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Investigation on phagocytosis index & serum (IGE, IGG, IGM) in patient of *Entamoeba histolytica*

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Abstract
The current study was conducted at the Al-Hussein Teaching Hospital in Baghdad province. It aimed to evaluate immune status amoebiasis of a patients by measuring the levels of immunoglobulin (IgE) by enzyme-linked immune sorbent assay (ELISA) and immunoglobulin (IgG, IgM) by single radial immune diffusion (SRID).
Its also included test phagocytic cells on phagocytosis (coefficient of phagocytosis). The study included a total of 100 patients with amoebiasis, they aged between 3-4 years. The results of the statistical analysis showed high significant increase ($P \leq 0.001$) in serum IgE, IgG of patients with amoebiasis compared with healthy control group, and noticeable significant difference in serum IgM of patients with amoeba compared with healthy control group. Phagocytosis coefficient were increased in patients and control group with significantly $P \leq 0.001$)

Keywords: Amoebiasis.IgE, IgG, IgM, Phagocytosis

Introduction

Entamoeba histolytica is an aerobic parasitic part the genus Predominantly infecting humans and other primates causing amoebiasis. It is an enteric tissue invasive protozoan parasite that causes amebic colitis and occasionally liver abscess in human (Kimka,. 2007) [1]. At extreme cases leads to ulcer disease as well as chronic colitis.

The trophozoite its natural habitat in the lumen of large intestine especially in cecal and sigmoid or rectal region causes symptomatic amoebiasis including diarrhea, abdominal pain and cramping or amoeba may produce, but patient show no clinical symptoms called Asymptomatic carriers(Variyam EP, 2007 [2], and Cuerrent *et al* 2000).Several means of transmitting *E. histolytica* are known, ingest of the infective cyst occurs in contaminated food and water(Aguiar *et al*, 2007).·The incubation period for infection with *E. histolytica* takes 1-4 weeks and the pathogenesis depends on parasite factors, host factors and transmission methods (Diamond, L.S. and Clar, C.G, 1993) [6]. Inflammatory diarrhoea produces a severe form of acute diarrhoea and requires additional medical and laboratory evaluation, in contrast non-inflammatory diarrhoeas generally are milder in immunocompetent hosts, although severe fluid loss with attendant morbidity and mortality can occur, especially malnourished individuals or low hygienic status (Eichinger, D. 1997, and Al-saad 2009) [5]. Inflammatory diarrhoea is caused primarily by invasive or toxin-producing organisms, including, *Clostridium difficile*, *Enterohemorrhagic* and *Enteroinvasive Escherichia coli* or Protozoa like *Entamoeba histolytica* beside the viral causes (Nari, G.A, *et al* 2008 [9], Butel *et al* 2004) [7] These organisms can produce obvious macroscopic and histologic alterations of the mucosal lining of the colon (Ordaz-Pichardo *et al* 2005) [8].

Amoebiasis is widely distributed all over the world, the infection with *E. histolytica* is increased in tropic and sub tropic regions, particularly in poor sanitation areas. It is responsible for at least 50 million cases of diarrhea and an estimated 100,000 deaths per annum and ranks second only to malaria as a cause of mortality, infection with *E. histolytica* leads to amoebic colitis, colonic ulceration and amoebic liver abscess according to report(WHO,2000). The World Health Organization/ Pan American Health Organization/ Unesco Expert Consultation on Amoebiasis held in 1997 (WHO,1997).

Immunoglobulins (Ig): The immune system generates billions of different antibody molecules by mature B cells which are capable of secreting antibodies and expressing B cell receptors on their cell surfaces. (Rolink, A.G. *et al*,1999 [10] Brekke, O.H. and Sandlie 2003) [11] Ig antibodies are large proteins composed of four polypeptide chains (two identical heavy chains and two identical light chains) joined together by disulphide bonds. Each Ig recognizes a specific antigen unique to its target and is used by the immune system to locate and destroy invading microorganisms (Takai T, 2002) [12]. Serum immunoglobulin levels provide key information on the humoral immune status. (Buckley, R.H., Dispenzieri, *A. et al*, 2001) [14]

Immunoglobulin E (IgE)
is a class of antibody or immunoglobulin (isotype) that has been found only in mammals. IgE's main function is immunity to parasites such as helminthes (Erb K, 2007) [15] also plays an essential role in type I hypersensitivity, (Gould H *et al*, 2003) [16] which manifests various allergic diseases, such as allergic asthma, food allergy, and some types of chronic atopic dermatitis

Immunoglobulin M (IgM): It is the first isotype to be generated during a primary immune response and it is predominates in immune response to most antigens. Its pentameric structure is a highly effective activator of complement. IgM is the first immunoglobulin class to be synthesized by the neonate. IgM is larger than IgG with a molecular mass of approximately 950 kDa that makes up about 8% of the antibody in the serum (Hayzer, D.J. and Jaton, 1985 [17], Klaus, D.E.1998) [18].

Immunoglobulin (IgG): Immunoglobulin G is a major effector molecule of the humoral immune response in man, accounts for about 80% of the total immunoglobulins in plasma of healthy individuals. The IgG (150 kD) is composed of two light chains and two heavy chains (g). The

four polypeptide chains are covalently held together by disulfide bonds. It's the major antibody in the blood. Human IgG consists of four subclasses (isotypes), which are numbered in order of their serum concentrations (IgG1, IgG2, IgG3, and IgG4). IgG express predominant activity during a secondary antibody response. IgG antibodies have a relatively high affinity and persist in the circulation for a long time. (Klaus, D.E *et al*, 1998) [18]

Phagocytosis: The process of phagocytosis involves the internalization of large particles (≥0.5μm), phagocytosis is limited to specific Phagocytic cells such as monocytes, macrophage and neutrophils. These cells are vital to both the innate and adaptive immune systems. The innate functions of these molecules (specifically, the internalization and digestion of pathogens bound to receptor on the cell surface) represent the first line of defense against invading microorganisms.
In the adaptive response, B cell produce antigen specific antibodies lead to the opsonization the pathogen (AbbasAK., 2007) [19]

Materials and methods

Study Design

This study was performed on of (100) Iraqi patients with intestinal amoebiasis, who attended the Al-Hussein Teaching Hospital. This study included too (24) person apparently healthy individuals as a control group., who have no history or clinical evidence of amoebiasis or any other chronic disease, and no obvious abnormalities.

Blood Samples Collection

Blood samples were collected by venipuncture from 100 patients and 24controls (five milliliters of venous blood) were drawn by disposable syringe under aseptic technique. Were placed in a sterile plane tube and allowed to clot, then serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum was stored at -10 C˚. These sera were used for estimating the concentration of Immunoglobulin's (Ig E, Ig G, Ig M).

Methods

Determination of serum levels of IgG and IgM, by single radial immune diffusion (SRID) plate. Principle

Kit of (Immunoglobulins, IgG, and IgM) provided by CUSABIO company. The total serum level of immunoglobulins (IgM, IgG) was determined by means of Single Radial Immune diffusion Assay. It is a single radial immune diffusion test, which was developed by (Mancini *et al*., 1965) [20] for quantitative determination of proteins in the serum. Test sample is added to a well in an agarose gel containing a monospecific antiserum. The sample diffuses radially through the gel and the substance being assayed forms a precipitation ring with the monospecific antiserum. Ring diameter is measured and the concentration is determined from the reference standard curve.

Determination of Serum Levels of IgE by enzyme-linked immunosorbent assay (ELISA)

Principle: This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IgE has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgE present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IgE is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IgE bound in the initial step. The color development is stopped and the intensity of the color is measured spectrophotometrically at 450 nm.

Hank s Balanced salt solution (HBSS): Prepare this solution according to (Gupta, p. k., 2009) [21] that contains calcium ions Ca + + and magnesium ion Mg + + dissolving substances in 1000 ml of distilled water and adjust the pH to (PH 7.2) and divided to the volumes are equal and then sterilized By autoclave then save in temperature (4 ° C) for use when we need.

Killed Yeast Suspension: was prepared for the purpose of studying the process of Phagocytosis and as the following steps:

1. Dry bread Saccharomyces cervisia yeast was used.
2. 10 grams of the killed yeast was suspended in 150 ml of normal saline.
3. The suspension was placed in a boiling water bath for an hour and then it lifted until get cold then filtered via a dual-layer sterile gauz
4. The stuck suspension was divided into many test tubes (5 mL), stored in (- 20 C°) until used, and The use melted when stuck in a water bath (37 m ˚) and washing twice before use by using a normal, saline. (Al-Obeidi, Nadia Ahmed. 2011) [22]

Wright' s stain

The testing equipment (kit) was used for Wright 's stain which consist of fixative solution and Eosine Stain and solution of methylene blue which is produced by syrbio company form republic of Arabia Syria.

Phagocytosis Procedure

The procedure carried out according (Met-Calf *et al*.,1986) [23] as follow: 0.025 ml of the collected blood was put in plane tube, then added for it 0.05 ml from Killed yeast suspension which prepared by soluble 10 grams of Saccharomyces ceversiae yeast made in Turkish pakamaya company in 150 milliliters of normal saline and put suspension in water bath for 60 minutes, then this suspension was filtered after it's cooling. 0.025 ml of HBSS were added to the mixture and incubated at 37 0C for 30 minutes. One drop of the mixture was placed on a slid and smeared, then left to dry, fixed by methyle alcohol (99%) for min and stained for 20 min with Wright stain. then, examined under oil immersion.

Phagocytosis index = No. of phagocytic cells\ Total number of cells × 100

Results

Serum Immunoglobulins concentration

The results of the current study, show a significant difference ($P \leq 0.001$) of IgA concentration (185.33 IU/ml) for patients compared with a group of control (1.188IU/ml) with significant difference (0.001), and IgM concentration reached (613.44 mg/dl) for patients compared with a group control (3.92mg / dl) with a significant difference is (0.001), while IgE concentration of patients are (290.33mg/dl) in comparison with the control group (1.65mg/dl) with significant difference.

Table 1: Shows some hematological parameters of intestinal amoebiasis patients and healthy control

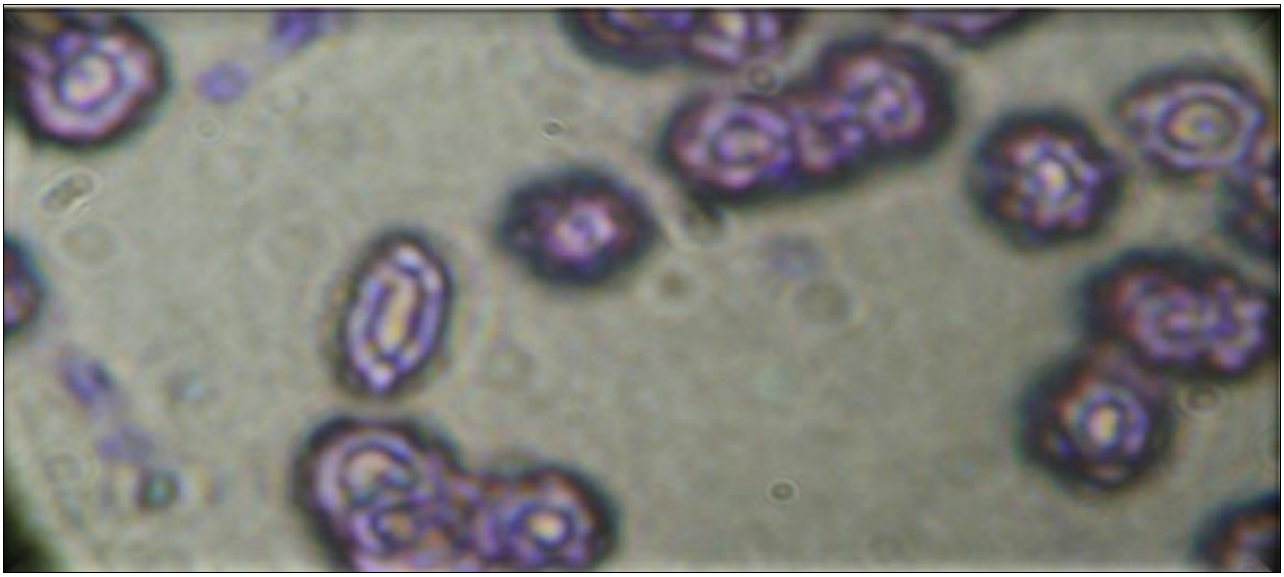
Parameter	Subject	No of Cases	Mean ± SD mg/dl	T-value	df	P-value
IgA	Study	60	185.33 ± 2.22	927	82	0.001
	Control	24	1.188 ± 0.01			
IgM	Study	60	613.44 ± 6.25	9.631	82	0.001
	Control	24	3.92 ± 0.33			
IgE	Study	60	290.33 ± 5.56	8.954	82	0.001
	Control	24	1.56 ± 0.10			

Phagocytic index

The results of the current study showed high significant difference ($p \leq 0.001$) in the rate of phagocytosis, as it decreased the rate of phagocytosis amoebiais patients to (302.34) compared to the healthy controls group, and that the rate of phagocytosis (71.37), as the table below shown (2).

Table 2: Shows phagocytosis index of entocyte amoboesis patients and healthy control

Pagocytosis Index	Subject	No Samples	Mean ± SD	T-value	df	P-value
	Patients	100	302.34 ± 8.64	5.67	122	0.001
	Control	24	71.37 ± 0.23			



The photo above shows the process of phagocytosis at patients infected with amoebiasis.

Discussion

In this study show, a high significant difference ($p \leq 0.001$) in the concentration of immunoglobulin's (IgA) in patients group with enterocyte amoebiasis (185.33 ± 2.22) compared a healthy control group (1.188 ± 0.01), also a high significant difference ($p \leq 0.001$) in the concentration of immunoglobulin's (IgM) (613.44 ± 6.25) of the patients group with asthma, while the healthy control is (3.92 ± 0.33). As well as witnessed Significant difference in the concentration of immunoglobulin's (IgE) in patients group with patient infected with entamoeba (290.33 ± 5.56) compared a healthy control group (1.56 ± 0.10).

Increasing levels of (IgA) antibody in patients' amoebiasis who suffered diarrhea. Humoral immunity plays a role in the immune response against the parasite despite the fact that this is less much that the role which plays the cellular immune in response against the parasite(Gosh *et al*,. 2007) This immunoglobulin plays an important role in humoral immunity against the parasite infection, the presence of this type of antibodies contributes to minimizing the frequency of infection, replication and gives the systemic stimulate to B-cell find a high- level antibodies amoeba such as IgA.Ig.

The reason of high level of IgE in our patients may be due to infestation of the parasite have led to stimulate large number of Lymphocyte B and thus increase in the antibody production especially the type IgE which plays arole in the control of intestinal parasitic infestation where pathological effects from a second reason for increased concentration of(IgE) liberated material histamine from mast cell thus(IgE) transferred antigen of sick cell to cell the immune system via receptor on the cell surface(CD41) and continuous scratch to the intestinal wall operate sensitive stimulates the production of senior amount of (IgE) (Valenzuela *et al*,.2001;Buss *et al*,.2008 [25] and Johansson S.G,.2006).The secretion of IgE by lymphocytes come into define against the parasitic state of an individual.

A high- level concentration of (IgM) have been observed, the reasons patients have an initially infected with amoebiasis, increased permeability the surface epithelial cell for tissue cell found in the intestines and presence this kind of antibodies help distinguish between acute and recent infection infectious that had been treated previously (Chandra, R.K. 2000) [26].

The current results show the significant difference ($p \leq 0.001$) as the percentage of phagocytosis in patients with amoebiasis (302.34 ± 8.64) compared with healthy control (71.37 ± 0.23). Our data showed that the phagocytic

capacity of phagocytic cells in patient with amoeba was lower than in the healthy control. A significant decrease in phagocytosis by monocytes and neutrophils (Alexis NE, Eldridge MW, Peden DB. 2003) [27], (Lavinskiene S, Jeroch J *et al*, 2012) [28] and alveolar macrophages (Fitzpatrick AM, 2008) [29] has also been shown in amoebiatc individuals in other studies.

Refrence

1. Kimka-Lee ya, Shin Mh. Calpain–Dependent CAL Pastatincle ava Gergulates. Caspase-3Activation During Apoptosis.of Julart T-cell induced by entamoeba histolytica. Intj parasitol,2007:37:1209-1210.
2. Variyam, IEP. Luminal Host-Defence mechanism against invasive amoebiasis. Trends Parasitol,2007:23:108-111.
3. Guerrant RL, Hughes JM, Lima NL, Crane J. Diarrhea in developed and developing countries magnitude special settings and etiologies. Rev Infect Dis,2000:12 Suppl,1:541-550.
4. Guiarj IA, Goncalvessodre F, Perei RS, Bona M, Lemose NR, Sanddaher RR. Intestinal Parasite protozoa and Helminths. J Biol Sci,2007:25(4):543-533.
5. Al-Saad S, Sara Muhand George GP. Fulminant Amoebic Colitis. Bahrain Medical Bulletin,2009:13(1):25-33.
6. Diamond LS, Clark CG. A rediscription of Entamoeba histolytica Haudinn, (Emende Walker Separation it form E. dispar Brumpt). J Eukaryot Microbiol,1993:40(3):340-440.
7. Butel GF, J.S. andmores SA. Invasive toxin lead amoebiasis. Medical-microbiology,23rd ed. New York: McGraw-Hill Companies, 2004, 771-774.
8. Ordaz-Pichardo, Shibayama M, Villa-Trevions, Arriaga M, Anglese G, Anglese E, Garza M. Antiamoebic and Toxicity Studies of crobonoic acid derivative and its therapeutic effect in a hamster model of hepatic amoebiasis. Antimicrob Chemother,2005:49(3):1160-1168.
9. Nari GA, Ceballos ER, Carrera G. Amoebic Liver Abscess. Revista Espanola de Enfer Meades Digestivas,2008:100(15):268-272.
10. Rolink AG, Ten Boekel E, Yamagami T, Ceredig R, Andersson J, Melchers F. B cell development in the mouse from early progenitors to mature B cells. Immunology Letters,1999:68:89-93.
11. Brekke OH, Sandlie I. Therapeutic antibodies for human diseases at the dawn of the twenty-first century. Nature Reviews Drug Discovery,2003:2:52-62.
12. Takai T. Roles of Fc receptors in autoimmunity. Nat Rev Immunol,2002:2(8):580-592.
13. Buckley RH. Humoral immunodeficiency. Clinical Immunology and Immunopathology,1986:40:13-24.
14. Dispenzieri A, Gertz MA, Therneau TM, Kyle RA. Retrospective cohort study of 148 patients with polyclonal gammopathy. Mayo Clinic Proceedings,2001:76:476-487.
15. Erb K. Helminths, allergic disorders and IgE-mediated immune responses: where do we stand? Eur J Immunol,2007:37(5).
16. Gould H, Sutton B, Beavil A, Beavil R, McCloskey N, Coker H, *et al*. The biology of IgE and the basis of allergic disease. Annu Rev Immunol,2003:21:579–628.
17. Hayzer DJ, Jaton Jr. C. Immunoglobulin M. Methods Enzymol,1985:116:26-30.
18. Klaus DE. Immunology: Understanding the Immune System. Antibody Structure And Function, chapter. John Wiley & Sons, Inc., 1998, 68-71.
19. Abbas AK, Andrew HL, Shiv P. Cellular and Molecular Immunology. 6th Edition. Saunders, 2007.
20. Mancini G, Carbonara AO, Hermans JF. Immunochemical quantization of antigen by single radial immunodiffusion. Immunochemistry,1965:2:235-254.
21. Gupta PK. Biotechnology and Genomics. Rastogi Publications, 2009, 187.
22. Al-Obeidi NA. Investigation of heat shock protein (HSP 70) in the blood of women abortifacients infected with toxoplasmosis [master's thesis]. College of Education, University of Dhi Qar, 2011.
23. Met-Calf JA, Gallin JI, Nanseef FW, Root RK. Laboratory Manual of Neutrophil Function. Raven Press, 1986, 84-90.
24. Ghosn PK, Gupta Ortiz-Ortiz L. Role IL-10 intestinal amoebiasis: delayed-type hypersensitivity response in child. J Health Popul Nutr,2000:18:109-114.
25. Buss S, Kabri M, Petri WA, Haque R. Comparison of two immunoassays for detection of Entamoeba histolytica. J Clin Microbiol,2008:46(8):2778-2779.
26. Chandra RK. Nutrition immunity and infection: present knowledge and future direction. Lancet,2000:1:688-691.
27. Alexis NE, Eldridge MW, Peden DB. Effect of inhaled endotoxin on airway and circulating inflammatory cell phagocytosis and CD11b expression in atopic asthmatic subjects. J Allergy Clin Immunol,2003:112:353–361. doi:10.1067/mai.2003.1651.
28. Lavinskiene S, Jeroch J, Malakaskas K, Bajoriuniene L, Jackute J, Sakalauskas R. Peripheral blood neutrophil activity during Dermatophagoides pteronyssinus-induced late-phase enterocyte inflammation in patients with Entamoeba inflammation. Inflammation,2012:35:1600–1609.
29. Fitzpatrick AM, Holguin F, Teague WG, Brown LA. Alveolar macrophage phagocytosis is impaired in children with poorly controlled asthma. J Allergy Clin Immunol,2008:121:1372–1378. doi:10.1016/j.jaci.2008.03.008.