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Protozoan Infections and Cytokine Profiles in Immunocompromised Cancer Patients

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ABSTRACT

Background: Immunocompromised cancer patients are highly susceptible to intestinal protozoan infections, which can exacerbate morbidity and complicate treatment outcomes. This study aimed to determine the prevalence of Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum infections among cancer patients in Wasit Province, Iraq, and to evaluate associated cytokine responses (IL-10 and IL-25).

Methods: A cross-sectional study was conducted on 90 cancer patients and 90 healthy controls. Stool samples were analysed using nested PCR for parasite detection, and serum levels of IL-10 and IL-25 were measured via ELISA. Statistical analyses were performed using SPSS version 28.

Results: The overall prevalence of intestinal protozoan infections was 61.59%. E. histolytica was the most prevalent (42.75%), followed by C. parvum (39.86%) and G. lamblia (17.39%). Significantly elevated levels of IL-10 and IL-25 were observed in infected patients compared to non-infected individuals ($p < 0.001$), indicating a strong immunomodulatory response. The elevated IL-10 suggests an immunosuppressive state that could facilitate parasite persistence and hinder anti-tumor immunity, while the rise in IL-25 indicates an active epithelial defense response. This dual cytokine response underscores the complex immune environment that may increase the risk of chronic infection and complicate the overall clinical management of cancer patients.

Conclusion: Intestinal protozoan infections are highly prevalent among cancer patients in Wasit Province and are associated with significant alterations in immune cytokine profiles. Routine screening and preventive measures are recommended to mitigate the impact of these infections on vulnerable populations.

KEYWORDS: Intestinal protozoa, cancer patients, IL-10, IL-25, immunocompromised, nested PCR, Wasit Province..

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INTRODUCTION

The non-cancerous enla
An immunocompromised host is typically characterized as a person with one or more deficiencies in the normal defines mechanisms that protect against infections, rendering them more susceptible to serious, life-threatening diseases. The specific immune response to parasites directs the production of antibodies (1).

According to the latest estimates from the World Health Organization's GLOBOCAN 2022 report, cancer remains a leading cause of mortality worldwide, responsible for nearly 10 million deaths annually. The global burden is disproportionately borne by low- and middle-income countries, which account for approximately 70% of cancer cases and deaths, often due to limited access to prevention, timely diagnosis, and treatment services. Current projections indicate a continued rise, with the annual number of new cancer cases expected to reach 28.4 million by 2040, underscoring the growing challenge it poses to global health systems (2).

Parasitic illnesses impact millions globally, causing considerable morbidity and mortality, particularly in low- and middle-income nations. The World Health Organization says that intestinal parasite infections impact about 67.2 million people, which is equal to 492,000 disability-adjusted life years (DALYs) (3).

In tropical nations, parasite infections are a common cause of illness and death in children. *Cryptosporidium parvum*, *Giardia lamblia*, and *Entamoeba histolytica* are the most prevalent parasite protozoa responsible for diarrhea (4).

Diarrhea occurs when you have three or more loose or watery stools a day. It can be caused by many bacteria, viruses, and parasites, and it can spread through food, drinking water, or from person to person because of poor hygiene(5).

Repeated exposures and the chronicity or persistence of infections enhance the development of the anti-infection immune response. The immune response was analysed based on age in uninfected, mono-infected, or polyparasitized individuals; it varied according to age and kind of parasitism (6).

The parasites possess numerous antigens due to the complexity of the host's bodily structure, enabling them to dodge the host's

immune response through several mechanisms, including alteration of their surface antigens, encystment, and migration(7).

IL-10 helps keep the host from getting tissue damage during the early stages of immune responses. IL-10 can be made by almost all immune cells, and it can also change how these cells work (8).

Regulatory T cells (Tregs) are one of the most abundant types of T cells in the gut. They help keep the immune system in balance and tolerant by releasing IL-10 and TGF-β (9).

People with healthy immune systems usually get rid of the virus on their own after a few weeks of being exposed. Some people never show any symptoms (10).

Furthermore, the cytokine IL-25, released primarily by intestinal epithelial tuft cells, plays a critical role in initiating type 2 immune responses characterized by the production of IL-4, IL-5, and IL-13, which are essential for expelling helminths and controlling certain protozoan infections. However, in immunocompromised hosts, such as cancer patients, this coordinated type 2 response may be impaired or dysregulated. This immunodeficiency can disrupt the critical balance between parasite clearance and immune regulation, potentially allowing parasites to establish persistent infections and contributing to the pathogenesis and severity of disease(11).

People can obtain intestinal parasite diseases directly or indirectly by eating or drinking infected food, water, fruits, or vegetables, or by touching anything that have faces on them, like fingers (12,13).

Intestinal parasite infections that last a long time are a big health problem all over the world. And how parasite infections are related to higher rates of sickness and death, especially in people who are malnourished and have weak immune systems(14).

Entamoeba histolytica, *Giardia intestinalis*, and *Cryptosporidium spp*. are three of the most common protozoan illnesses that make people have diarrhea. *E. histolytica* can cause diarrhea, amebic colitis, liver abscess, and anemia. It may also affect the development of newborns. Estimates say that at least 50 million people get invasive amebic infections every year, and between 40,000 and 100,000 people die from them(14).

The symptoms of an infection with the parasite *G. lamblia* can be different from person to person, and they may not show up until later. Some of the most common signs of this parasite infection are diarrhoea that is fatty, weight loss, stomach pain, vomiting, malnutrition, and nausea (15).

In a patient with a weak immune system, Cryptosporidium infection is marked by gastrointestinal symptoms like sudden, watery diarrhoea that may be accompanied by vomiting, abdominal pain or cramps, and weight loss. Other less specific symptoms include fatigue, malaise, nausea, muscle weakness, and fever(16).

The aim of present study was to determine the prevalence of intestinal protozoa and Association Cytokine Responses (IL-10 and IL-25) in cancer patients in wasit province.

METHODS

Study design and setting

This cross-sectional analytical study was conducted between August 2024 and February 2025 at the Oncology Department of Wasit Specialized Center for Cancer Treatment. A total of 90 cancer patients, aged between 9 and 85 years and clinically diagnosed by specialist physicians, were enrolled. A parallel control group of 90 age- and sex-matched apparently healthy individuals (AHC) with no history of cancer or immunosuppressive conditions was recruited from the same geographical region to provide a baseline for parasite prevalence and cytokine levels. A power calculation conducted prior to recruitment, assuming a medium effect size (Cohen's d = 0.5), an alpha error of 0.05, and a power of 80%, indicated a minimum required sample size of 64 per group. The chosen sample size of 90 per group exceeds this requirement to account for potential dropouts and to enhance the robustness of the findings. A questionnaire was administered to collect demographic and clinical data, including age, residence, gender, and duration of illness. Sterilized, airtight plastic containers were provided for stool sample collection to prevent desiccation and contamination.

Sample collection

From each participant (90 patients and 90 controls), 3 ml of venous blood was drawn using a sterile, disposable needle and syringe. The blood was placed in a gel clot activator tube, allowed to clot, and then centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum aliquots were stored at **-80 °C** until analyzed for cytokine levels.

DNA extraction

The Presto™ Stool DNA Extraction Kit was used to extract DNA from water samples. The process involved transferring 180-220 mg of stool to a Beadbeating Tube containing ceramic beads, adding 800 µl of ST1 Buffer, vortexing briefly, and centrifuging at 8,000 x g for 2 minutes. The resulting supernatant was transferred to a new 1.5 ml microcentrifuge tube. The required Elution Buffer (100 µl per sample) was preheated to 60ºC for DNA elution. The PCR inhibitor removal was done by adding 150 µl of ST2 Buffer, vortexing, incubating, centrifuging, and precipitating insoluble particles and PCR inhibitors. The DNA binding was done by adding 800 µl of ST3 Buffer, mixing it immediately, and placing a GD Column in a 2 ml Collection Tube. The column matrix was then washed with 400 µl of ST3 Buffer, washed again, and dried. Finally, the purified DNA was eluted by centrifuging at 16,000 x g for 2 minutes at room temperature.This extraction process was performed in the laboratory of the Faculty of Medicine, Wasit University.

Primer selection

The PCR and Nested PCR primers for detecting *Entamoeba histolytica*, *Giardia intestinalis*, and *Cryptosporidium parvum* were designed in this study based on the small subunit ribosomal RNA (SSU rRNA) gene sequences from NCBI GenBank (Accession numbers: MK332025.1, DQ157272.1, and AF308600.1, respectively) using Primer3Plus software. The primer sequences and their respective amplicon sizes are provided in table (1).

Table (1): The PCR primers for detection *E. histolytica, G. lamblia* , and *C. parvum* with their sequences and product size:

Primers	Sequence 5'-3'		Product size
PCR-ssrRNA gene *Entamoeba histolytica*	F	ATTGGAGGGCAAGTCTGGTG	616bp
	R	GCCTTGTGACCATACTCCCC	
PCR-ssrRNA gene *Giardia lamblia*	F	GGGCTAGAAGGCGATCAGAC	542bp
	R	GGCGCCTACAAGACATTCCT	
PCR-ssrRNA gene *Cryptosporidium parvum*	F	ATCTAAGGAAGGCAGCAGGC	670bp
	R	CCCCCAGAACCCAAAGACTT	

Table (2): The Nested PCR primers for detection *E. histolytica, G. lamblia* , and *C. parvum* with their sequences and product size:

Primers	Sequence 5'-3'		Product size
nPCR-ssrRNA gene *Entamoeba histolytica*	F	CGCGGTAATTCCAGCTCCAA	426bp
	R	ACGACGGTATCTGATCGTCT	
nPCR-ssrRNA gene *-Giardia lamblia*	F	TTGAAGGCATTGACGGAGGG	265bp
	R	ATCACAGACCTGCTATCGCC	
nPCR-ssrRNA gene *Cryptosporidium parvum*	F	TCAATTGGAGGGCAAGTCTG	510bp
	R	AGGTGCTGAAGGAGTAAGGA	

PCR analysis

The conventional thermal cycler (Germany) was used for PCR amplification; the total volume was about 25 µL, which included 12.5 µL of master mix, 5 µL of DNA template, 1.0 µL of each forward and reverse primers, and 5.5 µL of nuclease-free water.The Nested PCR reaction ,the total volume was about 25 µL, which included 12.5 µL of master mix, 3 µL of DNA template, 1.0 µL of each forward and reverse primers, and 7.5 µL of nuclease-free water. To ensure diagnostic accuracy, each PCR run included both positive and negative controls. For each parasite, a confirmed positive DNA sample from a previous study was used as a positive control. Nuclease-free water was used as a no-template negative control to monitor for contamination.

Human Interleukin (IL-25 and IL-10) ELISA Kit procedure

The serum concentrations of IL-25 and IL-10 were measured using commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits (e.g., SunRed Biotechnology, China), strictly following the manufacturer's protocols. The assays involved adding samples and standards to pre-coated wells, followed by biotin-conjugated detection antibodies and streptavidin-HRP. The optical density (OD) was read at 450 nm using a microplate reader (BioTek, USA). The intra-assay coefficient of variation (CV%), as provided by the manufacturer and confirmed with our pilot samples, was <10% for both cytokines. All samples were analyzed in duplicate.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, subject information, and consent form were reviewed and approved by a local ethics committee, according to document number 1509, dated August,5,2024, to get this approval.

Statistical analysis

Statistical analysis was performed using the SPSS version 28.0. Data were subjected to normality test. The normally distributed data are expressed as mean ± standard deviation and compared with one way analysis of variance (ANOVA) among three groups.

Data with abnormal distribution were compared with the non-parametric test. Qualitative data are expressed as number or percentage, and rates were compared using the $\chi 2$ test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

The result of this study was obtained by the analysis of 90 Cancer Patients who were recruited in this study. Table (1) shows the Age group (years). It is clear that the highest frequency Age is (41-56), with a frequency of (30) , i.e., a percentage of (33.3%), and the lowest frequency of Age is (9-24), with a frequency of (4), i.e., a percentage of (4.4%). and shows a frequency distribution table and percentages for the sex variable, as the number of males was (36) at a rate of (40%) and the number of females was (54) at a rate of (60%) of the sample under study. and shows the number and percentage of each type of cancer, as the numbers and percentages were equal for all types (Gastrointestinal, Breast and Hematology), i.e., with a number of (30) patients for each type and a percentage of (33.3) for each of the three

Table 3: Distribution of Cancer Patients by Age Group, sex, and Type of Cancer (Total n=90)

Variable	Category	Number (n)	Percentage (%)
Age Group (years)	**9-24**	**4**	**4.4%**
	25-40	**20**	**22.2%**
	41-56	**30**	**33.3%**
	57-72	**24**	**26.7%**
	73-88	**12**	**13.3%**
Sex	**Male**	**36**	**40.0%**
	Female	**54**	**60.0%**
Type of Cancer	**Gastrointestinal**	**30**	**33.3%**
	Breast	**30**	**33.3%**
	Hematological	**30**	**33.3%**

for **nested PCR** it was noted that the highest frequency is (38) and for the percentage (27.54%)for (Female) with *Entamoeba histolytica* and last place with a frequency of (7) and for the percentage (5.07%) For (Male) with *Giardia lamblia*, there was significant association (P-value=0.009) chi-square test criterion value (5.726).

Table (4) Sex-Specific Prevalence of Intestinal Protozoan Infections in Cancer Patients by nested PCR.

Sex	Nested PCR No. (%)			Total	P-Value
	Cryptosporidium parvum	*Giardia lamblia*	*Entamoeba histolytica*		
Male	25 (18.12%)	7 (5.07%)	21 (15.22%)	53 (38.41%)	**0.009**
Female	30 (21.74%)	17 (12.32%)	38 (27.54%)	85 (61.59%)	
Total	55 (39.86%)	24 (17.39%)	59 (42.75%)	138 (100%)	
χ^2 Value	**5.726**				

For nested PCR it was noted that the frequency is (59) and for the percentage (42.75%) with *Entamoeba histolytica* , the frequency is (55) and for the percentage(39.86%) with *Cryptosporidium parvum* and last place with a frequency of (24) and for the percentage (17.39%) with *Giardia lamblia*, there was significant association (P-value=0.027) chi-square test criterion value (6.046).

Table 5: Distribution of intestinal parasite species among study participants.

Age group (years)	Nested PCR No. (%)			Total	P-Value
	Cryptosporidium parvum	*Giardia lamblia*	*Entamoeba histolytica*		
9-24	2 (1.45%)	2 (1.45%)	1 (0.72%)	5 (3.62%)	**0.027**
25-40	16 (11.59%)	7 (5.07%)	17 (12.32%)	40 (28.99%)	
41-56	17 (12.32%)	8 (5.8%)	18 (13.04%)	43 (31.16%)	
57-72	15 (10.87%)	6 (4.35%)	17 (12.32%)	38 (27.54%)	
73-88	5 (3.52%)	1 (0.72%)	6 (4.35%)	12 (8.7%)	
Total	55 (39.86%)	24 (17.39%)	59 (42.75%)	138 (100%)	
χ^2 Value	**6.046**				

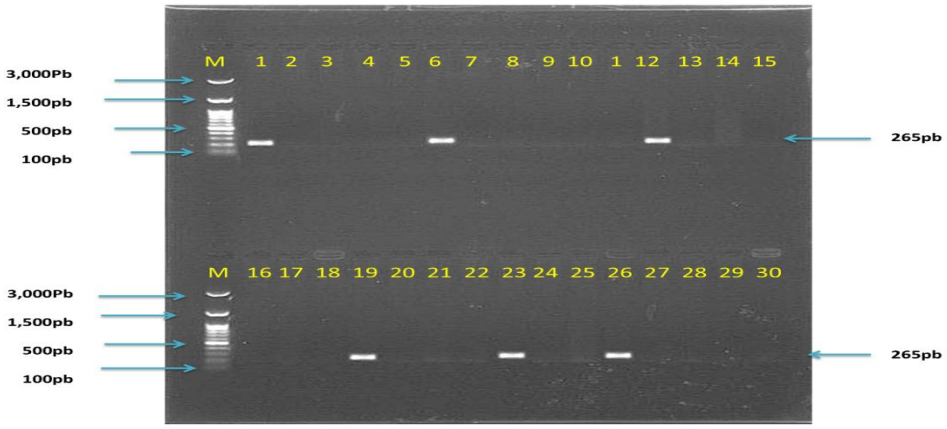
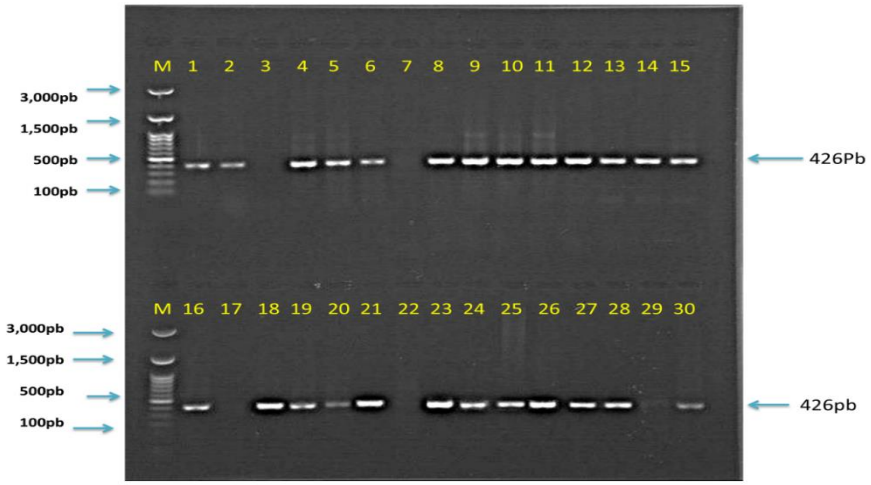
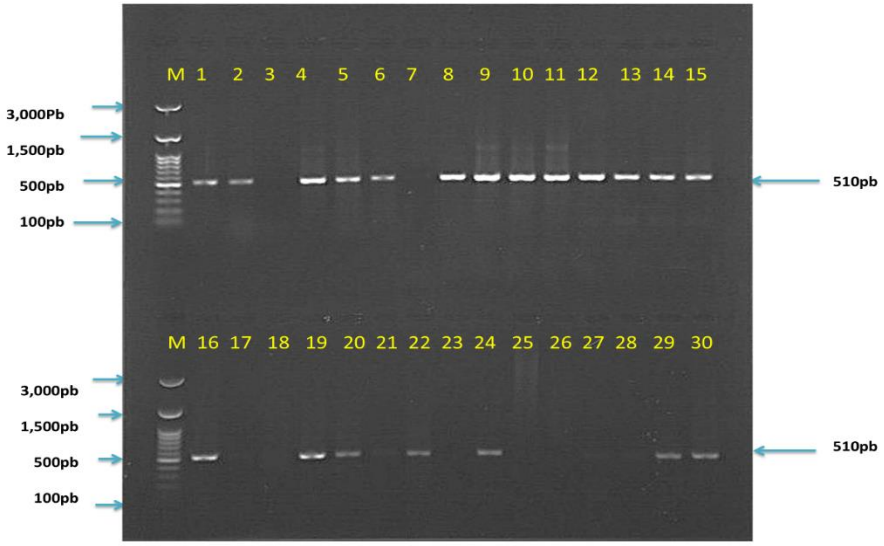


Figure 1: Agarose gel electrophoresis of nested PCR products for *Giardia lamblia*.
Lane M: 100-3000 bp DNA ladder. Lanes 1-5: Clinical stool samples from cancer patients. Lane 6: Positive control (*G. lamblia* DNA). Lane 7: Negative control (Nuclease-free water). The expected amplicon size for *G. lamblia* is 265 bp (indicated by the arrow). Electrophoresis was performed on a 0.8% agarose gel in 1X TPE buffer.



Figar2: Agarose gel electrophoresis of nested PCR products for Cryptosporidium *parvum*.
Lane M: 100-3000 bp DNA ladder. Lanes 1-4: Clinical stool samples from cancer patients. Lane 5: Positive control (*C. parvum* DNA). Lane 7: Negative control (Nuclease-free water). The expected amplicon size for *C. parvum* is 510 bp (indicated by the **arrow). Electrophoresis was performed on a 0.8% agarose gel in 1X TPE buffer.**



Figar3: Agarose gel electrophoresis of nested PCR products for *Entamoeba histolytica.*
Lane M: 100-3000 bp DNA ladder. Lanes 1-6: Clinical stool samples from cancer patients. Lane 8: Positive control (*E. histolytica* DNA). Lane 7: Negative control (Nuclease-free water). The expected amplicon size for *C. parvum* is 426 bp (indicated by the arrow). Electrophoresis was performed on a 0.8% agarose gel in 1X TPE buffer.

Table (6) shows the arithmetic mean, standard deviations and t-test for infection of each parasite and immune markers (IL10, IL25). It was noted that the arithmetic mean and standard deviation of IL10 with ***E. histolytica*** (501.63 ; 84.38) respectively and the t test and the P- value (45.66 , 0.000) respectively and through the results it was noted that they were statistically significant. It was noted that the arithmetic mean and standard deviation of IL10 with ***G. lamblia*** (536.38 ; 100.54) respectively and the t test and the P- value (26.136 , 0.000) respectively and through the results it was noted that they were statistically significant. It was noted that the arithmetic mean and standard deviation of IL10 with ***C. parvium***(508.80 ; 80.39)respectively and the t test and the P- value (46.46 , 0.000) respectively and through the results it was noted that they were statistically significant.It was noted that the arithmetic mean and standard deviation of IL25 with ***E. histolytica*** (1087.08; 390.87)respectively and the t test and the P- value (21.36 , 0.000) respectively and through the results it was noted that they were statistically significant. It was noted that the arithmetic mean and standard deviation of 25 with ***G. lamblia*** (1244.58; 553.66)respectively and the t test and the P- value (11.013, 0.000) respectively and through the results it was noted that they were statistically significant. It was noted that the arithmetic mean and standard deviationofIL25 with ***C. parvium***(1119.91; 386.98) respectively and the t test and the P- value (21.46, 0.000) respectively and through the results it was noted that they were statistically significant.

Table (6) Comparative Analysis of IL-10 and IL-25 Serum Levels in Protozoan Infections.

Protozoan Infections	N	IL10 (mean and Std. Deviation)	IL25 (mean and Std.Deviation)	t – test IL10	t – test IL25	P-Value IL10	P-Value IL25
E. histolytica	**59**	501.63 ∓ 84.38	1087.08∓390.87	45.663	21.363	.000	.000
G. lamblia	**24**	536.38 ∓ 100.54	1244.58∓553.66	26.136	11.013	.000	.000
C. parvium	**55**	508.80 ∓ 80.39	1119.91∓386.98	46.936	21.462	.000	.000

To further investigate the relationship between the two cytokines, a Spearman's rank-order correlation was performed on the data from all infected patients (n=138). The analysis revealed a statistically significant, moderate positive correlation between serum levels of IL-10 and IL-25 ($r_s(136) = 0.421$, $p < 0.001$). This result indicates that as the concentration of one cytokine increases, the concentration of the other tends to increase as well, suggesting a potential coordinated immunomodulatory response in cancer patients with protozoan infections.

Table (7): Spearman Correlation between IL-10 and IL-25 Serum Levels in Infected Cancer Patients (n=138).

Cytokine Pair	Correlation Coefficient (rs)	P-Value
IL-10 & IL-25	**0.421**	**< 0.001**

DISCUSSION

The present study aimed to determine the prevalence of three major intestinal protozoan parasites—*Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum*—in cancer patients in Wasit Province and to investigate the associated immune response through the serum levels of IL-10 and IL-25. Our findings reveal a high overall prevalence (61.59%) of intestinal protozoan infections among the studied cancer patients, as detected by nested PCR. *Entamoeba histolytica* was the most prevalent parasite (42.75%), followed by *Cryptosporidium parvum* (39.86%) and *Giardia lamblia* (17.39%). This high prevalence underscores the significant burden of parasitic co-infections in immunocompromised individuals, such as cancer patients, who often have impaired immune defenses due to both the malignancy itself and the immunosuppressive effects of chemotherapy or radiotherapy(6).In table (4) This study is agree with study in Iran University of Medical Sciences, the results of laboratory tests for 158 collected samples showed that the percentage of infection with parasites was the prevalence of protozoan infections was 24 (48%) in men and 26 (52%) in women among the cancer patients(15). This study is agree with study in University of Malaya Medical Centre, a convenient sampling method was utilised to gather 134 stool samples from cancer patients aged one and above at the Oncology Unit, UMMC(16). This study is agree with study in Sana'a University in Yemen, total samples were 436 collected from participants who were invited to participate voluntarily(17). On the other hand in Shahrekord University of Medical Sciences, Shahrekord, Iran disagreed Among the 250 cancer patients are admitted to the oncology ward of Kashani hospital every month samples, the total infection rate of *G.lamblia* was 5 (2%)(18). Also different with study in Jilin University(China)this study included 195 patients *Cryptosporidium* spp. was detected in 26 out of 195 patients with diagnosed gastrointestinal cancers (13.33%)(19). The significantly higher prevalence of protozoan infections observed among female participants (61.59%) compared to males (38.41%) may be attributed to several interrelated factors. Firstly, the higher proportion of females in our study sample (60%) inherently influences the overall rate. Beyond this sampling bias, behavioural factors are likely significant; in the local context, women are often primarily responsible for caregiving and food preparation, potentially increasing their frequency of exposure to contaminated water or food sources (12). Furthermore, the potential immunomodulatory effects of sexual hormones on immune responses to parasites cannot be ruled out and warrant further investigation.In table (5) this study is agree with study in Iran University of Medical Sciences, the results of laboratory tests for 158 collected samples showed that the percentage of infection with parasites was the prevalence of protozoan infections was 28 (56%) in the group under 50 years and 22 (44%) in those over 50 years(15). This study is agree with study in Sana'a University in Yemen, total samples were 436 collected from participants who were invited to participate voluntarily(17). On the other hand in Shahrekord University of Medical Sciences, Shahrekord, Iran disagreed Among the 250 cancer patients are admitted to the oncology ward of Kashani hospital every month samples, the total infection rate of *G.lamblia* was 5 (2%)(18). Also different with study in Jilin University(China)this study included 195 patients *Cryptosporidium* spp. was detected in 26 out of 195 patients with diagnosed gastrointestinal cancers (13.33%)(19).In table (6) this study is agree with study in University of Kufa, the study was conducted on350 potential clients the results of this investigation showed that the concentration of (IL-10) in patient infection with *E.*

histolytica (818.81± 99.03 ng/L)(20). Also result similarty in University of Thi-qar, A total of 80 patients . The results showed a significant difference ($p<0.001$) in the serum level of IL-25 in patients with *E. histolytica* infection (4275.19 pg/mL)(21). Also result similarty in Al-Mustansiriya University, A total of 86 diarrheal fecal samples were collected from children in age <1 year to 13 years suspected to be infected with *E. histolytica* A significant elevation in the serum level IL25 in relation to the expression of EhCRT Ag (delta value) in the stool samples of children whom strongly expressed EhCRT Ag, their serum levels of of IL25 was 4052.64 ng/l than that of EhCRT Ag not expressed(22). This study is agree with study in College of Science, Thi-Qar University, the result of present study revealed that 24 patients from 375 were positive for G. lamblia. In this study, the amount of IL-10 in infected patients was (14.71 Pg/ml)(23). Also result similarty in Kerman University of Medical Sciences, Further analyses showed that human subjects infected with *G. duodenalis* genotype AI had significantly elevated levels of serum IL-10(24).This study is agree with study in A total of 664 freshly collected stool samples from six different states in 517 Nigeria were mixed individually with an equal volume of 95% ethanol within an hour to 518 preserve the integrity of the DNA , enhanced production of IL-10, heightened goblet cell activity and increased mucus secretion, as well as elevated IL-25 levels in the small intestine(25).This study is agree with study in India levels The median IL-10 levels were found to be significantly higher for *Cryptosporidium*-infected (848.2)(26). The digestive tract, frequently compromised in gastrointestinal cancers, may provide a more susceptible environment for these opportunistic pathogens. The higher infection rate observed in females (61.59%) compared to males (38.41%) could be attributed to a combination of biological, social, and cultural factors. The higher number of female participants in our study sample (60%) is a contributing factor, potentially reflecting higher rates of certain cancers like breast cancer in this demographic. Furthermore, societal roles often associated with caregiving and food preparation may increase women's exposure to contaminated sources(12 ;13). The age group 41-56 years showed the highest frequency of infection (31.16%), which may coincide with the peak incidence of certain cancers and increased environmental exposure over time. The significantly elevated serum levels of both IL-10 and IL-25 across all three protozoan infections are a central finding of this study. IL-10, a potent anti-inflammatory cytokine, was markedly increased in infected patients. This aligns with its well-established role in modulating immune responses to prevent excessive tissue damage during infection(8). The significantly elevated IL-10 levels likely constitute a double-edged sword: while this immunosuppressive cytokine may protect the host from acute immunopathology, it also facilitates parasite persistence by dampening protective effector immune responses and, concurrently, may inhibit anti-tumor immunity by suppressing cytotoxic T-cell and NK-cell activity, potentially creating a permissive environment for both infection and cancer progression(16).However, in the context of cancer and parasitism, this immunosuppressive response may constitute a double-edged sword. While it may protect the host from acute immunopathology, it could also facilitate parasite persistence and potentially hinder the host's anti-tumor immunity, creating a more permissive environment for both the parasite and the cancer to thrive(9). Our results are consistent with previous studies that reported elevated IL-10 in patients infected with *E. histolytica*, *G. lamblia*, and *Cryptosporidium* spp(28 ;25 ;8). Similarly, IL-25, a cytokine primarily produced by intestinal epithelial tuft cells, was significantly elevated. IL-25 is a key initiator of type 2 immune responses, which are crucial for expelling helminths and controlling certain protozoan infections (11). The elevated IL-25 levels observed suggest an active attempt by the host's epithelial barrier to sense the parasitic threat and mobilize an appropriate defensive reaction, including the recruitment of regulatory and type 2 effector cells. This finding is supported by work from Khalaf et al. (2022) and AKh & FAb (2024), who also found significant increases in IL-25 in response to *E. histolytica* infection. The interplay between these elevated cytokines (IL-10 and IL-25), chronic parasitic infection, and a background state of cancer-related immunosuppression is complex. It is plausible that parasites exploit the host's immunoregulatory mechanisms, such as IL-10 production, to establish chronic infections. Conversely, the cancer-induced immunosuppression may render the patient more susceptible to these opportunistic infections in the first place. This vicious cycle can lead to worsened nutritional status, increased treatment complications, and potentially poorer overall outcomes for the cancer patient(14). When compared to other studies, our findings of a high prevalence of protozoan infections are consistent with research from Iran and Yemen (17; 19) but contrast with studies from Iran and China that reported lower rates (20; 21). These discrepancies highlight the importance of geographical location, local sanitation standards, dietary habits, and the specific diagnostic methods employed. The use of highly sensitive nested PCR in our study likely contributed to the higher detection rates compared to studies relying on conventional microscopy.

Conclusion and Recommendations

In conclusion, this study demonstrates a high prevalence of intestinal protozoan infections among cancer patients in Wasit Province, with *E. histolytica* and *C. parvum* being the most common. The infections are associated with a significant alteration of the host immune response, characterized by a sharp increase in the immunosuppressive cytokine IL-10 and the epithelial-derived alarmin IL-25. It is important to acknowledge that the cross-sectional design of this study captures data at a single point in time, which precludes the establishment of causal relationships between protozoan infections and the observed cytokine profiles. To definitively determine causality and elucidate the temporal dynamics of this interaction, future longitudinal cohort studies are recommended, tracking cancer patients from diagnosis through treatment to monitor incident infections and concurrent immune changes.

We recommend:

1. Routine Screening: Implementing routine molecular screening for intestinal parasites in cancer patients, especially before initiating immunosuppressive therapy.
2. Preventive Education: Enhancing patient education on hygiene practices to prevent fecal-oral transmission of parasites.
3. Further Research: Conducting larger longitudinal studies to elucidate the causal relationships between parasitic infections, cytokine modulation, and cancer progression and prognosis.
4. Clinical Consideration: Clinicians should maintain a high index of suspicion for parasitic infections in cancer patients presenting with gastrointestinal symptoms like diarrhea, as timely diagnosis and treatment could improve patient management and quality of life.

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