SEROLOGICAL DETECTION OF SERUM LEVELS OF IL-8 AND IL-37 IN CHILDREN INFECTED WITH AMOEBIASIS IN BAQUBAH CITY,IRAQ

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Abstract:

Amoebic disease in humans caused by Entamoeba histolytica parasite infection can be asymptomatic or lead to severe disease with amoebic colitis and amoebic liver abscess, amoebic disease is still an important cause of disease and mortality worldwide, Every year, this parasite causes the death of 40,000-100,000, ranking only next to malaria in patient mortality.This study proposes to find anti Entamoeba histolytica IL-8 and IL-37 antibodies in infants and kids from 1 year to 15 years old. ELISA was used to measure serum levels in 90 people (50 with E.histolytica and 40 healthy controls). Patients with clinical signs and symptoms including gastrointestinal upset and watery or bloody diarrhea in enteral amoebic disease at Albatool Teaching Hospital for Children in Baqubah, Diyala governorate, based on their infection severity.ELISA results showed that patients had anti-E.histolytica IL-8 serum levels of (88.2±6.3) while controls had levels of (160.3±23.2). IL-37 patients had blood levels of (162.2±14.9), while control subjects had serum levels of (64.4±14.1). The study found that the groups had a low percentage of IL-8 against the E.histolytica,and high percentage of IL-37 against the E.histolytica. Basic measures to prevent and control amoebiasis infection remain hygiene habits, use of toilets, hand washing, improvement of water purification systems and proper hygiene practices for food preparation, and avoidance of fecal-oral exposure, all of which can reduce the incidence of disease.These findings may help us better understand the transmission of amoebiasis amongchildren, potentially leading to fewer unnecessary antibiotic prescriptions in the community.

Key words: ALA ,IL-8,IL37

INTRODUCTION:

Amebiasis in humans caused by a parasite called Entamoeba histolyica (1) .The occurrence of amebiasis largely over a simple two-stage life cycle of E.*histolytica*, ecological gastric cyst and divided trovozoate found in the human intestine (2).It is an anaerobic invasive invasive intestinal protozoan, E. histolytica infection is associated with high mortality rates and the mother is satisfied (3). Every year, this parasite causes 40,000-100,000 deaths, ranking only next to malaria in patient mortality (4). The most common extraintestinal manifestation is an amoebic liver abscess (ALA), which is associated with significant morbidity and mortality (4). Some reports indicate that more than 55,000 people die each year from acute enteric amoebic disease (5).This study will look into the effects of the E.histolytica on interleukins, specifically IL-8 and IL-37. IL-8, also known as C-X-C motif chemokine ligand 8 (CXCL8), is a pro-inflammatory chemokine, a member of the CXC family of chemokines, produced under inflammatory conditions by immune and other cell types(6). The most prominent role of IL-8 is the attraction of neutrophils to the sites of inflammation, but also the

promotion of monocyte-macrophage growth and differentiation (7), endothelial cell survival, proliferation and angiogenesis (8).Interleukin-37 is an anti-inflammatory cytokine that appears in various inflammatory and autoimmune conditions.Immunofluorescence microscopy demonstrates that IL-37 serves both exterior and intracellular roles. IL-37 is present in both the cytoplasm and the nucleus, making it a dualfunctioning cytokine. The newly found antiinflammatory IL-37, a pro-inflammatory member of the IL-1 family, has been associated with (9).
Interleukin IL-37 test using ELISA technique.

Materials and Methods

This investigation was done from december 2023 to octoper 2024. (90) Blood samples were taken from both sexes. The study participants' ages ranged from 1year to 15 years. The study participants were residents of Diyala Governorate. The samples were obtained from national pathological investigation laboratories and Al-Batoul Maternity and Children's Hospital. The patients who participated in the study were diagnosed Clinical pediatric physicians accepted the chest x-rays used in the study, which were obtained from radiology records after patients were moved to the radiology department.

blood samples collection:

Using medical syringes sterilized with 70% ethanol, 2-5 milliliters of venous blood were taken from the children participating in the study and deposited in laboratory tubes containing clotting activators (Gel and Clot Activator Tube). After being numbered, the samples were placed in an ice box.

Test Principle:

Using the Biotin double antibody sandwich approach, this kit detects Human Interleukin 8 (IL-8) employing an enzyme-linked immunosorbent assay (ELISA). Incubate the pre-coated wells with the IL-8 monoclonal antibody. Next, mix biotintagged anti-IL-8 antibodies with streptavidin-HRP to form an immunological complex. Remove any enzymes that remain unattached after incubation and washing. Combine Substrates A and B. The acidic action causes the solution to become blue and yellow. Based on the color, there is a positive correlation between liquid and the interleukin quantity. This kit detects human interleukin 37 (IL-37) through an enzyme-linked immune sorbent assay (ELISA) with a Biotin double antibody sandwich technique. Incubate Interleukin 37 (IL-37) in wells coated with the IL-37 monoclonal antibody. For the creation of an immunological complex, mix biotinlabeled anti-IL-37 antibodies with streptavidin-HRP. After incubation and washing, remove any enzymes that are still unbound. Incorporate Substrates A and B. The acidic action causes the solution to become blue and yellow. The colors of the solution and the concentration of Human Interleukin 37 (IL-37) have a favorable association.

1. Results of the Serum level of Interleukin -8 (IL-8):

Table 1: Comparison of Mean Serum Concentration of IL-8 in children with amoebiasis and control group.

Variable	Study group Mean ± SD No:60(50)	Control Group Mean ± SD No:60(40)	p-value (Sig.)
IL-8	88.2±6.3	160.3±23.2	<0.05(HS)

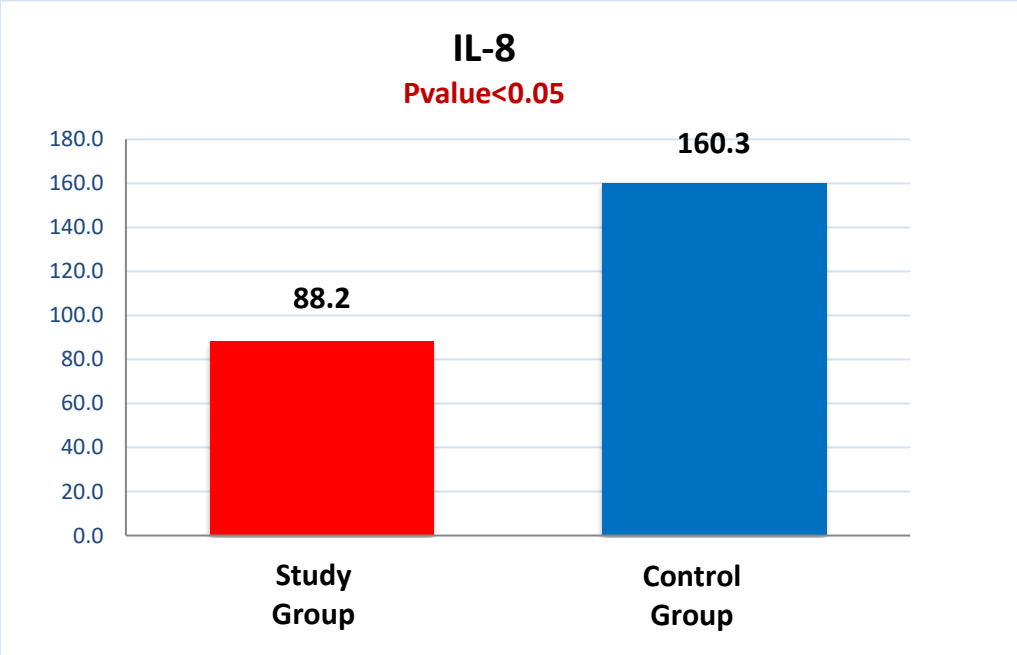

Figure (1): Boxplot of Mean Serum Concentration of IL-8 in children with amoebiasis and control group.

2. Results of the Serum level of Interleukin-37(IL-37):

Table 2: Comparison of Mean Serum Concentration of IL-37 in children with respiratory syncytial virus and control group.

Variable	Study group Mean ± SD No:60(50)	Control Group Mean ± SD No:60(40)	p-value (Sig.)
IL-37	162.2±14.9	64.4±14.1	<0.05(HS)

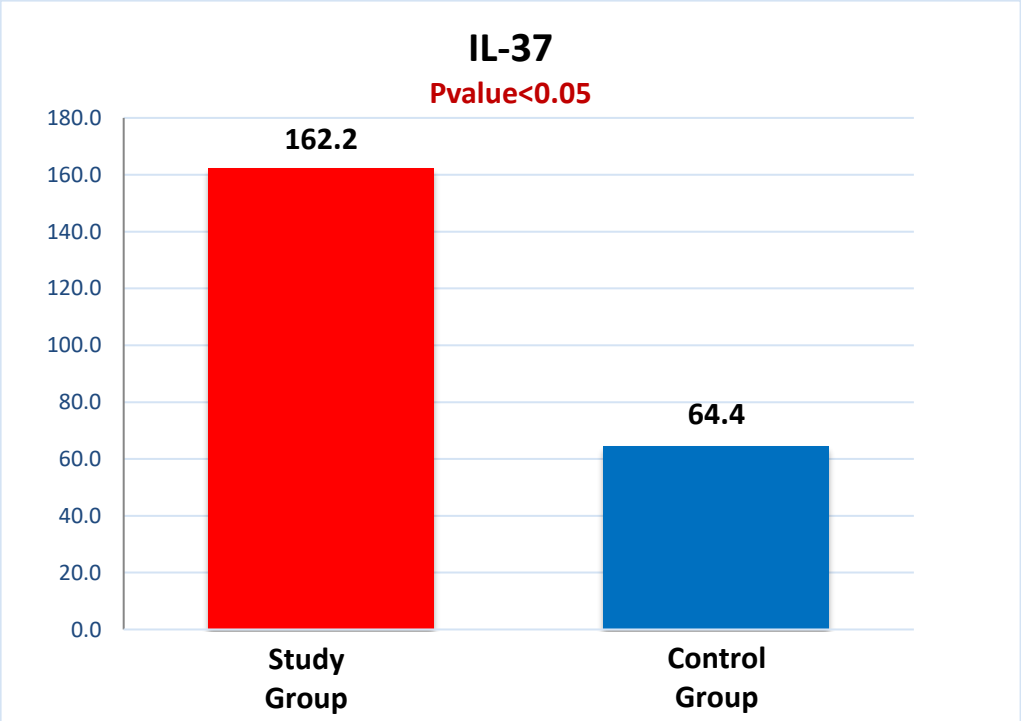

Figure (2): Boxplot of Mean Serum Concentration of Il-37 in children with amoebiasis and control group.

Results:

The study discovered that children infected with amoebiasis had lwoer levels of (IL-8) (88.2±6.3)than the control group (160.3±23.2). The statistical analysis found a significant difference (p-value < 0.05) as illustrated in Figure 1. Also we discovered that children infected with amoebiasis had more white blood cells (IL-37)(162.2±14.9) than the control group (64.4±14.1). Figure 2 indicates a significant difference (p-value < 0.05).

Discussion:

The conclusions of the present investigation Consistent those of this study. IL-8 levels were lower in the serum children infected with amoebiasis compared to children without amoebiasis , which are higher, differed with (10). Increase in IL-8 level in serum of people infected with histolytic amoeba parasite, reflecting the pro-inflammatory role of this cytokine in infection(11,12).The cellular immune response is the host's main defense against E*ntamoeba histolytica* During the first step of infection, histolytic amoeba interacts with the host's intestinal epithelial cell layer and leads to the production of inflammatory cytokines such as IL-8 (13,14).). IL-8 was strongly activated during severe amoebiasis and in relation to pus cells the more IL-8, the more pus cells there will be. Therefore, this reflects that in response to IL-8, neutrophils infiltrate the intestine as the first cells in the innate immune response to amoebic invasion (15,16). Chemo attraction in target cells stimulates primarily neutrophils but also other granulocytes, causing them to migrate towards the site of injury, and also stimulates phagocytosis once it reaches (17).The study discovered that children infected with amoebiasis had more white blood cells (IL-37) than the control group.These findings are consistent with previous reports that during Infection or inflammation increases IL-37 expression, which is an appropriate response to reduce disease severity(18).also In humans, IL-37 acts mainly as a regulatory mechanism for reducing excessive inflammation and cytokine production: accordingly, levels are abnormal in patients with inflammatory and autoimmune diseases.(19,20).

Conclusion:

The current study concludes that there is alow concentration in lL_8 levels in serum Pediatric patients infected with amoebiasis when compared to the increased control group. lL_37 levels have increased In the sera of pediatric patients infected with amoebiasis against the control group.

RFERENCES:

1. Saidin, S.; Othman, N.; Noordin, R. Update on Laboratory Diagnosis of Amoebiasis. Eur. J. Clin. Microbiol. Infect. Dis. 2019, 38, 15–38.
2. Ghosh S, Padalia J, Moonah S. "Tissue Destruction Caused by Entamoeba histolytica Parasite: Cell Death, Inflammation, Invasion, and the Gut Microbiome". Current Clinical Microbiology Reports PLoS Pathog 2019; 9(8): 39-44.

3. Rosales,C.Uribe;Querol,E.Phagocytosis:af undamental process in immunity. BioMed Res Int 2017: 9042851, 2017.
4. Marenga G, Traficante S, Ragonici S, Vincenzi C, Rocchetti M, De Rito G, Fonsi GB, Messineo D. Successful diagnosis of a longstanding giant amoebic liver abscess using contrast-enhanced ultrasonography (CEUS): a case report in a western country. The American Journal of Case Reports. 2019;20:493–498. doi: 10.12659/AJCR.914378.
5. Tharmaratnam, T.; Kumanan, T.; Iskandar, M.A.; D'Urzo, K.; Gopee-Ramanan, P.; Loganathan, M.; Tabobondung, T.; Tabobondung, T.A.; Sivagurunathan, S.; Patel, M.; et al. Entamoeba histolytica and Amoebic Liver Abscess in Northern Sri Lanka: A Public Health Problem. Trop. Med. Health 2020, 48, 2.
6. Matsushima,K.;Yang,D.;Oppenheim,J.J.In terleukin-8:An Evolving Chemokine. *Cytokine* 2022, *153*,155828.
7. Corre,I.;Pineau,D.;Hermouet,S.Interleukin -8:AnAutocrine/Paracrine Growth Factor for Human Hematopoietic Progenitors Acting in Synergy with Colony Stimulating Factor-1 to Promote Monocyte-Macrophage Growth and Differentiation. *Exp. Hematol.* 1999, *27*, 28–36.
8. Li, A.; Dubey, S.; Varney, M.L.; Dave, B.J.; Singh, R.K. IL-8 Directly Enhanced Endothelial Cell Survival, Proliferation, and Matrix Metalloproteinases Production and Regulated Angiogenesis. *J. Immunol.* 2003, *170*, 3369–3376.
9. Mei Y and Liu H. (2019) IL-37: An antiinflammatory cytokine with antitumor functions. Cancer Rep (Hoboken) 2(2): e1151.
10. Nunez, P.R.M, Honorio-França, A.C. , Geiger, S.M. , Guedes, M. , Fagundes, D.L.G , Magalhães, A.M , Gomes, M.A. and França, E.L.(2020). Tropical Biomedicine 37(3): 763–777 .
11. Aggarwal, B.B.: Gupta, S.C. and Kim, J.H. (2012). Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. Blood, 119: 651- 665.
12. Howerton, E. and Tarzami, S.T. (2017). Tumor Necrosis Factor-alpha and inflammation-mediated cardiac injury. J. Cell Sci. Ther., 8:1-4.
13. Nakada-Tsukui K, Nozaki T. Immune response of amebiasis and immune evasion by Entamoeba histolytica. Frontiers in immunology. 2016;7:175.
14. Snow,M. Chen,M. Guo,J. Atkinson,J. and Stanley Jr. S.L. (2008) Differences in Complement-Mediated Killing of Entamoeba histolytica between Men and Women—An Explanation for the Increased Susceptibility of Men to Invasive Amoebiaisis, American Journal of Tropical Medicine and Hygiene, 78, 922-923.
15. Dixit N and Simon SI. "Chemokines, selectins and intracellular calcium flux: temporal and spatial cues for leukocyte arrest". Front Immunol 2012; 10; 3: 188.
16. Mohsen , J, R. Maha, F,N. Thekra ,A ,I.(2019) evaluated serum levels for IgE, cytokines IL-6 and IL-10 in patients with eczema in city of baqubah.
17. Moonah SN, Jiang NM, Petri WA. (2013). Host immune response to intestinal amebiasis. PLoS Pathog 2013; 9(8).
18. Mao X, Zhu R, Zhang F, Zhong Y, Yu K, Wei Y, et al. IL-37 Plays a Beneficial Role in Patients With AcutE Coronary Syndrome.MediatorsInflamm(2019)2019: 9515346.doi:10.1155/2019/9515346.
19. Cavalli,G.Dinarello,C,A,Suppression of inflammation and acquired immunity by IL-37 *Immunol. Rev.* 2018; 281:179-190.
20. Maha,F,N.Zainab,A,M.Raghad,I,A,.Saad, A,J(2024) Serum level of female hormones Prolactin , FSH , LH and concentration of immune proteins C3,C4 in women with hyperprolactinemia of Muqdadiya city.