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Evaluation of Some Biomarkers Associated with Anemia in Patients who infected with *Giardia intestinalis*

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Abstract

Introduction: Protozoan infections cause a major part of morbidity in developing countries with poor sanitation and water systems. One of the most prominent protozoan infections is Giardia intestinalis, which is a well-known intestinal infectious agent, triggering a host of gastrointestinal problems and malabsorption syndromes, the infection causing structural and functional changes to the intestinal mucosa. These changes to the intestinal mucosa result malabsorption of critical nutrients, such as iron, folate, and the vitamin B_{12}. The study aims to identify the changes in some of hematological and biochemical parameters pertaining to anemia.

Methods: A 200 fecal samples were examinate from both sex (100 male and 100 female) in the same ages to reveal a *G. intestinalis* infection. The patients were classified into four groups: *G. intestinalis* infection, Anemia persons, *G. intestinalis* infection and anemia together, and healthy persons as a control; then a blood samples were collected from the forth groups, that attending to parasitological lab. At General health laboratories in Holy Karbala Province – Iraq, from October 2025 until March 2026. A five ml of blood samples were taken and divided into two parts: 2 ml of each sample placed in coagulant tubes and the remaining (3 ml) placed in anticoagulant tubes. The samples were measured and evaluated the mean ± SD and LSD test at $P \leq 0.05$ for hematological parameters (RBC, Hb, PCV, MCH, MCHC and WBCs), and some biochemical markers related with Hematopoiesis: Vit. D_3., Vit. B_{12}., Ferritin and serum iron.

Results: The results showed *G. intestinalis* infection effect on hematological parameters and biochemical markers related with hematopoiesis. Anemia in both male and female patients infected with Giardia and even some patients who did not present with anemia showed a reduction in RBC, Hb and PCV compared to the control group. Biochemical analysis showed that the infected and anemic group had low values of serum iron, ferritin and vitamin B_{12} compared to the control group, who had higher levels. Vitamin D3 levels were also showed a lower values in the group with *G. intestinalis* infection plus anemia compared with control group.

Conclusion: The study suggests that infection with *G. intestinalis* causes notable changes in some of hematological and biochemical parameters pertaining to anemia. These changes mirror the relationships between intestinal protozoan infection with blood parameters and nutrient absorption. Even in some patients the combination of *G. intestinalis* infection with anemia presented with the most changes in some parameters. All this changes and the already existing altered blood biochemical and hematological parameters in patients with giardiasis underline the infection's complications.

Keywords: Intestinal protozoa, Anemia, *Giardia intestinalis*, Vit. D_3., Vit. B_{12}

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Introduction

In developing nations, intestinal protozoan infections are a major cause of food and water-borne illnesses. Protozoan infections cause a major part of morbidity in developing countries with poor sanitation and water systems (1) .One of the most prominent protozoan infections is *Giardia intestinalis* (*Giardia lamblia*), which is a well-known intestinal infectious agent, triggering a host of gastrointestinal problems and malabsorption syndromes. Giardia lamblia infections primarily occur in the small intestine, leading to parasitic attachment to the epithelial layer of the intestine, causing structural and functional changes to the intestinal mucosa (2, 3). These changes to the intestinal mucosa result in the malabsorption of critical nutrients, such as iron, folate, and the vitamin B12 (4, 5). Intestinal protozoan infections cause chronic inflammation of the intestines which contribute to nutrient deficiencies and affect the blood (6).

There have been multiple research reports that appear to suggest the presence of intestinal protozoan parasites results in noticeable alterations of certain hematological markers that is, hemoglobin levels and mean corpuscular volume as a consequence of their effects on the nutrition and physiology of the infested individual (7). Further, impaired iron absorption and the inflammatory response can ultimately contribute to the development of anemia which is considered a worldwide public health concern across many demographic groups (5, 8).

Holy Karbala province is a region where the aforementioned environmental and demographic elements may aid in the transmission of intestinal protozoan parasites via means of population mobility and problems associated with sanitary systems (9,10).

Therefore, it will be quite relevant to evaluate the various biomarkers associated with anemia in regards to *G. intestinalis* infection within this particular population (2).

This study highlights to effectiveness of *G. intestinalis* infection in some of hematological and biochemical parameters pertaining to anemia, such as the number

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of RBCs, Hb, PCV, MCH, MCHC and WBCs, and, and some biochemical markers related with Hematopoiesis Vit. D_3., Vit. B_{12}, Ferritin and serum iron, compared with healthy persons (control group).

Materials and Methods

Study Design and Population

This study aims to assess certain biomarker related to anemia in G. intestinalis infected patients in Karbala. The study population consisted of patients visiting health centers and laboratories and was divided into four categories: patients with Giardia, patients with anemia, patients with both Giardia infection and anemia, and a healthy control group. The study included both males and females in the same ages. The grouping of participants was done based on lab identification of Giardia infection and a blood test for anemia.

Sample Collection and Diagnosis

Stool samples were taken from participants and analyzed using standard lab methods for Giardia infections. Identification of Giardia infections was based on sample detection of stages of parasites. All participants provided blood samples for hematological and biochemical analysis. Hematology parameters were analyzed through routine laboratory techniques.

Hematological Parameters

The following hematological parameters were evaluated (7, 9):

1. Red Blood Cell count (RBC)
2. Hemoglobin concentration (Hb)
3. Packed Cell Volume (PCV)
4. Mean Corpuscular Hemoglobin (MCH)
5. Mean Corpuscular Hemoglobin Concentration (MCHC)

White Blood Cell count (WBC)The parameters listed were employed to evaluate the hematological condition of the subjects and to identify the existence and classification of anemia. Biochemical parameters In addition to the hematological indices, the following biochemical parameters related to anemia were also assessed.

Biochemical Parameters

In addition to hematological indices, the following biochemical markers associated with anemia were measured: Serum Iron, Serum Ferritin, Vitamin D3 and Vitamin B12 (6, 7).Theoretical frameworks cite the association of the selected biomarkers with iron metabolism and anemia.

Statistical Analysis

The means and standard deviations are presented as MSD. The statistical evaluations of the study groups were done and the Least Significant Difference (LSD) method was applied to identify the significant differences between the groups. The significance level was set based on the P-values in the statistical tables (11).

Results

Hematological Parameters in Male Patients

Mean ± standard deviation (MSD), LSD and P values evaluating statistical significance are provided for the four groups: Giardia infected patients, anemic patients, patients with Giardia plus anemia, and the healthy (control group), for comparison of the male participant hematological parameters.

Compared to other groups, the control group had the highest values in RBC, Hb, PCV, MCH, MCHC, and WBC. The control group had the marker showing a significant difference from the Giardia infected and anemic groups. In the Giardia only infected group, the control group had marked b due to significant reductions in RBC, Hb, and PCV. The anemia group had similar reductions in RBC, Hb, and PCV compared to the control group, and the differences were significant in light of the reported LSD and P values.

The group with both Giardia infection and anemia showed further reduction in hematological parameters compared with the control group. The statistical analysis indicated a significant differences among the groups, as demonstrated by the different letter annotations in the Table 1, These values indicate significant differences between the study groups according to the reported P values.

Biochemical Parameters in Male Patients

The biochemical parameters related to anemia were also evaluated among male participants.

These included Vitamin D3, Vitamin B12, Ferritin, and serum Iron.

According to results in Table 2, Vitamin D3, the control group recorded the highest value (42.9), marked with the letter (a), while the Giardia infection plus anemic group showed lower values (13.8) marked with (b), indicating a significant differences among groups.

Vitamin B12 levels in the control group reached 484.7 and were marked with (a), while the other groups showed lower values marked with (b), indicating a statistical significance.

Participants in the control group showed the highest ferritin levels (71.6) and lower values in the infected and anemic groups in (b). The control group also showed a serum iron level of 83.9, and the remaining groups showed lower values marked with (b). The results show a significant changes in both metabolic and hematological biomarkers in male patients infected with Giardia intestinalis and especially when there's anemic co-morbidity.

Hematological Parameters in Female Patients

The hematological parameters of female participants were analyzed and compared among four groups: patients infected with Giardia, patients with anemia, patients with both Giardia infection and anemia, and the healthy control group. The results are expressed as

mean ± standard deviation (MSD), with LSD and P values used to determine statistical significance.

The control group (Table 3) showed the highest values in all hematological parameters and was marked with the letter (a), indicating a significant differences compared with the other groups. In the group infected with Giardia only, the values of RBC, Hb, PCV, MCH, MCHC, and WBC were lower than those of the control group and were marked mainly with the letter (b), indicating statistically significant differences.

The anemia group also showed reduced hematological values compared with the control group. Some parameters were marked with (bc), indicating intermediate values between the infected and control groups but still significantly different according to the reported statistical analysis.

The group with both Giardia infection and anemia demonstrated further reductions in several hematological indices compared with the control group, and these were marked with different letters (b or c), confirming the presence of significant differences among groups.

Biochemical Parameters in Female Patients

In the results which showed in Table 4, there was notable inconsistency for the levels of vitamin D3 among the groups. The control group recorded the highest value (41.5), labeled (a), while the infected and anemic groups showed lower values labeled (b) or (c) showing a significant differences.

For vitamin B12, control group values reached a total of 458.3 and were labeled with an (a). The other groups showed lower values, mostly labeled with a (b) showing a significant difference statistically compared to controls. Ferritin values were highest for the control group (69.8), where the infected and anemic groups had lower values labeled (b) showing significant differences. The levels of serum iron was also different among the groups where the control group had 82.8, while the infected and anemic groups had lower values labeled (b) or (c) showing a significant difference statistically.

Table 1. Effects of G. intestinalis infection on Hematological Parameters in Male Patients According to Study Groups

Measured hematological parameters	Study groups (Mean ±SE)				LSD P≤0.05
	G. intestinalis + Anemia	G. intestinalis	Anemia	Control	
Total RBCs (x10³/ ml)	3.46 ± 1.28 B	4.12 ± 1.82 B	3.41 ± 1.47 B	5.14 ± 1.81 a	0.83
Hb (gm / dl)	9.0 ± 0.9 C	11.5 ± 1.9 B	10.1 ± 1.3 B	14.5 ± 2.1 a	2.3
PCV %	30.2 ± 1.6 C	38.2 ± 4.1 B	33.6 ± 3.6 Bc	45.1 ± 5.1 a	7.5
MCH (x10³/ ml)	20.73 ± 2.2 B	24.92 ± 3.8 B	23.68 ± 3.6 B	32.18 ± 4.1 a	5.1
MCHC (x10³/ ml)	298.9 ± 10,9 B	322.8 ± 14.8 B	312.3 ± 13.4 Bc	355.6 ± 19.8 A	22.8
WBCs (x10³/ ml)	13.68 ± 2.95 B	12.39 ± 2.92 B	9.13 ± 2.1 B	7.45 ± 1.83 A	1.93

Table 2. Effects of G. intestinalis infection on Biochemical Biomarkers in Male Patients According to Study Group

Biochemical Parameters	Study groups (Mean ± SE)				LSD P≤0.05
	G.intestinalis + Anemia	G.intestinalis	Anemia	Control	
Vit. D3	13.8 ± 3.81 B	22.7 ± 4.16 B	21.3 ± 4.23 B	42.9 ± 5.92 A	9.15
Vit. B12	228.1 ± 63.7 B	305.4 ± 85.8 B	320.3 ± 97.6 B	484.7 ± 128.7 A	123.4
Ferritin	11.8 ± 2.7 B	21.3 ± 4.2 B	12.4 ± 2.9 B	71.6 ± 9.3 A	17.6
Iron	39.7 ± 11.4 B	55.6 ± 12.7 B	37.1 ± 9.4 B	83.9 ± 16.1 A	23.1

Table 3. Effects of G. intestinalis infection on Hematological Parameters in Female Patients According to Study Groups

Measured hematological parameters	Study groups (Mean ±SE)				LSD P≤0.05
	G. intestinalis + Anemia	G. intestinalis	Anemia	Control	
Total RBCs (x10³/ ml)	3.39 ± 1.14 B	3.94 ± 1.56 B	3.32 ± 1.35 B	4.98 ± 1.63 A	0.9
Hb (gm / dl)	8.9 ± 0.8 C	11.1 ± 1.6 B	9.9 ± 1.4 BC	13.9 ± 1.7 A	2.15
PCV %	29.8 ± 1.4 C	36.1 ± 3.9 B	33.1 ± 1.9 BC	43.2 ± 4.8 A	5.9
MCH (x10³/ ml)	20.13 ± 1.9 B	24.38 ± 3.6 B	22.36 ± 3.1 B	31.82 ± 3.5 A	5.3
MCHC (x10³/ ml)	299.2 ± 11.5 B	320.1 ± 15.2 B	308.3 ± 11.7 B	354.2 ± 19.1 A	24.3
WBCs (x10³/ ml)	13.36 ± 2.82 B	12.23 ± 3.16 B	8.75 ± 1.69 A	7.12 ± 1.92 A	1.78

Table 4. Effects of G. intestinalis infection on Biochemical Biomarkers in Female Patients According to Study Group

Biochemical Parameters	Study groups (Mean ±SE)				LSD $P \leq 0.05$
	G.intestinalis + Anemia	G.intestinalis	Anemia	Control	
Vit. D3	11.8 ± 3.59 C	20.3 ± 3.98 B	17.4 ± 4.23 BC	41.5 ± 5.83 A	8.22
Vit. B12	219.3 ± 59.2 B	298.7 ± 81.6 B	315.2 ± 91.4 B	458.3 ± 112.4 A	125.6
Ferritin	10.5 ± 2.4 B	19.4 ± 3.9 B	11.3 ± 2.8 B	69.8 ± 9.1 A	18.2
Iron	37.6 ± 10.3 C	59.8 ± 10.8 B	35.1 ± 8.3 BC	82.8 ± 15.3 A	21.5

Compared with the control group, the findings indicate that female patients infected with *G. intestinalis*, especially those with combined anemia, showed changes in the hematological and biochemical markers.

Discussion

This research focused on the blood and biochemical markers associated with anemia due to the infection of Giardia intestinalis among individuals in Karbala. The findings indicated a noteworthy distinction across many variables in relation to infected and anemic individuals, those with comorbidities, and the healthy controls. In both genders, there were notable hematological findings indicating a decreased number of red blood cells (RBC), hemoglobin (Hb) levels, and packed cell volume (PCV) in the infected and anemic groups as opposed to the control group (10,12).The lowest levels of these values were seen in those patients who were coinfected with anemia and the G. intestinalis. This leads to the conclusion that the malabsorption due to the intestinal protozoa (i.e., Giardia) infection and the nutritional blood parameters impact those individuals (8).

Given that a significant number of malnourished individuals have mucosal damage in the small intestine, the impaired absorption of iron and other essential nutrients, as indicated in the theoretical background, supports the infection of intestinal protozoa as the mechanism to impair the absorption of iron, folate, and vitamin B12, which are essential for the healthy production of red blood cells (4, 13, 14).

Furthermore, the inflammatory response that occurs during infections caused by intestinal protozoa could affect the body's processing of iron and subsequently lead to anemia (15).

Describe an effect of intestinal protozoa on changes of certain hematological parameters, which indicates a negative effect on the nutritional and physiological condition of the subjects. The biochemical data affirm this (12).

When comparing the infected and anemic groups to the control groups, females and males were found to have a lower serum iron and a lower serum ferritin. The finding agrees with the theory of chronic intestinal infection causing a negative iron balance and subsequently causing anemia (4, 15).

Infected groups also had a lower serum vitamin B12 compared to controls. Previous studies have shown that intestinal infections can impede the absorption of vitamin B12, which is critical in the process of erythropoiesis (7, 16).

Multiple groups showed differing levels of vitamin D3 within the study, revealing lower levels of the vitamin in both the infected and anemic groups compared to the control group (17).

These results could potentially be due to intestinal disorders that affect the absorption of nutrients and disrupt the balance of metabolism (4, 18).

The male-female differences for particular groups could be due to some physiological differences along with variation in the nutritional status and the overall effect of the infection and anemia combined. Yet, in both groups, the control set showed much higher levels of the studied parameters compared to the infected and anemic groups (19).

The study showed that Giardia intestinalis infection caused changes in certain parameters of anemia. It also showed that giardiasis and anemia together caused greater changes in certain parameters which showed the importance of early diagnosis and assessment of nutritional and hematological parameters from the bloodstream of the infected individuals (20-26).

Conclusion

The present study suggests that infection with Giardia intestinalis in Karbala causes notable changes in some of the hematological and biochemical parameters pertaining to anemia. Anemia in both male and female patients infected with Giardia and even some patients who did not present with anemia showed a reduction in RBC, hemoglobin (Hb), packed cell volume (PCV), and red blood cell indices compared to the control group. Biochemical analysis showed that the infected and anemic group had low values of serum iron, ferritin and vitamin B12 compared to the control group, who had higher levels. Variations of the Vitamin D3 levels were also exhibited in the study groups. These changes mirror the intestinal protozoan infection and blood parameters and nutrient absorption as previously described in the study. Even in some patients the combination of Giardia intestinalis infection with anemia presented with the most changes in some parameters. This and the already existing altered blood biochemical and hematological parameters in patients with giardiasis underline the infection's complications. Because of this, early infection

control measures should be implemented to lessen the complications of anemia in the affected patients.

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Competing Interests
The authors declare that they have no known competing financial interests that could have appeared to influence the work documented in the study.

Data Availability Statement
Data are available upon reasonable requesting.

Ethical Approval
The present study was conducted in accordance with the ethical standards of biomedical research involving human participants. Approval for the collection and analysis of fecal and blood samples was obtained from the General Health Laboratories Ethical Committee in Holy Karbala Province, Iraq. All participants were informed of the study objectives, procedures, and potential risks prior to sample collection. Written informed consent was obtained from each participant (or their legal guardian when applicable). Confidentiality of patient data was strictly maintained, and all samples were anonymized to protect participant identity. The study adhered to the principles outlined in the Declaration of Helsinki (2013 revision) regarding medical research ethics. No invasive procedures beyond routine blood sampling were performed, and all laboratory analyses were carried out under biosafety protocols.

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