Biochemical and nutritional evaluation of patients with visceral leishmaniasis before and after treatment with leishmanicidal drugs

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ABSTRACT

Introduction: Visceral leishmaniasis (VL) is caused by the intracellular protozoan Leishmania donovani complex. VL may be asymptomatic or progressive and is characterized by fever, anemia, weight loss and the enlargement of the spleen and liver. The nutritional status of the patients with VL is a major determinant of the progression, severity and mortality of the disease, as it affects the clinical progression of the disease. Changes in lipoproteins and plasma proteins may have major impacts on the host during infection. Thus, our goal was to evaluate the serum total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, glucose, albumin, globulin and total protein levels, as well as the body composition, of VL patients before and after treatment.

Methods: Nutritional evaluation was performed using the bioelectrical impedance analysis (BIA) to assess body composition. Biochemical data on the serum total cholesterol, HDL, LDL, triglycerides, glucose, albumin, globulin and total protein were collected from the medical charts of the patients.

Results: BIA indicated that both pre-treatment and post-treatment patients exhibited decreased phase angles compared to the controls, which is indicative of disease. Prior to treatment, the patients exhibited lower levels of total body water compared to the controls. Regarding the biochemical evaluation, patients with active VL exhibited lower levels of total cholesterol, HDL, LDL and albumin and higher triglyceride levels compared to patients after treatment and the controls. Treatment increased the levels of albumin and lipoproteins and decreased the triglyceride levels.

Conclusions: Our results suggest that patients with active VL present biochemical and nutritional changes that are reversed by treatment.

Keywords: Visceral leishmaniasis. Nutritional status. Leishmanicidal drugs.
such as protein-calorie malnutrition (PCM), or a micronutrient deficiency. The infectious process is usually followed by hypercatabolism, which is aggravated by anorexia, resulting in the loss and the consequent depletion of the body’s nutrient reserves, thereby causing great changes in the metabolism of the host.

Some studies have reported differences in body mass index (BMI), glucose, triglycerides and lipoproteins in patients with active VL. Studies performed on patients with VL showed high triglyceride levels and low high-density lipoprotein (HDL), low-density lipoprotein (LDL) and total cholesterol levels. Additionally, VL patients showed reduced serum cholesterol concentrations as a function of their splenic parasite burden. Elevated levels of triglycerides and low levels of HDL were observed in canine VL. Changes in lipoproteins have also been reported in children with active VL. Lipoproteins have the ability to modulate the immune response. Low density lipoproteins, such as very low-density lipoprotein (VLDL) and LDL, can inhibit lymphocyte proliferation in vitro, and another study in VL patients found that lipoproteins altered the immune response and the pathogenesis of the disease by modulating cytokine production.

Changes in cholesterol levels can have a major impact on the host during infection. In addition to playing an important role in the maintenance of membrane fluidity, cholesterol is essential for the proper function of antigen presenting cells and is also a prognostic indicator of increased morbidity and mortality associated with pathological conditions. This lipid is responsible for the formation of membrane rafts, which are essential for Leishmania entry. In malnourished animals infected with Leishmania chagasi, decreases in serum albumin, globulin and total protein were observed, and these changes were associated with an increased parasite burden and a non-response to the vaccine.

Interest in body composition has grown substantially in the last few years due to the association between increased body fat and metabolic disorders, including cardiovascular diseases, diabetes, hypertension and dyslipidemia. Bioelectrical impedance analysis (BIA) has been used to analyze body composition for more than 25 years. Additionally, BIA is a portable, noninvasive method to measure body composition that is practical, reproducible and relatively inexpensive. BIA can be repeated often and does not require patient cooperation.

Although some studies have reported changes in lipoproteins in patients with VL, studies concerning the serum total protein, albumin, globulin and BIA body measurements are absent in these patients, and there are few published papers that examine a wider range of nutritional status factors. Thus, our goal was to evaluate the serum total cholesterol, HDL, LDL, triglycerides, glucose, albumin, globulin and total protein, as well as body composition using BIA, in LV patients before and after treatment with leishmanicidal drugs.

**METHODS**

**Patients**

We evaluated 13 patients pre-and post-treatment at an interval of 18 months. The patients were of both genders and were 18 years or older. The age of the patients ranged from 18 to 53 years, and the patients were predominantly male (11 individuals). We excluded patients with other infectious or granulomatous diseases, as well as HIV positive patients and pregnant women. The patients were enrolled in the study when they contacted health services for diagnosis and treatment of the disease. The patients came from the State Hospital of Bauru, State of São Paulo and the Hospital of Marília, State of São Paulo. We evaluated 16 healthy, age- and sex-matched subjects as controls. The age of the control subjects ranged from 21 to 58 years, and the group included 3 women and 13 men.

**Diagnosis and treatment of visceral leishmaniasis**

The diagnosis of visceral leishmaniasis was performed through the confirmation of the parasites in bone marrow aspirate smears. Patients were treated with N-methylglucamine (Glucantime), amphotericin B deoxycholate or amphotericin B liposome. Data were collected from the patients in the hospital where they were admitted prior to starting treatment for VL. The post-treatment period ranged from 1 to 3 months after the end of the drug regimen, and the patient data were included when the patients returned to the hospital for monitoring. However, two patients did not return to the hospital after the treatment. Post-treatment data were collected only from patients that were cured according to the serological and clinical tests.

**Biochemical and nutritional analysis**

The biochemical and nutritional evaluation was performed in patients before and after treatment and was performed once in the control subjects. BIA was used for body measurements in the control subjects and in patients pre-and post-treatment using a Biodynamics BIA, model 450 device (TBW). For this procedure, the patients and the control subjects laid down on a non-conductive surface, with their legs apart and their arms parallel and apart from the trunk. Immediately prior to the placement of the electrodes, the contact areas were cleaned with alcohol. An emitter electrode was placed near the metacarpal-phalanx of the dorsal surface of the right hand, and another was placed near the distal transverse arch of the upper surface of the right foot. An electrode detector was placed between the distal prominences of the radius and ulna of the right wrist, and another was placed between the medial and lateral malleolus of the right ankle. The following data were acquired from the patients and control subjects: lean mass (%), body fat (%), body mass index (kg/m²), phase angle (°), resistance (ohms), reactance (ohms) and total body water (L). In addition to the BIA, the patient’s weight (kg) and height (m) were also recorded.

The analyses of the total cholesterol, HDL, LDL, triglycerides, glucose, total protein, albumin and globulin in the control subjects were performed in the Clinical Laboratory of the Faculty of Medicine of Botucatu (Universidade Estadual Paulista - UNESP). The patient analyses were performed at the hospital where they were admitted, as these are routine tests for the treatment and follow-up of the disease. These determinations were performed using enzymatic, colorimetric and dry-chemistry methods. To evaluate these parameters, blood was collected from the patient in the morning after fasting for 12h, after no rigorous physical activity for the last 24h and after no alcohol consumption within the last 72h.
RESULTS

TABLE 1 - Bioelectrical impedance analysis of patients with visceral leishmaniasis (difference estimates and 95% confidence intervals).

<table>
<thead>
<tr>
<th></th>
<th>Pre- vs post-treatment estimate (95% CI)</th>
<th>Pre-treatment vs controls estimate (95% CI)</th>
<th>Post-treatment vs controls estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>-5.662 (-14.35-3.03)</td>
<td>-10.84 (-22.25-0.567)</td>
<td>-5.184 (-16.47-6.1)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>0.006 (-0.02-0.03)</td>
<td>-0.02 (-0.09-0.03)</td>
<td>-5.184 (-16.47-6.1)</td>
</tr>
<tr>
<td>Resistance (ohms)</td>
<td>4.59 (-61.33-70.51)</td>
<td>12.36 (-63.16-87.87)</td>
<td>16.95 (-68.17-102.07)</td>
</tr>
<tr>
<td>Reactance (ohms)</td>
<td>2.52 (-10.14-15.18)</td>
<td>9.124 (-0.671-18.91)</td>
<td>6.599 (-1.884-15.082)</td>
</tr>
<tr>
<td>Phase angle (º)</td>
<td>0.28 (-0.82-1.38)</td>
<td>1.5 (0.31-2.7)*</td>
<td>1.22 (0.17-2.27)*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>2.03 (-1.61-5.67)</td>
<td>2.9 (-0.641-6.45)</td>
<td>0.86 (-2.89-4.63)</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>4.18 (-3.02-11.38)</td>
<td>4.6 (-2.24-11.45)</td>
<td>0.42 (-6.08-6.92)</td>
</tr>
<tr>
<td>Total body water (L)</td>
<td>-2.57 (-8.787-3.633)</td>
<td>6.825 (0.417-13.23)*</td>
<td>4.73 (-1.944-11.40)</td>
</tr>
</tbody>
</table>

vs: versus; CI: confidence interval; BMI: body mass index. *p value <0.05.

Statistical analysis

Non-continuous variables were analyzed using Fisher’s test. Comparisons for the continuous variables were performed using an analysis of variance (ANOVA) with a repeated measures design between the pre- and post-treatment groups, followed by an adjusted Tukey’s test if the distribution were normal. Otherwise, the same design was used to adjust a generalized linear model with gamma distribution, followed by a multiple comparison test (Wald type). Comparisons between the pre- and post-treatment groups and the control group were made using separate Student’s t-tests (control vs. pre and control vs. post). All of the analyses were made using the Statistical Analysis System (SAS) software, v.9.3. The significance level was fixed at 5% or the correspondent p-value.

Ethical considerations

All enrolled patients and control subjects were fully informed of the study and signed the consent form. This study was approved by the Ethics in Research of the Faculty of Medicine of Botucatu, Bauru State Hospital and the Hospital of Marilia and is in accordance with the Declaration of Helsinki of 1964.

Evaluation of body measurements by bioelectrical impedance analysis in patients pre- and post-treatment

There was a male predominance in the patients and control subjects in this study, and there was no significant difference in the mean age of the patients and the control subjects. The results obtained from the BIA analysis showed that there were no significant differences in the phase angle between the patients before and after treatment; however, the phase angle in the patients pre-treatment and post-treatment were significantly lower (p-value <0.05) than the phase angle of the control subjects. In relation to total body water, there were no significant differences between pre- and post-treatment or between post-treatment and the controls. However, patients with active VL showed a decreased (p-value <0.05) amount of total body water than the control subjects. No significant differences in weight (kg), height (m), body mass index (kg/m²), percentage of lean mass, percentage fat, resistance (ohms) and reactance (ohms) were detected between the patients pre-treatment and post-treatment or between these two groups and the control subjects. However, even without significant differences between the pre- and post-treatment measurements or between these groups and the control group, the pre-treatment patients tended to have smaller reactance, BMI, fat percentage and weight values than the post-treatment patients and the controls. The percentage of lean mass tended to decrease with treatment and tended to be higher in the control subjects. Data are shown in Table 1.

Biochemical assessment of patients with visceral leishmaniasis before and after treatment

The pre-treatment patients showed significantly lower levels of total cholesterol, HDL and LDL compared to the post-treatment patients and control subjects (p-value <0.05). However, with exception of LDL, the treated patients also showed significantly lower levels of these variables when compared to the controls (p-value <0.05). Prior to treatment, patients exhibited significantly higher levels of triglycerides compared to post-treatment and the control subjects; even after treatment, these levels still remained elevated when compared to the controls (p-value <0.05). Pre-treatment patients also had lower albumin levels compared to the post-treatment and the control groups (p-value <0.05). However, the treated patients showed higher albumin levels than the controls (p-value <0.05). No differences were found in the levels of glucose, total protein and globulin between these groups. Data are shown in Table 2.
### DISCUSSION

According to our results, BIA demonstrated a decreased phase angle in patients before treatment and after treatment compared to the control subjects. The phase angle is formed when the electric current is stored across cell membranes and depends on the permeability of the membranes; this measurement is associated with tissue hydration and cellularity, as well as cell size and body cell mass. Thus, the phase angle is associated with cell balance. The phase angle measurement can vary between 0° and 90°, and the reference values are between 4° and 10°. The phase angle has been used as an indicator of the general state of health and nutrition in various diseases, as nutrition is interconnected with changes in membrane integrity and the balance of body fluids. Our findings showed that both groups were within the reference values; however, the pre-treatment patients had lower phase angle values than the control group. According to our results, phase angle in patients with infectious diseases and inflammation is lower than the reference values. Therefore, studies in patients co-infected with human immunodeficiency virus (HIV) and tuberculosis also exhibited low phase angles when compared to the control subjects.

Although no statistically significant difference was found, our results showed that treatment for VL tended to increase the phase angle. However, even after treatment, the phase angle was still lower than in the control subjects. A possible explanation could be that the patients in the study period had not yet fully recovered. Furthermore, we have to consider that the phase angle is positively associated with reactance, which in our study tended to be smaller in patients with active VL compared to the controls. While the phase angle is decreased, the reactance is low, which indicates the presence of a disease. Reactance values inversely correlate with the percentage of lean body mass, which is highly conductive to electrical current, as resistance values directly correlate to the percentage of fat mass, which is poor electrical conductor. In the present study, although no significant difference in weight was detected between the groups, the amount of lean mass tended to be higher in the pre-treatment patients, while the percentage of fat mass tended to be lower in these patients. Taking into account these results, the pre-treatment patients also exhibited normal BMI values, as malnutrition is considered a BMI below 18.5 kg/m². Contrary to our findings, one study reported that children with active VL had lower BMI values than healed children and uninfected children; additionally, children that breastfeed for longer period of time have asymptomatic VL, which shows that nutritional status plays a crucial role in the pathogenesis of visceral leishmaniasis. Thus, we can conclude that patients with active VL showed no difference in weight compared to the post-treatment patients and the controls and, therefore, displayed no difference in BMI, percentage of lean mass and fat mass.

According to our results, glucose levels did not differ between the groups. Unlike our results, previous studies have shown that experimental infection with *L. chagasi* leads to a reduction in glucose levels compared to control mice. Concerning lipid levels, our results showed that pre-treatment patients had lower HDL, LDL and total cholesterol levels and higher levels of triglycerides compared to the post-treatment patients and the controls. However, it is worth noting that all groups were within the normal range for total cholesterol (reference values <200 mg/dL) and LDL (reference value <130 mg/dL). The HDL levels (reference values >35 mg/dL) and triglycerides levels (reference value <150 mg/dL) were below and higher than normal in patients before treatment, respectively. These results are in agreement with Soares et al., who showed that patients with VL had high triglycerides levels and low HDL, LDL and total cholesterol levels. Other studies of visceral leishmaniasis and other infectious diseases, such as HIV and schistosomiasis, have reported the same lipid changes. Our findings show that patients with active VL, which most likely have a high parasite load, have total cholesterol levels lower than the control group, and after treatment, these levels increase. In this sense, Ghosh et al. showed that patients with VL have cholesterol concentrations that were inversely correlated with

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**TABLE 2 - Biochemical assessment of patients with visceral leishmaniasis (difference estimates and 95% confidence intervals).**

<table>
<thead>
<tr>
<th></th>
<th>Pre- vs post-treatment estimate (95% CI)</th>
<th>Pre-treatment vs controls estimate (95% CI)</th>
<th>Post-treatment vs controls estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cholesterol (mg/dL)</strong></td>
<td>58.82 (11.14-66.5)*</td>
<td>94.27 (59.5-129)*</td>
<td>35.44 (0.33-70.55)*</td>
</tr>
<tr>
<td><strong>HDL (mg/dL)</strong></td>
<td>30.44 (22.26-38.62)*</td>
<td>46.8 (32.5-61.17)*</td>
<td>16.4 (1.38-31.41)*</td>
</tr>
<tr>
<td><strong>LDL (mg/dL)</strong></td>
<td>38.29 (14.01-62.57)*</td>
<td>62.55 (33.15-91.96)*</td>
<td>24.26 (-6.71-55.23)</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dL)</strong></td>
<td>0.0016 (0.00007-0.0032)*</td>
<td>0.001672 (0.000072-0.003272)*</td>
<td>0.005 (0.003105-0.007285)*</td>
</tr>
<tr>
<td><strong>Glucose (mg/dL)</strong></td>
<td>0 (0)</td>
<td>0.00068 (-0.000852-0.002212)</td>
<td>-0.00148 (-0.00061-0.00357)</td>
</tr>
<tr>
<td><strong>Albumin (g/dL)</strong></td>
<td>1.47 (0.99-1.95)*</td>
<td>0.9 (0.39-1.42)*</td>
<td>0.56 (0.12-0.99)*</td>
</tr>
<tr>
<td><strong>Globulin (g/dL)</strong></td>
<td>-1.04 (-2.52-0.44)</td>
<td>0.91 (-0.19-2.01)</td>
<td>0.13 (-0.77-1.03)</td>
</tr>
<tr>
<td><strong>Total protein (g/dL)</strong></td>
<td>0.38 (-0.88-1.64)</td>
<td>0.04 (-0.94-1.04)</td>
<td>0.43 (-0.29-1.15)</td>
</tr>
</tbody>
</table>

vs: versus; CI: confidence interval; LDL: low-density lipoprotein; HDL: high-density lipoprotein. *p value <0.05
their splenic parasite load. Due to the high parasitic loads in the spleen, patients with active VL experience dysfunctions in this organ, which is responsible for cholesterol biosynthesis, thus increasing the morbidity of this disease. Our results showed that treatment appeared to normalize the HDL and triglyceride levels and increase the LDL and total cholesterol levels.

The changes in the levels of lipoproteins can be directly related to the modulation of the immune response, as the plasma membrane, which is composed of lipids, is essential for antigen presentation and phagocytosis. Internalization involves interaction between the parasite and the plasma membrane of the cell host. Low cholesterol levels in humans decrease the number of circulating lymphocytes, thus increasing the chances of mortality. The decrease in lipoproteins may affect the immune response and the pathogenesis of the disease, as lipoproteins are related to the production of TNF-α, IL-6 and IL-10. In addition to being targets for the immunomodulatory activity of lipoproteins, macrophages express lipoprotein receptors and are the host cells of the *Leishmania* parasite. However, the study demonstrated that macrophage-depleted lipids decreased their ability to interact with *Leishmania donovani* and internalize promastigotes by 45%, thus impairing the replication of the parasite. On the other hand, when depleted macrophages are treated with cholesterol, there is an increased chance that the parasites will destroy the cell membrane.

In relation to the plasma proteins, it was demonstrated in our study that patients with active VL have lower albumin levels compared to the post-treatment patients and the controls, and no differences were observed in the levels of total protein and globulin between the groups. Contrary to the findings of our study, Malafaia et al. reported decreased globulin and total protein in mice infected with *L. chagasi* compared to uninfected mice. However, according to our results, animals infected with *L. chagasi* also exhibit a decrease in serum albumin, which is associated with increased parasite load, a negative response to the vaccine and reduced IFN-γ production. Another study reported that the albumin/globulin ratio was lower in VL patients compared to post-treatment patients and the controls, and no differences were observed in the levels of total protein and globulin between the groups of patients with active VL and those with healed VL. Our results show that patients with active VL were eutrophic, had a lower phase angle and exhibited changes in their albumin and lipoprotein levels. Treatment changed the biochemical frame and the nutritional status of the patients, tending to return to normal levels. The data presented in this study suggest an association between the biochemical and nutritional alterations and leishmaniasis. However, our study does not conclude that the changes were responsible for the worsening of the VL or if the infection led to the biochemical and nutritional changes, thus aggravating the clinical manifestations of VL. Thus, we are developing new studies to have a better understanding of the involvement of biochemical and nutritional parameters in the pathogenesis of LV.

**ACKNOWLEDGMENTS**

We gratefully acknowledge all of the patients, the State Hospital of Bauru and the Hospital of Marilia.


