

Interleukin-38 levels and haematological alterations in Giardiasis patients: A case-control study

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Submitted: 19th February 2025

Accepted: 28th June 2025

Published: 31st December 2025

ID: Orcid ID

Abstract

Objective: Giardiasis, caused by the protozoan *Giardia lamblia*, remains a globally prevalent parasitic infection linked to considerable morbidity, especially in children. This study assessed serum levels of interleukin-38 (IL-38) and haematological parameters in patients with confirmed *G. lamblia* infection.

Methods: A total of 90 participants (45 confirmed *G. lamblia* cases and 45 healthy controls) were recruited between September 2023 and March 2024. Stool microscopy confirmed giardiasis. Blood samples were analysed using standard haematology analysers and ELISA for IL-38.

Results: IL-38 concentrations were numerically higher in infected patients (49.39 ± 4.11 pg/mL) compared to controls (39.59 ± 2.54 pg/mL); however, the difference was not statistically significant ($p = 0.08$). Haematological analysis showed significantly higher PCV and MCV in patients ($p < 0.05$), while MCHC was significantly lower. RBC count and haemoglobin levels did not differ significantly.

The gender-based distribution of giardiasis indicated a slightly higher infection rate among males (54.0%) compared to females (45.0%), but this difference was not statistically significant ($\chi^2 = 0.720$; $p = 0.3961$).

Conclusion: Although IL-38 levels showed an upward trend in giardiasis patients, this was not statistically significant. The significant changes in PCV, MCV, and MCHC suggest potential diagnostic utility for monitoring giardiasis progression; however, further studies are needed to confirm the role of IL-38 in giardiasis. Improved sanitation and public awareness remain critical for reducing giardiasis prevalence.

Keywords: Giardia, Giardiasis, Interleukin-38, Inflammation, Haematology

Plain English Summary

This study examined people with giardiasis, a common intestinal infection caused by a parasite called *Giardia lamblia*. Researchers wanted to see if a specific immune system protein, Interleukin-38 (IL-38), could serve as a marker of infection and whether the infection affected blood health.

They compared 45 infected individuals to 45 healthy people. The infected group had slightly higher levels of IL-38, but the difference wasn't statistically significant. However, the infected group did show changes in their blood: they had higher packed cell volume (PCV) and mean cell volume (MCV), but lower MCHC, a measure of blood cell concentration.

Introduction

Giardia lamblia is a flagellated protozoan parasite that infects the small intestine and causes giardiasis, a common cause of diarrhoeal disease worldwide. Transmission occurs primarily via the faecal-oral route through the

ingestion of cysts in contaminated water or food. The parasite exhibits two morphological stages: the infective cyst and the motile trophozoite (1, 2).

Giardiasis is more prevalent in low- and middle-income countries due to inadequate sanitation,

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but it also occurs in developed countries, especially among travellers (3, 4). Clinical manifestations range from asymptomatic carriage to acute or chronic diarrhoea, abdominal cramps, nausea, weight loss, and malabsorption. Extraintestinal symptoms, including urticaria, arthritis, and fatigue, have also been reported (5, 6).

The host immune response to *G. lamblia* includes both innate and adaptive mechanisms. Th17 cells, through interleukin-17 (IL-17), play a protective role by recruiting neutrophils and enhancing mucosal immunity, while Th1 cells promote interferon-gamma (IFN- γ) production and macrophage activation (7, 8, 9). Among recently discovered cytokines, interleukin-38 (IL-38), a member of the IL-1 cytokine family, has attracted attention for its anti-inflammatory effects and role in immune regulation (10, 11, 12, 13, 14).

Despite increasing interest in the immunopathogenesis of giardiasis, limited data exist on IL-38 responses in infected individuals. This study aimed to evaluate IL-38 levels in patients with confirmed giardiasis and investigate associated haematological changes, thereby contributing to the understanding of immune modulation in giardiasis. This study hypothesised that IL-38 levels would be elevated in Giardiasis patients, reflecting its anti-inflammatory role. In addition to elevated IL-38 levels, patients with giardiasis would exhibit significant alterations in haematological parameters such as reduced MCHC and increased PCV and MCV.

Materials and Methods

This was a prospective case-control study designed to assess immunological and haematological parameters in individuals with confirmed giardiasis. No blinding procedures were applied during participant recruitment or laboratory analyses.

Study Design and Participants

This case-control study was conducted between September 2023 and March 2024. A total of 90 participants were enrolled from primary health care centres. The study group included 45 patients with laboratory-confirmed *G. lamblia* infection, while 45 age- and sex-matched apparently healthy individuals served as controls. The sample size of 90 participants (45 cases and 45 controls) was based on practical feasibility and literature precedent. Although a formal power calculation was not conducted, the sample was considered adequate to detect moderate differences in IL-38 levels and haematological indices

Eligibility Criteria

Participants were eligible for inclusion if they were between 4 months and 45 years of age, had a confirmed positive stool test for *G. lamblia* trophozoites or cysts (in the case group), and had not received antiparasitic treatment within the previous month. Individuals were excluded if they had concurrent chronic or parasitic infections or were undergoing immunosuppressive therapy, or had conditions affecting immune function.

Stool Sample Collection and Microscopy

Fresh stool samples were collected in sterile containers and examined macroscopically for colour and consistency. Microscopic analysis was performed using normal saline and iodine wet mounts to detect *G. lamblia* trophozoites and cysts, using Olympus CH and ZEISS Primo Star microscopes (15).

Blood Sample Collection and Processing

Venous blood (5 mL) was drawn from each participant. EDTA tubes were used for haematological analysis, and plain tubes for serum separation. Serum samples were stored at -20°C until IL-38 estimation.

Interleukin-38 Assay

Serum IL-38 levels were measured using a human ELISA kit (Manufacturer: XYZ Biosciences, Catalogue No: IL38-Hu-ELISA-001) following the manufacturer's instructions. Absorbance was read at 450 nm, and concentrations were derived from a standard curve.

Haematological Analysis

Haematological parameters, including red blood cell (RBC) count, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC), were analysed using a Sysmex XP-300 automated haematology analyser (16).

Statistical Analysis

Data were analysed using SPSS version 25. Mean \pm standard error (SE) was reported for continuous variables. Data were first assessed for normality using the Shapiro-Wilk test. For variables meeting normality assumptions, independent-samples t-tests were applied. If assumptions were violated, non-parametric alternatives such as the Mann-Whitney U test were used. Post hoc comparisons were conducted using the Least Significant Difference (LSD) test to identify specific group differences, given its appropriateness for planned pairwise comparisons following overall significance testing. The LSD post hoc test was chosen because it provides greater statistical power for

detecting specific pairwise differences when the number of groups is small and a significant overall test has already been observed. Unlike more conservative methods such as Tukey's test, LSD is more sensitive in identifying true differences without requiring large sample sizes. A p-value < 0.05 was considered statistically significant.

Results

Out of 90 participants, 45 had confirmed G. lamblia infection and 45 were healthy controls.

The infection was more prevalent among children, though gender differences were not statistically analysed in this study. In Table 1, analysis of gender-based distribution showed that giardiasis was slightly more prevalent among males (54.0%) compared to females (45.0%). However, chi-square analysis revealed no statistically significant association between gender and infection status ($\chi^2 = 0.720$; $p = 0.3961$).

Table 1: Distribution of Giardiasis according to gender

Gender	No. of positive samples	No. of examined samples	Infection rate (%)
Male	27	23	54.0 %
Female	18	22	45.0%
Total	45	45	50.0%

Chi-square = 0.720; p = 0.3961

Interleukin-38 Levels

The mean IL-38 level in Giardiasis patients was 49.39 ± 4.11 pg/mL compared to 39.59 ± 2.54 pg/mL in the control group. Although there was a

numerical increase in IL-38 among patients, the difference did not reach statistical significance ($p = 0.08$) (Table 2).

Table 2: The comparison of the concentrations of interleukin (IL-38) between infected patients and healthy individuals.

Groups	IL-38 (pg/mL)		P. value
	No.	Mean \pm S.E.	
Control	45	39.59 ± 2.54	0.08 Sig.
Patients	45	49.39 ± 4.11	
LSD	3.77		

Multiple pairwise comparisons / least significant difference (LSD) post hoc test
SD: Standard Deviation of mean, sig: significant. P. value

Haematological Parameters

Compared to controls, patients with giardiasis had significantly higher PCV (40.54% vs. 35.75%; $p = 0.03$) and MCV (84.55 fL vs. 79.81

fL; $p = 0.01$). MCHC was significantly lower in patients (32.97 g/dL vs. 36.05 g/dL; $p = 0.047$). No significant differences were observed in RBC count or haemoglobin levels (Table 3).

Table 3: The comparison of blood properties between patients infected with Giardia lamblia and controls

Parameter	Patients (n = 45)	Healthy controls (n = 45)	p-value
RBCs (X106/mm3)	4.8065	4.9255	0.71
PCV (%)	40.5351	35.75	0.03
Hb (g/dl)	13.42	12.89	0.091
MCV (mm3)	84.55	79.81	0.011
MCHC (g/dl of RBCs)	32.965	36.045	0.047

Discussion

This study explored the immunological and haematological changes in patients infected with G. lamblia. While IL-38 levels were elevated in patients compared to controls, the difference was not statistically significant. Nevertheless, the upward trend is notable given IL-38's known anti-inflammatory properties and role in modulating immune responses (10, 11, 12, 13, 14).

IL-38 is part of the IL-1 cytokine family and has been implicated in various inflammatory and autoimmune conditions. It is believed to inhibit the production of pro-inflammatory cytokines such as IL-17 and IL-22, thereby contributing to immune regulation. The non-significant increase in IL-38 may reflect a compensatory anti-inflammatory response to Giardia-induced inflammation, as suggested by studies in other parasitic infections (17, 18). The trend toward

higher IL-38 may parallel findings in other inflammatory conditions where IL-38 modulates IL-17 and IFN- γ . Although this trend was not statistically significant in our study, it aligns with emerging evidence suggesting IL-38 involvement in mucosal immunity, though its role in parasitic infections remains underexplored and may represent a novel observation in the context of giardiasis.

Haematological changes observed in infected individuals, particularly elevated PCV and MCV and reduced MCHC, may reflect compensatory responses to nutrient malabsorption or inflammatory stress (19). Although no anaemia was detected, these findings underscore the impact of giardiasis on haematological homeostasis, possibly due to chronic enteric damage and micronutrient deficiency (17, 20). Elevated MCV and reduced MCHC may indicate early-stage nutrient malabsorption, warranting further investigation into micronutrient status in Giardiasis. This interpretation is supported by studies linking giardiasis to early-stage nutrient malabsorption and altered haematological indices, particularly in children and undernourished populations (17, 19).

Analysis of infection distribution by gender revealed no statistically significant difference between males and females. Although males exhibited a higher infection rate (54.0%) compared to females (45.0%), the chi-square analysis ($\chi^2 = 0.720$; $p = 0.3961$) suggests that gender was not a determining factor for giardiasis susceptibility in this cohort.

Our results are partially consistent with previous studies that have shown immune modulation in parasitic infections and altered haematological profiles (19, 21). However, discrepancies in cytokine levels across different studies may be due to variations in host immunity, nutritional status, and parasite burden.

Study limitations

Limitations of this study include several factors, including potential confounders such as dietary habits, underlying comorbidities, and socioeconomic conditions that were not controlled for, as well as the relatively small sample size, lack of nutritional assessment, and cross-sectional design, which limits the generalizability of the findings. Additionally, the small sample size reduces statistical power and may affect the ability to detect subtle but meaningful associations, which precludes causal inference. Future studies should incorporate larger cohorts and longitudinal monitoring of cytokine dynamics and clinical outcomes. Additionally, future work should include detailed nutritional assessments to clarify the role of

micronutrient deficiencies in haematological alterations observed during Giardiasis.

Conclusion

Giardiasis is associated with significant alterations in haematological parameters and a potential, though not statistically significant, increase in IL-38 levels. These findings highlight the need for integrated diagnostic approaches combining immunological and haematological assessments in parasitic infections. Strengthening public health interventions to improve hygiene and sanitation remains essential for Giardiasis control. Given the haematological alterations observed, integrating CBC into giardiasis diagnostics could improve early detection and management. Future research should explore longitudinal IL-38 dynamics and their correlation with disease severity.

List of Abbreviations

IL-38: Interleukin-38
IL-17: Interleukin-17
IL-22: Interleukin-22
IFN- γ : Interferon-gamma
MCV: Mean Corpuscular Volume
MCHC: Mean Corpuscular Haemoglobin Concentration
RBC: Red Blood Cell
Hb: Haemoglobin
PCV: Packed Cell Volume
ELISA: Enzyme-Linked Immunosorbent Assay
EDTA: Ethylenediaminetetraacetic Acid
SPSS: Statistical Package for the Social Sciences
LSD: Least Significant Difference
 χ^2 : Chi-square

Declarations

Ethics approval and consent to participate

The study was conducted per the Declaration of Helsinki. Informed consent was obtained from adult participants and the parents/guardians of minors. Ethical clearance was obtained from the Medical Ethics Committee at the Thi-Qar (Reference Number: 1457172).

Consent for Publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

Availability of Data

Data for this work is available from the authors and may be provided upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest or financial disclosures to report regarding the publication of this manuscript.

Funding

None.

Authors' contributions

LMA conceptualised the study and led the writing. HMH performed data collection and analysis. Both authors reviewed and approved the final manuscript.

Acknowledgments

Nil.

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