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## **Study of some haematological and immunological parameters in patients infected with giardia lamblia in Najaf Governorate**

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**Abstract**---The current study aimed at finding the relationship between infection of *Giardia lamblia* and some hematological (complete blood count, concentration of ferritin) and immunological parameters (interleukin-6, interleukin-10), The present study was carried out from June, 2020 to May, 2021. A total of 509 out suspected patients samples and 50 healthy persons , whom have meant the laboratory of AL-Hakeem hospital ,Al Sader General Hospital ,AL-Zahra maternity and pediatrics and Al Furat General Hospital in AL-Najaf province.The results of the current study showed a high incidence of injuries among the age group (10-20) As the percentage (52.06%) , While the lowest rate of infection was in the age group (51-60) As the percentage (37.52%),The results of the current study showed a clear decrease in most of the blood parameters in people infected with the parasite *Giardia lamblia* Compared to the healthy group While the results showed an increase in eosinophilic blood cells in patients infected with the parasite *Giardia* compared to the healthy group, Result of study revealed that concentration of (IL-6) in male and female patients infected with *G. lamblia* were significant decrease ( $P < 0.05$ ) ( $211.92 \pm 13.07$  pg /ml), ( $263.75 \pm 21.73$ ) respectively in compared to the control group ( $481.54 \pm 19.83$  pg /ml), ( $394.66 \pm 32.98$  pg/ml) respectively , Result of study revealed that concentration of (IL-10) in

male and female patients infected with *G. lamblia* were significant decrease ( $P < 0.05$ ) ( $372.04 \pm 19.21$  pg /ml), ( $217.91 \pm 22.010$ ) respectively in compared to the control group ( $631.84 \pm 44.111$  pg /ml), ( $532.11 \pm 49.103$  pg/ml) respectively, The statistical analysis exhibited significant decrease ( $P < 0.05$ ) in patients ( $22.31 \pm 2.911$ ) and ( $16.33 \pm 1.6$ ) respectively compared with control group ( $194.12 \pm 42.05$ ) and ( $169.46 \pm 33.18$ ) respectively, infected with *G. lamblia* parasite .

**Keywords**---giardiasis, haematological parameters, ferritin, IL-8, Najaf Governorate.

## Introduction

*Giardia lamblia* is a pathogenic protozoan that colonizes in the small intestine of humans which attachment strongly to the host intestine and caused severe gastrointestinal disease [1],[2] . This microorganism a worldwide parasite may be leads to chronic diarrhea and malabsorption of human [3].The cyst of this parasite has ability to resistance the unsuitable condition and adaptations with external environment to survival, whereas trophozoite responsible on virulence properties and clinical symptomates in host[4] .

Giardiasis is a disease caused by *G. lamblia* parasite and may be acute or chronic infections, several symptoms associated with chronic infections such as vitamin deficiencies, lactase deficiency and fatty diarrhea as well as cramping intestine irritable bowel syndrome and fatigue [5]. Also this infection may be lead to malnutrition, weight loss, growth impairment and even poor cognitive development due to persistent diarrhea but not in acute diarrhea [6],[7],[8] [9] recorded that several pathological changes occur in the small intestine of human resulting in the malabsorption of nutrients similar to different non-infectious intestinal sicknesses like irritable bowel syndrome, celiac Crohn's and disease .Also iron status, vitamin A status and growth cognitive development were influences by this disease.[10], [11] Clinical symptoms of giardiasis depend on different factors such as virulence of the *Giardia* strain, number of mature cysts swallowed, age of the human, and host's immune system [12]. The several studies revealed the role of human's giardiasis in nutrient malabsorption and micronutrient deficiencies such as zinc, vitamin B-12, vitamin A and iron [13],[14],[15] Transmission occurs by ingestion of the cyst in contaminated food and water with feces. As few as 10 - 25 cysts are able to cause an infection in some humans(16). Excystation occur in the duodenum, where the trophozoite attach to the gut wall but does not invade, The trophozoite causes inflammation of the duodenal mucosa leading to malabsorption of protein and fat. Many of dividing trophozoite enter the colon and encyst as a response to bile salts and other stimuli (17). *Giardia lamblia* can produce a wide spectrum of clinical manifestations, from asymptomatic to acute or chronic diarrhoea with malabsorption of fat, vitamins A, B12 , iron and weight loss, nausea, anorexia, flatulence and abdominal cramps persisting from weeks or months. (18)(19).

## Materials and Methods

The present study was carried out from June, 2020 to May, 2021. A total of 509 out suspected patients samples and 50 healthy persons , whom have meant the laboratory of AL-Hakeem hospital ,Al Sader General Hospital ,AL-Zahra maternity and pediatrics and Al Furat General Hospital in AL-Najaf province This study was designed to determine the effects of *Giardia lamblia* infection on some biomarkers such (complete blood count, concentration of ferritin and IL-6, IL-6 )

### Blood Specimens collection

The samples of stool were collected into clean, wide-mouth specimen bottles, from patients and blood samples were also drawn from the same patients by vein puncture into specimen tubes and remains for 30 minutes at room temperature. After that the samples were centrifugation at 3000 rpm for 5 minutes (Backman/counter, Germany) to separate the serum and collected in other sterile tubes, each sample of serum was divided into five parts; each of them was kept in deep freeze at -20C° till used for the determination of IL-6, IL-10 and level of ferritin. were collected from aged (10-60) years belonging to both the gender and processed by Direct Smear method(59) and Formol Ether concentration technique. About 1.5 mL of blood were drawn with the help of sterile disposable syringes and collected in labeled sterile EDTA tubes

Examination of blood was done to estimate Hb value, PCV, TLC and DLC. Estimation of Hb value and hematocrit was done by Sahli's acid hematin method and Wintrobe's method(20), respectively. The WBC count was done with the help of hemocytometer(21) (Sigma Aldrich, St. Louis, Mo, USA) and differential counting was done by using Leishmann's staining method(22).

### Wet mount Examination

Freshly voided stool specimens were processed and examined microscopically using X40 objective lens for intestinal parasites as described by <sup>[23]</sup>. Before a slide was considered negative, ten X40 objective fields of the stool smears were examined.

### The Kits

The biomarker s in the current Study were estimated by Eliza Kits such as Human p-selectin (SELP) ELISA/ Kono Biotech/ Bulgaria (catalogue number KN0432Hu), Human Interleukin8 (IL-8) ELISA Kit/ Kono Biotech/ Bulgaria (catalogue number KN0923Hu), Immunoglobulin E(IgE)ELISA Kit/ Kono Biotech/ Bulgaria (catalogue number T1244A(69 Tests), Ferritin ELISA/ Monobind/ USA( product Code:2825-300) and spectro Kits such as Iron/ Biolabo/ France (02160Maizy France).

## Results

### Age group

The results of the current study showed a high incidence of injuries among the age group (10-20) As the percentage (52.06%) , While the lowest rate of infection was in the age group (51-60) As the percentage (37.52%)

Tabel (1) . Distribution of Giardia lamblia according to patient's age groups

Age group	No. examined	No. positive	Percentage (%)
10-20	121	63	52.06
21-30	107	45	42.05
31-40	118	38	32.20
41-50	84	27	32.14
51-60	79	18	22.78
Total	509	191	37.52

The study revealed that the Giardia lamblia prevalence rate ranged in males 118(42.44%) was higher than females 73(31.60 %)

Table ( 2). Distribution of Giardia lamblia according to patient's gender

Patients gender	No. examined	No. positive	Percentage (%)
Male	278	118	42.44
Female	231	73	31.60

### Hematological parameters

The results of the current study showed a clear decrease in most of the blood parameters in people infected with the parasite Giardia lamblia Compared to the healthy group While the results showed an increase in eosinophilic blood cells in patients infected with the parasite Giardia compared to the healthy group

Table (3): Comparative between hematological parameters in infected patient group and control group

Hematological parameters	patient group	control group
Hb (g/dl)	10.64±1.2*	13.08±0.79*
RBCs (X10 <sup>6</sup> /mm <sup>3</sup> )	3.55±0.72	5.71±0.33*
PCV (%)	31.03±3.83*	39.86±2.92
PLT (X10 <sup>3</sup> /mm <sup>3</sup> )	471.41±140.58*	323.84±60.16*
MCV (fL)	69.59±7.75	80.02±4.09
MCHC (g/dl)	35.19±2.17	35.71 ±1.27
WBCs (cells/mm <sup>2</sup> )	13.11±5.09*	6.85±1.49*
Neutrophils (%)	60.71 ±18.18*	51.04±9.42*
Basophils (%)	1±0.33*	0.48±0.27*
Eosinophils (%)	4.05±3.73*	2.31 ±1.38*
Lymphocytes (%)	34.57±12.39	34.35 ±4.82
Monocytes (%)	5.33 ±3.97	5.71 2.07

### Interleukin – 6 (IL – 6)

Result of study revealed that concentration of (IL-6) in male and female patients infected with *G. lamblia* were significant decrease ( $P < 0.05$ ) ( $211.92 \pm 13.07$  pg /ml), ( $263.75 \pm 21.73$ ) respectively in compared to the control group ( $481.54 \pm 19.83$  pg /ml), ( $394.66 \pm 32.98$  pg/ml) respectively, as seen in figure (2).

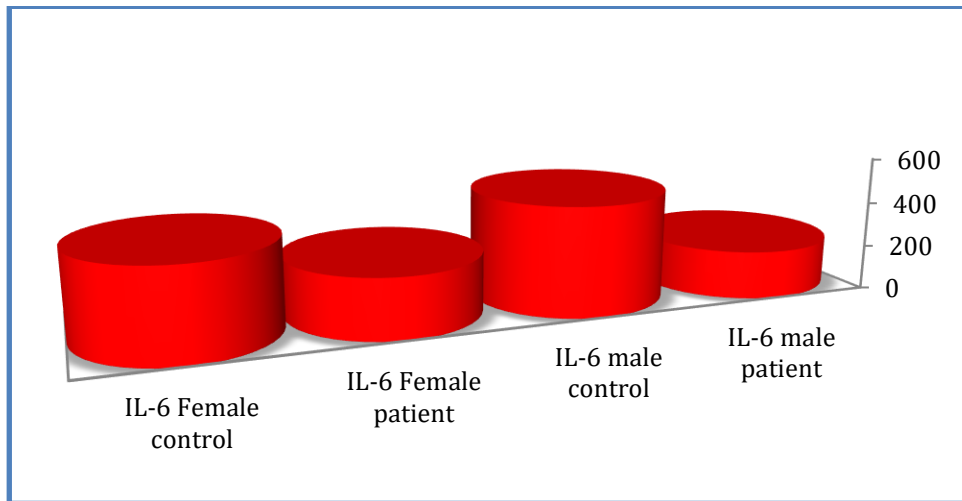


Figure 3: Concentration of IL-6 (pg/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and Control Group.

### Interleukin – 10 (IL – 10)

Result of study revealed that concentration of (IL-10) in male and female patients infected with *G. lamblia* were significant decrease ( $P < 0.05$ ) ( $372.04 \pm 19.21$  pg /ml), ( $217.91 \pm 22.010$ ) respectively in compared to the control group ( $631.84 \pm 44.111$  pg /ml), ( $532.11 \pm 49.103$  pg/ml) respectively, as seen in figure (2).

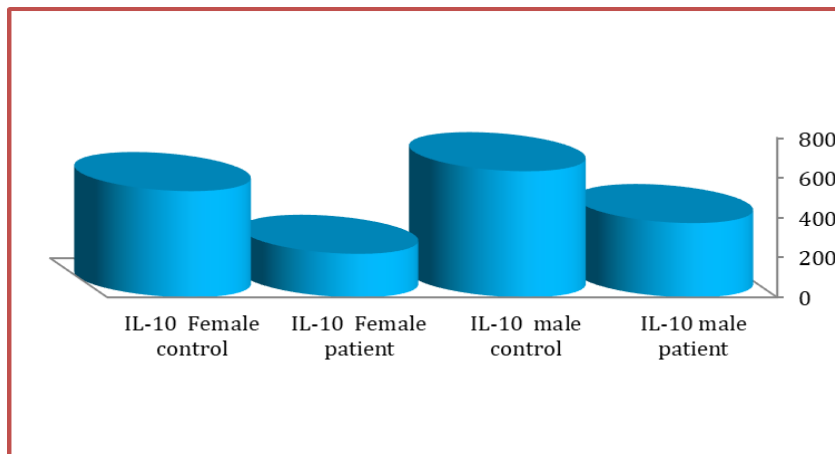


Figure 4: Concentration of IL-10 (pg/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and Control Group.

### Serum ferritin

The statistical analysis exhibited significant decrease ( $P < 0.05$ ) in patients ( $22.31 \pm 2.911$ ) and ( $16.33 \pm 1.6$ ) respectively compared with control group ( $194.12 \pm 42.05$ ) and ( $169.46 \pm 33.18$ ) respectively, infected with *G. lamblia* parasite, as seen in Figure (5).

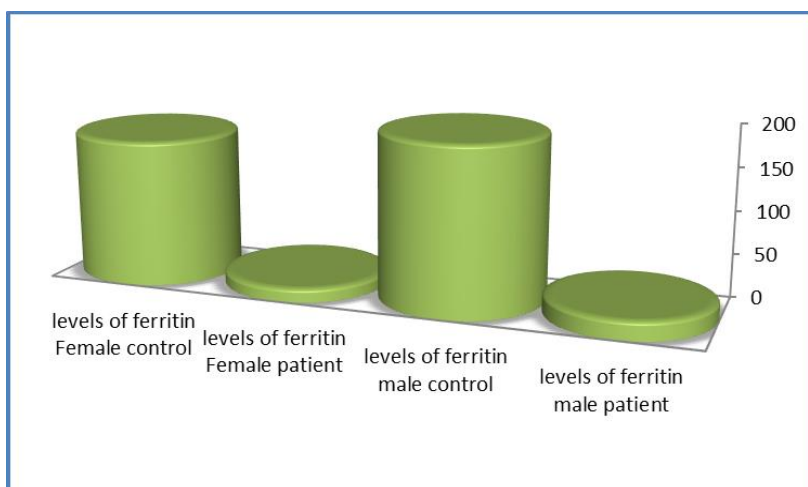


Figure (5 ): Concentration of levels ferritin Comparison between Patients Suffering from *Giardia lamblia* Infection and Control Group.

### Discussion

In the present study some epidemiological factors were associated with the rate of infection with *Giardia lamblia*, such as age groups and sex distributions should also be taken in to consideration in this study . Some other parameters may be affected by parasitic infections such as hematological status are studied. [24] *Giardia lamblia* infection exhibits a high rate among our community especially among children., such high rate of infection among children could be related to a number of factors such as low socioeconomic status and climatic conditions, overcrowding, poor health hygiene, low education of children, another important factor which affects the rate of giardiasis is the presence of asymptomatic patients in the community who can be considered as the main source of infection through continuously excreting the cysts stages with their stools. [25].

The results showed a significant decrease serum concentration of IL-6 and IL-10 in *G. lamblia* infection patients in compared to control group. This may be due to the impairment of cell-mediated immune response leading to decrease cytokine production by immunologically effector cells which is characterized produce cytokines leading to further damage of the host defense against infection this in turn badly affects all the biological processes in with IL-6 is involved in particular, activation of neutrophils and chemotaxis of different leukocytes[26,27].

In parasite infection Interleukin- 10 is produced by eosinophil and mast cell. It triggers the activation, differentiation, growth and chemotaxis of eosinophils. This

may be due to a pathogenic role of parasite infection, also causes increase in intestinal necrosis. The presence of eosinophils in human congenital toxoplasmosis probably related to the production of IL-5<sup>[28]</sup>. reported that tumor necrosis factor- $\alpha$  and interleukin-5 produced by macrophages, mast cells and lymphocytes, and these cytokines increase the cytotoxic activity of eosinophils. Many specific cytokine synthesized by Th2 lymphocytes such as "(IL-4, IL-5, IL-6, IL-10, IL-13, and IL-14)" the major cytokine responsible for the increase in the eosinophil population in parasitoses is interleukin-5 and play an important role in the pathogenesis of parasitic diseases<sup>[29]</sup>

Also<sup>[30]</sup> showed that these chemokines may have no influence on immunity to giardiasis and explain the chronic nature of this disease as *G. lamblia* usually extracellular parasite does not able to penetrate the epithelial layer therefore removal this parasite depends on the immune response of the host.

The results showed Decrease in the serum level of iron in patients infected with *G. lamblia* may be due to significant effect of giardiasis on iron malabsorption as it infects the duodenum the main site of iron absorption another possible reason for this significant change may be due to the possible high load of parasites in the intestine <sup>[31]</sup>. The result of study agree with study of <sup>[32]</sup> whom showed reduced iron absorption and reduced iron levels in children with symptomatic giardia in Turkey and Egypt respectively showed iron level were decreased during giardiasis due to malabsorption these conclusion were also suggested in a rat model <sup>[33]</sup> . <sup>[34]</sup> show over on quarter 26.4% of the children were identified as having iron deficiency anemia also<sup>[35]</sup> showed through his study among children from endemic areas of intestinal parasitic infection that the population was found to have iron deficiency and appeared to be the dominant cause of anemia.

All the study cases have wasting followed by abdominal distention and the dehydration. The reason why of with malnutrition presented with different signs and symptoms affecting different part of the body is that malnutrition is one of the most common acquired immune deficiency disorder that make the patient vulnerable to different types of infections affecting many systems in the body so present with different signs and symptoms<sup>(37)</sup>. In the <sup>[38]</sup> study, parasite infections were insignificantly associated with anemia which was found in only 12.3% of girls infected with *G. lamblia* and in the <sup>[39]</sup> study malabsorption of iron was reported in the children with symptomatic giardiasis, however asymptomatic giardiasis did not affect the intestinal absorption of iron but <sup>[40]</sup> showed in endemic setting there was no evidence that giardia infection impair iron status.

The results showed a significant decrease ( $P < 0.05$ ) in serum concentration of ferritin and in infected with *G. lamblia* parasite in compared to control group The decrease in serum ferritin level in patients infected with giardiasis may be due to depletion in iron stores in body as result of chronic giardia infection where the mean concentration of serum ferritin reflects the iron body stores. This result corresponds with study of <sup>[41]</sup> that showed decrease in ferritin level in patient with *G. lamblia* compared with control group.

Also <sup>[42]</sup> showed the level of ferritin in both human and animals are significantly decreased in giardiasis infection. Study achieved by <sup>[43]</sup> showed lower level ferritin

in patients with giardiasis as result of damage to the intestinal mucosa. In other intestinal parasitic infection showed decrease in ferritin level in children infected Also <sup>[44]</sup> who showed that ferritin was in it is lower level in children infected with some intestinal parasites such as *Ascaris* and *Trichuris*. <sup>[45]</sup> Who show that ferritin serum concentration was higher in infected children with *G. lamblia* than non-infected. Also <sup>[46]</sup> showed that the levels of ferritin in infected children with giardiasis are higher than non-infected children. The difference between this result and other result which showed increased level of ferritin may be due to age and sample size.

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