

## GENE EXPRESSION OF TLR2 AND TLR4 RECEPTORS AND LEVEL OF IL-10 AND IL-23 IN PATIENTS WITH CYSTIC ECHINOCOCCOSIS

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(Received 26 July 2019, Accepted 24 August 2019)

**ABSTRACT :** The current study was conducted for the period from May 2018 to April 2019 in the Department of Biology, Faculty of Education for Girls, University of Kufa, which aims to detect the amount of genetic expression of the mRNA for the receptors TLR2 and TLR4 in peripheral blood and levels of IL-10 and IL-23 in serum of patients before and after the removal of hydatid cysts. The results of the gene expression test for TLR2 and TLR4 in peripheral blood cells of patients with hydatidosis using flow cytometry techniques showed a significant increase ( $P \leq 0.05$ ) in the amount of the gene expression of receptors before removing the hydatid cysts, which amounted  $20.84 \pm 1.38$  and  $13.55 \pm 0.88$ , respectively, compared to the quantification of gene expression after the removing of the hydatid cysts, which was  $15.56 \pm 1.41$  and  $8.53 \pm 0.73$  respectively and healthy  $15.21 \pm 1.18$  and  $8.11 \pm 0.72$ , respectively. The results of the ELISA test showed a significant increase ( $P \leq 0.05$ ) in the levels of IL-10 and IL-23 in patients with hydatidosis prior to removal of the cysts, which reached  $22.84 \pm 1.14$  and  $125.52 \pm 5.58$  pg/ml respectively compared with the group of patients after removal of hydatid cysts was  $11.02 \pm 0.24$  and  $92.72 \pm 1.29$  pg/ml respectively and healthy group of  $14.59 \pm 0.65$  and  $74.17 \pm 1.93$  pg/ml, respectively. The conclusion from this study, the presence of TLR2 and 4 TLR receptor genes in most blood samples and occurrence the cellular immune response by increasing the levels of IL-10 and IL-23 in people with hydatidosis.

**Key words :** Cystic echinococcosis, hydatidosis, TLR2, TLR4, IL-10, IL-23.

### INTRODUCTION

Hydatidosis is a widespread disease in all parts of the world. It is a common disease between humans and animals (Zoonotic disease) (Wahlers *et al*, 2012). The disease is one of the parasitic pathogens transmitted from carnivorous to humans and herbivorous animals and there are two types are the most medical important and most widespread are the *Echinococcus granulosus*, its larval stage is called the unilocular hydatid cyst and *E. multilocularis*, the causative agent of alveolar echinococcosis (Nourbakhsh *et al*, 2012). The life cycle of *E. granulosus*, includes two types of hosts, namely, final hosts represented by dogs and other members of the Canidae. The second host is known as the intermediate host in which the parasite proliferates in organs and tissues of animals such as sheep, cows, goats, pigs and camels. The human is an accidental intermediate host (Filippou *et al*, 2007). The hydatidosis is a danger to public health as the cysts develop in most organs of the body (Kose and Sevimli, 2008; Kaplan *et al*, 2001). Particularly in areas where sheep and cattle are raised, causing significant economic losses (Eddi *et al*, 2006). Toll-like receptors (TLRs) are cellular receptors that are recognize pathogens - associated molecular patterns

(PAMPs) and these receptors contribute to innate immunity of the pathogens (Kaye and Aebischer, 2011), 13 of these receptors were described in the Mammals. 9-1 is functional receptors between humans and mice. The TLR10 is functional only in human and TLR11 is only used in mice. TLRs are divided into extracellular receptors (TLR1, TLR2, TLR4, TLR6, TLR11) (Pifer *et al*, 2011) and intracellular receptors (TLR3, TLR9, TLR7, TLR3) (Blasius and Beutler, 2010). The innate response begins with TLRs expressed by macrophages, monocytes, and various types of dendritic cells (DCs) (Kawai and Akira, 2011). Neutrophils also express these receptors but lack receptors within the cell, (TLR3 and TLR7). This response results in the production of inflammatory cytokines (Prince *et al*, 2011). Adaptive immunity is influenced by the two helper T cells (Th2 and Th1). This results in the production of cytokines by these cells. The naive (non-activated T cell), which are stimulated by antigens presented by antigen-presenting cells (APCs), as a result, naive T cells differentiate to Th1 and Th2. Th1 cells produce  $IFN\gamma$ , which mainly promote cellular immunity while Th2 cells produce IL-4, IL-5, IL-10 and IL-13, which in turn enhances humoral immunity (Aderem and Ulevitch, 2000). The IL-12 produced by APCs has a

key role in stimulating the differentiation of Th1 cells while IL-4 stimulates differentiation of Th2 cells (Abbas *et al*, 1996). The present study aimed to investigate the amount of genetic expression of the mRNA for the receptors TLR2 and TLR4 in peripheral blood and levels of IL-10 and IL-23 in serum of patients before and after the removal of hydatid cysts.

## MATERIALS AND METHODS

The study included 66 patients, who were diagnosed with cystic echinococcosis through clinical examination, radiography and magnetic resonance imaging, and 30 healthy people who are not infected with cystic echinococcosis or any other medical condition have been confirmed by clinical, radiological and magnetic resonance examination, who attended the General Surgery Consultant at Al-Sadr Medical City Hospital and Al-Hakeem General Hospital for the period from December 2018 to December 2019 and their ages ranged from 10 to over 60 years. Patients were followed up before the removal of the hydatid cysts by surgical operations and after the removal of the hydatid cysts with a period of 5-9 months for the purpose of performing the tests of the blood and immunological and to determine the pathological condition of them. A questionnaire was adopted including the name of the patient, sex, housing, age, other diseases and the date of the operation. A 5 mL blood sample was withdrawn from the patient and healthy groups under study with a 5 ml syringe. 2 ml of blood was placed in a container containing Ethylene Diamine Tetra Acetic Acid (EDTA) (1 ml to measure the amount of gene expression of TLR2, TLR4 receptors and 1 ml to measure the percentage of eosinophils), while placing 3 ml of blood in test tubes that did not contain anticoagulant and was discarded in the centrifuge at 3000 cycle / minute for 20 minutes to obtain serum for the purpose of measuring the level of IL-10 and IL-23 and keeping the serum at a temperature of  $-20^{\circ}\text{C}$  until it is used in tests. The gene expression of the TLR2 and TLR4 receptor was measured in blood samples of patients with cystic echinococcosis and healthy individuals (control) by flow cytometry technique. A cell suspension is prepared from the cells to be studied. This is done by incubating the cells with monoclonal antibodies that are labeled with fluorochrome and directed against specific surface proteins. Many antibodies can be applied simultaneously, provided they are marked with different fluorescence colors. After preparing the cell suspension, it is transmitted in a monotonous frame of cells in front of the light source (Givan, 2001). The light then collides with the cell, leading to:

1. Light reflection or refraction (light scatter), which is

either a frontal scatter light (FSC), gives us an idea of the size of the cell, or side scattering caused by the reflection of the light at the side scatter light (SSC), which gives us an idea about the inner components of the cell (granularity).

2. Fluorescence Light: The spark is triggered by the excitation of the light source of the wavelength-specific light source, which produces a light with a different wavelength (light of brilliance). In the case of glare, this means that the monoclonal antibody binds to its specific receptor.

The concentration of IL-10 and IL-23 was estimated using ELISA technique and according to the instructions of Elabscience Company. This test is based on the use of the designated capture antibodies that cover the 96 pits in a measuring plate. The specimens to be tested and the standard solutions are added to the pits as the human cytokine is binding with the capture antibody. The pits is then washed and biotin-conjugated antibodies are added. The washing is done again and Avidin-horseradish peroxidase (Avidin-HRP) is added. Consists of antigens-antibodies complex. Then wash, TMB is added to form a blue color that is proportional to the concentration of the specific cytokine. The stop solution is added to the reaction to form yellow. The absorption coefficient (OD) of each hole is measured at a wavelength of 450 nanometers.

## Statistical analysis

Statistical package for Social Sciences (SPSS) version 16 was used to analyze the data by calculating the mean and standard error and using the T-test. The P-Value was calculated to find the significant differences between the treatments (Morgan *et al*, 2010).

## RESULTS AND DISCUSSION

Results in Table 1 showed a significant increase in the levels of gene expression of TLR2 and TLR4 receptors in the group of patients with hydatidosis before to removal of the hydatid cysts, which amounted to  $20.84 \pm 1.38$  and  $13.55 \pm 0.88$ , respectively, compared with the healthy group, which reached to  $15.56 \pm 1.41$  and  $8.53 \pm 0.73$ , respectively. The statistical analysis showed significant differences at  $P \leq 0.05$ , where Shan *et al* (2011) showed high levels of gene expression for TLR2 and TLR4 receptors in peripheral blood cells and hepatic tissue in patients with cystic echinococcosis (CE) compared to healthy group with significant differences at  $P < 0.01$ . Apaer *et al* (2017) showed that high levels of gene expression for TLR2 and TLR4 receptors were observed in mice infected with *E. multilocularis* compared with the group of mice treated with albendazole,

explaining the role of albendazole in mitigating the parasite's vitality, thereby reducing the immune system response and removing parasite. The TLR2 and TLR4 immune receptors may be able to bind to worms or their products, thus stimulating inflammatory response by secreting inflammatory cytokines from dendritic cells. During parasitic infections, TLR2 and TLR4 support and maintain Th2 immune responses to create favorable conditions of the worm and then develop the function of the lesion. The TLR2 and TLR4 receptors play an important role in initiating a different immune response that may help the parasite survive and sustain chronic inflammation. Lawn *et al* (2004) confirmed that IgG or IgG1 and IgG2 concentrations were significantly increased in patients with cystic echinococcosis, compared to IgG3 and IgG4 antibodies, during the surgical and therapeutic period. The IgG2 antibody response showed the best correlation to disease activity and parasite protection, allowing the hydatid cysts survive and grow. The difference in the level of gene expression receptor, may be due to the severity of the disease, which changes the gene expression of these receptors, causing a change in the production of cytokines and allowing the parasite to evade the host's immune system and promote chronic infection. Zhongguo *et al* (2016) showed changes in the levels of TLR2 and TLR4 in peripheral blood in patients with hepatic echinococcosis and healthy patients. The TLR2 and 4 TLR gene expression levels were high  $0.100 \pm 0.048$  and  $0.004 \pm 0.003$ , respectively, compared with the healthy group  $0.055 \pm 0.040$  and  $0.0034 \pm 0.002$ , respectively. The gene expression of the TLR2 and 4 TLR receptor mRNA was found to be high in the infected tissue compared to the normal tissue. The reason for the role of different receptors such as TLR2 and TLR4 TLR to recognition the molecular patterns associated with *E. granulosus* parasites that play an important role in initiating different immune responses that help maintain the survival of the parasite and the persistence of chronic inflammation.

The results in Table 1 showed a decrease in the levels of TLR2 and TLR4 gene expression after removal of the hydatid cysts  $15.56 \pm 1.41$  and  $8.53 \pm 0.73$ , respectively, compared with the preoperative group of  $20.84 \pm 1.38$  and  $13.55 \pm 0.88$ , respectively. The statistical analysis showed significant differences at  $P < 0.05$ . Versteeg *et al* (2009) referred to the key role of Toll-like receptors in induction of postoperative immune endurance and decreased levels of TLR2 and TLR4 gene expression clearly, after surgery. Studies have shown that Th2 cells are predominant in patients with hydatidosis, who have chronic and active untreated cysts. In contrast, Th1 cells

are more effective in patients with treated and ineffective cysts (Rigano *et al*, 2007; Rigano *et al*, 2004).

The results in Table 2 showed high levels of IL-10 and IL-23 in the serum of patients with hydatidosis prior to removal of cysts ( $22.84 \pm 1.14$  and  $125.52 \pm 5.58$  pg/ml), respectively, compared with the healthy group that was ( $14.59 \pm 0.65$  and  $74.17 \pm 1.93$ ) pg/ml, respectively. The statistical analysis showed significant differences at  $P \leq 0.05$ . Tuxun *et al* (2015) reported a rise in levels of IL-10 and IL-23 in patients with hydatidosis compared with healthy group. Bayraktar *et al* (2005) reported a significant increase in IL-2, IL-4 and IL-10 values in patients with cystic echinococcosis in the Netherlands ( $6.5 \pm 5.7$ ,  $3.2 \pm 2.7$  and  $4.1 \pm 2.7$  pg/ml) respectively compared to control group ( $1.2 \pm 1.1$  and  $0.19 \pm 0.11$  and  $2.1 \pm 1.1$  pg/ml) respectively. The increase in the level of cytokines in the serum of patients may be due to the size of the cyst and the survival time of the parasite. Both IL-10 and IL-23 affect cellular immune response inhibition as they are anti-inflammatory cytokines. As well as many factors that affect the level of cytokines such as the location of the cyst in the liver. Torca *et al* (1996) confirmed that the significant increase in levels of IFN $\gamma$ , IL-2 and IL-4 in patients with hydatid cysts located in the central region of the liver is more likely than the peripheral region. In addition, Piccoli *et al* (2011) assessed levels of Th1 (IFN $\gamma$ ) and Th2 (IL-4, IL-10, IL-13) in serum for patients with cystic echinococcosis in San Mateo Hospital in Italy. Results showed no significant difference in IFN $\gamma$ , IL-4 and IL-13, levels in patients compared to the control group, and may be due to the fact that cytokines is not specific to a particular disease, and its formation occurs in all inflammatory processes mediated by Th1 and Th2. Diaz *et al* (2011) showed the structure of laminated layer and indicated their role in reducing the responses of Th1 and Th2 cells, thus allowing the parasite to survive. The studies indicate that the IL-23 activates adaptive immunity and innate immunity to the production of IFN $\gamma$ , IL-17A, IL-17F and IL-22. These cytokines in turn help stimulate epithelial cells to produce antimicrobial agents (Floss *et al*, 2015). Dematteis *et al* (2003) showed that the response of experimental stimulation mice depends on the dose of injected protoscoleces as it was found that the reduced dose of injected protoscoleces stimulates an immune response towards Th1. The high doses of injected protoscoleces stimulates an immune response toward Th2. The cause of the rise in the level of cytokines may be due to the continued stimulation of parasite antigens, leading to increased activation of immune cells produced of these cytokines or may be due to the mechanisms of escape from the immune system

**Table 1 :** Gene expression of receptor TLR2, TLR4 before and after removal of Hydatidcysts.

Receptors	Healthy (M ± SE) n=30	Patients before the removal of Hydatidcysts (M ± SE) n=66	Patients after the removal of Hydatidcysts (M ± SE) n=66	P-value
TLR2	15.21±1.18	20.84±1.38	15.56±1.41	0.043*
TLR4	8.11±0.72	13.55±0.88	8.53±0.73	0.00032*

\*Significant differences at  $p \leq 0.05$

**Table 2 :** Levels of IL-10 and IL-23 before and after removal of the Hydatidcysts.

Cytokines	Healthy (M ± SE) n=30	Patients before the removal of Hydatidcysts (M ± SE) n=66	Patients after the removal of Hydatidcysts (M ± SE) n=66	P-value
IL-10(pg/ml)	14.59±0.65	22.84±1.14	11.02±0.24	0.009*
IL- 23(pg/ml)	74.17±1.93	125.52±5.58	92.72±1.29	0.00024*

\*Significant differences at a  $p \leq 0.05$

used by the parasite through its effect on the dendritic cells, which play a key role in providing the antigen either inhibits the proliferation and differentiation of these cells or interfere with their function and inhibits their ability to produce IL-12, which is the key to stimulate the immune response towards Th1 and thus deviate the immune response towards Th2 causing an increase in cytokines, resulting in the establishment of hydatid cysts and continued parasite growth (Kanan and Chain, 2006; Rigano *et al*, 2007). The results in Table 2 showed a significant decrease of ( $P \leq 0.05$ ) in the levels of IL-10 and IL-23 in the group of patients after removal of the hydatid cysts where they were ( $11.02 \pm 0.24$  and  $92.72 \pm 1.29$ ) pg / ml respectively, compared to the group of patients before the removal of cysts which amounted to  $22.84 \pm 1.14$  and  $125.52 \pm 5.58$  pg / ml, respectively, where Rigano *et al* (2004) recorded high level of IL-10 in patients with cystic echinococcosis who did not respond to treatment compared to patients who respond to treatment. Bayraktar *et al* (2005) showed a significant decrease in levels of IL-2, IL-4 and IL-10 in patients with cystic echinococcosis in the Netherlands after surgery ( $2.8 \pm 1.8$ ,  $0.98 \pm 0.61$  and  $2.6 \pm 1.5$  pg / ml) respectively compared with the pre-operative group ( $6.5 \pm 5.7$ ,  $3.2 \pm 2.7$  and  $4.1 \pm 2.7$  pg/ml) respectively. Naik *et al* (2016) assessed the relationship between levels of cytokines in serum and outcome of chemotherapy and surgical intervention by ELISA before and after drug and surgical treatment. Levels of IFN $\gamma$ , IL-4 and IL-10 were elevated in patients during the active phase of the disease prior to pharmacological and surgical treatment compared with the group of patients after treatment and surgical intervention, where these levels decreased at a  $P < 0.05$ . Tuxun *et al* (2018) also confirmed that levels of IL-10

and IL-23 in serum were alternative indicators of metabolic activity in patients with hepatic alveolar echinococcosis. The study included 30 patients divided into 15 patients with metabolic active cysts and 15 metabolic inactive cysts and 22 control group. The results showed an increase of (96.53) and (7.48) pg/ml, respectively, in the levels of cytokines in the group of patients who were metabolic active cysts in comparison to patients with metabolic inactive cysts and control group. The conclusion from this study, the presence of TLR2 and 4 TLR receptor genes in most blood samples and occurrence the cellular immune response by increasing the levels of IL-10 and IL-23 in people with hydatidosis.

## CONCLUSION

The presence of TLR2 and 4 TLR receptor genes in most blood samples and occurrence the cellular immune response by increasing the levels of IL-10 and IL-23 in people with hydatidosis.

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