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Comparison of Some Immunological Parameters Associated with *Entamoeba Histolytica* and *Giardia Lamblia* Infections in People with Diabetes Mellitus.

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Abstract

The current study was conducted on people with type 2 diabetes, *E. histolytica* and *G. lamblia*, in Salah El-Din General Hospital and some private laboratories in Tikrit city in Salah El-Din governorate, on age groups ranging from (20-50) years, during the extended period. From 09/20/2020 to 05/1/2021. The cases of infection with *E. histolytica* and *G. lamblia* parasites were diagnosed by examining faecal samples using a direct swab method using a light microscope to detect the trophic and cystic stages.The results showed a significant increase in the concentration of IgA, IgM, IL-8 and TNF-α in all the studied groups compared with the control group, while there was a significant decrease in the concentration of IgA and IgM among people with diabetes, while there was no change in the concentration of TNF-α in people with diabetes compared with the control group.

Keywords: *E. histolytica; G. lamblia*;type II diabetes.

Introduction

Intestinal parasites of Entamoeba histolytica and Giardia lamblia are common causes of Diarrhea and Malabsorption in humans, accompanied by chronic infections that lead to growth retardation and weight loss (Cook, 1995).Entamoeba histolytica is an Intestinal protozoan parasites that causes what is known as Amoebiasis (Ali, 2015), which causes Colitis amoeba and leads to liver abscess (Choi *et al*., 2005). Infection begins with ingestion of infective stags, represented by the cystic form found in contaminated food and drink (Pereira *et al*., 2014). The parasite attacks tissues by attaching to the intestinal epithelial lining, and the adhesion occurs through virulence factors (Tanyuksel & Petri, 2003; Stanley & Reed, 2001; Shegal *et al*., 1996).The

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parasite G. lamblia is one of the flagellate parasites of the eukaryotes Eukaryote(Adam, 2001), and it causes Giardiasis (Kumar & Singh, 2016), which is considered a Zoonosis common between humans and animals (Thompson *et al*., 2009). The parasite infects the small intestine (jejunum and ileum) of the teeth (Faubert, 2000). Infection with G. lamblia occurs after ingestion of cysts of the parasite, and the infection can cause significant damage to the lining of the intestine as a result of the parasite's attachment with the help of its bilobed sucking disc to the lining of the intestine (Gardner & Hill, 2001); Faubert, 2000).People with diabetes are more likely to have many infections. As a result of decreased immunity in these patients, due to increased levels of sugar in the bloodstream (Joshi et al., 1999).The current study aimed to detect the biochemical and immunological changes associated with *E. histolytic* and *G. lamblia* for diabetes patients.

Materials & Methods

Collection of samples

The study included the examination of 500 faeces samples from the auditors of Salah El-Din General Hospital and some private laboratories in Tikrit in Salah El-Din Governorate. The collection period lasted from 20/9/2020 to 1/5/ 2021. The study included examining samples from both males and females of different ages. The collected faecal samples were kept in sterile, dry plastic bottles with a tight seal prepared for this purpose. A paper sticker was placed on one of its sides to record (patient's name and sample number, gender, age, presence of diarrhea, diabetes) and according to the attached questionnaire form

The studied samples were divided into five groups, namely: the control group included 20 people, the group with type 2 diabetes only included 20 people, the group with parasites (*E. histolytica*and *G. lamblia*) included 20 people, the group with type 2 diabetes and dysentery amoeba infection Together it included 20 people, the group with type 2 diabetes and *G. lamblia* infection together included 20 people

Measurement of the level of immunoglobulin IgA and IgM

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The kit contains the necessary reagents needed to calculate or perform a quantitative measurement of the human IgA and IgM levels from serum samples or plasma. The kit is supplied by the Spanish company LiNEAR

Determination of IL-8 and TNF-αconcentration

The kit contains all necessary reagents needed to calculate or quantify the level of human IL-8 and TNF-α cytokinesis from serum samples, plasma, culture medium, or other biological fluids. The kit is supplied by the Korean company KOMA BIOTECH INC.

Statistical analysis

The results were statistically analyzed using the statistical program Minitab for the studied data and the significant differences between the means were compared with the least significant difference test L.S.D. As for the percentages, the differences between them were compared with the Chi-squar test.

Results & Disccusion

Percentage of parasites *E. histolytica* and *Giardia lamblia* in faecal samples examined by microscope, infections of the parasite *E. histolytica* and *G. lamblia* were diagnosed in only 60 (12%) samples out of 500 (88%) samples (Fig. 1).

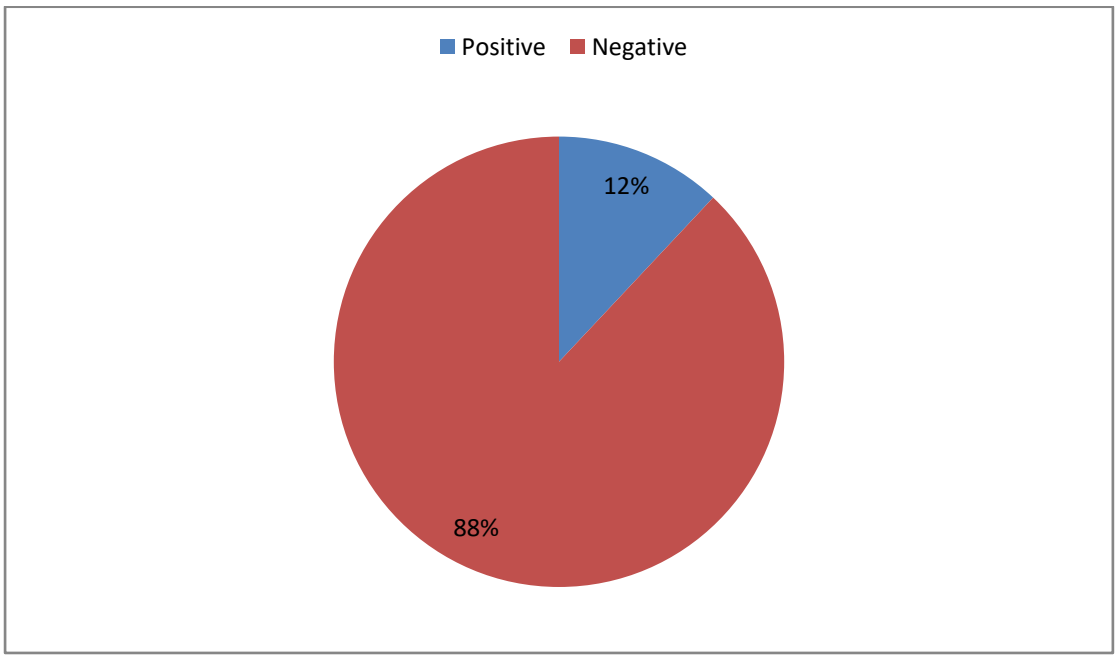

Figure (1): Percentage of parasites *E. histolytica* and *G. lamblia* in faecal samples

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The results of the current study agree with Jaeffer (2010), who recorded a parasite infection rate of 13.64%, while the results of the current study differ with Anwar (2014), who recorded a rate of 30.22%. The similarity in the rates of infection that we have reached with previous studies is due to the similarity between these areas in terms of the cultural, social and health level. The difference in the percentage of parasite infection is due to several factors, including the total number of samples examined, geographical location, age groups taken, and the accuracy of the microscope examination , method of diagnosis, study period, climate influences, cultural awareness and personal hygiene, population density, and the difference in the level of sanitation services (Kurt et al., 2007).

Measurement of the level of IL-8 and TNF-α

The results of the current study showed a significant increase in the rate of IL-8 cytokinesis in people with type 2 diabetes and *E. histolytica* infection together (98.9 ± 36.2), as well as in people with type 2 diabetes and G. lamblia infection together (50.18 ± 14.1) The same was observed in people infected with *E. histolytica* and G. lamblia (93.81±19.07). While the results of the current study showed a significant difference in the rate of IL-8 cytokinesis in people with type 2 diabetes only (33.11 ± 8.83) compared to the control group (17.61 ± 2.98).The results of the current study showed a significant decrease in the rate of IL-8 cytokinesis in people with type 2 diabetes and G. lamblia infection together (50.18 ± 14.1), as well as a significant decrease in the rate of IL-8 cytokinesis in people with type 2 diabetes only (33.11 ± 8.83) compared with the group of people with type 2 diabetes and *E. histolytica* infection together (98.9 ± 36.2), as well as with the group of people infected with *E. histolytica* parasites Amoeba and G. lamblia (93.81 ± 19.07).The results of the current study showed a significant increase in the level of TNF-alpha in people with type 2 diabetes and *E. histolytica* infection together (28.98 ± 5.65), as well as in people with type 2 diabetes and Giardia lamblia infection together (24.85 ± 5.37) The same was observed in people infected with *E. histolytica* and G. lamblia (26.75 ± 5.66). While the results of the current study showed no significant difference in the level of TNF-alpha among people with type 2 diabetes only (11.77 ± 7.46) compared with the control group (6.09 ± 2.03).While the results of the current study showed a significant decrease in the level of TNF-alpha in people with type 2 diabetes only (11.77 ± 7.46) compared with people with type 2 diabetes and HMF together (28.98 ± 5.65), as well

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as in those with type 2 diabetes mellitus and TNF-alpha. Persons with type 2 diabetes mellitus and G. lamblia infection together (24.85±5.37), as well as in persons infected with the *E. histolytica* and G. lamblia (26.75±5.66). And as in the following table (1)

Table (1): Concentration of IL-8 and TNF in study groups

Parameters / Groups	IL-8	TNF
Control group	17.61±2.98 c	6.09±2.03 b
Diabetes and *E. histolytica*group	98.9±36.2 a	28.98±5.65 a
Diabetes and *G. lamblia*group	50.18±14.1 b	24.85±5.37 a
Diabetes only group	33.11± 8.83 bc	11.77±7.46 b
E. histolytica and *G. lamblia*group	93.81±19.07 a	26.75±5.66 a
L.S.D. (0.05)	100.28	10.147

The increase in the rate of cytokinetic IL-8 secretion when infected with *G.lamblia* may be explained by the parasite's possession of secretory-exogenous antigens that stimulate the production of cytokinetic IL-8 in humans by colonic epithelial cells. Cytokinetic IL-8 has an essential role in the immune response during infection with *G. lamblia*, as it summons neutrophils to the site of inflammation and infection (Al-Cantara et al., 2003). Also, the feeding phase of *G. lamblia* is able to stimulate neutrophils and mononuclear cells to produce IL-8 cytokinesis (Ahmed et al., 2015).While many studies have shown a decrease in the rate of cytokinetic IL-8 during infection with G. lamblia, the reason for the decrease in the rate of cytokinetic IL-8 during infection with G.lamblia is due to the weakness of the cellular immune response that leads to a decrease in the production of cytokines by target cells.The results of the current study confirm that there is a significant increase in the rate of IL-

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8 cytokinesis in people with type 2 diabetes compared to people without diabetes, and it agreed with Boroujeni et al (2016), and agreed with Al-Tuma et al (2011).TNF-alpha is associated with an increased risk of diarrhea in people with dysentery, as the amoeba destroys the intestinal mucosal barrier, and this facilitates the parasite's penetration and destruction of tissues, and thus the parasite will stimulate the production of many inflammatory initiators such as TNF-alpha in The site of injury (Noor et al., 2017). TNF-alpha increases the permeability of blood vessels and this leads to the parasite getting red blood cells that will exude from the damaged vessels into the tissue, and red blood cells are the best food source for the parasite (Silvestre et al., 2015 ; Ankri, 2015 ; Noor et al., 2017).

Measurement of the level of IgM and IgA in the study groups

The results of the current study showed a significant increase in IgA concentration in people with type 2 diabetes and*E. histolytica* infection together (412.15 ± 21.37), as well as in people with type 2 diabetes and *G. lamblia* infection together (420.1 ± 23.64), as well as among people with type 2 diabetes and *G. lamblia* infection together (420.1 ± 23.64). Persons infected with *E. histolytica* and Giardia lamblia (396.85±25.81). While the results of the current study showed a significant decrease in the concentration of IgA in people with type 2 diabetes only (132.65 ± 11.87) compared to the control group (251.85 ± 41.04).

The results of the current study showed a significant increase in the concentration of IgM immunoglobulin in people with type 2 diabetes and hemolytic amoeba infection together (243.5 ± 18.27), as well as in people with type 2 diabetes and *G. lamblia* infection together (252.25 ± 20.17), as well as among people with type 2 diabetes and *G. lamblia* infection together (252.25 ± 20.17). Persons infected with *E. histolytica* and *G. lamblia* (255.1±20.06). While the results of the current study showed a significant decrease in the concentration of IgA in people with type 2 diabetes only (129.85 ± 8.77) compared to the control group (183.15 ± 16.21), as in the following table (2).

Table (2) Concentration of IgA and IgM Immunoglobulins in Study Groups

Parameters / **Groups**	**IgM**	**IgA**

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Control group	183.15±16.21 b	251.85±41.04 b
Diabetes and *E. histolytica*group	243.5±18.27 a	412.15±21.37 a
Diabetes and *G. lambliia*group	252.25±20.17 a	420.1±23.64 a
Diabetes only group	129.85±8.77 c	132.65±11.87 c
E. histolytica and *G. lambliia*group	255.1±20.06 a	396.85±25.81 a
L.S.D. (0.05)	10.81	16.626

The results of the current study showed an increase in the concentration of immunoglobulins IgA and IgM among people with type 2 diabetes and dysentery amoeba infection together, as well as people with type 2 diabetes and Giardia infection together, as well as people infected with the dysentery amoeba and Giardia lamblia parasite. This is consistent with several studies that showed an increase in the level of IgA and IgM antibodies in acute and chronic *E. histolytica* infections, as Al-Quraish&sultany (2017) indicated an increase in the concentration of both IgA and IgM in the sera of infected persons.The reason for the increase in the level of IgA is due to the fact that the epithelial cells lining the intestines produce these antibodies, and their surfaces have special receptors for binding with IgA, IgM, and that these cells produce IgA depends on the presence of microorganisms in the intestine, and B lymphocytes in the nodes Peyer's production of these antibodies, as these lymphocytes differentiate into plasma cells that produce local IgA antibodies, and 90% of the cells in the lamina propria layer located in the duodenum and ileum produce IgA, and IgA antibodies play a key role in mucosal humoral immunity , where IgA can neutralize toxins and bind with pathogenic microorganisms, thus losing the ability to bind to mucous surfaces through its ability to transmit across mucous membranes, and when injuries occur in the mucous membranes, an increase in the level of immune antibodies is observed in the mucosal sites and a decrease in the serum (Macpherson et al. , 2008).

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References

1. Adam, R. D. (2001). Biology of *Giardia lamblia*. Clinical Microbiology Reviewes, 14 (3): 447-475.
2. Ahmed, N. S.; Al- Khayat, F. A. and Abdullah, F. T. (2015). Interleukin IL-6, IL-8, IL-10 and Tumor necrosis factor (TNF) Expression in human infection with Giardia duodenalis. *American Journal of Medicine and Medical Sciences*, 5 (1): 15-19.
3. Alcantara, C. S. ; Yang, C. H. ; Steiner, T. S. ; Barrette, L. I. & Lima-Guerrant, R. L. (2003). Interleukin-8, tumor necrosis factor and lactoferrin in immunocompetent hosts with experimental and Brazilian children with acquired cryptosporidiosis. *The American Journal of Tropical Medicine and Hygiene*, 68: 325-328.
4. Ali, I. K. M. (2015).Intestinal amebae. Clinics in laboratory medicine, 35(2), 393-422.
5. Al-Tuma F. J., Ibraheem A. A., and Al-Khayatt T. H. (2011). Adiponectin Insulin and Interlukin-8 in Type-2 Diabetic Obese Patients in Kerbala Province: Iraq Professor of Clinical Biochemistry Department of Biochemistry – College of Medicine – University of Babylon / Hilla – Iraq. Karbala *J. Med.4*(3 4) Dec
6. Ankri, S. (2015). *Entamoeba histolytica* – tumor necrosis factor: a fatal attraction. Microbial Cell, 2 (7): 216- 218.
7. Anwar, Shaylan Akbar. (2014). Immuno-epidemiological study of E. histolytica/E. dispar infection among children attending Kirkuk Children's Hospital with a therapeutic trial using Deferoxamine and zinc as an alternative treatment for amoebic dysentery. PhD thesis, College of Education for Pure Sciences, Tikrit University.
8. Boroujeni M., Tavangar A., Khozeimeh F., and Ghoreishian F. (2016). Serum level of Interleukin-8 in subjects with diabetes plus oral lichen planus and oral lichen planus: A biochemical study. *Dental Research Journal* 13(5) 413.
9. Choi, M. ; Sajed, D. ; Pool, L. ; Hirata, K. ; Herdman, S. ; Torian, B. E. & Reed, S. L. (2005). An unusual surface peroxiredoxin protects invasive *Entamoeba histolytica* from oxidant attack. Molecular and Biochemical Parasitology, 143 (1): 80-89.
10. Cook, G. C. (1995). *Entamoeba histolytica* and *Giardia lamblia* infections: current diagnostic strategies. Parasite, 2: 107-112.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 184 - 193
Received 05 May 2021; Accepted 01 June 2021.

11. Faubert, G. (2000). Immune response to *Giardia duodenalis*. Clinical Microbiology Reviews, 13 (1): 35-54.
12. Gardner, T. B. and Hill, D. R. (2001). Treatment of Giardiasis. Clinical Microliology Reviews, 14 (1): 114-128.
13. Jaeffer, H. S. (2010).prevalence of Giardia lamblia and Endameba histolytic / Endameba dispare infection among children in AL-shulaa and AL-khdimya-Baghdad –Iraq ,*J. of university of Anbar for pure science :* 5: (2).
14. Joshi, N. ; Caputo, GM. ; Weitekamp, MR.; and Karchmer, AW. (1999). Infections in patients with diabetes mellitus. *N. Engl. J. Med. 341* : 1906-1912.
15. Kumar, S. and Singh, V. A. (2016). Prevalence of Entamoeba histolytica and Giardia lamblia infection in arural area of Haryana, India. *International Journal of Current Microbiology and Applied Sciences,* 5 (6): 204-209.
16. Kurt Z., Jorge L., Yura M., Maria L., et al. (2007). Effect of Vitamin A and Zinc Supplementation on Gastrointestinal *Parasitic Infections Among Mexican Children. Pediat.* ;120:846- 554.
17. Noor, Z. ; Watanabe, K. ; Abhyankar, M. M. ; Burgess, S. ; Buonomo, E. L. ; Cawardin, C. A. & Petri, W. A. (2017). Role of eosinophils and tumor necrosis factor alpha in interleukin- 25- mediated protection from amebic colitis. *American Society For Microbiology*, 8 (1):1-10.
18. Pereira, V. V.; Conceicao, A. D. S.; Maximiano, L. H. S.; Belligoli, L. Q. G.; andSilva, E. S. D.;(2014). Laboratory diagnosis of amebiasis in a sample of student from southeastern Brazil and a comparison of microscopy with enzyme=linked immunosorbent assay for screening of infection with *Entamoeba sp.* Revista da Sociedade Brasileira de Medicina Tropical.47(1):52-56.
19. Stanley, S. L. and Reed, S. L. (2001). Microbes and microbial toxins: paradigms for microbial mucosal interactions. *American Journal of Physiology Gastrointest Liver Physiology*, 280: 1049-1054.
20. Shegal, D.; Bhattacharya, A. and Bhattacharya, S. (1996). Pathogenesis of infection by *Entamoeba histolytica*. *Journal of Biosciences*, 21 (3): 423-432.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 184 - 193
Received 05 May 2021; Accepted 01 June 2021.

21. Silvestre, A. ; Plaze, A. ; Berthon, P. ; Thibeaux, R. ; Guillen, N. & Labruyère, E. (2015). In *Entamoeba histolytica*, a BspA family protein is requiredfor chemotaxis toward tumour necrosis factor. *Microbial Cell*, 2 (7): 235- 246.
22. Tanyuksel, M. and Petri, W. A. (2003). Laboratory diagnosis of amebiasis. *Clinical Microbiology Reviews*, 16 (4): 713-729.
23. Thompson, R. C. A.; Kutz, S. J. and Smith, A. (2009). Parasite zoonoses and wildlife: emerging issues. International *Journal of Environmental Research*, 6: 678-693.
24. Al-Quraishi, M. A., & Al-Sultany, S. H. (2017). Immunoglobulin profile of E. histolytica and E. dispar in human sera. *J. Bio. Innov*, *6*(2), 203-213.
25. Macpherson, A. J., McCoy, K. D., Johansen, F. E., &Brandtzaeg, P. (2008). The immune geography of IgA induction and function. Mucosal immunology, 1(1), 11-22.