

Hormonal Disturbances in Visceral Leishmaniasis (Kala-Azar)

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Abstract. This study presents a cross-sectional analysis of the hormonal alterations of patients with visceral leishmaniasis. The diagnosis was established by the bone marrow aspiration and polymerase chain reaction test. Primary adrenal insufficiency was observed in 45.8% of patients; low aldosterone/renin plasma ratio in 69.4%; low daily urinary aldosterone excretion in 61.1%; and low transtubular potassium gradient in 68.0%. All patients had normal plasma antidiuretic hormone (ADH) concentrations, hyponatremia, and high urinary osmolality. Plasma parathyroid hormone was low in 63%; hypomagnesemia was present in 46.4%, and increased Mg^{++}_{EF} in 100%. Primary thyroid insufficiency was observed in 24.6%, and secondary thyroid insufficiency in 14.1%. Normal follicle-stimulating hormone plasma levels were present in 81.4%; high luteinizing hormone and low testosterone plasma levels in 58.2% of men. There are evidences of hypothalamus-pituitary-adrenal axis abnormalities, inappropriate aldosterone and ADH secretions, and presence of hypoparathyroidism, magnesium depletion, thyroid and testicular insufficiencies.

INTRODUCTION

Visceral leishmaniasis (VL) or kala-azar is a worldwide parasitic disease and in South America it is caused by the protozoa *Leishmania donovani*, sub-specie *chagasi*. In Brazil, VL manifests as a zoonosis with dogs, foxes, and some marsupials (*Dedelphis ambeventris*) as natural reservoirs. Visceral leishmaniasis has expanded greatly in recent years, taking place in large urban centers. It has an endemic character in 12 of the 27 states of Brazil, mostly in the Northeastern region where its prevalence is between 5% and 25%. In the state of Ceará, where the research was conducted, the incidence of kala-azar is 4.6%. In its mammalian host *Leishmania* survives within the severe environment of the phagolysosome and evades the defense mechanisms induced during the immune response. Kala-azar patients, primarily children and young adults, present with dark skin and brittle and dry hair of varying color tones in areas of their heads.¹ Low blood pressure levels are common during the progression of the disease, even in patients with signs of renal disease, such as renal functional alterations, proteinuria, and abnormalities of the urinary sediment.²

Histopathological studies have evidenced parasitism of endocrine glands, particularly the pituitary, the adrenal, the thyroid, and the sex glands.³ Puberal retardation among these patients is frequent in both sexes, a complication of long-term progression of the disease in young people. The testicles are frequently severely compromised with a reduction in overall size, fewer Sertoly cells, and scanty Leydig cells lacking spermatogenesis.⁴ Data show the necessity for an investigation of the main hormonal alterations in VL.

MATERIALS AND METHODS

This is a cross-sectional analysis of 72 patients with clinical and laboratorial diagnosis of chronic VL, admitted from January 2005 to February 2008 to the Hospital São José de Doenças Infecciosas and to the Hospital Geral de Fortaleza, in Fortaleza, Ceará, Brazil. All patients were adults, from rural

areas, and were natives of the state of Ceará. Sixty-one patients were male between 15 and 58 years of age, and 11 were female, between 16 and 32 years of age. They all provided informed consent. The selection criterion for the study was the diagnosis of VL through the identification of *Leishmania chagasi* in smears obtained from sternal bone marrow and by the identification of anti-*Leishmania* antibodies using polymerase chain reaction (PCR). All patients tested negative for human immunodeficiency virus (HIV). The presence of arterial hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg), diabetes mellitus, recurrent urinary tract infections, or previous history of renal disease were conditions for exclusion of the patients. No specific treatment (N-methylglucamine antimoniate) was administered before the investigation. The work did not include laboratorial control after the specific treatment. The control group was composed of 20 hospital workers, 17 were men between 16 and 54 years of age and three were women 16, 21, and 29 years of age. They were all submitted to the same protocol and received the same diet as the patients. They all gave informed consent. The study protocol was registered and approved by the Ethics Committees of both Hospital São José de Doenças Infecciosas and Hospital Geral de Fortaleza.

Clinical and laboratory parameters. The following parameters were investigated: history of chronic disease, heart failure, high blood pressure, diabetes mellitus, and drug use. All patients, at admission, presented with an impaired general state, weight loss, hepatosplenomegaly, daily fever, frequent diarrhea, anemia, leucopenia, and occasional edema of lower limbs; no patient had ascites. The rural origins of the patients and their low socio-economic status were the main factors of a diagnostic delay. The control group was evaluated at the hospital yearly and the subjects were considered clinically normal. They did not have a history of diabetes mellitus, renal pathology, or arterial hypertension.

Analytical methods. The patients and controls were kept on a general hospital diet for 5 days (sodium chloride intake between 7 and 10 g/day). The studies began at 7 AM after an overnight fast, with patients in the supine position for 12 h. All blood analyses were done in duplicates. For the electrolyte study, urine was collected daily in a polyethylene recipient using 5 mL of 5% thymol-isopropanol solution as a preservative. For aldosterone analysis, two 24-h urine collections were

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made with the addition of 20 mL of 50% hydrochloric acid. Blood samples were taken at 7 AM for plasma hormone determinations. The chemiluminescence method was used to measure ACTH, cortisol, intact parathyroid hormone molecule (PTH), thyroid-stimulating hormone (TSH), tri-iodothyronine (T_3), free thyroxine (T_4), and testosterone. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured by immunofluorometry. Plasma levels of renin, aldosterone, antidiuretic hormone (ADH), and daily urinary aldosterone excretion were measured by radioimmunoassay. For PTH determination, frozen syringes were used during blood sampling and serum was kept frozen until analysis. Sodium and potassium were measured in an AVL ion-selective photometer, ISE 9180 model (AVL Scientific Corporation, Roswell, GA). A Becman Analyzer II spectrophotometer (Toronto, Ontario) was used for creatinine, chloride, inorganic phosphate, and magnesium determinations. Phosphate was measured by the molybdenum reaction. Serum creatinine was determined by the alkaline picrate technique using Lloyd's reagent. While on a hospital diet, a daily urine sample was collected for osmolality, sodium, urea, and uric acid determinations. Creatinine clearance was determined in supine position and with previous fluid overload. Blood was collected initially for creatinine measurement. Patients then received 1,500 mL of water during 30 minutes and the bladder was emptied. The clearance procedure was timed and followed by three urine collection periods (1 hour each). The mean creatinine clearance was calculated if the difference between two consecutive periods was lower than 10%. Finally, creatinine clearance was adjusted to a 1.73-m² body surface area. The normal values for creatinine clearance were 97–137 mL/min/1.73 m² for men and 85–127 mL/min/1.73 m² for women.

Ionic calcium was measured with ion-selective electrode. Magnesium was measured by the xilidil blue method. Plasma ADH, serum sodium, serum osmolality, and first urine osmolality were measured simultaneously. Serum and urinary osmolalities were measured with an Osmette A osmometer (Precision Systems, Natick, MA). Transtubular potassium gradient was calculated by the relation: $U_{K^+}/U_{osm}/P_{osm}/P_{K^+}$. Maximal tubular phosphate reabsorption was calculated by the Bijvoet nomogram, with a normal range of $TmP_{O_4^-}$ /glomerular filtration rate (GFR) = 2.5 to 4.2 mg/100 mL of GFR.

Statistical analysis. Data were expressed as mean \pm SD; $P < 0.05$ was considered statistically significant. The patients were compared with a control group. Differences between two independent variables were evaluated using the student's *t* test or the Mann-Whitney test as appropriate. The analysis of variance (ANOVA) test was used to verify the statistical difference between patients with low creatinine clearance and patients with normal or increased GFR. The Games-Howell test was used to study the statistical difference between multiple comparisons of both groups. Both Epi Info 2002 (Centers for Disease Control and Prevention, Atlanta, GA) and SPSS 10.0 (SPSS Inc., Chicago, IL) were used in all analyses.

RESULTS

Male patients had a mean age of 24.6 ± 9.0 years (range 15–58 years) versus controls 28 ± 11 years (range 16–54 years), $P = 0.325$, and female patients had a mean age of 17.1 ± 8.0 years (range 16–32 years) versus controls 16.0 ± 13.0 years (range 16–29 years), $P = 0.152$ (Table 1). No pediatric

TABLE 1
Demographic and clinical data of kala-azar patients and controls

	Kala-azar		Controls	
	N = 61	N = 11	N = 17	N = 3
Sex	Male	Female	Male	Female
Age (years)	24.5 ± 9.0 †	19.7 ± 10.0 †	28.0 ± 11	16 ± 13
Systolic B; pressure (mm of Hg)	105.4 ± 10.1 ***	90.3 ± 12.3 †	120.0 ± 9.0	71.2 ± 6.0
Diastolic B; pressure (mm of Hg)	61.8 ± 10.4 **	52.7 ± 11.2 †	72.8 ± 5.4	65.0 ± 5.0
Weight (Kg)	51.0 ± 7.1 ***	39.6 ± 10.3 *	63.5 ± 11.6	60.0 ± 7.9

Results expressed as mean \pm SD; descriptive level: * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0001$.
†Not significant.

patient was included in this study. At the time of admission, all patients had weight loss and 41 patients (56.9%) had severe signs of malnutrition. Systolic and diastolic blood pressures were significantly lower in kala-azar patients than in the control group ($P < 0.0001$, $P < 0.005$, respectively). Supine blood pressure lower than 100/60 mmHg was detected in 40% of the patients; no patients had orthostatic hypotension. Male kala-azar patients had greater body weight loss ($P < 0.0001$) than female patients ($P < 0.05$) when compared with the respective control group. Because the diagnosis of kala-azar was made in the advanced stages of the disease, usually after weeks or months of daily fever and weight loss, the patients frequently had important signs of chronic disease.

Plasma ACTH (corticotrophin) was significantly higher in kala-azar patients, $P < 0.05$ (Table 2). Thirty-three patients (45.8%) had high ACTH plasma levels and normal plasma cortisol concentrations. Twenty-nine patients (40.2%) presented with both normal ACTH and normal cortisol plasma levels, whereas 16 patients (22.2%) had both high plasma ACTH and high plasma cortisol levels. Plasma cortisol was moderately higher in kala-azar patients (19.2 ± 6.1 versus 14.0 ± 3.9 μ g/dL, $P < 0.05$). No patient had plasma cortisol levels below the physiological range. Plasma renin activity was higher in kala-azar patients: 2.9 ± 3.1 versus 1.0 ± 0.2 ng/mL/h, $P < 0.05$. Twenty-eight patients (38.8%) had increased renin plasma levels (mean 5.5 ± 4.5 ng/mL/h), whereas plasma aldosterone concentrations remained within physiological range

TABLE 2
Laboratory findings in plasma and urine of kala-azar patients and controls

	Kala-azar	Controls
	N = 72	N = 20
ACTH (pg/mL)	39.8 ± 0.2 *	30.2 ± 0.3
Cortisol (μ g/dL)	19.9 ± 6.1 *	14.0 ± 3.9
Renin (ng/mL/h)	2.9 ± 3.1 *	1.0 ± 0.2
Aldosterone (ng/dL)	6.1 ± 3.6 †	5.5 ± 1.5
Aldosterone/Renin ratio	3.8 ± 4.9 **	5.8 ± 2.3
Sodium (mEq/L)	129.2 ± 4.1 ***	139.4 ± 1.6
Potassium (mEq/L)	3.7 ± 0.4 *	4.0 ± 0.3
ADH (pg/mL)	5.4 ± 1.0 †	5.1 ± 3.7
Osmolality (mOsm/kg H ₂ O)	269.6 ± 8.0 ***	290.2 ± 5.3
U. osmolality (mOsm/kg H ₂ O)	785.4 ± 139.2 *	859.3 ± 80.4
U. aldosterone (μ g/day)	5.7 ± 3.4 **	12.1 ± 3.2
U. sodium (mEq/L)	88.3 ± 40.1 *	110.3 ± 34.7
U. potassium (mEq/L)	24.2 ± 11.0 ***	39.4 ± 10.7
Transtubular potassium gradient	3.0 ± 1.1 **	5.5 ± 2.3
Cr. clearance (mL/min/1.73 m ²)	114.7 ± 31.2 †	110.4 ± 20.7

Descriptive level: U = urine; results are expressed as mean \pm SD. Significant * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0001$.
†Not significant.

(6.3 ± 3.7 ng/dL). Twenty-two patients (30.5%) had inappropriately low serum aldosterone levels (< 3 ng/dL) relative to their respective plasma renin concentrations. Plasma aldosterone was not significantly different in kala-azar patients and controls (6.1 ± 3.6 versus 5.5 ± 1.5 ng/dL, *P* = 0.3637). Eighteen patients (25.0%) had high plasma renin and high plasma aldosterone levels. A low aldosterone/renin plasma ratio was present in 50 kala-azar patients (69.4%) and was significantly different to that of controls (3.8 ± 4.9 versus 5.8 ± 2.3, *P* < 0.005). Daily urinary aldosterone excretion was significantly lower in kala-azar patients (*P* < 0.005). Forty-four patients (61.1%) had urinary aldosterone levels < 6 µg/day. No patient had increased urinary aldosterone excretion. The mean transtubular potassium gradient was low (< 4.0) in 49 kala-azar patients (68.0%) and significantly different to the controls (3.0 ± 1.1 versus 5.5 ± 2.3, *P* < 0.005).

All kala-azar patients had hyponatremia (*P* < 0.0001) and urinary sodium excretion > 40 mEq/L. Even though urinary sodium excretion was lower in kala-azar patients than in the control group (88.3 ± 40.1 versus 110.3 ± 34.7 mEq/L, *P* < 0.05), it was sufficiently high to exclude a state of volume depletion.

Plasmatic ADH, measured simultaneously with the first urine osmolality, was not significantly different among kala-azar patients and the controls (*P* = 0.3859). All kala-azar patients had a plasma osmolality significantly lower than the control group (*P* < 0.0001), and an inappropriately high first morning urine osmolality.

Serum potassium was significantly lower in kala-azar patients (*P* < 0.05). Hypokalemia (K⁺ < 3.5 mEq/L) and inappropriately increased urinary potassium excretion fraction were observed in 26.4% of patients. Urinary potassium excretion of kala-azar patients was also significantly lower than that of the control group (*P* < 0.0001).

Plasma PTH was significantly lower in kala-azar patients (10.6 ± 6.9 versus 32.1 ± 10.5 pg/mL, *P* < 0.0001), (Table 3). Plasma PTH was < 12 pg/mL in 63% of the patients. Hypomagnesemia and low PTH levels were present in 37% of patients. Serum magnesium was significantly lower in kala-azar patients (1.8 ± 0.3 versus 2.2 ± 0.1 mg/dL, *P* < 0.005), with hypomagnesemia (< 1.8 mg/dL) present in 46.4% of them. All kala-azar patients had increased daily urinary magnesium excretion (*P* < 0.05) and a higher urinary magnesium excretion fraction compared with the controls (*P* < 0.05).

TABLE 3

Laboratory findings in plasma and urine of kala-azar patients and controls

	Kala-azar	Controls
	N = 72	N = 20
Parathormone (pg/mL)	10.6 ± 6.9***	32.1 ± 10.5
Total calcium (mg/dL)	8.5 ± 0.7***	9.9 ± 0.3
Ionic calcium (mEq/L)	1.0 ± 0.1*	1.2 ± 0.0
Albumin (g/dL)	2.5 ± 0.7***	4.6 ± 0.5
Globulins (g/dL)	5.3 ± 1.9***	3.1 ± 0.9
Phosphate (mg/dL)	3.5 ± 0.7†	3.6 ± 0.5
TmP _{O4} ⁻ /GFR mg.100 mL	3.4 ± 0.8†	3.2 ± 1.8
Magnesium (mg/dL)	1.8 ± 0.3**	2.2 ± 0.1
U. calcium (mg/day)	156.7 ± 81.6†	179.6 ± 51.0
U. phosphate (mg/day)	565.8 ± 250.3*	734.6 ± 190.5
U. magnesium (mg/day)	103.6 ± 54.5†	99.7 ± 20.1
Magnesium _{EF} (%)	4.8 ± 3.3*	3.1 ± 1.0

Descriptive level: U = urine; results are expressed as mean ± SD. Significant * *P* < 0.05, ** *P* < 0.005, *** *P* < 0.0001. † Not significant.

Decreased total serum calcium was observed in all kala-azar patients. Ionic serum calcium was decreased in 34% of the patients. Daily urinary calcium excretion of kala-azar patients showed no significant difference to the controls (*P* = 0.6216), but hypercalciuria (U_{Ca²⁺}/U_{Cr} > 0.2) was observed in 26.4% of the patients.

Serum inorganic phosphate was not significantly different, but kala-azar patients had lower daily urinary phosphate excretion (*P* < 0.05). By using the nomogram for derivation of the renal phosphate concentration threshold, maximal tubular phosphate reabsorption (TmP_{O4}⁻/GFR) was decreased in 26.4% of patients (< 2.5 mg/100 mL GFR), but increased in 20.0% of them (> 4.2 mg/100 mL GFR). Maximal tubular phosphate reabsorption was in the normal range in 33.6% of patients.

Normal creatinine clearance values (85–130 mL/min/1.73 m²) were observed in 47.3% of kala-azar patients. Low creatinine clearance was found in 17 patients (23.6%), ranging from 49 to 74 mL/min/1.73 m². Creatinine clearance was higher than 130 mL/min/1.73 m² in 29.1% of kala-azar patients. Patients with low creatinine clearance (*N* = 17) were compared with kala-azar patients with normal or increased renal function (*N* = 55) using the ANOVA test. There were statistical differences between both groups for the following parameters: Ccr (64.3 ± 8.7 versus 115.5 ± 22.0 mL/min/1.73 m², *P* < 0.0001); PTH (12.2 ± 6.9 versus 13.7 ± 8.0 pg/mL, *P* < 0.0001); cortisol (28.0 ± 5.8 versus 21.0 ± 6.0 µg/dL, *P* < 0.0001); U_{Ca²⁺} (91.1 ± 35.4 versus 180.6 ± 127.0 mg/day, *P* < 0.0005). By using the Games-Howell test, patients with low creatinine clearances showed a correlation between plasma PTH levels and daily urinary calcium excretion (U_{Ca²⁺}), *P* < 0.005. In patients with normal or increased glomerular filtration rate there was a correlation between Ccr and TmP_{O4}⁻.

Thyroid-stimulating hormone plasma levels of kala-azar patients (Table 4) were not statistically different to the controls (*P* = 0.6274). Increased plasma TSH levels and decreased plasma T₃ and free-T₄ concentrations were found in 24.6% of patients. Low TSH, low T₃, and low free-T₄ plasma levels were present in 14.1% of patients. Plasma tri-iodothyronine and plasma-free thyroxine were significantly lower in 32.0% of patients compared with the control group (*P* < 0.0001, *P* < 0.05, respectively). No patient had increased T₃ and free-T₄ plasma levels.

The FSH was not significantly different in kala-azar patients (*P* = 0.5) and remained within the normal range in 81.4% of the patients. Increased FSH plasma levels were observed in 18.6% of patients. The LH plasma levels were not significantly

TABLE 4

Laboratory findings in plasma of kala-azar patients and controls

	Kala-azar	Controls
	N = 72	N = 20
TSH (µIU/mL)	2.8 ± 2.1†	2.5 ± 2.0
T ₃ (ng/mL)	81.2 ± 26.1*	88.6 ± 12.5
Free-T ₄ (ng/dL)	1.2 ± 0.6†	1.5 ± 2.5
FSH (U/L)	6.1 ± 5.5†	5.2 ± 1.5
LH (U/L)	7.2 ± 7.4†	6.8 ± 1.9
Testosterone man (pg/mL)	3011 ± 2074***	7461.6 ± 852.1
Testosterone woman (pg/mL)	134.2 ± 80.2***	376.0 ± 78.8

Descriptive level: IU = international units; results are expressed as mean ± SD. Significant * *P* < 0.05, *** *P* < 0.0001.

† Not significant.

TSH = thyroid-stimulating hormone; FSH = follicle-stimulating hormone; LH = luteinizing hormone.

different in kala-azar patients ($P = 0.0853$). Increased plasma LH was observed in 21.8% and low testosterone plasma levels ($< 2,410$ pg/mL) were observed in 58.2% of patients. Normal plasma LH and normal plasma testosterone concentrations were present in 41.8% of patients. Testosterone plasma levels of kala-azar patients were significantly lower than that of the control group ($P < 0.0001$). All women had significantly lower plasma testosterone levels than the controls ($P < 0.0001$).

DISCUSSION

Patients with chronic forms of kala-azar frequently present with anemia, hepatosplenomegaly, weight loss, malnutrition, and daily fever, because of the prolonged stress situation resulting from the systemic parasitic infection and widespread inflammatory process involving vital organs.

Long-standing stress situations are characterized by increased cortisol secretion under pituitary stimulus,⁵ inhibitions of thyrotropin release, low T_3 , and T_4 plasma levels,^{6,7} and interference with the regulation of gonadotropin secretion in both sexes.⁸

Evidences of hypothalamus-pituitary-adrenal axis dysfunction were observed in nearly half of the patients, presenting with increased ACTH plasma levels and normal cortisol plasma concentrations. The inability to appropriately elevate the levels of plasma cortisol under ACTH stimulus is characteristic of a state of primary adrenal insufficiency.⁹ Although kala-azar patients show significantly higher cortisol plasma levels, the values may be considered very moderate when compared with plasma cortisol levels of patients with other similar chronic infectious diseases. These patients have plasma renin and cortisol concentrations many times higher than that observed in kala-azar patients.¹⁰ Kala-azar patients with lower glomerular filtration rates (< 85 mL/min) had higher plasma cortisol concentrations than those patients with normal or increased creatinine clearances. This finding is perhaps caused by a more advanced stage of the disease and a more stressful situation. Plasma levels of ACTH and cortisol within the physiological range do not necessarily exclude a dysfunction of the hypothalamus-pituitary-adrenal axis, particularly because they are observed in patients with chronic debilitating diseases, where increased values for these hormones are already expected.

Most kala-azar patients presented with moderately increased plasma renin concentrations, without elevation of aldosterone plasma levels and daily urinary aldosterone excretion. The aldosterone/renin plasma ratio was lower than in the control group, suggesting inappropriate adrenal aldosterone production in kala-azar patients. This hormonal pattern has been described in chronic stress situations and hospitalized critically ill patients.¹¹

Low transtubular potassium gradient values suggest decreased aldosterone activity at the distal convoluted tubules and collecting ducts¹² and are in accordance with the decreased aldosterone plasma values and low daily urinary aldosterone excretion. Some prolonged stressful situations can trigger a complex endocrine response, with high plasmatic renin concentrations and no increase of aldosterone levels.^{13,14}

Chronic body potassium depletion may have an important inhibitory effect on the secretion of aldosterone by the zona glomerulosa of the adrenal glands.^{15,16} Parasitism and atrophy of the cortical layers of the adrenal glands, especially of the zona glomerulosa, have been observed in histological studies,

and can exert an additional influence in the adrenal response resulting in low aldosterone production even after renin and hypothalamus-pituitary-adrenal axis stimuli.⁴

Kala-azar patients presented with hyponatremia, plasma hypo-osmolality, persistent high urinary concentration capacity, and normal ADH plasma levels, all characteristic features of the syndrome of inappropriate ADH secretion.¹⁷ This incapacity of free water formation determines an expansion of body hydric space, clinically manifested by hyponatremia and hyposmolality.^{18,19}

The presence of anemia, hyponatremia, hypoalbuminemia, and the relatively frequent episodes of diarrhea and vomiting in kala-azar patients would suggest intravascular volume contraction.² Nevertheless, these patients have high sodium urinary excretion, excluding a renal sodium conservation situation.¹⁷ Evidence of body water expansion has been observed during the progression of the disease.^{20,21}

Two-thirds of the patients presented with low parathormone (PTH) plasma levels. Lower levels of PTH were observed in the plasma of patients with moderately decreased glomerular filtration rates, but no difference was observed between the serum magnesium concentration of these patients and that of patients with normal renal function. A dysfunction in the secretion of PTH is frequently observed in patients with hypomagnesemia and intracellular magnesium depletion.^{22,23} All studied kala-azar patients presented with an increased urinary magnesium excretion fraction, indicating renal loss of magnesium.²⁴ These patients present some aspects that contribute to intensifying the hypomagnesemia and body magnesium depletion: 1) the inflammatory parasitic process at the jejunum and ileum and frequent diarrheic episodes²⁴; 2) the presence of interstitial nephritis with an inflammatory infiltrate of the renal cortex and medulla reducing the tubular reabsorption of magnesium²⁵; and 3) the volume expansion state with a reduction of the reabsorption of sodium, potassium, calcium, and magnesium at the thick ascendant loop.^{25,26}

Hypocalcemia is mainly caused by a reduction in the fraction of albumin bound to calcium in Kala-azar patients. Low PTH levels may be responsible for the decreased ionic calcium concentrations found in the plasma of these patients. Impaired PTH bone response, reduced calcium bone removal, magnesium depletion, hypercalciuria, and the reduction of intestinal calcium absorption in patients with diarrhea are important factors that contribute to the reduction in ionic calcium plasma levels.²⁷

A third of the patients presented with an increased GFR, a result of the expanded water space. An increase in glomerular ultrafiltration load to the proximal tubule may contribute to hypercalciuria and increased urinary excretion of magnesium, potassium, and calcium.²⁸

The patients also presented with reduced urinary phosphate excretion, which can be a consequence of lower daily food ingestion or decreased PTH secretion. Employing the Bjvoet nomogram,²⁹ one-fourth of kala-azar patients have an increased maximal tubular reabsorption of phosphate, which may indicate a response of the renal tubules to the lower PTH levels and/or a lack of response to it. Chronic mixed alkalosis, respiratory, and metabolic has been described in 75.5% of a group of 55 kala-azar patients.² Chronic respiratory alkalosis may contribute to an increased maximal renal tubular reabsorption of phosphate, hypercalciuria, and defective PTH secretion.³⁰

A direct correlation between TmP_{04}^- and creatinine clearance values was observed in patients with normal and increased GFRs. Reduction of the maximal tubular phosphate reabsorption rate may result from the effect of body water expansion at the proximal tubule.

As part of the inflammatory and immune responses of *Leishmania*-infected cells, particularly the macrophages, are responsible for increased secretion of arachidonic acid metabolites and the synthesis of prostaglandins.^{31,32} The systemic inflammatory state and the presence of interstitial nephritis act as important sources of prostaglandin and nitric oxide secretions, resulting in arterial hypotension, increased medullary blood flow, elevated interstitial pressure, and decreased renal tubular reabsorption of sodium, potassium, calcium, and magnesium in these patients.^{33,34}

Characteristics of primary thyroid insufficiency were observed in patients with high TSH, and low T_3 and free- T_4 plasmatic concentrations. Evidences of primary pituitary hypofunction and secondary hypothyroidism were observed in patients with low TSH and low free- T_4 plasma levels.

Thyroid and reproductive axis dysfunctions have been detected as part of an endocrine response to stress, with a reduction in thyroid hormone production and development of gonadotropic hypogonadism.³⁵

Although there is no data regarding the spermatogenesis of kala-azar patients, normal FSH plasma levels are found in the majority of them suggesting a secondary gonadal insufficiency. Elevation of FSH levels observed in kala-azar patients may be evidence of damage to the seminiferous tubules, deregulation of spermatogenesis, and lack of response to pituitary gonadotropic stimuli. High LH levels and low plasmatic testosterone concentrations were found in more than half of the male patients, indicating primary gonadal insufficiency. Testicular parasitism and malnutrition may also contribute to lower testosterone production. Low plasmatic testosterone levels were also found in female patients. Normal levels of LH and testosterone in plasma do not exclude a dysfunction of the pituitary-gonadal axis.

Malnutrition is a common aspect among these patients, despite preserving a healthy appetite during the entire course of the disease.¹ Daily urinary phosphate excretion, a result of food intake, showed no difference between kala-azar patients and controls.²

The presence of caloric-proteic malnutrition may contribute to the reduction of thyroid hormone production by suppressing TSH secretion.⁶ High LH and FSH plasma levels and low testosterone plasma concentrations may be present in malnourished patients.³⁶

The hormonal alterations in kala-azar patients may be reproduced experimentally by the administration of inflammatory cytokines: interleukin-1 (Il-1), interleukin-2 (Il-2), interleukin-6 (Il-6), and tumor necrosis factor (TNF- α). The widespread inflammatory process of the disease produces cytokines,³⁷ whose administration to laboratory animals and human subjects are potent stimulus to the hypothalamic-pituitary-adrenal axis, with an increased production of ACTH and cortisol, while suppressing both the hypothalamic-pituitary-thyroid and gonadal axis. Suppression of the production of aldosterone and stimulation of renin secretion are observed after administration of Il-1 and TNF- α .³⁸⁻⁴⁰

The Il-6 acts stimulating the secretion of corticotrophin-releasing hormone by the hypothalamus, and the production

of arginine vasopressin by the posterior pituitary through activation of the magnocellular nuclei.⁴¹⁻⁴³

CONCLUSION

Endocrine system alterations are evident in patients with the chronic form of kala-azar. Signs of dysfunction are observed in ADH secretion by the posterior pituitary, and in hypothalamus-pituitary-adrenal, pituitary-thyroid, and pituitary-gonadal axis. The PTH secretion can be affected by magnesium depletion. Anemia, reduction in systemic blood pressure, and decreased blood glandular circulation may influence glandular metabolic activities. The inflammatory status may alter water and electrolyte excretion. Hypoalbuminemia and hepatic dysfunction are important features of low total plasma hormone concentrations.

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