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Measurement of IL-10 levels in spleen tissue of experimentally immunosuppressive (BALB/ C) mice infected with *Cryptosporidium* spp.

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Abstract

Cryptosporidium spp. which can cause weight loss and severe watery diarrhea, is often found in the intestinal tracts of both animals and humans. However, little is known about the host immune responses elicited upon infection with *Cryptosporidium* spp. Although cryptosporidiosis due to *Cryptosporidium* spp. generally self-limiting in immunocompetent individuals, it can be lethal in immunocompromised or in immunosuppressed hosts, such as infants and HIV/AIDS. Current study aimed to investigate of IL-10 Level in spleen homogenate for immunosuppressant BALB/ C mice by dexamethasone and infected with *Cryptosporidium* spp. Level of IL-10 appeared to increase in infected group (infected with *Cryptosporidium* spp.) (1446.93 ±161.02 pg/ml) compared to control group (non-infected) (641.20±40.42pg/ml), while it get decreased in immuno suppressant group (immuno suppressed by dexamethasone and infected with *Cryptosporidium* spp.) (872.26 ±46.20 pg/ml) P≤0.001. The results of the current study may provide benefit to future research on the immune response to *Cryptosporidium* spp. infection in temporary immunosuppressed populations.

Key words:- *Cryptosporidium spp.*, spleen homogenate, Dexamethasone, IL-10

Introduction

Malnutrition is caused by the loss of appetite, decreased absorption of food, and a higher catabolism of nutrient stores due to the inflammation and diarrhea caused from enteric protozoal infections by Cryptosporidium spp., which represent a significant global health concern. Cryptosporidiosis is a virulent endemic parasite which notably, infects individuals with immunocompromised illnesses as well as children under four years old . It annually accounts for >7.5 million disability-adjusted life-years and >4.2 million reported cases (1). Cryptosporidium species causing the infection include (*C. parvum and C. hominis*). The fecal–oral pathway is the route of spread for Cryptosporidium spp. from sources such as drinking water or recreational waters contaminated with raw sewage and/or animal faeces(1).

The result and severity of cryptosporidiosis, as well as the host's vulnerability to infection with this parasite, are significantly influenced by their immunological condition. Infections in immunocompetent hosts are frequently self-limited, mild to moderate, or asymptomatic (2). Nonetheless, infection can cause chronic, crippling, and potentially lethal diarrhea and wasting in immunocompromised hosts, including those with HIV/AIDS, congenital immunodeficiencies, and transplant recipients (2,3). The majority of symptomatic infections in regions where cryptosporidiosis is endemic happen in young children and those with compromised immune systems (4).

Intestinal epithelial cells (IECs) lining the small intestine serve as the first line of defense against invasive *Cryptosporidium* spp. via a variety of physical and physiological mechanisms. Sporozoites, and severity of cryptosporidiosis is directly related to the immunologic status of the host (5). Key stages in the infection cycle of *Cryptosporidium* spp. Nitric oxide, dendritic cells (DCs), phagocytes, natural killer (NK) cells, and complement system protect against them. The primary function is to protect the host from infection (6), however, CD4+ T cells IFN-γ is also required during early infection for suppressing parasite replication (6), while Th2 cytokines such as IL-4 are important for limiting pathogenesis (6). Nonetheless, the IL-4 and IFN-γ–deficient mice cleared infections with *Cryptosporidium* spp. (7). The spleen is a large secondary lymphoid organ found in the blood with the function primarily as a large blood filter. It was used to sort settled damaged cells, apoptotic bodies, antigen-antibody complexes, effector red blood cells, etc. The spleen has a wealth of micro- anatomical diversity reflecting the complexity and variety of its functions. The spleen is a distinct immunologic organ of the body and plays an important role in both the innate and adaptive immune systems. Certain types of innate immune cells, for instance, B cells, natural killer (NK) cells and macrophages reside in special areas of the spleen. In particular, the spleen acts as a peripheral site for immune tolerance to complement central tolerance mechanisms (8).

The spleen is unique in that it contains both innate and adaptive immune systems. There will be many infections that will follow splenic excision, the most important being an increased susceptibility to infections. However, in recent years, several studies have shown that, in contrast to the other secondary lymphoid organs, the spleen is a particularly important organ in immunoregulation, besides playing a role during the immune response. This unique function is basically mediated by regulating splenic cell migration and proliferation (9). Leukocytes, which include T cells, B cells, monocytes,

macrophages, and dendritic cells (DCs), are the main producers of the anti-inflammatory cytokine IL-10 (10). IL-10 is a significant immunomodulatory factor that is essential for the immune response's negative control. Considering the paucity of research on the spleen's function in the immunological response to *Cryptosporidium* spp. Therefore, the aim of the current investigation was to find out how IL-10 functions in immunosuppressed mice's spleen homogenate in the immune response against *Cryptosporidium* spp.

Materials & Methods

The animal used in this investigation were sixty male of BALB/c mice were used in this investigation their aged three to six weeks, weighing around 20 g, which were acquired from the Central Health Laboratory in Baghdad. Every mouse was produced and kept in settings that were specific pathogen-free (SPF), with unrestricted access to sterile food and water.

The following groups were created from the experimental animals:

Group I: (n=20) orally infected with *Cryptosporidium spp.* oocysts.

Group II: (n=20) immuno suppressed and infected mice.

Group III: (n=20) non infected mice (Control group).

Animals stool were examined by used the modified Zeihl-Neelson technique.

Immuno suppression : Before the animals were inoculated with *Cryptosporidium* spp. oocysts, experimental immunosuppression was achieved by administering a synthetic corticosteroid (Dexamethasone) intraepithelially at a dose of 0.25 mg/g/day for 14 days in a row. Throughout the experiment, the mice were given the identical doses of dexamethasone.

The oocyst: Using a modified Ziehl-Neelson staining method, fecal samples from slaughterhouses were analyzed for the presence of Cryptosporidium spp. oocysts, which were isolated from naturally infected calves. Samples were mixed with an equal volume of 2.5% potassium dichromate (K2Cr2O2) in order to observe them (11).

Infective inoculum was prepared according with Suresh & Rehg (12), and the number of *Cryptosporidium spp.* oocyst in the concentrated stock inoculums were determined using heamatocytometer (13).

The infection: The prepared inoculums of *Cryptosporidium* spp. oocysts were administered orally to all of the mice in our investigation, with the exception of the control groups. This happened in the immunosuppressed group on day 14 of dexamethasone.

Using a tuberculin syringe attached to a polyether tube, the animals were injected intra-esophageally with the prepared inoculums after being deprived

of water for the whole night. The amount administered to each mouse was adjusted to include roughly $1x10^5$ oocysts.

Tissue homogenate: According to Costa *et al.* (14), spleen tissue was homogenized using cold lysis buffer (50 mM Hepes, 1% Triton x 100, and 1:100 protease inhibitor). Then, centrifuged at 4C° at full speed for 10 minutes, and the supernatant was collected and stored at -80C°. The cytokine levels in the spleen were assessed using the Enzyme-linked immunosorbent assay (ELISA) to measure the amount of IL-10 (PeproTech; USA) in each sample obtained from each study group at 14 days after infection (post infection).

Statistical analysis

To determine the impact of the difference factor in the study parameters, data were gathered, tabulated, and statistically examined using the SAS (2012) program. This study's means were significantly compared using the Least Significant Difference (LSD) test (Analysis of Variance, or ANOVA).

Results and Discussion

IL-10 is a cytokine was assessed in the spleen of three groups: infected mice, immunosuppressed infected mice, and uninfected control mice. This was done to evaluate the immune condition of immunocompromised persons, who are more vulnerable to infection by *Cryptosporidium* spp.

Levels of IL-10 appeared to increase in infected mice (1446.93 ±161.02 pg/ml) compared to uninfected control group (641.20±40.42pg/ml), while it get decreased in immuno suppressant (872.26 ±46.20 pg/ml) P≤0.001 (Table 1).

Table 1: Comparisons between difference groups in IL-10 level in Spleen homogeneous

Group	Mean ± SE of IL-10 (pg/ml)
Group I	1446.93 ±161.02 a
Group II	872.26 ±46.20 b
Group III	641.20 ±40.42 b
LSD value	247.58 **
Means having with the different letters in same column differed significantly. ** (P≤0.01).	

In healthy people, cryptosporidiosis is a self-limiting condition; in immunocompromised patients, it can be chronic and deviant (15), when infection with *C. parvum*, intestinal epithelial cells initiate a series of innate

defense reactions that lead to infection and activate immune effector cells at the infection sites (16). Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine that is necessary for maintaining normal tissue homeostasis, preventing and inflicting damage to host tissue, and regulating the host immune response to infections (17). Th2-cells are believed to be the primary generator of IL-10 *in vivo*. They mediate tissue homeostasis and suppress the immune response by delivering IL-10 to the site of inflammation (18). It plays a variety of roles in both acquired and innate immunity. In order to elicit a potent inflammatory response, it supports B-cell differentiation and antibody secretion (18). Although the host's immunological condition has a significant impact on the infection's prognosis and severity, little is known about the nature of the immune response in cryptosporidiosis, especially in humans. The majority of our knowledge regarding immunological responses to *Cryptosporidium* is founded on research conducted on animals, specifically mice (19, 20). It's crucial to remember that the intestines are the main location of cryptosporidiosis infection. It's possible that the spleen has a more indirect function in this infection by bolstering the infection's overall immune response (21,22). To completely comprehend the unique function of the spleen in cryptosporidiosis, more investigation is required. Research on the immune responses in patients who have had a splenectomy or have splenic dysfunction may shed light on how the spleen helps combat this infection(23).

Due to the lack of studies showing the immune role of the spleen in infection with cryptosporidiosis so, the researcher will discuss of studies that are similar with the current study. According to a study by Lacroix *et al.* (24), IL-10 (and IL-4) was present when adult GKO mice of C57BL/-6 were infected, but it did not eliminate the chronic infection of *C. parvum*. This implies that, despite elevated IL-10 levels, the Th1 cytokine response is essential for the parasite's removal. Additionally, Robinson *et al.* (25) reported that mice and humans infected with *C. parvum* had intestinal mucosa that was upregulated in IL-10.

Unlike mice models, calves, who are natural hosts of *C. parvim*, showed a correlation between the course of infection and the generation of IL-10 by gut mucosal lymphocytes at 8 days post-infection. The absence of IL-10 expression in any uninfected calves implies a connection between the response and recovery, as IFN-γ boosts expression by gut mucosal cells (26). According to Tessema *et al.* (6), both neonatal and adult groups of GKO mice and wild type neonates showed a rising IL-10 response during the peak of

infection on day 9 (post infection). IL-10 levels were completely reduced after 5 weeks (p.i.) of infection challenge and 2 weeks (post infection) of infection resolution, indicating that following the peak of oocyst shedding, the expression of the virus has decreased as a result of resolution in the infected group as compared to the healthy control group. Contrary to our findings, Lean et al. (27) revealed that IL-10 regulates the activation of iELc by IFN-γ. This may not come as a surprise, but it also failed to prevent the upregulation of iNOS-induced.

Conclusion

Owing to the paucity of research in the field (and in Iraq specifically) on the application of homogenization to evaluate the immunological status of cryptosporidiosis, this technique offers a more precise evaluation of the intestinal infection site and also tracks the proliferation of CD4+ T-cells in the spleen. Th1, Th2, and Th17 cytokines have comparable effects on the immune response during an infection, which allows us to use them as a foundation for more in-depth research in this area.

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قياس مستويات إنترلوكين-10 في نسيج الطحال لدى الفئران البيضاء المثبطة مناعيا تجريبيا والمصابة بطفيلي *Cryptosporidium* spp.

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مستخلص البحث:
طفيلي داء الابواغ الخبيئة والذي يمكن أن يسبب فقدان الوزن والإسهال المائي الشديد، وغالبًا ما يتواجد في الأمعاء لدى كل من الحيوانات والبشر. ومع ذلك، لا يُعرف سوى القليل عن الاستجابات المناعية للمضيف التي تنشأ عند الإصابة بالطفيلي . على الرغم من أن داء الابواغ الخبيئة بشكل عام، يمكن ان يشفى ذاتيًا لدى الأفراد ذوي الكفاءة المناعية، ويمكن أن يكون مميتًا لدى الأشخاص الذين يعانون من نقص المناعة أو في الاشخاص الذين يعانون من ضعف المناعة، مثل الرضع وفيروس نقص المناعة البشرية/الإيدز. هدفت الدراسة الحالية إلى تقصي مستوى الإنترلوكين - 10 في متجانس الطحال لدى الفئران البيضاء المثبطة مناعيا بواسطة الديكساميثازون والمصابة بطفيلي داء الابواغ الخبيئة. تظهر النتائج ارتفاع مستوى الإنترلوكين - 10 في المجموعة المصابة بالطفيلي اذ بلغ التركيز 1446.93 ±161.02بيكوغرام/مل مقارنة بمجموعة السيطرة 40.42 ± 641.20 بيكوغرام/مل ، بينما انخفض في المجموعة المثبطة مناعيا بالديكساميثازون والمصابة بطفيلي الابواغ الخبيئة اذ بلغ 46.20± 872.26 بيكوغرام/مل وبمستوى احتمالية P ≥0.001. قد توفر نتائج الدراسة الحالية فائدة للبحث المستقبلي حول الاستجابة المناعية للإصابة بداء الابواغ الخبيئة في السكان الذين يعانون من ضعف المناعة المؤقتة.

الكلمات المفتاحية: طفيلي داء الابواغ الخبيئة ، متجانس الطحال ، الديكساميثازون، انترلوكين – 10