

Impact of Intestinal *Entamoeba histolytica* on Sera of Leukotreins D4, Interleukin -6, Acid phosphatase and other Some Trace Elements

Khalil Ismail A.Mohamed¹, Mohammed Sami Khadhum²,
Huda Q. Mohammed Abu-Al-ess¹, Saad Hasan Mohammed Ali¹,
Suha A.AL. Fukhar¹, Wifaq M. Ali AL-Wattar¹ and Jinan M. Mousa¹

¹Clinical Communicable Diseases Research Unit, College of Medicine,
University of Baghdad, Baghdad-Iraq.

²Department of Basic Sciences, College of Dentistry, University of Baghdad, Baghdad-Iraq.

<http://dx.doi.org/10.22207/JPAM.11.3.17>

(Received: 21 June 2017; accepted: 19 August 2017)

The study was carried out during the period of February 2016 - September 2016 for the detection of *Entamoeba histolytica* in (66) patients with age group ranges from 21 -60 years who attended Al -Fayed clinical laboratory in Baghdad. The diagnosis done by microscopic examination and Triage (Micro parasite panel test) methods. Blood samples were taken from patients as well from other (30) healthy control matching in age and gender. The study included measurement of concentration of Leukotrein D4, Interleukin-6, Acidphoshatase activity, Zinc and Copper in sera of patients and control. The results indicated presence of parasites in all patients in both methods. The concentration of LTD4, IL-6, ACP increased significantly. The concentration of Zinc decreased significantly. The concentration of copper statistically non significant in both interval ages in patients sera in comparison with healthy control

Keyword: *Entamoeba histolytica*, LeukotreinsD4, Acidphosphatase, Interleukin-6, Copper, Zinc.

Entamoeba histolytica is the causative organism of amoebic dysentery, a disease which affects a large number of people every year in the tropical regions of the world. This organism invades the human gut by first adhering to the intestinal mucosa and then secreting enzymes for cytolysis¹.

Most cases are intestinal and asymptomatic. Symptoms, when occur, are multiple and varied, ranging from mild abdominal discomfort and diarrhea (often with blood and mucus) alternating with periods of remission or constipation, to severe illness with fever, chills, and significant bloody or a mucoid diarrhea. Amoebic

colitis may be confused with inflammatory bowel disease such as ulcerative colitis². The infection of *E. histolytica* occurs invade colonic crypts lamina propria and cause flask shaped ulcer, this ulcer may activate apoptosis in the target cells³. *Entamoeba histolytica* trophozoites reaching the liver create their unique abscesses, which are well circumscribed region of cytolysed cells, liquefied cells, cellular debris, the lesions are surrounded by connective tissue enclosing few inflammatory cells paranchymal cells adjacent to the lesion are often unaffected, however lysis of neutrophil by *E. histolytica* trophozoites might release mediators that lead to death of liver cells, and extended damage to hepatocyte⁴. of liver, ruptured into the lung; the other way is by lymph channels from amoebic hepatitis, and the last way is through the systemic circulation⁵. Cell mediated response have been characterized by lymphocytes proliferation

* To whom all correspondence should be addressed.
E-mail: dr.alkarkhi@gmail.com

and lymphokines secretion especially in patients with amoebic liver abscess. Suggestion a role of body immune system cells. the production of inflammatory cytokines, including IL-1 β , IL-6, IL-8, IL-12, IFN- γ , and TNF- α ^{6,7,8}. IECs are the second line of barriers against pathogens after the mucosal layer and the first line of host cells to encounter microbial/parasite antigens, they express an array of pathogen recognition receptors (PRRs), including TLRs⁹.

Recently, experimental studies show that zinc alter functionality of *E. histolytica* and reflect decrease in replication and adhesion manifested by inhibition of amoebic pathogenicity¹⁰. *E. histolytica* acid phosphatase activity is significantly inhibited by copper suggesting a possible roles in amoebic dysentery¹¹. In this study the level of Leukotreins D4, Interleukin-6, Acidphosphatase and Copper with Zinc determined in patients with acute Amoebiasis as inflammatory mediators in patients with healthy control group matched in age and gender.

MATERIAL AND METHODS

Studied groups

The study carried out during the period from (February 2016- November 2016), the age of patients extended from (21 – 60) years, two studied groups were involved Suspected patients: Blood and stool samples were obtained from a total of 66 patients clinically suspected with amoebic dysentery that had been examined and defined as suspected cases by specialized physician and healthy control

Samples collection

Stool sample from each patient was collected in a clean, dry tight cover container and examined with a half an hour. The samples were examined for the presence of *E. histolytica*.

Stool sample examination

Macroscopic examination

It was performed by observing the consistency of stool, presence of blood, mucous and other substances.

Microscopic examination

For each stool sample, wet mount preparation slide was examined by clean, dry slides by obtaining one drop of normal saline and small

amount of stool from different places of stool by using clean wooden stick, especially when blood or mucous were noticed, then mixed gently with normal saline and covered with cover slip, the slide was examined under the low (10x) and high power (40x) of microscope¹².

Specific test for *E. histolytica* (Triage) Cassette

This test is based on quantitative Immunochromatographic assay for determination of *Entamoeba histolytica* in stool samples.

Assay Procedure

1. The cap of the stool collection tube was taken out and used the stick to pick up sufficient sample quantity.
2. Introduced the stick once into 4 different parts of the stool sample (\approx 100mg) and added it to the stool collection tube.
3. For liquid samples (\approx 100mg) was added in the stool collection tube by using a micropipette, closed the tubes of the diluents and stool samples.
4. Proceeded to shake the stool collection tube in order to assure good sample dispersion.
5. The *Entamoeba* card test was removed from its sealed bag just before use.
6. The stool collection tube was taken, cut the end of the cap, and dispensed 4 drops in the circular window marked with letter S. Avoid adding solid particles with the liquid.
7. Read the results at 10 minutes.

Blood samples

Five mL of Venus blood was obtained from each patient and collected in sterilized screw cap plastic tube, blood samples were left for 30 min. at room temperature, then centrifuge at 3000 rpm for five minute, then the serum for each sample was collected in eppendorf tubes and stored in deep freeze at -20°C until the time for using. The current study included Immunological & Clinical biochemical aspects. the level of interleukin -6(IL-6) estimated by ELISA according to manual procedure of cusabio Biotech(Germany) and Leukotreins D4 were estimated by ELISA according to the manual procedure of Creative – Diagnostic Company. Copper, Zinc and acidphosphatase Concentration determined according to manufactures instructions of Biosystem(Spain).

Statistical Analysis

The results were analyzed using statistical system SPSS version -18 (T-testing).

RESULTS

Diagnosis of *E.histolytica*

The result of *E.histolytica* show prevalence using direct microscopic Examination and Triage (Micro parasite panel test) that 66 patient with a percent of 100% infected with Entamoebiasis (Table 1).

LeukotreinsD4

The level of Leukotreins D4 increased significantly ($p \leq 0.05$) in patients with *E.histolytica* in comparison with healthy control in both interval ages till reach to 48.61,37.71 for patients and 31.75,23.25 for healthy control respectively (Table-2).

Interleukin-6

The level of IL-6 Increased significantly ($p \leq 0.05$) in patients with *E.histolytica* in comparison with healthy control in both interval ages the value 21489,21449 pg/ml for patients and 11470,11430 pg/ml for healthy control respectively (Table 3).

Zinc and Copper

The concentration of zinc decreased significantly ($p \leq 0.05$) in both interval ages of patients with Entamoebiasis in comparison with healthy control (Table 4). While the result of copper statistically non significant in both interval ages of patients and healthy control.

Acidphosphatase activity

The activity of acid phosphatase increased significantly $p \leq 0.05$ in both interval ages of patients

in comparison with healthy control (Table-5).

DISCUSSION

The presence of *E.histolytica* by using direct microscopic examination and Triage (Table 1). the result show no difference between the two methods. In spite of the microscopic examination of stool samples considered to be the gold standard for diagnosis of Entamoebiasis and other parasites¹³. However, microscopy has several important disadvantages among these (I) Correct identification depend greatly on experience and skills of microscopist (II) Sensitivity is low and therefore, examination of multiple samples is required (III) *E.histolytica* cannot be differentiated from the other nonpathogenic *E.disapr* simply on the basis of the morphology of the cyst and small trophozoites¹⁴. The Triage is immunoassay to diagnosis the stool for antigens for the parasites¹⁵. The increasing level of LeukotreinsD4(LTD4) in patients with *E.histolytica* in comparison with healthy control may be associated with the impairment of the immune system especially during the acute phase of disease by the appearance of suppressor CD8 lymphocyte, than, defect in cell mediated immune response which occur in amoebic infection. However, the mechanism of immunosuppression in Entamoebiasis occur by induce macrophages eicosanoides in both Cyclooxygenase and 5-Lipoxygenase pathway to produce prostaglandins and Leukotreins¹⁶ which

Table 1. Distribution of *Entamoeba histolytica* infection according to microscopic examination and Triage

Methods	No. of samples	No. of positive	%
Microscopic examination	66	66	100 %
Triage	66	66	100 %

Table 2. Concentration of Leukotreins-D4 in patients with *E.histolytica* and healthy control

Parameters	Age categories	Leukotreins D4(ng/ml)
20-40	Patients	48.61±3.06
	Control	31.65±6.78
40-60	Patients	37.71±1.82
	Control	23.25±1.75

Table 3. Concentration of Interleukins-6 in patients with *E.histolytica* and healthy control

Parameters	Age categories	IL -6(pg/ml)
20-40	Patients	21489±2.76
	Control	1470±5.30
40-60	Patients	21449±4.50
	Control	1430±2.90

Table 4. Zinc and Copper concentration(mmol/L) in patients with *E.histolytica* and healthy control

Parameters Age categories	Zinc		Copper	
	Patients	Control	Patients	Control
20-40	9.8±0.6	12.4±2.3	18.3± 4.1	11.5±1.6
40-60	10.6±0.4	13.8±3.1	17.6±2.8	11.9±2.3

Table 5. Acid phosphatase activity in patients with *E.histolytica* and healthy control

Age categories	Patients	Acid phosphatase (IU/ml)
20-40	Patients	0.9±0.3
	Control	0.4±0.2
40-60	Patients	0.1±0.12
	Control	0.5±0.1

play important role in regulation of cellular and humoral immune response included the suppression of macrophages derived TNF- α and gene expression of interleukin-1 production and the MHC-II and other signal peptide necessary for cell-to cell communication would alter macrophage-lymphocyte driven reaction and down regulate the local immune response^{17,18}. The increasing level of IL-6 in patients with Entamoebiasis in comparison with healthy control(Table-4) may be due to ability of *E.histolytica* to up regulate of Th2 and down regulate of Th1 to inhibit INF- γ (19)INF- γ involved in clearance of infection and correlated with the protection from *E.histolytica* infection^{20,21} The result of the study demonstrate that serum level of zinc decreased in patients with acute *E.histolytica* were a significant difference was not observed for serum copper level (Table-4). However, acute phase of infection with Entamoebiasis causes increased metallothionein mediated hepatic uptake of serum zinc ,leading to hepatic accumulation of zinc than decreased serum zinc level via interleukin -1 mediated mechanism²² than increase the severity of disease ,altered immune status and impaired antioxidant system²² on the other hand the immune response up regulate of Ceruloplasmin gene and synthesis Ceruloplasmin -CU complex in the blood. A Ceruloplasmin contain 95% of total serum copper and this may at least partly explain lack of a significant increase or decrease in serum Copper concentration. Acid phosphatase increased

significantly in patients with Entamoebiasis (Table 5) in a general ,ACP considered as a virulence factor in some pathogenic microorganism or may be important for management of disease severity²⁴.

CONCLUSION

The result indicated presence the parasites in all patients in both methods .The concentration of LTD4, IL-6, ACP increased significantly. The concentration of Zinc decreased significant. The concentration of copper statistically non-significant in both interval ages in patients sera in comparison with healthy control .

REFERENCES

1. Talamas-Rohana, P. and Meza, I. Interaction between amebas and ϵ bronectin: substrate degradation and changes in cytosolic organization. *J. Cell Biol.*, 1988; **106**: 1787-1794.
2. Raha, S., Giri, B.B., Bhattacharya, B. and Biswas, B.B. Inositol (1,3,4,5)-tetrakis phosphate plays an important role in calcium mobilization from *Entamoeba histolytica*. *FEBS Lett.* 1995; **362**: 316-318.
3. Halla, A. C.; Haidar, K. Z. and Ahmed K. O. Biochemical Changes of Liver That Infected with *Entamoeba histolytica* In White Rats. *J. Babyl. Universe.* 2014; **22**(9):2360-2352.
4. Stanley, J. R. Amebiasis. *Lancet.*, 2003; **361**:1025-34.
5. Zaki, H. A. Pulmonary Amoebiasis. Journal. Publications.chestnet.org.
6. Guide to Surveillance, Reporting and control. Amoebiasis. Massachusetts Department of public Health, Bureau of Communicable disease Control., 2006; 29-35.
7. Bansal D, Ave P, Kerneis S, Frileux P, Boché O, Baglin AC, et al. An ex-vivo human intestinal model to study *Entamoeba histolytica* pathogenesis. *Plops Negl Trop Dis*, 2009; **3**:e551. doi:10.1371/journal.pntd.0000551
8. Galván-Moroyoqui JM, Del Carmen Domínguez-Robles M, Meza I. Pathogenic bacteria prime

- the induction of toll-like receptor signalling in human colonic cells by the Gal/GalNAc lectin carbohydrate recognition domain of *Entamoeba histolytica*. *Int J Parasitol*, 2011; **41**:1101–12. doi:10.1016/j.ijpara.2011.06.003
9. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. *Int Rev Immunol*, 2011; **30**:16–34. doi:10.3109/08830185.2010.529976
 10. Vega, R.G, Carrero, J.C., Ortiz, L. Effect of zinc on *Entamoeba histolytica* pathogenicity. *parasitol. Res.*, 1999; **85**:487–492.
 11. Agrawal, A., Prasad, H.C, Pandey, V.C, Sagar, P. Specific features of phosphomonoesterase of *Entamoeba histolytica*. *India. J. Exp. Biol.*, 1990; **28**: 141–143.
 12. Frances, F. and Marshall B. D. A Manual of Laboratory and Diagnostic Tests. Lippincott Williams & Wilkins. 8th ed, 2009; 286–290.
 13. Jaco, J.V.; Eric, A.B. ; Marianne, A.; Avan, R. and Lisetta, V. L. Simultaneous detection of *Entamoeba histolytica*, *Giardia lamblia* and *Cryptococcus parvum* in fecal samples by using multiplex Real time PCR. *J. of clinical microbiology.*, 2004; **42**(3):1220–1223.
 14. Hove, R.T.; Schurman, T.; Moller, L.; Lieshout, L. and Verweij, j. j. Detection of diarrhea-causing protozoa in general practice patients in the Netherlands by multiplex real time PCR. *Clinical microbiology and infection*, 2007; **13**(10): 1002–1007.
 15. Susan, E. S.; Clarisa, A. S.; Yolanda, D. Robert, J. P. Evaluation of the Triage Micro Parasite Panel for Detection of *Giardia lamblia*, *Entamoeba histolytica*/ *Entamoeba dispar* and *Cryptosporidium parvum* in patient stool specimens. *Amer. Soci. Microbiol. J. Clin. Microbiol.*, 2001; **39**(1): 332–334.
 16. Wang, w, Chadee, k. *Entamoeba histolytica* alters arachidonic acid metabolism in macrophages *in vitro* and *in vivo immunology*, 1992; **76**: 242–250.
 17. Bansal D, Ave P, Kerneis S, Frileux P, Boché O, Baglin AC, et al. An ex-vivo human intestinal model to study *Entamoeba histolytica* pathogenesis. *PLoS Negl Trop Dis*, 2009; **3**:e551. doi:10.1371/journal.pntd.0000551
 18. Galván-Moroyoqui JM, Del Carmen Domínguez-Robles M, Meza I. Pathogenic bacteria prime the induction of toll-like receptor signalling in human colonic cells by the Gal/GalNAc lectin carbohydrate recognition domain of *Entamoeba histolytica*. *Int J Parasitol*, 2011; **41**:1101–12. doi:10.1016/j.ijpara.2011.06.003
 19. Guo X, Stroup SE, Houpt E. Persistence of *Entamoeba histolytica* infection in CBA mice owes to intestinal IL-4 production and inhibition of protective IFN- γ . *Mucosal Immunol*, 2008; **1**:139–46. doi:10.1038/mi.2007.18
 20. Ghadirian E, Denis M. In vivo activation of macrophages by IFN- γ to kill *Entamoeba histolytica* trophozoites in vitro. *Parasite Immunol*, 1992; **14**: 397–404. doi:10.1111/j.1365-3024.1992.tb00014.x
 21. Lin JY, Chadee K. Macrophage cytotoxicity against *Entamoeba histolytica* trophozoites is mediated by nitric oxide from L-arginine. *J Immunol*, 1992; **148**:3999–4005.
 22. Min, k.s, Terano, y, Onosko, s, Tanaka, k. Induction of hepatic metallothionein by non metallic compounds associated with acute phase response in inflammation *Toxicol. Appl. pharmacol.*, 1991; **111**:152–162.
 23. Franco, E, De Arajo soares, R.M., Meza, I. Specific and reversible inhibition of *Entamoeba histolytica* cysteine-proteinase activities by zinc implication for adhesion and cell damage. *Arch. Med. Res.*, 1999; **30**: 82–88.
 24. Chandhuri, s., Choudhury, N. and Raha, S. Growth stimulation by serum in *Entamoeba histolytica* is associated with protein tyrosine dephosphorylation. *FEMS. Microbiology letters*, 1999; **178**: 241–249.