Abstract

The current study aimed to reveal the evaluation of the level of some immune chemokines and liver function in infected with the parasite Entamoeba Histolytica. Blood samples were collected during the period from the beginning of August 2021 until the month of April 2022. The separated sera were kept at -20 temperature until use. Enzyme-linked immunosorbent adsorption (ELISA) technique was used to detect dysentery amoeba infection. CCR2 and CCL2 levels were measured using ELISA technique, while ALP, ALT, AST and TSP were measured using Spectrophotometric method. In the current study, 250 samples were taken, and the number of samples infected with the dysentery amoeba parasite was 120 samples, at a rate of 48%. The results of the current study showed a significant increase of P≤0.05, as it was found that there were significant differences in the group with diarrhea only without the presence of the dysentery amoeba parasite, where there was an increase in the levels of ALT, AST, ALP and globulin compared to the control group. It was also found that there were no significant differences in AST levels between the group infected with the dysentery amoeba parasite compared to the control group.

Keywords: chemokines, blood proteins, E.histolytica

1. Introduction

During his life, a person is exposed to many infections caused by parasites, and these parasites are: E.histolytica is a primary human intestinal parasite. There are several species of the genus Entamoeba parasite in humans. These include: Entamoeba histolytica, Entamoeba dispar, Entamoeba coli, Entamoeba hartmanii, Entamoeba polecki, and Entamoeba gingivalis. (Ash and orihel, 1980). Of these, only E. histolytica is a pathogen and the disease caused by amoebiasis is called amoebiasis or dysentery (Sateriale et al., 2011). It is an intestinal parasite that colonizes the lumen of the human intestine and has the ability to invade the epithelium. Amoebic dysentery occurs when it invades the walls of the intestine and multiplies in the mucosal layer, causing ulcers and the epithelial layer of the intestine.

The most common infections caused by the parasite E.histolytica are dysentery, colitis, flatulence, weight loss, fatigue and abdominal pain. A common consequence of amoeba invasion of tissues is liver abscesses that can be fatal and secrete the pathogen histolysin (1988, Luaces), which causes problems to the intestines of the affected individual hence the Latin name histo (tissue) Lytica (destroy) (Stanely, 2003).

Eating contaminated food or water containing pouches causes them to grow in the intestines. Each cyst produces eight numbers of active phase Trophozoite which colonize the host colon. In cases where the infection is not limited, amoebic dysentery and the formation of liver abscesses can occur. Ninety percent of injuries are asymptomatic and self-healing, and the scientist Sateriale and his group (2011) showed that there are about 50 million injuries annually. According to the World Health Organization report (2010) that amoebic dysentery ranks third among the most important parasitic diseases and 100,000 deaths occur annually due to the disease. Infection and deaths are observed primarily in
developing countries and the main cause of disease transmission is poor sanitation, especially with regard to food and water, in addition to ignorance and lack of access to clean food and drink due to frequent wars and floods, according to (AMREF, 2009), to the increased prevalence of diarrhea and infections globally. Interleukins (IL6, IL1 beta, IL2) in addition to their effect on the levels of chemokines in the body such as (CCL2, CCL1) and others.

2. Sample collection:
Samples were collected randomly from both sexes for the period from (12/8/2021) to (2/4/2022), where samples were taken from children who visit specialized doctors in outpatient clinics and health centers in the city of Samarra. Where 250 samples were collected to detect the presence of the parasite E.histolytica. Samples of the parasite E.histolytica were obtained from patients and patients in Samarra General Hospital who suffer from moderate to severe diarrhea and in most cases they suffer from bloody diarrhea. The samples were placed in sterile containers with wide openings fitted with a tight seal. To maintain the sample’s moisture and prevent its drying, the samples were examined within half an hour of their arrival in the laboratory, with care taken when examining blood or mucus-containing areas, as their presence often indicates an infection of the tissue-limiting amoeba parasite (Clark & Diamond, 2002).

3. Study design:
The study groups were divided into three groups: (the group with diarrhea 'group with the parasite Amoeba dysentery', the control groups) where the serum albumin concentration was measured and the concentration of chemokine receptor type 2 (CCR2) was calculated. The concentration of chemokine ligand 2 (CCL2) was calculated. Total serum protein (TSP) and globulin count

4. Statistical analysis
The results obtained from the current study were analyzed using the SPSS statistical program and Duncan's polynomial test was used to compare between four groups at the level of significance P≤0.05 to identify the degree of significant differences between the groups' averages (Al-Rawi, 1989).

5. Results and discussion
Samples were collected from patients coming to external laboratories, private clinics, and Samarra Hospital. And 250 samples were collected from patients. Table Epidemiological studies on the prevalence of parasitic intestinal infections in different regions usually aim to identify communities at risk and diseases that pose risks to human populations, which makes it necessary to conduct further studies on infections that threaten human health in all regions. around the world (Saida et al., 2016). Many environmental, biological, behavioral, social, economic and health factors influence parasitic infection directly or indirectly. The quality of city or village infrastructure, income, occupation, and level of education are also important aspects that affect the spread of infection, transmission of disease and mortality (Coulibaly et al., 2012). The total positive samples that were represented by individuals carrying E. Histolytica parasite 120 (48%) were the same It is illustrated in Figure (4-1), while the rest of the samples were considered negative for the disease. This result is consistent with that reported by (2016) Al-Daerchi & El-Ebrahim and Hamza and his group (2021) in Iraq and the year 2020 witnessed the lowest infection rate during the study period, which may have been due to the Corona Virus 2019 (COVID-) epidemic 19) in Iraq, which led people to fear hospitals and health centers.

Table (1) shows the percentage of samples examined for E. histolytica

<table>
<thead>
<tr>
<th>Total number of samples</th>
<th>The number of infected samples</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>120</td>
<td>%48</td>
</tr>
</tbody>
</table>
5.1. Effect of E. histolytica on the level of blood proteins:

Table (1) shows some biochemical parameters between the infected and the uninfected. There was a significant increase in globulin (0.616 ± 0.176) and albumin (6.456 ± 0.424) of the patients in comparison with the control group, as for the total protein values, there was no significant difference between the injured (6.423 ± 0.490) and the control group at a probability level, P < 0.01. In similar studies in Tikrit city (Muhsin, 2009) significantly lower level of total protein in serum and albumin was recorded, but in E. histolytica infected patients compared to control. In Erbil (Ahmed, 2004), there was no significant difference in serum total protein and albumin levels in the affected and uninfected groups (6.82 ± 0.59, 3.8 ± 0.3) (7.23 ± 0.4, 3.82 ± 0.27 g/dL), respectively.

The results of the study did not agree with the study of Mohammad and Kadir ((2011) in the town of Kala in northern Iraq, where the results of the two researchers showed that the level of globulin in the serum did not differ significantly between children infected with E. histolytica and uninfected children in the study. The blood serum contains large amounts of protein.

The two main groups of proteins in blood serum are albumin and globulin. The serum total protein test measures the total amount of protein in the blood serum as well as the amount of albumin and globulin. Because the total protein represents the sum of albumin and globulin. It is important to know which portion of protein is high or low of total protein. Low albumin may also be explained by poor nutrition or a low-protein diet. G. lamblia E. histolytica has been associated with the consequences of delirium that may result from poor nutrition in protein energy (Kadir & Mohammad, 2011).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patient Mean ±S.D</th>
<th>Diarrhea Mean ±S.D</th>
<th>Control Mean ±S.D</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Globulin</td>
<td>0.706±0.2737a</td>
<td>0.616±0176b</td>
<td>0.063±0.287c</td>
<td>0.0001</td>
</tr>
<tr>
<td>Albumin</td>
<td>6.774±0.554a</td>
<td>6.456±0.424b</td>
<td>0.5699±5.306c</td>
<td>0.0008</td>
</tr>
<tr>
<td>Total Protein</td>
<td>6.999±0.260a</td>
<td>6.423±0.490b</td>
<td>6.699±0.3286b</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Similar English letters indicate that there are no significant differences at a significant level (P<0.05).

5.2. Effect of the parasite E. histolytica on the level of chemokine ligands 2

The results in Table (2) showed that the concentration of chemokines in patients and patients was significantly higher than in the control group, (a 91.74 ± 9.13) and (79.24 ± 8.14 b) and (c 29.28 ± 4.04), respectively. The only classical first wave of host defense against pathogenic microorganisms but can lead to immunopathology if not adequately controlled (Shi & Pamer, et al., 2011). C–C chemokine ligand 2 (CCL2), which is secreted by many cells in infected or inflamed tissues, stimulates the exocytosis of classical CCR2+ monocytes from the bone marrow, allowing migration to sites of infection22. To date, it is unclear whether CCL2 directs classical monocytes directly to infected or inflamed sites in a concentration gradient-dependent manner (Winkler et al., 2018; Gschwandtner et al., 2019).

TNF stimulates CCL2 production by epithelial and endothelial cells; Thus infection is associated with high serum levels of TNF and CCL210,22. Another TNF-induced chemokine, chemokine (C-XC motif) ligand 1 (CXCL1), is also responsible for the recruitment of leukocytes to sites of inflammation (Griffith et al., 2014). CXCL1 binds to its receptor, CXCR2, and recruits neutrophils to sites of infection. (Lo et al., 2014) However, CXCR2 is also expressed by classical monocytes, indicating that monocytes are also recruited by sensing CXCL1 (Wang et al., 2016).
A previous study in a murine model infected with ALA showed that CCL2 participates in the immunopathology of ALA by recruiting tissue-destroying inflammatory monocytes (Helk et al., 2013) since men have higher monocyte counts than women (Lefevre, et al., 2012). An additional increase in the monocyte chemical CCL2 may indicate that men are more likely than women to recruit inflammatory tissue-destroying monocytes before E. histolytica spreads in the liver. Furthermore, the percentage of circulating mononuclear cells in the peripheral blood is higher in men than in women and male monocytes show a stronger innate immune response than that in females. However, once E. histolytica begins liver damage, CCL2 equally responsible for the recruitment of monocytes in both men and women. (Aulock et al., 2006).

Table 2: Effect of E. histolytica infection on CCL2 level.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patient Mean ±S.D</th>
<th>Diarrhea Mean ±S.D</th>
<th>Control Mean ±S.D</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemokine ligand 2</td>
<td>9.13±91.74 a</td>
<td>79.24±8.14 b</td>
<td>29.28±4.04 c</td>
<td>0.00004</td>
</tr>
</tbody>
</table>

Similar English letters indicate that there are no significant differences at a significant level (P<0.05).

5.3. Effect of the parasite E. histolytica on the level of chemokine receptor type 2:

The results in Table 3 showed that the concentration of chemokine 2 receptors in patients and patients was significantly higher than in the control group where it was (a 1.3891 ± 0.3067), (b 1.4521 ± 0.1443) and (0.8265 ± 0.05109 c), respectively. C-C chemokine receptor type 2 (CCR2 or CD192 differentiation cluster 192) is a protein that in humans is encoded by the CCR2 gene. CCR2 is a CC chemokine receptor.

In an observational study of white blood cell gene expression in humans, Harries et al. found evidence of a relationship between CCR2 expression and cognitive function (assessed using the Mini Mental State Examination, MMSE). (Harries et al., 2012).

Higher CCR2 expression was associated with worse performance in the MMSE assessment of cognitive function. The same study found that CCR2 expression was also associated with cognitive decline over 9 years in a sub analysis of inflammatory-related transcripts only. Harries et al suggest that CCR2 signaling may have a direct role in human cognition, in part because expression of CCR2 was associated with the ApoE haplotype (previously associated with Alzheimer's disease), but also because CCL2 is expressed at high concentrations in macrophages present in Atherosclerotic plaques and microglia in the brain (El-Khoury et al., 2007).

The difference in observations between mice (CCR2 depletion causes cognitive decline) and humans (high CCR2 associated with decreased cognitive function) could be due to the increased demand for macrophage activation during cognitive decline, associated with increased β-amyloid deposition (a key feature of Alzheimer's disease progression). With E. histolytica, the development of ALA cannot be explained solely by the invasion of E. histolytica activators alone, but primarily by an unbalanced innate immune response to the host. The appearance of ALA is characterized by the formation of a capsule of immune cells around the E. histolytica activators. This cellular organization is defined by monocytes and macrophages, which are mainly located next to the lysed tissue at the edge of the abscess. In the center can be found E. histolytica activators surrounded by neutrophils (Helk et al., 2013).

Due to initial tissue damage caused by invading parasites, liver-resident cells, eg KPVR cells begin to release cytokines such as CCL2 and TNF. CCL2 concentration is further increased by the initiation of the IL-23/IL-17 immune axis, (Noll et al., 2016) with IL-17 involved in neutrophil recruitment and modulation (Stark et al., 2005). CCL2 leads to the recruitment of CCR2-expressing Ly6Chi monocytes from the bone marrow to the site of infection. These monocytes in turn express TNF, which results in the expression of CXCL1 in an autocrine manner. CXCR2-expressing monocytes as well as neutrophils are attracted by CXCL1, thus promoting ALA-specific immunopathology. (Sellau et al., 2020).
Similar English letters indicate that there are no significant differences at a significant level (P<0.05).

References:

Table (3): Effect of E. histolytica infection on CCR2 level.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patient Mean ±S.D</th>
<th>Diarrhea Mean ±S.D</th>
<th>Control Mean ±S.D</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemokine Receptor type 2</td>
<td>1.3891±0.3067 a</td>
<td>1.4521±0.1443 b</td>
<td>0.8265±0.05109 c</td>
<td>0.00004</td>
</tr>
</tbody>
</table>

Table 3: Effect of E. histolytica infection on CCR2 level.