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The effect of immunizing laboratory rabbits with O-antigen purified from *Escherichia coli* and infected experimently with *Giardia lamblia* on some Cytokines

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Abstract

Background: This study was conducted for the period from January 2022 until the end of December 2022 at Samarra University / Graduate Studies Laboratory of the College of Applied Sciences, **Methods:** 50 New Zealand white rabbits with weight ranged from (1500-1800) gm obtained from the National Center for Drug Control and Research in Baghdad, while their ages ranged from (10-14) months.The present study included the effect of Outer Membrane Proteins (OMPs) or (O-Antigen) extracted from *Escherichia coli* on the immune response in male rabbits infected with *G. lamblia* parasite, by measuring the levels of cytokines (IL-4, IL-6 and IL-10). **Results**: The cytokines under study, its level increased in the serum of laboratory rabbits, and our study showed that the level of IL-4 for the O-antigen group increased significantly compared with the negative control group, where its average of concentration was (12.35 and 8.71) pg/ml, respectively. An increase in the level of IL-4 occurred in Challenge group, compared to the positive control, where its average of concentration reached (14.11 and 10.98) pg/ml, respectively. The results of the current study indicated that the level of IL-6 of the O-antigen group increased significantly compared with the negative control group, where its average of concentration was (5.24 and 3.37) pg/ml, respectively, and also the level of IL-6 in Challenge group, it increased non-significantly when compared with the positive control, where its average of concentration was (6.11 and 5.82) pg/ml, respectively. As for IL-10, there was a significant increase in its level in the O-antigen group compared with the negative control group, where average of concentration was (17.17 and 10.41) pg/ml respectively, and the IL-10 levels of Challenge group increased, and it was noted that the IL-10 level increased more than previous compared with the positive control to reach (21.84 and 14.79) pg/ml respectively. **Discussion**: Immunization of laboratory rabbits with outer membrane proteins purified from *E. coli* had a clear effect on the levels of under study cytokines, and this led to a reduction in the effect of infection with *Giardia lamblia*. **Conclusions:** Elevation of interleukins
concentration under study after immunization with O-antigen. The outer membrane proteins extracted from *E. coli* and at under study concentrations can be considered as non-specific immunomodulators and non-toxic compounds at the specified concentrations and as stimulators of the immune system against infection with the *G. lamblia* parasite in white laboratory rabbits.

Key words: *Giardia lamblia,* O- antigen, Cytokines.

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Introduction

Giardiasis is one of the common zoonosis diseases between humans and animals,[1] and it is caused by parasites of the genus *Giardia* spp.[2] *Giardia lamblia* (syn. *Giardia intestinalis, Giardia duodenalis*) is a cosmopolitan flagellated,[3] and one of the top ten protozoan parasite infecting small intestine (duodenum and jejunum) of humans, and this type of pathogenic agents that cause diarrhea in humans and mammals,[4-6] where infection with parasites of this type spread all over the world.[7] The clinical signs associated with infection with *G. lamblia* vary from the absence of pathological symptoms to the appearance of various pathological symptoms.[8] Symptoms of infection usually vary depending on the parasite strain and the host's immune response.[9] Steatorrhea, abdominal cramping, nausea, anorexia and weight loss are among the most obvious clinical signs of giardiasis.[10].

Giardiasis is a major cause of diarrhea all over the world, affecting 280 million people annually.[9] As the parasite globally caused the death of 125 thousand children in the last five years.[11] The parasite spreads in different regions of the world, especially tropical and subtropical regions, crowded industrial areas and poor environments. Generally, the parasite spreads in developing countries.[12,13] Many studies conducted in Iraq confirmed the prevalence of the disease in many governorates of the country, as well as studies that indicated many epidemiological aspects of the disease.[14-18] The presence of the *G. lamblia* parasite in the gastrointestinal tract stimulates the host's immune response, and that response may be associated with the disease with its various symptoms.

A lot of information has been reached about the immune response of the host through studies conducted on experimental animals (mice and rats), where these studies indicated the possibility of recovery from infection within a period of time ranging between 3-4 weeks.[19] Cellular and humoral immunity contributes in the immune response against giardiasis infection, and the primary immune response is likely to occur in Peyer's patches of the small intestine, and studies by electron microscopy have confirmed the presence of the parasite inside macrophages cells in Peyer's smear,[20] where the trophozoite of the parasite is swallowed by opsonization.[21] Cytokines such as IL-6, IL-17, and others play an important role in regulating innate and acquired immunity against infection with *G. lamblia*.[22] IL-10 has been found in some studies to be significantly elevated in the case of parasitic infections such as malaria.[23] The outer membrane is a distinctive structure of the cell wall in Gram-negative bacteria, the cell wall consists of the outer membrane and one or very few layers of peptidoglycan. The peptidoglycan is covalently linked to lipoproteins in the outer membrane, and the last one consists of lipopolysaccharides (LPS), lipoproteins and phospholipids.[24]

In Gram-negative bacteria, the outer membrane proteins are of the β-barrel type, the most important of which are OmpA proteins.[25] OMPs act as antigens that stimulate the immune response in the body.[26]

E. coli has long known as a commensal bacterium colonizing the gastrointestinal tract.[27] *E. coli* possess enzymatic proteins such as glutaminase that stimulate each T-cells and B- cells.[28] The outer membrane proteins are very effective vaccines that stimulate both Innate immunity and acquired immunity are long-term, because they represent antigenic determinants that are displayed on the surface of the bacterial cell, and they are highly stable among different types of Gram-negative bacteria.[25,29]

Based on the above, the objectives of the current study were identified as follows:

1. Experimental infection with *G. lamblia* in laboratory rabbits.

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2. Testing the ability of outer membrane proteins (O-antigen) extracted from *E. coli* to modify the immune response in laboratory rabbits against passive infection with giardiasis based on several criteria including changes in the levels of cytokines, (IL-4, IL-6 and IL-10).

Materials and Methods

The study was conducted for the period from January 2022 until the end of December 2022 at Samarra University / Graduate Studies Laboratory of the College of Applied Sciences, and 50 rabbits obtained from the National Center for Drug Control and Research in Baghdad were used in this study, and the weights of the animals used ranged from (1500-1800) gm, while their ages ranged from (10-14) months, and they were given water and food continuously throughout the study period.

The study included the effect of Outer Membrane Proteins (OMPs) or (O-Antigen) extracted from *Escherichia coli* bacteria on the immune response in male New Zealand white rabbits infected with *G. lamblia* parasite that causes giardiasis, by measuring the levels of cytokines (IL-4, IL-6 and IL-10). The parasite was isolated from patients attending Samarra General Hospital according to the method mentioned later.

Preparation of media

The culture media (Nutrient agar, MaCconkey agar and Brain heart infusion broth), this media was later used to grow the bacteria under study,[30] and were prepared according to the manufacturer's instructions, which were attached to the box and the pH was adjusted. They were autoclaved at a temperature of (121) °C under a pressure of (15) bar for a period of (20) minutes and incubated at a temperature of (37) °C for a period of (24) hours in order to ensure that it is not contaminated and to get rid of excess moisture on the surface of the medium.

Sucrose solution

A saturated sugar solution was prepared according to the Peakman and Vergani method, consisting of (500) grams of sucrose in (320) ml of tap water, then (6.5) grams of phenol was added to the solution.[31]

Samples collection

First: Collecting parasite samples

The parasite *G. lamblia* and its two phases (trophozoite and cyst) were obtained from the faeces of infected persons only and those attending the general hospital and primary health care centers in Samarra, where the samples were collected in sterile plastic bottles, and the cysts samples were used to infect the laboratory rabbits under study.

Type of sampling and reasons for selection

Randomized stratified sampling techniques to remove bias and sampling errors.

1- Examination of stool samples

Microscopic Examination Direct

Faecal samples taken from people infected with the *G. lamblia* parasite were examined by direct wet mount preparation according to Ali *et al.* [4]

2- Preparation of the parasite cyst suspension used in the dose of laboratory animals

Isolation of the parasite from fecal samples obtained from patients with diarrhea who had confirmed the presence of the parasite by direct microscopy, then, the Clark & Diamond method was

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followed in preparing the parasite cyst suspension, which became ready to dose laboratory animals and cause infection.[32]

3- Experimental infection of Rabbits

The number of parasite cysts was calculated using a hemocytometer, in order to determine the oral infection dose, which was 5×10^3 cysts. The parasite cysts were investigated in the feces of rabbits daily for two weeks after the dose of the cysts was given to ensure that parasite infection occurred. The infection was confirmed by preparing a swab of the infected rabbit's feces on a glass slide and examining it under a microscope, observing the parasite and its different stages. Logal's Iodine and eosin dyes were used.

Sample of bacteria

Pure *E. coli* isolates were obtained from the microbiology laboratory at the College of Applied Sciences and the diagnosis was confirmed using the VITEK 2 device as well as using Eosin Methylene Blue Agar (EMB) medium.

First: preservation and perpetuation of the bacterial isolation

The bacterial isolate (after diagnosis) was grown in Brain heart infusion Broth (BHIB) at a temperature of (37) °C for a period of (24) hours, then it was grown on nutrient broth under the same development conditions, and then kept at a temperature of (4) °C These isolates were used in daily work, taking into account their monthly renewal and maintenance.

Second: Extraction and purification of outer membrane proteins (O-Antigen)

The outer membrane proteins were extracted according to Murphy *et al.* method. [33]

Third: prepare and estimate the amount of protein

The standard curve was prepared and the protein concentration in the samples was estimated according to Lowry *et al.* [34] and as shown in Figure (1).

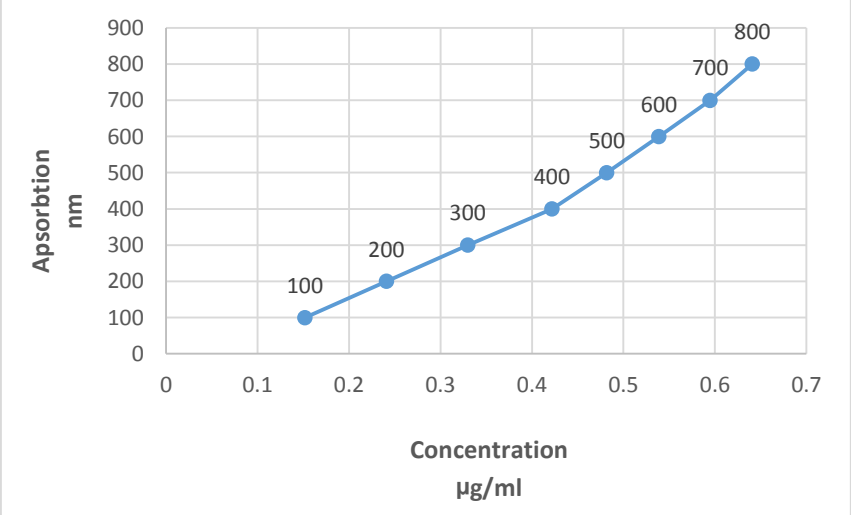

Figure 1: The standard curve for the determination of protein concentration in the purified protein solution

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Toxicity test for outer membrane proteins (O-Antigen)

The used concentrations of outer membrane proteins were tested for the treatment of experimental rabbits, which are (100, 200, 400, 800, 1000, 1200 and 1400) µg/ml according to what was mentioned by Al-Refaei.[35] Thirty-five male rabbits aged 10-14 months were used in this experiment. It was divided into eight groups, each group consisting of (5) rabbits, and the antigen concentrations were injected into the intraperitoneum, at a rate of (1) ml for each concentration.

Immunization of rabbits with the O-antigen

(5) rabbits were vaccinated with O-antigen, and immunization was carried out according to the method Griffiths and Tomas, [36,37] where the method of subcutaneous and intramuscular injections was used to give doses to animals at a rate of (1) ml per animal, and concentrations (100, 200, 400 and 800) µg/ml were used, depending on the toxicity test, blood samples were drawn from rabbits by heart stab a week after the end of immunization, and it was used in the subsequent immunological examinations.

Expermental design

(15) male rabbits, whose ages ranged between (10-14) months, were used after making sure that they were safe from apparent diseases and free from infection with intestinal parasites. They were divided into three groups, and each group included (5) rabbits:

Group I: the negative control group

They were injected with physiological salt solution (0.9%) subcutaneously at an amount of (1) ml per day for two weeks, after which blood was drawn for the purpose of obtaining serum and measuring IL-4, IL-6 and IL-10.

Group II: O-Antigen/ outer membrane proteins group

Immunization was done with O-antigen according to the method of [36,37] for a period of (14) days with an amount of (1) ml of antigen and as indicated in the above paragraph, and after the end of immunization, blood was drawn for the purpose of obtaining serum and measuring IL-4, IL- 6 and IL-10.

The second group was dosed above after completing the immunological experiments with *G. lamblia* was treated orally at a dose of 5×10^3 cysts /ml and was considered a challenge group, and the stool was examined daily to verify the occurrence of infection, and two weeks after infection, the immunological tests were repeated to compare the immunization with the O-antigen before and after infection with the parasite.

Group III: The positive control group

Animals of this group were infected with *G. lamblia* were treated with an oral dose of $5 \text{ X } 10^3$ cysts of the parasite. Animal feces were examined daily to confirm the occurrence of infection. Two weeks after infection, blood was drawn for the purpose of obtaining serum and measuring IL-4, IL-6 and IL-10.

Immunological Study

First: Estimation of the concentration of interleukins (IL-4, IL-6 and IL-10) in blood serum.

The concentration of interleukins (IL-4, IL-6 and IL-10) in the blood serum of laboratory rabbits was estimated using a special analysis kit prepared by the Chinese company SUNLONG, where the

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concentration of each of the above interleukins in the blood serum was measured using the Sandwich-ELISA method, which is based on The principle of color change resulting from the binding of specific antibodies specific to the antigen.

Statistical Analysis

The present results were statistically analyzed by using Duncan¼s multiple range test; the means of the groups were measured with $P \leq 0.05$ and by using the SPSS program. [38]

Results

Diagnosis and isolation of the parasite

G. lamblia was diagnosed by direct examination using physiological saline (0.9%), iodine-allocale solution and eosin dye. trophozoites and cysts appeared in the examined smears.

Diagnosis of *Escherichia coli*

Identified bacteria E. coli using the VITEK 2 device, as well as the use of Eosin Methylene Blue Agar (EMB) medium, which gives colonies with a green metallic sheen. [17]

Estimation of the concentration of proteins in the outer membrane protein extract sample

The concentration of the extracted outer membrane proteins was estimated according to the method of Lowry *et al*,[33] and by using the standard curve that shows the linear relationship between the absorbance at a wavelength of (750) nm and the standard protein concentrations estimated at (µg/ml), it was found that the protein concentration in the outer membrane proteins solution was 280 µg/ml.

Estimation of toxicity of outer membrane proteins

The results of the O-antigen toxicity experiment on rabbits treated with concentrations (100, 200, 400, 800, 1000 and 1400) µg /ml showed the following:

- ❖ No disease sign was observed in the animals treated with concentrations (100, 200, 400 and 800) µg/ml compared to the negative control group and they were monitored for two weeks.
- ❖ Lethargy and general weakness were observed in animals treated with a concentration of 1000 µg/ml.
- ❖ It was observed (general weakness, hair loss from different parts of the body, and poor movement) on animals treated with a concentration of 1200 µg /ml.
- ❖ There were deaths in a number of animals treated with a concentration of 1400 mcg / ml on the fifth day, and based on these results

The used concentrations were tested (100, 200, 400 and 800) µg /ml under study.

Immunological tests

Effect of O-antigen on the level of interleukin-4 in serum

The results of the current study showed, as shown in Figure (2), that the IL-4 level of the O-antigen group increased significantly compared with the negative control group, where its concentration was (12.35 and 8.71) pg/ml, respectively.

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The current results (Figure 2) showed a significant increase in the level of IL-4 in the challenge group, and it was found that the level of IL-4 increased more than before compared to the positive control, where its concentration averaged (14.11 and 10.98) pg/ml, respectively.

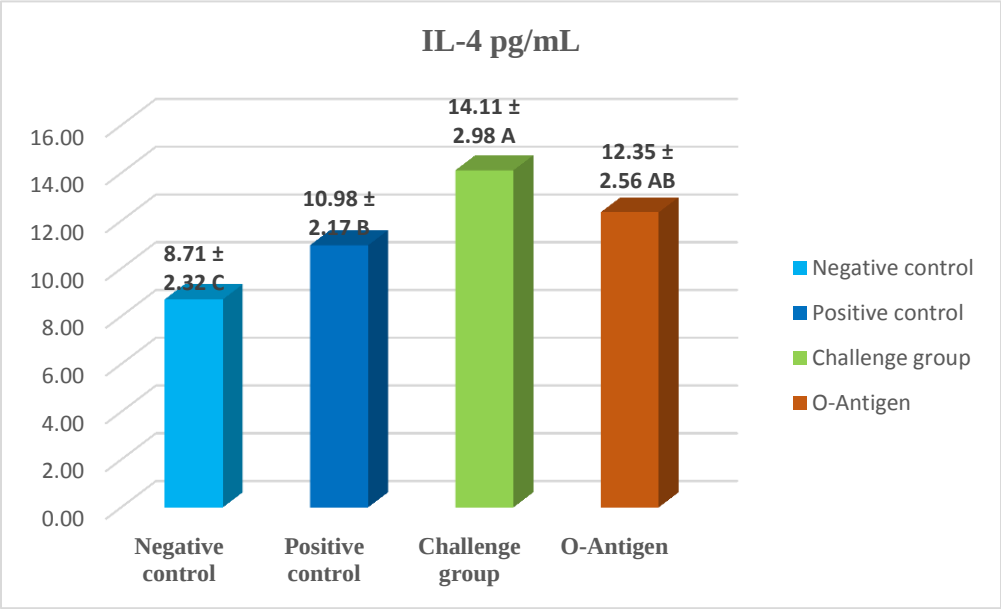

Figure 2: Interleukin-4 level of rabbits in the study groups, Similar letters indicate to non-significant differences at $P > 0.05$**,** while Different letters indicate to significant differences at $P \leq 0.05$

The effect of O-antigen on the level of interleukin-6 in serum

The results of the current study (Figure 3) indicated that the level of IL-6 of the O-antigen group was significantly increased compared with the negative control group, where its concentration was (5.24 and 3.37) pg/ml, respectively.

It was found through the current results (Figure 3) that the level of IL-6 in the challenge group increased in a non-significant manner compared to the positive control, as its concentration averaged (6.11 and 5.82) pg / ml, respectively.

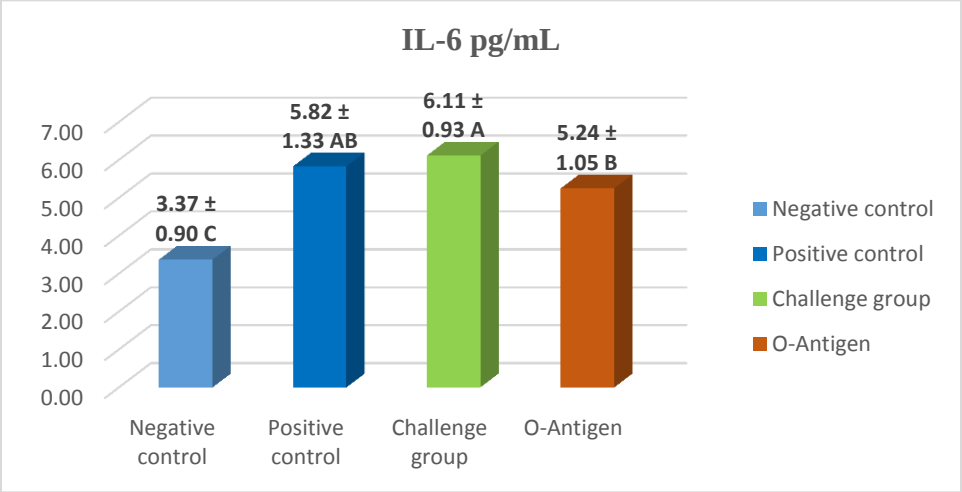

Figure 3: Interleukin-6 level of rabbits in the study groups, Similar letters indicate to non-significant differences at $P > 0.05$**,** while Different letters indicate to significant differences at $P \leq 0.05$

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The effect of O-antigen on the level of interleukin-10 in serum

The results of the current study (Figure 4) showed a significant increase in the level of IL-10 in the O-antigen group compared with the negative control group, where its concentration was (17.17 and 10.41) pg/ml, respectively.

The current results, as shown in Figure (4), indicated a significant increase in the levels of IL-10 in the challenge group, and it was noted that the level of IL-10 increased more than previously compared with the positive control to reach (21.84 and 14.79) pg/ml, respectively.

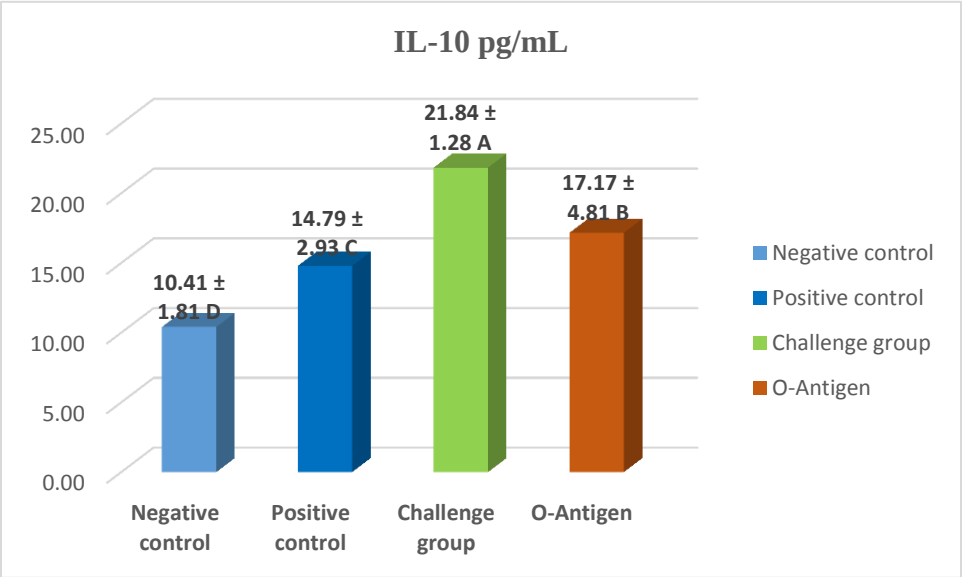

Figure 4: Interleukin-6 level of rabbits in the study groups, Similar letters indicate to non-significant differences at $P > 0.05$**,** while Different letters indicate to significant differences at $P \leq 0.05$

Discussion

The current results of IL-4 were consistent with the results of Majeed, who indicated that inoculation with *K. pneumoniae* vaccine caused a significant increase in the level of IL-4 compared with the control group, as its concentration reached (74.76 and 61.36) pg/ml, respectively, [39] and also agreed with the results of Schaut, where they confirmed that there was a significant increase in the level of IL-4 in laboratory mice after they were vaccinated with E. coli vaccine, where its concentration reached (4.80 and 0.90) pg / ml, respectively.[40] Antigenic proteins purified from the outer membrane of *E. coli* stimulate a T-cell-dependent cellular and humoral immune response. [41,42] The reason for the increase in the level of IL-4 when immunizing with O-antigen can be explained by the fact that the immune system includes T cells, which are classified into two subgroups (based on the function and discrimination of MHC molecules and the expression of cell surface proteins), which are the helper cells (CD4+ T) and cytotoxic cells (CD8+ T), in addition to classifying T cells into two secondary classes (Subset) depending on the cytokines, they produce, where different types of cytokinesis are secreted in response to antigen stimulation, the first class (Th1) produces IL-2 and IFN-γ. The second (Th2) produces interleukins (4, 5, 10 and 13). [43,44]

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For the challenge group we did not find studies published in the literature indicating agreement or lack thereof with the current findings. Jiménez reported an elevated level of IL-4 in the case infection with *G. lamblia*,[45] Cotton showed that infection with *G. lamblia* causes increased expression of several cytokines, including IL-4, and the reason may be due to the activity of the parasite's cysteine protease enzyme, which stimulates the functional differentiation of Th2 cells, [46] The infection with *G. lamblia* stimulates the immune response by T helper cells of both types (Th1 and Th2),[47] and since T cells (Th2) when activated produce IL-4 (which is the main cytokine produced by activated Th2 during infection with Giardiasis), the reason for the high level of IL-4 can be attributed to it, and it was found that the cellular and humoral immune response dependent on T cells It is stimulated when infection with the *G. lamblia*, and that stimulation of T cells (CD4+T) causes B cells to produce specific antibodies to parasite's antigens.[22]

Galdiero and Gupta, confirmed outer membrane proteins of *Salmonella typhimurium* stimulate the production of several cytokines, including IL-6.[48,49] The ability of outer membrane proteins to stimulate IL-6 is probably attributed to the presence of so-called loops that confer many biological properties on these proteins.[48] The current results agreed with the results of Turkan, where he indicated to significant increase in the level of IL-6 in people infected with the same parasite under study compared with healthy subjects, as its concentration averaged (18.60 and 8.34) pg/ml, respectively.[50] Das-Neves indicated that *G. lamblia* stimulates the expression of several cytokines, including IL-6.[51] The control on infection with *G. lamblia* parasite requires both humoral and cellular immune response, which includes the release of cytokinesis (IL-4, IL-6, IFN-γ and TNF-α), and IL-6 is the main cytokinesis whose concentration is elevated during infection with Giardiasis, as well as a pro-inflammatory cytokine, stimulate the innate immune response against *G. lamblia*.[22]

The current results for IL-10 are agreement with the results of Al-Refaei, which showed that outer membrane proteins purified from *K. pneumoniae* resulted in a significant increase in the level of IL-10 after its use in immunizing laboratory rabbits compared with the negative control, where the concentration of IL-10 reached (34.58 and 18.72) pg/ml, respectively,[35] and agreed with the results of Majeed, who indicated that vaccination with the vaccine *K. pneumoniae* caused a significant increase in the level of IL-10 compared with the control group, and it was revealed that the level of IL-10 reached (132.93 and 109.56) pg/ml, respectively, [39] while the current results were not agreement with those of Schaut, where they confirmed that there was a significant decrease in the level of IL-10 in laboratory mice after they were given a vaccine of *E. coli* bacteria compared with the control, where the concentration of interleukin-10 was (2.5 and 8.5) pg/ml, respectively.[40] IL-10 is an important molecule in the control of viral, bacterial, and parasitic infections, allergic conditions, and autoimmune diseases. [52]

Jiménez showed a significant increase in the level of IL-10 in the case of immunization of mice with the enzyme cysteine protease of the *G. lamblia* parasite compared with the control, where the concentration of IL-10 was (34.58 and 18.72) pg/ml, respectively.[45] The experimental infection with *G. lamblia* leads to increased IL-10 production.[46] Perhaps the reason for the increase in the level of IL-10 in the challenge group can be explained by the presence of T cells, which are components of the immune system, and are classified into two subgroups (based on the function and discrimination of MHC molecules and the expression of cell surface proteins) into helper cells (CD4+ T) and Cytotoxic cells (CD8+ T), in addition to classifying T cells into two secondary classes (Subset) depending on the cytokines they produce, where different types of cytokines are secreted in response to antigenic

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stimulation, the first class Th1 produces IL-2 and IFN-γ, while the second class is called Th2 produces Interleukins (4, 5, 10 and 13), and each type of T cells has a different immune response from the other, as Th1 stimulates cellular immunity responsible for intracellular pathogens such as some bacteria and viruses, while Th2 cells stimulate humoral immunity responsible for the production of specific antibodies against extracellular pathogens (fungi, bacteria and parasites),[43,44] Since the parasite *G. lamblia* is an extracellular parasite, the immune response towards it may be stimulated by Th2, and this was confirmed by the results of the current study and some studies that indicated that the parasite antigens stimulate the production of different cytokines (IL-4, IL-5, IL-10 and IFN-γ), that have a role in parasite resistance.[22] Changes in the growth of epithelial cells of the intestinal lining are an essential response to intestinal infections and facilitate the removal of damage and cells infected with the pathogen. *G. lamblia* inhibits the regeneration of intestinal epithelial cells and induces their apoptosis.[46] Therefore, IL-10 is very important because it increases host resistance by maintaining the intestinal epithelial barrier.[53] IL-10 has a significant role in protecting the host from direct damage caused by protozoan or helminth infection, but the mechanism is unclear. [54]

The current results for IFN-γ were agreement with the results of Al-Refaei, showing that outer membrane proteins purified from *K. pneumoniae* resulted in a significant increase in the level of IFN-γ after immunization of laboratory rabbits compared with the negative control, where the concentration of IFN-γ was (29.18 and 20.36) pg/ml, respectively, [35] It also agrees with the results of Schaut, where who indicated a significant increase in the level of IFN-γ in laboratory mice vaccinated with *E. coli*, where the concentration of IFN-γ reached (212.0 and 101.0) pg/ml, respectively.[49] Protein antigens purified from the outer membrane of *E. coli* stimulate a T-cell-dependent cellular and humoral immune response, [42,43] and protein nature of antigens stimulate a T-cell-dependent humoral immune response, and antigens are phagocytized by macrophages (antigen-presenting cells) and presented in the form of a peptide carried on MHC Class II that interferes with T cell receptor (TCR).[43] As mentioned above, the immune system includes two secondary classes of T cells (Th1 and Th2), depending on the cytokinesis that they produce. Different types of T cells are secreted in response to antigen stimulation. The first class (Th1) produces IL-2 and IFN-γ.[43,44]

Turkan, indicated that there was a significant increase in the level of IFN-γ in people infected with the same parasite under study compared with healthy subjects, as its concentration averaged (39.51 and 21.98) pg/ml, respectively,[50] and also agreed with the results of Brune, indicated that the level of IFN-γ was higher in patients with giardiasis than in healthy subjects, as its concentration averaged (14.50 and 4.40) pg/ml, respectively.[55] The infection with *G. lamblia* parasite stimulates the immune response by T-helper cells of both types (Th1 and Th2), [47,56] as host control of infection with *G. lamblia* parasite requires both humoral and cellular immune response, which includes liberation of cytokines (IL- 4, IL-6, IFN-γ and TNF-α).[44] Since T cells (Th1) when activated produce IFN-γ, [22] the reason for the high level of IFN-γ can be attributed to them. The action of IFN-γ in Giardiasis infection is also associated with the production of active oxygen against the parasite by macrophage cells, but its mechanism of action is not clear. [55]

Conclusions

1- Elevation of interleukins concentration under study after immunization with O-antigen.

2- Elevation of gamma-interferon concentration after immunization with O-antigen.

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3- The outer membrane proteins extracted from *E. coli* bacteria and at the concentrations used in the current study can be considered as non-specific immunomodulators and non-toxic compounds at the specified concentrations and as stimulators of the immune system against infection with the *G. lamblia* parasite in white laboratory rabbits.

Limitation of the study

Patients infected with the *Giardia lamblia* parasite did not refuse to give a stool sample in support of scientific research, in addition to that we did not need more than three samples to cause an experimental infection of laboratory rabbits.

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Nil.

Conflicts of interest

There are no conflicts of interest

References

1. Park, Y., Cho, H., Jang, D., Park, J. and Choi, K., Multilocus genotyping of *Giardia duodenalis* in pre-weaned calves with diarrhea in the Republic of Korea. PLoS ONE, 2023; 18(1): 1-16.

2. Davoodi, J. and Abbasi-Maleki, S. Effect of *Origanum vulgare* Hydroalcoholic Extract on *Giardia lamblia* Cysts Compared with Metronidazole in Vitro. Iran J Parasitol 2018; 13(3):486-492.

3. Fusaro, C., Chávez-Romero, Y.A., Prada, S.L.G., Serrano-Silva, N., Bernal, J.E., González-Jiménez, F.E. and Sarria-Guzmán,Y. Burden and Epidemiology of Human Intestinal *Giardia duodenalis* Infection in Colombia: A Systematic Review. Trop. Med. Infect. Dis., 2022, 7(325): 1-18.

4. Ali, I.Y., Mero, W.M.S. and Mohammed, A.B.Prevalence and risk factors of *Giardia lamblia* among infants and children in Duhok province/Kurdistan Region, Iraq. Academic Journal of Nawroz University (AJNU), 2022; 11(4): 147-152.

5. Ryan, U.M., Feng, Y., Fayer, R., Xiao, L. Taxonomy and molecular epidemiology of Cryptosporidium and Giardia–a 50-year perspective (1971–2021). Int J Parasitol 2021; 51 (13–14): 1099–1119.

6. Trelis, M., S´aez-Dur´an, S., Puchades, P., Castro, N., Miquel, A., Gozalbo, M. and Fuentes, M.V. Survey of the occurrence of *Giardia duodenalis* cysts and *Cryptosporidium* spp. oocysts in green leafy vegetables marketed in the city of Valencia (Spain). International Journal of Food Microbiology, 2022; 379: 1-7.

7. Meng, X., Kang, C., Wei, J., Ma, H., Liu, G., Zhao, J., Zhang, H., Yang, X., Wang, X., Yang, L., Geng, H,. and Cao, H. Meta-Analysis of the Prevalence of *Giardia duodenalis* in Cattle in China. FOODBORNE PATHOGENS AND DISEASE, 2023; 20(1): 17-31.

8. El-Gendy, A.M.L., Mohammed, M.A.A. Ghallab, M.M.I., Abdel Aziz, M.O., Ibrahim, S.M. Therapeutic Effect of Chitosan Nanoparticles and Metronidazole in Treatment of Experimentally Giardiasis Infected Hamsters. Iran J Parasitol 2021;16(1): 32-42.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 02, 2023

9. Aziz, A.F.E., Roshidi, N., Othman, N., Hanafiah, K.M. and Arifin, N. Application of Proteomics to the Study of the Therapeutics and Pathogenicity of Giardia duodenalis. Diagnostics 2022, 12, 2744): 1-14.

10. Farthing, M.J.G. Giardiasis as a disease. *In*: *Giardia:* from molecules to disease. Thompson, R.C.A. Reynoldson, J.A. and Lymbery, A.J. (eds.). Wallingford, Oxon, CAB Int England, 1994; 394pp.

11. Kilinç, Ö.O. Ayan, A., Çelik, B.A., Çelik, Ö.Y., Yüksek, N., Akyildiz, G. and O˘guz, F.E. The Investigation of Giardiasis (Foodborne and Waterborne Diseases) in Buffaloes in Van Region, Türkiye: First Molecular Report of Giardia duodenalis Assemblage B from Buffaloes. Pathogens, 2023;12(106):1-9.

12. Squire, S.A. and Ryan, U. Cryptosporidium and Giardia in Africa: current and future challenges. Parasites & Vectors, 2017; 10(195): 1-32.

13. Meningher, T., Boleslavsky, D., Barshack, I., Tabibian-Keissar, H., Kohen, R., Gur-Wahnon, D., Ben-Dov, I. Z., Sidi, Y., Avni, D., & Schwartz, E. *Giardia lamblia* miRNAs as a new diagnostic tool for human giardiasis. PLoS negl trop dis 2019; 13(6): 1-16.

14. Al-Ammash, M.S.J. Study on prevalences of *Entameoba histolytica & Giardia lamblia* in Samarra city. Kufa Journal for Veterinary Medical Sciences. 2015; 6(2): 194-204.

15. Bazzaz, A.A., Shaker, O.M. and Alabbasy, R.H. Prevalence of Two Gastrointestinal Parasites *Entamoeba histolytica* and *Giardia lamblia* within Samarra City, Iraq, Advances in Bioscience and Biotechnology, 2017; 8: 399-410.

16. AL-Khikani, F.H.O., Almosawey, H.A.S., Hameed, R.M., Abid alhussain, B.A.A.A., Ayit, A.S., Al-Ibraheemi, M.K.M. and Alsalami, M.M.A.Y. Prevalence of *Entamoeba histolytica* and *Giardia lamblia* Associated with Infectious Diarrhea in Al-Shomally Population, Babil, Iraq. Biomedical and Biotechnology Research Journal, 2019; 3(4): 245-248.

17. Sabry, N.N., Owaied, Y.H. and Mahmoud, A.J. Epidemiological Study of Common Bacterial and Parasitic Infections in Some Areas of Salah Al-Din Governorate, Iraq. IOP Conf Ser Earth Environ Sci 2021; 923:1-9.

18. Zaki, D.M. Prevalence of *Entamoeba histolytica* and *Giardia Lamblia* Associated with Diarrhea in Children referring to lbn Al-Atheer Hospital in Mosul, Iraq. Archives of Razi Institute 2022; 77(1): 63-69.

19. Roberts-Thomson, I.C., Stevens, D.P., Mahmoud, A.A.F. & Warren, K.S. Giardiasis in the mouse: an animal model. Gastroenterology, 1976b; 71: 57–61.

20. Owen, R.L., Allen, C.L. and Stevens, D.P. Phagocytosis of *Giardia muris* by macrophages in peyer's patch epithelium in mice. Infect Immun 1981; 33(2): 591–601.

21. Hill, D. R. *Giardia lamblia*. *In*: Principles and practice of clinical parasitology. Gillespie, S. H. and Pearson, R. D. (eds.). John Wiley & Sons, Ltd Chichester 2001; Pp: 219–241.

22. Lopez-Romero, G., Quintero, J., Astlazarán-Garcia, H. and Velazquez, C. Host defences against *Giardia lamblia*. Parasite Immunology 2015; 37(8): 394–406.

23. Osazuwa, F. and Abdul, I.O. Association between C‐reactive protein and interleukin‐10 levels and malaria severity among children in Warri, Southern Nigeria. CHRISMED J Health Res 2021;8:59-60.

24. Tortora, G.J., Funke, B.R. and Case, C.L. Microbiology An Introduction. 13thedn. Pearson 2018; 960pp.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 02, 2023

25. Maiti, B., Dubey, S., Munang'andu, H.M., Karunasagar, I., Karunasagar, I. and Evensen, Ø. Application of Outer Membrane Protein-Based Vaccines Against Major Bacterial Fish Pathogens in India. Frontiers in Immunology 2020; 11(1362): 1-12.

26. Anwar, M., Muhammad, F., Akhtar, B., Anwar, M.I., Raza, A. and Aleem, A. 2021. Outer Membrane Protein- Coated Nanoparticles as Antibacterial Vaccine Candidates. International Journal of Peptide Research and Therapeutics 27:1689–1697.

27. Maniha, S.M. and Noor, R. Genetic makeup and associated virulence posed by the enteropathogenic *Escherichia coli* and the enterotoxigenic *Escherichia coli* pathotypes. Biomed Biotechnol Res J 2020;4:280-4.

28. Parmar J, Tripathi S, Kumar A. Immunogenicity assessment of antileukemic agent glutaminase from *Escherichia coli*, *Pseudomonas* sp., and *Bacillus* sp. Biomed Biotechnol Res J 2022;6:138-44.

29. Mirzarazi, M., Bandehpour, M., Hashemi, A., Vahidi, M., Taghavi, A., Bashiri, S., *et al.* Antibiotic resistance, phylogenetic group, and genotyping investigation in *Escherichia coli* strains of gut flora in patients with colorectal cancer in Iranian population. Biomed Biotechnol Res J 2021;5:313-319.

30. Malik, S., Rana, J.S. and Nehra, K. Prevalence and antibiotic susceptibility pattern of uropathogenic *escherichia coli* strains in Sonipat region of Haryana in India. Biomed Biotechnol Res J 2021;5:80-87.

31. John, D.T. and Petri, W.A. Markell and Voge's Medical Parasitology. 9th edn., Saunders, Elsevier Inc., 2006; 463pp.

32. Clark, C.G. and Diamond, L.S. Methods of Cultivation of huminal parasitic protists of Clinical importance. Clin Microbiol Rev 2002; 15(3): 329-341.

33. Murphy, T.F., Dudas, K.C., Mylotte, J.M. and Apicella, M.A. A subtyping system for nontypable *Haemophilus influenza* based on outer membrane proteins, J Infect Dis 1983; 147(5): 838-846.

34. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randal, R.J. protein measurement with the folin phenol reagent. J Biol Chem 1951; 193(1): 265 - 275.

35. Al- Refaei, O.M.S. Effect some antigens of *Klebsiella pneumonia* as a immune catalyzer for laboratory rabbits against infected by *Entamoeba histolytica*. . Ph.D. Thesis. Faculty of Education, Tikrit University. 2016; 172pp.

36. Griffiths, E., Stevenson, P., Thorpe, R. and Chart, H. Naturally occuring antibodies in human sera that react with the iron-regulated outer membrane protein of *Escherichia coli*, Infect. Immun., 1985; 47(3): 808-813.

37. Tomas, J.M., Benedi, V.J., Ciurana, B. and Jofre, J. Role of Capsule and O-Antigen in Resistance of *Klebsiella pneumoniae* to Serum Bactericidal Activity. Infect Immun 1986; 54(1): 85-89.

38. Cleophas, T.J. and Zwinderman, A.H. SPSS for starters and 2nd levelers. 2nd edn. Springer Cham Heidelberg, New York, 2016; 375pp.

39. Majeed, A.A. Preparation of attenuated vaccine for *Klebsiella pneumoniae* isolated from respiratory tract infection by using nanobiotechnology. M.Sc. Thesis, Col Sci Univ Baghdad, 2019; 136pp.

40. Schaut, R.G., Boggiatto, P.M., Loving, C.L. and Sharma, V.K. Cellular and Mucosal Immune Responses Following Vaccination with Inactivated Mutant of *Escherichia coli* O157:H7. Scientific Reports, 2019; 9:1-11.

41. Guan, Q., Wang, X., Wang, X., Teng, D., Mao, R., Zhang, Y. and Wang, J. Recombinant outer membrane protein A induces a protective immune response against *Escherichia coli* infection in mice. Appl Microbiol Biotechnol 2015; 99, 5451–5460.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 02, 2023

42. Pen, G., Yang, N., Teng, D., Hao, Y., Ruoyu Mao, R. and Wang, J. The Outer Membrane Proteins and Their Synergy Triggered the Protective Effects against Pathogenic *Escherichia coli*. Microorganisms 2022; 10(982): 1-15.

43. Punt, J., Stranford, S.A., Jones, P.P. and Owen, J.A. Kuby immunology. 8th edn. W.H. freeman, New York, 2019; 1904pp.

44. Penman, I.D., Ralston, S.H., Strachan, M.W.J. and Hobson, R.P. Davidson's Principles and Practice of Medicine. 24thedn. Elsevier. 2022; 1357pp.

45. Jiménez, J.C., Fontaine, J., Grzych, J.M., Capron, M. and Dei-Cas, E. Antibody and cytokine responses in BALB/c mice immunized with the excreted/secreted proteins of Giardia intestinalis: the role of cysteine proteases. Ann Trop Med Parasitol 2009; 103(8): 693–703.

46. Cotton, J.A., Motta, J., Schenck, L.P. Hirota, S.A., Beck, P.L. and Buret, A.G. Giardia duodenalis Infection Reduces Granulocyte, Infiltration in an In Vivo Model of Bacterial Toxin-Induced Colitis and Attenuates Inflammation in Human Intestinal Tissue. PLOS ONE 2015; 9(10): 1-15.

47. Jiménez, J.C., Fontaine, J., Creusy, C., Fleurisse, L., Grzych, J., Capron, M. and Dei-Cas, E. Antibody and cytokine responses to *Giardia* excretory/secretory proteins in *Giardia intestinalis*-infected BALB/c mice. Parasitol Res 2014; 113: 2709-2718.

48. Galdiero, M., Cipollaro de l'Ero, G., Donnarumma, G., Marcatili, A. and Galdiero, F. Interleukin-1 and interleukin-6 gene expression in human monocytes stimulated with *Salmonella typhimurium* porins. Immunology 1995; 86: 612–619.

49. Gupta, S. Priming of T-cell responses in mice by porins of *Salmonella typhimurium*. Scand J Immunol 1998; 48: 136–143.

50. Turkan, A.H.H. Epidemiological, immunological and molecular study of the effect of *Giardia lamblia* infection in children attending health centers in Tikrit. Ph.D. Thesis. Faculty of Education for pure science, Tikrit University 2020; 120pp.

51. Das-Neves, I.M.P. Host cell response upon exposure to *Giardia intestinalis* glycoproteins *in vitro.* M.Sc. Thesis. Uppsala University, Sweden 2016; 35pp.

52. Couper, K.N., Blount, D.G. and Riley, E.M. IL-10: The Master Regulator of Immunity to infection. J Immunol 2008; 180: 5771-5777.

53. Kyou-Nam, C., Stephen, M. and Eric, R. The NF-KB p50 Subunit Is Protective during Intestinal *Entamoeba histolytica* Infection of 129 and C57BL/6 Mice. Infect Immun 2010; 78(4): 1475-1481.

54. Ouyang, W. and O'Garra, A. IL-10 Family Cytokines IL-10 and IL-22: from Basic Science to Clinical Translation. Immunity 2019; 50(4): 871-891.

55. Brune, M.W., França, E.L., Moraes, L.C.A., Ribeiro, V.P., Gomes, M.A. and Honorio-França, A.C.H. Effects of Cytokines IFN-γ and TGF-β on the Functional Activity of Blood Mononuclear Cells against *Giardia lamblia*. Iran J Parasitol 2021; 16(2): 209-218.

56. Berger, A. "Th1 and Th2 responses: what are they?," BMJ 2000; 321(7258); 424.