Venous Endothelial Dysfunction in Chagas' Disease Patients without Heart Failure

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OBJECTIVE

To analyze the venous endothelial function in Chagas' disease patients without heart failure.

METHODS

The Chagas' disease Group (G1) was composed by 14 women and 2 men aged $46 \pm 2,7$ and the Control Group (G0) by 7 women and 1 man matched by age, weight and height. Dorsal Hand Vein Compliance Technique was used to evaluate the venous endothelial function. Crescent doses of phenylephrine were infused to get a 70% pre-constriction of the vein; after that, acetylcholine and sodium nitroprusside were respectively administrated to analyze the endothelium-dependent and -independent venodilation.

RESULTS

No significant systemic hemodynamic changes were observed in both groups during the experiment. The necessary phenylephrine dose to reach 70% pre-constriction of the vein was significantly higher in the G1 ($1116 \pm 668,2$ ng/ml) compared to G0 (103 ± 28 ng/ml) $p = 0,05$. The endothelium-dependent venous dilation was significantly lower in G1 ($65,5 \pm 8\%$) compared to G0 (137 ± 20 %) $p = 0,009$. No difference was observed in the endothelium-independent venous dilatation between groups.

CONCLUSION

Patients with Chagas' disease without heart failure presented venous endothelial dysfunction.

KEY WORDS

Endothelium, Chagas' disease, system venous, cardiovascular disease.

Chagas disease is still a major public health problem. It is estimated that between four and six million Brazilians have the disease. It is a chronic debilitating disease that provokes premature disability and is one of the main causes of early retirement in Brazil[1,2]. Considering the clinical seriousness of its evolution and the socioeconomic impact, Chagas disease has been targeted as a study topic by numerous, renowned researchers seeking to improve the available information about the disease[3-9].

It has been clearly established that Chagas disease is extremely heterogeneous in respect to the severity of the myocardial condition. Even though significant physiopathological aspects related to the onset and progression of the Chagas cardiopathy have been established, there are still many factors that need to be clarified. Various studies on Chagas patients without heart disease have demonstrated the presence of lesions in the area of the heart's innervation and others have indicated deficiencies in the autonomic control of heart rate and blood pressure[9-12]. More recent studies have demonstrated that the reflex control of peripheral vascular reactivity is also compromised in Chagas patients with preserved ventricular function[9,12].

We have to consider that the control of peripheral tonus and reactivity does not only depend on neural control but also on adequate endothelial cell function. Endothelial dysfunction has been proposed as one of the mechanisms for the onset, maintenance and progression of various cardiovascular diseases such as hypertension, atherosclerosis and heart failure[13-15].

During the course of the Chagas disease infection, parasites have been observed in the microvascular endothelia of the aorta and coronary arteries before detection of the parasitemia[10,16,17]. Cultures of endothelial cells infected with *T. cruzi* show complex alterations in mechanisms that are critical to maintain normal vascular function[18-20]. Based on this, endothelium cell dysfunction in the coronary arteries has been determined to be one of the primary mechanisms observed in patients with Chagas disease, and could be related to the myocardial lesion.

The evaluation of endothelial function using concentrations of endothelium produced substances and inflammatory markers suggests the presence of endothelial dysfunction in Chagas patients with preserved vascular function[21-24].

There are few documented studies using endothelium-dependent vasodilation analysis to evaluate peripheral endothelial function in Chagas patients. Two studies conducted on Chagas patients without heart failure are controversial as to whether or not there is endothelial dysfunction in the peripheral artery region (brachial artery)[4,5]. There are no reports that confirm whether or not Chagas patients present venous endothelial dysfunction.

The objective of this study is to investigate whether or not Chagas patients with preserved ventricular function present venous endothelial dysfunction.

METHODS

This study was approved by the Research Ethics Committee of the São Paulo Federal University – Unifesp and the Heart Institute - InCor (Protocol 0425/02).

The individuals with Chagas disease were selected from the patients at the outpatient clinic of the Heart Institute's (InCor) Cardiomyopathy Unit of the University of São Paulo School of Medicine between February 2003 and February 2005. The endothelial function evaluation was conducted at the Laboratory of Clinical Investigation of InCor's Hypertension Unit. All study participants were briefed on the objectives of the study and signed the free and informed consent form.

All the Chagas patients had positive serological tests for Chagas disease (indirect hemaglutination and immunofluorescence) and were from the disease's endemic zone. Sixteen patients were selected based on the following inclusion criteria: did not present symptoms related to the cardiac or gastrointestinal forms of Chagas disease or any other disease; were not using medication; had a clinical examination within normal limits; had left ventricular function greater than 60% of the ejection fraction on the Doppler echocardiogram test (Teichholz and associates method[25]); had CBC and biochemical (glucose, cholesterol and triglycerides) test results within normal ranges.

From the sixteen Chagas patients, eight presented a normal electrocardiogram and eight presented an altered electrocardiogram: right bundle branch block, bradycardia and/or block of the anterosuperior division of the left bundle branch (Minnesota code modified for Chagas disease[26]).

Venous endothelial function was evaluated using the dorsal hand vein compliance technique[27-30]. During the evaluation, the individuals remained in the supine position with one of the forearms placed on a support forming an upward 30 degree slope from the horizontal. A 23G butterfly needle was inserted in a vein on the dorsum of the hand and saline solution was administered using a Harvard infusion pump (Harvard Apparatus Inc. South Natick, Mass) for 30 minutes at 0.3 ml/min, to allow the vein to restore its tone following the constriction caused by the insertion of the needle.

The transducer, sensitive enough to detect minimal linear displacements (TDLV, Shaevitz Engineering, Pennsauken, NJ) was placed on dorsum of the hand and fixed securely to the skin with adhesive tape. One of the ends of the metallic rod that slides through the transducer was placed over the vein to be studied, less than 1 cm from the end of the intravenous needle.

Vertical displacement of the rod according to the degree of vein dilation or constriction generated a transducer

signal that was amplified and recorded on graph paper.

Measurements of the vein diameter were conducted after inflating a sphygmomanometer cuff to 40 mmHg which was placed on the same arm. The vein diameter variations were calculated by the percentage difference of the metallic rod positioning and graphic recording was generated before and after cuff inflation.

After the saline infusion, phenylephrine was infused in progressive doses until a 70% constriction was obtained (VC70%). Each of the progressive doses of phenylephrine (75 to 25,000 ng/ml) were administered for seven minutes. Once the desired venoconstriction was obtained, which established the base line for venous vascular response evaluation, the phenylephrine dosage was maintained and administered for the duration of the study.

To evaluate endothelium-dependent venodilation, seven progressive doses of acetylcholine (3.6 to 3,600 ng/ml) were administered at an infusion rate of 0.3 ml/min, for a period of three minutes each. Maximum venodilation and the required dosages to attain this effect were compared between the groups.

At the end of this phase, a 30 minute interval was taken for the acetylcholine effect to wear off. Next, three progressive doses (156 to 3,125 ng/ml) of sodium nitroprusside were administered at an infusion rate of 0.3 ml/min, for a period of three minutes each, using the method described earlier to obtain preconstriction (VC70%). Maximum venodilation and the dosages required to achieve this effect were compared between the groups.

RESULTS

Demographic characteristics of the Chagas and control groups - In relation to age, weight, height and body mass index (BMI) there were no significant differences between the control (G0) and Chagas (G1) groups. Additionally, the BMI values were within the healthy range.

In relation to gender, the distribution was similar between the groups with 12.5% male and 77.5% female in the control (G0) and Chagas (G1) groups.

The values of fasting glucose, total cholesterol, triglycerides, hemoglobin and hematocrit did not present any significant statistical differences between the groups.

Both groups attained similar venous preconstriction values, in the range of 70%, that was adequate for the subsequent tests. However, there was a significant difference between the groups in relation to the dosages of phenylephrine required to obtain the desired level of venoconstriction. In comparison to the control group, the Chagas patients required significantly higher doses of phenylephrine to obtain the satisfactory degree of venoconstriction.

In relation to the administration of acetylcholine, it was observed that the maximum venodilation values for the Chagas patients were roughly 50% lower than the control group. The average acetylcholine dosages in relation to maximum venodilation were similar for the two groups.

Maximum venodilation values with the administration of sodium nitroprusside (endothelium-independent dilation evaluation) did not reveal any significant differences between the groups or the dosages of agent used.

DISCUSSION

The major finding of our study was the confirmation that patients with Chagas disease without heart failure present significantly lower maximum venodilation values with the administration of acetylcholine and normal maximum venodilation values with the administration of sodium nitroprusside. Combined analysis of these data indicates venous endothelial dysfunction in Chagas patients with preserved ventricular function.

Another item that deserves emphasis was the observation that Chagas patients require significantly

Table 1 – Demographic and biochemical characteristics of the study groups: control group (G0) and Chagas group (G1)

	Group 0 (n = 8)	Group 1 (n = 16)	p < 0.05
Age (years)	43 ± 4.5	46 ± 2.7	ns
Gender	1M/7F	2M/14F	ns
Weight (kg)	65.3 ± 3.4	63.3 ± 2.3	ns
Height (m)	1.66 ± 0.02	1.63 ± 0.01	ns
BMI (kg/m²)	23.5 ± 0.8	24 ± 0.7	ns
Systolic BP (mmHg)	115.8 ± 4.6	122.8 ± 6.53	ns
Diastolic BP (mmHg)	73.5 ± 3.4	76 ± 4.2	ns
Fasting Glucose (mg/dl)	89 ± 1	97.2 ± 2.8	ns
Total Cholesterol (mg/dl)	209.2 ± 10	201.4 ± 3.2	ns
Triglycerides (mg/dl)	101.1 ± 25.5	121.6 ± 25	ns
Hemoglobin (g/100ml)	13.4 ± 0.29	13.1 ± 0.3	ns

The values represent the mean ± standard error, Student's t-test, ns = not significant; BMI – body mass index

Table 2 – Average of the maximum venodilation and required dosages of phenylephrine, acetylcholine and sodium nitroprusside for the study groups: control group (0) and Chagas group (1)

	Group 0 (n = 8)	Group 1 (n = 16)	p
VC70%	68.5 ± 4.5	76.8 ± 2.4	0.15
Phenylephrine doses for VC70%	103 ± 28	1116 ± 668 **	0.05
Max.VD with Acetyl.	137 ± 20	65.5 ± 8 **	0.009
Acetyl. doses (ng/ml) for Max.VD	1980 ± 390	2092 ± 366	0.85
Max.VD with SNP	169 ± 25.5	163 ± 19	0.83
SNP doses (ng/ml) for MaxVD	1425 ± 436	1004 ± 223	0.39

The values represent the mean ± standard error; VC = venoconstriction; MaxVD = maximum venodilation; Acetyl. = acetylcholine; SNP = sodium nitroprusside

higher doses of phenylephrine to obtain venoconstriction, suggesting lower alpha-adrenergic receptor sensitivity in the venous system of these patients.

The endothelial cells produce the most potent vasodilator ever discovered, nitric oxide (NO) that was described by Furchgott and Zawadzki in 1980 and identified as a nonprostanoid, labile and diffusible substance that mediates the endothelium-dependent vasorelaxation. The formation of NO by endothelium cells performs a critical role in maintaining the balance between vasoconstriction and vasodilation in the arterial and venous homeostasis process[31].

Numerous studies describe the vasodilation changes in the dorsal hand vein after local infusion of acetylcholine or bradykinin in patients with cardiovascular risk factors that are known to affect artery endothelium[27-30]. Additionally, treatments that improve artery endothelial function also improve endothelial function in the venous system[29]. Studying the venous endothelia enables a better understanding of the circulatory regulation process and the effect of treatments on the modulation of peripheral regulation.

The venous system plays an important role in the homeostasis of the circulatory system and alterations in this system can lead to compromised venous return and circulation, since the changes in the vascular system could potentially affect cardiac function regulation and cause heart failure[32]. To date no previous studies had detected peripheral venous system alterations, or more importantly, venous endothelial responses in Chagas disease patients.

Recent studies have demonstrated increased plasma levels of the cytotoxic endothelial moderators, tumor necrosis factor alpha (TNF-α) and interleukin 10 (IL-10) in asymptomatic patients with Chagas disease, suggesting that a prolonged production of these factors could be related to the cardiomyopathy progression in these patients[21-23]. Circulating anti-cholinergic receptor antibodies have also been found in chronic Chagas cardiopathy patients[33-36]. Considering these findings

and their repercussions on vascular tonus modulation, we can speculate a possible relation between them and the results in our study since these factors can lead to endothelial dysfunction.

Experimental studies on the distribution and different responses to pharmaceutical agents are fundamental to understand the venous system physiopathology. In this study we demonstrate that the alpha adrenergic sensitivity, evaluated during the administration of phenylephrine, was diminished in the Chagas patients when compared to the control group. Comparisons of receptor distribution and venous response to the administration of adrenergic agents using noradrenaline (non-selective α agonist), phenylephrine (α-1 agonist) and azepexol (α-2 agonist) demonstrated that vasoconstriction was induced by all the agents and suggests that the veins are responsive to all alpha receptors. Since the response to phenylephrine was more accentuated, the author suggested that the distribution of α-1 receptors was prominent in the veins [37]. In humans with Chagas disease the sympathetic innervation of the heart, evaluated using scintigraphy with radio-isotopes, was found to be diminished as reported by Simões and associates[38]. An indirect study of peripheral sympathetic innervation using catecholamine concentrations or blood pressure variations indicates alterations in the sympathetic function[39]. Data from an earlier study conducted by our group with a similar population of Chagas patients revealed an increased peripheral chemoreflex response[9], indicating alterations in the peripheral sympathetic response of these patients. This alteration in sympathetic activity could be related to the reduced sensitivity to adrenergic agonists, as observed in this study.

Based on our findings, we can conclude that venous alpha adrenergic sensitivity is diminished and that endothelial response mediated by muscarinic receptors is lowered, which is indicative of venous endothelial dysfunction in Chagas patients with preserved ventricular function.

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