RESEARCH ARTICLE

The Role of TLR2 and TLR4 Immune Receptors in the Immune Response against the Giardia Lambila Parasite

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ABSTRACT

Patients with Giardia lamblia infections who visited Samarra General Hospital and a few private labs in Samarra and the Salah al-Din Governorate were the subjects of the study. The age range of the groups was 5 to 22 years old. The project was finished between November 28, 2023, and February 2, 2024. Using an optical microscope to examine stool samples and identify the feeding and encysted stages, cases of parasite infection were identified. The impact of the current study on many immunological variables, interleukin ten, or IL_10, and both Toll_like2 and Toll_like4 was investigated in (90) stool samples, and the investigation examined its impact on a few immunological variables, including interleukin ten, or IL_10, and Toll_like2 and Toll_like4. The results showed a significant decrease in the levels of interleukin 10 (122.423±29.788 pg/ml compared to the control group (62.312±345.934) pg/ml, while the Toll_like2 immune receptor increased (8.374±2.133) pg/ml compared to the control group (0.814) ± ± 2.472) as well as the Toll_like4 immune index increased (3.566 ± 1.231) pg/ml compared to the control group (0.432 ± 1.181) pico grams/ml.

INTRODUCTION

Giardiasis is a common parasitic disease that spreads across the globe. Giardiasis is an infection of the human small intestine caused by the protozoan parasite Giardia lamblia (1). It induces giardiasis, a prevalent ailment shared by both people and animals (2). The infection spreads throughout the world, and is responsible for more than 280 million cases of gastrointestinal complaints annually worldwide (3).

The life cycle of Giardia includes two stages: non-pathogenic trophozoite form, and the infectious, environmentally resistant form, the cyst (4).

Giardia infections can spread through the mouth and feces, as well as by direct contact with sick animals and ingestion of contaminated water, food, or soil. Within 1-3 weeks, the parasite cysts are ingested through contaminated food and drink, resulting in infection (5). A Giardia infection can cause diarrhea, nausea, vomiting, abdominal pain, and weight loss. It also compromises the integrity of the intestinal epithelial barrier (6). Intestinal cells and the intestinal lumen finally form a physical barrier as a result of the fast growth of Trophozoite stages. Since the human body has numerous systems that function to naturally maintain it, the penetration of the parasite into the intestinal tissues of the host during the feeding stage induces the creation of a cellular immune response. The
immune system, which functions to prevent foreign objects from entering the body through specialized cells with the aid of the thymus gland, is the most significant of these systems (7).

White blood cells derived from the bone marrow play a role in acquired immune responses by utilizing their inherent defense mechanisms, such as the secretion of mucus and enzymes that cover the linings of the digestive tract, bowel movements, and the acidic environment of the stomach(8). Through these innate immune responses, the body combats invasion by parasites. The body has several defense mechanisms in place to combat parasite invasion, including large and mesenteric lymph nodes, secondary lymphoid organs in the colon, and other immune physiological barriers. These barriers, such as Peyer's spots, White blood cells are located in the spaces between epithelial cells, and the tightness of the epithelial cells is maintained by protein complexes(9), work together to protect the body. The immune receptors play a crucial role in this defense mechanism. The cellular immune response is triggered when the parasite's feeding stage enters the host's intestinal tissues. The parasite tries to attach to the epithelial cells in the small intestine to form colonies, which then activate the inflammatory process. This, in turn, stimulates the production and release of cellular cytokines like interleukin 10 (IL-10) by these cells. The recruitment of immune cells such as macrophages, neutrophils, and mononuclear cells towards the inflammatory region is facilitated by a regulator. The cells undergo phagocytosis, enveloping the parasite and then breaking it down by digestion (10).

MATERIALS AND METHODS

The samples collecting

From November 2023 to February 2024, scientists at Samarra General Hospital and several commercial labs collected 90 samples from individuals who had been positively diagnosed with *Giardia lamblia*, a parasitic organism known to induce diarrhea, sometimes accompanied by steatorrhea and stomach discomfort. Their ages ranged from five to twenty-two.

Hygienic, spacious receptacles were used to maintain the samples in a moist state. The desiccation of the material was halted. The specimens were meticulously examined within thirty minutes of their arrival at the laboratory. Blood or mucous in these locations indicates a giardia infection.

After confirming the presence of a parasite infection by direct swabbing, blood samples were taken from Administer 5 milliliters (ml) intravenously to each patient using a disposable syringe. A volume of five milliliters of blood was subjected to centrifugation for a duration of fifteen minutes using specialized test tubes. The serum that was created was dispersed using a micropipette, which operated at a speed of 3000 rpm as the following:

1. 1 milliliter of the resultant serum was placed into five milliliter sterile plastic tubes that have been labeled, and then was freezing at -20 degrees Celsius to preserve them in case the results need to be reexamined or more serum is needed.

2. The remaining serum was stored into plain plastic tubes free of any Coagulation substance for the purpose of detecting interleukin 10, and the immune receptors TL2 and TL4. The samples exhibiting hemolysis were disregarded. In order to ensure precision in the outcomes, every sample was assigned a number.

The procedure:

1. Detection of the parasite by direct smear method

A drop of physiological saline solution was used to evaluate the samples using the direct smear technique. Before setting the protozoa cysts on the glass slide and staining their nuclei with Lugol's iodine, collect a tiny quantity of stool—about the size of a matchstick head—from several locations using clean wooden sticks. After the balanced salt solution and the specified quantity of feces were thoroughly mixed, a sliding cover was delicately put over the mixture to avoid the creation of air.
bubbles. After that, the cover was put on the slide and the optical microscope was used with a 10x and a 40x objective lens to verify that the parasite was present. (8).

2. Concentration method

3- Zinc sulphate flotation method

1 - 2 Grams were used as a unit of measurement for quantifying the volume of feces. After completely diluting with sterile water using a glass rod, the cysts should be separated from the feces. Subsequently, filter the completed product by using four layers of cheesecloth. Subsequently, transfer the filtrate into test tubes and subject them to centrifugation at a speed of 1000 revolutions per minute for a duration of 5 minutes in order to separate and collect the liquid. Once the buoyant object has been taken out of the test tubes, a little amount of distilled water is introduced to dissolve the solid particles. Then, the tube is filled with water and then reinserted into the centrifuge. There is a recurrence of sedimentation. The cleaning and sedimentation process continues until the float achieves a condition of cleanliness. Subsequently, a solution of zinc sulfate is introduced to the silt, irrespective of its level of purity. Subsequently, the test tubes are returned to the centrifuge and undergo rotation at an identical velocity for an equivalent duration. The supernatant is obtained by using a Pasteur pipette to deposit the liquid onto a glass slide that is previously coated with a drop of iodine solution. The slide is then seen using a microscope.

Flotation method with sucrose solution

Follow the same steps as in the previous procedure and use the sucrose solution instead of the Zinc sulfate solution (11)

The content of interleukin-10 and TLR2, 4 immune receptors was measured using an ELISA kit manufactured in Spain.

RESULTS AND DISCUSSION

The current study showed that the level of interleukin 10 in people infected with the Giardia lamblia parasite decreased compared to uninfected people, as in Table (1)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Value P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected with parasite</td>
<td>122.423± 29.788</td>
</tr>
<tr>
<td>Control</td>
<td>62.312 ± 345.934</td>
</tr>
</tbody>
</table>

Similar English letters indicates that no significant differences at p <0.05 level comparison between groups

These results were in agreement with study by (4, 10) and not in agreement with (11, 12.13, 14).

As for the immune receptors TLR 2 and TLR 4, they increased significantly compared to the control group, as shown in Tables 2 and 3.
The Role of TLR2 and TLR4 Immune Receptors

Table 2: Toll like Receptor TLR 2- level in infected with giardia

<table>
<thead>
<tr>
<th>Groups</th>
<th>(pg/ml) TLR2</th>
<th>Value P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average ± standard deviation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M ± S.D</td>
<td></td>
</tr>
<tr>
<td>Infected with parasite</td>
<td>8.374 ± 2.133</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>0.814 ± 2.472</td>
<td>B</td>
</tr>
</tbody>
</table>

Similar English letters indicates that no significant differences at p <0.05 level comparison between groups

Table 3: Toll like Receptor TLR 4- level in infected with giardia

<table>
<thead>
<tr>
<th>Groups</th>
<th>(pg/ml) TLR4</th>
<th>Value P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average ± standard deviation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M ± S.D</td>
<td></td>
</tr>
<tr>
<td>Infected with parasite</td>
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</tr>
<tr>
<td>Control</td>
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These findings were in conflict with both (15) and (16).

The innate and acquired immune responses are what determine how the body reacts to microbiological pathogens, such as Giardia. Several investigations have shown the emergence of acquired immune systems, despite the fact that the defense mechanisms in place to manage Giardia remain poorly understood (17, 18).

IL-10 has both positive and negative effects due to its regulatory role, and its production by various immune cells classifies it as multidirectional. One of the pleiotropic effects of this substance is its ability to enhance the activity of phagocytic cells in the process of phagocytosis. Additionally, it promotes the synthesis of TNF-α and IFN-γ by natural killer cells (NK). Furthermore, it serves the purpose of controlling the immune response, safeguarding the intestinal epithelial barrier, and limiting the demise of host cells and tissue damage caused by inflammation. Additionally, it stimulates the proliferation of activated B cells and facilitates their transformation into cells that secrete antibodies (19).

Each type of T cell carries out a distinct immunological response. Th1 cells promote humoral immunity, which is in charge of manufacturing antibodies, whereas Th2 cells promote cellular immunity, which is in charge of intracellular parasites like bacteria and viruses. The immune system reacts to infections that are outside of cells. Th2 stimulates the immune response because Giardia lamblia are extracellular parasites (20).
The findings indicated a rise in immune receptor levels because TLR2, a membrane protein that identifies foreign objects and transmits signals to immune system cells, is thought to be the foundation of natural immunity. An immunological reaction is triggered when the host becomes infected with the Giardia parasite. Activating Toll-like receptor 2 (TLR2), a kind of pattern recognition receptor that is essential for identifying microbial infections, is one method to achieve this (21).

When TLR2 detects specific molecular patterns associated with pathogens such as *Giardia lamblia*, it initiates a signaling cascade within immune cells, leading to the production of pro-inflammatory cytokines and other molecules that help fight infection. Activation of TLR2 by *Giardia lamblia* is an important step in the host immune response to the parasite (20, 21). Understanding the mechanisms involved in this process can provide insight into both host defense mechanisms and potential targets for therapeutic intervention in giardiasis (22).

*Giardia* infection of the intestine can be used to explain the rise in both immune receptors because Toll-like receptor 4 (TLR4) on the surface of immune cells recognizes particular chemicals on the intestine's surface, such as lipopolysaccharides (LPS) or glycoproteins. The cell's signaling cascade is started by this recognition. Activation of TLR4 attracts adaptor proteins, like TRIF and MyD88, which start signaling pathways downstream (23).

These pathways involve various protein kinases, such as the IRAKs RAF6, which ultimately lead to the activation of transcription factors such as NF-kB and AP-1. Once activated, NF-kB and AP-1 translocate to the nucleus and stimulate the expression of pro-inflammatory cytokines (24). For example, TNF-α, IL-1β, IL-6 and chemokines (e.g., IL-8, MCP-1). These molecules attract immune cells to the site of infection and promote inflammation, which helps eliminate the parasite (25).

However, this immune response can also lead to tissue damage and contribute to symptoms of giardiasis, such as diarrhea and abdominal pain. Therefore, while TLR4 activation is necessary to mount an immune response against *Giardia lamblia* (26). The interleukin 10 level decreased in the current study's results, which could be the result of weakening.

The cytokine production of immune cells is reduced as a result of the cellular immune response, which further weakens the host's defenses against infection. This ultimately has detrimental effects on all biological processes, with interleukin 10 being one example (27).

IL-10 has both positive and negative effects due to its regulatory role, and its production by many immune cells makes it a multidirectional factor. One of its pleiotropic effects is the stimulation of phagocytic cells to engage in the process of phagocytosis. Additionally, it promotes the production of TNF-α and IFN-γ from natural killer cells (NK). Furthermore, it serves the purpose of controlling the immune response, safeguarding the intestinal epithelial barrier, and limiting the demise of host cells and tissue damage caused by inflammation. Additionally, it stimulates the proliferation of activated B cells and facilitates their transformation into cells that secrete antibodies (28).

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