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Assessment of the Level of Interleukin IL 5 and IL 17 in Patients Infected with some Intestinal Parasites and *H. pylori*

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ABSTRACT

The current study aimed to assess the level of interleukin IL 5 and IL 17 in patients infected with *E. histolytica, G. lamblia* parasites and *H. pylori* who suffer from diarrhea and some intestinal disorders, who attended Shirqat General Hospital / Salah al-Din Governorate, during the period from October 2020 to March 2021. The number of faecal samples examined was 409. The parasite infection were detected and investigated using direct wet smear and ether-formalin precipitation methods using light microscopy. All positive samples were examined for microscopic examination by antigen test for faecal samples and antibody test for rapid serum samples for detection of *H. pylori* co-infection of intestinal protozoa. The results showed that the total infection rate was 46 (11.24%) positive sample for the intestinal protozoa and 363 (88.7%) negative sample, *E. histolytica* was the higher with 67.38%, followed by *Giardia lamblia* with 32.61%. The results of the bacterial detection showed that 13 (28.26) positive samples and 33 (71.73%) negative samples, and the rate of co-infection between *E. histolytica* and *H. pylori* 9 (29.03%) was higher than the infection rate between *Giardia lamblia* and *H. pylori* 4 (26.66%). As for evaluating the level of Cytokines studied, the concentrations of some anti-inflammatory kinetics such as interleukin-5 and some pro-inflammatory kinetics such as interleukin-17 were estimated using ELISA technique. The results showed an increase in the concentration of interleukin-5 in the serum of patients with intestinal protozoa infections, as its concentration reached (10.956 ± 2.381 ml/Pg) compared to the control group (4.334 ± 1.434 ml/Pg), As for the patients infected with *H. pylori* (4.284±1.260 ml/Pg), there was no increase in the concentration of interleukin-5 compared to the control group (the healthy ones), While the concentration of IL-17 was lower in patients with intestinal protozoa infections (30.114 ± 4.877 ml/Pg) compared to the control group (35.665 ± 3.198 ml/Pg), As well as in patients infected with *H. pylori* bacteria (31.097±2.001 pg/ml) compared to the control group. The results of the statistical analysis showed that there were significant significant differences in the concentration of each of interleukin 5 and 17, which were statistically significant at the level of probability ≤ 0.01 P.

Keywords- *H. pylori*, IL 5, IL 17, protozoa, ELISA, fecal sample, serum.

I. INTRODUCTION

The parasites *Entamoeba histolytica* and *Giardia lamblia* of the intestinal protozoa cause diarrhea and malabsorption in humans, Where the infection leads to severe diarrhea in children, especially less than five years of age, and is accompanied by chronic infections that lead to low weight and delayed growth (1).

H. pylori is characterized by its helical or spiral shape, gram-negative microaerophillic that suffers morphological transformation when exposed to unsuitable conditions ranging in width (0.9-0.5) microns and length (4-2) microns (2). The enzyme urease is produced in large quantities, so it is positive for the urease test, it affects the stomach lining These bacteria have phenotypic and physiological characteristics, as well as pathological factors that enable them to penetrate the stomach lining, especially the pyloric region, and are resistant to high acidity of the stomach (3). Infection may be accompanied by the appearance of symptoms or asymptoms in patients It is noted that 70% of people who carry this bacteria do not show symptoms of disease, and this depends on the person's ability and immunity, Epidemiological studies also confirmed the spread of *H. pylori* infection and its associated diseases throughout the world and in different age groups, In general, infection is more common in underdeveloped countries than in developed countries, reflecting the fact that infection is linked to an individual's socioeconomic standing and health awareness (4).

Interleukins They are cytokine aggregates made up of proteins that are expressed by white blood cells (5). Interleukins play an important role in the immune system's overall function. Because helper lymphocyte T cells produce a significant number of interleukins, they can also be produced by macrophages, monocytes, and endothelial cells, and they encourage the development and differentiation of B and T lymphocytes as well as hematopoietic cells. (6) .

II. MATERIALS AND METHODS

Faecal sample collection:

409 feces samples were obtained from patients suffering from diarrhea and other intestinal illnesses who were attending Al-Sharqat General Hospital / Salah Al-

Din Governorate between the October 2020 and March 2021.To maintain moisture and avoid dehydration or bacterial contamination, feces samples were collected in clean, open plastic containers with an airtight seal. Direct smear examination, as well as ether-formalin precipitation, are used to analyzed samples in less than half an hour or an hour after they arrive at the lab.

Microscopy:

Fecal samples were examined microscopically to detect the trophic or cystic phases using direct smear method and ether-formalin precipitation method.

Blood sample collection:

Venous blood samples were collected from patients who had been confirmed to be infected with intestinal parasites and *H. pylori* bacteria using a medical syringe with a capacity of 3 ml and then placed in clean plastic tubes for a period of time before being centrifuged (4000 rpm) for 10 minutes to obtain blood serum for immunological tests (control group).

Blood sample examination:

Serum IL-5 and IL-17 Concentration Measurement: Using ELIZA technology using the kit supplied by Sunlong Biotech of China to measure IL-5 and IL-17 in Pg/ml units according to the manufacturer's instructions. Standard curve is plotted to extract antibody values in affected patients. As well as the uninfected.

III. STATISTICAL ANALYSIS

The results of the study were analyzed using the Anova test and t-test under the probability level $P \leq 0.05$, $p \leq 0.01$ independent of statistical significance (7).

IV. RESULTS AND DISCUSSION

409 stool samples were examined for patients with diarrhea by the direct wet smear method using 0.9% normal saline and local iodine 1%, and by ether-formalin precipitation method. It was noted that 46 faeces samples (11.24%) contained the *E. histolytica* and *G. lamblia*, and no infection with the parasite was recorded in 363 (88.7%) faeces samples, as shown in Table (1).

Table 1: The total percentage of parasitic infections for the study group

Statistical Function P value	The number of Negative samples (%)	The Number of Positive samples (%)	Total number o F samples
≤ 0.01**	363 (88.7%)	46 (11.24%)	409
0.018*	378 (92.42%)	31 (7.57%)	Parasite *E. histolytica*
0.02*	394 (96.33%)	15 (3.66%)	Parasite *G. lamblia*

P ≥ 0.05: Non-significant; *: Significant at p ≤ 0.05; **: Highly significant at p ≤ 0.01

The *E. histolytica* got 7.57% higher than the infection rate of *G. lamblia* 3.66%, and this can be explained that the *E. histolytica* can survive in the cool humid environment for at least 12 days, and maybe up to 30 days in water. It can resist the chlorination process used to sterilize drinking water, while *Giardia* bags do not have this specificity (8). The percentage of *E. histolytica* is almost in agreement with the percentage recorded by (9) in the city of Tikrit, where it recorded an infection rate of 9.7%, It does not agree with what (10) recorded in the city of Erbil, where the infection rate was 14%. As for *G. lamblia* parasite, it got the highest infection rate of 3.66%, This percentage is close to what was recorded by (11) in Tikrit, where the infection rate was 5.26%, While the percentage in this study was less than what was found by (12) when studying the epidemiology of Giardia in the city of Samarra, where the infection rate was 22.12%.

H. pylori infection was diagnosed by performing a test to detect antigens for faecal samples positive for intestinal parasite and Ab test for blood serum samples, The infection was distributed as follows: 9 samples (29.03%) were co-infested with the *E. histolytica* parasite and 4 samples (26.66%) were co-infected with the *Giardia lamblia* parasite *G. lamblia*. As per the table below.

Table 2: Shows the percentage of *H. pylori* infection among parasitic infections

Statistical function p value	The rate of co-infection with *H. pylori*	The number of positive samples	Parasite type
≤ 0.01**	9 (29.03%)	31 (67.38%)	*E. histolytica*
0.011*	4 (26.66%)	15 (32.61%)	*G. lamblia*
-	13 (28.26%)	46	The total
	0.165	0.018*	P value

P ≥ 0.05: Non-significant; *: Significant at p ≤ 0.05; **: Highly significant at p ≤ 0.01

This study is in agreement with the report of (13) in Baghdad governorate, where out of 118 stool samples only 35 (29.7%) were infected with *H. pylori*. It also agrees with (14) in the United States and Mexico when they examined 386 faeces samples that found *H. pylori* with a percentage of 38.2%. The results of the current study do not agree with (15) in Erbil governorate, as they found that out of 311 faecal samples only 173 (55.8%) were infected with *H. pylori*. This difference and compatibility with previous studies may be due to the characteristics of the study population or due to the different stage of the disease (16). The infection associated with some intestinal parasites with *H. pylori* occurs as a result of *H. pylori* production of the enzyme urease, which in turn leads to reducing stomach acid, this condition provides a suitable environment for some intestinal parasites (17). The prevalence of *H. pylori* in children aged 11 to 15 years is 54% associated with age and poor socioeconomic status, It is a number very close to the prevalence of protozoa, which leads to a strong possibility of co-infection. *H. pylori* may influence the presence protozoa, severity, and pathophysiology of *H. pylori* infection (18). *H. pylori* and protozoa are known for their high prevalence all over the world (16). These studies showed that the *E. histolytica* is one of the most common intestinal parasites compared to other parasites, approximately one third of the patients had a Co-infection with the E. histolytica and *H. pylori* bacteria (19*). G. lamblia, E. histolytica and C. parvum* are among the most prevalent protozoa in the world, especially in developing countries. Most of the infections are related to diarrhea, irritation of the intestines and abdominal pain, especially in children, as most cases are related to contaminated drinking water (20). People infected with *H. pylori* are about twice as likely to be infected with *G. lamblia* than those without *H. pylori* although the difference was not statistically significant (21). *G. lamblia* and *H. pylori* are known to have a high prevalence in human hosts all over the world. The prevalence of both organisms is known to peak in densely populated and under-resourced settings and to infect children early in life. However, there is not much data available regarding the prevalence of different *G. lamblia* groups and their possible association with *H. pylori* infection (22). Several studies have reported co-infection with *G. lamblia* and *H. pylori* all over the world and in Egypt, however the coexistence of these two pathogens among mentally retarded individuals remains unexplored (21). *H. pylori* coexisted in more than half of the patients with giardiasis. It is recommended in patients with giardiasis who have reached late childhood and early childhood to have an easy, non-invasive test for *H. pylori* antigen. Protozoa such as *C. parvum*, which causes cryptosporidiosis, have come to the fore as a clinically important human parasite associated with *H. pylori* because of their strong association with gastroenteritis (23).

In Table No. (3) the results of the current study showed that there was an increase in the concentration of interleukin-5 IL among the group of patients with intestinal protozoa infections (10.956 ± 2.381 ml/Pg) and that this increase formed a significant difference with a high statistical significance at the level of probability (≤ 0.01). p). As for patients infected with *H. pylori* bacteria (4.284±1.260 ml/Pg), there was no increase in the concentration of interleukin-5 compared to the control group (the healthy) (4.334±1.434 ml/Pg), and this concentration did not form a significant difference Statistical if the p-value is equal to 0.905 and Figure (1) illustrates this.

Table 3: Interleukin concentration (5) for the study group

P value (Bacterial / Control)	P value (parasite / Control)	Statistical function p value	Control number (16)	Bacterial Infections Number (32	Parasitic Infections Number (40)	-
			Mean± SD	Mean± SD	Mean ± SD	
0.905	≤ 0.01**	≤ 0.01**	4.334±1.434	4.284±1.260	10.956±2.381	-
-	-	-	-	≤ 0.01**		P value

P ≥ 0.05: Non-significant; *: Significant at p ≤ 0.05; **: Highly significant at p ≤ 0.01

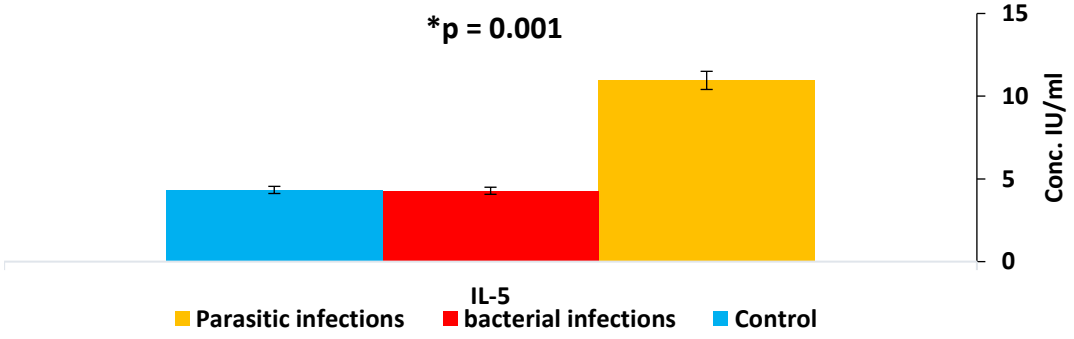

Figure 1: Interleukin-(IL-5) 5 concentrations in patients infected with protozoa and *H. pylori* bacteria, as well as in healthy subjects (control group).

The reason for the significant increase in the concentration of interleukin IL5 in the sera of patients with protozoa compared to healthy people (control group) is due to the immune response of the host against the protozoa through T helper2 cells. The response of these cells is characterized by the production of high levels of interleukins (IL 4, IL 5, IL 9, IL 10, IL 13, IL21, IL33). These interleukins regulate hypersensitivity reactions that ensure that B lymphocytes alter their production from antibodies to IgM and IgG4 only, eosinophilia, goblet cell and mast cell hyperplasia, alternate activation of macrophages, and influx of inflammatory cells such as eosinophilic white blood cells that contribute to parasite killing, Activation and recruitment of eosinophils at sites of parasite infection where they release toxic granule proteins that destroy parasites. Such an immune response can control the parasite's population by killing them with tissue or expelling them from the intestinal lumen (24,25).

This result is in agreement with the result of (13) in Baghdad, the capital of Iraq, as well as the result of (26) in Egypt.

In Table No. (4), the results of the current study showed that there was a decrease in the level of interleukin IL-17 concentration in patients with protozoa infections (30.114 ± 4.877 ml/Pg) and patients infected with *H. pylori* bacteria (31.097 ± 2.001 ml/Pg) together compared to the control group (healthy) 35.665 ± 3.198 (ml/Pg), and this high decrease formed a significant difference with statistical significance at the level of probability ($p \leq 0.01$), Figure (2) illustrates this.

Table 4: Interleukin-IL 17 concentration for the study group

p value Bacterial/(Control)	p value (parasite/ control)	Statistical function p value	control number (8)	Bacterial Infections No. (9)	Parasitic Infections No. (25)	-
			Mean ± SD	Mean ± SD	Mean ± SD	
≤ 0.01**	**≤ 0.01****	**≤ 0.01****	35.665±3.198	31.097±2.001	30.114±4.877	-
-	-	-	-	**0.564**		P value

P ≥ 0.05: Non-significant; *: Significant at p≤ 0.05; **: Highly significant at p ≤ 0.01

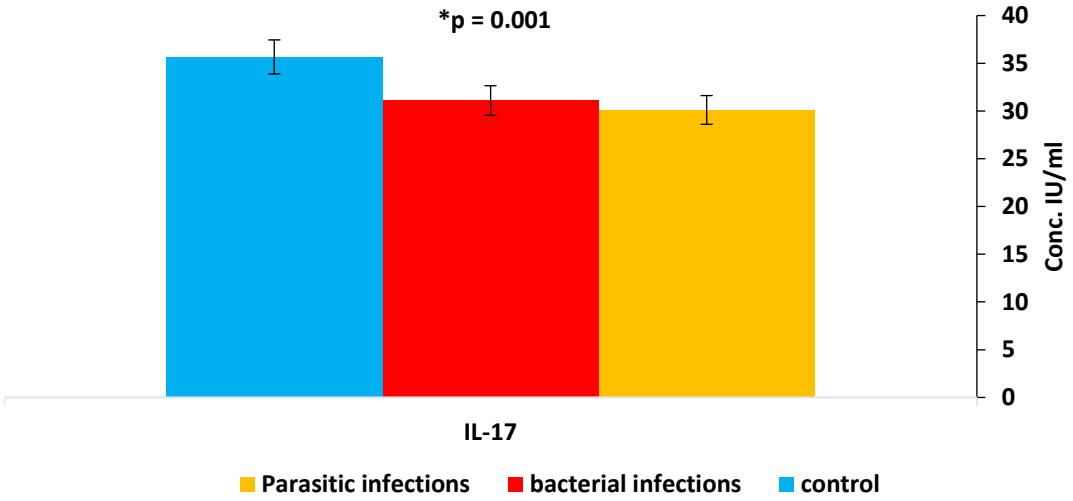

Figure 2: Interleukin-(IL-17) concentrations in patients infected with protozoa and *H. pylori* bacteria, as well as in healthy subjects (control group).

Interleukin IL-17 is produced by T-helper 17 cells and stimulates the production of nitrogen oxide and cyclooxygenase-2 High concentrations of this interleukin can be seen in chronic conditions such as chronic inflammation in rheumatoid arthritis (27). A decrease in the level of interleukin IL-17 concentration was observed in various parasitic infections, including Schistosoma mansonis and Fasciola hepatica (28). We would like to point out that the research that dealt with the relationship of interleukin-17 with the infection of *E. histolytica* is very few and the study of the level of this Cytokines in rats. This study is somewhat in agreement with other studies, as the level of interleukin IL-17 showed little effect during infection with *E. histolytica* parasite (29). The results of this study are inconsistent with (30) study where persistent infection with *E. histolytica* was found to be associated with elevated levels of Th-17 cytokines. The mechanism of IL-17-mediated protection involves recruitment of neutrophil cells to sites of inflammation, regulation of dendritic cell function and Th1 responses through IL-17-induced cytokines and chemokines (31,32).

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