Tikrit Journal of Pure Science
ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)
Journal Homepage: http://tjps.tu.edu.iq/index.php/j

Assessment of some immunological parameters for patients with diabetes mellitus infected with *Entamoeba histolytica*

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https://doi.org/10.25130/tjps.v27i4.26

ARTICLE INFO.
Article history:
-Received: 4 / 7 / 2022
-Accepted: 3 / 8 / 2022
-Available online: / / 2022
Keywords: diabetes mellitus, Immunological parameters, *Entamoeba histolytica*,
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ABSTRACT

The current study was conducted on patients with diabetes mellitus and those infected with *Entamoeba histolytica* of those who were admitted and arrived in Salah El-Din General Hospital and some civil laboratories in the city of Tikrit/Salah El-Din Governorate for the period from 1/3/2021 to 1/4/2022 at ages ranging from 20-60 years. The Microscopic examination was used to detect the infection with Amoebiasis in 300 diabetes pations, and also immunological methods were used to measure some criteria (IL-10, TNF-α, CRP, IgM, IgG, IgA), the results indicated a significant increase in The concentration of antibodies (IgM, IgG, IgA) was 14.5 ± 273.3, 116.0 ± 1345.0 and 38.5 ± 441.9 mg/dl, respectively, compared with the control group. The total and complements (C3, C4) were 8.35±65.0, 21.4±222.0 mg/dl, respectively, compared with the control group. 11.6±142.2, 3.94±28.80 mg/dl, respectively, and C-reactive protein (CRP) was 6.300±22.30 mg/dl, 0.937± 2.975 mg/dl in comparison to the control group, and cytokinetics (IL-10, TNF- α) were 9.32± 46.00 and 31.8 ±92.9 pg/ml, respectively, in comparison to the negative control group, 1.21 ±6.48 and 7.18 ±19.60 pg/ml, respectively.

1- Introduction

E. histolytica is one of the intestinal protozoan parasites that causes what is known as Amoebiasis, and it is the third leading cause of death after malaria and Schistosoma[1]. The transmission of the parasite depends mainly on the contamination of water and food with the cyst, which is one of the most important means that contribute to its transmission, and therefore prevention depends on the cleanliness of food, boiling drinking water, and sterilization with iodine is one of the means that contribute to the elimination of the cysts in endemic areas [2]. The infection is spread all over the world and poses a serious threat to health in tropical and subtropical developing regions as well as in developed countries [3]. Diarrhea can be defined as the expulsion of loose stool or liquid three times or more per day, and it is one of the most common health problems [4]. Diabetes mellitus is a group of metabolic disorders characterized by elevated blood sugar levels resulting from defects in insulin secretion, insulin action, or both Diabetes [5]. The infection is spread all over the world and poses a serious threat to health in tropical and subtropical developing regions as well as in developed countries [6]. The aim of study: Determination of the levels of antibodies (Immunoglobuling M, Immunoglobuling G, ImmunoglobulingA), Complement3, Complement4, C-reactive protein, Interleukine-10) and Tumor necrosis factor-α) in subjects' samples. Infections with type 2 diabetes mellitus and the parasite *Entamoeba histolytica*.

2- Materials and methods

2-1: Collection of sample

In total, 300 faecal samples were collected from patients in Salah al-Din General Hospital and civil laboratories in the city of Tikrit for the period from the beginning of March 2021 to April 2022 who suffered from severe to moderate diarrhea, and in most cases, they suffered from bloody diarrhea .The samples are in sterile containers with a wide opening equipped with a tight cover to maintain the sample's moisture and prevent it drying out. The focus method was used to investigate the infectious phases, and 90 blood samples were drawn from patients with *E. histolytica* and type 2 diabetes, and 30 samples were drawn from people without infection and were used as a control group. The blood was placed in gel tubes, and the blood was left in them for a period of

minutes. It was noted that the blood serum was separated, then it was placed in a centrifuge for 10 minutes at a speed of 3000 cycles. For minutes, serum is withdrawn using a micropipette and stored in Eppendorf tubes at a temperature of -20°C for the purpose of performing serological tests.

2-2 : Microscopic Examination of stool samples direct wet mount method

The faecal samples were examined by preparing a wet direct swab to investigate the infectious stages of the parasite by taking a quantity of faeces with wooden sticks special for this purpose and from different areas of the sample and placing them on clean glass slides with a drop of normal saline on one end of the glass slide and a drop of Logule solution Iodin 1% on the other end of the slide and mixing them together well, then putting the cover of the slide on and examining the samples under the light microscope under magnification powers of 10% and 40% [7].

2-3: method for measuring IgA, IgM, and IgG immunoassays, and measuring the concentrations of C3, C4, and C-reactive protein protein:

Procedure:

The analysis was conducted in lab Salah aldin on an American-made chemistry analyzer smart -150 device, which is a full-automated biochemical analyzer device that has its own blocks produced by the manufacturer of the same device. Its working mechanism depends on placing 300 micrometer of blood serum in a small tube and putting it in the place designated for it in the device, specifying the required tests (IgA, IgG, IgM, CRP, C3, C4) and writing the name of the patient on the calculator connected to the device itself; then the device is turned on, and after a period of 10 minutes, the result appears on the calculator screen. Then it was printed and pulled [8].

2-4: Enzyme–linked Immunosorbent Assay (ELISA)

It is one of the most applied options of German to detect infection, and it depends on the work of adding soluble antibodies and then adding the serum sample, as the antigen-antibody complex is formed in the presence of parasite antibodies in the serum and detects the complex by adding enzyme-linked antibodies [9].

2-4-1 Determination of IL-10 concentration

The enzyme-linked immunosorbent assay (ELISA) technique was used to measure the level of cytokinetic IL-10 options of china in human serum. fixed on drilling surfaces. In the second stage, the biotin conjugated antibody prepared specifically for IL-10 cytokinesis was added, then the streptavidin-HRP-conjugated enzyme was added to all the pits and then placed in the incubator for 60 minutes at a temperature of 37°C. The substrate was added to all the pits to give color fluorescence. The reaction is chromogenic (the addition leads to a color change in the craters) and the reaction is stopped by adding a stop solution. The resulting color change is measured by an ELISA reader with a wavelength of 450 nanometers**.**

2-4-2: Determination of TNF-α concentration

An ELISA was used to measure the level of TNF-alpha options in china in human blood serum. The surfaces of the pits in the micro-standard plate are covered with TNF-alpha specific antibodies, and standard solutions and samples are added. TNF-alpha present in the samples binds to the specific antibodies fixed on the surfaces of the pits. In the second stage, biotin conjugated antibody and the prepared specifically for tumor necrosis factor-alpha, then the enzyme associated with Streptavidin-HRP is added to all the pits and then placed in the incubator for 60 minutes at a temperature of 37-65 °C. Then the substrate is added to all the pits to give a chromogenic brilliance, as the addition leads to a color change in the pits, The reaction is stopped by adding a stopping solution, and the resulting color change is measured by an ELISA reader with a wavelength of 450 nm.

2-5 Statistical analysis

The data of all tests were statistically analyzed by means of a calculator and using the (SPSS) Statistical Package of Social Science program, as it depends on the t-test (T-test) at a probability level of $0.01 \leq p$ to identify the degree of significant differences between transaction rates.

3- Results and Discussion:

The results of the current study showed a significant increase in people infected with the histolytic amoeba parasite and diabetes mellitus, and the cysts were the contagious phase as in Figure (1).

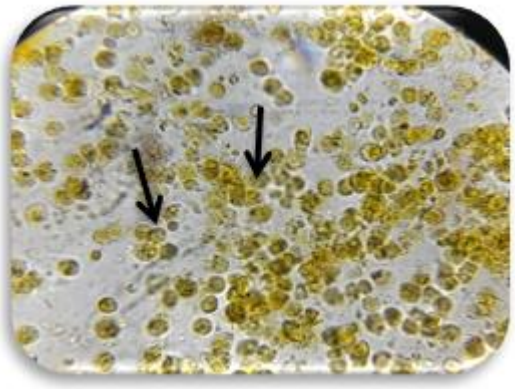

Fig. 1: Cyst of *E .histolytica* under the microscope unstained under the power of magnification 40X

3-1: Immunological tests

Measurement of the level of immunoglobulins for people with type 2 diabetes mellitus and histoplasmic amoeba infections.

1. Rate of IgA Concentration Measurement

The results of the current study showed a significant increase in the average concentration of IgA in people with type 2 diabetes and *E. histolytica* infection together, 38.5 ± 441.9 mg/dl compared with the control group, 34.3 ± 296.6 mg/dl, and significant differences of 0.01 were recorded between the infection group and the control group. Table 1

2 . Measurement of IgG Concentration Rate

In this study, a significant increase in the average concentration of IgG immunoglobulin was recorded for people with type 2 diabetes and tissue amoeba infection together,116.0±1345.0 mg/dl compared with the control group, 75.9±873.0 mg/dl. The results of the statistical analysis showed a significant difference of 0.01 between the infection group and the control grou table1

3. Measuring the rate of the concentration of IgM

In this current study, a significant increase in the concentration of IgM immunoglobulin was observed in people with type 2 diabetes and tissue amoeba infection together, 14.5 ± 273.3 mg/dl compared with the control group, 23.0 ± 170.0 mg/dl. The statistical analysis revealed that there were significant differences between the infected and control groups 1.

Table 1: shows the concentration of immunoglobulins IgG, IgA, and IgM in the study groups.

Mean ± S.D			Parameters / Groups
IgM mg/dl	**IgG mg/dl**	**Mg/dl IgA**	
170.0±23.0 A	873.0±75.9 A	296.6±34.3 A	Control
273.3±14.5 **B**	1345.0±116.0 **B**	441.9±38.5 **B**	Diabetes and *E.histolytica* infection together
19.05	21.53	16.24	P-value

*Different letters indicate significant differences (probability ≥ 0.01) between the averages of the different groups

This is consistent with many studies that showed an increase in the level of antibodies (IgM, IgG, and IgA) in acute and chronic *E .histolytica* infections, as [10] indicated an increase in the concentration of IgA, IgG, and IgM in the sera of infected persons compared to non-infected persons. injured, and also agrees with the results of [11] in Tikrit and with the results of [12] in Kirkuk, while the results of the current study did not agree with [13] as the results of his study recorded no significant differences.

A decrease in the efficiency of the immune system has been observed in people with diabetes, especially in the elderly, in addition to genetic factors that play a major role in protecting the body against parasitic pathogens[14].

The reason for the increase in the level of IgA is that the epithelial cells lining the intestines produce these antibodies, and their surfaces have special receptors for binding with IgA, IgG, and IgM. By producing these antibodies, these lymphocytes differentiate into plasma cells that produce local IgA antibodies, and 90% of the cells in the thin layer in the duodenum and ileum produce IgA. Antibodies play a key role in mucosal humoral immunity, where IgA can neutralize toxins and link with pathogenic microorganisms, causing them to lose the ability to bind to the mucous surfaces. Through their ability to transmit through the mucous membranes, when mucous membrane injuries occur, an increase in the level of immune antibodies is observed in the mucous sites and a decrease in the serum [15].

The reason for the rise in IgM because it is the first antibody formed in the immune response against infections. IgM formation begins at the beginning of infection and continues to increase for several weeks before beginning to decline when IgG begins to form. Since most of the cases in this study were of the acute type, IgM is one of the most immunoglobulins that caused an increase in its concentration [16], and indicated a significant increase in the concentration of IgG when injecting the rabbit's body with large quantities of the parasite, and indicated that IgG from Most of the antibodies in the serum play a major role in the immune system by neutralizing toxins, and due to their long stay in the blood, they are able to protect the host in cases of repeated infection with the parasite.

3-2: Measurement of the level of complement proteins C4, C3 and C-reactive protein (CRP) in study group

The results of the study showed a significant increase in the rate of complement proteins C3, C4 and C-reactive protein 21.4 ± 222.0, 8.35 ± 65.0 mg/dl and 6.300 ± 22.30 mg/l, respectively, in people with type 2 diabetes and tissue amoeba infection compared with the control group. Table 4-13 shows that the concentrations were 11.6±142.2, 3.94± 28.80 mg/dl, and 0.937± 2.975mg/dl, respectively.

Table2: C3, C4, and C-reactive protein (CRP) concentrations in the study groups.

Mean ± S.D			Parameters / Groups
CRP mg/dl	**C4 mg/dl**	**C3 mg/dl**	
2.975±0.937 **A**	28.80±3.94 **A**	142.2±11.6 **A**	Control
22.30±6.300 **B**	65.09 ±8.35 **B**	222.0 ±.21.4 **B**	Diabetes and *E .histolytica* infection together
24.72	27.26	21.95	P-value

*Different letters indicate significant differences (probability ≥ 0.01) between the rates of the different groups.

The results of the current study agreed with [17] through its study of the levels of serum proteins, as its results showed an increase in the levels of the third and fourth complement concentrations, C3 and C4 in the sera of people infected with the parasite, and differed with [18], as it was noted that there were no significant differences between the two groups, as it was noted that Cystine proteinase enzyme secreted by the active phase has the ability to activate the alternative pathway of complement [19].

3-3: Measurement of serum cytokinetic IL-10 and TNF-α levels in study groups.

The current study found a significant increase in the average concentrations of IL-10 and TNF- α cytokines in diabetic mellitus and *Entamoeba histolytica* at 9.32±46.00 92.9 ± 31.8 pg /ml , respectively, when compared to the control group at 6.48 ± 1.21 and 7.18±19.60 pg/ml, respectively. Meanwhile, significant differences of 0.01 were recorded between the infection group and the control group Table 3.

Table 3: shows the cytokinetic rates of IL-10 and TNF- α in the study groups.

Mean ± S.D		Parameters / Groups
TNF- α pg/ml	**IL-10 pg/ml**	
19.60 ± 7.18 **A**	6.48 ± 1.21 **A**	Control
92.9 ±31.8 **B**	46.00 ± 9.32 **B**	Diabetes and *E. histolytica* infection together
17.67	34.47	P-value

*Different letters indicate significant differences (probability ≥ 0.01) between the averages of the different groups.

The result of cytokinetic IL-10 agreed with [20] and differed with [21] as some studies indicated that the balance between pro inflammatory and anti-inflammatory produced by macrophage and monocyte cells regulates the innate immune response and is important for immune stability, as some studies indicated that dysregulation The inherent immune response is harmful to the host of any disease. Therefore, IL-10 is a cytokine that maintains immune homeostasis and stability by controlling the function of the macrophage cell and through its inhibition of NF-KB (the gene expression factor responsible for cytokine) activity. Its inhibition of the production of TLR (receptors on cell surfaces), inhibits the activity of phagocytes and limits the exacerbation of the immune response, preventing tissue damage and the continuation of inflammation [22] Also, IL-10 stimulates the secretion of mucin by goblet cellS in the epithelium layer of the intestine, thus preventing Thus, from the adhesion of the trophozoite to the epithelial layer, it was observed in mice that have a deficiency of IL-10 that the epithelial cell cells are unable to produce mucus, which is an essential compound to reduce the adhesion of the amoeba to the intestine [23].

A decrease in the efficiency of the immune system has been observed in people with diabetes, especially in elderly people. In addition, genetic factors play a major role in protecting the body against parasitic pathogens[24].

The results of the current study agreed with the results of [25] in Al-Amarah and with the results of [26] in Tikrit, TNF-alpha is associated with an increased risk of people suffering from *Entamoeba histolytica* diarrhea, as the amoeba works to destroy the intestinal mucosal barrier and this leads to facilitating the parasite's penetration and destruction of tissues. Thus, the parasite will stimulate the production of many initiators of inflammation, such as TNF-alpha, at the site of infection [27]. TNF-alpha increases the permeability of blood vessels, and this leads to the parasite getting into red blood cells that will exude from the damaged vessels and into the tissue, and red blood cells are the best food source for the parasite [27], as TNF-alpha is a mediator. As shown [28] that mast cells are directly stimulated by the parasite or its antigens, resulting in the secretion of histamine, in addition to the ability of parasite antigens to stimulate the secretion of tumor necrosis factor-alpha from cells, the inflammatory response plays an important role in the control of parasitic diseases.

Conclusions

Diabetes and *Entamoeba* infection led to a significant increase in the levels of antibodies (IgM, IgG, IgA), complements (C3, C4), C-reactive protein (CRP) and cytokinetics (IL-10, TNF-α).

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التحري عن بعض المعايير المناعية لمرضى داء السكري والخمجين بطفيل اميبا الحالة للنسج

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الملخص

أجريت الدراسة الحالية على مرضى داء السكري والخمجين بالاميبا الحالة للنسج للراقدين والوافدين في مستشفى صلاح الدين العام وبعض المختبرات الأهلية في مدينة تكريت/ محافظة صلاح الدين للفترة من 2021/3/1 ولغاية 2022/4/1 وبأعمار تراوحت من 20−60 سنة, تناولت الدراسة الحالية الفحص المجهري للكشف عن الاطوار المعدية لـ 300 عينة غائط مصابين بإمراض مزمنة وتحديد نسبة الخمج بالطفيليات المعوية وداء السكري بالنسبة لعدد من المعايير (IgA , IgG , IgM, CRP, TNF-α , IL-10), اشارت النتائج إلى وجود ارتفاع معنوي في تركيز الاضداد(IgM, IgG, IgA) حيث بلغت 273.3±14.5 و 1345.0±116.0 و 441.9±38.5 mg/dl على التوال مقارنة مع مجموعة السيطرة 170.0±23.0 و 873.0±75.9 و 296.6±34.3 mg/dl على التوال والمتممات (C4 ,C3) اذ بلغت 65.0±8.35 , 222.0 ±21.4 mg/dl على التوال مقارنة مع مجموعة السيطرة 142.2±11.6, 3.94 ± 28.80 mg/dl على التوال, وبروتين سي التفاعلي (CRP) حيث بلغ 22.30±6.300 mg/l مقارنة مع مجموعة السيطرة 2.975±0.937 mg/l والحركيات الخلوية (T NF-α,IL-10) لدى الأشخاص المصابين بطفيل الاميبا الحالة للنسيج وداء السكري بلغت 46.00±9.32 و 92.9±31.8 pg/ml على التوال مقارنة مع مجموعة السيطرة السالبة 6.48±1.21 و 19.60±7.18 pg/ml على التوال.