0.05$ as compared with healthy adults or with the indeterminate or symptomatic group and with the cardiac asymptomatic group.

DISCUSSION

To our knowledge, this is the first study on Se status determination in Chagas' disease and its relationship to the pro-

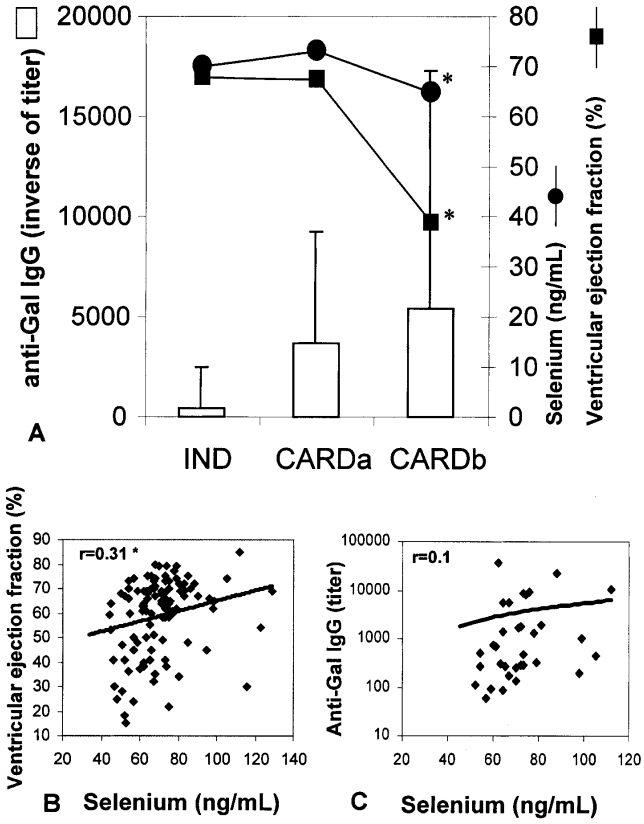

FIGURE 2. Selenium (Se) levels, anti-galactose (anti-Gal) immunoglobulin (Ig) G levels, and ventricular ejection fraction (VEF) values in chronic chagasic patients in three groups: indeterminate/asymptomatic; cardiac asymptomatic patients, with electrocardiographic alterations and absence or only slight heart dysfunction; and patients with cardiac symptomatic with moderate or severe heart dysfunction. Progression of Chagas' disease cardiomyopathy is associated to high anti-Gal IgG immunoreactivity **(A, open bars),** to the decrease in Se levels **(A, circle-linked lines)** and decrease in VEF **(A, square-linked lines).** A significant positive correlation can be observed between Se levels and VEF **(B)** and a nonsignificant correlation between Se and anti-Gal IgG titers **(C).** r = Pearson coefficient of correlation. *$P < 0.05$.

gression of the pathology. Of concern to us were, first, the absence of endemic Se deficiency in the areas studied, and second, the existence of a significant proportion of patients with moderate to severe ongoing chagasic cardiomyopathy (chronic CARDb) and of patients with MS who presented a decrease in blood Se concentration, with lower mean or median levels.

The Se supply found in the population depends on its presence in the soil and in the food chain. Its nutritional status in human populations is related to geography.[30] Both the cardiomyopathy of Keshan disease and the degenerative osteoarthropathy of Kashin-Beck disease have been associated with extremely severe Se deficiency: serum Se is < 20 ng/mL, and serum GPx is < 200 U/L.[12,21,28] An overall Se deficiency was excluded for both Brazilian chronic patients in the region of Rio and for Bolivian asymptomatic infected children from the region of Cochabamba; we excluded the notion of a deficiency because healthy subjects had a Se status similar to that observed in areas with sufficient Se. This indicated that both populations were living in Se-nondeficient areas and had a nutritional profile quite different from the one observed in Se-deficient regions of rural China. This indicates also that endemic nutritional Se deficiency is not a risk factor for chagasic cardiomyopathy in the areas of Brazil and Bolivia that we studied here.

However, a moderate Se deficiency was detected in the group of samples from BH, where 40% of the healthy subjects had Se < 50 ng/mL. In the absence of a regional reference range for Se in Brazil, we adopted the international one (50–120 ng/mL), but a review in the Brazilian veterinary literature showed that it is frequent to find animals with low Se levels in the liver, associated to myopathies and even cardiomyopathies, related to soil or food Se deficiency.[24] Because Minas Gerais state is an important endemic area for Chagas' disease in Brazil, it would be interesting if the present observations could be confirmed in studies conduced directly in patients living in other endemic areas of Central and Latin America.[31]

Historically, endemic Chagas' disease in Brazil was associated with endemic goiter by Carlos Chagas himself: he rightly observed the occurrence of both endemic diseases in the same areas.[32,33] More recently, geographical distribution of Se deficiency has been shown to overlap partly the map of iodine deficiency in Central Africa and in China.[30] Hypothyroidism was excluded in adult Brazilian patients because their serum thyrotropin concentration was normal or marginally elevated (< 10 mU/L). This excluded severe iodine deficiency.

Because geographical Se deficiency has not been observed in Rio but not necessarily in patients coming from BH, the decrease in Se levels in symptomatic chagasic patients was further explored. Usually, serum Se concentration and GPx activity evolve in parallel in case of deficiency, and there is a linear relationship between Se concentration and GPx under a threshold of serum Se at ~ 50 ng/mL; for greater Se concentrations, GPx remains at a plateau.[12] In chagasic patients, the decrease of Se levels was not associated with a concomitant decrease of GPx activity. The paradoxical decrease of serum Se concentration without parallel decrease in GPx activity confirmed that there was not a nutritional deficiency in these patients because they comprised the samples coming from Rio. It thus raised the hypothesis that it might be related to the severity of the progression of Chagas' disease pathology. Other chronic inflammatory diseases were associated with a decrease of serum Se concentration, such as acquired immunodeficiency syndrome, and also another chronic parasitic disease with intracellular parasites, cutaneous leishmaniasis.[34,35] In the latter case, in contrast with our observation, a decrease of erythrocyte GPx was also observed.[35]

Decrease of serum Se concentration is associated with cardiac infarction and with infectious cardiomyopathy, but a clear association to chronic NIC is not well established.[22,36] Because our study was designed to focus on chagasic cardiomyopathy, only a small sample of patients who experienced NIC was comparatively studied; in this group, we observed that Se concentration and GPx activity were normal. Thus, degenerative cardiomyopathy is probably not associated with alterations in Se status, and the decrease of serum Se concentration in cardiac symptomatic chagasic patients (CARDb) is one more biological marker of chronic *T. cruzi* infection and is related to progression of the pathology. This conclusion is reinforced by the finding that Se levels also decrease in chagasic patients with MS without cardiomyopathy, and also by the finding that *T. cruzi*–infected children in an asymptomatic acute phase had also normal Se. However, the low levels of Se in patients with MS could also be interpreted as a consequence of long-term reduced absorption. Therefore, we can now establish a clear association of Se decrease only with progressive heart dysfunction in Chagas' disease—not with the development of MS.

An important parameter indicating the intensity of cardiomyopathy is the VEF determined in echocardiogram analysis, which indicates the effective contraction power of the heart. The heart function is compromised by heart fibrosis in chagasic patients, who have low VEF (< 50%). We found a significant positive correlation between Se and this parameter, indicating that higher the Se level, the better the heart function. Maybe with the analysis of progression of the disease in individual patients with low Se levels over a long period of time, an inverse correlation would be found. This hypothesis remains to be tested. The decrease of Se could be associated with ongoing cardiomyopathy damage involving mononuclear infiltration linked with parasites antigens, to microvascular alterations, and to fibrosis.[3,37] We plan to follow the small percentage of patients from the IND group that had Se levels < 60 ng/mL to ascertain whether a more pronounced decrease would in fact occur, in parallel to the development of cardiac symptoms. A therapeutic approach—providing patients with Se levels below the normal range with selenate supplements—will also help to determine whether low Se is a cause or a consequence of disease severity.

Data in the literature support the notion that anti-Gal levels are high in chagasic patient with cardiomyopathy.[38] These antibodies are associated with the presence of active ongoing infection (live parasites) and with a protective immune response elicited against the parasite because anti-Gal levels decrease when chronic patients are treated with benznidazole; thus, the presence of these antibodies could be used as an indirect marker of parasite load.[39,40] We also found a higher immunoreactivity of anti-Gal IgG in patients in the chronic CARDa and CARDb groups, with a trend of higher levels in CARDb but without significant difference as related to CARDa; no significant correlation was observed between Se levels and anti-Gal immunoreactivity. It remains to be established whether there is a causal relationship between serum Se decrease and parasite load increase. Further inves-

tigations on this matter are being conducted by use of experimental models.

In conclusion, we confirmed the hypothesis that chronic Chagas' cardiomyopathy is associated with a decrease in Se, but questions regarding the causal nature of the association arose as a result of the lower Se concentrations in more severe cases of Chagas' disease. The more plausible explanation is that low Se level is a biological marker of a long-standing inflammatory process that may lead to progressive heart damage and dysfunction in chronic chagasic patients.

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