

Study of the enzymatic antioxidants and trace elements level in the people infected by leishmaniasis

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ABSTRACT

Leishmaniasis is a protozoa parasite, unicellular, obligatory intracellular parasitism, bi-host; it has two phases in his life cycle the first phase it called Promastigote that live in the intestines of female sandflies belonging to genus. The second stage Amastigote grows and multiplies inside the macrophages of the vertebral storage host. The purpose of this study was to evaluate the effect of *L. tropica* on the antioxidants indicators and trace elements level. The current study included the collection of 55 serum samples from patients infected by the parasite *L. tropica*. The results showed a decrease ($p < 0.05$) in the concentration of glutathione, the level of zinc and iron, while it was noted that the concentration of peroxynitrite radical and the level of magnesium increased ($p < 0.05$) in patients by leishmaniasis compared to the control. The information gathered can be used to lessen the effect of *L. tropica* on human, by treating patients with cutting-edge methods.

Keywords: *leishmaniasis, Antioxidants, Trace elements*

INTRODUCTION

Leishmaniosis is a disease caused by a group of obligate intracellular protozoa parasites belonging to the genus *Leishmania*. These parasites cause various diseases in humans and mammals, as they infect the reticulo-endothelial system and multiply in its different cells [1]. The sand fly is the host that transmits the parasite through the bite of an insect that contains the promastigote phase of the parasite while absorbing blood from the host through the skin. This leads to transmission of the disease to humans and other mammals [2].

Cutaneous leishmaniasis (CL), the most common type that affects the skin, and Mucocutaneous leishmaniasis (MCL), which has the ability to destroy mucous tissues, is widespread in Central and South America, and the dangerous type is Visceral leishmaniasis (VL), which causes enlargement of the liver and spleen and can be fatal if not treated quickly [3]. The World Health Organization (WHO) considers leishmaniasis one of the neglected tropical diseases that spread widely in societies, threatening about 350 million people in 88 countries around the world, and new infections range between one and two million new cases annually and occur as a result of infection, especially visceral leishmaniasis. Deaths reach (30,000) thousand deaths annually and the incidence of infection were linked to malnutrition, population displacement, weak immune system and poverty [4].

Classification of leishmanial

Leishmania parasites are classified according to [5]

Kingdom: Protista

Subkingdom: Protozoa

Phylum: Sarcostylozoa

Subphylum: Mastigophora

Class: Zoomastigophora

Order: Kinetoplastida

Suborder: Trypanosomatina

Family: Trypanosomatidae

Genus: *Leishmania*

Leishmaniosis Types

Cutaneous Leishmaniasis

Cutaneous leishmaniasis is one of the most typical types, especially at tropical countries [6]. The annual number of cases of this type of leishmaniasis worldwide is estimated at 1.5-1 million cases [7], it spreads widely in the countries surrounding the Mediterranean, North and Central Africa, as well as West and South Asia, and the cure for the disease is automatic [8]. The infection leads to the enlargement of the dermis area and the occurrence of a cellular infiltration consisting of lymphocytes and serous cells, where the ulcer initially appears in the form of a node and grows in size, taking the form of a papilla. The papilla has a raised center and is covered with a thin layer of skin in the form of an ulcerated vesicle, after which it bursts and a purulent substance emerges from it, leaving in its place a distorted brown area with irregular edges called a scar which it spreads in different areas of the body, especially in the face, arms and legs [9]. The forms of cutaneous leishmaniasis:

Cutaneous Urban

This parasite caused by *L. tropica* as it is found in urban areas in parts of India and Pakistan and in the countries of the Mediterranean Basin and the Middle East [10]. This type causes dry skin ulcers of small size, slow developing, painless, usually with a dry, unstable shape. The incidence of this type of cutaneous leishmaniasis increases in urban areas. It recovers after a year or more. Its incubation period ranges between (8-2) months [11]. Skin ulcers appear on the exposed parts of the body [12].

Rural cutaneous leishmaniasis

This parasite caused by *L. major* leads to the occurrence of painless ulcers where the ulcer is of a wet skin type similar to the dry type, but it is distinguished from it by the fact that the central area, tends to necrosis, produces a hemorrhagic abscess, is characterized by being larger than the dry form, similar to it in that the edges gradually flatten, the ulcerated area has a diameter. 2-5 (cm) and cured within 3-6 months [13]. The incubation period for this species takes at least two weeks, and this species is the most widely distributed species in Iraq [14], as shown in Figure 1.



FIGURE 1: shown *L. major* [8]

Leishmania aethiopsica

This disease spreads in Ethiopia and Kenya. The symptoms appear in the exposed parts of the body (the face, neck, hands and legs) [15]. The wild rodents types Hyray are the parasite's storage hosts and a source of infection for humans. Symptoms appear in the form of a small point resembling a bed bug bite and then turn into a small scar centered on the face. It remains red and painless, but it causes itching. If it is not treated with specific treatment, the scar will gradually increase in size [16-17].

Mucocutaneous leishmaniasis

Mucocutaneous leishmaniasis is caused by the parasite *L. braziliensis*, *L. Mexicana*, *L. amazonensis* and *L. panamensis* in addition to other species include *L. infantum*, *L. major*, *L. tropica*, identified from a few mucosal leishmaniasis patients [18-19]. This disease causes skin lesions in the area where the mucous membranes meet around the nose and may spread to the inside, leading to tissue destruction and complete deformation of the face. In the case of successful treatment and healing of the ulcers, the lesions leave permanent scars and congenital abnormalities, causing the occurrence of the so-called camel's nose and in some cases secondary bacterial infections occur [20]. Rodents and some wild animals are considered reservoirs for this parasite [21], as shown in Figure 2. a; b.



FIGURE 2: shown (a) *L. infantum* (b) *L. tropica* [8].

Diffuse cutaneous leishmaniasis

This disease occurs due to the presence of an immune deficiency inside the body that allows the leishmaniasis parasite to spread within the subcutaneous tissues. For this disease, it is destructive to tissues, leading to complete deformation, and it is resistant to treatment and may be mistakenly diagnosed as leprosy [22-23].

Paper title

Paper titles should be written in upper-case and lower-case letters, not all upper-case, e.g., "Instructions for preparing papers for International Journal of Design & Nature and Ecodynamics". Do not use capital letters for prepositions, articles or conjunctions unless one is the first word.

Avoid writing long formulas with subscripts in the title; short formulas that identify the elements are fine (e.g., "Nd-Fe-B").

Leishmania parasite stages

Leishmania parasites go through two phases

Promastigote

This stage is characterized by being elongated to spindle in shape and its size ranges from (20-14 x 4-1.5) microns. It contains the motility generator, which is located 2 microns from the front end, from which extends a free flagellum with a length that sometimes reaches about 22 microns by the length of the parasite's body approximately. The nucleus is located in the center [24- 27] as shown in Figure (1).

Amastigote

This phase is found within macrophages only in the vertebral host. It is characterized by being oval to round, colorless, and its size ranges between (3-5) microns. This phase consists of one nucleus near the center, in addition to the motility generator, which is characterized by its variable shape between oval, circular and rod. Curved and very close to the basal granule (Blephoroplast) from which the axoneme extends [28-30], as shown in Figure 3.

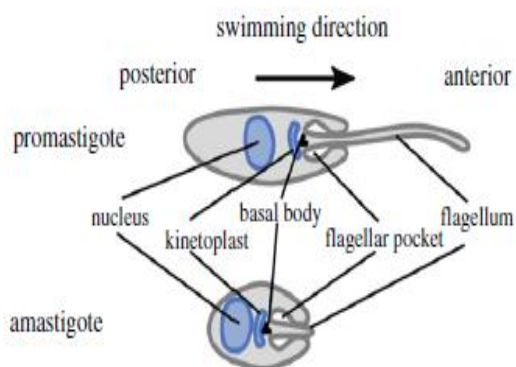


FIGURE 3. shown Promastigote & Amastigote phases [26]

Life cycle of Leishmania

The life cycle of Leishmania parasites passes through two hosts, the first one is the Invertebrate host and the second one is the Vertebrate host. The female sand fly sucks the blood from the infected vertebral host, thus entering the flagellumless phase of the parasite into the vector. Then the first step begins, which is the transformation of the flagellar phase into the anterior flagella, which divides to give the procyclic stage, and after 72 hours of feeding the

insect moves anterior to the promastigote and heads towards the anterior part of the middle intestine of the insect [31, 32]. The promastigote actively divides within the middle intestine of the insect to produce large numbers of the parasite, then it moves towards the head of the fly to settle in the pharynx and thus it is called salivarian and then moves towards the mouth parts to become ready for injection into the blood of the vertebral host with saliva. During the feeding of the fly, and when the fly stings a healthy human, the anterior flagella is devoured by the macrophages in the skin and quickly turns into the amastigote stage, as the latter multiplies by binary fission to produce large numbers of them until the cell explodes, thus infecting other cells, and thus the process continues. This period is called nursery [33-35], as shown in Figure 4.

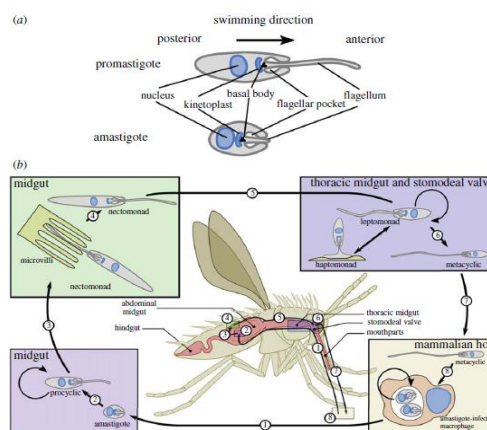


FIGURE 4. shown life cycle of Leishmania [26]

Methodology and Experimental design

The study was conducted from October 2021 until January 2022. The study included 55 samples cases of cutaneous leishmaniasis were diagnosed in Samarra General Hospital. The information of each patient with cutaneous leishmaniasis was written according to the information of each patient (age - gender - type of ulcer). The infection was diagnosed by a dermatologist at Samarra General Hospital. Blood samples were collected in a volume of (5) ml in clean dry tubes and placed in an incubator at 37°C for (5) minutes, then the coagulated fraction was separated from the clear solution using a centrifuge at a speed of (2000 cycle/min) for (15) minutes.

The clear solution represents the blood serum that was drawn using a micropipette, which was divided into (5) parts using plastic tubes. Serum samples were used directly for detection the glutathione peroxidase, peroxyntirite, zinc, magnesium and iron test by using the kit for each analysis.

Statistical analysis

All data were expressed as the mean SD of at least three independent experiments. Using the Origin 6.0 program, the statistical analysis for the parametric data was computed using an ANOVA (Microcal software, Inc. Northampton, MA, USA). After performing an ANOVA, post hoc analysis (multiple comparison t- test) was used to see how different each group was from the others.

RESULTS

L. tropica and GPx

The outcomes of blood samples taken from the patient infected by L. tropica were analyzed at the experiment conclusion to ascertain the impact of L. tropica on GPx. The results show significance (p < 0.05) (Table 1) decreased of the blood GPx in the patients diagnosed by L. tropica compared to the control group.

TABLE 1. L. tropica and GPx level

	M ± S.d	P value
Control group	55 ± 0.735	A
Patient group	10 ± 0.0386	B

Table 1: Changes in the blood total GPx level in the patient diagnosed with L.tropica. Each bar represents the Mean ± SD (n = 55) at a significance differences (p < 0.05) as compression to the control group

ONOO and L. tropica

The outcomes of blood samples taken from the patient infected by L. tropica were analyzed at the experiment conclusion to ascertain the impact of L. tropica on -ONOO. The results show significance (p < 0.05) (Table 2) decreased

of the blood -ONOO in the patients diagnosed by L. tropica compared to the control group.

TABLE 2. ONOO and L. tropica

	M ± S.d	P value
Control	10 ± 21.78	a
Patient	55 ± 14.34	b

Table 2: Changes in the blood total -ONOO level in the patient diagnosed with L.tropica. Each bar represents the Mean ± SD (n = 55) at a significance differences (p < 0.05) as compression to the control group.

Zn level and L. tropica

The outcomes of blood samples taken from the patient infected by L. tropica were analyzed at the experiment conclusion to ascertain the impact of L. tropica on Zn level. The results show significance (p < 0.05) (Table 3) decreased of the blood Zn level in the patients diagnosed by L. tropica compared to the control group.

TABLE 3. Zn level and L. tropica

	M ± S.d	P value
Control	55± 125.8	a
Patient	10 ± 117.8	b

Table 3: Changes in the blood Zn level in the patient diagnosed with L. tropica. Each bar represents the Mean ± SD (n = 55) at a significance differences (p < 0.05) as compression to the control group.

Mg and L. tropica

The outcomes of blood samples taken from the patient infected by L. tropica were analyzed at the experiment conclusion to ascertain the impact of L. tropica on Mg level. The results show significance (p < 0.05) (Table 4) increased of the blood Mg level in the patients diagnosed by L. tropica compared to the control group.

TABLE 4. Mg and *L. tropica*

	M ± S.d	value P
Control	10 ± 2.012	a
Patient	55 ± 2.1521	b

Table 4: Changes in the blood Mg level in the patient diagnosed with *L. tropica*. Each bar represents the Mean ± SD (n = 55) at a significance differences (p < 0.05) as comparison to the control group.

Fe and L. tropica

The outcomes of blood samples taken from the patient infected by *L. tropica* were analyzed at the experiment conclusion to ascertain the impact of *L. tropica* on Fe level. The results show significance (p < 0.05) (Table 5) decreased of the blood Fe level in the patients diagnosed by *L. tropica* compared to the control group

TABLE 5. Fe and *L. tropica*

	M ± S.d	P value
Control	55 ± 81.2	a
Patient	10 ± 61.8	b

Table 5: Changes in the blood Fe level in the patient diagnosed with *L. tropica*. Each bar represents the Mean ± SD (n = 55) at a significance differences (p < 0.05) as comparison to the control group.

DISCUSSION

Leishmania need GSH to survive, but it is unclear whether the parasite needs to maintain its GSH homeostasis in order to survive or not. The existence of any physiological and chemical variations between parasite and their host is so important of parasites survived and their infection [36-37]. An experiment of those paths plays significant roles of discovering procedure of appropriate drugs or vaccine candidate. In *Leishmania* parasite, redox metabolism ways are special protection techniques to induce reactive oxygen species (ROS), that helps the parasite to survive in the hostile environments for activate host macrophages. Redox metabolism path of *Leishmania* is completely different from human

host redox path. At the human host, GSH (glutathione) performing much decisive chemical or physiological operation, includes removing oxidative stress and saving cellular redox balance, stimulate by every intracellular or extracellular agents [38]. The current study found a decrease in the level of glutathione in patients with leishmaniasis compared to the control group. The significant decrease in glutathione peroxidase (GSH-Px) activity in the leishmaniasis patients may be due to the overproduction of ROS and RNS that result in oxidative stress and acceleration of lipid peroxidation in leishmaniasis patients [39].

It has been demonstrated the huge susceptibility of *Leishmania* to reactive nitrogen species (RNS) [40]. Actually, RNS is a major significance through the function of the natural immune system [41]. Under physiographic and pathophysiological circumstances, NO as a signal molecule, is capable to react with special cellular components to form RNS, such as peroxynitrite (ONOO⁻) nitrogen dioxide (NO₂), dinitrosyl iron compound, and nitrosothiols. Each of these types has different targets and biological impacts [42]. The results of the current study showed a high level of peroxynitrite in people with leishmaniasis compared to the control group. The significance of radical nitric oxide in *Leishmania* had been confirmed in several animal models and macrophage cell cultures [43-44]. Studies have reached a relevance among reduced levels of NO[•] during suppression of nitric oxide synthesis and reduced impedance against *Leishmania* [45]. The rise in oxidants found as a result of this survey is in conformity with all the preceding studies, all confirmed the excess in reactive types produced and the imbalance of oxidative-anti-oxidative [46-48].

The present results showed a significant reduction of the blood zinc element in the infected patients by leishmaniasis compared to the control group. The previous studies which conducted on *L. tropica* indicated that the function of certain inflammatory products at completely regulator zinc equilibrium, as well as that a cellular movement formative of leucocytes (interleukins) releasing through effective

phagocytosis, stimulation hypoglycemia in patients infected by rerouted plasma zinc to the liver, as well as the cause of zinc deficiency may be due to resulting from the synthesizing almathalutaonin at the liver and else tissues. Methallothionein binding by 7g for each atoms of Zn / mL [49].

Magnesium is other trace elements that increased in leishmaniasis patients. Mg is involved in immune responses and has anti-inflammatory properties. Mg reduces inflammatory responses [50, 51] and their deficiency increased inflammatory reactions [52]. Hence, the decreased levels of Mg in leishmaniasis could perturb the efficient immune responses against the parasite and could help in the persistence [53].

Iron is necessary for metabolic processes cellular diseases being contributory in the composition of numerous protein components including Fe2OD [54], ascorbate peroxidase, cytochrome b5 (CytB5) and cytochrome p450 (CYP) that are contributory in detoxification of reactive oxygen species (ROS) [55, 56]. The finding in a present survey, which showed the iron levels reduction in the patients infected by the leishmaniasis parasite compression to the control group, a reduction iron blood levels in the patients infected by the leishmaniasis with a proportion of transferrin concentration and an raise in the ferritin saturation, that indicated the inflammatory state pattern, through inflammation, the hepcidin hormone expression raised, especially during raise in interleukin IL-6 and IL-1 between acute phase proteins in the patients infected by the leishmaniasis, where hepcidin Performs a vital role as a coordinator of iron metabolism [57].

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