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Study of the Oxidative Status and Some Cytokines in Children with Giardiasis

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Abstract: Acute and chronic diarrheal illnesses are frequently caused by Giardia lamblia in people all over the world. Studying the variations in interleukins (ILs) in giardiosis patients is especially important because ILs directly impact an immune response. In order to expose the oxidative state and certain cytokines in children with giardiasis, the current study was conducted. To find out if the child has a G. lamblia infection, a general stool investigation was performed. The parasitology unit saw 150 youngsters under the age of 15 between May and August of 2024. Abdominal discomfort and diarrhea plagued the chosen children. The results showed 17(13.1%) samples were found positive for giardiasis, while 113(86.9%) samples were negative. The gender of patients showed non-significant (P≤0.05) differences. The percentage of infected male patients at (52.9%) while, the percentage of female patients at (47.1%). The age of patients showed significant (P≤0.05) differences. 1-3-year group showed high percentage of infected patients at (52.9%) while, the percentage was showed in 10-12 year which reached 5.9%. The results found that the concentration of IL-2 (34.19± 3.88), IL-4 (19.53± 1.73) and IL-10 (16.52± 0.36) showed significant (P≤0.05) elevated between G. lamblia patients compared to the control group (12.04± 1.47; 8.91± 1.61; 9.04± 0.42 respectively). We

conclude that pro-inflammatory and anti-inflammatory interleukins play a significant role in Giardia lamblia infection because the levels of interleukins (IL-2, IL-4, and IL10) were elevated in the infected patient relative to healthy individuals.

Keywords: G. lamblia, Giardiasis, interleukin, cytokines.

Introduction

Giardia duodenalis, also referred to as Giardia lamblia, is the causative agent of Giardiasis and one of the most prevalent intestinal protozoan flagellates in humans. Two active trophozoite and cystic forms are part of the basic life cycle of Giardia species [1]. After cysts are consumed, the incubation period lasts anywhere from nine to fifteen days. Weight loss, nausea, epigastric pain, and severe watery diarrhea are all possible signs of an infection [1, 2, 3]. It can spread from person to person through the fecal oral route in low-quality child care facilities [7]. Giardiasis is most commonly contracted by consuming water contaminated with G. lamblia, which is found in lakes, swimming pools, spas, and other bodies of water. Diapers, agricultural runoff, and animal feces are also sources of contamination. The infection can be spread by ingesting infectious cysts [4,5], which are resilient and protect against varying degrees of heat, cold, desiccation, and infection from other organisms [6]. Because the parasites are killed by heat, giardiasis is less commonly contracted by food [8]. Although it is thought that humans can contract giardiasis with just a little amount of infection (10–25 cysts), giardiasis symptoms include diarrhea, stomach aches, bloating, weight loss, and malabsorption in at least 15% of filtered water samples [9]. Giardia lamblia, which is found in up to 80% of raw water sources—most of which originate from lakes, streams, ponds, and rivers—can cause both acute or chronic diarrheal disease and asymptomatic colonization [10]. The immune system of the host is stimulated and modulated by parasites [11]. The humoral and cellular responses are two of the various ways the immune response acts [12]. The host's body may experience oxidative stress as a result of several components of this immunological response [13]. An imbalance between oxidants and antioxidants is referred to as oxidative stress. It could be the outcome of multiple bodily functions. It results from either an increase in oxidant production or a decrease in antioxidants [14]. It was discovered that G. lamblia can trigger immunological responses that result in the production of several serum immunoglobulins and a variety of cytokines, including interleukin-12, tissue necrotic factor-alpha, and interferon-gamma (IFNɣ) [15]. Therefore, the current work was aimed to revealing of the oxidative status and some cytokines in children with giardiasis.

Materials & Methods

Patients

A routine stool examination was performed on the child to determine for the presence of a G. lamblia infection. 150 kids younger than 15 years old visited the parasitology section between May and August of 2024. Children that were chosen experienced stomach ache and diarrhea.

Samples collection

Fresh stool samples were taken and placed in a sterile, screw-disposable plastic container. For wet mount analysis, a piece of the material was treated directly. Serum samples were obtained from patients whose G. lamblia test results were microscopically positive.

Blood collection

Using a sterile syringe, 5 ml of each patient's venous blood was extracted, and the sample was placed in sterile gel tubes. The blood was separated by centrifuging it for five to ten minutes at 3000 rpm. After that, the serum was divided into four Eppendorf tubes and stored at -20 C in a deep freezer until it was needed [16-17].

Inclusion criteria

The study included all children aged 1-13years who did not receive any treatment.

Exclusion criteria

Children over 12 years of age were excluded, and every child received treatment or suffered from other health problems.

Measurements

The ELISA kits used a technique called sandwich-ELISA. The Microelisa stripplate that comes with this kit has already been coated with an interleukin-specific antibody, or samples can be added to the appropriate Microelisa stripplate wells and combined with the specific antibody. After that, an interleukin-specific Horseradish Peroxidase (HRP)-conjugated antibody is added to each Microelisa stripplate well, and the plates are incubated. Washing removes any free pieces. The TMB substrate solution is added to each well. Only the wells containing interleukin and HRP conjugated interleukin antibodies will initially look blue before becoming yellow with the addition of the stop solution. The optical density (OD) is measured at a wavelength of 450 nm using spectrophotometry.

Statistical analysis

The Minitab statistical software was used to evaluate the data. One-way analysis of variance was used to examine a statistical difference between the experimental groups' means (ANOVA) [18-19].

Results & Discussion

Sample distribution

130 samples were directly examined by using microscopic (wet mount) examination for parasite diagnosis. whereas, 17(13.1%) samples were found positive while other, 113(86.9%) samples were negative, table (1)

Table (1): Distribution of study group according to results

Procedures	Samples	Positive samples	
		No.	%
Direct examined (wet mount)	130	17	13.1

The results of the current study are consistent with some studies conducted in different governorates in Iraq. The current percentage was 11.45%, which was comparable to the findings of studies conducted in Kirkuk Governorate [20]. The current study's results were higher than those of another study conducted in Baghdad [21], which recorded 3.78%, and lower than those of [22], which obtained 48% in Wasit Governorate. Giardia prevalence in stool samples submitted for parasite analysis, on the other hand, has been shown to range from 2% to 5% in wealthy nations to 20% to 30% in underdeveloped nations [23]. The socioeconomic level, personal and communal hygiene, educational attainment, nutritional status, and the techniques employed for examining fecal samples could all contribute to the variations in prevalence [22].

The relation of gender with infection

Patient gender differences were not statistically significant (P≤0.05). The proportion of male patients with the infection was 52.9%, whereas the proportion of female patients was 47.1% (Table:2).

Table (2): the gender of patients in current study

Gender	Positive results	
	No.	%
Male	9	52.9%
Female	8	47.1%
Total	17	100.0%
P-value	0.391	

There were no significant differences (p≤0.05) between the male (52.9%) and female (47.1%) infectivity rates, according to Table 2. This finding was in line with Berrilli et al. [24], who also found no differences in the distribution of sexes. Men and women have similar employment and educational opportunities, and exposure to Giardia and other parasites may not differ significantly between the sexes. However, other studies found a significant correlation between gender and the Giardia infectivity rate. Some of these studies suggested that the female had the highest infectivity rate because of household activities like cleaning and food preparation that could expose them to parasites [25], However, some said that the male had the highest prevalence of contagious disease. These researchers hypothesized that the higher rate of intestinal parasite infections in males could be due to the fact that males engage in more activities and are more exposed to environmental conditions than females [26].

The relation of gender with *E. histolytica* infection

The age of patients showed significant (P≤0.05) differences. 1-3-year group showed high percentage of infected patients at (52.9%) while, the percentage was showed in 10-12 year which reached 5.9% (Table:3).

Table (3): the gender of patients in current study

Age (year)	Positive results	
	No.	%
1-3	9	52.9%
4-6	5	29.4%
7-9	2	11.8%
10-12	1	5.9%
Total	17	100.0%
P-value	0.045	

According to Table 3, the highest infection rate in the age range of 1-3 years was 52.9%, and there was a very significant correlation between age and infection rate (p≤0.01). These results are in line with the majority of earlier studies conducted in our nation or its neighbors [27, 28]. Giardiasis is common in children for a number of reasons, including a lack of cultural and health knowledge and playing in areas with parasite-contaminated soil [29].

Interleukins

The table (4) shows some immunological parameters significant (P≤0.05) differences between the study groups. The results found that the concentration of IL-2 (34.19± 3.88), IL-4 (19.53± 1.73) and IL-10 (16.52± 0.36) showed significant (P≤0.05) elevated between *G. lamblia* patients compared to the control group (12.04± 1.47; 8.91± 1.61; 9.04± 0.42 respectively).

Table (4): the concentrations of some immunological parameters in *G. lamblia* patients compared with control group

Parameter	*G. lamblia*	Control	P value
	Mean ± SD	Mean ± SD	
IL-2 pg/ml	34.19± 3.88 a	12.04± 1.47 b	0.001
IL-4 pg/ml	19.53± 1.73 a	8.91± 1.61 b	0.001
IL-10 pg/ml	16.52± 0.36 a	9.04± 0.42 b	0.001

* Different letters indicate significant (P≤0.05) differences, while the same letters indicate non-significant (P≤0.05) differences.

The significance of interleukins in giardiosis has not received much attention in the literature or in clinical studies, despite the fact that the immune response in parasite infection has been thoroughly described. By comparing the patients' levels of IL-2, IL-4, and IL-10 to those of the healthy control group, this study attempted to address the problem. The generation of IL-2, IL-4, and IL-10 are among the several mechanisms that have been put out to explain the immune response against parasite infections. It was discovered that the patients' interleukin levels were greater than those of the healthy control group (Table 4). A statistically significant rise in the ratios of IL-2, IL-4, and IL-10 was observed (P≤0.05). Numerous research [30,31] concurred with the current study's findings. According to other research, pro-inflammatory cytokines produced by T-helper 1 (Th1), like IL-2, may be crucial in the removal of protozoa from the human body [32, 33]. The potent anti-inflammatory cytokine IL-10, which is produced by innate immune cells, regulatory T cells (Tregs), and B cells, has been shown to inhibit IFN-γ, inhibit macrophages' ability to kill intracellular and extracellular parasites, and prevent the destruction of host tissue by the immune system in reaction to pathogenic pathogens [34]. However, this result differed from the findings of Mitra et al. [35], who confirmed that giardiosis patients had lower levels of IL-4. Additionally, we disagreed with the authors [35,36] who agreed that there was no discernible difference between the giardiosis patients' and the control group's levels of IL-10.

Conclusions

We conclude that pro-inflammatory and anti-inflammatory interleukins play a significant role in Giardia lamblia infection because the levels of interleukins were elevated in the infected patient relative to healthy individuals.

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