



RESEARCH ARTICLE

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THE ROLE OF IL-6 AND IL-8 IN THE SEVERITY OF BLOODY DIARRHEA CAUSED BY PARASITIC INFECTIONS: A CASE CONTROL STUDY

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Abstract

Background: Bloody diarrhea due to parasitic infections is an important health issue in developing regions around the world, and intestinal protozoa are a major contributor to the global burden of morbidity. The host immune response, and specifically the levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-8 (IL-8), is an important determinant of disease pathogenesis and severity.

Aims: The objective of this study was to assess the serum level of IL-6 and IL-8 among parasitic-induced bloody diarrhea patients and compare them with healthy controls. It also aimed to evaluate the distribution of cytokine levels by genus stage infected. Patients and methods: A case-control study was conducted at Al-Sadr Medical City, Najaf, Iraq, during the period from June to December 2025.

Patients and methods: A total of 62 patients with parasitic bloody diarrhea, clinically and laboratory-confirmed, and 68 healthy controls were enrolled. Patients who received antimicrobial therapy within the previous 2 weeks, had chronic systemic diseases, or had mixed infections were excluded. Stool examination has been utilized to determine some of the anal parasites, including, anal Entamoebahistolytica, anal Giardia lamblia, anal Balantidium coli. Serum IL-6 and IL-8 level was assessed by enzyme linked immunosorbent assay (ELISA).

Results: IL-6 and IL-8 levels were significantly higher in patients than in controls ($p < 0.001$). Entamoeba infections had the highest cytokine levels compared to other parasitic groups followed by Balantidium and the lowest levels were found in Giardia infections.

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Statistical analysis confirmed differences between the groups for both IL-6 ($p = 0.04$) and IL-8 ($p = 0.002$).

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Conclusions: IL-6 and IL-8 are significantly increased in parasitic-associated bloody diarrhea and differ by type of parasite. This histogram represents the spectrum of possibilities that can be developed, where we can see that both of these cytokines may be good biomarkers for disease severity and/or parasitism-specific immune responses.

Introduction:-

Bloody diarrhea have been an important public health problem, especially in developing countries with endemic parasitic infections, including Iraq. This is usually linked with invasive enteric pathogens, which include *Entamoeba histolytica*, *Giardia lamblia* and other protozoan parasites that compromise intestinal mucosa integrity and trigger inflammatory response (Dhubyan Mohammed Zaki, 2022). The clinical severity of bloody diarrhea can vary from mild self-limiting illness to severe life-threatening disease characterized by dehydration, anemia and systemic inflammation. Identifying immunopathological mechanisms that drive disease severity is key to advancing diagnostic and therapeutic approaches (Ibraheem, 2016).

Host cytokines plays an integral role in regulating intestinal inflammation and tissue repair during parasitic infections. Among these, interleukin-6 (IL-6) and interleukin-8 (IL-8) are some of the most important pro-inflammatory mediators which have significant roles in mucosal immunity. IL-6 is a pleiotropic cytokine playing important role in the acute-phase response, immune regulators and hematopoiesis; while IL-8 acts mainly as a chemokine and controls neutrophil recruitment and activation at infection sites (Zaki et al., 2020).

In the case of gastrointestinal infections, intestinal epithelial cells and immune cells release IL-6 and IL-8 in response to pathogen invasion. These cytokines play a role in the inflammatory cascade where they promote leukocyte infiltration, increase vascular permeability, and amplify local immune responses. As such, elevated levels of IL-6 and IL-8 have been naturally associated with acute gastroenteritis in patients since they act as a key determinant of disease. However, the exact role of these factors in exacerbating bloody diarrhea due to parasitic infections is not fully understood (Adumitrăchioaiei et al., 2024).

Additional studies has suggested that IL-6 and IL-8 may also serve as prognostic biomarkers in gastrointestinal infections. Higher serum concentrations of IL-6 have been linked to severe inflammatory disease and may distinguish between non-bacterial and bacterial causes of diarrhea (Zaki et al., 2020). An analogous association has also been reported with IL-8, which is associated with the extent of mucosal inflammation and clinical symptom severity, likely related to its role in neutrophil chemotaxis and activation. These findings indicate that profiling of cytokines may be helpful in evaluating the severity of disease (Adumitrachioaie et al., 2024).

Additionally, new data suggest that host factors (e.g. nutritional status, age and immune competence) may also influence cytokine responses. For instance, a recent case-control study showed that malnourished children with acute diarrhea had significantly different levels of IL-6 and IL-8 compared to their well-nourished equivalents; hence altered immune responses may impact disease outcomes. This variability highlights the need for additional studies to clarify which factors drive cytokine expression within various clinical contexts such as that of parasitic infections (Al-Masoudi et al., 2024).

While there is growing interest in the role of cytokine-mediated immune responses, most studies have addressed cases of viral and bacterial gastroenteritis, although relatively little attention has been paid to parasitic etiologies. Chronic or recurrent inflammation is a characteristic feature of many parasitic infections and can yield unique cytokine profiles different from acute infection. Moreover, parasites have evolved specialized mechanisms to escape host immunity, which may modify the production of cytokines as well as disease progression. Hence, exploring whether IL-6 and IL-8 are involved in parasitic-associated bloody diarrhea will enhance our knowledge of host-parasite interactions (Xing et al., 2024).

Considering these factors, we are here to assess the role of IL-6 and IL-8 for severity of bloody diarrhea caused by parasitic infection in a case-control study. This study aims to determine if cytokine levels in affected patients differ from healthy controls and contribute to the existing body of research investigating immunological responses in parasitic GI diseases; potentially uncovering their applicability as biomarkers of disease severity.

Patients and Methods: -

A case-control study at Al-Sadr Medical City in Najaf in Iraq was conducted from June 2025 to December 2025. After excluding 3 patients with nonparasitic infections, we enrolled a total of 62 bloody diarrhea cases with confirmed parasitic infections. Ages varied from 18 to 60 years. Diagnosis was clinical, confirmed by laboratory detection of parasitic infection. The control group included 68 healthy individuals with no history of gastrointestinal disorders or recent infections, matched as closely as possible to patients by age and sex.

Inclusion and Exclusion Criteria: -

Hard and soft data were used to identify patients with acute diarrhea (defined as ≥ 5 stools per day) and bloody diarrhea confirmed by laboratory methods, along with the underlying cause of parasitic infections. Exclusion criteria included use of antibiotics, antiparasitic, or immunosuppressive therapy in the two weeks prior to enrollment and chronic systemic diseases (eg, diabetes mellitus, autoimmune disorders), inflammatory bowel disease or malignancy. Mixed infections (either bacterial or viral coinfection) were also excluded to avoid possible observed confounding effects on cytokines.

Classification of Parasitic Infections: -

Cases of bloody diarrhea were further classified by the parasitic genus identified from stool samples.

For all these reasons, many studies are focusing on three genera closely related to intestinal pathology:

- Entamoeba spp. (particularly Entamoeba histolytica)
- Giardia spp. (notably Giardia lamblia)
- Balantidium spp. (primarily Balantidium coli)

This classification allowed cytokine responses to be correlated with distinct parasitic etiologies.

Sample Collection and Laboratory Analysis: -

5 mL of venous blood was taken aseptically from each participant (patients and controls) in plain tubes for serum separation. Blood samples were kept at room temperature to allow clotting followed by centrifugation at 3000 rpm for 10 minutes. Separate serum aliquots were essentially frozen at -20°C until assayed. Concentrations of serum IL-6 and IL-8 were accurately quantified using enzyme-linked immunosorbent assay (ELISA) with commercially available kits according to manufacturer's instructions. Data were analyzed in duplicates for reproducibility and accuracy of the results.

Stool Examination and Diagnosis: -

Direct wet mount microscopy (saline and iodine preparations) and concentration techniques (formalin-ether sedimentation method) were performed on fresh stool samples for all patients to screen for parasitic cysts, trophozoites or ova. Identifications of the parasites were based on standard morphological criteria. In low case numbers, staining methods were applied to improve diagnostic sensitivity.

Assessment of Disease Severity:-

The severity of bloody diarrhea was clinically evaluated based on the number of bloody stools, dehydration status and abdominal pain with systemic symptoms such as fever. Patients were included based on the clinical criteria used for PRC infection, just as with mild, moderate and severe groups. It has been applied this classification to examine the association of cytokine levels with disease severity.

Ethical Considerations:-

This study had obtained an ethical approval from the institutional review board of Al-Sadr Medical City. Informed consents were obtained from all participants before sample collection. The study was performed according to the Good clinical practice and principles enounced in the Declaration of Helsinki.

Statistical Analysis:-

Statistical analysis was conducted using SPSS, version 26. Results Continuous variables were summarized as mean \pm standard deviation (mean \pm SD), and categorical variables were described as frequencies and percentages. Cytokines levels (IL-6 and IL-8) were compared between patients and subjects using independent samples t-test. Cytokine levels between parasitic genera and severity groups were analyzed using one-way analysis of variance

(ANOVA) Associations between categorical variables were evaluated using the chi-square (χ^2) test. A p-value less than 0.05 was defined as statistically significant.

Results:-

Table 1 demonstrates the distribution of age groups and residence among patients with bloody diarrhea and healthy controls. The age distribution appears relatively comparable between the two groups, with the highest proportion observed in the 30–39-year category for both patients (29.0%) and controls (29.4%). Similarly, other age categories showed only minor variations between groups. Statistical analysis revealed no significant difference in age distribution ($\chi^2 = 1.07$, $p = 0.785$), indicating that both groups were well matched in terms of age. Regarding residence, a slightly higher proportion of participants in both groups resided in urban areas compared to rural areas. Specifically, 54.8% of patients and 58.8% of controls were from urban settings. However, this difference was not statistically significant ($\chi^2 = 0.21$, $p = 0.64$).

Table 1. Age and residence distribution of both control and study

Indicators		Patients (No. = 62)		Control (No. = 68)		Chi Square	P value (Sig.)
		Freq.	%	Freq.	%		
Age/Years	20-29	16	25.8	18	26.5	1.07	0.78 (NS)
	30-39	18	29	20	29.4		
	40-49	14	22.6	11	16.2		
	> 50	14	22.6	19	27.9		
Residence	Urban	34	54.8	40	58.8	0.21	0.64 (NS)
	Rural	28	45.2	28	41.2		

Table 2 shows the clinical characteristics of patients with parasitic bloody diarrhea. Most patients (71.0%) were not treated with antiparasitic therapy prior to enrolment, showing that most cases were investigated during the active phase of infection. In terms of hydration status, patients were dehydrated in 62.9% of cases, which corresponds to the high percentage of fluid loss related with persistence diarrheal episodes. Dehydration is a frequent complication of parasitic gastrointestinal infections, which may increase disease severity and lead to worse clinical outcomes. Of the patients, 50.0% reported a duration of diarrhea for 3–5 days and 27.4% had symptoms lasting more than 5 days. Chronic diarrhea may suggest treatment-resistance mucosal injury, and ongoing inflammatory activity in the intestine. This chronic periodical stimulation of the immune system can lead to enhanced levels of production of pro-inflammatory cytokines like IL-6 and IL-8. Fever was noted in 58.1% of cases to indicate that systemic inflammatory activation occurs in a significant number of patients.

Table 2. Clinical characteristics of patients group

Characteristics		Patients (No. = 62)	
		Freq.	%
Antiparasitic drugs	Yes	18	29
	No	44	71
Dehydration Status	Yes	39	62.9
	No	23	37.1
Duration of Diarrhea	< 3 days	14	22.6
	1-5 days	31	50
	> 5 days	17	27.4
Fever	Yes	36	58.1
	No	26	41.9

Figure 1 shows the distribution of cases of bloody diarrhea by parasitic etiology. The infections were caused by *Entamoeba* spp in the majority of cases, that is 47% of the total. *Giardia* spp secondly at 37% followed by *Balantidium* spp at 16%.

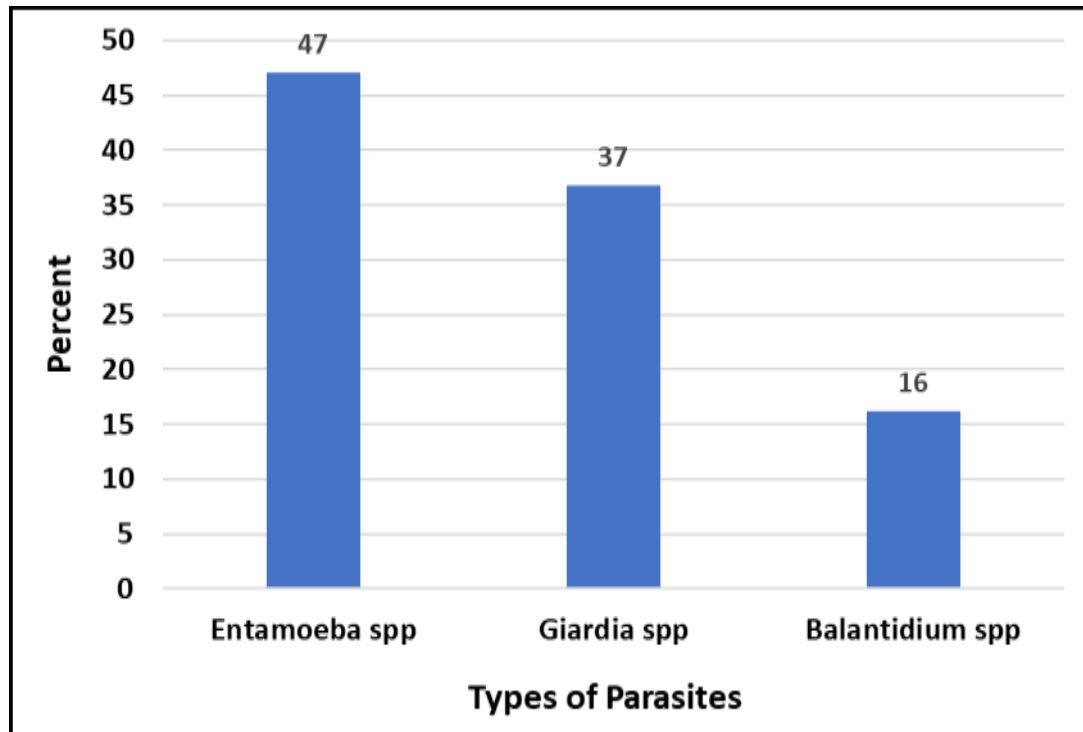


Figure 1. Distribution of bloody diarrhea according to parasitic etiology

Table 3. Measurement IL-6 and IL-8 levels in patients and control groups

Groups	Patients Mean \pm SD	Control Mean \pm SD	T Test (P Value)
IL-6	28.6 \pm 12.4	14.8 \pm 5.6	0.02 (S)
IL-8	72.3 \pm 18.7	21.5 \pm 7.9	0.000 (HS)

S: Significant at $p < 0.05$; HS: High Significant at $p < 0.01$

Patients with parasitic bloody diarrhea showed significantly higher serum IL-6 and IL-8 levels than healthy controls (Table 3). The results show a significantly higher level of both cytokine factors in the patient population than controls. The serum IL-6 level in patients (28.6 \pm 12.4 pg/mL) was significantly greater than that of control (14.8 \pm 5.6 pg/mL). Likewise, the concentrations of IL-8 were significantly elevated in patients (72.3 \pm 18.7 pg/mL) when compared with controls (21.5 \pm 7.9 pg/mL). Statistical analysis was performed using the independent samples t-test, which found highly significant differences between both IL-8 levels of both groups ($p < 0.001$) and found a significant differences between both IL-6 levels of both groups ($p < 0.02$). Together these results show a strong correlation between elevated pro-inflammatory cytokines and parasitic-induced bloody diarrhea. Increased levels of IL-6 suggest greater mediation of both the acute-phase inflammatory response and immune activation which occur as a result of parasitic infection. Conversely, the increased IL-8 levels represents an adaptive response in the gut that promotes recruitment and activation of neutrophils to sites of mucosal inflammation and leads to mucosal damage resulting in a more severe clinical phenotype (table 2).

Table 4. Assessment of IL-6 levels in patients' groups according to parasitic etiology

Groups	Freq.	IL-6 (pg/ml) Mean \pm S.D	F test	T test P-value
Entamoeba	29	55.8 \pm 11.6 A	24.6	<0.000 (HS)
Giardia	23	42.3 \pm 10.2 B		
Balantidium	10	50.6 \pm 12.1 AB		
Control	68	14.8 \pm 5.6 C		

A, B Different letters refer to significant difference at $p < 0.05$; HS: High Significant at $p < 0.01$

As shown in Table 4, a very significant difference in serum IL-6 levels between the studied groups in relation to parasitic etiology ($F = 24.6$, $p < 0.0001$). The mean of IL-6 concentration in Entamoeba infected patients (55.8 ± 11.6 pg/ml) was significantly more than in the Giardia (42.3 ± 10.2 pg/ml) and the healthy control group (14.8 ± 5.6 pg/ml). The IL-6 level in the Balantidium group (50.6 ± 12.1 pg/ml, AB) was intermediate between the Entamoeba and Giardia groups, but it did not differ significantly from either group as indicated by the letter designation (AB).

Table 5. Assessment of IL-8 levels in patients' groups according to parasitic etiology

Groups	Freq.	IL-8 (pg/ml) Mean \pm S.D	F test	T test P-value
Entamoeba	32	85.4 \pm 17.2 A	26.85	<0.000 (HS)
Giardia	25	60.8 \pm 14.5 B		
Balantidium	11	74.6 \pm 16.1 B		
Control	68	21.5 \pm 7.9 C		

A, B Different letters refer to significant difference at $p < 0.05$; HS: High Significant at $p < 0.01$

Table 5 shows a highly significant group difference in IL-8 Levels Between Groups from the study by parasitic etiology ($F = 26.85$, $p < 0.0001$) The mean IL-8 concentration was significantly higher in Entamoeba (85.4 ± 17.2 pg/ml) than Balantidium (74.6 ± 16.1 pg/ml) and Giardia (60.8 ± 14.5 pg/ml) groups, and was lowest in controls (21.5 ± 7.9 pg/ml) ($P < 0.05$). Post hoc analysis revealed that the Entamoeba group had greater IL-8 levels compared to both the Giardia and Balantidium groups (pA); however, no significant difference was found between the latter two groups (B), which are marked with the same letter designation. Compared with the healthy controls, levels of IL-8 were significantly higher in all infected groups.

Discussion:-

In this study, we aimed to evaluate the importance of IL-6 and IL-8 in bloody diarrhea severity due to parasitic infections. Levels of IL-8 and IL-6 were significantly higher in patents compared to healthy controls, $p < 0.001$ Moreover, subjects' parasitic etiology was shown to affect cytokine levels. The highest cytokine levels were associated with Entamoeba infections which was then followed by Balantidium and Giardia infections, albeit that they were in statistically lower levels compared to each other. These results have significant implications for the immunopathogenesis of gastrointestinal parasitic infections.

The significant increase in IL-6 observed in patients of this study is in line with the well-established function of IL-6 as a major mediator of inflammation. While IL-6 is released by a variety of immune cells as well as by macrophages and intestinal epithelial cells in response to infection, it is now established that it serves to drive the acute-phase response through the amplification of inflammatory signaling (Tanaka et al., 2014). But while in gastrointestinal infections, high levels of IL-6 have been associated with mucosal inflammation and disease severity. For instance, Chen et al. inflammatory biomarker (2014), they showed that children with acute gastroenteritis had significantly higher serum concentrations of IL-6 compared to healthy controls.

These current findings establish a relationship to prior data indicating higher levels of both IL-6 and IL-8 were noted in the plasma from patients with diarrhea, and increased expression on membrane-bound cell lysates was also noted confirming its role as an important chemokine inducing recruitment of neutrophils and activation. IL-8 would obviously be critical for neutrophil accumulation at sites of infection to drive pathogen clearance but also lead to tissue destruction and clinical manifestations such as bloody diarrhea. IL-8 levels have been shown to be markedly

upregulated in infectious diarrhea and correlated with the degree of intestinal inflammation (Zaki et al., 2020). The present results are consistent with these reports and suggest that IL-8 is highly correlated to the inflammatory response in parasitic infections (figure 2.).

The pathogens included in this study also differed in terms of cytokine levels, further reinforcing the role pathogen-specific mechanisms play on host responses. Patients infected with *Entamoeba* spp. had the greatest amount of both IL-6 and IL-8 (Lin et al., 2006). This results from the invasive property of *E. histolytica*, which penetrates the intestinal mucosa, and induces tissue destruction with ulceration and bleeding. This invasive procedure triggers a robust immune response driven by the release of pro-inflammatory cytokines. IL-6 and IL-8 production from intestinal epithelial cells has been shown to be induced by *E. histolytica* in vitro via activation of inflammatory signaling pathways (Petri et al., 2002).

In contrast, *Giardia* spp. infections were linked to much lower levels of IL-6 and IL-8. This result is in accord with the low invasive property of *G. lamblia*, as it does not invade the intestinal epithelial cells extensively but rather colonizes and topically attaches to luminal surfaces. Additionally, *Giardia* has been observed to induce downregulation of pro-inflammatory cytokine production in hosts (Cotton et al., 2011), again allowing for the parasite's persistence. This immune modulation could account for the relatively moderate inflammatory response and lower cytokines levels in giardiasis as compared to amoebiasis.

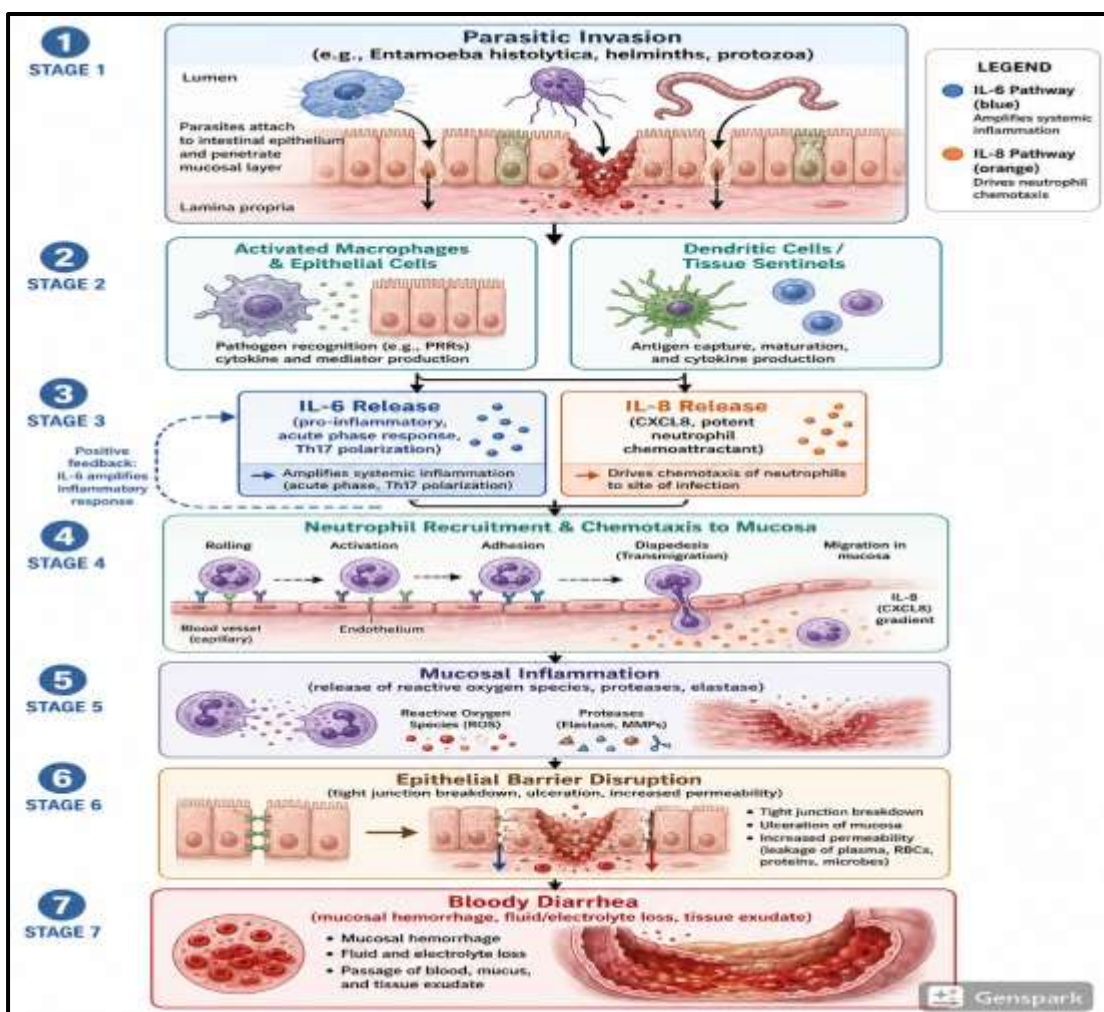


Figure 2. The cascade from parasitic invasion through IL-6/IL-8 release to bloody diarrhea (Kang et al., 2022)

Intermediary levels of cytokines were found in case of *Balantidium* infections, consistent with the moderate inflammatory response. Less well studied is the fact that *Balantidium coli* can invade colonic mucosa and cause

ulcerative lesions producing clinical signs which resemble amoebiasis, but which are less severe. The relative intermediate pathogenic potential of *Balantidium* is reflected in the percentage overlap in cytokine levels between this group and the others (Ismail et al., 2025).

The present findings support and validate the role for IL-6 and IL-8 as potential biomarkers of disease severity in parasitic infections. Reference "Clinical Implications of the Cytokine Network during COVID-19 Infection" found high levels of these cytokines correlated with severity of inflammatory response and may be useful for assessing disease progression. The combination of these findings and the intermediate level of ri-IL-6 suggests that by facilitating transcytosis, GI infections probably out liquidate other infections in systemic IL-6 IS. In another report, Tanaka et al observed lower or higher level of ri-IL-6 correlated with severity of GI infection and systemic inflammatory response (Tanaka et al., 2014). Moreover, neutrophil infiltration induced by IL-8 has been directly linked to mucosal damage and symptom severity in infectious diarrhea (Zaki et al., 2020).

From an impressively immunopathological point of view, the elevated release of IL-6 and IL-8 represents a homosexual activation of the innate immune system to assist against parasitic invasion. While this response is necessary for controlling infection, an excessive cytokine response can contribute to tissue damage and exacerbate the clinical features. In addition, parasites have evolved strategies to manipulate host immune responses as the release of immunomodulatory molecules that induce changes in cytokine expression and promote immune evasion (Ghosh et al. 2019). Interactions between parasite virulence determinants and host immune responses lead to a diverse disease spectrum and are important for the resulting pathology.

Although these findings are significant, there are several limits that must be acknowledged. If sample size was larger, and likely did impact statistics power of the study. Moreover, this study determined the induction of only IL-6 and IL-8 that has occurred since other cytokines like TNF- α and IL-10 may also be reflected in the inflammatory reaction. Further studies with larger number of samples and a wider panel of cytokines should be performed to better demonstrate the immune response in parasitic diseases.

Conclusion:-

In parasitic bloody diarrhea, the levels of Interleukin 6 (IL-6) and Interleukin 8 (IL-8) are higher in the blood compared to healthy subjects; and the concentrations of both interleukins is different depending on the parasite that causes the bloody diarrhea. High concentrations in *Entamoeba* infections indicates act of tissue-dependence and strong inflammatory potential, while low concentration in *Giardia* infections is consistent with low tissue-invasiveness. This study identifies that IL-6 and IL-8 play an important role in parasitic infections, and also enables both of them to be potential biomarkers for the prediction of disease severity.

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