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Assessement of interleukin 37 and some immune parameters in patients with giardiasis : A Case Control Study

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Abstract

Background: Giardia lamblia is the cause of giardiasis, one of the most prevalent parasitic illnesses in the world that affects millions of people annually. Although the majority of infections are most likely asymptomatic, some are linked to intestinal irritation and subacute or chronic diarrhea, which can lead to malabsorption and nutritional deficiencies, particularly in children.Aim of study :is to Assessment of interleukin 37 and some immune parameters in patients with giardiasis .Patients and methods:The stool samples were collected and labeled with the patient's information in sterile plastic containers and checked via the naked eye for colour, consistency, after that, they were analyzed using light microscopy to check for the presence of Giardia trophozoite and cyst stage.(45)blood samples were taken from suspected G. lamblia patients, and (45) blood samples from healthy individuals as a control group. Blood samples were captured to enable to establish the white blood cell (WBC) count. Results: The outcomes of the research demonstrated that both male and female patients infected with G. lamblia had significantly lower levels of IL-37 ($p > 0.05$) in their 72.36 ±10.87 concentrations than in the control group (86.51 ±11.83). WBC levels It reveals a substantial rise in the number of infected patients (8.94 ± 4.36) in contrast to 7.44 ± 2.4 of the control collective. ($P<0.05$) . Conclusion **:** the spread of the disease depends on the environmental conditions as well as on factors specific to the host and the strength of occurrence affected by these conditions.

Introduction:

The Giardia duodenalis parasite is among the most prevalent parasites in the world and causes serious health problems in the human digestive system [19]. Over three billion people worldwide are impacted by the high intestinal protozoan parasite prevalence, which is one of the main health issues [36]. Children and infants make up the majority of those infected; as dictated by the World Health Organization, 450 million children and infants worldwide are infected with these parasites [2]. Infection rates are highest in developing nations, where they primarily affect individuals living in closed communities, immigrants, and tourists returning from endemic nations [25]. Giardia lamblia sometimes called Giardia intestinalis, is a small parasite that causes giardiasis by colonizing and reproducing in the small intestine [6], it can be found on contaminated surfaces, food, or water with animal or human feces [15], the highest prevalence of Giardia lamblia occurs in tropics and sub tropics where sanitation is poor. Giardiasis is spread via the fecal-oral route, which can be gained directly from an infected host or indirectly by consuming infectious cysts in food or water tainted with feces [1]. Depending upon its morphological

forms the life cycle of Giardia is also divided into two distinct phases. These two phases include a proliferating stage of the trophozoite phase and an infectious stage of the cyst [14]. Symptoms of giardiasis differ from intestinal symptoms as diarrhea, abdominal discomfort, nausea and mild weight loss to extraintestinal symptoms like fever, lymphadenopathy, urticaria, maculopapular rash, polyarthritis, and pulmonary infiltrate [26]. *G. lamblia* is a very common causative agent of diarrhea, favorably colonizes the duodenum and jejunum a hostile environment in which few microorganisms survive due to the high levels of digestive enzymes and bile present during digestion [24]. Giardiasis is reported to be significantly associated with micronutrient deficiency, particularly vitamin A deficiency, protein-energy malnutrition, and iron deficiency anemia. These factors contribute to stunting, malnutrition, and wasting, which in turn raise vulnerability to other infections [28]. Giardia duodenalis carries a high level of genetic diversity and has been grouped into genotypes A to H [32]. Although all of the assemblages have been documented in humans [34], assemblages A and B are the most dangerous because they primarily infect humans and have a wide range of hosts [5]. In general, in developing countries, 33% of the population is claimed to be infected. In developed countries, giardiasis is often associated with travelling, with prevalence of 2-5% [11]; [35]. A complicated interaction between inflammatory and anti-inflammatory processes characterizes the immunological reaction to an infection with G. lamblia. The creation of pro-inflammatory cytokines and chemokines is one of the processes that the host's immune system initiates to manage the parasite after infection. The production of IL-17A, for example, by Th17 cells is essential for the defense of the immune system against G. lamblia [13]. Nonetheless, the parasite takes a variety of strategies to alter the host's immune system, which helps it avoid detection and survive in the host [37]. Increased levels of IgA, nitric oxide (NO), AMPs, mucus, and a protective Th17 immune response are some of the characteristics of host responses against G. intestinalis [21]; [29]. Both humoral and cellular immunological systems are involved in the adaptive response against G. lamblia. Th1 and Th17 cells in particular, which are CD4+ T lymphocytes, are essential for coordinating this response. Th1 cells release IFN-γ, which activates macrophages, while Th17 cells promote the migration of neutrophils and induce intestinal epithelial cells to create antimicrobial peptides via the production of IL-17 [18]. Giardiasis can be confirmed by a faecal examination of the suspected individual. If Giardia trophozoites or cysts are seen during the microscopic examination of the stool sample, infection is confirmed [22].

Materials and Methods:

Study design and ethical approval

A case control study was carried out to assess interleukin 37 and some immune parameters in patients with giardiasis . The study was conducted from September 2024, to the end of March 2025 at Almosawi Hospital , Bint Al-Huda Teaching Hospital, and Private Al-sabah laboratory Ethical approval for this study based on with the guidelines established by the Iraqi Ministry of Health and ethical considerations. A committee of ethical standards of Thi-Qar University's College of Science, one of the colleges in Iraq's Ministry of Scientific Research and Higher Education, also approved the research. Additionally, prior to sampling, consent was obtained from both healthy individuals and patients .

Sampling Technique and Sample Size Determination

The purposive sampling technique was employed to recruit participants who met the inclusion criteria for this study. The inclusion criteria were: Involved patient had a range of age from infants and above diagnosed with Giardia lamblia . Exclusion criteria involved patients who declined participation. The sample size was determined based on previouse similar studies and standard practices in immunological reserach. Ultimately, a total of 90 participants were included in the final analysis.

Blood Samples Collection

When suspected G. lamblia patients went back to the laboratory to obtain the outcomes of the investigation and emerged to have the parasite, from them, 45 blood samples were obtained. and 45 blood samples were discovered in healthy individuals as a control group. Male and female samples were collected via a range of age groups, including adults aged 20 to 45 and children aged 4 months to 10 years [3].

Stool Samples for Testing

The stool samples were inspected for color and consistency via the naked eye before being detected for the presence of Giardia trophozoite and cyst stages as well as other parasite stages via light microscopy (Primo star ZEISS Microscopy-Germany and Olympus CH series Japan). Giardia lamblia trophozoite and cyst phases were observed using a light microscope after the mixing of mixed physiological saline (solution of normal saline) double-blind test was implemented by dissolving 8.5 grams of pure sodium chloride in 1 liter distilled water [17].

Human Interleukin 37 Enzyme-Linked Immunosorbent Assay (ELISA) Principle

Interleukin 37 is an ELISA kit. The human IL37 antibody has already been employed for the plate. Post being presented, the sample's IL37 connects itself to the antibodies clothed with the wells. Afterwards, its addition, the biotinylated human IL37

antibody attaches itself to the IL37 present in the sample. Post it was included, streptavidin-HRP connects itself to the biotinylated IL37 antibody. Freed Streptavidin-HRP is discarded via a washing step seeing incubation. With the selection of the substrate solution, color shifts occur in response to the concentration of human IL37. By as well as a stop liquid that is acidic, the process has been halted, and at 450 nm, the absorbance is tracked.

Determination of blood parameters

After thoroughly mixing blood samples in Ethylene Diamine Tetraacetic Acid tubes in a mixing apparatus, the detector (Sysmex, XP 300 Japan) sucked 0.2 milliliters of blood per sample to determine the WBC count. Primary health center patients who had no history of giardiasis infection and who appeared healthy based on physical examination were the subjects of 45 blood samples. Along with the stool examination outcomes, Giardia lamblia stage, and related parasites, each child's age, sex, and place of residence were noted on a separate form.

Results:

Table 1: Distribution of Giardiasis according to gender

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

The study included 90 blood participants, of whom 45 were diagnosed with giardia lamblia. The prevalence of Giardia was found to be 50% with a higher incidence in children. The data suggest that the factors such as study the effect of some factor like age, sex, months, socioeconomic status, hygiene practices and exposure to contaminated water on infection rate of parasite.

Table 2: Comparison of the concentrations of the interleukins (IL-37) in healthy control and infected patients

IL-37 (Pg/ml)			P. value
Groups	**No.**	**Mean ± S.E.**	
Control	**45**	**86.51 ±11.83 A**	**0.007 Sig.**
Patients	**45**	**72.36 ±10.87 B**	
LSD	**9.33**		
Multiple pairwise comparisons and the post hoc test for the least significant difference (LSD)			
SD: mean standard deviation; sig: significant. P. value (≤ 0.05)			

The outcomes of the research demonstrated that both male and female patients infected with G. lamblia had significantly lower levels of IL-37 ($p > 0.05$) in their 72.36 ±10.87 concentrations than in the control group (86.51 ±11.83).

Table 3: Displays a comparison between the control group and the mean level of WBCs in Giardia lamblia-infected patients.

Parameter	Patients N = 45	Healthy N = 45	P.value
WBCs (X 103/mm3)	8.94 ± 4.36	7.44 ± 2.4	0.09
Neutrophile	2.59 ± 0.93	2.69 ± 0.83	0.14
Lymphocyte	34.27±11.38	34.05±3.81	0.75

Table 3 compares the mean WBC levels of Giardia lamblia-infected patients with those of the control group. It reveals a substantial ($p<0.05$) rise in the number of infected patients (8.94 ± 4.36) in contrast to 7.44 ± 2.4 of the control collective.

Discussion

The adult and infant blood samples with a Giardia infection parasite lamblia, which numbered 45 samples, and 45 samples of healthy people missing the parasite, were subjected to a study of cytokines (IL-37) and a blood parameters study. The outcomes showed a higher rate of infection in males than females (54.0%), (45.0%) respectively (Table 1). The outcomes of the present study demonstrated a decrease in the level of IL-37, as shown in Table 2. In response to an infection with Giardia lamblia, the release of IL-37 was crucial for starting the production of inflammatory cytokines [31]. IL-37 appears particularly well suited for this function since it differs in several aspects from most other proinflammatory cytokines. Through three mechanisms (reducing transcriptional cytokine expression, blocking kinase signaling activation, and lowering the making of pro-inflammatory cytokines), IL-37, a novel cytokine, modulates immune responses and has anti-inflammatory properties [30]. IL–37 is subsequently released by macrophages [10]; [33]. Numerous human tissues and organs, such as the heart, skin, gut, kidney, lymph node, bone marrow, thymus, testis, placenta, lung, and uterus, have widespread expression of IL-37 [20]. Barring the activation of kinase signaling, limiting the expression of transcriptional cytokines, and decreasing the production of pro-inflammatory cytokines are the three ways that IL-37 works [9]; [34]. However, depending on the particular tissues and organs involved, different subtypes express themselves differently. The thymus, lymph nodes, bone marrow, placenta, colon, testicles, lung, and brain are the primary locations for IL-37a under physiological conditions, while the peripheral lymph nodes, blood, placenta, lung, colon, testicles, and kidney are the primary locations for IL-37b. In contrast to IL-37d, which is mostly stated in bone marrow, the testis, blood system, adipose tissue mesenchymal stem cells, and umbilical cord tissue, IL-37c is primarily stated in the lymph nodes, placenta, colon, lung, testis, and heart [23]; [37]. The main locations for IL-37e expression are the bone marrow and testicles. The main function of the anti-inflammatory cytokine IL-37 is to secrete proteins to the outside of cells that engage the IL-18 receptor and operate as ligands for A structure of a functioning sensor on the membrane of the target cell [27]; [16].The results demonstrated that, in comparison to the control group, patients with G. lamblia infections had a significantly lower serum concentration of IL-37. The host's defense against infection may be further weakened as a result of the impairment of the cell-mediated immune response, which lowers the generation of cytokines by immunological effector cells. The findings demonstrated that, in comparison to the healthy control group, the IL-37 concentration in the patients had decreased (Table 2). A statistically significant drop in the ratio of IL-37 was observed ($P>0.05$). Numerous investigations [4] concurred with the current study's findings. Pro-inflammatory and anti-inflammatory cytokines, which evoke various responses to immunogens at different phases of infection, are among the crucial biological products that are vital to the development and outcome of infection. Acute or chronic disease, its etiology, and the type of disease all influence how cytokines react to the same stimulus. Recently identified as a member of the IL-1 family of cytokines, IL-37 is a significant regulator of both innate and adaptive immunity that interacts with the IL-18 receptor (IL-18R).The anti-inflammatory cytokine IL-37 suppresses the expression of pro-inflammatory genes by blocking NF-kB and MAPK signaling [8]; [12]. Giardia intestinalis infection induces harm to enterocytes and loss of the brush margin of intestinal epithelial cells, which minimizes microvilli and alterations the function of the epithelial barrier. Increased enterocyte apoptosis rates, intestinal barrier disruption, host lymphocyte activation, CD8+ lymphocyte-mediated brush boundary microvilli shortening with or without concomitant villous atrophy, small intestinal malabsorption, disaccharidase deficiencies, elevated intestinal transit rates, and anion hypersecretion are all linked in the pathophysiology of Giardiasis-related acute diarrhea [7].When the mean WBC levels of Giardia lamblia-infected patients and the control group are compared in Table (3), the infected patients' WBC levels are significantly more powerful ($p<0.05$) than those of the healthy control group.The presence of certain parasites in the human gut may be the cause of the poor absorption of carbohydrates, lipids, vitamins, folic acid, zinc, and iron. As iron deficiency affects the composition of hemoglobin, the primary component of red blood cells, the presence of nutrient phases that adhere to intestinal villi and absorb large amounts of blood, as well as hemolytic factors generated by parasites and other factors, anemia results. The immune system's robust reaction to fight and

eradicate parasites could be the cause of the increase in WBC and some differential WBC. When parasites enter the body, these cells adhere to surfaces, start to peel off their granules, then fight and eliminate the parasites.

Conclusion

We conclude from this study that the spread of the disease depends on the environmental conditions as well as on the factors specific to the host and the strength of occurrence affected by these conditions. This indicates the need for long-term control measures for improving health and living conditions, in particular in areas of high prevalence.There are statistically significant differences in the prevalence of infection between males and females. The prevalence of infection is higher in the age group (15–29) years. The study was also able to demonstrate, by examining the serum of patients infected with G. lamblia, that there are significant changes in the level of a number of hrmatological parameters among infected patients infected with giardiasis that may be specifically recognized as a consequence of malabsorption during infection and may lead to transient or chronic malnutrition status that can be specifically identified as a consequence of malabsorption during infection. This study also concludes that the level of interleukin (37) is decreased in patients with giardiasis. This is a result of the immune response during infection.

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