Short Report: Preliminary Study on Tubuloglomerular Dysfunction and Evidence of Renal Inflammation in Patients with Visceral Leishmaniasis

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Abstract. Visceral leishmaniasis (VL) is a re-emerging zoonosis of worldwide distribution. Monocyte chemotactic protein-1 (MCP-1) and malondialdehyde (MDA) are inflammation biomarkers that have never been investigated in VL. The aim of this study is to investigate the association between renal abnormalities and inflammation biomarkers in VL. This study is a preliminary prospective study with 16 VL adult patients evaluated before treatment compared with a group of 13 healthy volunteers and 5 VL patients evaluated after treatment. Urinary concentration and acidification tests were performed. MCP-1 and MDA were quantified in urine. Urinary concentration deficit was found in all VL patients before (100%) and four VL patients after (80%) treatment. Urinary acidification deficit was found in nine cases before (56.2%) and two cases after (40%) treatment. Urinary MCP-1 (374 ± 359 versus 42 ± 29 pg/mg creatinine, $P = 0.002$) as well as urinary MDA (5.4 ± 2.6 versus 2.0 ± 0.8 $\mu\text{mol/mL}$) showed significant differences between VL patients and controls. These data show that VL patients present urinary concentration and acidification deficit, which can persist even after specific treatment. Urinary MCP-1 and MDA are elevated in patients with VL, which suggests renal inflammation and incipient renal damage.

Visceral leishmaniasis (VL) is a re-emerging zoonosis of worldwide distribution.^{1,2} VL can involve multiple organs, including the kidneys, by different and complex mechanisms, which may lead to tubulointerstitial and glomerular changes.^{3,4} Interstitial involvement is more pronounced, and it is presented mainly through tubular changes as urinary acidification deficit and reduction in urinary concentration capacity.^{5,6} In humans, renal involvement is usually caused by subclinical inflammatory interstitial infiltrate induced by the parasite, and discrete changes may also occur in glomerular basal membrane.^{7,8} There is evidence that VL is associated with systemic inflammatory response syndrome, probably because of high production of proinflammatory cytokines.⁹ The aim of this study is to investigate the association between renal abnormalities and inflammation biomarkers in VL.

A prospective preliminary cohort study with 16 adult patients with confirmed diagnosis of VL undergoing treatment in a public health service was conducted in Fortaleza city in the northeast region of Brazil from April of 2011 to April of 2012. Patients were treated with pentavalent antimony (*N*-methylglucamine antimoniate) at a dose of 20 mg/kg per Sb^{+5} per day for 20 days.

The protocol of this study was revised and approved by the Ethical Committee of the São José Infectious Disease Hospital, Fortaleza, Brazil. Patients were included in the study after signing the informed consent form. Inclusion criteria were age of 18–65 years and VL diagnosis done by searching the parasite in bone marrow and serological tests (use of recombinant antigen K-39 or indirect immunofluorescence). Patients were selected from the infectious diseases outpatients clinics, and 16 patients were compared with 13 healthy volunteers

(randomly selected among blood donors). Ninety days after specific treatment with antimonial pentavalent, five patients were assessed and underwent a novel renal function evaluation. The following laboratory parameters were evaluated: total blood count, plasma urea (P_{Ur}), creatinine (P_{Cr}), sodium (P_{Na^+}), potassium (P_{K^+}), osmolality (P_{osm}), venous pH, and bicarbonate (HCO_3^-). A 24-hour urinary volume (UV) sample was collected for creatinine (U_{Cr}), sodium (U_{Na^+}), potassium (U_{K^+}), microalbuminuria, and proteinuria measurements. Urine samples were collected for osmolality (U_{osm}) and pH (U_{pH}). To assess tubular function, fractional excretion of sodium (FE_{Na}) was calculated through a standard formula. Solute free water reabsorption (TcH_2O) was calculated by the equation $\text{TcH}_2\text{O} = \text{Cosm} - V$ (where $\text{Cosm} = U_{\text{osm}} \cdot V / P_{\text{osm}}$; V is urinary flow), and the transtubular potassium gradient (TTKG) was calculated by the equation $\text{TTKG} = (P_{\text{osm}} \times U_{\text{K}}) / (P_{\text{K}} \times U_{\text{osm}})$. All patients underwent food and water deprivation for 12 hours. Urinary concentration ability was evaluated through the ratio between urinary and serum osmolality ($U_{\text{osm}} / P_{\text{osm}}$) after 12 hours of water deprivation and use of desmopressin acetate (DDAVP) at a dose of 20 mcg in the form of nasal spray at time zero (T_0) and 4 hours later (T_4). Urinary concentration defect was considered when $U_{\text{osm}} < 700$ mOsm/L and/or $U_{\text{osm}} / P_{\text{osm}} < 2$.^{8,10–12} Urinary acidification was evaluated through the measure of urinary pH before and after administration of oral CaCl_2 (2 mEq/kg; T_0 and T_4).¹³ Acidification defect was determined by the inability to decrease U_{pH} to less than 5.5 after the administration of the acid load.¹⁴ Urinary monocyte chemotactic protein-1 (MCP-1) measurement was performed through enzyme-linked immunosorbent assay (ELISA) using the R&D Systems Kit (R&D Systems Kit, Inc., Minneapolis, MN). Urinary malondialdehyde (MDA) was isolated and quantified using the thiobarbituric acid (TBARS) test. The MCP-1 and MDA results were correct by urinary creatinine.

Fisher's exact test and χ^2 test were used to analyze allele frequencies in the patient group. Differences between two

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independent variables were evaluated using Student's *t* test or Mann-Whitney test as appropriate. Wilcoxon test was used in the group after treatment. Data were expressed as means $\pm$ SDs, and $P < 0.05$ was considered statistically significant. The SPSS software for Windows, release 10.0 (SPSS Inc., Chicago, IL) was used in all analysis.

In total, 16 VL patients were studied before treatment, and there was no significant difference regarding age, sex, mean systolic blood pressure, and body weight between the VL patients and the control group. A comparison between five VL patients before and after treatment was also done. Glomerular filtration rate (GFR) was similar in VL patients before treatment and the control group (93.5 ± 20.2 versus 94.6 ± 22.4 mL/min per 1.73 m^2 , $P = 0.889$), and there was no difference after treatment (82.4 ± 12.2 versus 83.1 ± 22.7 mL/min per 1.73 m^2 , $P = 0.593$). Microalbuminuria > 30 mg/day was found in three cases before treatment (18.7%), and no patients had microalbuminuria after treatment. Proteinuria was significantly higher in VL patients before treatment compared with the control group (250.6 ± 375.5 versus 83.7 ± 49.2 mg/24 hours, $P = 0.022$). These data are in Tables 1 and 2. In basal conditions, the VL group before treatment had a significantly higher FE_{Na} ($0.95 \pm 0.52\%$ versus $0.54 \pm 0.21\%$, $P = 0.01$). Patients in the VL group before treatment had a significantly higher FE_{Na} ($0.95 \pm 0.52\%$ versus $0.54 \pm 0.21\%$, $P = 0.01$). When the maximum urinary concentration ability was assessed, patients in the VL group before treatment had lower U_{osm} (478 ± 107 versus 744 ± 182 mOsm/kg, $P < 0.001$) after a 12-hour period of water deprivation compared with controls as well as lower $\text{U}_{\text{osm}}/\text{P}_{\text{osm}}$ (1.63 ± 0.37 versus 2.60 ± 0.67 , $P < 0.001$). Despite using DDAVP, VL patients persisted with lower U_{osm} (516 ± 113 versus 743 ± 189 mOsm/kg, $P < 0.001$) and lower $\text{U}_{\text{osm}}/\text{P}_{\text{osm}}$ (1.78 ± 0.40 versus 2.54 ± 0.63 , $P = 0.001$) compared with controls. After treatment, only one patient presented $\text{U}_{\text{osm}} > 700$ mOsm/kg in T_4 . The U_{pH} before CaCl_2 load was higher in the VL patients before treatment compared with controls (5.71 ± 0.84 versus 5.08 ± 0.43 , $P = 0.018$). The inability to decrease urinary pH to less than 5.5 after use of CaCl_2 was observed in nine patients before treatment (56.2%). After treatment, two of five patients (40%) persisted with urinary pH less than 5.5 after

TABLE 2
Comparison of renal function parameters between patients with VL before and after treatment

	VL group (before treatment; $N = 5$)	VL group (after treatment; $N = 5$)	<i>P</i>
P_{Cr} (mg/dL)	1.1 ± 0.1	1.1 ± 0.2	0.414
GFR (mL/min per 1.73 m^2)	82.4 ± 12.2	83.1 ± 22.7	0.593
Proteinuria (mg/day)	268.1 ± 259.4	113.3 ± 50.1	0.043
Microalbuminuria (mg/day)	14.5 ± 14.0	8.1 ± 8.9	0.465
Urine output (mL/day)	$1,803 \pm 1,068$	$1,263 \pm 583$	0.043

Data are shown as means $\pm$ SDs. $P < 0.05$ was considered significant.

the use of CaCl_2 . HCO_3^- was lower in VL patients before (22 ± 2 versus 29 ± 4 mEq/L, $P < 0.001$) and after (21 ± 2 versus 26 ± 1 mEq/L, $P < 0.001$) CaCl_2 test. These data are in Tables 3 and 4. The search for urinary MCP-1 showed significant difference between VL patients before treatment and the control group (374 ± 359 versus 42 ± 29 pg/mg creatinine, $P = 0.002$). Urinary MDA levels were also significantly higher in VL patients before treatment than in controls (5.4 ± 2.6 versus 2.0 ± 0.8 $\mu\text{mol/mL}$).

Renal involvement in VL has been the subject of great importance because of complications and increased morbidity and mortality. A retrospective study by our group with 224 patients has shown that one-third of the patients developed acute kidney injury (AKI) during hospitalization and that mortality was higher in this group of patients.¹⁵ The assessment of GFR showed no significant difference comparing patients before treatment with the control group. The group of five patients re-evaluated after treatment also showed no statistical differences compared with their pre-treatment results. It was found that six patients before treatment had a GFR below 90 mL/min per 1.73 m^2 , and only two patients had a value greater than 120 mL/min per 1.73 m^2 . Patients in this study were evaluated for the presence of microalbuminuria before and after treatment, and there was no statistical difference. Microalbuminuria is considered as one early marker of glomerular damage in several studies. Data from meta-analyses have shown the cost-effectiveness of biomarkers, such as microalbuminuria, in high-risk populations, such as hypertensive, diabetic, and elderly populations. There is no

TABLE 1
Baseline characteristics of patients with VL compared with controls

	VL group (before treatment; $N = 16$)	Control group ($N = 13$)	<i>P</i>
Age (years)	42 ± 17	40 ± 15	0.75
Sex			0.19
Male (%)	15 (93.7)	10 (76.9)	—
Female (%)	1 (6.3)	3 (23.1)	—
Systolic blood pressure (mmHg)	112 ± 12	122 ± 9	0.05
Diastolic blood pressure (mmHg)	71 ± 10	80 ± 7	0.04
Weight (kg)	65 ± 10	71 ± 9	0.16
Sick animal in peridomicile (%)	8 (50)	—	—
Hematocrit (%)	26.7 ± 5.9	45.1 ± 2.7	< 0.001
Hemoglobin (g/dL)	9.0 ± 2.0	15 ± 1.0	< 0.001
White blood count (mm^3)	$3,075 \pm 2,688$	$6,173 \pm 221$	0.001
Platelets (mm^3)	$76,400 \pm 35,538$	$285,000 \pm 36,756$	0.004
P_{Cr} (mg/dL)	1.0 ± 0.2	1.0 ± 0.2	0.802
P_{Ur} (mg/dL)	32 ± 11	26 ± 8	0.200
GFR (mL/min per 1.73 m^2)	93.5 ± 20.2	94.6 ± 22.4	0.889
Proteinuria (mg/day)	250.6 ± 375.5	83.7 ± 49.2	0.022
Microalbuminuria (mg/day)	17.3 ± 23.8	6.7 ± 6.3	0.135
Urine output (mL/day)	$2,008 \pm 1,118$	$1,351 \pm 487$	0.059

Data are shown as means $\pm$ SDs or numbers with percentages in parentheses. $P < 0.05$ was considered significant.

TABLE 3

Comparison of concentration and acidification test results between patients with VL before treatment and controls

	VL group (before treatment; N = 16)	Control group (N = 13)	P
U _{Osm} T ₀ (mOsm/kg)	478 ± 107	744 ± 182	< 0.001
U _{Osm} T ₄ (mOsm/kg)	516 ± 113	743 ± 189	< 0.001
U _{Osm} /P _{Osm} T ₀	1.63 ± 0.37	2.60 ± 0.67	< 0.001
U _{Osm} /P _{Osm} T ₄	1.78 ± 0.40	2.54 ± 0.63	0.001
pH: venous T ₀	7.37 ± 0.05	7.39 ± 0.05	0.508
pH: venous T ₄	7.36 ± 0.05	7.35 ± 0.05	0.932
HCO ₃ T ₀ (mEq/L)	22.0 ± 2.0	29.0 ± 4.0	< 0.001
HCO ₃ T ₄ (mEq/L)	21.0 ± 2.0	26.0 ± 1.0	< 0.001
U _{pH} T ₀	5.99 ± 0.94	5.55 ± 0.54	0.149
U _{pH} T ₄	5.71 ± 0.84	5.08 ± 0.43	0.018

Data are shown as means ± SDs. *P* < 0.05 was considered significant.

consensus about its use in the general population without comorbidities as an early marker of kidney disease.¹⁶

All VL patients studied before treatment presented urinary concentration deficit, and among those who were revalued after treatment, the majority persisted with lower urinary osmolality to 700 mOsm/L, despite fluid deprivation and use of desmopressin acetate. In this study, the ability to acidify the urine was also evaluated before treatment, and it was found that just over one-half of the patients had urinary acidification deficit after overload of CaCl₂. A previous study with 50 patients with cutaneous leishmaniasis detected deficit of urinary concentration in 77% and 88% of patients before and after specific treatment, respectively and detected urinary acidification deficit after overload of CaCl₂ in 17 patients before treatment with pentavalent antimony. After treatment, six patients had persistent deficit (considering the inability to reduce urinary pH < 5.5) similar to the approach used in our study.¹⁴

In this study, the urinary MCP-1 and MDA levels were significant elevated compared with the control group. This study is the first study showing an increase in MCP-1 and MDA in VL. Urinary MCP-1 is a biomarker that has been associated with kidney damage and inflammation in chronic and acute diseases.¹⁷⁻¹⁹ Previous data showed a correlation between albuminuria, elevated levels of urinary MCP-1, and inflammation represented by macrophages in the renal tissue.²⁰ There are few data in the literature that report the association between infectious and parasitic diseases and MCP-1. A recent study with patients with schistosomiasis has shown that this infection can induce a chronic renal inflammatory status shown by an increment in urinary MCP-1, and

it is not interrupted by specific treatment, even in patients with subclinical infection. These data suggest that MCP-1 can be an early sensitive marker of renal injury in patients with schistosomiasis.²¹ Urinary MDA levels were also higher in VL patients before treatment compared with the control group, suggesting increased oxidative stress. Previous study with canine VL showed significant correlation between serum MDA, blood urea nitrogen, and creatinine and showed that symptomatic dogs showed more severe oxidative stress than asymptomatic and non-infected dogs.²² Costa and others,⁹ in a case-control study, compared VL patients who died with those who survived and found the occurrences of bacterial infection and bleeding as main risk factors for death. Additionally, Costa and others⁹ suggest that a kind of immune paralysis leads to bacterial infection and that the multiorgan involvement is explained by the high production of proinflammatory cytokines in the setting of a slowly developing systemic inflammatory response syndrome.

The main limitation of our study was the inability to perform the evaluation of urinary MCP-1 and MDA after treatment because of the small number of patients who have returned for a re-evaluation. Early biomarkers of renal damage are important factors that could assist clinical practice in preventing or attenuating the severity of kidney injury.

In summary, this preliminary data showed that VL patients present urinary concentration and acidification deficit, which can persist even after specific treatment. Urinary MCP-1 and MDA are elevated in patients with VL, which may suggest inflammation and incipient renal damage, although other classical markers, such as creatinine, are not changed. Additional studies are required for additional information, including the search for new markers of early tubuloglomerular injury in VL and other infectious and parasitic diseases.

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TABLE 4

Comparison of concentration and acidification test results between patients with VL before and after treatment

	VL group (before treatment; N = 5)	VL group (after treatment; N = 5)	P
U _{Osm} T ₀ (mOsm/L)	518 ± 51	557 ± 69	0.500
U _{Osm} T ₄ (mOsm/L)	581 ± 84	622 ± 89	0.686
U _{Osm} /P _{Osm} T ₀	1.77 ± 0.24	1.90 ± 0.25	0.500
U _{Osm} /P _{Osm} T ₄	1.96 ± 0.27	2.14 ± 0.31	0.345
pH: venous T ₀	7.35 ± 0.05	7.38 ± 0.02	0.593
pH: venous T ₄	7.33 ± 0.04	7.35 ± 0.02	0.593
HCO ₃ T ₀ (mEq/L)	20.0 ± 2.0	27.0 ± 1.0	0.109
HCO ₃ T ₄ (mEq/L)	21.0 ± 3.0	25.0 ± 2.0	0.068
U _{pH} T ₀	5.54 ± 0.42	5.49 ± 0.32	0.684
U _{pH} T ₄	5.61 ± 0.55	5.25 ± 0.53	0.715

Data are shown as means ± SDs. *P* < 0.05 was considered significant.

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