Study role of hematological and leptin biomarkers in human infected with Entamoeba histolytica parasite

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Abstract. The study was conducted on 58 out patients with Entamoeba histolytica parasite and 28 healthy people as control group to determine the influences of infected on levels of iron, ferritin and leptin in patients infected with E. histolytica in compared with healthy group. Who have visited Al-Sadder medical city and Al- Hakeem Hospital in Al- Najaf Province during the period from January till August 2016. This disease diagnosis by using the wet amount microscope for stool from patients. The results showed significant decrease (P<0.05) iron and ferritin in E. histolytica infection patients in compared to control group. Also the results showed significant decreased (P<0.05) in serum leptin of patients with E. histolytica in compared to control group.

Keywords: Leptin, Iron, Entamoeba histolytica

1. Introduction

Entamoeba histolytica is a gastrointestinal protozoon caused amoebiasis disease for persons by contaminated food and water with cyst or trophozoites. The introduction of the parasite does not necessarily develop into symptoms, a bigger percentage of infected persons are asymptomatic and they can be in this state for as long as a year but after signs seem, the disease can be dangerous because it may present diarrhea which may lead to acute dehydration [1, 2,3,4].

Phagocytosis starts with the ligation of the Galactose/ N-Acetylgalactosamine lectin (Gal/GalNAc lectin) [5] and in order to promote degradation, amoeba pores and cysteine proteinases are secreted to the phagosome [4]. There are evidences of the main role of cysteine proteinases as a virulence factor for E. histolytica being involved in the breach of the mucus barrier, which is crucial in the pathogenesis of amoebiasis [6,7].

E. histolytica is needs a high level of iron to continue, this protozoan pathogen is capable to get iron from host proteins, example hemoglobin, ferritin, Lactoferrin and transferrin. That E. histolytica trophozoites endocytose ferritin by clathrin-coated pits and degrade this protein by means of specific cysteine proteases in the endosome/lysosome pathway [8,9]. Ferritin is a heteropolymer composed of 24 subunits of two types, H and L, and the proportion of each subunit depends on the main function of the protein. For example, in the liver and spleen [10].
This pathogenic parasite capable of removing and acquiring iron from Ferritin can obtain a plentiful source of this crucial metal to survive, colonize and invade the host [11]. Ferritin is a versatile protein, mainly cytosolic, but localized also in other structures such as mitochondrion, nucleus, and in mammalian serum (in this case poor in iron). Ferritin is a heteropolymer composed of 24 subunits of two types, H and L, and the proportion of each subunit depends on the main function of the protein. For example, in the liver and spleen [10]. *E. histolytica* is an enteric parasitic protozoan that causes amoebiasis, a cosmopolitan infection that affects only human beings. Cyst is the infective stage transmitted by the fecal-oral route through the intake of contaminated water and foods. When cysts are ingested they can pass throughout the acidic pH of stomach, and in terminal ileum excystation occurs producing the invasive stage or trophozoites.

The aim of this study to estimate the levels of iron, ferritin and leptin in serum of patients infected with *E. histolytica* parasite. Due to its toxicity, iron is not soluble within the cell. In humans, most iron is found within intracellular proteins (60.5% in Hb, 8.3% in myoglobin, 26.3% in storage reserves (e.g., ferritin and hemosiderin) and 4.8% is part of diverse enzymes) [12].

2. **Material and methods**

2.1. **Patients & healthy group:**

From January till August, 2016, 58 samples were collected from patients and 28 healthy people who attended the clinics in AL-Sadder teaching Hospital and AL-Hakeem Hospital in AL-Najaf province. Stool samples were collected into clean, wide-mouth specimen bottles, from patients and control and examined microscopically using 40X objective lens for intestinal parasites as described by [13]. 3ml of blood samples were drawn by vein-puncture into specimen tubes and Separated for two parts the first 0.5ml with EDTA tube for detection of blood parameters and the second 2.5ml remains for 30 minutes at room temperature. After that the second part was centrifugation at 3000 rpm for 5 minutes (Backman/counter, Germany) to separate the serum and collected in other sterile tubes, each sample of serum was divided into three parts; each of them was kept in deep freeze at -20°C till used for the determination of iron, ferritin and leptin.

2.2. **Serum Iron**

The colorimetric test method was used to estimate the serum of iron via randox reagents, code HB012. (RANDOX Kit, U.K) by cypress diagnostics biochemistry analyzer, ferritin estimated by Ferritin ELISA/ Monobind / USA(product Code:2825-300) and serum levels of leptin estimated by using ELISA Kit supplied by Ray Biotech, Inc. China with number code Cat#: ELH-Leptin-001.

2.3. **Statistical analysis**

Data were analyzed using the software packages Graph pad prism for Windows (5.04, Graph pad software Inc. USA). Data are presented as the mean ± standard error (SE). The comparison between the patients and healthy groups were analyzed by one-way ANOVA. A p-value < 0.05 was considered significant.

3. **Results**

3.1. **Serum iron**

The Result of the current showed significant decrease (P<0.05) in concentration of serum iron in patients with amoebiasis 30.719± 0.013 ng /ml in comparison with control group 63.968 ± 0.603 ng/ml where as seen in figure (1).
Figure 1. Comparison between Iron levels of amoebiasis patients and control group.

3.2. Serum ferritin

The Result of the current showed significant decrease (P<0.05) in concentration of serum ferritin in patients with amoebiasis 13.321 ± 0.086 ng/ml in comparison with control group 26.906 ± 0.021 ng/ml where as seen in figure (2).

Figure 2. Comparison between ferritin levels of amoebiasis patients and control group.

3.3. Serum leptin

The Result of the current showed significant decrease (P<0.05) in concentration of serum leptin in patients with amoebiasis 5.632 ± 0.192 ng/ml in comparison with control group 9.281 ± 0.021 ng/ml where as seen in figure (3).
4. Discussion

The results revealed the serum iron, ferritin leptin significantly decrease in *Entamoeba histolytica* infection patients compared to healthy group. The decrease in iron level in patients with *E. histolytica* may be due to the pathogenicity of this parasite dependent on the relationship between iron concentration and adhesion of parasite on epithelial cell [14]. Whereas the source of iron maybe from the hemolysis of red blood cells from lesion occur by the parasite. The consuming of iron by *E. histolytica* may cause a decrease in the iron levels. The decrease in ferritin levels may be due to an increase in consuming iron by this parasite and this leads to decrease in the storage of iron as ferritin or increased utilized by parasite whereas some studies describing *E. histolytica* as an iron source [15]. As confirmed by the data from Lopez-Soto et al. (2009)[11] that *E. histolytica* use the ferritin as an iron source; therefore, ferritin decreased when an infectious process occur. The current study agrees with the study of Weinberg (1999)[16] who recorded that the *E. histolytica* uses the ferritin as source of iron and these lead to decrease in the ferritin in serum of men infected with *E. histolytica* compared to healthy group. The results has revealed that the serum leptin significantly decrease in *E. histolytica* infection patients compared to healthy group. The decrease in leptin level in patients with *E. histolytica* may be due to a role of leptin in host resistance to infection had been suggested in children and adult with congenital deficiency of the leptin receptor [17,18,19,20,21] or maybe due to deletion of the leptin receptor in the intestinal epithelium and increased susceptibility to amoebiasis [22,23,24,25,26].

5. Conclusion

From this study conclusion that serum levels of leptin decrease in patients infected with *E. histolytica* due to effects of early iron deficiency on the dopamine system and due to effects occur in receptor of leptin by this parasite.

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*Significant difference (P<0.05) between control group and patients

**Figure 3.** Comparison between leptin levels of amoebiasis patients and control group.
6. Significance statements

This study is the first clinical study in Iraq.

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8. References

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Abbreviations:

ANOVA- Analysis of variance
E. histolytica-Entamoeba histolytica
EDTA- Ethylene Diamine Tetra-acetic Acid
Gal/GalNAc lectin- Galactose/ N-Acetylgalactosamine lectin
Hb- Hemoglobin concentration
ELISA- Enzyme-Linked Immunosorbent Assays
SE-Standard error