Immunological Study of Patients Infected with Entamoeba histolytica in Al-Najaf Province

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Abstract Protozoa infections represent a major health burden in the developing world and contribute significantly to morbidity and mortality. Entamoeba histolytica is among the most common intestinal parasites, causing many pathological symptoms that may develop into amoebic abscesses in different regions in the body and Due to the importance of this parasite, the current study was conducted to find out the effect of the parasite on the immune responses of the patients. Serum was collected from 520 patients and 20 healthy volunteers. The percentage of parasite infection in females was 51.43%, which is slightly higher than that of males. IL-11 , IL-15 and adiponectin levels in the serum were estimated by enzyme-linked immunosorbent assay . IL-11 , IL-15 levels were significantly higher in patients compared to that of healthy subjects , While a significant decrease in the concentration of adiponectin was observed in patients compared to healthy subjects. The correlations between the interleukins under study were also studied. The results showed a positive correlation between Interleukin 11.15, with a significant difference of 0.000 at the significance level of 0.01.

Keywords: Entamoeba histolytica, Interleukin11, Interleukin15, Adiponectin

Introduction

Entamoeba histolytica is a tissue-invading protozoan and pathogen that causes amebiasis and an important cause of diarrhea in developing countries, Amoebas normally survive on intestinal bacteria and partially digested host food but are capable of invading tissues and spreading (Petri and Haque, 2014). The virulence of the E. histolytica parasite depends on its ability to secrete enzymes, enabling it to penetrate the colonic mucosa by invading epithelial cells and thus interfering with the humoral immune response of the host (Anaya-Velazquez and PadillaVaca, 2011).Cellular immune responses are also important for the host's defense against the parasite during the first phase of infection, Intestinal epithelial cells bind to Gal/GalNAc via special receptors, which activate NFκB and trigger the production of inflammatory cytokines, including IL-1β, IL-6, IL-8, IL-12, IFN-γ, and TNF-α (Gay and Gangloff, 2007).

Interleukin 11(IL11) Among all the anti-inflammatory cytokines, IL-11 is a cytokine with potent anti-inflammatory properties. Initially, IL-11 was described as a hematopoietic cytokine with haemostatic activity. It is expressed in the central nervous system and the gastrointestinal tract. Its role is In the process of hematopoiesis by stimulating and increasing primordial stem cells, it works synergistically with other cytokines to support the proliferation and differentiation of all hematopoietic stem cell lineages (Pine and Hill, 2011).

Interleukin 15 (IL15) is a novel cytokine similar to IL-2 in its biological activity (Bamford et al., 1994), stimulating macrophages, natural killer cells, T-cell receptor (TCR) αβ T cells, and B cells to proliferate and secrete cytokines, lead to an increase in cytotoxicity (Carson et al., 1994), and produce antibodies (Armitage et al., 1995). A large number of T cells, which appear in the early stage of infection, may preferentially use IL-15 from stimulated macrophages as a growth factor and play an important role in protection in the early stage of infection before the appearance of IL-2-producing αβ T cells. The functions of IL-15 also include the regulation of cellular expression by antigen-presenting cells and the production of chemokines by intestinal epithelial cells.(Ohtek et al., 2001) activates IL-15 and maintains innate and adaptive immune cells, stimulates the secretion of chemokines by neutrophils and monocytes, and activates these In rodent models of intracellular bacterial infection, IL-15 attracts natural killer cells to infected sites and reduces Bacterial colonization) and natural killer cells have also been studied in mouse models of malaria and toxoplasmosis (Umemur et al., 2001).

Adipokines (also known as adipocytokines) are cytokines produced by adipose tissue that have a role in the body's energy/metabolic state, inflammation, obesity, and other functions. Adipokines include leptin, adiponectin, interleukin-6, and other factors (Fasshauer and Blüher, 2015). Some intestinal parasites can cause anorexia by disrupting the

secretion of leptin and adiponectin, as well as affecting the absorption of certain nutrients. Anorexia caused by parasites is an acute phase reaction to infection mediated by cytokine-induced leptin production (Yahya et al., 2018). which may affect BMI and cause anorexia (Raida et al.,2018; Kyriazakis et al.,1999). Adiponectin is generally considered an anti-inflammatory agent although there are studies also suggesting pro-inflammatory properties (Tilg and Moschen, 2008). Adiponectin and TNF-α suppress each other's production and antagonize each other's actions in target tissues, and tend to upregulate IL-6 (Bruun et al., 2003), IL-10 and IL-8 (Goldfine and Kahn, 2003). Considering the the limiting of information on the exact relationship between IL-11 ,IL-15 and adiponectin levels with patients infected with Entamoeba histolytica, the present study was conducted .

Materials And Methods :

Study population:

The present study consisted of 520 patients samples suffering from abdominal pain, diarrhea, and vomiting, and 20 of healthy subjects as a control group , with ages from 20-70 years and for both sexes. All participants were recruited between November 2021 to April 2022 from Al-Hakim Teaching Hospital, Al-Sadr Teaching Hospital, Al-Najaf General Hospital, Al-Sajjad General Hospital, and Al-Furat Al-Awsat Hospital. Approval for the study was taken from these hospitals.

Serum sampling

Five ml of venous blood were obtained from each individual participating in this study and the serum separation from blood was performed by centrifugation

at 3000 rpm for approximately 15 minutes. Then the serum was collected in sterile appendorf tube and the serum isolated, aliquoted and stored at -80°C until subsequent ELISA.

ELISA analysis of IL-11 ,IL-15 and Adeponectin

Levels of **IL-11 ,IL-15 and Adeponectin** was estimated by using human Enzyme-Linked Immunosorbent Assay (ELISA) Kits (Sunlong company ,China).ELISA kit uses Sandwich-ELISA in accordance with the manufacturer's instructions. Absorbance in each well was read on an ELISA reader using 450 nm as the primary wavelength. Concentrations of **IL-11 ,IL-15 and Adeponectin** were estimated using the standard curve.

Statistical analysis

All statistical analyses were performed by using SPSS version 26 software program. (Paulsan, 2008). In this study, non-parametric tests were used using the manwhitney test to extract the values of the arithmetic mean and standard deviation at the level of significance ($p<0.05$) among the groups under study, because the data of the study are not subject to a normal distribution.

Results and Discussion

This study enrolled 20 healthy volunteers and 520 patients samples suffering from abdominal pain, diarrhea, and vomiting,70 samples were positive for Entamoeba histolytica, with a percentage of 13.46, as shown in Table (1).

Table 1: Percentage of infection with Entamoeba histolytica

Sample	total samples	Positive sample	percentage	Negative sample	percentage
Patient	520	70	13.46	450	86.54
Control	20	0			

A study of the effect of sex on the percentage of infection with Entamoeba histolytica

The results of our current study showed a similar percentage of infection with the E. histolytica parasite according to sex, as it reached 48.57 and 51.43 for both males and females, respectively, despite its slight increase in females, as shown in Table (2).

Table (2): Percentage of infection with Entamoeba histolytica by sex

Sample	total samples	male	percentage	female	percentage
Patient	70	34	48.57	36	51.43
Control	0	5	0	15	0

Perhaps the reason for the high rate of infection among females is due to the lack of observance of public health and the lack of health education and work with animals, especially among women in the countryside. Including laundry rooms, bedrooms, and meadows. All these job responsibilities expose these females to rubbish and pollution because they keep various pathogens with their bodies and become victims of them. Also, these people do not take serious measures to address this problem. When the disease seriously affects their health, they go to the doctor to receive Treatment In addition, they are not taking the right medication for an appropriate period. They stop using the drugs as soon as they feel the end of the symptoms of the disease. Also, females are often in direct contact with food, especially vegetables that may contain the parasite.

Level of interleukin11 in patients infected with Entamoeba histolytica

The results of the current study showed significant increasing in level of IL-11 in patients(71.26 ± 13.3) compared to that of healthy subjects (62.2± 11.98) (P-value = 0.01), as shown in Table 2.

Table (3) : Levels of IL-11 in patients and healthy control group

Sample	**N**	**Mean**	**Std. Deviation**
Patient	70	71.2606	13.30376
Control	20	62.2000	11.98754
Asymp. Sig. (2-tailed)	.010		

***Significant differences at 0.05 between patients and control.**

The high concentration of IL-11 may be attributed to its being considered a disease mediator. Previous studies have shown that IL-11 can play a protective role, by fighting apoptosis, promoting proliferation and trophicity, and reduce regulating pro-inflammatory cytokines, in intestinal epithelial cell damage induced by various factors ((Kuenzler et al., 2002) . One of the interesting effects of IL-11 is its ability to protect at the site of mucosal injury. These effects are more pronounced in the gastrointestinal tract where IL-11 protects small intestinal cells after combined radiotherapy and chemotherapy, and reduces of experimental mucositis and attenuates infection in inflammatory bowel disease models (Keith et al.,1994). It was also recently shown that IL-11, a novel multifunctional bone marrow tissue-derived growth factor, can induce rapid repair of small intestinal villous structures in mice treated with radiation and chemotherapy (Orazi et al., 1996).

IL-11 protects barrier integrity during oxidative stress, and is a common endpoint for many types of intestinal injury including ischemia and immune inflammation. Since heat shock proteins (hsp) protect cells in the intestinal epithelium, it was hypothesized that the cellular protection conferred by IL-11 is mediated by inducible hsps. Interleukin induces hsp25 in an intestinal epithelium-specific manner that significantly preserves cell viability in the presence of monochloramine. Induction of hsp25 by IL-11 confers epithelial-specific cellular protection independent of the phosphorylation-dependent co-localization of hsp25 to F-actin, thus contributing to Protective effects of IL-11 in models of intestinal epithelial injury (Robleski etal., 2003).

Level of interleukin15 in patients infected with Entamoeba histolytica

It was recognize significant higher level of Interleukin15 in patients (12.57±5.20) compared to the control group (10.39±0.88), (P-value= 0.002) as shown in Table (4).

Table (4): Levels of IL-15 in patients and healthy control group

Sample	N	Mean	Std. Deviation
Patient	70	12.5729	5.20498
Control	20	10.3975	.88205
Asymp. Sig. (2-tailed)	.002		

***Significant differences at 0.05 between patients and control.**

Human infection with Entamoeba histolytica leads to an increase in the expression of IL-15 in the intestinal tissues. This is consistent with (Robinson et al., 2001). Dann et al. (2005) noted that infection with Cryptosporidium parvum increases the expression of IL-15 within cells in a human intestinal cell line. Similarly, quantification of IL-15 protein from human jejunal biopsies revealed that IL-15 levels increase within hours of exposure to the parasite and are maintained over the following week.In the intestine, IL-15 stimulates NK cells and NK cell, T, γδ, and T cell (Inagaki-Ohara et al., 1997). After IL-15 treatment, both intraepithelial T cells (Taunk et al., 1997) and cells expressing NK markers have a greater killing potential (Kinoshita et al., 1997). Dann et al., 2005 showed that depletion of NK cells from the population of effector cells significantly reduces lysis of infected cells. Thus, data suggest that IL-15, which is produced within hours of exposure to the parasite invivo, may activate natural killer cell clearance from Cryptosporidium parasites.

Hirose et al.(1998) investigated whether IL-15 is involved in intestinal immune responses against intestinal infection, examining IL-15 production by intestinal intraepithelial lymphocytes after oral infection of mice with Listeria monocytogenes. Invitro experiments revealed that L. monocytogenes invasion in intestinal epithelial cells (a rat small intestine epithelial cell line) induced activation of nuclear factor-κB (NF-κB, which is essential for IL-15 gene expression, and thus upregulated expression of IL-15 mRNA in intestinal epithelial-6 cells, and oral inoculation with L. monocytogenes enhanced IL-15 synthesis by intestinal epithelial cells in conjunction with an increase in the number of TCR+ cells, CD3+ CD8αα+ cells, and NKR.P1+ cells in intra-intestinal lymphocytes at the early stage of infection,Lymphocytes within the intestinal epithelium showed enhanced IFN-γ-producing activity upon stimulation. Overall, these results indicate that IL-15 can be produced by intestinal intraepithelial lymphocytes after oral infection with L. monocytogenes and that early IL-15 production may be involved in protection against intestinal infection through induction of a large fraction of intraepithelial lymphocytes in order to produce IFN-γ. As IL-15 is secreted from intestinal epithelial cells in response to invasion by L. monocytogenes and T cells and natural killer cells are activated in intestinal intraepithelial lymphocytes to produce IFN-γ in the early stage of oral infection with L. monocytogenes, our findings indicate that IL -15 produced by epithelial cells in response to Listeria invasion may have a role in the early activation of intravitreal lymphocytes in the intestine, contributing to the first immune barrier of host defense against oral infection by invading bacteria.

Level of adiponectin in patients infected with Entamoeba histolytica

The results of the current study showed that the mean concentration of adiponectin in patients was lower than that in the control group, with a significant difference of 0.029 at a significance level of 0.05, as shown in Table (5).

Table (5): Levels of Adiponectin in patients and healthy control group

Sample	N	Mean	Std. Deviation
Patient	70	5.5950	1.91236
Control	20	6.0175	1.60224
Asymp. Sig. (2-tailed)	.029		

***Significant differences at 0.05 between patients and control.**

The results of the current study were in agreement with (Valentini et al., 2009), where low levels of adiponectin were recorded in inflammatory bowel disease, which the decrease was related to the body mass index of patients in addition to hyperinsulinemia, as the recorded decrease in adiponectin levels represented an actual mechanism of protection against Colitis This is consistent with the study (Nakajima et al., 2002).

In a study conducted by Maeda et al.(2001) through an experiment conducted on animals with Dextran Sulfate Sodium-induced colitis (DSS), few peroxisome proliferator-activating gamma bonds (PPARg), known regulators of adiponectin, were found in colitis patients.

In a study conducted on laboratory mice to study the concentration of adiponectin, Bohn et al.(2006) noted that colonization of Escherichia coli can induce colitis in IL-2 deficient mice by suppressing adiponectin expression. Studies also revealed that treatment Corticosteroids were associated with a decreased rate of adiponectin secretion by mesenteric white adipose tissue, in Crohn's patients (Paul et al., 2006). Elisavet et al., 2011 showed alterations caused by Trypanosoma cruzi (the causative agent of Chagas' disease), which include reactivity Severe inflammation of adipocytes and decreased levels of adiponectin.

Correlation study between interleukin 11,15 and Adipnictin concentration among patients

Table (6) shows a positive correlation between the concentration of interleukin 11,15 among the patients

Table (6) Correlation of interleukin 11,15 and Adipnictin concentration among patients

Spearman's rho Correlation Coefficient	interleukin11	interleukin15	Adipnictin
Adipnictin	.092	-.146-	1.000
Sig. (2-tailed)	.389	.170	.
N	90	90	90
interleukin11	1.000	.501**	.092
Sig. (2-tailed)	.	.000	.389
N	90	90	90

**** Correlation is significant at the 0.01 level (2-tailed)**

Interleukin 15 is a pro-inflammatory cytokine characterized for its effects on T lymphocyte and natural killer cell activation, proliferation, and survival (Kennedy et al.,2000) In inflammatory bowel disease, increased expression of IL-15 on peripheral blood leukocytes has been reported. A significant increase in the expression of IL-15 mRNA was found in the inflamed rectal mucosa of IBD patients. (Sakai et al.,1998). Several types of cells can produce IL-15, including macrophages, dendritic cells, and intestinal epithelial cells. The finding that enteric cells can produce and respond to IL-15 (Reinecker et al., 1996) and that IL-15 effectively stimulates intraepithelial lymphocytes (Kennedy et al., 2000), has focused attention on its role in intestinal inflammation. IL-15 also has a number of other activities, including recruitment and activation of T cells, maintenance of memory T cells, stimulation of proliferation and immunoglobulin synthesis by B cells, natural killer (NK) cell proliferation, activation of neutrophils, and inhibition of apoptosis. (Lodolce et al., 1996)

IL-11 effectively inhibits the expression of the nuclear transcription factor NFκB and, by extension, interleukin-1, tumor necrosis factor-α and other pro-inflammatory peptides (Neurath et al., 2000). In addition, IL-11 promotes intestinal mucosal integrity in the face of a combination of (Keith et al.,1994) Therefore, interleukins 11 and 15 may participate in being pro-inflammatory cytokines and have similar properties in their activities against disease through their high concentration when infected with the parasite. The researcher's data (Dickinson et al., 2000) indicate that the positive effects of IL-11 result from its ability to stimulate intestinal cell proliferation and differentiation and prevent epithelial cell apoptosis. Human IL-11 may also modulate the immune system by stimulating an increase in immunoglobulins that B lymphocyte secreted cells and our results confirm those of Orazi et al.,1996 who showed that IL-11 inhibits apoptosis in the small intestine after radiotherapy and protects intestinal stem cells, which definitively determine the integrity of the intestinal mucosa. Thus, IL-11 -11 not only maintains the integrity of the intestinal mucosal barrier, but it may also enhance the immune function of the intestine.

Conclusion

On the basis of the results obtained, it was found that infection with the Entamoeba histolytica parasite leads to immune responses in the human body represented by an increase in the concentration of interleukins 11 and 15 and a decrease in the concentration of adiponectin.

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