Biological Trace Element Research
https://doi.org/10.1007/s12011-020-02505-0

Leishmaniasis and Trace Element Alterations: a Systematic Review

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Received: 6 October 2020 / Accepted: 17 November 2020

Abstract

Leishmaniasis is a worldwide prevalent parasitic infection caused by different species of the genus *Leishmania*. Clinically, the disease divided into three main forms, including visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL). There is no vaccine for human leishmaniasis and their treatment is challenging. Trace elements (TEs) alteration, including the selenium (Se), zinc (Zn), copper (Cu), ron (Fe), and magnesium (Mg) have been detected in patients with CL and VL as well as canine leishmaniasis. Because TEs play a pivotal role in the immune system, and host immune responses have crucial roles in defense against leishmaniasis, this systematic review aimed to summarize data regarding TEs alteration in human and animal leishmaniasis as well as the role of these elements as an adjuvant for treatment of leishmaniasis. In a setting of systematic review, we found 29 eligible articles (any date until October 1, 2020) regarding TEs in human CL ($N = 12$), human VL ($N = 4$), canine leishmaniasis ($N = 3$), and treatment of leishmaniasis based on TEs ($N = 11$), which one study examined the TEs level both in CL and VL patients. Our analysis demonstrated a significantly decreased level of Fe, Zn, and Se among human CL and canine leishmaniasis, and Zn and Fe in patients with VL. In contrast, an increased level of Cu in CL patients and Cu and Mg in VL patients and canine leishmaniasis was observed. Treatment of CL based zinc supplementation revealed enhancement of wound healing and diminished scar formation in human and experimentally infected animals. The results of this systematic review indicate that the TEs have important roles in leishmaniasis, which could be assessed as a prognosis factor in this disease. It is suggested that TEs could be prescribed as an adjuvant for the treatment of CL and VL patients.

Keywords Leishmaniasis · Trace elements · Zinc · Iron · Selenium · Copper · Magnesium

Introduction

Leishmaniasis is a zoonotic disease caused by an obligate intracellular protozoan parasites of the genus *Leishmania* [1, 2]. This parasite is transmitted through the bite of the sand fly vectors to mammalian hosts, including humans [3]. The three main clinical patterns of the disease in the human host include visceral leishmaniasis (VL), cutaneous leishmaniasis (CL),

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Published online: 06 January 2021

and mucocutaneous leishmaniasis (MCL) [4, 5]. According to the World Health Organization (WHO) reports, the annual incidence of CL and VL is estimated at (600 000 to 1 million) and (50 000 to 90 000) new cases worldwide, respectively (https://www.who.int/news-room/fact-sheets/detail/leishmaniasis). Moreover, over 90% of MCL cases occur in Bolivia, Brazil, Ethiopia, and Peru (https://www.who.int/news-room/fact-sheets/detail/leishmaniasis).

Host immune responses play a pivotal role in protecting and pathogenesis against leishmaniasis, while an immunoprotected response to leishmaniasis required a potent T helper 1 (Th1) cells development and production of pro-inflammatory cytokines, such as interleukin (IL)-2, IL-12, gamma interferon (IFN-γ), and tumor necrosis factor (TNF)-α. Activation of this pathway alongside with macrophages and neutrophils activations leads to parasite killing and protection against the infection [6–9]. Other factors, such as co-infection with other infectious agents (i.e., helminth infection), biochemical and physiological disorders, lead to changes in this response and can increase the duration of treatment and the severity of the leishmaniasis [10, 11]. IFN-γ and TNF-α play a bifunctional role in immunoprotection and immunopathology of leishmaniasis [8, 12]. IFN-γ is mainly produced by both CD4+ and CD8+ T cells and natural killer (NK) cells. TNF-α is mostly secreted by macrophages. IFN-γ and TNF-α increase in macrophage activity to secrete nitric oxide (NO) against *Leishmania* parasite [8, 12]. Moreover, IFN-γ augments the differentiation of CD4+ T cells to the Th1 subset and impedes the expansion of Th2 and Th17 cells [8, 13]. Lack of IFN-γ increases susceptibility to human CL and VL [14]. In animal models, lack of IFN-γ resulted in susceptibility to *L. amazonensis* with increased in parasite burden, larger lesions, and expansion of Th2-type immune responses and their anti-inflammatory cytokine IL-4 than wild-type mice [15]. IFN-γ was used as an adjuvant for treatment of human CL [16] and VL [17]. Treatment of VL patients with recombinant human IFN-γ and pentavalent antimony was effective in seriously ill patients who were unresponsive to pentavalent antimony alone [17]. TNF-α promotes Th1-type immunity expansion and IFN-γ production against *L. major* infection [18], while lack of TNF-α in C57BL/6 mice (which are resistant to *Leishmania* infection) resulted in fatal visceral infection despite the production of IL-12 and IFN-γ by macrophages [19]. Neutralization of TNF-α receptor 1 in resistant C57BL/6 mice resulted in non-healing lesions following *L. major* infection [20]. By the way, susceptibility to leishmaniasis is linked to the Th2 pathway and their anti-inflammatory cytokines (e.g., IL-4, IL-5, and/or IL-13), which lead to parasite persistence and replication [6–9]. Furthermore, overstimulation of Th1 cells alongside with Th17 (which is a highly inflammatory cell) resulted in severe immunopathology [8, 21]. Therefore, a balance between type 1 and type 2 immunity is required for successful immunoprotection against the parasite with minimal side-effects [8, 9].

Trace elements (TEs) such as sulfur, iron, chlorine, cobalt, copper, zinc, manganese, molybdenum, iodine, and selenium have a broad range of metabolic and physiological processes in the human body [22]. Alteration (deficiency or excess) in serum levels of TEs can modulate various activities of the immune system, such as antibody production, T-cells proliferation to mitogen, B-cells activity, and natural killer cells (NK cells) [23, 24]. In this regard, low or high level of these elements in comparison with the optimal level can lead to increased susceptibility to infectious diseases [24].

Since the development of *Leishmania* infection and their severity depends on the status of the immune system, maintaining the optimum level of TEs in residents of endemic areas is important. On the other hand, there are numerous epidemiologic and experimental evidences to suggest that *Leishmania* infection is a risk factor for the changes of TEs. As well, TEs deficiency may be a risk factor in the severity of *Leishmania* infection [25, 26]. However, there is a lack of a systematic review paper to understand the role of *Leishmania* in changing TEs and vice versa. Hence, we conducted a systematic review of leishmaniasis and TEs in order to present the TEs type and how to changes (increasing or decreasing) before and after treatment, and outcomes of subjects with leishmaniasis.

Methodology

Search Strategy

In this systematic review, we applied the standard protocol of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) to design, report, and interpret our study [27]. To survey the status of TEs in subjects with leishmaniasis, four international databases (PubMed, Web of Science, Scopus, and Google scholar) were systematically searched by two independent investigators (AT and AA), for peer-reviewed online articles up to October 1, 2020. In this study, we used a combination of the following search terms: ("*Leishmania*" OR "leishmaniasis") AND ("trace element" OR "selenium" OR "zinc" OR "copper" OR "magnesium" OR "calcium").

Eligibility Criteria, Study Selection, and Data Extraction

Following the initial search, the relevant records were screened by the titles and abstracts and then were saved in a clean Microsoft Word file. Afterward, duplicate citations were removed completely. In the next step, the remaining records were carefully evaluated by two trained investigators (AT and AA). Finally, the full-texts of high potentially eligible papers were evaluated for final eligibility and inclusion criteria. Any disagreements were resolved by consensus and discussion

with a third reviewer (AR). In this systematic review, a strict protocol for the inclusion of papers was defined by our research team, so we included only those articles if they had all of the following inclusion criteria: (1) original research articles and short reports with case-control design that studied the status of TEs in subjects with leishmaniasis and control groups; (2) investigations that used animal models and human studies; (3) the papers with full-text or abstract in English without geographical restriction; (4) papers published online from the inception up to July 25, 2020, with a Digital object identifier (DOI); (5) specimens collected from the blood/serum of animals and human; and (6) those papers that provided the exact total sample size and changes of TEs in case and control groups. Articles without any of aforementioned criteria, including reviews, editorials and/or letters, those with confusing/unclear analysis, and investigations of animals which had been experimentally infected for other purposes, were excluded. The reference lists of eligible articles were meticulously checked manually to retrieve the other citations not found through database searching.

Following reviewing the eligibility criteria, A.T. extracted the requisite data into a Microsoft Excel sheet, and AA rechecked them for accuracy before data-report and interpretation. The discrepancy and inconsistency between the authors

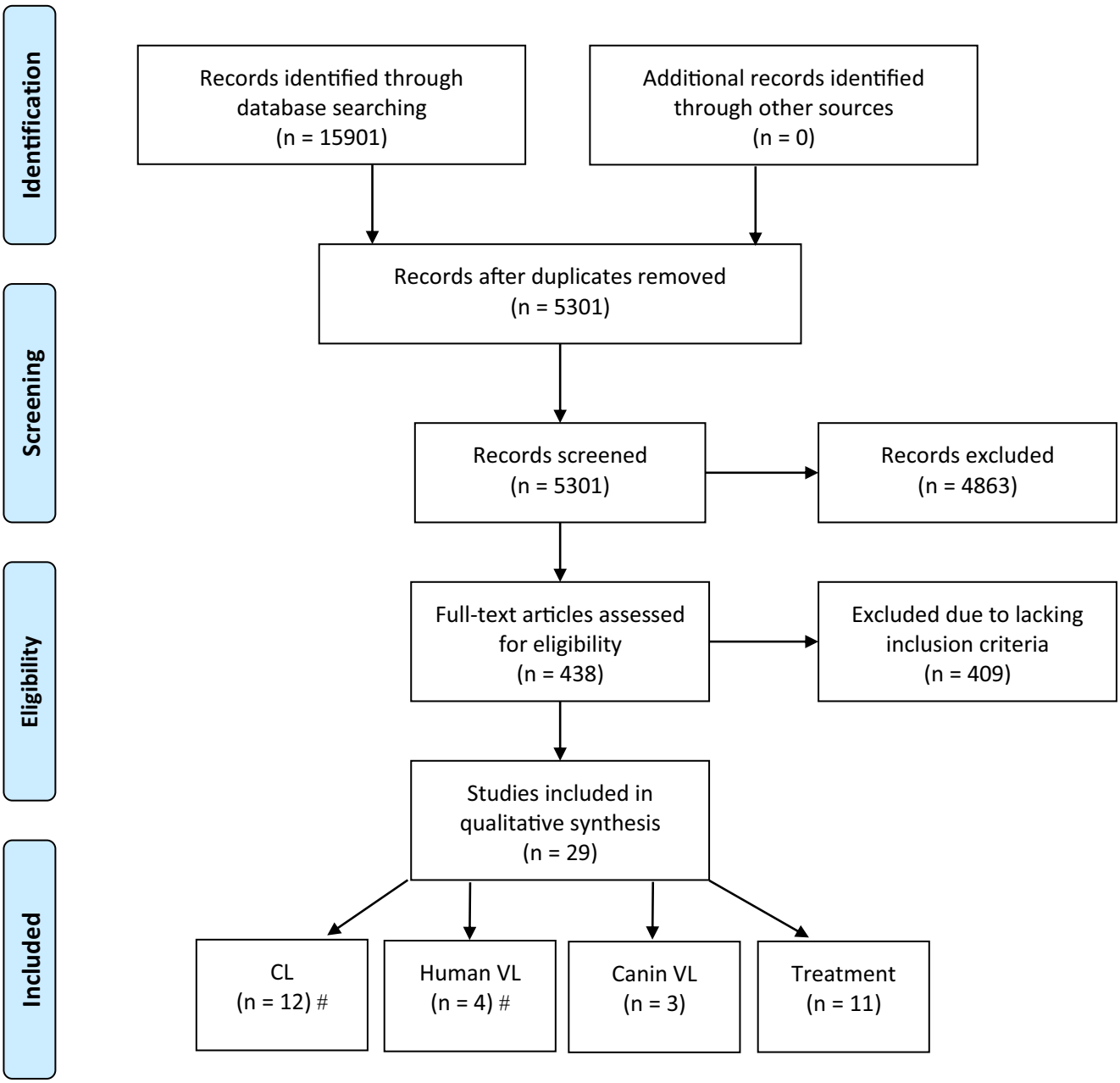

Fig. 1 PRISMA diagram through the different phases of the review. #One study examined the TEs level both in CL and VL patients

Table 1 Trace element alterations in human and animal models of cutaneous leishmaniasis

Ref	Country (province or city)	Study aims and design	Types of trace element (single or mix) and other factors	Methods	The main findings during the disease or after treatment
[28]	Iran (Qom, Northern Khorasan, and Esfahan provinces)	• To evaluate the serum antioxidant trace elements Se, Zn, and Cu in patients with CL. • Case–control • 95 cases/100 control	• Se • Zn • Cu	• *Leishmania* diagnosis: paraclinically • Trace element detection: Determining of Se, Zn, and CU in human serum by atomic absorption spectrometry	▶ The level of Se in patients (4.33 ± 1.06 μg/dl) was significantly lower than the control group (11.10 ± 2.37 μg/dl) with ($P < 0.0001$). ▶ The level of Zn in patients (70.23 ± 19.12 μg/dl) was significantly lower than the control group (119.61 ± 26.18 μg/dl) with ($P < 0.01$). ▶ No significant difference in status of Cu was observed between cases (107.68 ± 29.16 μg/dl) and healthy subjects (91.42 ± 27.54 μg/dl) with ($P > 0.05$)
[29]	Iran (Qom)	• To assess the levels of Zn, Cu, Fe, and Zn/Cu ratio in the sera of patients with CL in Qom Province, center of Iran • Case–control • 60 cases/100 control	• Zn • Cu • Fe • Zn/Cu ratio	• *Leishmania* diagnosis: direct smears (with Giemsa stain) • Trace element detection: by atomic absorption spectrometry and colorimetric methods	▶ The levels of Zn, Fe, and Zn/Cu ratio in patients were significantly lower than the control group. ▶ The level of Cu in patients (133.65 ± 9.1 μg/dl) was significantly higher than the control group.
[30]	Iran (Qom, Northern Khorasan, Esfahan, and Kerman province)	• To evaluate the serum levels of Se, Zn, and Cu change in leishmaniasis patients and their comparison between CL and VL patients	• Se • Zn • Cu	• *Leishmania* diagnosis: paraclinically • Trace element detection: by atomic absorption spectrometry	▶ The levels of Se and Zn were significantly lower in leishmaniasis patients than the healthy control group. Cu level was not a significant change in patients than control group. ▶ Among the CL patients, the levels of Se (4.33 ± 1.06) and Zn (70.23 ± 19.12) were significantly decreased in comparison of healthy control group (Se: 11.10 ± 2.37 and Zn:119.61 ± 26.18). Cu was increased (but non-significant) in CL patients than the control group (107.68 ± 29.16 *vs* 91.42 ± 27.54, respectively). ▶ Among the VL patients, the levels of Se (2.57 ± 0.64) and Zn (62.5 ± 18.19) were significantly decreased in comparison of healthy control group (Se: 11.10 ± 2.37 and Zn:119.61 ± 26.18). Cu was increased CL patients than the control group (115.9 ± 29.39 *vs* 91.42 ± 27.54, respectively).
[26]	Iran (Tehran)	• To measure the alterations in serum zinc copper and iron in patients with acute and chronic CL • Case–control • 36 cases (18 cases with acute and 18 cases with chronic CL)/18 control	• Zn • Fe • Cu	• *Leishmania* diagnosis: direct smears (with Giemsa stain) • Trace element detection: by atomic absorption spectrometry	▶ Serum Cu level was significantly higher in the patients with acute and chronic CL than those of control group ($P < 0.05$). ▶ Zn and Fe levels were significantly lower in patients with acute and chronic CL than the control group ($P < 0.001$).

Table 1 (continued)

Ref	Country (province or city)	Study aims and design	Types of trace element (single or mix) and other factors	Methods	The main findings during the disease or after treatment
[31]	Iran (Abadan and Khorramshahr)	• To assess the levels of Se, Zn, Cu, Fe, and Zn/Cu ratio in CL patients and uninfected healthy individuals. • Case–control • 80 patients with CL (cases group) and 80 healthy individuals (control group)	• Zn • Cu • Fe • Se • Zn/Cu ratio	• *Leishmania* diagnosis: confirmed cases of CL who referred to the health centers • Trace element detection: by atomic absorption spectrometry	► The mean ± SD concentrations of Zn, Fe, and Se were statistically lower ($P < 0.001$) in the case than the control group. ► Serum levels of Cu were significantly higher in CL patients than the controls ($P < 0.001$). ► A significantly lower Zn/Cu ratio was observed among CL patients than controls ($P < 0.001$).
[32]	Turkey (Sanliurfa)	• To assess the levels of some trace element, their related antioxidant enzyme activities, and carrier proteins in patients with CL • Case–control • 42 cases/38 control	• Se • Zn • Cu • Fe • Erythrocyte Cu-Zn SOD (U/gr Hb) • GSH-Px-Se • Albumin- Se • Cu-Cp • Htc (%)- Se • Cu-Zn SOD - Cp • Cu-Zn SOD-Cu • CAT-Fe	• *Leishmania* diagnosis: direct smears (with Giemsa stain) and cultures (with Novy- Mac Neal-Nicolle (NNN)) • Trace element detection: Determining of Se, Zn, and CU in human serum by atomic absorption spectrometry, Tf level was measured by turbidimetric method, other factors were measured with enzymatic colorimetric methods.	► The levels of Se, Zn, and Fe in patients were significantly lower than the control group. ► The levels of Cu, erythrocyte "Cu-Zn SOD", were higher in patients than the control group. ► Positive correlations were found between: Se and GSH-Px ($r = 0.703$, $P < 0.0001$) Se and albumin ($r = 0.344$, $P < 0.05$) Cp and Cu ($r = 0.67$, $P < 0.001$) Htc% and Se ($r = 0.289$, $P < 0.05$) Cu-Zn SOD and Cp ($r = 0.365$, $P < 0.01$) Cu-Zn SOD and Cu ($r = 0.255$, $P < 0.05$) CAT and Fe ($r = 0.43$, $P < 0.01$)
[33]	Turkey (Sanliurfa)	• To evaluate the effects of CL infection on total levels of Zn, Cu, and Fe and the effects of antimonial therapy on these factors • Case–control • 40 cases/32 control	• Zn • Cu • Fe	• *Leishmania* diagnosis: direct smears (with Giemsa stain) and cultures (with Novy- Mac Neal-Nicolle (NNN)) • Trace element detection: by atomic absorption spectrometry and colorimetric methods • Therapeutic method: in the patient's group, the drug pentavalent antimonial compounds "glucantime" was administrated intramuscularly once a day for 21 days (20 mg/kg) in a single injection. venous blood was withdrawn before and three times during the treatment at one-week intervals following overnight fasting.	• Before antimonial therapy, Zn and Fe concentrations were significantly lower and Cu was significantly higher in the patient group an those of healthy subjects. • During treatment, Zn and Fe levels were significantly increased and Cu was significantly decreased in the patient group than the control group.
[34]	Turkey (Sanliurfa)	• To examine the levels of essential trace elements and their association with immunoregulatory cytokines in patients with CL • Case–control	• Zn • Se • Fe • Cu • IL-1β	• *Leishmania* diagnosis: direct smears (with Giemsa stain) and cultures (with Novy- Mac Neal-Nicolle (NNN))	► The levels of Zn, Se, Fe, and IL-2r in patients were significantly lower than the control group. ► The levels of Cu, as well as the cytokines IL-1β, IL-8, IL-6, and TNF-α, were significantly higher in patients than in the control group.

Table 1 (continued)

Ref	Country (province or city)	Study aims and design	Types of trace element (single or mix) and other factors	Methods	The main findings during the disease or after treatment
		• 28 cases/22 control	• IL-2r • TNF-α • IL-6 • IL-8 • albumin	• Trace element detection: by atomic absorption spectrometry and colorimetric methods • The cytokines were determined by a chemiluminescence	▶ There was no significant difference in plasma albumin levels between the two groups. ▶ There were positive correlations between Se and IL-2r, copper and IL-6, and copp5er and IL-1β. ▶ There were negative correlations between Se and IL-8, Fe and TNF-α, and Zn and IL-1β contents in patients with CL.
[35]	Turkey (Sanliurfa)	• To examine the status of selenium and GSH-Px in patients with CL • Case–control • 52 cases/38 control	• Se • albumin	• *Leishmania* diagnosis: direct smears (with Giemsa stain) and cultures (with Novy- Mac Neal-Nicolle (NNN)) • Selenium and GSH-Px detection: by atomic absorption spectrometry and colorimetric methods	▶ The level of Se and GSH-Px activities in patients was significantly lower than the control group ($P < 0.0001$). ▶ The levels of albumin and hematocrit % (Htc%) were not different in patients than in controls. ▶ There were positive correlations between Se and GSH-Px activities ($r = 0.703$, $P < 0.0001$), Se and albumin ($r = 0.344$, $P < 0.05$), and GSH-Px and Htc% ($r = 0.48$, $P < 0.01$) in CL patients.
[36]	Brazil (Corte de Pedra)	• To investigate the levels of zinc and copper in different clinical forms of leishmaniasis, and their role in the immune response against the infection. • Case–control • 31 patients with either localized cutaneous (LCL), mucosal (ML) or visceral, (VL) leishmaniasis and 25 control	• Zn • Cu	• *Leishmania* diagnosis: Diagnosis was confirmed by Montenegro skin test, serology, direct culture of parasites from lesions. • Trace element detection: by atomic absorption spectrometry • Therapeutic method: During a one-year period, 14 patients with LCL (single lesion with less than 4 weeks of duration) were selected and treated (20 mg/kg of glucantime during 20 days).	▶ A significant decrease in Zn was observed in all three patient groups ($P < 0.01$ for LCL and ML, $P < 0.001$ for VL), compared to controls, but only VL (7/10) and ML (1/7) patients displayed overt Zn deficiency. ▶ The level of Cu was significantly higher in LCL and VL ($P < 0.001$) but not in ML patients. ▶ The level of Cu was strongly correlated to anti-*Leishmania* IgG (Spearman $r = 0.65$, $P = 0.0028$). ▶ Ex vivo production of parasite-induced IFN-γ was negatively correlated to plasma Cu levels in LCL patients ($r = -0.57$, $P = 0.01$). ▶ After 3 months of treatment, plasma Zn increased and Cu decreased in LCL patients, resulting in values indistinguishable from endemic controls.
[37]	Iraq (Najaf)	• To examine the status of trace elements in the sera of patients with CL and their correlation with anti-oxidant elements	• Zn • Cu	• *Leishmania* diagnosis: paraclinically • Trace elements and anti-oxidant detection: by atomic absorption spectrometry and ELISA	▶ The levels of Zn, Se, and Fe were significantly decreased in CL patients compared to the control group ($P < 0.05$). ▶ The level of Cu was significantly increased in CL patients compared to the control group ($P < 0.05$). ▶ The levels of GSH-Px and catalase were significantly decreased and SOD significantly increased in CL patients compared to the control group ($P < 0.05$).
[38]	Iran	● Animal model/CL caused by *L. major*	• Zn • Cu	• The animals divided into three groups: healthy group	• Serum Cu levels were significantly higher in the test group than in the control and naive groups

Table 1 (continued)

Ref	Country (province or city)	Study aims and design	Types of trace element (single or mix) and other factors	Methods	The main findings during the disease or after treatment
		• To evaluate the effect of trinitroglycerin (TNG) as nitric oxide donor agent on serum Cu, Zn and liver enzymes in BALB/c mice infected with *L. major*		(uninfected naive mice), control group (infected with *L. major*), and test group (*L. major* infected mice treated with TNG) •Trace elements and live enzymes: by atomic absorption spectrometry and colorimetry	(P value < 0.05), while Zn levels were higher in the test group than in the control group with no significant difference.

SOD superoxide dismutase, *GSH-Px* glutathione peroxidase, *Cp* ceruloplasmin, *Htc* hematocrit, *CAT* catalase

were consulted and resolved by consensus. Briefly, the following characteristics of each eligible article were extracted using a data extraction form based on study characteristics country, province or city, type of TEs, the number of case and control groups, alteration (high or level) in serum levels of TEs in case and control groups, leishmaniasis diagnostic methods, TEs detection methods, therapeutic methods, and outcome.

Results

As shown in Fig. 1, a total of 15900 records were found following the initial search of databases; after removing duplicates and/or non-eligible papers, 29 papers had eligibility to be included in this systematic review (Fig. 1). The main characteristics of each study are categorized in Tables 1, 2, 3, and 4, respectively.

TEs Alterations in Human CL

A total of 12 studies were assessed TEs alterations in human CL (Table 1). The studies were from Turkey (four studies), Iran (six studies), Brazil (one study), and Iraq (one study). Datasets were related to four different TEs, including the Se, Zn, Cu, and Fe. As shown in Table 1, the main diagnostic methods for CL were paraclinical examination, direct smears (with Giemsa stain) and cultures (with Novy-Mac Neal-Nicolle (NNN)). Moreover, all studies have used the atomic absorption spectrometry method to identify TEs. Interestingly, serum concentrations of Fe, Zn, and Se were significantly decreased in all patients with CL than in the control group [26, 28–37]. In contrast, seven of the eight studies have shown that the level of Cu was increased in the sera of patients compared to the control group [26, 29–37]. In addition, two studies evaluated the TEs concentration after CL treatment [33, 36]. In this regard, Kocyigit et al. reported increased concentrations of Fe and Zn and decreased levels of Cu after treatment with glucantime [33]. In agreement with them, Weyenbergh et al. showed an increased and decreased concentration of Zn and Cu after treatment, respectively [36]. Kocyigit et al. [34] found that the levels of Zn, Se, Fe, and IL-2r were significantly lower patients than the control group. As well, the levels of Cu and cytokines IL-1β, IL-8, IL-6, and TNF-α were significantly higher in patients than the control group. There were positive correlations between Se and interleukin-2 receptor (IL-2R), copper and IL-6, and copper and IL-1β and negative correlations between Se and IL-8, Fe and TNF-α, as well as Zn and IL-1β in patients with CL [34]. Two studies also indicated the levels of some antioxidants altrations in human CL [35, 37]. Accordingly, a significantly decreased levels of glutathione peroxidase (GSH-Px) [35, 37] and catalase [37] and an increased level of superoxide

Table 2 Trace element alterations in human visceral leishmaniasis

Ref	Country (province or city)	Study aims and design	Types of trace element (single or mix) and other factors	Methods	The main findings during the disease or after treatment
[39]	India (Bihar)	• To evaluate the relationship between zinc level in visceral leishmaniasis (VL) patients in endemic and non-endemic regions of India. • Case–control • 16 case/37 control 1((non-endemic region) and 35 control 2 (endemic region)	• Zn	• *Leishmania* diagnosis: (1) the kala-azar serology and detection of *Leishman*-Donovan (LD) bodies. Antibody detection using recombinant antigens from *L. chagasi*, (Lc-rK39), and another from *L. donovani*, (LdrKE-16); (2) the diagnosis is confirmed by microscopic demonstration of LD bodies in the bone marrow and splenic aspirates • Trace element detection: by colorimetry	▶ A significant decrease in serum zinc levels was observed in VL patients (8.1 ± 2.7 μM/l) compared to control group 1(12 ± 3 μM/l) and control group 2(8.8 ± 3.1 μM/l).
[40]	India (Patna)	• To compare serum trace elements concentrations in acute and chronic VL patients. • Case–control • 22 chronic case and 22 acute case/22 control	• Cu • Zn • Fe • Ca • Mg	• *Leishmania* diagnosis: by microscopic examination of *Leishmania* parasite in the splenic aspirate of the patients • Trace element detection: by calorimetric assay.	▶ The mean level of Cu was significantly different among three groups ($P = 0.001$) without significant difference between chronic and acute VL patients ($P = 0.114$). It was significantly higher in both chronic and acute VL patients when compared with healthy controls ($P = 0.001$) indicating increased Cu level in both chronic VL and acute VL patients. ▶ A statistically significant difference of Zn level among three groups ($P = 0.001$) and also significantly different when compared with each other. The level of Zn was found decreased significantly in both chronic and acute VL patients compared to healthy controls ($P = 0.001$). There was a significant indication of more decreasing trend of Zn levels as the disease becomes chronic. ▶ There was significantly lower mean level of Fe in both chronic ($P = 0.001$) and acute ($P = 0.001$) VL patients compared to healthy controls. But we did not observe any statistically significant difference of mean Fe level between chronic and acute VL patients ($P = 0.817$), almost having the same level of Fe.

Table 2 (continued)

Ref	Country (province or city)	Study aims and design	Types of trace element (single or mix) and other factors	Methods	The main findings during the disease or after treatment
					▶ The mean level of Ca was almost similar in three groups showing no statistically significant difference between chronic, acute VL patients, and control group ($P = 0.096$). ▶ There was statistically significant difference of Mg mean level among three groups ($P = 0.001$), and also between chronic and acute VL patients ($P = 0.002$). The level of Mg was significantly higher in chronic VL patients compared to healthy individuals ($P = 0.006$), but the level was almost similar in acute VL patients compared to healthy individuals ($P = 0.928$).
[36]	Brazil (Corte de Pedra)	• To investigate the levels of zinc and copper in different clinical forms of leishmaniasis, and their role in the immune response against the infection. • Case–control • 31 patients with either localized cutaneous (LCL), mucosal (ML), or visceral (VL) leishmaniasis and 25 control	• Zn • Cu	• *Leishmania* diagnosis: Diagnosis was confirmed by Montenegro skin test, serology, direct culture of parasites from lesions. •Trace element detection: by atomic absorption spectrometry • Therapeutic method: During a one-year period, 14 patients with LCL (single lesion with less than 4 weeks of duration) were selected and treated (20 mg/kg of glucantime during 20 days).	▶ A significant decrease in Zn was observed in all three patient groups ($P < 0.01$ for LCL and ML, $P < 0.001$ for VL), compared to controls, but only VL (7/10) and ML (1/7) patients displayed overt Zn deficiency. ▶ The level of Cu was significantly higher in LCL and VL ($P < 0.001$) but not in ML patients. ▶ The level of Cu was strongly correlated to anti-*Leishmania* IgG (Spearman $r = 0.65$, $P = 0.0028$). ▶ Ex vivo production of parasite-induced IFN-γ was negatively correlated to plasma Cu levels in LCL patients ($r = -0.57$, $P = 0.01$). ▶ After 3 months of treatment, plasma Zn increased and Cu decreased in LCL patients, resulting in values indistinguishable from endemic controls. ▶ Cu/Zn ratios significantly increased in VL patient group,

Table 2 (continued)

Ref	Country (province or city)	Study aims and design	Types of trace element (single or mix) and other factors	Methods	The main findings during the disease or after treatment
[30]	Iran (Qom, Northern Khorasan, Esfahan, and Kerman province)	• To evaluate the serum levels of Se, Zn, and Cu change in leishmaniasis patients and their comparison between CL and VL patients	• Se • Zn • Cu	• *Leishmania* diagnosis: paraclinically • Trace element detection: by atomic absorption spectrometry	reaching a three-fold molar excess of Cu to Zn in VL patients. ▶ The levels of Se and Zn were significantly lower in leishmaniasis patients than the healthy control group. Cu level was not a significant change in patients than control group. ▶ Among the CL patients, the levels of Se (4.33 ± 1.06) and Zn (70.23 ± 19.12) were significantly decreased in comparison of healthy control group (Se: 11.10 ± 2.37 and Zn:119.61 ± 26.18). Cu was increased (but non-significant) in CL patients than the control group (107.68 ± 29.16 *vs* 91.42 ± 27.54, respectively). ▶ Among the VL patients, the levels of Se (2.57 ± 0.64) and Zn (62.5 ± 18.19) were significantly decreased in comparison of healthy control group (Se: 11.10 ± 2.37 and Zn:119.61 ± 26.18). Cu was increased CL patients than the control group (115.9 ± 29.39 *vs* 91.42 ± 27.54, respectively).

Table 3 Trace element alterations in canine visceral leishmaniasis

Ref	Country (Province or city)/type of visceral *Leishmania*	Study aims and design	Types of trace element (single or mix) and other factors	Methods	The main findings during the disease or after treatment
[41]	Brazil (Minas Gerais)/*Leishmania infantum chagasi*	• To evaluate the alterations and potential associations of antioxidant enzymes, trace elements, and histopathology in canine visceral leishmaniasis (CVL) •Case–control •19 cases (with symptomatic) /11 control or asymptomatic	• Cu • Fe • Zn • Se • catalase (CAT) • GSH-Px • SOD	• *Leishmania* diagnosis: 1- indirect immunofluorescence antibody testing (IFAT) and enzyme-linked immunosorbent assay (ELISA); 2- bone marrow impression smears, ear biopsies for histology and immunohistochemistry, and conventional PCR using spleen samples. •Trace elements and antioxidant detection: by atomic absorption spectrometry and colorimetric methods.	► The levels of Fe and Zn were significantly lower in blood of symptomatic dogs than in either asymptomatic or controls ($P < 0.01$). ► The Se concentration was significantly lower in infected dogs than in controls and asymptomatic ($P < 0.001$ and $P < 0.01$ respectively). ► Serum Cu concentrations were higher in symptomatic dogs than in asymptomatic and control dogs ($P < 0.01$ and $P < 0.001$ respectively). ► The levels of catalase (CAT) and GSH-Px were significantly lower in plasma ($P < 0.001$ and $P < 0.01$, respectively) of both symptomatic and asymptomatic infected dogs than in controls. ► A significantly higher SOD activity was detected in infected dogs than controls ($P < 0.01$). ► CAT, GSH-Px, and SOD were significantly lower ($P < 0.05$) in liver, spleen, and lymph node in infected dogs than in controls, with no difference between asymptomatic and symptomatic. ► Lipid peroxidation was higher in plasma of all symptomatic and asymptomatic dogs than in controls ($P < 0.0001$ and $P < 0.01$, respectively) and higher in symptomatic than in asymptomatic dogs ($P < 0.001$).
[42]	Iran (Mashhad)/*Leishmania infantum*	• To investigate the role of oxidative stress in the pathology of canine visceral leishmaniasis (CVL) • Case–control • Leishmania infected dogs (14 asymptomatic and 16 symptomatic) and 30 non-infected (control)	• Cu • Fe • Zn • Se • Total antioxidant status (TAS) • Malondialdehyde (MDA) • Urea nitrogen (BUN) • Albumin	• *Leishmania* diagnosis: serological (IFAT) and parasitological methods • Trace element detection: by atomic absorption spectrometry and colorimetric methods.	► A significantly decreased Zn ($P < 0.001$) and Cu ($P < 0.001$) were detected in asymptomatic group, when compared to control group, while the symptomatic group presented a significantly decreased their levels ($P < 0.001$) when compared to control and asymptomatic animals. ► A significantly increased level of Fe was detected in asymptomatic dogs when compared to control group,

Table 3 (continued)

Ref	Country (Province or city)/type of visceral *Leishmania*	Study aims and design	Types of trace element (single or mix) and other factors	Methods	The main findings during the disease or after treatment
					while the symptomatic group presented decreased levels compared to control and asymptomatic animals. ▶ A significant decrease ($P < 0.001$) in serum TAS and albumin concentration ($P < 0.05$) and a significant increase in serum MDA and BUN concentrations ($P < 0.001$), in the asymptomatic dogs were observed compared to control group and asymptomatic dogs.
[43]	Turkey (NR)/NR	• To determine the zinc, iron, copper, calcium, phosphorus, and magnesium levels in blood serum and zinc and copper levels in hair of dogs with canine visceral leishmaniasis • Case–control • 10 case/8 control	• Cu • Fe • Zn • Se • Mg • Ca • Phosphorus (P)	• *Leishmania* diagnosis: clinical signs including skin lesions, weight loss, lymphadenopathy, and anemia and indirect immunofluorescence assay • Trace element detection: by atomic absorption spectrophotometry	▶ The serum levels of Zn and Fe in case group were significantly lower than the control group with ($P < 0.05$ and $P < 0.001$, respectively). ▶ The serum level of Cu was significantly higher than the control group with ($P < 0.05$). ▶ The serum level of Mg in case group was higher than the control group (1.06 ± 0.1 mg/dl) with ($P > 0.05$ not significant). ▶ No significant differences in the Zn and Cu levels were detected in hair. ▶ No significant differences were observed for serum levels of Ca and P in case than control group.

dismutase (SOD) in the sera of patients with CL compared to healthy control group [32] were reported. In an animal model, treatment of BALB/c mice infected with *L. major* with trinitroglycerin (TNG) led to a significantly increased level of Cu and Zn than in the non-treated infected group [38] (Table 1).

TEs Alterations in Human VL

As shown in Table 2, four studies were evaluated TEs alterations in human VL. The studies were conducted in Brazil (one study) [36], India (two studies) [39, 40], and Iran [30]. Five TEs, including Zn, Cu, Fe, Ca, and Mg, have been examined in patients with VL. The diagnostic methods of VL and the methods of evaluating TEs are listed in Table 2. Similar to patients with CL, serum concentrations of Zn and Fe in patients with VL were lower than in the control group. Likewise, serum concentrations of Cu and Mg in patients were higher than the control group (Table 2).

TEs Alterations in Canine VL

Three studies from Brazil [41], Iran [42], and Turkey [43] were examined TEs alterations in canine VL. Five TEs (Cu, Fe, Zn, Se, and Mg) were evaluated. As shown in Table 3, immunofluorescence antibody testing (IFAT) methods have been used to diagnose VL and spectrometry methods have been used to find serum concentrations of TEs. Like human CL and VL, serum concentrations of Fe, Zn, and Se were significantly decreased in case groups compared to the control groups. Moreover, in agreement with CL and VL, serum concentrations of Cu and Mg in case group were higher than the

Table 4 Trace elements as an adjuvant for treatment of leishmaniasis

Ref	Country Aims of the study	Animal or human studies/type of *Leishmania* (*Leishmania* species)	Treatment/prescription method/ duration of follow up	Grouping/sample size (*n*)/ dose of each group	Main findings
[44]	• Iraq • To assess the efficiency of oral zinc sulfate in the treatment of CL patients	• Human/acute cutaneous leishmaniasis (both *L. major* and *L. tropica*)	• Zinc sulfate/orally (powder)/45 days	Group 1/(*n* = 31)/2.5 mg/kg Group 2/(*n* = 29)/5 mg/kg Group 3/(*n* = 32)/10 mg/kg Control/(*n* = 12)/a control group of patients did not receive any treatment	► Results have shown that the cure rates for the 2.5, 5, and 10 mg/kg group were 83.9%, 93.1%, and 96.9%, respectively. *Mean duration of treatment (days ± SEM): Group 1: 30.8 ± 1.82 Group 2: 29.96 ± 1.65 Group 3: 28.32 ± 1.35 Control: none *Side-effects (n patients): Group 1: Nausea and vomiting (*n* = 1), Edema (*n* = 2) Group 2: Nausea and vomiting (*n* = 1), Edema (*n* = 2), Leishmanid reaction with a macular rash overexposed parts of the body (*n* = 1) Group 3: Nausea and vomiting (*n* = 5), Edema (*n* = 1) Control: none
[45]	• Iraq • To assess the efficiency of intralesional Zinc sulfate, Sodium chloride, and pentavalent antimony in the treatment of CL patients	• Human/cutaneous leishmaniasis (both *L. major* and *L. tropica*)	• Zinc sulfate (2%)/injection intralesionally/45 days • Sodium chloride (7%)/injection intralesionally/45 days	• Zinc sulfate Case: (*n* = 19, with 38 lesions)/2% zinc sulfate Control: (*n* = 9, with 38 lesions)/a control group of patients did not receive any treatment • Sodium chloride Case: (*n* = 17, with 40 lesions)/2% zinc sulfate Control: (*n* = 9, with 38 lesions)/a control group of patients did not receive any treatment	► Zinc sulfate Cure rate of zinc sulfate: 94.8% *Mean duration of treatment (days ±SEM): Case/6.89 ± 0.70 (36 cured lesions and 2 mild lesions) Cure rate of pentavalent antimony: 88.6% Control: - ► Sodium chloride Cure rate of Sodium chloride: 85% *Mean duration of treatment (days ±SEM): Case/7.647 ± 0.59 (34 cured lesions and 6 mild lesions) Cure rate of pentavalent antimony: 88.6% Control: -
[46]	• Iraq (Baghdad,) • Comparison of topical zinc sulfate solution (25%) to be with topical podophyllin solution (25%) in treatment of patients with CL lesions	• Human/cutaneous	• 25% zinc sulfate solution • Forty patients with a total 88 lesions were enrolled.	• Group A treated with topical 25% podophyllin solution, once weekly for a maximum of 6 weeks • Group B was treated with topical 25% zinc sulfate solution, twice daily for 6 weeks	► The total cure rate in the group A and B were 82% and 73.4%, respectively. ► There was no statistically significant difference between the cure rates of both groups. No important local or systemic side effects were seen in any patients. ► Topical zinc sulfate 25% is an effective simple non-invasive non-costly safe topical therapy for cutaneous leishmaniasis.

Table 4 (continued)

Ref	Country Aims of the study	Animal or human studies/type of *Leishmania* (*Leishmania* species)	Treatment/prescription method/ duration of follow up	Grouping/sample size (*n*)/ dose of each group	Main findings
[47]	• Iraq (Baghdad,) • Comparison of oral zinc sulfate and oral ketocanazole singly and in combination against CL lesions	• Human/cutaneous	• Zinc sulfate • Seventy-five patients with acute CL were enrolled in this study.	• Group A: 24 patients treated with oral zinc sulfate capsules 10 mg/kg/day for 6 weeks • Group B: 24 patients treated with ketoconazole tablets 200 mg twice daily for 6 weeks • Group B: 27 patients treated with a combination of zinc sulfate and ketoconazole for 6 weeks	▶ The cure rate was 60% and 50% in Groups A and B, respectively ($P = 0.146$) ▶ The cure rate of combination therapy was 96% ($P < 0.04$). ▶ The combination therapy using oral zinc sulfate and oral ketoconazole gave a high cure rate.
[48]	• Brazil • To evaluated the role of zinc supplementation alongside with conventional antileishmanial therapy on regression of hepatosplenomegaly and recovery of hematological parameters in children with VL	• Human study	• Zinc sulfate • Therapeutic methods: All patients were treated with amphotericin B (0.5–1 mg/kg/day administered intravenously) or meglumine antimoniate (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.	• 23 case 1 (with Zn) and 29 case 2 (without Zn) /15 control	▶ Plasma Zn levels were lower in patients supplemented with Zn than in patients who did not receive Zn supplementation. ▶ After Zn supplementation, plasma Zn levels gradually increased, while it remained unchanged in patients who did not receive Zn supplementation. ▶ Administration of zinc during treatment with amphotericin B or glucantime accelerates the regression of the spleen enlargement without interfering with the recovery of hematological parameters.
[49]	• Iran (Kerman) • To compare the efficacy of intralesional glucantime plus niosomal zinc sulfate in comparison with intralesional glucantime plus cryotherapy in the treatment of patients with acute CL	• Human study	• Therapeutic methods: • Group A: weekly intralesional meglumine antimonite plus twice daily niosomal topical zinc sulfate • Group B: weekly intralesional glucantime plus every other week cryotherapy	• Case–control study on 64 patients with CL	▶ Partial response rate was 16.6% and 12.9% in group A and B, respectively ($P = 0.784$). ▶ Complete response rate was 73.3% and 80.6% in group A and B, respectively ($P = 0.784$). ▶ Complete response rate was achieved in 4.73 ± 0.29 weeks and 4.69 ± 0.28 weeks in group A and B, respectively ($P = 0.925$).
[50]	• Iran (Kerman) • To compare the efficacy of intralesional injections of 2% zinc sulfate solution with glucantime in the treatment of acute Old-World CL	• Human study	• Seventy-two patients with CL lesions treated with 6 weekly intralesional injections of either drug (2% zinc sulfate solution and glucantime)	• Randomized, double-blind, clinical trial on 72 patients with CL	▶ Complete re-epithelialization was observed among 10.5% and 61.3% of the lesions one week after the end of treatment in the ZnSO4 and glucantime groups, respectively ($P < .05$).

Table 4 (continued)

Ref	Country Aims of the study	Animal or human studies/type of *Leishmania* (*Leishmania* species)	Treatment/prescription method/ duration of follow up	Grouping/sample size (*n*)/ dose of each group	Main findings
					► In conclusion, a 6-week intralesional injections of ZnSO4 solution was less effective than glucantime in the treatment of acute Old-World CL.
[51]	• Iran (Mashhad) • Comparison of the efficacy of intralesional injection of 2% zinc sulfate with glucantime in treatment of acute CL	• Human study	• 24 out of 35 patients in group A (treated with 2% zinc sulfate) and 10 out of 15 patients in group B (treated with glucantime) completed the study.	• Randomized, double-blind, clinical trial	► The healing rates were 80% and 33.3% after 8 weeks among the patients who received glucantime and zinc sulfate, respectively ($P = 0.009$). ► In conclusion, intralesional injection of 2% zinc sulfate was less effective in treatment of CL than glucantime.
[52]	• Iran • To evaluate the effectiveness of sodium selenite and zinc sulfate in combination with glucantime® in treatment of CL lesions by *L. major* in susceptible BALB/c mice.	• Animal model/cutaneous Leishmaniasis (*L. major*)	• Zinc sulfate • Sodium Selenite • Control	• Zinc sulfate/($n = 11$)/2 mg/kg injection (NR) • Sodium Selenite//($n = 11$)/0.35 mg/kg • Control/($n = 11$)/control group treated by distilled water • All groups received glucantime as a standard antileishmanial agent, 30 days	► Zinc sulfate: *Lesion size (mm) before intervention Case: 2.8 ± 0.3 Control: 1.8 ± 0.4 *Lesion size (mm) after intervention Case: 3.4 ± 0.5 Control: 3.4 ± 0.4 *Not significant: P value = 0.57 ► Sodium Selenite: *Lesion size (mm) before intervention Case: 2.4 ± 0.3 Control: 1.8 ± 0.4 *Lesion size (mm) after intervention Case: 3.4 ± 0.2 Control: 4 ± 0.4 *Not significant: P value = 0.73
[53]	• Iran • To evaluate the influence of zinc sulfate on Th1 and Th2 immune responses in BALB/c mice with *L. major* infection	• Animal model/cutaneous Leishmaniasis (*L. major*)	• Zinc sulfate	• Test group: 37 mice that treated orally with 50 μl Zn sulfate syrup • Control group: 23 mice that injected with 50 μl of a 10-fold diluted glucantime (1.5 g/5 ml) every day for 1 month • Untreated control: 12 mice were left untreated as a control	• A significant decrease in parasite loads and lesion sizes was observed in Zn sulfate treated group compared to the untreated group. • IFNγ significantly upregulated in mice treated with Zn sulfate compared to the control. • The ratio of IFNγ /IL-4 mRNA expression was increased in Zn sulfate-treated compared to glucantime-treated animals. • Zn Sulfate has the ability to induce strong Th1 responses against the *L. major* in susceptible BALB/c mice.

Table 4 (continued)

Ref	Country Aims of the study	Animal or human studies/type of *Leishmania* (*Leishmania* species)	Treatment/prescription method/ duration of follow up	Grouping/sample size (n)/ dose of each group	Main findings
[54]	• Iraq • To evaluate the effectiveness of zinc sulfate in vitro and in an animal model against *L. major* and *L. tropica*	• Animal model/cutaneous leishmaniasis/-cutaneous leishmaniasis (both *L. major* and *L. tropica*)	• Zinc sulfate/orally/56 days	Group 1/(n = 10)/100 mg/kg Group 2/(n = 10)/200 mg/kg Group 3/(n = 10)/400 mg/kg Control/(n = 10)/control group treated by distilled water	▲ Results showed that oral zinc sulfate was effective in both treatment and prophylaxis for cutaneous leishmaniasis. ▲ The results show that the two forms (promastigote and amastigote) of both strains were sensitive to zinc sulfate and their respective LD50 were lower compared to the pentavalent antimony compound. ▲ Zinc sulfate administered resulted in a dose dependent decrease in the mean lesion score of the treated mice when compared to sham treated controls.

control group; however, in one study, dogs in the asymptomatic and symptomatic groups showed an outcome of heterogeneity in Cu, Zn, and Fe concentrations compared with the control group [42].

Trace Elements as an Adjuvant for Treatment of CL

Overall, 11 studies were evaluated the effects of TEs as adjuvant in the treatment of CL. Of them, 8 studies were related to human [44–51] and three were to the animal model [52–54]. The mode of administration and the period of treatment are shown in Table 4. In a human study, Sharquie et al. [44] have shown that treatment with a high dose of zinc sulfate shortened the duration of treatment compared to lower doses; however, side effects increase in high doses [44]. In another human study Sharquie et al. [45] demonstrated that the cure rates of zinc sulfate and sodium chloride against CL lesions were 94.8% and 85%, respectively, while the cure rate of Pentavalent antimony was 88.6% [45]. Farajzadeh et al. [49] compared the efficacy of intralesional injection of glucantime plus niosomal zinc sulfate (group A) and glucantime plus cryotherapy (group B) for treatment of patients with acute CL in Keran, Iran. The result revealed that complete cure rate in the groups A and B was 73.3% and 80.6%, respectively (P = 0.784). In a randomized, double-blind, clinical trial in Iran [50], 72 patients with CL received 6-week intralesional injections of zinc sulfate solution (2%) or glucantime. The results have shown that zinc sulfate solution was less effective than glucantime (10.5% and 61.3% re-epithelialization rates, respectively) [50]. In another clinical trial in Iran, the effectiveness of an 8-week intralesional injections of zinc sulfate (2%) was significantly less than glucantime in treatment of acute CL lesions (80% and 33.3%, respectively; P = 0.009) [51]. Sharquie et al. [47] compared the efficacy of oral zinc sulfate and oral ketocanazole for treatment of CL lesions in Iraq. They reported that the cure rates of zinc sulfate and ketocanazole were 60% and 50%, respectively (P = 0.146), while combination therapy had a cure rate of 96% (P < 0.04) [47]. Another study by the same group of researchers compared the efficacy of topical zinc sulfate solution (25%) and podophyllin solution (25%) in treatment of patients with CL lesions [46]. The results have shown total cure rates of 82% and 73.4% for podophyllin solution and zinc sulfate, respectively, which was not statistically significant [46]. Moreover, a study among Brazilian children with VL [48] revealed that zinc supplementation during treatment with amphotericin B or glucantime accelerated the regression of the spleen enlargement than in patients who did not receive Zn supplementation [48].

In animal models, the results of Najim et al. [54] revealed that oral zinc sulfate was effective in both treatment and prophylaxis of animal model of CL [54]. In contrast, the results of Sorkhroodi et al. [52] found that zinc sulfate and sodium selenite did not significantly improve the wound size of CL lesions in a mouse model [52]. Afshari et al. [53] found that oral

treatment of BALB/c mice with zinc sulfate led to a significant reduction in parasite loads and lesion sizes in *L. major*-infected animals. Indeed, zinc sulfate induced a strong T helper 1 (Th1) responses against the *L. major* with upregulation of the IFNγ and IFNγ /IL-4 ratio in the draining lymph nodes of treated mice [53].

Discussion

Leishmaniasis is a neglected disease with a wide range of clinical manifestations from self-healing to severe infection [55]. There is no vaccine for human leishmaniasis, and their treatment is often ineffective [55]. Several host factors are involved in the severity of the infection, including genetic background, immune responses, and nutrition status [21, 56]. Immune responses play a pivotal role both in protection and pathogenesis of leishmaniasis [8, 21]. It is well documented that the type 1 immunity and their pro-inflammatory cytokines (e.g., IL-12, IFN-γ, and TNF-α) have a crucial role for parasite elimination, and the type 2 immunity and their anti-inflammatory cytokines (e.g., IL-4, IL-5, IL-10, IL-13, and transforming growth factor beta (TGF-β)) facilitate the parasite persistence by downregulating the effector elements of the type 1 immunity [8, 21]. Nevertheless, aggravation of each type 1 or type 2 immunity can lead to severe inflammation and tissue damage or chronic-nonhealing infection, respectively [8, 21]. Hence, a balance between type 1 and type 2

Table 5 Summary of the study's results regarding relationship between TEs alterations and immune responses in leishmaniasis

Ref	Country (province or city)	Study aims and design	The main findings during the disease or after treatment
[34]	Turkey (Sanliurfa)	• A case–control (28 cases and 22 controls) study to examine association between TE2 and immunoregulatory cytokines in patients with CL	▶ The levels of Zn, Se, Fe, and IL-2r in patients were significantly lower than the control group. ▶ The levels of Cu, as well as the cytokines IL-1β, IL-8, IL-6, and TNF-α, were significantly higher in patients than the control group. ▶ There were negative correlations between Se and IL-8, Fe and TNF-α, and Zn and IL-1β contents in patients with CL. ▶ There were positive correlations between Se and IL-2r, copper and IL-6, and copp5er and IL-1β. ▶ There was no significant difference in plasma albumin levels between two groups.
[36]	Brazil (Corte de Pedra)	• A case–control study to investigate the levels of zinc and copper in different clinical forms of leishmaniasis, and their role in the immune response against the infection. • 31 patients with either localized cutaneous (LCL), mucosal (ML), or visceral (VL) leishmaniasis and 25 control	▶ A significant decrease in Zn was observed in all three patients compared to controls, but only VL (7/10) and ML (1/7) patients displayed overt Zn deficiency. ▶ The level of Cu was significantly higher in LCL and VL but not in ML patients. ▶ Ex vivo production of parasite-induced IFN-γ was negatively correlated to plasma Cu levels in LCL patients ($r = -0.57$, $P = 0.01$). ▶ The level of Cu was strongly correlated to anti-*Leishmania* IgG (Spearman $r = 0.65$, $P = 0.0028$). ▶ After 3 months of treatment, plasma Zn increased and Cu decreased in LCL patients, resulting in values indistinguishable from endemic controls.
[53]	● Iran ● To evaluate the influence of zinc sulfate on Th1 and Th2 immune responses in BALB/c mice with *L. major* infection	● Animal model/cutaneous Leishmaniasis (*L. major*). ● Test group: 37 mice that treated orally with 50 μl Zn sulfate syrup ● Control group: 23 mice that injected with 50 μl of a 10-fold diluted glucantime (1.5 g/5 ml) every day for 1 month ● Untreated control: 12 mice were left untreated as a control	● A significant decrease in parasite loads and lesion sizes was observed in Zn sulfate treated group compared to the untreated group. ● IFNγ significantly upregulated in mice treated with Zn sulfate compared to the control. ● The ratio of IFNγ /IL-4 mRNA expression was increased in Zn sulfate-treated compared to glucantime-treated animals. ● Zn Sulfate has the ability to induce strong Th1 responses against the *L. major* in susceptible BALB/c mice.

immunity is needed to eliminate the infection with minimal side effects [8, 9]. Interestingly, there is a direct relationship between malnutrition and the parasite endemicity, while the intensity of *Leishmania* infection can be intensified owing to malnutrition [56]. In addition to the protein and energy deficiency, microelement alterations have been detected in patients with CL and VL as well as canine leishmaniasis (Tables 1, 2, and 3). Our analysis revealed a significantly decreased level of Fe, Zn, and Se among human CL patients and canine leishmaniasis (Table 1 and 3) and Zn and Fe in patients with VL (Table 2). In contrast, an increased level of Cu in CL patients and Cu and Mg in VL patients and canine leishmaniasis was detected (Tables 2 and 3).

TEs alterations could modulate various immune responses, such as antibody production, T cells, B cells, and NK cells activities [23, 24]. TEs have various roles in immune cells, including synthesis of metalloproteins, which participate in housekeeping procedures such as energy production and protection against reactive oxygen species [57]. For instance, energy production requires Fe and Cu, while Fe is needed for production of cytochromes a, b, and c, Nicotinamide adenine dinucleotide (NADH) and succinate dehydrogenases, and Cu is needed for production of for cytochrome c oxidase in the mitochondrial electron-transport chain [57]. As such, Cu, Zn, Se, and Fe are involved in protection against reactive oxygen species (ROS) (e.g., Se for glutathione peroxidases (GSH-Px), Fe for catalase, and Cu and Zn for superoxide dismutase (SOD)) [57]. Although increased levels of ROS can destructively effect in tissues and organs, the optimal levels of them are needed to defend against intracellular infections [58]. In our analysis, a significantly decreased level of GSH-Px [35, 37] and catalase [37] and an increased level of SOD [32] were detected among patients with CL than healthy control groups. Secretion of ROS by phagocytic cells, such as macrophages and neutrophils, is an important mechanism of intracellular parasite killing [8]. Hence, modulation of TEs in leishmaniasis could alter ROS production and mitigate parasite killing. Synthesis and secretion of cytokines and chemokines are influenced by TEs status [57]. Zinc deficiency in humans is associated with T cell dysfunction [59], imbalance of cytokine secretion by peripheral blood mononuclear cells [60]. Zinc deficiency influences on cytokine production, including IL-1β, IL-2, IL-6, TNF-α, and IFN-γ [61, 62] and their supplementation diminished the incidence of infections in the elderly [63]. Zinc deficiency also compromised wound healing and is extensively used in skincare [64, 65]. Zinc supplementation as an adjuvant for CL treatment enhanced wound healing and diminished scar formation in human and experimentally infected animals (Table 5).

Iron is an important TEs for nearly all infectious agents, including *Leishmania* parasite [66, 67], and iron deprivation is one of the successful mechanisms of host defense against microbial pathogens [68]. The *Leishmania*-infected macrophage restricts iron and heme availability to the parasite to impede parasite replication [69]. Hence, the decreased levels of Fe in CL and VL patients as well as canine leishmaniasis may be an off-target mechanism of parasite replication.

Selenium is another important TEs that declined in CL and VL patients (Tables 1 and 2). Selenium supplementation promotes the differentiation of $CD4^{+}$T cells into Th 1 cells and boosts immunity against pathogens [70]. In vitro and in vivo studies have shown anti-*Leishmania* activity of selenium nanoparticles alone [71, 72] or in combination with amphotericin B [73] or glucantime [74].

Copper and magnesium are other TEs that increased in CL and VL patients and canine leishmaniasis (Tables 1, 2, and 3). Cu and Mg are involved in immune responses and have anti-inflammatory properties [75–77]. Mg reduces inflammatory responses [78, 79] and their deficiency increased inflammatory reactions [80]. Hence, the decreased levels of CU and Mg in leishmaniasis could perturbate the efficient immune responses against the parasite and could help in the persistence of the parasite.

Conclusion

The results of this review emphasize the important roles of TEs in leishmaniasis. Hence, TEs could be assessed as a prognosis factor in leishmaniasis. Also, TEs could prescribe as an adjuvant for the treatment of leishmaniasis.

Funding Amir Abdoli is supported by National Institute for Medical Research Development (NIMAD) grant number: 978507. This study is financially purported by Jahrom University of Medical Sciences, Jahrom, Iran.

Data Availability Not applicable.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Code availability Not applicable.

Abbreviations VL, visceral leishmaniasis; CL, cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis; TEs, trace elements; Se, selenium; Zn, zinc; Cu, copper; Fe, iron; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; Gpx, glutathione peroxidase enzyme; Cp, ceruloplasmin; Htc, hematocrit; CAT, catalase; Th1, T helper 1; Th2, T helper 2; IFNγ, interferon gamma; TNF, tumor necrosis factor; TGF-β, transforming growth factor beta

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