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The Exploring of Pro- Inflammatory Cytokine IFN- γ , IL-17 and TNF- α in Patients with Amoebic Dysentery from Kerbala Province

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Abstract

This research investigated the correlation between pro-inflammatory cytokines (IFN- γ , IL-17, and TNF- α) and *Entamoeba histolytica* infection in amoebic dysentery. The study examined the role of pro-inflammatory cytokines in preventing *Entamoeba histolytica* parasite infection. It measured IFN- γ , IL-17, and TNF- α concentrations in amoebiasis patients and compared them to healthy participants, demonstrating their importance. The study found a significant difference in IFN- γ , IL-17, and TNF- α concentrations in affected individuals (15.3 ± 1.5 , 980.5 ± 23.4 , and 29.4 ± 0.5 pg/ml) compared to the control group (6.5 ± 1.026 , 658.6 ± 653 , and 6.4 ± 0.9 pg/ml, respectively) ($p \leq p \leq 0.05$). In addition, children ages 3-9 (78.5%) had the greatest infection rate, followed by 9-17 (71.4%), 24-30 (66.6%), and 17-24 (56.2%). This research shows that amoebic dysentery patients had higher cytokine levels than healthy people. Age affects amoebic dysentery prevalence. Younger age groups, particularly children under 10, are more vulnerable to infection.

Introduction

Amoebic dysentery, caused by the parasite Entamoeba histolytica, affects 40,000 to 110,000 people worldwide. The number of infections is estimated at (480) million infections and deaths annually in the world. This disease is ranked third after malaria and schistosomiasis in Causing deaths (Lampel et al.,2012).

This ancient protozoan shows how parasites have co-evolved with mankind, using host resources for growth, division, and encystment; evading immune responses; and interacting with gut microorganisms to suit nutritional and protective requirements. Unbalanced trophozoite-cyst life cycle. If unchecked, trophozoites become virulent and invade the gut, causing amoebiasis. The highly contagious *E. histolytica* must cross mucus, epithelial connective tissues, and maybe blood (Guillén, 2023).

Amoebic infections are generally more common in countries with poor public health and worse socioeconomic situations (Haque et al., 2003). *Entamoeba histolytica* is spread via amoebic cysts in contaminated food or drink. (Billet et al., 2019; Salit et al.2009)

E. histolytica or *E. dispar* are the most common causes of *Entamoeba* infections. Amoebic colitis and amoebiasis, which occur outside of the intestines, may be caused by the pathogenic strain of *E. histolytica*. Infection occurs when trophozoites attach to colonic epithelial cells via a galactose-N-acetyl galactosamine lectin. According to Stanley SL's study (2003), when trophozoites stick to colonic epithelial cells, these cells experience cytolysis and death, which results in the release of interleukin and precursor interleukins. These interleukins activate NF- κ B in distal cells, which in turn leads to the production of cytokines and further inflammatory chemicals.

Entamoeba histolytica infection stimulates numerous cytokines, which activate white blood cells to govern the immunological response. Previous investigations have indicated that cytokines regulate monocyte function and activate amoebicides. According to Gordon (2010), the cytokines IFN- γ and TNF- α play a role in triggering and regulating various immune responses.

The aim of this study was to explore the pro-inflammatory cytokines IFN- γ , IL-17, and TNF- α in patients with amoebic dysentery from Kerbala Province.

Methods and Materials

Specimens Collection: At Al-Emam AL-Hussain Teaching Hospital, 60 blood samples were taken from people who had the *Entamoeba histolytica* parasite. The ages of the people who took part ranged from 6 to 30 years old, and there were both men and women. There were also 26 control samples.

Sample preparation: Following the placement of blood samples into EDTA anticoagulant tubes, a centrifuge was operated at a speed of 6,000 cycles for 10 minutes to obtain serum. This serum was then utilized for the analysis of cytokines IFN- γ , IL-17, alongside TNF- α using the ELISA technique. The EDTA was utilized for performing a comprehensive blood count analysis and an evaluation of white blood cells.

We used the ELISA method to find out how much IFN- γ , IL-17, and TNF α were in serum, following the instructions from SUNLONG, a Chinese business that makes the kits. The absorbance measurements was done at 450 nm, and the normal ranges were between 15.6 pg/ml and 1000 pg/ml.

Statistical Analysis: Using the statistical program SPSS, Version 22, the findings were put

| Patients Age | Examined samples | | | % of Examined samples | | | p- value |
|--------------|------------------|---------|-------|-----------------------|---------|-------|----------|
| | infected | Healthy | Total | infected | healthy | Total | |
| 3- 9 years | 22 | 8 | 28 | 78.5 % | 28.5% | 100% | P=0. 002 |
| 9-17 years | 15 | 6 | 21 | 71.4 % | 28.5% | 100% | |
| 17-24 years | 9 | 5 | 16 | 56.2% | 31.2% | 100% | |
| 24-30 years | 14 | 7 | 21 | 66.6% | 33.3% | 100% | |

through a statistical analysis. To identify statistically significant differences across the study groups, the (L.S.D.) test was used (Morgan et al., 2010).

Results And Discussion

1- Amoebic Infection Distribution According to Patients Age

Table 1: Amoebic infection distribution according to patients Age

Table 2 shows a significant difference in amoebic infection rates by age group ($P = 0.002$). The greatest infection prevalence was in children ages 3-9 (78.5%), followed by 9-17 (71.4%) and 24-30 (66.6%). The 17-24 age group had the lowest infection incidence (56.2%). This suggests that endemic *Entamoeba histolytica* amoebiasis in Karbala province is linked to the consumption of food contaminated with the parasite's cystic phase, socioeconomic factors, untreated water sources, and poor hygiene. Environmental, dietary, socioeconomic, geographical, demographic, sampling, study duration, and data collection months may explain the observed variance in this parasitic infection rate across age groups in the current study.

2- Analysis of Cytokine Levels in Serum of Amoebiasis-Infected Patients

Table 2 shows that symptomatic *E. histolytica* patients had higher blood levels of all three cytokines (IL-17, IFN- γ , and TNF- α) compared to the healthy control group, as determined by ELISA. IL-17 serum levels were higher in infected individuals than controls. IFN γ , IL-17, and TNF- α levels significantly increased with $P \leq 0.05$, reaching 15.3 ± 1.5 ,

980.5 ± 23.4, and 29.4 ± 0.5 pg/ml, respectively, compared to the control group (6.5 ± 1.026, 658.6 ± 653, and 6.4 ± 0.9 pg/ml). *E. histolytica* sequentially disrupts the mucosal barrier. Through its Gal/GalNAc lectin, the parasite first attaches to intestinal epithelial cells. After this, amoebic trophocytosis kills epithelial cells, penetrating the epithelium and destroying submucosal tissue (Peterson, 2011).

E. histolytica induces pro-inflammatory cytokines such interleukin-17 (Kissoon-Singh, 2013). Inflammasome activation stimulates cytokine production. Zainab Abdul (2013), Noor (2017). Stomach acid helps fight *E. histolytica* by killing trophozoites, although amoebic cysts are resistant (Mondal et al., 2012). According to Lidelle et al. (2006), cysts excyst in the lumen of the colon, and trophozoites stick to the tissues of the intestinal tract, causing the muscular layer to be disrupted and increasing tissue invasion. Here, powerful cytokines are produced, luring immune cells to the invasion site. As stated by Bansal et al. (2009).

63 TNF-α and INF-γ, which are induced by IL-12 from natural killer cells, cause macrophages to release reactive oxygen species (ROS) and nitrogen oxides (NO), which ultimately leads to the death of the parasite (Al-Ubaydi, 2019). Recently, TNF-α production has been connected to diarrhea induced by *E. histolytica* (Peterson, 2011). TNF-α activates neutrophils and macrophages, releasing ROS and NO to fight parasites and promote phagocytosis. The pro-inflammatory cytokine TNF-α is essential for mediating inflammation and activating acute phase proteins (Jari and Yousif 2020). This research supports Uddin et al. (2021) results linking higher TNF-α production to amoebic dysentery. Overactive TNF-α triggers an aggressive immune response, resulting in inflammation and illnesses.

Table 2: Levels of IFN-λ, IL-17, and TNF-α cytokines in the serum of patients with amoebiasis infection

| Study groups | IFN- λ (pg /ml) | IL-17(pg/ml) | TNF-á (pg/ml) |
|---------------------|-----------------|--------------|---------------|
| Positive (infected) | 18.5 ± 1.5 | 991.4 ± 23.4 | 16.6 ± 3.5 |
| Negative(healthy) | 7. 5± 1.026 | 664.6± 55.6 | 9.4 ± 0.9 |
| L.S.D. | 0.8 | 23.2 | 1.7 |

Conclusion

The present study shows:

1. Amoebic dysentery patients have much higher cytokine levels than healthy persons.
2. Age correlates with amoebic dysentery prevalence. According to research, children under 10 are more vulnerable to illness than older people.

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