Biomarker Profiling of Calprotectin and Lactoferrin in Children Under Five years with Protozoal Intestinal Infections in Kirkuk

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Abstract

Background & Aim: Intestinal protozoal infections are prevalent intestinal infection and common infectious disease causing many health problems and impaired growth and physical development. So, the present investigation was aimed to evaluate some inflammatory biomarkers among children under 5 years of age infected with intestinal protozoal infection. **Materials and Methods:** A 260 children under 5 years of age whom attended a governmental hospital were included in the study, after receiving an agreement from their parents before starting the study. Across sectional study was done on 260 children from period starting from 1/9/2024 till end of 1/2/2025 in Kirkuk city Iraq. **Results:** The results showed that the number of positive samples for parasitological detection assays was 160 samples, while the number of negative samples was 100 samples. The findings showed a relationship between age and the type of parasite, as it was found that the highest infections were with the *E. histolytica* parasite, especially in the age group (4-5) years, which showed significant ($P \leq 0.05$) differences with the rest of the parasite types in the different age groups. While regarding gender, there was no significant difference in the proportion of each parasite between males and females. Regarding residency, a significant ($P \leq 0.05$) difference was found (51.25%), with the most cases being rural disease, and the most prevalent parasite was E. histolytica, accounting for 51.25% of the total cases. The same was observed with respect to the type of water consumed, with the highest incidence of intestinal protozoal infection especially *E. histolytica* and *C.parvum* being found among patients who consumed tap water. A similar pattern was evident in relation to the type of nutrition, with the highest incidence of *E. histolytica* in the bulky food group. Finally, regarding hospitalization, the highest incidence was *E. histolytica*, which reached 35%, which showed significant ($P \leq 0.05$) differences for the rest of the detected parasite species. Among all single infections of intestinal protozoa, *E. histolytica* showed the highest calprotectin levels (6.7130 ± 2.91 ng/ml) and elevated morphonuclear (6.06 ± 3.0 cells/µL) and lymphocyte counts (4.3 ± 2.0), with reduced Hb (11.0 ± 1.6 g/dL). *C. parvum* showed lower levels of calprotectin (4.34 ± 1.31) but relatively high lactoferrin levels (4.68 ± 2.62) and reduced morphonuclear and lymphocyte counts. For *Giardia lamblia* infections presented the lowest inflammatory biomarker levels, especially calprotectin (4.38 ± 1.21) and lactoferrin (3.63 ± 0.91), showed that 112 patients with fever, 52 with bloody diarrhea, and 64 with dehydration had a positive calprotecrtin result, while 110 patients with fever, 44 with bloody diarrhea, and 70 with dehydration had a positive lactoferrin result. The patients with positive results for the fecal occult blood test showed significant differences ($p<0.05$) compared to those who showed negative results for the fecal occult blood test. **Conclusion:** This comparative analysis underscores the diagnostic utility of fecal biomarkers in children under 5 years the intestinal protozoal infections and highlights the distinct inflammatory profiles elicited by different protozoa. Future research should focus on integrating these biomarkers into routine screening to enhance early detection and optimize treatment approaches, especially in endemic areas.

Keywords: Inflammatory Biomarker, Calprotectin, Lactoferrin, Fecal Occult Blood, Intestinal Protozoa, Infection, 5 Years of Age.

Introduction

Intestinal protozoal infections are among the most common infectious disease especially in developing countries with poor hygienic conditions and one of the main causes of health problems worldwide including diarrhea, weakness, abdominal pain, malaise and growth retardation. Most common intestinal protozoa include *Entamoeba histolytica*, *Blastocystis hominis, Giardia lambilia* and *Cryptosporidium parvum*.[1] Children under 5 years are at high risk for

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intestinal protozoal infections and they often undergo reinfection due to immature immune systems. Development of the immune system in children, innate Immunity is the first line of defense, phagocytic cells (neutrophils, macrophages), natural killer cells, and the complement system. In children under 5 years, innate immunity is immature, neutrophil function, complement levels, and cytokine responses are reduced. Cell-mediated immunity, T-cell responses are underdeveloped in early life, with a bias toward Th 2 responses, reducing the ability to clear intracellular pathogens like protozoa.[2] Children under five years old are said to be in their "golden years" and requires special attention due to immune system is still developing and is not yet fully developed.[3]

Intestinal protozoae has two stage, infective stage (cyst) and vegetative stage (trophozoite). Infection results by ingestion cyst. The cyst of intestinal protozoa invasion the intestine followed by tissue damage and inflammation. During this invasive process, intestinal protozoa kill and phagocytose epithelial cells ,immune cells and erythrocytes.[4] Although in intestinal tract the mucosal layer usually works as a prime physical barrier in contradiction to pathogens of the intestine, the immune response to the intestine is the secondary protection against intestinal protozoal infection. [5] Inflammatory biomarkers play significant role in the immune system which are evaluated for assessing intestinal protozoal infection. Inflammatory biomarkers used in this study are CALP (Calprotectin) and Ltf/Lf (Lactoferrin). Calprotectin, a 36-kDa protein and a member of the S100 family, comprises calcium binding proteins. Calprotectin, primarily derived from neutrophils and macrophages, serves as a potent neutrophil chemotactic factor with inherent anti-inflammatory and antimicrobial properties.[6] This protein is suggested as a marker for primary screening and follow up of intestinal protozoal infection in children.[7] Lactoferrin is an iron- binding nonheme glycoprotein. In mucosa, Lf displays antimicrobial activity against a wide range of pathogen. Lf in feces is also due to the neutrophils action and its concentration increase in intestinal protozoal infection.[8] Finally to distinguish between invasive protozoa (e.g., E. histolytica) and non-invasive ones like *Entamoeba dispar*, *Giardia lamblia,* or *Cryptosporidium parvum,* used immunochromatography tests, Fecal Occult Blood (FOB), FOB refers to a nonvisible blood in the stool.[9] The current study was aimed to evaluate some inflammatory biomarkers among children under 5 years of age infected with intestinal protozoal infection.

Materials and Methods

Study Population

A total of 260 children under 5 years of age were included in this cross –sectional study after obtaining informed consent from their parents prior to participation. The study was conducted between September 1, 2024 till the end of February 1, 2025.

Sample Collection and Technique Tests

A representative sampling was taken from Pediatric Hospital, General Kirkuk Hospital, Azadi Teaching Hospital as showed in the table below:

Hospital	Total no. of Patients = 260	No. %
1. Azadi Teaching Hospital	18	6.9%
2.Pediatric Hospital	212	81.5%
3.General Kirkuk Hospital	30	11.5%

Different sample was obtained (blood, stool) to detect protozoal infection by performing the following tests:

- **Wet preparation:** Fresh stool samples (2 mg of stool) were put on a slide with a wooden applicator stick, the stool was emulsified with a drop of physiological saline (0.85%) for diarrheic and semi-solid samples. For formed stools, iodine was used. Then, they were covered with a cover slide and examined under a microscope using first 10x objectives and then 40x objectives.[10]
- **Modified Ziehl Neelsen Technique (hot technique):** fecal samples were analyzed utilizing the adapted modified Ziehl-Neelson staining procedure under a light microscope. Cryptosporidium oocysts appear circular, spherical, small, pink-red color and the rest of the stool is blue or green.[11]
- **Enzyme - linked immunesorbent assay (ELISA):** fecal lactoferrin and calprotectin concentrations were determined uses the Sandwich-ELISA. The preserved stool (0.1g) in potassium dichromate solution are first homogenised with (0.9 ml) phosphate buffer saline PH 7.4, then centrifuged for 20 min at (2000-3000) rpm. Supernatants were then used in the ELISA[12,13] which was carried out using the Human Lactoferrin (FLTF) (SUNLONG BIOTECH, China) and calprotectin (SUNLONG BIOTECH, China) ELISA Test Kits according to the manufacturer's instructions.
- **Fecal Occult Blood (FOB):** Rapid Test Cassette (feces) BIOZEK medical, following the manufacturers instruction, a small amount of preserved fecesin potassium dichromate solution was homogenized in a liquid buffer. Two drops of stool suspension were applied to a test cassette and results were visually read after 5 min and categorized as positive (+), negative (-), trace (+,-).[14]

Statistical analysis

The data collected in this study were analyzed using Statistical Package for the Social Sciences (SPSS) program. One way ANOVA and Chi- square test were applied and a p-value is regarded as a significant of (0.05).

Result and Discussion

Table 1 shows the number of participants according to gender and result. It shows that the number of males was 115(44.2%) and the number of females was 145(55.8%) and three were non-significant (P≤0.05) differences. Table 2 shows the distributive of samples according to result, it was found that the number of positive samples was 160 samples, while the number of negative samples was 100 samples and three were non-significant (P≤0.05) differences between the results.

Table 1: Distributive of Study Samples According to Gender.

Gender	No.	%
Male	115	44.2%
Female	145	55.8%
Total	260	100%
P value	0.184	

Table 2: Distributive of Study Samples according to Results of Parasite Detection Techniques.

Result	No.	%
Positive for intestinal protozoa infection	160	61.5%
Negative for intestinal protozoa infection	100	38.5%
Total	260	100%
P value	0.071	

Table 3: Demographic Characteristic of Positive Study Subjects, (260) samples Examined by Wet Preparation and Modified Ziehl Neelsen Stain (M.Z.N) Hot Technique.

Demographic Parameters			Intestinal Protozoa*				P value
			E.histolytica	*G.lambilia*	*C.parvum*	*B.hominis*	
	Mean± SD		30 ± 12,25	3,33 ± 3,4	8 ± 7,5	12 ± 9,4	
Ages	(0-1) years		15(9.3%)	0(0%)	0(0%)	3(1,8%)	0.001
	(2-3) years		30(18,75%)	2(1,25%)	6(3,75%)	8(5%)	
	(4-5) years		45(28,12%)	8(5%)	18(11,25%)	25(15,6%)	
Sex	Male		35(21,8%)	2(1,25%)	15(9,37%)	16(10%)	0.094
	Female		55(34,3%)	8(5%)	9(5,6%)	20(12,5%)	
Residency	Rural		82(51.25%)	7(4.4%)	21(13.13%)	31(19.4%)	0.001
	Urban		8(5%)	3(1.9%)	3(1.875%)	5(3.13%)	
Type of consumed water	tap water		85(53%)	10(6,25%)	21(13,13%)	34(21,3%)	0.001
	filtered water		5(3,12%)	0(0%)	2(1,25%)	3(1,8%)	
Type of nutrition	Feeding (Bulky food)		55(34,3%)	10(6,25%)	20(12,5%)	30(18,75%)	
	Milk	breast feeding	2(1,25%)	0(0%)	0(0%)	1(0,625%)	0.001
		bottle feeding	33(20,6%)	0(0%)	4(2,5%)	5(3,12%)	
Hospitalization			56(35%)	4(2.5%)	17(10.63%)	19(11.875%)	0.001
Total			90(56,3%)	10(6,3%)	24(15%)	36(23%)	

*prevalence of protozoal infection among the studied sample = No. of Protozoal Infection/Total * 1000 .

This study examined the demographic characteristics and associated risk factors for intestinal protozoal infections, specifically *Entamoeba histolytica, Giardia lamblia, Cryptosporidium parvum, and Blastocystis hominis*, among children under five years of age. The results demonstrate significant variation in prevalence patterns according to age, sex, residency, type of water consumed, nutritional habits, and hospitalization history. The highest prevalence was recorded for *E. histolytica* (56.3%), followed by *B. hominis* (23%), *C. parvum* (15%), and *G. lamblia* (6.3%). These findings align with previous literature indicating that *E. histolytica* remains a leading protozoal pathogen in developing countries due to its wide environmental distribution and poor sanitation conditions.[15]

Age Distribution: The majority of infections were identified in children aged 4–5 years, particularly for *E. histolytica* (28.1%) and *B. hominis* (15.6%). This may be attributed to increased environmental exposure, poor personal hygiene practices at this age.The study recorded in Jassim *et al.*[16] the highest infection rate of *Giardia lambilia* was 5.8% in the age group 2-4 years. Notably, younger children (0–1 years) exhibited the lowest infection rates, possibly due to higher rates of breastfeeding and parental supervision as had seen in interested study. While sex differences, a slight female predominance was observed in *E. histolytica* and *G. lamblia* infections, while male children were more affected by *C. parvum* and *B. hominis.* These differences may reflect gender-based behavioral patterns or differences in immune responses, though the variations were not substantial enough to suggest a strong sex-linked susceptibility.[17]

Residency and Environmental Exposure; Children residing in rural areas showed significantly higher infection rates compared to those in urban settings, especially for *E. histolytica* (51.25%) and *B. hominis* (19.4%). This highlights the role of environmental contamination, lack of access to safe water, and inadequate sanitation infrastructure in rural communities. Corresponding water sources; tap water was the most common source of drinking water among infected children, particularly in *E. histolytica* (53%) and *B. hominis* (20.6%) cases. The minimal infection rates among children consuming filtered water suggest that waterborne transmission is a critical pathway for these protozoa. Contaminated or improperly treated tap water likely serves as a reservoir for cysts and oocysts.[18] In recent studies; infections were more prevalent among children consuming bulky foods compared to those exclusively breastfed. Breastfeeding appeared to offer a protective effect, as evidenced by the minimal infection rates among this group. Bottle-fed children had a higher prevalence of *E. histolytica* and *C. parvum*, possibly due to contamination of feeding equipment or preparation with unsafe water. These findings are consistent with prior research emphasizing the protective role of breastfeeding against gastrointestinal infections.[19] Hospitalization; a considerable proportion of infected children required hospitalization, particularly those infected with *E. histolytica* (35%) and *B. hominis* (11.9%). This underscores the clinical significance of protozoal infections in young children, which can lead to serious outcomes such as dehydration, anemia, and malnutrition if not promptly managed.[20] As showed in table (4), This study analyzed the concentrations of fecal calprotectin and

fecal lactoferrin , immune cell profiles (morphonuclear cells, lymphocytes, macrophages), and hemoglobin (Hb) levels that analyzed whole blood (EDTA blood) which performed by auto-analyzer Swelab Alfa Plus Standard hematology analyzer among children under five years of age with single or mixed intestinal protozoal infections.[21]

Table 4: Association between Calprotectin and Lactoferrin Levels with some Immune Cells Levels in Children Undere 5 Years Infected with Intestinal Protozoal Infection (total positive No. =160).

Type of Parasiteinfection Total Positive No.160	Cal. 3-200ng/ml (Mean ± SD)	Lact. 0.5-40ng/ml (mean± SD)	Frequency of Cal.	Frequency of Lact.	Morphonuclear 1.2-8.0(cells/µL) (Mean ± SD)	Lymph.con. 0.9-5.0 (cells/µL) (Mean ± SD)	Macrophage conc. 0.1-1.5(cells/µL) (Mean ± SD)	Hb con. 11.5-16.5g/dl (Mean ± SD)	p-value
Single Infection No.= 134									
E.histolytica No.=83	6.7130± 2.9056	4.6313 ± 2.6813	5.6320	4.2310	6.06 ± 3.0	4.3 ± 2.0	1.6 ± 1.2	11.0 ± 1.6	0.000
B.hominis No.=22	6.3312±2.6631	4.1933 ± 2.2650	5.7621	4.0224	6.2± 2.11	4.7 ± 1.71	1.16 ±0.89	11.0 ± 1.2	0.000
C.parvum No.=21	4.3401±1.3108	4.6762 ± 2.6182	4.3250	4.0321	4.05 ± 1.3	3.6 ± 1.7	1.46 ± 1.4	11.2± 1.4	0.000
G.lambilia No.=8	4.3821±1.2152	3.6260 ± 0.9059	4.1032	3.5410	4.41 ± 1.3	4.6 ± 1.7	1.35 ±0.6	11.3 ± 1.19	0.000
Mixed Infection No.= 26									
E.histolytica+ B. hominis No. =18	7.2640±2.394	4.765 ±2.448	6.7230	4.354	6.90 ± 2.04	5.9± 2.06	1.6±0.93	10.6± 0.74	0.005
E.histolytica + C.parvumNo.=6	6.1442±2.652	6.421 ±2.485	5.1267	5.342	8.18 ±4.41	6.9± 1.87	1.13± 0.75	10.25±1.25	0.000
E.histolytica + G.lambiliaNo. =2	6.7620± 2.320	5.928 ±2.316	5.6310	4.834	8.9 ± 0.35	6.35± 0.45	1.3± 0.35	10.8± 0.4	0.000

Mo: Morphonuclear, Ly: Lymphocyte, Lac: Lactoferrin, Cal: Calprotectin, Mac: Macrophage

The data reveal significant variations in inflammatory and hematologic responses depending on the type and combination of protozoan species. Calprotectin is a well-established biomarker for intestinal inflammation, predominantly derived from neutrophils, while Lactoferrin is a sensitive marker of intestinal inflammation, particularly reflecting neutrophilic activity in the gut mucosa.[7,20] Among all single infections, E. histolytica showed the highest calprotectin levels (6.7130 ± 2.91 ng/ml) and elevated morphonuclear (6.06 ± 3.0 cells/µL) and lymphocyte counts (4.3 ± 2.0), with reduced Hb (11.0 ± 1.6 g/dL). This is consistent with E. histolytica's known invasive nature, which leads to mucosal inflammation and intestinal bleeding, hence increased inflammatory markers and mild anemia.[12,22] Blastocystis hominis infections showed moderate calprotectin (6.33 ± 2.66 ng/ml) and lymphocyte counts (4.7 ± 1.71), with no significant drop in hemoglobin (11.0 ± 1.2 g/dL). While B. hominis has been considered non-pathogenic, recent studies suggest some strains may cause low-grade inflammation, especially in immunocompromised or malnourished children.[6,23] C. parvum showed lower levels of calprotectin (4.34 ± 1.31) but relatively high lactoferrin levels (4.68 ± 2.62) and reduced morphonuclear and lymphocyte counts. Cryptosporidiosis induces epithelial disruption, and although systemic inflammation may be less severe, local neutrophil response can be significant.[24] While Giardia lamblia infections presented the lowest inflammatory biomarker levels, especially calprotectin (4.38 ± 1.21) and lactoferrin (3.63 ± 0.91), indicating a lesser degree of mucosal damage. However, lymphocyte counts remained relatively high (4.6 ± 1.7), which aligns with G. lamblia's tendency to cause chronic immune stimulation without acute inflammation.[25]

Table (5) demonstrates a significant association between elevated inflammatory biomarkers—calprotectin and lactoferrin—and the severity of gastrointestinal symptoms in children under 5 years and outcomes. As shown in the Table 5, children with positive calprotectin levels exhibited a notably higher frequency of defecation (6 ± 1.92 times/24 hrs), more days of diarrhea (6 ± 1.12), and longer hospitalization periods (4 ± 1.11 days) compared to those with negative calprotectin (3.5 ± 1.11; 4 ± 0.84; and 3 ± 1.20 respectively). The p-value of 0.0021 indicates a statistically significant difference, affirming that calprotectin is a reliable marker of intestinal inflammation. Similarly, lactoferrin-positive patients experienced more frequent defecation (4 ± 1.77 vs. 3 ± 1.56), longer diarrhea duration (5 ± 1.31 vs. 3 ± 1.36), and slightly reduced hospitalization days (3.6 ± 1.88 vs. 4 ± 1.63) compared to lactoferrin-negative patients. The statistically significant p-value (0.0034) reinforces the role of lactoferrin as an indicator of active intestinal inflammation, although the hospitalization duration did not differ markedly.

Table 5: Association between Calprotectin and Lactoferrin levels in Positive and Negative Groups in Subjects with Intestinal Protozoal Infection in Children Under 5 Years.

Inflammatory Biomarkers	Frequency of Defecation /24 hr (Mean ± SD)	Diarrhea /Days (Mean ± SD)	Hospitalization /Days (Mean ± SD)	p-value
Calprotectin (+ve) No. = 146	6 ± 1.92	6 ± 1.12	4± 1.11	0.0021
Calprotectin (-ve) No. =14	3.5± 1.11	4± 0.84	3 ± 1.20	
Lactoferrin (+ve) No.=143	4± 1.77	5 ± 1.31	3.6 ± 1.88	0.0034
Lactoferrin (-ve) No. =17	3± 1.56	3± 1.36	4± 1.63	

These findings support previous research suggesting that both calprotectin and lactoferrin are sensitive non-invasive fecal biomarkers of mucosal inflammation. Elevated levels often reflect neutrophil activation and

migration into the intestinal lumen, typically observed in infectious gastroenteritis, inflammatory bowel disease, or protozoal infections. The increased stool frequency and prolonged diarrhea in biomarker-positive groups may be attributed to the inflammatory response disrupting intestinal absorption and promoting mucosal damage. Importantly, the prolonged hospital stays associated with positive calprotectin levels may reflect more severe clinical presentations or complications necessitating extended medical care. Although lactoferrin-positive cases had slightly shorter hospitalization periods, this could be due to variability in infection types, response to treatment, or sampling size differences, which may influence the outcome.[26]

Table 6: Correlation between Some Clinical Findings and Inflammatory Biomarkers (Calprotectin and Lactoferrin) in Children Under 5 Years old with Diarrheal Cases (160 = Total number of positive intestinal protozoa).

Clinical Finding	Calprotecrtin Positive No=146	Calprotectin Negative No=14	p- value	Lactoferrin Positive No =143	Lactoferrin Negative No =17	p-value
Fever	112	10	0.302	110	15	0.656
Bloody diarrhea	52	9	0.181	44	8	0.271
Dehydration	64	7	0.901	70	5	0.143

The current study evaluated the correlation between inflammatory biomarkers—calprotectin and lactoferrin—and clinical symptoms such as fever, bloody diarrhea, and dehydration in children. Among the 146 participants who tested positive for calprotectin, 112 presented with fever compared to 10 out of 14 in the calprotectin-negative group ($p = 0.302$). Similarly, among lactoferrin-positive individuals (n=143), fever was reported in 110 cases, compared to 15 in the lactoferrin-negative group ($p = 0.656$). These differences were not statistically significant, indicating that the presence of fever may not be strongly linked to elevated levels of calprotectin or lactoferrin in this cohort. In the case of bloody diarrhea, 52 of the calprotectin-positive participants reported the symptom, compared to 9 in the negative group ($p = 0.181$). A comparable trend was observed for lactoferrin, with 44 positives and 8 negatives reporting bloody diarrhea ($p = 0.271$). Though the number of cases was higher among biomarker-positive groups, the lack of statistical significance suggests that while these biomarkers may be elevated in some cases of bloody diarrhea, they are not consistently predictive of its presence.

Interestingly, dehydration was reported in 64 calprotectin-positive cases and 7 calprotectin-negative ones ($p = 0.901$), while among lactoferrin-positive individuals, 70 had dehydration compared to 5 in the negative group ($p = 0.143$). These findings again fail to reach statistical significance, though a slight trend toward more dehydration in the lactoferrin-positive group could suggest a weak correlation.

Taken together, the findings imply that elevated fecal calprotectin and lactoferrin levels may be indicative of underlying intestinal inflammation, yet do not strongly correlate with specific acute clinical symptoms like fever, bloody diarrhea, or dehydration in a statistically significant way. These biomarkers are more reflective of subclinical or chronic mucosal inflammation rather than acute symptomatology alone. This result is consistent with earlier studies which reported that while fecal calprotectin and lactoferrin are reliable non-invasive markers for detecting inflammatory bowel conditions and intestinal infections, their levels do not always correlate directly with the severity or presence of clinical symptoms. For instance the studied that observed that fecal calprotectin was elevated in patients with IBD, but did not always correlate with fever or bloody stools and similarly noted that lactoferrin and calprotectin were useful for screening inflammation, though their clinical predictive value was limited when used in isolation.[27,28] The nonsignificant p-values may also reflect the small sample size in the biomarker-negative groups, which limits statistical power and warrants cautious interpretation.

Table (7) shows a correlation between fecal occult blood and some blood parameters, where it was found that the hemoglobin concentration 10.1 ± 1.10 g/dl , MCV 72.3 ± 2.05 fl, and MCHC 34 ± 0.81g/dl in patients who showed positive results for the fecal occult blood test, and they showed significant differences ($p<0.05$) compared to those who showed negative results for the fecal occult blood test

Table 7: Hematological Parameters in Positive and Negative Group of Fecal Occult Blood.

Parameters Total no.= 113	Frequency of (Mean ± SD)	Hb (11.5-16.5g/dl) (Mean ± SD)	MCV (75-100 fl) (Mean ± SD)	MCHC (31-38g/dl) (Mean ± SD)	p-value
Positive for fecal occult blood n=8	4.3 ±1.24	10.1 ±1.10	72.3± 2.05	34 ±0.81	< 0.001
Negative for fecal occult blood n= 95	5± 1.41	11.6±1.01	78.4 ±3.8	34.6± 2.15	

The results of the current study also showed in (Table 7) a correlation between hematological parameters and positive group of fecal occult blood, where a decrease in hemoglobin concentration was found. This result was not consistent with the study of Behera and Bulliyya[29] reported that there was no difference in the hemoglobin (g/dL) concentrations of male and female preschool children in Odisha, India (10.57 ± 3.01 and 10.27 ± 2.99, respectively). The size of the RBCs, reflected by mean corpuscular volume (MCV) was less than 80 fL; this was smaller than the normal size of RBCs. Almost all of the enrolled children (100%) were suffering from microcytic anemia. This shows the decreased

production of hemoglobin due to the short supply of iron in the body. Engidaye *et al.*[30] reported that 50.4% of preschool children in Ethiopia had microcytic hypochromic anemia and 12.2% had macrocytic anemia. Furthermore, these authors reported that the reason underlying the dominant type of microcytic hypochromic anemia was the greater consumption of cereals by the studied community; these cereals have a low iron content. Our results suggest the cause of some clinical finding and reduced Hb in children is due to the enteric infection of parasites. This has a harmful effect on nutritional status and interferes with the use of many nutrients including iron, vitamin B12, folic acid, thus contributing to anemia. The main cause of disease transmission may be socioeconomic condition (education, occupation, income) along with personal hygiene (hand washing habit before food, after using the latrine, along with the type of water and water storage utensils).

Table (8) shows while the correlation have revealed between fecal occult blood and some clinical findings that 5 of the patients who showed a positive result for the fecal occult blood test were suffering from fever, while 3 patients who showed a positive result for the fecal occult blood test were suffering from bloody diarrhea and showed significant differences ($P<0.05$) compared to those who showed a negative result for the fecal occult blood test as showed in the study of Kwasi *et al.*[31].

Table 8: Clinical Finding in Positive and Negative Groups of Fecal Occult Blood.

Parameters Total no .= 113	Fever	Bloody Diarrhea	p-value
Positive fecal occult blood n=8	5	3	0.008
Negative fecal occult blood n= 95	63	5	0.043

Conclusions

This comparative analysis underscores the diagnostic utility of fecal biomarkers in children under 5 years with intestinal protozoal infections and highlights the distinct inflammatory profiles elicited by different protozoa. Future research should focus on integrating these biomarkers into routine screening to enhance early detection and improve clinical management, especially in endemic areas. In regions with poor sanitation, malnutrition, and limited access to healthcare, such as some parts of Iraq especially rural areas, undiagnosed and untreated protozoal infections contribute significantly to morbidity, growth retardation, Iron deficiency anemia and even mortality among children under the age of five.

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