



The Relationship Between the Intestinal Parasitic Infections with Leptin Concentration and Some Biochemical Parameters in Babylon Province/Iraq

Nebras A. J. Hussien*, Hussein J. Al-Harbi, Kassim A. H. Al- Morshidy

University of Babylon, College of Science, Department of Biology-Iraq

*Corresponding Author: Nebras A.J. Hussien

Abstract

The current study aimed to determination of the relationship between intestinal parasitic infections with leptin, reactive oxygen species (ROS) and total antioxidants in patients in Babylon province, Iraq from the December 2015 to April 2016. The total exanimated person who visited the hospitals (male and female) are 863 person, 85 (9.8%) persons were infected with intestinal parasites (*Entamoeba histolytic* and *Giardia lamblia*) included 56 females and 29 males. The patients were divided by age into three groups included 52 (1-10) years (35 females and 17 males), 19 (11-20) years (13 female 6 males) and 14 (20>) years (8 females and 6 males). The healthy group included 40 persons which divided to the same groups of patient 20 (1-10) years (10 females and 10 male), 10 (11-20) years (5 females and 5 males) and 10 (20>) years (5 females and 5 males).The results showed significant increase ($p<0.05$) in serum ROS level in patients infected with parasite infections compare to healthy group, and significant elevated ($p<0.05$) in leptin level in female patients compared to male patients. When compare between age groups, the results obtained significant decrease ($p<0.05$) in leptin level in patients aged (1-10 & 11-20) years and elevated in age group (>20) years compare to healthy group in the same age, also significant elevated in ROS level in patients aged (1-10 & >20) years compare to healthy groups. The results of parasite type showed significant increased ($p<0.05$) in ROS level in patients infected with *E. histolytic* compared to patients infected with *G. lamblia*. The result showed the infected with parasite effected both leptin and ROS levels, but the total antioxidant did not affect parasitic infections.

Introduction

Intestinal parasites constitute major health problems, especially in the tropical and subtropical regions. In developing countries, it is estimated that some 3.5 billion people are affected, and that 450 million is ill as a result of these infections, the majority being children [1].

The main intestinal parasites that infect man is *Entamoeba histolytic* a, *Giardia lamblia*, *Cryptosporidium* species, *Taenia saginata*, *Hymenolepis nana*, *Ascaris lumbricoides*, *Trichuris trichura*, *Enterobius vermicularis*, Hookworms [1].

The leptin hormone has a wider regulatory role of fatty tissue mass may be because it seems that this hormone involved in fertility control at the beginning of puberty, in regulating the immune response. Lepton harms one synthesized by fat tissue mass [2-3]. Some studies on leptin levels that showed

an increase during parasitic infections. The suggested of leptin results that seem not responsible for anorexia caused by parasites. Serum leptin concentration in children, showed low concentrations in many forms of malnutrition, including delayed intrauterine growth, UN treated of anorexia nervosa, and showed increased in the adult [4].

Free radical was defined as a molecular fragment that contains one or more unpaired electrons in its furthest atomic or molecular orbital and capable of free existence [5]. A number of studies have pointed to an increase in the amount of reactive oxygen species in the host cells infected with the parasite.

The increased in free radical in cells infected with parasites relies on the nutritional status of the host, the degree of parasitic infestations and on the devastating impact

on tissue. [6]. Antioxidants are any molecule compounds or systems exist in low concentration, delayed oxidation (proteins, carbohydrates, fats and DNA) by Inhibiting the formation of free radicals[7-8]. The relationship between oxidative stress and antioxidants among humans with parasitic infections may help to explain the effect of asymptomatic intestinal parasitic infection. Oxidative stress has benefit to host inflammatory cells, stimulating the manufacture of reactive oxygen species (ROS) to invading parasites [9-10]. Oxidative enzymes arisen in individuals with intestinal parasitic infections [11].

Materials and Methods

Collecting 863 stool samples for the period from December 2015 to April 2016 in Babylon province. 85 patients were founded infection with intestinal parasites (*E. histolytic a* and *G. lamblia*).

The age of the infected patients with intestinalis parasites were between (1-60) years 56 female and 29 male, the patients divided according to age involved 52 (1-10) years (35 female and 17 male), 19 (11-20) years (13female and 6 males), 14 (20>) years (8 female and 6 male), the healthy group were 40 samples aged between (1-60) years, divide according to age involved 20 (1-10) years (10 female and 10 male), 10 (11-20) years (5 female and 5 male), 10 (20>) years (5 male and 5 female), The samples were examined by using a direct smear method [11].

Sample Macroscopic Examination

Examine stool samples include considering the amount of feces, shape, consistency and color. In liquid or soft specimens often trophozoites to appear while the Cystic stages appear in semisoft samples.

The stool may contain blood or mucus, so these parts should be examined separately and carefully because it may contain trophozoite stage of parasite. At the same time, if you access more than one sample to the lab, the first examine the liquid sample and mucus [12].

Microscopic Examination (Direct smear method)

It was during this way putting a drop of normal saline (0.9%) on one side of the glass

slide clean and dry and again from Lugol's iodine on the other side of the same glass slide, then took a small amount by a wooden stick head and mix well with a drop of solution and back with iodine swab are thin, have taken samples from different parts of the model to increase the possibility of a parasite, if one put glass slide cover on the two samples are sloping to avoid air bubbles and large particles were removed from the sample because they prevent glass slide cover to be in direct contact with the sample and therefore difficult to test and diagnosis [13].

Blood Sample Collection

Withdraw 5 ml of blood of 85 patients under study from patients that infection with *E. histolytic a* and *G. lamblia* and also withdraw 5 ml of blood of 40 blood samples from healthy children and adult (sample control) mediated medical sterile needle (G23) 5 ml usable once disposable it put 3 ml of blood in a gel tube, for use once and without material for clotting no anticoagulant left for a period of time, then placed in a centrifuge type (T30) German origin quickly 3000 r/min serum placed in the append off tubes and stored for use later.

Biochemical Parameters Estimation

Leptin, ROS and total antioxidants were estimated in sera of patients and healthy persons by using ELIZA technique and using kits from Elab science Company / China.

Statistical Analysis

The data were analyzed using the SPSS program, standard version 23. Quantitative data are presented as mean \pm standard error. Study t- test and a p- value \leq 0.05 was considered to be statistically significant.

Results

The result of biochemical parameters of patients infected with *G. lamblia* & *E. histolytic a* showed significant change in most parameters of the study.

Table (1) reveled no significant difference ($P>0.05$) in Leptin and antioxidants levels, while finding significant increased ($p<0.05$) in ROS levels patients infected with *G. lamblia* & *E. histolytic a* compare with healthy groups.

Table 1: Comparison in biochemical parameters between patients that infected with *E. histolytica* & *G. lamblia* and healthy groups

Standard	Patient	Healthy	Sig.
Leptin pg./ml	4104.41±381.80	4527.54±465.04	0.31
ROS ng/ml	9.51±0.12	8.85±0.28	0.05
Antioxidant Mm/ml	527.56±10.62	464.54±12.93	0.56

When compared between female and male patients, the result showed significantly elevated ($p<0.05$) in leptin level in female

Compared with male, but the ROS and Antioxidant did not significantly difference ($P>0.05$) in Table (2)

Table 2: Comparison between male & female infected with (*E. histolytica* & *G. lamblia*) according to biochemical parameter concentrations

Standard	Female	Male	Sig.
Leptin pg./ml	4332.33±526.92	3822.86±561.97	0.05
ROS ng/ml	9.49±0.14	9.53±0.22	0.16
Antioxidant Mm/ml	523.88±15.23	543.85±15.41	0.28

When compared between different age groups among patients and healthy, the result obtain significant decrease in leptin levels in patients aged (1-10 & 11-20) years and significantly increased with age >20 in patients compared with healthy group. Also

significant elevated in ROS level in patients aged (1-10 & >20) years compared with healthy group, while the antioxidant level showed no significant changes in all age groups in patients compared with the healthy group (Table 3,4,5).

Table 3: Comparison between patient aged (1-10) years infected with intestinal parasites and healthy groups according to biochemical parameters concentration

Standard	Patient (1-10) years	Healthy	Sig.
Leptin pg./ml	3885.97±654.26	5283.45±341.09	0.00
ROS ng/ml	9.70±0.14	8.93±0.59	0.02
Antioxidant Mm/ml	527.14±15.41	476.40±15.98	0.24

Table 4: Comparison between patient aged (11-20) years infected with intestinal parasites and healthy groups according to biochemical parameters concentration

Standard	Patient (11-20)years	Healthy	Sig.
leptin pg./ml	3492.28±665.96	4530.77±913.25	0.05
ROS ng/ml	9.32±0.24	9.38±0.22	0.25
Antioxidant Mm/ml	514.90±21.77	439.11±19.24	0.28

Table 5: Comparison between patient aged (20<) years infected with intestinal parasites and healthy groups according to biochemical parameters concentration

Standard	Patient(>20)years	Healthy	Sig.
leptin pg./ml	5141.62±601.19	4084.40±782.94	0.05
ROS ng/ml	9.17±0.49	8.31±0.45	0.05
Antioxidant Mm/ml	553.06±23.83	471.36±27.67	0.90

The result in Fig (1,2 and 3) showed significant elevated ($p<0.05$) in ROS level in patients infected with *E. histolytica*

compared to patients with *G. lamblia*, but the level of Leptin & Antioxidant did not affect significantly ($P>0.05$).

Leptin level

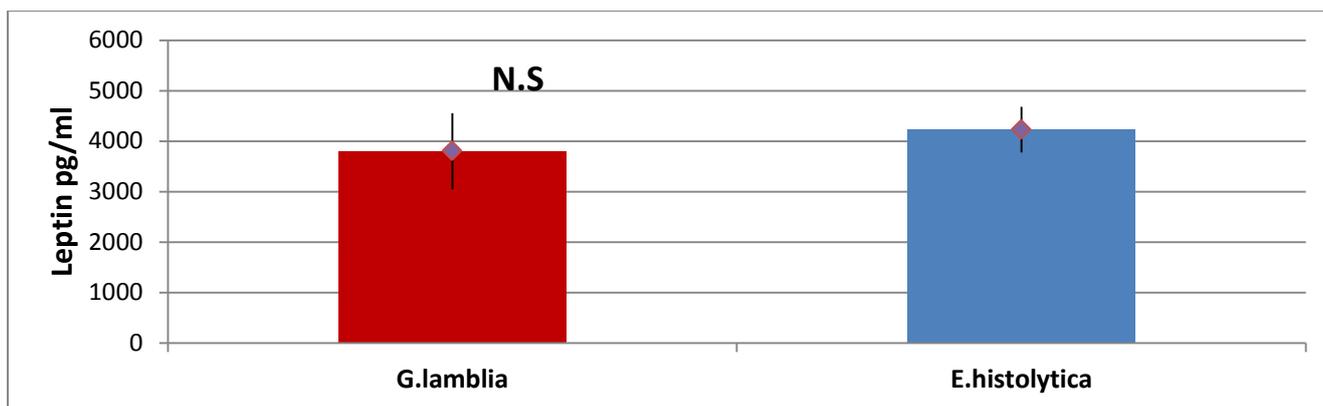


Fig 1: Effect of parasite type on a Leptin level in patient infected with *G. lamblia* & *E. histolytica*
 N.S: Non significant

ROS Level

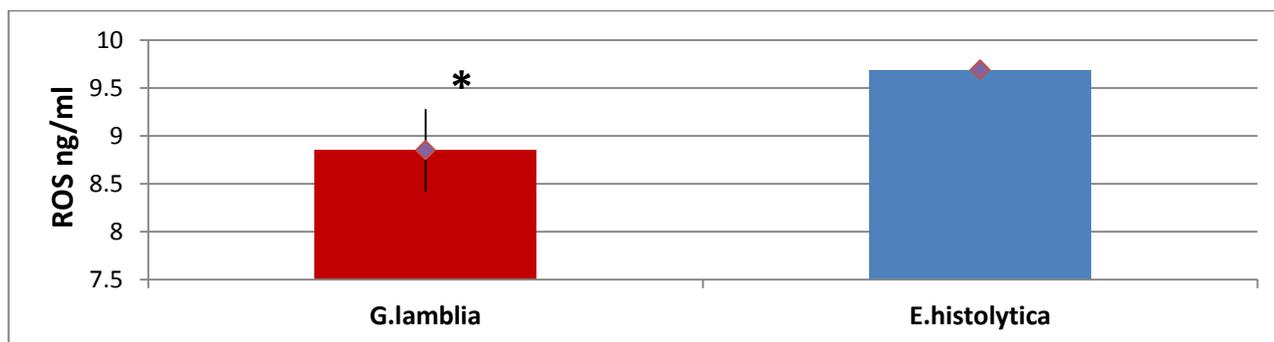


Fig 2: Effect of parasite type on a ROS level in patient infected with *G. lamblia* & *E. histolytica* a): (p<0.05)

Antioxidant Level

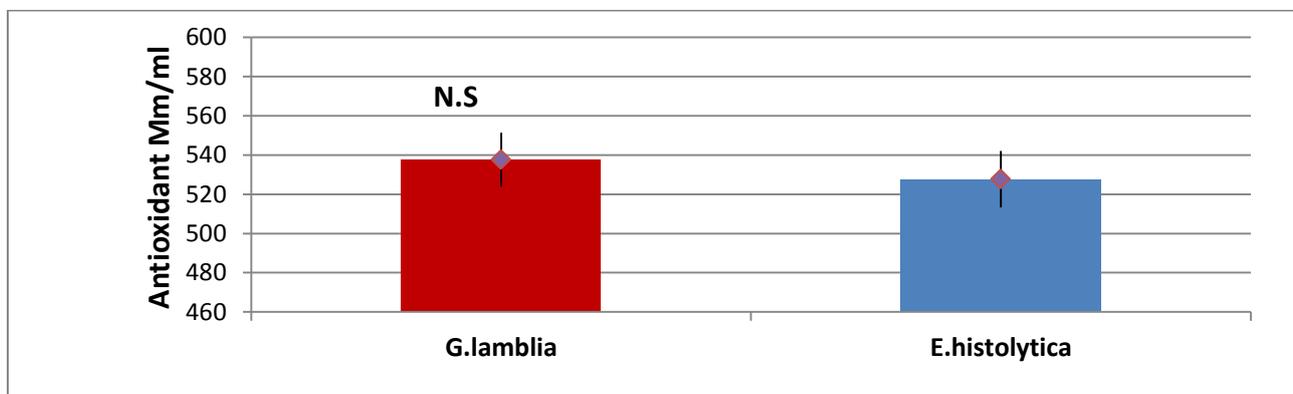


Fig 3: Effect of parasite type on an Antioxidant level in patient infected with *G. lamblia* & *E. histolytica* a N.S: Non significant

Discussion

The results in this study showed no significant difference ($P > 0.05$) in Leptin in patients infected with *G. lamblia* & *E. histolytica* compared with healthy groups (table 1), while showing leptin significantly decreased ($p < 0.05$) in patients with age (10-11), (11-20) years compared to healthy (Table 3- 4), the reason may be in several forms of malnutrition, leptin concentrations is lower, involved untreated anorexia nervosa, intrauterine growth retardation and children are susceptible to opportunistic infection and

the risk of end parasite infections increased in patient with malnutrition, which are causally related with a chain of events including inflammatory reaction, malabsorption, digestive problems, losses of nutrients and anorexia (2, 4).

And in (Table 2) leptin level significantly increased ($p < 0.05$) in female patient compared to male patient, also shows in (table 5) leptin level significantly increased ($p < 0.05$) in patients with age (20>) years, the reason may be due to During puberty, the percentage of body fat, increase in girl,

and this process regulates by sex hormones and growth hormone, also Leptin concentrations that relate to puberty, leptin levels reflect the total adipose tissue, which is closely linked with the fat under the skin (14). And leptin increase in *E. histolytica* compared to *G. lamblia* but no significantly difference ($p>0.05$) (Fig 1) may be *E. histolytica* causing tissue invasion and damage of intestinal mucosa such as ulceration, inflammation, pathological changes in the villi of epithelial cells in the acute period of infection lead to shortening of intestinal villi and dilation of the crypts which can activate mesenteric lymph nodes, then activates adjacent adipose tissues to secrete leptin, and The absence of this significant increase in *Giardia*, which has no or minimal tissue invasion [15].

And significantly increased ($p<0.05$) in ROS levels in patients compared to healthy group (table 1,3,5) may be increased of free radicals in the parasite cells determined by the degree of parasitic infestations, the destructive effect on tissue and the nutritional status of the host. End parasitic infection is amongst the main causes of disrupted in the balance between antioxidants and free radicals lead to oxidative stress, this disruption may be recognized by several factors such as the

increased manufacture of reactive oxygen species, the inability of the cells to produce sufficient amounts of antioxidants and the nutritional deficiency of minerals or vitamins [6,16]. And in (Fig 2) showing ROS significantly increased ($p<0.05$) in *E. histolytica* compared *G. lamblia*, The reason of *E. histolytica* trophozoites are exposed increased to reactive oxygen ROS during tissue invasion, colonization in the intestine, and extra intestinal propagation compared to *G. lamblia*, also increased ROS was generated during the consumption of hemoglobin by *E. histolytica* [17, 18].

The mean of antioxidant level increased, but

not significant ($p>0.05$) in patients infected with *G. lamblia* & *E. histolytica* compared with healthy groups. The reason of antioxidant increased in the patient compared healthy group may be suggesting that elevation of these antioxidant enzymes provides mainly protection against ROS-induced tissue injury during intestinal parasite [19], also antioxidant levels was higher among children with pathogenic intestinal parasitic infections compared to the healthy may be due to parasitic infection may result in higher susceptibility to lipid per oxidation [20].

References

- Gabbad AA, Elawad MA (2014) Prevalence of Intestinal Parasite Infection in Primary School Children in Elengaz Area, Khartoum, Sudan. Academic Research International. 5 (2):86-90.
- Büyükgebiz B, Öztürk Y, Yılmaz S, Arslan N (2003) Serum leptin concentration in children with mild-to-moderate protein energy malnutrition. *Pediatr Int*, 45: 550-554.
- Zaralis K, Tolkamp BJ, Houdijk JK, Wylie AR, Kyriazakis I (2009) Consequences of protein supplementation for anorexia, expression of immunity and plasma leptin concentrations in parasitized ewes of two breeds. *Br J Nutr*;101 (4): 499-509
- Karul A, Ertabakar H, Karatas E, Ertug s (2009) Serum Leptin Concentrations in Patients with Intestinal Parasites. *Türkiye Parazitoloji Dergisi.*; 33 (3): 207 – 211.
- Sinniah B, Hassan, A.K.R., Sabaridah, I., Soe, M.M., Ibrahim Z. and Ali, O.(2014). Prevalence of intestinal parasitic infections among communities living in different habitats and its comparison with one hundred and one studies conducted over the past 42 years (1970 to 2013) in Malaysia. *Tropical Biomedicine*, 31(2): 190–206.
- AbdEllah MR (2013) Involvement of free radicals in parasitic infestations. *Journal of Applied Animal Research*.41 (1):69-76.
- Brewer MS (2011) Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. *Comprehensive Reviews in Food Science and Food Safety*; 10:221-247.
- Sindhi V, Gupta V, Sharma K, Bhatnagar S, Kumari R, Dhaka N (2013) Potential applications of antioxidants-A review. *Journal of Pharmacy Research.*; 7 :8 2 8-8 3 5.

9. Gookin JL, Allen J, Chiang S, Duckett L, Armstrong MU (2005) Local peroxynitrite formation contributes to early control of *Cryptosporidium parvum* infection. *Infect Immun* .73: 3929-36.
10. El-Taweel H, El-Zawawy LA, Said DE, Sharara GM (2007) Influence of the antioxidant drug (Antox) on experimental giardiasis and microsporidiosis. *J Egypt Soc Parasitol* .; 37: 189-204.
11. Chandramathi S, Suresh K, Shuba S, Mahmood A, Kuppusamy UR (2010) High levels of oxidative stress in rats infected with *Blastocystis hominis*. *Parasitology* 137: 605-11.
12. Al-Ammash MJ (2015) Study on prevalence's of *Entameoba histolytica* & *Giardia lamblia* in Samarra city. *Kufa Journal for Veterinary Medical Sciences*.6 (2):194-204.
13. World Health Organization (1991) Basic laboratory methods in medical parasitology. Geneva.
14. Wauters M, Considine RV, Van Gaal, LF (2000) Human leptin: from an adipocyte hormone to an endocrine mediator. *European Journal of Endocrinology*: 143 293-311.
15. Yahya RS, Awad SI, Kizilbash N (2015) Enteric parasites can disturb leptin and adiponectin levels in children.
16. AbdEllah, MR (2010) Involvement of free radicals in animal diseases. *Comp Clin Pathol*, 19:615–619.
17. Prasanna chandra, D' Souza V, D 'Souza B (2006). Comparative study on lipid peroxidation and antioxidant vitamins E and C in *falciparum* and *vivaxmalaria*, *Indian Journal of Clinical Biochemistry*., 21 (2): 103-106.
18. Jeelani1 G, Nozaki T (2014) Metabolomic analysis of *Entamoeba*: applications and implications, *Current Opinion in Microbiology*. 20:118–124.
19. Jafari M, Salehi M, Shirbazou S et al.(2014) Evaluation of gender-related differences in responseto oxidative stress in *toxoplasma gondii* positive serum, *Annals of Military & Health Sciences Research*., 12(2): 64-69.
20. Mahittikorn A, Prasertbun R, Mori H, Popruk S (2014) Antioxidant enzyme activity among orphans infected with intestinal parasites in Pathumthani province, Thailand., 45 (6):1252-1263.