DOI:10.22059/IJVM.2024.374186.1005546 Iranian Journal of Veterinary Medicine Original Article

Online ISSN: 2252-0554`

Investigation of specific calprotectin and immunological markers linked with intestinal infections induced by the parasite *Entamoeba histolytica*

Hamssa M. Mustafa[1*] , Anas Abdullah Hamad[2], Osama A. Mohsein[3,4]

1- Department of Medical Laboratory Techniques, Shatra Technical College , Southern Technical University, Shatra, Thi-Qar, Iraq

2- Department of Microbiology, College of Veterinary Medicine, University of Fallujah, -Iraq.

3- Department of Medical Laboratory Techniques, Mazaya University Collage, Thi-Qar, Iraq.

4- Main Laboratory Unit, Thi-Qar Health Directorate, Al Habbobi Teaching Hospital, Thi-Qar, Iraq

Abstract

***Background**: Entamoeba histolytica is a parasitic organism that infects the intestines and causes dysentery, which is characterised by intestine inflammation.*

***Objectives:** Estimating calprotectin levels and their relationship to the severity and progression of intestinal inflammation*

***Methods**: Al-Habbobi Teaching Hospital conducted a cross-sectional study from March 10 to October 1, 2023. The sample included 50 men and 50 women aged 25–29, 30-34, and > 34. microscopy, direct wet method, formal-ether concentration approach to detect parasites, ELISA for Faecal Calprotectin FC and CRP, and XP-Sysmex for WBC.*

***Results**: The frequency Calprotectin levels below 50 were more common in the second age group. The third-age group had calprotectin values of 50. In the first age group (25–29), calprotectin frequency was similar. CRP levels were equal in the first age group. In the second age group (30–34 years), negative CRP outnumbered positive CRP. Positive CRP was more common than negative in the third age group (> 34). Above 35 years old had the highest GSE and antigen Ag detection rates, while 30–34 years old in the control group had the highest.*

***Conclusion**: Calprotectin levels less than 50 were more frequent in the age group of 30-34 years, while calprotectin levels greater than 50 were more frequent in the age group <35 years. CRP levels were equal in frequency in the first-age group. Negative CRP was more common than positive CRP in the second-age group. Positive CRP was more common than negative CRP in the third age group.*

Keywords: *Entamoeba histolytica*, C-reactive protein, Calprotectin, Gastroenteritis**,** *intestine inflammation*

1. Introduction

Entamoeba histolytica is a type of single-cell organism that is responsible for causing a disease called intestinal amebiasis. It can also lead to other health problems outside of the intestines. Most cases of *Entamoeba histolytica* infection do not show symptoms (Stanley, 2003; Aqeele et al., 2023). There are 50 million people who suffer from clear clinical symptoms, which leads to about 100,000 deaths annually (Mewara et al., 2023; Dhubyan et la., 2022). The infection occurs in countries characterised by poor sanitation and low economic standing (Stanley, 2003; Al-Samarrai et al., 2022). The prevalence of Entamoeba varies from one city to another. As results have shown in Thi-Qar governorate, of the 341,554 cases of intestinal parasitic infections, 38,004 (11.1%) individuals were recorded as having amoebiasis, which accounted for the highest proportion of infections in 2015 (26.1%) and the lowest in 2020 (8.1%). Amoebiasis was distributed among all age groups (Flaih et al., 2021). Research in Baghdad Governorate and the prevalence of Entamoeba histolytica have also shown that the prevalence of Entamoeba histolytica was 15.89% among 497 patients. The two stages of parasitism found in the same patient are 50.63%, while one stage was 49.36%. In relation to sex, the incidence was higher in males 51.89% than females 48.10% (Nayyef et al 2022). *E. histolytica* is commonly transmitted through the consumption of cysts present in contaminated food and water (Salit et al., 2009; Billet et al., 2019; Gosh et al., 2019; Hassan et al., 2022). It causes inflammation of the digestive system and, in advanced stages, can lead to liver abscesses **(**Stauffer and Ravdin, 2003**)**. It is still a major global health concern, ranking as the third-leading cause of death due to parasitic infections (Dezfouli et al., 2022). The virulent variant, *E. histolytica*, can cause amoebic colitis

and extraintestinal amoebiasis. *E. dispar* is considered nonpathogenic and does not cause disease symptoms (Haque et al., 2003; Shirley et al., 2018; Quail et al., 2009). In response to inflammatory bowel disease (IBD) pathogens, neutrophils produce faecal calprotectin FC antibodies. Because these antibodies bind to serum albumin and form antigen-antibody complexes, determining the serological evaluation of these antibodies is simple using an ELISA reader. An FC antibody level greater than 50 ng/mL indicates significant gastrointestinal damage, necessitating endoscopy to determine the agents responsible. Local inflammation also causes FC degranulation within the intestinal lumen (Shirley et al., 2018). Recently, faecal calprotectin has emerged as an important diagnostic tool in gastroenterology practices for distinguishing inflammatory bowel disease from other non-inflammatory conditions (Mewara et al., 2023). In addition, it can be used as an indicator of the activity of the disease and also the effectiveness of treatment, especially in cases of diarrhoea caused by parasitic sources (Van Rheenen et al., 2010; Spadafora et al., 2016; Hosseini et al., 2022). Amoebic colitis affects males and females equally, however, in different stages of life. In addition to the variety of stages of disease transmission, especially faecal matter, direct contact between healthy and sick people, and contamination of water and food, there are several risk factors that can lead to an increased incidence of severe infection and death, such as pregnancy, the use of corticosteroids, malignancy, and malnutrition (Van Rheenen et al., 2010). *Amoebic* liver abscess infections have a higher likelihood of occurring in middle-aged men, specifically those between the ages of 18 and 50, at least three times (Saidin et al., 2019; Garg et al., 2018). Faecal calprotectin FC is secreted by neutrophils and is a calcium- and zinc-binding protein. Calprotectin levels are measured in various types of bacterial, parasitic, and viral infections, in addition to colorectal

cancer and intestinal inflammatory diseases (Saidin et al., 2019). Measuring calprotectin levels in stool is used as a diagnostic marker for gastrointestinal diseases, especially intestinal infections. When there are harmful microorganisms that transport neutrophils to the cells of the digestive system, the neutrophils, in turn, secrete calprotectin, which leads to tissue damage (Garg et al., 2017). Patients with gastrointestinal symptoms suffer from severe diarrhoea, which is accompanied by increased intestinal permeability and, chlorine imbalance (Garg et al., 2017; Duman et al., 2015). The aim of the study is to estimate calprotectin and CRP levels and their relationship to the severity and progression of intestinal inflammation.

2. Materials and Methods

The study designs

A cross-sectional study that included 100 samples from people infected with *Entamoeba histolytica*. The samples were collected in the period between March 10, 2023, and October 1, 2023, in Alhabbobi Teaching Hospital, Thi-Qar, Al-Nasseriah, Iraq. Fifty males and fifty females were enrolled from three different age groups: 25–29, 30–34, and more than 34.

Laboratory tests

Macroscopic examination

The stool's consistency, colour, smell, and the presence of mucus or blood were all included in the macroscopic examination. The concentration technique (formal ether), wet mount smears, and iodine-stained smears were conducted.

Fecal calprotectin FC and CRP assay using ELISA test

ELISA was used to examine the levels of calprotectin in the faeces of the patient using the immunological principle of ELISA. The sandwich technique was used to determine the antibodies according to the working principle of the kit used.

Blood sample collection and processing

A total of 5 mL of blood was drawn from each participant; 3 mL of blood was placed in a gel tube; and 2 mL was placed in an EDTA tube. The first tube was left at room temperature for 10 minutes for the purpose of coagulating the blood; the serum was separated for the purpose of conducting the required tests; and the other tube was shaken well. After adding blood to prevent clotting, a CBC test was performed, and the number of white blood cells was counted using an automated haematology machine called XP-Sysmex. And identify the presence of *E. histolytica* antigen using a rapid test.

Ethical approval

Every parent of the patients who participated signed a written illustrative consent form. The ethical guidelines for medical research with patients outlined in the 1964 Declaration of Helsinki were followed in the conduct of this investigation. The ethics and research committee of the Department of Medical Laboratory Techniques at Shatra Technical College, Southern Technical University, gave its approval.

Statistical analysis

The statistical analysis of the data was done by the Statistical Package for the Social Sciences (SPSS) version 26 (IBM, SPSS Inc., USA). For parametric variables, an independent sample t-test was used, and a Mann-Whitney U-test was used for nonparametric variables. The correlation

between dependent variables was evaluated using Pearson's correlation coefficient analysis. The result was statistically significant at $P \leq 0.05$.

3. Results

The socio-demographic characteristics of study group

In **Table** 1, the results showed that there was no statistically significant difference in age among the group infected with *Entamoeba histolytica* compared to the control group ($P = 0.80$). While there was statistical significance in WBC, levels of WBC increased significantly in patients compared to the control group (P <0.001). The 95% confidence intervals of WBC in the control group were 6.25 ± 0.149**,** while in the patients group they were 10.88 ± 0.135.

Table 1. The relation between the ages and level of WBC for control and patient groups

Characteristic	Control group (n=100)	Patients group (n=100)	*P*-value
Age (year) Mean ± SD	32.37 ± 3.12	32.48 ± 3.19	0.80 ^{NS}
WBC Mean ± SD 95%confidence intervals	6.25 ± 0.76 (6.25 ± 0.149)	10.88 ± 0.69 (10.88 ± 0.135)	<0.001

NS: None significant

The frequency and percent of age among male and female, Clinical Sign and Symptoms and diagnosing of E. histolytica in GSE among to the study groups.

Regarding males, the proportion comprised 21 individuals within the age range of 25–29, accounting for 43.8% of the overall total. 47 individuals in the age group 30-34 accounted for 52.2%. The proportion comprised 32 individuals in the age category of 35 and older, accounting for 51.6% of the overall total. According to females, the percentage comprised 27 individuals in

the age group 25–29, which accounts for 56.3% of the total. 43 individuals in the age group 30-34 accounted for 47.8%. The percentage comprised 30 individuals within the age bracket of 35 and older, accounting for 48.4% of the total, as shown in **Table 2 and Figure 1**. The frequency of diarrhoea and abdominal pain was observed in 24 cases within the age group of 25–29, accounting for 50% of the total. Additionally, there were 44 cases within the age group of 30-34, representing 48.9% of the total. Furthermore, there were 32 cases in the age group of 35 and older. Constituting 51.6% of the entire amount. Regarding signs and symptoms that are not related to clinical conditions, there is a lack of observable indications and symptoms. 24 cases were in the age group 25–29, accounting for 50% of the total. 46 instances were observed in the age group of 30-34, accounting for 51.1% of the total. The proportion comprised 30 instances within the age category of 35 and older, accounting for 48.4% of the overall total **(Table 2 and Figure 2).** There were 24 cases of *E. histolytica cyst* formations in people aged 25–29, accounting for 50% of the total. 24 cases of people with normal formations in the same age group, accounting for 50% of the total. 44 cases of *E. histolytica* cyst formations occurred in people aged 30-34, accounting for 48.9% of the total. 46 cases in people with normal formations in the same age group, accounting for 51.1% of the total. 32 cases of E. histolytica cyst formations in people aged 35 and up account for 51.6% of the total. 30 cases in people with normal formations in the same age group, accounting for 48.4% of the total, **(Table 2 and Figure 3).**

Table 2. The relation between the ages and genders, Clinical Sign and Symptoms and diagnosing of E. histolytica in GSE

Parameters	**Sex**	
	Male	**Female**

		Frequency	Percent	Frequency	Percent
Age	25-29	21	43.8%	27	56.3%
	30-34	47	52.2%	43	47.8%
	35>	32	51.6%	30	48.4%
Parameters		Clinical Sign and Symptoms			
		Diarrhea and abdominal pain		Non clinical sign and symptom	
		Frequency	Percent	Frequency	Percent
Age	25-29	24	50%	24	50%
	30-34	44	48.9%	46	51.1%
	35>	32	51.6%	30	48.4%
Parameters		GSE			
		E. histolytica cysts		Normal	
		Frequency	Percent	Frequency	Percent
Age	25-29	24	50%	24	50%
	30-34	44	48.9%	46	51.1%
	35>	32	51.6%	30	48.4%

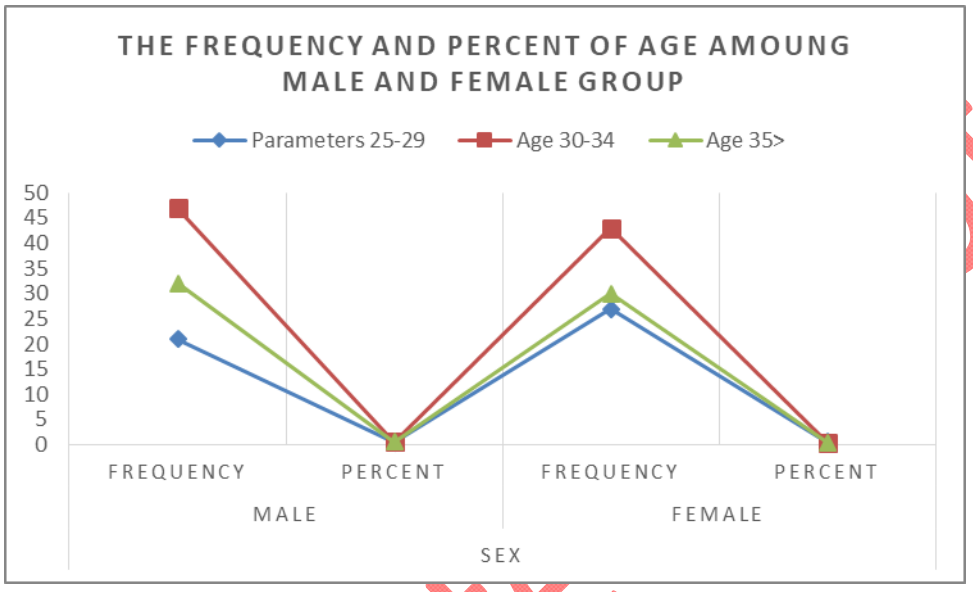

Figure 1. The frequency and percent of age among male and female group. The frequency and percent of clinical sign & symptoms among to the study groups

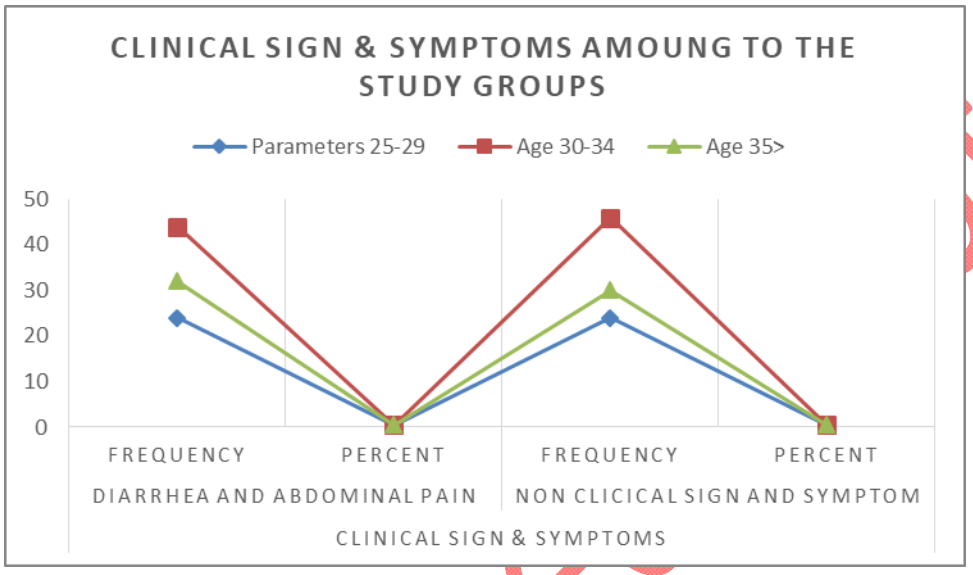

Figure 2. Signs and symptoms among the research groups

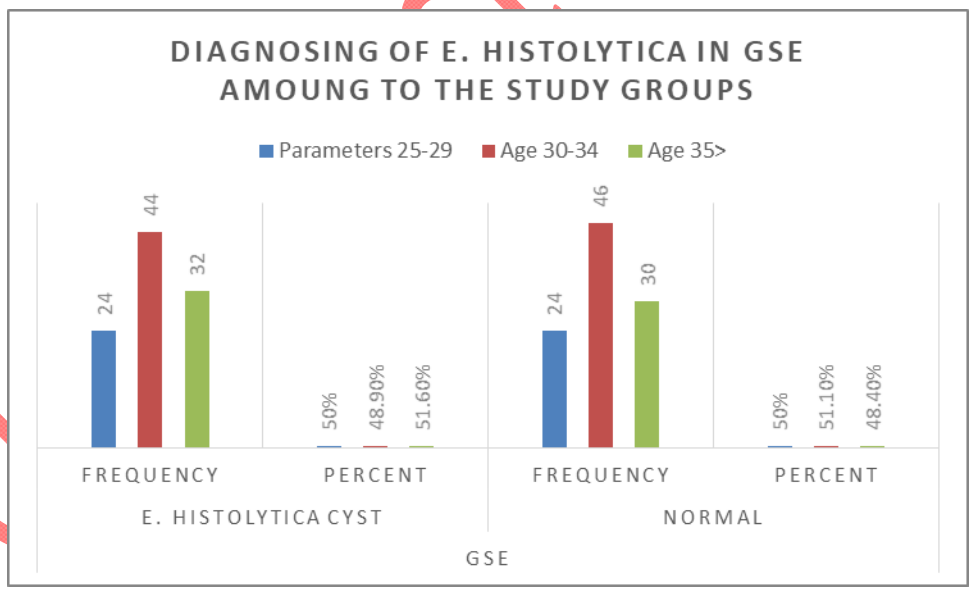

Figure 3. Detection of *E. histolytica* in GSE for the research groups

The frequency and percent of diagnosing of *E. histolytica* in immunology rapid test among to the study groups

In the 25-29 age group, there were 24 *E. histolytica* antigen Ag positive cases, representing 50% of the total. In the same age group, there were also 24 *E. histolytica* Ag negative cases, representing 50% of the total. In the 30-34 age group, there were 44 *E. histolytica* Ag positive cases, representing 48.9% of the total. In the same age group, there were also 46 *E. histolytica* Ag negative cases, representing 51.1% of the total. In the age group of 35 and older, there were 32 *E. histolytica* Ag-positive cases, representing 51.6% of the total. In the same age group, there were also 30 *E. histolytica* Ag negative cases, representing 48.4% of the total **(Table 3 and Figure 4).**

Table 3. The association between age and *E. histolytica* Ag infection

Parameters		***E. histolytica* Antigen**			
		Positive		**Negative**	
		Frequency	**Percent**	**Frequency**	**Percent**
Age	**25-29**	**24**	**50%**	**24**	**50%**
	30-34	**44**	**48.9%**	**46**	**51.1%**
	35>	**32**	**51.6%**	**30**	**48.4%**

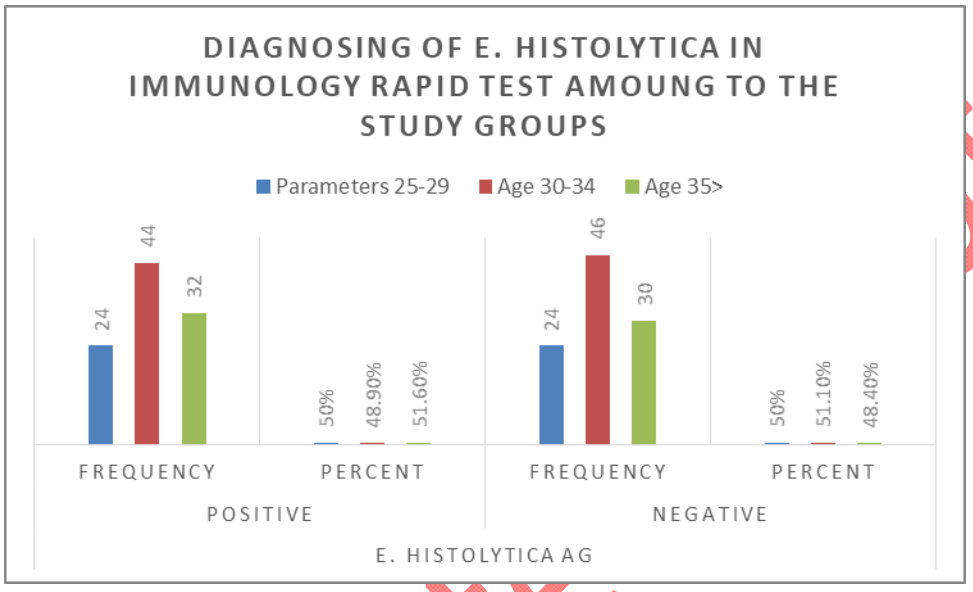

Figure 4. Identification of *E. histolytica* in immunology rapid test among research groups

The frequency and percent of calprotectin and CRP test among to the study groups

There were 24 cases with a calprotectin value of less than 50 in the 25–29 age group, accounting for 50% of the total. In the same age group, there were 24 cases with a calprotectin value greater than 50, accounting for half of the total. There were 46 cases with a calprotectin value of less than 50 in the 30-34 age group, accounting for 51.1% of the total. In the same age group, there were 44 cases with a calprotectin value greater than 50, accounting for 48.9% of the total. There were 30 cases with a calprotectin value of less than 50 in the age group of 35 and older, accounting for 48.4% of the total. In the same age group, there were 32 cases with a calprotectin value greater than 50, accounting for 51.6% of the total **(Table 4 and Figure 5)**. There were 24 cases with a positive CRP result in the age group of 25–29, accounting for 50% of the total. In the same age group, 24 cases had a negative CRP result, accounting for 50% of the total. There

were 44 cases with a positive CRP result in the 30-34 age group, accounting for 48.9% of the total. There were 46 cases with a negative CRP result in the same age group, accounting for 51.1% of the total. There were 32 cases with a positive CRP result in the age group of 35 and up, accounting for 51.6% of the total. In the same age group, 30 cases had a negative CRP result, accounting for 48.4% of the total **(Table 4 and Figure 6).**

Table 4. The correlation between calprotectin levels and age

Parameters		Calprotectin			
		Less than 50		More than 50	
		Frequency	Percent	Frequency	Percent
Age	25-29	24	50%	24	50%
	30-34	46	51.1%	44	48.9%
	35>	30	48.4%	32	51.6%
Parameters		CRP			
		Positive		Negative	
		Frequency	Percent	Frequency	Percent
Age	25-29	24	50%	24	50%
	30-34	44	48.9%	46	51.1%
	35>	32	51.6%	30	48.4%

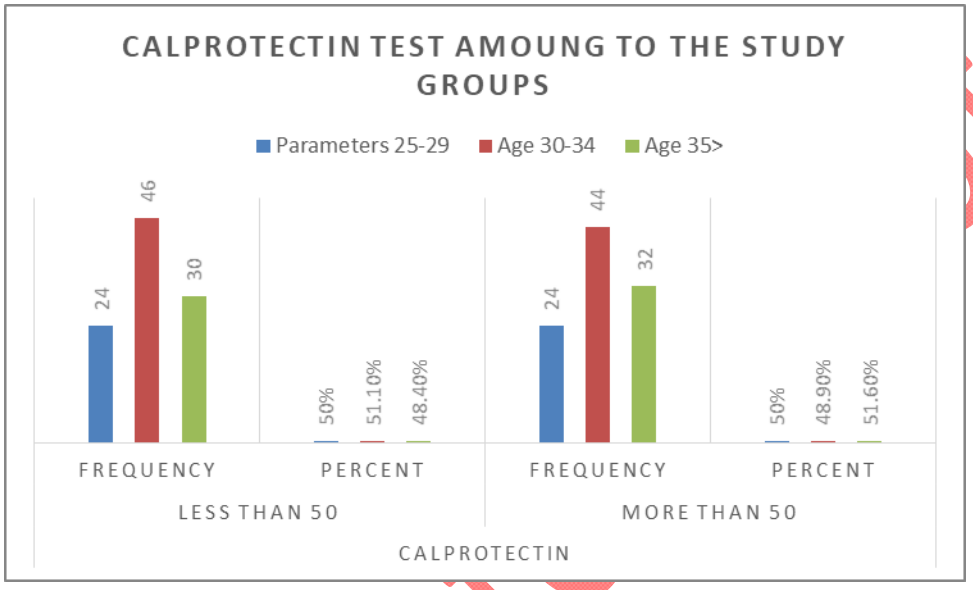

Figure 5. Testing for calprotectin in the research groups

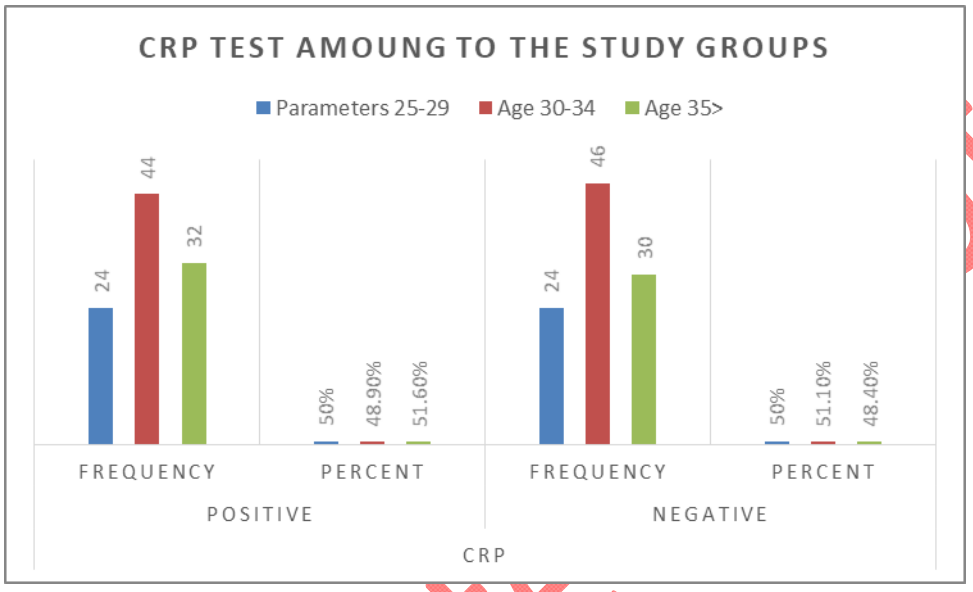

Figure 6. CRP test amongst the research groups

4. Discussion

For age, $P = 0.80$ indicates that there are no statistically significant differences on average or standard deviation between the two groups. Therefore, it can be believed that age does not have a significant impact on the other characteristics studied. For the number of white cells in blood (WBC), the value $P<0.001$ indicates statistically significant differences between the two groups. The average number of white cells in the patient population (10.88 ± 0.69) is markedly higher than the average of the control group (6.25 ± 0.76). This indicates an increase in the number of white cells in patients compared to healthy people. And that's because *E. histolytica* causes gastrointestinal infections, which leads to a high rate of white blood cells (Saidin et al., 2019). Data are provided in the form of frequency and percentage per age group for both clinical and non-custodial symptoms. For clinical symptoms, diarrhoea and abdominal pain were found in

50% of patients in each age group (25–29, 30-34, and more than 34 years). For non-clinical symptoms, the percentages show that there is no significant difference between the two groups. The percentage of non-clinical symptoms ranges from 48.4% to 51.6% in different age groups (Kantor et al., 2018). Colonic epithelial cells stick to trophozoites because of a certain galactose-N-acetyl galactosamine lectin, which leads to infection. When trophozoites adhere to colonic epithelial cells, they cause cytolysis and apoptosis, leading to the release of interleukin-1α and the precursor interleukin-1β. Distal cells produce cytokines and inflammatory mediators like COX-2, interleukin-1, and interleukin-8 when IL-1β activates NF-κB. Also, amoebic cysteine proteinases can convert precursor IL-1β to active IL-1β, enhancing the process (Kantor et al., 2018). Cytokines and inflammatory mediators attract neutrophils and macrophages. Direct contact with trophozoites damages neutrophils, which damages colonic epithelial cells and releases more mediators. TNFα and other mediators released by macrophages further contribute to inflammation (Stanley, 2003). Data were provided in the form of frequency and percentage per age group and each calprotectin level (less than 50 and higher than 50). It can be noted that there is an equal distribution of the calprotectin level among patients in different age groups. In each age group, 50% of patients have a calprotectin level below 50, and 50% of patients have a calprotectin level above 50. Thus, it can be said that the level of calprotectin does not differ significantly among the age groups included in the study. This indicates that the calprotectin level is not significantly affected by age and may be a strong indicator of intestinal inflammation, regardless of age group. Calprotectin levels were high in all people infected with *Entamoeba histolytica* compared to the control group. Calprotectin can affect the severity of inflammation because it is released by neutrophils and has an important role in the inflammatory immune

response in the body (Ali et al., 2018; Salman et al., 2017). The specific mechanisms that cause the occasional invasion of the mucosal epithelium by Eh are still not understood, and neither are the elements from the host-parasite relationship that contribute to this invasion. The prognosis of an invasive *Entamoeba histolytica* infection is unpredictable and might result in symptoms such as amebic diarrhoea, colitis, and/or the spread of parasites through the portal circulation, leading to the formation of hepatic abscesses. An invasion by *Entamoeba histolytica* causes a profound pro-inflammatory response and the destruction of host tissue, both of which exacerbate the illness. Currently, there is no vaccination that effectively counteracts this condition. However, parasites that live in tissues can be successfully treated using nitroimidazoles, such as metronidazole. Administration of metronidazole can lead to the emergence of severe side effects and an elevated susceptibility to drug resistance. Neutrophils, which are part of the host immune system, release significant amounts of the protein calprotectin near the infection site as a defensive response to kill the parasite. However, continued morbidity and mortality point out that this parasite is capable of escaping host defence responses to maintain its own survival. Thus, an understanding of the human immune response to the parasite and the strategies used by the parasite to evade host defence will deeply improve the development of effective immunotherapies (Salman et al., 2017). Calprotectin levels are high in people infected with Entamoeba histolytica, as well as other intestinal infections such as worms, because the mechanism by which calprotectin protein can increase is similar among all infections because parasitic intestinal infections lead to inflammation and damage to the intestinal tissue, which leads to increased levels of blood cells. White blood, especially neutrophils, raises calprotectin levels as a defence method to get rid of infection or disease developments (Ali et al., 2018).

There is no statistical significance in the percentage of positive and negative results between age groups. C-reactive protein levels were also high in all patients infected with Entamoeba histolytica compared to the control group. Due to the pathological condition caused by the parasite, in addition to inflammation, the body produces C-reactive protein as a first stage of innate immunity for the purpose of getting rid of the infection and protecting the damaged tissue. Therefore, CRP levels were higher in patients compared to the control group; these results are consistent with Saheb et al. **(**Saheb et al., 2020**)**. One study also found that C-reactive protein levels are high in patients infected with both *Giardia lamblia and Entamoeba histolytica*, which makes it a non-specific immunological marker to evaluate clinical symptoms during infection. In reaction to interleukin 6, the liver produces the pentameric protein kwon as a C-reactive protein during inflammation. When inflammation and infection are at their most severe, they are released (Saheb et al., 2020). As soon as membrane-associated macrophages get activated during inflammation, particularly when infections like *Entamoeba histolytica* are present, C-reactive protein is produced due to this process (Tinuade et al., 2006; Haque, 2007). There are a number of reasons why the C-reactive protein levels could be low, but the amount of antigen or the causative agent that enters the body is the primary one (Tinuade et al., 2006).

The liver releases C-reactive protein in response to intestinal parasite attachment to the intestinal wall or membrane. This protein then combines with macrophages and mucosal immunoglobulin A to combat infection. Thus, CRP levels significantly rise during the acute phase of *G. lamblia* and *E. histolytica*-related diarrhoea (Saheb et al., 2020)**.** It can be believed that age does not have a significant impact on the other characteristics studied. The increased number of WBC may be in relation to the defence mechanism and immunological responses against parasites (Safi., 2012;

Macy et al., 1997). In addition to intestinal parasitic infections that lead to increased levels of calprotectin, other parasitic infections, especially Helminth, can lead to increased release of the protein calprotectin. Beyond IBD, infections are another significant contributor to elevated calprotectin levels. These include bacterial infections like Salmonella and Clostridioides difficile, which often lead to much higher calprotectin levels than viral infections such as rotavirus or norovirus. Infection with some parasite protozoa, such as Dientamoeba fragilis, can lead to the infiltration of neutrophils in the intestines, resulting in an elevation of faecal calprotectin levels. Faecal calprotectin is a chemical associated with inflammation. Levels of faecal calprotectin were higher in both adults and children with acute bacterial diarrhoea compared to viral diarrhoea. Furthermore, the severity of the diarrhoea was directly proportional to the increase in these levels.

5. Conclusion

It can be believed that age does not have a significant impact on the other characteristics studied. The number of white cells in the patient population was significantly higher than the control group, indicating an increase in white cells due to gastrointestinal infections. The study also found that calprotectin levels were high in all age groups infected with *E. histolytica* compared to the control group. The CRP test results were high in all patients infected with *E. histolytica* compared to the healthy, uninfected control group. Suggesting that gastrointestinal tract inflammation caused by *Entamoeba histolytica* has an effective role in immune responses and high CRP levels. To further investigates the role of immune responses and their relevance to inflammation resulting from infection with *E. histolytica*, the observed impact of this infection

on CRP and calprotectin levels. These studies can be expanded to understand the biochemical and cellular mechanisms that drive these processes and how these elements interact with various factors such as diet, overall body health, and environmental factors.

Abbreviations

CRP: C-Reactive Protein; GSE: General Stool Examination; Ag: Antigen; IBD: Inflammatory Bowel Disease; FC: Faecal Calprotectin; ELISA: enzyme-linked immunosorbent assay; EDTA: thylenediaminetetraacetic acid; CBC: complete blood count; WBC: white blood count; IL-1β: Interleukin 1 beta; COX-2: Cyclooxygenase-2 inhibitor; NF-κB: Nuclear Factor Kappa B; TNFα: Tumour Necrosis Factor Alpha; IgA: Immunoglobulin A.

-Consent for publication Informed written consent was obtained from all the study participants.

-Competing interests The authors declare that they have no competing interests

-Funding No funds were received to fulfil this work.

-Acknowledgements: We would like to thank the environmental health units' workers in the teaching hospital of the southern governorates, and we are most grateful to the laboratory staff of the department for protecting and improving the environment in the southern governorates. We express our gratitude to the College of Veterinary Medicine at the University of Fallujah for providing the motivation to do this research.

References

Ali, O.S., Mohammad, S.A. and Salman, Y.J., (2018). Relationship between Entamoeba histolytica and Fecal Calprotectin in Patients with Gastroenteritis in Kirkuk City-Iraq. Egyptian Journal of Medical Microbiology, 27(2), 49-56. https://doi.org/10.21608/ejmm.2018.285525

Al-Samarrai, A. S. M., Al-Samarrai, R. R. H., & Hamdi, B. I. (2022). An Investigation of Parasitic Protozoa in Drinking Water in Samarra, Iraq. Volume 77(2), 821-825. https://doi.org/10.22092/ARI.2022.357106.1977

Aqeele, G., Hraija, B. A., & Kadhim, D. K. (2023). Molecular-Genotyping Detection of Entamoeba histolytica in Diarrheic Patients. Volume 78(1), 337-343. https://doi.org/10.22092/ARI.2022.358947.2336

Billet, A.C., Rousseau, A.S., Piroth, L. and Martins, C., (2019). An underestimated sexually transmitted infection: amoebiasis. BMJ Case Reports CP, 12(5), e228942. https://doi.org/10.1136/bcr-2018-228942. PMCID: PMC6536245.

de Gier, B., Pita-Rodríguez, G. M., Campos-Ponce, M., van de Bor, M., Chamnan, C., Junco-Díaz, R., ... & Wieringa, F. T. (2018). Soil-transmitted helminth infections and intestinal and systemic inflammation in schoolchildren. Acta tropica, 182, 124-127. https://doi.org/10.1016/j.actatropica.2018.02.028

Dezfouli, Mohammad Reza Mokhber, et al. "Protection of Calves Against Cryptosporidiosis by Hyperimmunization of Pregnant Cattle Colostrum using Oocyst Whole Antigens." Iranian Journal of Veterinary Medicine 16.4 (2022). D10.22059/IJVM.2021.315021.1005144

Dhubyan, Z. M. Z. (2022). Prevalence of Entamoeba histolytica and Giardia Lamblia Associated with Diarrhea in Children referring to Ibn Al-Atheer Hospital in Mosul, Iraq. Volume 77(1), 73-79. https://doi.org/10.22092/ARI.2021.356312.1820

Duman, M., Gencpinar, P., Biçmen, M., Arslan, N., Özden, Ö., Üzüm, Ö., Çelik, D., Sayıner, A.A. and Gülay, Z., (2015). Fecal calprotectin: can be used to distinguish between bacterial and viral gastroenteritis in children. The American journal of emergency medicine, 33(10),1436-1439. https://doi.org/10.1016/j.ajem.2015.07.007.PMID: 26233616.

Flaih MH, Khazaal RM, Kadhim MK, Hussein KR, Alhamadani FAB. The epidemiology of amoebiasis in Thi-Qar Province, Iraq (2015-2020): differentiation of Entamoeba histolytica and Entamoeba dispar using nested and real-time polymerase chain reaction. Epidemiol Health. 2021;43:e2021034. doi: 10.4178/epih.e2021034.

Garg, M., Leach, S.T., Coffey, M.J., Katz, T., Strachan, R., Pang, T., Needham, B., Lui, K., Ali, F., Day, A.S. and Appleton, L., (2017). Age-dependent variation of fecal calprotectin in cystic fibrosis and healthy children. Journal of Cystic Fibrosis, 16(5),631-636. https://doi.org/10.1016/j.jcf.2017.03.010. PMID: 28416415.

Garg, M., Leach, S.T., Pang, T., Needham, B., Coffey, M.J., Katz, T., Strachan, R., Widger, J., Field, P., Belessis, Y. and Chuang, S., (2018). Age-related levels of fecal M2-pyruvate kinase in children with cystic fibrosis and healthy children 0 to 10 years old. Journal of Cystic Fibrosis, 17(1),109-113. https://doi.org/10.1016/j.jcf.2017.07.011. PMID: 28754328.

Ghosh, S., Padalia, J. and Moonah, S., (2019). Tissue destruction caused by Entamoeba histolytica parasite: cell death, inflammation, invasion, and the gut microbiome. Current clinical microbiology reports, 6: 51-57. Current Clinical Microbiology Reports, 6,51–57 https://doi.org/10.1007/s40588-019-0113-6. PMCID: PMC6449278.

Haque, R., (2007). Human intestinal parasites. Journal of health, population, and nutrition, 25(4),387. PMID: 18402180.

Haque, R., Huston, C.D., Hughes, M., Houpt, E. and Petri, W.A., (2003). Entamoeba histolytica. N Engl J Med, 348,1565-73. DOI: 10.1056/NEJMra022710. PMID: 12700377.

Hassan, Nuzhat, Charanjit Singh Randhawa, and Deepti Narang. "Diagnostic Workup in Relation to Odds of Clinico-Fecal Predictors in Adult Dairy Cattle and Buffalo with Chronic Diarrhea." Iranian Journal of Veterinary Medicine 16.4 (2022). DOI: 10.22059/IJVM.2022.340527.1005257

Hosseini, Mona, et al. "Morpho-Molecular DOI:Characterization of Cattle Haemonchus Nematodes From Southeast of Iran." Iranian Journal of Veterinary Medicine 16.4 (2022). DOI: 10.22059/IJVM.2022.318533.1005157

Kantor, M., Abrantes, A., Estevez, A., Schiller, A., Torrent, J., Gascon, J., Hernandez, R. and Ochner, C., (2018). Entamoeba histolytica: updates in clinical manifestation, pathogenesis, and vaccine development. Canadian Journal of Gastroenterology and Hepatology, 2018. https://doi.org/10.1155/2018/4601420. PMID: 30631758.

Macy, E.M., Hayes, T.E. and Tracy, R.P., (1997). Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clinical chemistry, 43(1),52-58. PMID: 8990222.

Mewara, A., Khunger, S., Sharma, C., Krishnamoorthi, S., Singh, S., Yadav, R., ... & Sehgal, R. (2023). A rapid multiplex loop-mediated isothermal amplification (mLAMP) assay for detection of Entamoeba histolytica and Giardia duodenalis. Letters in Applied Microbiology, 76(10), ovad114. https://doi.org/10.1093/lambio/ovad114

Nayyef, H. J., Abo-Alhur, F. J., Mohammed, S. W., Taqi, E. A., & Kahdim, S. S. (2022, January). Prevalence of Entamoeba histolytica among enteric infection in Al-Furat general hospital in Baghdad/Iraq. In AIP Conference Proceedings (Vol. 2386, No. 1). AIP Publishing. https://doi.org/10.1063/5.0066967

Quail, M.A., Russell, R.K., Van Limbergen, J.E., Rogers, P., Drummond, H.E., Wilson, D.C. and Gillett, P.M., (2009). Fecal calprotectin complements routine laboratory investigations in diagnosing childhood inflammatory bowel disease. Inflammatory bowel diseases, 15(5),756-759. https://doi.org/10.1002/ibd.20820. PMID: 19107785.

Safi, S., (2012). Acute phase proteins-analysis, clinical applications and potentials. Inflammatory Diseases-Immunopathology, Clinical and Pharmacological Bases, pp.351-381.

Saheb, E. J., Kuba, R. H., & Musa, I. S. (2020). The Role of C-Reactive Protein in the Infections Caused by Parasites. DOI:https://doi.org/10.26505/DJM.19025550828

Saheb, E. J., Kuba, R. H., & Musa, I. S. (2020). The Role of C-Reactive Protein in the Infections Caused by Parasites. http://148.72.244.84:8080/xmlui/handle/xmlui/5617

Saidin, S., Othman, N. and Noordin, R., (2019). Update on laboratory diagnosis of amoebiasis. European Journal of Clinical Microbiology & Infectious Diseases, 38:15-38. European Journal of Clinical Microbiology & Infectious Diseases, 38,15–38.https://doi.org/10.1007/s10096-018-3379-3. PMID: 30255429.

Salit, I.E., Khairnar, K., Gough, K. and Pillai, D.R., (2009). A possible cluster of sexually transmitted Entamoeba histolytica: genetic analysis of a highly virulent strain. Clinical Infectious Diseases, 49(3),346-353. https://doi.org/10.1086/600298; PMID: 19580413.

Salman, Y.J., Ali, C.A. and Razaq, A.A.A., (2017). Fecal calprotectin among patients infected with some protozoan infections. Int J Curr Microbiol App Sci, 6(6),3258-3274.PMID: 19640387

Shirley, D.A.T., Farr, L., Watanabe, K. and Moonah, S., (2018). A review of the global burden, new diagnostics, and current therapeutics for amebiasis. In Open forum infectious diseases, 5(7),161. https://doi.org/10.1093/ofid/ofy161. PMCID: PMC6055529.

Spadafora, L.J., Kearney, M.R., Siddique, A., Ali, I.K., Gilchrist, C.A., Arju, T., Hoffstrom, B., Nguyen, F.K., Petri Jr, W.A., Haque, R. and Cangelosi, G.A., (2016). Species-specific immunodetection of an Entamoeba histolytica cyst wall protein. PLoS neglected tropical diseases, 10(5),e0004697. https://doi.org/10.1371/journal.pntd.0004697. PMCID: PMC4859568.

Stanley, S.L., 2003. Amoebiasis. The lancet, 361(9362),1025-1034. https://doi.org/10.1016/S0140-6736(03)12830-9. PMID: 12660071.

Stauffer, W. and Ravdin, J.I., (2003). Entamoeba histolytica: an update. Current opinion in infectious diseases, 16(5),479-485. doi: 10.1097/00001432-200310000-00016. PMID: 14502002. PMID: 14502002.

Tinuade, O., John, O., Saheed, O., Oyeku, O., Fidelis, N. and Olabisi, D., (2006). Parasitic etiology of childhood diarrhea. The Indian Journal of Pediatrics, 73,1081-1084. https://doi.org/10.1007/BF02763049. PMID: 17202634.

Van Rheenen, P.F., Van de Vijver, E. and Fidler, V., (2010). Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. Bmj, 341. https://doi.org/10.1136/bmj.c3369.