The Role of IL-25 and IL-35 in Amoebiasis

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Abstract

Bacground: Amoebiasis is a protazon infection of the human intestine spread through the world the most prevelant form of the disease is amebic dysenterywhich characterizes acute diarrhoea with observable blood and mucus in stools.

Aims: This article aimed to detects the role of IL-25 and IL-35 in the immune response against amebic dysentery.

Methods: This study was conducted in Thi-Qar province-Al-Nasiriyah city in Muhammad Al-Mousawi Hospital for Children, the study included collection of (60)blood samples from amebiasis patients and (30) apparently healthy children at a period from September 2019 to March 2020 with the age less than one year to 15 years that divides to four age groups, the levels of IL-25 and IL-35 were determined by ELISA technique.

Results: The results indicate that the IL-25 and IL-35 concentrations in serum samples from amoebiasis patients were significantly higher when compared with that from healthy controls. The highest level of IL-25 was recordsin the third age group of patients with level 1677.2 ± 867.2ng /ml,compare with the high level in the third age group of control with level 450.40 ± 97.31ng /ml.Also, the findings indicates the highest level of IL-35 records35 in the second age group of patients with level 291.0 ± 62.3ng /ml, while the high level recorded in the first age group of control with level 8.246± 0.60 ng /ml.

Keywords: *IL-25, IL-35, Amoebiasis, cytokine*

Introduction

Amoebiasis is an infection of the human intestine by*E. histolytica* protazoen**,** also called amoebic dysentery is acute diarrhoea with observable blood and mucus in stools and the existence of haematophagous trophozoites in stools or tissues. Non-dysenteric amoebic colitis presents as recurring seizures of diarrhoea with or without mucus and no visible blood with the presence of *E. histolytica* cysts or non-haematophagoustrophozoites [(1)]. *E. histolytica* stimulate both the innate and adaptive immunity such as the resistance of the mucosal barrier and lymphocytes of the class Cluster of differentiation 4 (CD4), Cluster of differentiation 8 (CD8) and the presence of amoeba antibodies such as immunoglobulin Class A secretory (SIgA) [(2)].

The cells in the intestine adhere to and distinguish the Gal/GalNAc lectin of parasiteby (TLR)-2/4, as these cells work to send pro-inflammatory signals and leads to the formation of inflammatory cytokines, including IL-1β, IL-6, IL-8, IL-12, IFN-γ and TNF-α, these signals lead to attraction of the defensing cells like: neutrophils and macrophages to the position of amoebaeinfection[(3)]. IL-25 belongs to the IL-17 family; the name for the alternative is IL-17E. The protein contains 177 amino acids, which are secreted by many human cells, such as T cells, macrophages, masts, epithelium, dendritic and other [(4)]**.** A study by [(5)] treating infected mice with intestinal IL-25 showed that it stimulates the immune response against *Trichinella spiralis* infection.

Also, observed that intestine IL-25 would be inhibited during CDI infection in humans and mice.

Therefore they concluded that an IL-25 induces eosinophil to defend against *Clostridium difficile* colitis CDI [6]. IL-35 was first known in 2007, has been added in the IL-12 family for similarity in the unique heterodimeric structure. It is mainly provided by CD4 Treg as well as by stimulated B cell and a smaller extent by motivated endothelial cells and monocyte cells [7].Li and his colleagues enrolled in an interleukins study that IL-35 and IL-37 ratio are higher in inflammatory bowel disease (IBD) patients, while serums IL-35 and IL-37 level were suggestively low in the ulcerative colitis (UC) patients compared with healthy controls (HC) [8].

Material and Methods

The study involved collection of 3 ml of blood for each samples from 60 children suffering from amebic dysenteriae and 30 samples from appearantly healthy children as control with the age ranging from less than 1 year to 15 years during the period from September 2019 to march 2020 in Al-Musawe Hospital for childrenin Al-Nasiriyah city-Thi-Qar province-Iraq. Serum cytokine concentrations of IL-25 and IL-35 were determined in the serum samples by using enzyme linked immunosorbent assay (ELISA) technique according to manufacturer's instructions(BIO-TEC-China) using microplate spectrophotometric reader.

Statistical Analysis

All data of the present study were statistically analyzed by using Microsoft windows 10 Excel (version2010) and SPSS version 24 (ANOVA for Leas Significant Difference LSD and Independent T. test).

Results

The study showed higher level of IL-25 and IL-35 concentrationsin patients who infected with amoebiasis in comparison to health control and recorded statistically significant difference in levels of IL-25 and IL-35between all age groups of patients compared with corresponding age groups of health control at P. value < 0.05**(Tables1 &2)**

Table (1)Level of IL-25 for patient and control according to age groups

Parameter / Grups		No. of Cases	IL-25 M ± SD ng /ml	P. value
Patient	< 1 year	18	1233.1 ± 405.3	< 0.0001
Control		5	378.20 ± 48.97	
Patient	1–5 years	47	1504.6 ± 643.0	< 0.0001
Control		14	444.91 ± 114.1	
Patient	6–10 years	21	1677.2 ± 867.2	< 0.0001
Control		8	450.40 ± 97.31	
Patient	11–15 years	11	1672.4 ± 700	< 0.0001
Control		3	431.9 ± 157.1	

Table (2)Level of IL-35 for patient and control according to age groups

Parameter / Groups		No. of Cases	IL-35 M±SD ng /ml	P. value
Patient	< 1 year	18	240.6 ± 39.5	< 0.0001
Control		5	8.246 ± 0.60	
Patient	1–5 years	47	291.0 ± 62.3	< 0.0001
Control		14	6.746 ± 1.39	
Patient	6–10 years	21	256.0 ± 72.8	< 0.0001
Control		8	7.170 ± 1.30	
Patient	11–15 years	11	249.8 ± 52.2	< 0.0001
Control		3	6.718 ± 1.59	

Level of IL-25 and IL-35 According to Statues of Infection

According to status of infectionthe results of current study showed the high levels of IL-25 and IL-35 in triple infections, followed bydouble and single infections in comparison health control**Table (3)**

Table (3) Level of IL-25 and IL-35 according to statues of infections

Parameters / Infection Status	No. of Cases	IL-25 M ± SD ng /ml	IL-35 M ± SD
Single	1463.2 ± 645.4b	264.3 ± 62.4b	227.2 ± 28.7b
Double	1483.1 ± 605.7c	276.4 ± 60.7b	218.8 ± 26.6b
Triple	2631.0 ± 536.6d	305.1 ± 69.3c	263.0 ± 35.7c
Control	433.92 ± 104.0a	7.106 ± 1.34a	4.790 ± 0.7a
P. Value		< 0.0001	< 0.0001
LSD		172.86	30.16

Discussion

Level of IL-25 for Patient and Control According to Age Groups

The current study showed rising levels of IL-25 in all age groups and differences between them, significant differences were observed. These results indicate the impact of IL-25 with *E. histolytica* infection.In the present study, it is possible to find a role for IL-25 with amoebiasis by noting several things, High levels of IL-25 when we examined and compare patients of

amoebiasis with healthy control serum of all ages. This means that interleukins concentration does not affect with the patient's age,but rather depends on increasing the number and concentration of parasites, also the immune status of patients,and the evidence is that the interleukin concentration increased in triple infections more than double and single infections.

This compatible with study by [9]pointed out that epithelial cells in the human intestine have the ability to produce IL-25, which has an important role in balancing defensive barriers in the intestine, as well as commensal bacteria in the intestine, which have the ability to stimulate intestinal epithelial cells to produce IL-25. The increase levels of IL-25 in this study may due to the ability of many types of cells to produce this cytokines such as T. cells, dendritic,macrophage, eosinophil and epithelial cells [4]. IL-25 levels associated with increase the inflammation during intestinal infection because its ability to stimulate the production of some immunological mediators like: IL-8, chemotactic factors for neutrophil and induced inflammation also that IL-25 has been proved to enhance mucus production in intestinal[10], and production of Th2 cytokines such as: IL-4 which help in humeral defense mechanism against pathogens including parasite infection [11][12]. So, IL-25 plays an important role in implicating immune response against amebiasis. As [13]mentioned that IL-25 provided protection from *E. histolytic*a in an eosinophil-dependent, as demonstrated by abrogation of protection by depletion of eosinophils.

A role for eosinophil's in amebiasis is show that decline eosinophil products (Charcot-Leiden crystals) exist along with trophozoites in the stool of patients with amebiasis [14]. In addition [11] was also shown IFN-γ, IL-17(IL-25) contribute to vaccine-induced protection in murine studies, these findings suggest an important role for cell-mediated cytokine production in protection from ameobiasis. Camelo and his colleagues found that IL-25-induced inflammation is typically characterized by elevated levels of type-2 cytokines which lead to pathological changes in the lungs and digestive tract, such as elevated serum IgE and IgG1, increased mucus secretion, and epithelial cell hyperplasia [15].

Level of IL-35 for Patient and Control According to Age Groups and **Statues of Infection**

The present study recorded the higher level of IL-35 in all age groups and statistically significant difference in levels of IL-35 between all age groups of patients compared with corresponding groups of health control. We showed distinctly the high level of IL-35 concentration with infection more than one parasite, where the highest infections were triple, double and single infections respectively. Also, the high level of IL-35 concentration does not depend on a specific age group, and we also recorded a rise in both sexes, as there are no statistical differences between them. This means that interleukins concentration does not affect by the patient's age, but rather depends on the patients 'immune status.

The presence study agree with Cao and his colleagues in Chongqing, China ,they found that IL-35 levels in serum samples from adult or child patients with sepsis was significantly higher compared with healthy controls and progressively increased according to sepsis severity [16]. Also our study agree with study by Fonseca [17] in Mexico City recorded results suggest that down-regulation of inflammation in active Inflammatory Bowel Disease (IBD) patients might be based on the increased expression of IL-35 and IL-37.As in a study by[18] they found T cells that secrete IL-35 and have suppressive functions can be induced in the intestines of mice infected with the intestinal parasite Trichuris muris. Also agree with Choi found that IL-35 has an immunosuppressive effect on inflammation through induction of Treg cells and suppression of Th1 and Th17[7]. These results agree with [19] has been shown IL-35 in other chronic inflammatory diseases and parasitic/ bacterial infections, where the inhibitory cytokines.

Depending on the results achieved in our study of patient's children with ameobiasis and comparing them with healthy children, and according to published research, we saw that the level of IL-35rises in gastrointestinal infections, as it is considered an immune suppressant.

Conclusion

The cytokines under this study produces in high levels during the course of intestinal amoebic infections and they play important role in the development severity of the symptomatic form of amoebic dysentery.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval ofboth environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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