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BRIEF COMMUNICATION

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Potential role of zinc in the visceromegaly regression and recovery of hematological parameters during treatment of visceral leishmaniasis in children from an endemic area

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ABSTRACT

Leishmaniasis is a disease complex with various clinical symptoms caused by different species of parasites of the genus Leishmania. The visceral form of the disease, characterized by severe symptoms is fatal, if not treated. The high toxicity of current antileishmanial drugs and the need for long-term treatment make the therapy complicated, especially in a large number of infected children. Hence, the search for new therapies must be intensified. Oral administration of the trace element zinc has been considered in alternative treatments against different clinical forms of leishmaniasis. This study revealed that the administration of zinc in children with visceral leishmaniasis, during treatment with amphotericin B or glucantime, accelerates the regression of the spleen enlargement without interfering with the recovery of hematological parameters.

KEYWORDS: Zinc. Visceral leishmaniasis. Splenomegaly. Leishmaniasis treatment.

INTRODUCTION

Visceral leishmaniasis (VL) is a zoonotic infectious disease which is caused in Americas by the protozoan parasite *Leishmania infantum* (*L. infantum*) transmitted through the bite of female infected phlebotomine sand flies *Lutzomyia longipalpis* (*L. longipalpis*)[1]. According to the World Health Organization (WHO), VL causes more than 20,000 deaths each year worldwide[2]. Among symptomatic patients, the main clinical signals of VL include intermittent fever, weight loss, adenopathy, anemia and substantial enlargement of the spleen and liver. In advanced stages of the disease, a consequence of the increase in the spleen and liver is intense abdominal distention and pain[3]. If patients are left untreated, the mortality rate can reach 100% within 2 years[4].

Despite the high toxicity, the need for intravenous administration and the need for hospitalization and monitoring during treatment, the antileishmanial drugs amphotericin B and glucantime are used as first-line drugs in several countries[5,6]. Nowadays, many studies are conducted to determine alternative drugs to treat neglected diseases with lower toxicity and costs and to identify methods to increase the efficacy of current drugs, decreasing the side effects[7,8]. The efficacy of the trace element zinc (Zn) has been tested in a murine model of infection by *L. major*[9] and in human cases of cutaneous leishmaniasis[10]. Since Zn plays an essential role in improving the nutritional and immunological status[11], considered crucial

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parameters for the clinical progression of patients with VL in response to treatment, in this study we evaluated the role of zinc supplementation, combined with conventional antileishmanial therapy, in the regression of hepatomegaly and splenomegaly and recovery of hematological parameters in children with VL.

PATIENTS AND METHODS

Patients and Ethics Statement

Fifty-two patients with VL and fifteen healthy children aged 9–144 months were recruited to this study from the Hospital Maria Aparecida Pedrossian – HUMAP, Universidade Federal do Mato Grosso do Sul, Campo Grande, MS, Brazil. VL was suspected in patients with fever and splenomegaly. Diagnosis was confirmed through detection of amastigote forms of the parasite in bone marrow aspiration samples. The healthy children included in this study were recruited during pediatric queries for routine examination. Patients with other diseases were excluded. Prior to the recruitment of patients, the study was approved by the Research Ethics Committee of the University (Protocol Nº 2207/0305.0.049.000-11). Written informed consents were obtained from parents or legal guardians of the minors.

Treatment and zinc supplementation

All patients were treated with amphotericin B (0.5–1 mg/kg/day administered intravenously) or meglumine antimoniate (glucantime®) (20 mg/kg/day administered intravenously) for 20 days. During treatment, patients were divided into two groups: one group received conventional treatment alone, while the other group received both conventional treatment and a syrup containing zinc in the form of a chelate solution (10 mg/ml). The group received a total dose of 2 mg/kg/day in 14 days.

Plasma zinc analysis

On days 1, 7 and 14 of treatment, blood samples were collected in polypropylene syringes and immediately transferred to vacuum tubes free of trace elements (BD Vacutainer Systems). Plasma samples were separated by centrifugation and stored at -20 °C for analytical analysis. All plastic or glass materials were previously treated for decontamination. The calibration curves were constructed using four different zinc concentrations with values ranging from 0.25-2.0 mg/L. Atomic absorption analyses for plasma zinc were performed using a Perkin Elmer AA100 spectrometer (Waltham, MA, USA).

Liver and spleen measurement

On days 1, 7 and 14 of treatment, liver and the spleen enlargement were measured using percussion methods and a flexible measuring tape. Measurements for all patients were performed by the same researcher during the entire study period. The liver was measured at the level of the right hemiclavicular line, and the spleen was measured at the level of the transverse line drawn perpendicular to the left costal margin. The longitudinal and transversal lengths of the spleen were used to calculate the area of the organ using the formula of an elliptic area.

Classification of the nutritional status

The nutritional status was classified based on the World Health Organization (WHO) weight-for-age growth charts[12] used for monitoring the growth of children.

Serum albumin quantification

On days 1, 7 and 14 after the beginning of treatment, blood samples were collected in collection tubes (BD microtainer®), serum samples were separated by centrifugation and the albumin levels were measured using a specific kit (ALB2 Roche®) according to the manufacturer's instructions.

Total blood count

On days 1, 7 and 14 after the beginning of treatment, blood samples were collected intravenously from all patients using specific blood collection tubes (BD microtainer®). Hematologic parameters were assessed in a Sysmex XN3000™ hematology analyzer.

Statistical analysis

Data were expressed as means ± SEM. The statistical significances between different time points were calculated using the Student's t test. All analyses were performed using the PRISM 5.0 software. Results were considered statistically different when the p value was < 0.05.

RESULTS AND DISCUSSION

Before the beginning of treatment, plasma Zn levels in all patients was measured (Figure 1A). Some studies showed that plasma Zn levels in patients with VL were lower than those of healthy individuals[13,14]. Our results have shown that plasma Zn levels were lower in patients supplemented with

Zn than in patients who did not receive Zn supplementation, despite this, none of the groups presented plasmatic Zn levels lower than healthy children (Figure 1A). Even when Zn levels are within the normal range, patients can receive the mineral supplementation as a preventive measure[15]. Moreover, less than 0.2% of the total body Zn content circulates are present in plasma samples[16]; hence, it may not be a reliable indicator to assess the Zn levels in tissues. The supplementation of Zn was performed in a blind manner, and the quantification of the trace element on days 1, 7 and 14 after the beginning of supplementation showed that plasma Zn levels gradually increased (Figure 1B), while it remained unchanged in patients who did not receive Zn supplementation (Figure 1C).

The mean age and gender were not significantly different between children who received Zn supplementation and those who did not receive Zn supplementation during treatment of VL (Table 1). Previous studies showed that

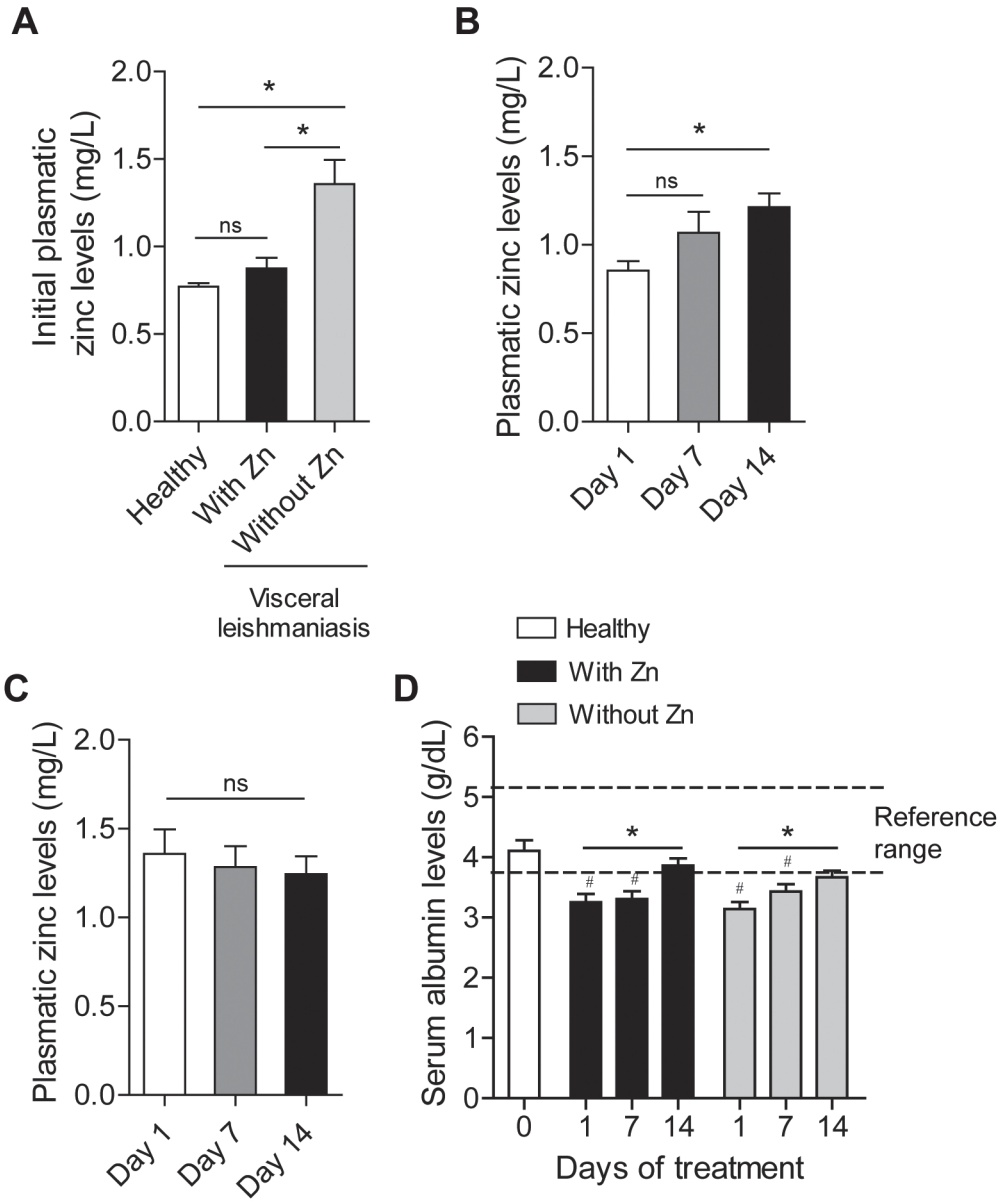

Figure 1 - A) Initial plasma zinc levels quantificated in healthy children (n=15) and in patients with visceral leishmaniasis before the beginning of zinc supplementation (n=23) or in those who did not receive zinc supplementation (n=29); B) Plasmatic zinc levels quantificated on days 1, 7, and 14 of zinc supplementation and treatment with amphotericin B or glucantime in patients with visceral leishmaniasis (n=23); C) Plasmatic zinc levels quantificated on days 1, 7, and 14 of treatment with amphotericin B or glucantime in patients with visceral leishmaniasis who did not receive zinc supplementation (n=29); D) Serum albumin levels of healthy children and patients with visceral leishmaniasis with and without zinc supplementation on days 1, 7, and 14 of treatment with amphotericin B or glucantime. The reference interval was delimited by dashed lines according to the values proposed by the kit manufacturer. *$p< 0.05$ in A, B and D, #$p< 0.05$ compared with healthy controls in D and ns=not statistically different in A–C

Table 1 - Age, gender and nutritional status of patients with VL and healthy children enrolled in the study

	With Zn	Without Zn	Healthy
Mean age (months)	46.20 ± 9.66	43.76 ± 6.50	44.60 ± 10.20
Male	n=11	n=11	n=06
Female	n=12	n=18	n=09
Nutritional status		Number/Total (%)	
Overweight	1/23 (4.35)	1/29 (3.45)	0
Overweight risk	2/23 (8.69)	3/29 (10.34)	0
Normal nutrition	18/23 (78.26)	24/29 (82.76)	15/15 (100)
Nutritional risk	1/23 (4.35)	1/29 (3.45)	0
Malnutrition	1/23 (4.35)	0	0

VL in Brazil has a great incidence in children[17,18]. Although literature suggests predominance of the disease in male gender[19], our data are in accordance with the results of Queiroz *et al.*'s study[20], who found the same distribution of the disease between children of both genders. An important risk factor of severe VL is malnutrition[21]. In this study, most of the recruited patients had normal nutritional status based on the weight-for-age indicator in the WHO growth charts (Table 1). Although serum albumin levels were used to evaluate the nutritional status, albumin levels are not specific to detect malnutrition and can be affected by some factors, such as inflammation[22]. Similarly to the results obtained by Gatto *et al*.[23], here we showed that serum albumin is slightly reduced during VL and the treatment of children, whether using amphotericin B or glucantime alone or in combination with Zn supplementation, increases serum albumin to normal levels (Figure 1D).

The earliest signs of improvement in the condition of patients with VL are the regression of clinical symptoms and splenomegaly. This study revealed that patients who received Zn supplementation during treatment with amphotericin B or glucantime showed rapid reduction in the spleen size compared with those patients who did not receive Zn (Figure 2A). On day 7 of treatment, patients supplemented with Zn had a significant reduction in the area of spleen, while patients without Zn supplementation presented considerable regression of splenomegaly only 20 days after the beginning of treatment (Figure 2A). The reduction of hepatomegaly was also evaluated in patients. On day 7 of treatment, patients supplemented with Zn presented a slight reduction in the liver size (Figure 2B); however, a significant reduction occurred in both groups of patients on day 14 of treatment (Figure 2B). In our model, we found that Zn supplementation only accelerates the splenomegaly regression but not the hepatomegaly in patients with VL. However, we believe that these data are still interesting as most studies showed that splenomegaly is more commonly observed than hepatomegaly among patients with VL. Enlargement of spleen is reported in about 90% of patients[24], while the liver involvement is observed in less than 30% of patients[25].

During the treatment of VL, recovery of hematological parameters were among the signs that indicated an improvement in patients' conditions[26]; hence, in this study, leukocytes and hemoglobin levels were evaluated in blood samples of patients. Treatment with amphotericin B or glucantime increased leukocyte and hemoglobin levels in both groups (Figures 2C and 2D). Moreover, Zn supplementation did not interfere with hematological parameters of patients during treatment of VL (Figures 2C and 2D). Our data are not in agreement with those of Dunchateau *et al.*[27], who showed that Zn supplementation increased the circulating number of T lymphocytes in elderly people. This disagreement can be explained by the high concentration of Zn administrated by Dunchateau *et al.*[27] (400 mg/day) and because elderly patients were used in that study. On contrary, the results of our study are in accordance with those of Bonham *et al.*[28] who found that supplementation with 30 mg/kg/day of Zn in adult healthy men had no effect on the circulating levels of peripheral blood leukocytes subsets.

Previous and recent studies have shown that, although both antileishmanial drugs are effective, children treated with amphotericin B showed faster clinical improvement and shorter hospital stay than those children treated with glucantime[29,30]. The main advantage of glucantime compared with amphotericin B is the lower cost, for this reason most patients in our study were treated with glucantime. Only a few patients received amphotericin B treatment, thus, we could not evaluate whether the choice of drug interfered with the results. Future studies are necessary to evaluate whether Zn supplementation has different results when used with amphotericin B or with glucantime alone.

Our data corroborate the importance of zinc supplementation favoring the regression of splenomegaly

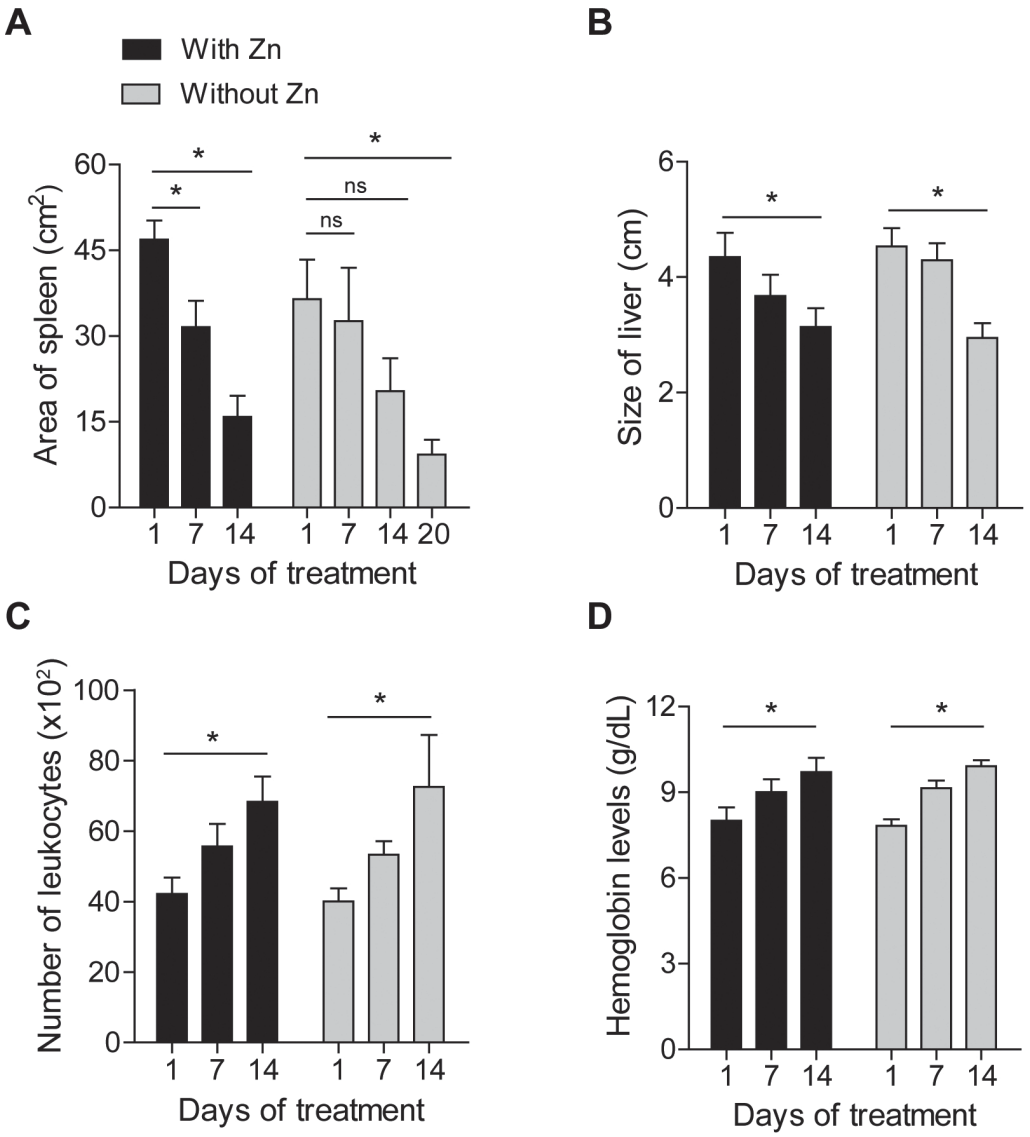

Figure 2 - A) Area of spleen; B) size of liver; C) number of leukocytes; D) hemoglobin levels evaluated in patients with visceral leishmaniasis with (n=23) or without zinc supplementation (n=29) during treatment with amphotericin B or glucantime on days 1, 7, and 14 of treatment. *p< 0.05 and ns=not statistically different

in children during treatment of VL. These findings open new perspectives to increase the effectiveness of the current treatment of VL.

CONFLICT OF INTERESTS

The authors declare that the research was conducted without conflicts of interest.

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AUTHORS' CONTRIBUTIONS

Conceived and designed the experiments: DCBC, LZGZ, MCS; patient's recruitment and collection of human samples: DCBC, LZGZ; writing – original draft: MCS, DCBC; writing – review and editing: DCBC, LZGZ, FZC,

SCS, VQR, KTCM, CMEC, MCS; analyzed the data: MCS, DCBC; statistics: MCS.

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