Some immunological parameters of patients with *Entamoeba histolytica* in Baghdad city

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Abstract

The current study was conducted during the period from October 1, 2020 to September 30, 2021 for the purpose of diagnosing infection with the *Entamoeba histolytica* parasite in the city of Baghdad. Where 986 clinically diagnosed cases of diarrhea were examined from patients to the Child Protection Hospital and Baghdad Teaching Hospital in Medical City. Where the percentage of positive infections of the amoeba parasite was recorded at 53.448% of the total samples clinically infected with diarrhea when the samples were examined microscopically using the direct wet mount sample. And studied the effect of infection with the *Entamoeba histolytica* on some immune parameters of the infected, a significant increase was observed in patients with infection when compared with the control group at p – value 0.001 in each of the IL-4, interferon gamma and CD4 ratios (200.095±55.016). (104.645 ± 37.645), (11.562 ± 3.680) respectively.

1. Introduction

Parasites are among the pathogenic biological causes whose pathogenic effect is no less important than the effect of bacteria, viruses and fungi, and man has known them for their relationship to diseases that affect him and animals, so infection limits the activity of the organism causing various diseases. Parasites in general, especially intestinal parasites, spread in various societies on a large scale in different parts of the world. Its spread depends on the environmental conditions, so it can be observed that the infection of intestinal parasites spreads in the tropics and subtropics, due to the availability of suitable climatic conditions for them, such as heat, soft soil and moisture that help to sustain the growth of parasite stages such as cysts, eggs and larval stages. Entamoeba histolytica is one of the most important primary intestinal parasites that cause amoebiasis or what is known as amoebic dysentery [1].

Amoebic dysentery affects a large number of people around the world, and the number of people infected with it varies annually Between 40 - 50 million people, and amoebic dysentery occupies the third place in the death rate for parasitic infections after malaria and schistosomiasis, with a death rate of 40 - 100 thousand people annually [2].

Diagnosis of infection with *E. histolytica* depends on direct microscopic examination of the stool sample, as well as the use of culture media, which have low sensitivity, so methods are used to detect antibodies in the patient's serum [3], so resorting to use of modern methods of diagnosis using ELISA technology or by detecting the nucleic acids of the parasite, using the PCR Polymerase chain reaction [4].

The cellular immune response appears, which is stimulated by breaching the nutritional phases of the intestinal tissues, which stimulates the formation of colonies and thus the occurrence of the inflammatory process, these cells stimulate the production and release of cellular dynamics known as cytokines, for example, between Interleukin and Tumor necrosis factor, which works on the factor Proinflammatory factors that work to resist inflammation before reaching the immune cells such as white cells and phagocytes, which attack the parasite and ingest and digest it [5]. So, the current study aims to know the extent of amoebic dysentery and the relationship of amoeba infection with some immunological parameters of the infected in the city of Baghdad.

2. Material and method

Collect of sample

Sample of stool

In the current study, 986 stool samples were examined for incoming patients in Baghdad Teaching Hospital and Child Protection Hospital in Medical City during the period from October 1, 2020 to September 30, 2021. Excrement samples were collected from infected patients, provided that the examination did not exceed an hour upon their arrival at the laboratory. The samples were placed in sterile containers with a wide opening and a tight lid to maintain the samples' moisture and prevent their damage and exposure to dehydration. After that, the samples were diagnosed by the eyes, Macro examination to determine the nature of the stool, whether solid, liquid or semi-solid, as well as determining whether it contained mucous or blood. Then a small sample was taken for the purpose of microscopic examination. The sample is often taken from the areas that contain mucus or blood, because its presence is often associated with infection with the *Entamoeba histolytica* parasite [6].

Sample of blood

A total of 120 blood samples were collected from the patient. where 10 ml of blood of patients infected with amoeba was withdrawn by a medical syringe and placed in gel tubes containers and then left to coagulate and then placed in the freezer at a temperature of 20 - centrifuge at a speed of 3000 rpm for 5 minutes Then the separation was done by the automatic pipette, and the serum was placed and distributed in 4 Eppendorf tubes and kept in

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the refrigerator until the immunological tests were performed. Another 60 samples of serum from healthy people were collected and considered as a control group.

Laboratory diagnosis

Direct wet mount

The infection was diagnosed microscopically by using a direct wet mount by taking a glass slide and placing a sample of stool on it, and mixed with drops of physiological saline solution NaCl at a rate of 0.9%, and the slide cover was slowly placed over it for the purpose of preventing the formation of bubbles Usually this method is used for the purpose of diagnosing the active phases. The samples were examined at 4X and 40X powers [7].

Iodine Staining direct Wet Mounts

This method was used for the purpose of confirming the presence of cysts, where the iodine dye works to dye the glycogen and nucleated vacuoles, so an iodine solution (iodine dye) is placed instead of the physiological saline solution and prepared in the same way as before.

Immunological tests

Immunological serological tests were performed on 120 cases of tissue-forming amoeba infection in external private laboratories. The samples collected in Eppendorf tubes were pre-frozen and placed in a water bath, thawed and prepared for the purpose of starting the immunological examinations, which included the following tests.

Level of IL – 4 \ INF- Y \CD4

The enzyme-linked immunosorbent assay, called ELISA, was used to measure the levels of cellular kinetics under study of the blood serum of patients with *Entamoeba histolytica* and compare them with the control group. The principle of action depends on the surfaces of the pits that are found in the micro-standard plate, which are covered with specific antibodies specific to the cytokine specific for each assay.

3. Result and dissection

Diagnosis of *Entamoeba* in direct smear

The results showed Table (1), after examining 986 stool samples, that there were 527 cases of *E.histolytica* infection through direct examination of the wet swab sample, with a percentage of 53,448%, a positive sample of the parasite out of the total number of samples examined.. The study also recorded 459 negative samples of the amoeba parasite at a rate of 46.552%. The higher infection compared to negative samples is due to the deterioration of the sewage system, which leads to pollution of the environment through excreta and the absence of safe and clean sources of drinking water, and this consequently leads to the availability of Conditions that favor the perpetuation of the pathogenic parasite, which may lead to death for many individuals, especially children [8].

This study agrees with Hamzah et al. [9] in Al-Diwaniyah governorate showed an increase in the incidence rate of 61.26% of amoeba cases [9].

The similarity and difference in results with other studies is due to differences in regions and years, as well as climatic, social, economic and cultural conditions, or to experience and accuracy in the examination. Medicines. These percentages within the country also differ from the percentages recorded in some other Arab, Asian, European, American and Australian countries as a result of the development that has taken place in these countries on various health economic levels in recent years.

Table (1): The number of cases and percentages of *Entamoeba histolytica*

percentage	Number	Group
% 53.448	527	positive samples
% 552.	459	Negative sample
%100	986	Total

Effect of infection on some immunological parameters

Level of IL-4

The results of the current study Table (2) indicate a significant increase in the IL-4 rate in patients with amoeba (200.095±55.016 pg/ml) compared with the control group (92.177±24.613 pg/ml) at p – value 0.001.

This increase is attributed to the fact that this species enhances the natural resistance against intestinal amebiasis [10]. In addition, IL-4 is one of the most important cytokines, as it is called a typical immune regulatory cytokine, as it has the ability to target infected cells, and the infection penetrated into the colon tissue that contains a variety of cell types, which have the ability to develop as fibroblast cells. Immune cells result in a specific inflammatory response when the presence of *E. histolytica*. Where it was observed in most studies after taking measurements and quantitative sorting of multiple cytokines, a discrepancy in their values was observed in various studies conducted on culture media, mice, human intestinal xenografts and patient samples. The most important ones are IL-1b, IL-2, IL-4, IL-6, GM-CSF, IL-8, IL-10, IFN-c, and TNF [11].

These results were consistent with [3, 12], where these studies recorded a high percentage of IL-4 in patients with dysentery amoeba, as well as a study conducted with[13] on mice infected with *E. histolytica* where An increase in IL-4 was observed in mice treated with interleukin IL-25 and then gradually decreased until it was completely inhibited. This was attributed to the fact that IL-25 induction of IL-4 was blocked when eosinophils were depleted of all monoclonal antibodies. In a study conducted withGuo et al. [14] who characterized the kinetic pattern of cytokine responses during normal infection in mice and showed that amoebic infection led to rapid and sustained upregulation of Th2 IL-4, IL-5, IL-13.

Table (2): Average levels of interleukin 4 among patients with *E. histolytica* and compare it with the control group.

Group	IL- 4 S.D ± Mean	F- value	P – value
Patient group	200.095±55.016	144.268	0.001*

Control group	92.177±24.613		
* Indicates that there are significant differences between the injury group and the corresponding control, at a level of <0.001.			

Level of $IFN-\gamma$

The results of the current study in Table (3) indicate a significant increase in the rate of IFN-γ in patients with amoeba disease (104.645 ± 37.645 pg/ml) compared with the control group (39.790 ± 11.640 pg/ml) at p – value 0.001.

The rise is often attributed to the role of IFN-γ in the elimination of parasites and the *E. histolytica* parasite in particular, as the high rate of IFN-γ has a role in providing protection against amoeba by stimulating macrophages, especially neutrophils inside the organism's body In vivo, interferon like IFN-γ cannot fulfill this role without the assistance or immunological enhancement of the individual's immune system [5].

The current study agrees with [14] which showed a significant increase in the level of interferon IFN-γ, which could be considered a successful step for the manufacture of vaccine against amoeba, and its results recorded high rates of IFN-γ and most types of cytokines that were studied. Also [11] found that acute amoeba infection is associated with excessive release of pro-inflammatory cytokines in culture media, as it showed increased levels of cytokine in the presence of pathogenic strains of amoeba and no increase of cytokine with non-pathogenic strains. Cytokines have also been shown to have profound effects on biological signaling and important regulation of physiological processes and cytokines can also be linked to the activation of phagocytosis and production of active oxygen species [15].

A study conducted with [16-22]s on the effect of IFN-γ on the functional activity of mononuclear cells (MN) in infection with *E. histolytica*, where they found a difference in the concentrations of interferon gamma in the culture media containing the activators of the amoeba parasite, and also found that the immune cells Regardless of the cytokines, when the amoeba is present, it increases the release of superoxide, but in the absence of cytokines, there was an increase in the rate of ingestion of amoeba by immune cells. While it was observed that in the presence of IFN-γ or less ingestion of amoeba by immune cells. The immune cells also showed that treatment with cytokines had the highest rates of amoeba annihilation and thus apoptosis, and this indicates the efficiency of cytokines as therapeutic materials.

Table (3): Average levels of interferon gamma among patients with *E. histolytica* and compare it with the control group.

Group	$IFN-\gamma$ S.D ± Mean	F- value	P – value
Patient group	104.645±37.645	121.902	0.001*
Control group	39.790±11.640		
Indicates that there are significant differences between the injury group and the corresponding control, at p – value <0.001			

Level of CD4

The results of the current study were recorded in Table (4) to a significant increase in the rate of Cluster differentiation 8 (CD4) in patients with amoeba disease (11.562 ± 3.680 ng/ml) compared to the control group (4.924 ± 1.993 ng/ml) at 0.001 p – value.

Studies have confirmed that CD4 cells exert protection with the dysentery vaccine. The mechanism of protection mediated by immune cells may involve direct activity of amoebae and are useful alternatives for immune protection. In a study conducted [17] on the prevention of amebiasis in laboratory animals by immunization with Gal/GalNAc lectin through a T-cell-dependent mechanism which is a multifunctional virulence factor for the human parasite *Entamoeba histolytica* through the use of CD3+ or CD4+ CD8 cells, where The results showed that the recipients of CD4 immune cells had significantly less infection with amoeba compared with the recipients of the control, as it was noted that CD4 cells are the main source of interferon-gamma. This is mostly due to the fact that CD4-T cells have a great role in protection [18].

In a study conducted by [19] on those infected with intestinal parasites and the calculation of The number of T-CD4 cells, as it was found that the infection of the histiocytic amoeba parasite in patients with From anemia, it leads to an increase in the number of CD4-producing cells, with a number of more than 500 cells / microliter, and compared to the standard ratios established by the World Health Organization, the increase in cell production is clear.

In several studies conducted on patients with HIV and simultaneous infection with intestinal parasites, especially the *E. histolytica* parasite, as the study of [20, 21] on patients with *E. histolytica* who were diagnosed Recently, chronically and coexisting with HIV, they noticed a difficulty in the work of CD4-producing lymphocytes, where a decrease in the number of cells was recorded to less than 200 cells/microliter, using FACS count technique, a count of T-CD4 T lymphocytes. This may be attributed to the fact that infection with HIV leads to a gradual decrease in the number of T cells that express CD4, as most medical professionals have found that the number of CD4 has a major role in determining when to start treatment for HIV infection [22].

Table (4): Average levels of CD4 among patients with *E. histolytica* and compare it with the control group.

Group	CD4 S.D ± Mean	F- value	P – value
Patient group	11.562±3.680	113.166	0.001*
Control group	4.924±1.993		
Indicates that there are significant differences between the injury group and the corresponding control, at a level of <0.001			

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