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Research Article

Critical Roles of IL-40 and IL-41 in the Determination of the Severity of Hydatid Cyst Infection in Iraqi Patients

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Abstract

Background: Hydatid cystic disease is a serious public health concern worldwide, and the link between the intermediate host (human) and parasite is important to the disease's etiology and severity. ***Objective***: Determine the levels of IL-40 and IL-41 in the serum of Iraqi patients infected with hydatid disease, as well as their function in disease severity. ***Methods***: The quantified ELISA was used to assess the levels of IL-40 and IL-41 in serum from 35 persons in Baghdad, Iraq, who had been diagnosed with hydatid cysts and were undergoing surgery to remove them. In addition, control samples from forty healthy individuals were taken for comparison purposes. ***Results***: The patients' ages ranged from 8 to 63. Females had higher infection rates than males. Rural areas have a higher rate of infection than urban areas. The lungs and liver were the most severely injured. The majority of lung infections resulted in coughs and chest discomfort, whereas liver damage resulted in stomach pain as well as substantial changes in hematological parameters (white blood cells, hemoglobin, and erythrocyte sedimentation rate), liver enzyme, and C-reactive protein in patients compared to controls. Patients had lower IL-40 levels than the control group, but significantly higher IL-41 levels (p<0.05) than healthy controls. ***Conclusions***: Patients with hydatid cysts showed higher serum levels of IL-41 and lower levels of IL-40, which may correlate with disease severity and act as biomarkers for chronic hydatid cyst infection.

Keywords: Cytokines, Hydatid cystic disease, IL-40; IL-41, Immune response.

الأدوار المهمة لـ IL-40 و IL-41 في تحديد شدة الاصابة بالاكياس العدرية لدى المرضى العراقيين

الخلاصة

الخلفية: يعتبر مرض الاكياس العدرية مشكلة صحية عامة كبيرة في العالم، و تلعب العلاقة بين المضيف الوسطي (الإنسان) والطفيلي دورا حاسما في احداث المرض و شدته. **الهدف** تقييم مستوى السيتوكينات الحديثة الاكتشاف IL-40 و IL-41 في مصل المرضى العراقين المصابين بمرض الاكياس العدرية، للوقوف على دور هذه السيتوكينات في احداث المرض وشدته. **الطريقة:** استخدمت الايزا الكمية لقياس المستوى المصلي للانترلوكين 40 و41 في امصال 35 مريضا تم تشخيص إصابتهم بالاكياس العدرية قبل اجراء عمليةالاستئصال الجراحي للكيس من مختلف مستشفيات بغداد / العراق. بالإضافة إلى ذلك، تم جمع عينات السيطرة من أربعين متطوعًا أصحاء لغرض المقارنة. **النتائج:** اظهرت النتائج بان الفئة العمرية للمرضى للمصابين تراوحت مابين 8 إلى 63 سنة. وكانت نسبة الإصابة بين الإناث أعلى من الذكور. وقد شكلت معظم الاصابات في المناطق الريفية تلتها المناطق الحضرية. ويعد كلا من الرئة والكبد الأعضاء الأكثر إصابة وإن معظم الإصابات في الرئة عانت من السعال وألم في الصدر امافي حالات إصابات الكبد، فقد شعر غالبية المرضى بألم في البطن، هذا وقد بينت النتائج تغايرا كبيرا في الجانب الدموي من خلايا الدم البيضاء، و مستوى الهيموجلوبين، ومعدل ترسيب كرات الدم الحمراء، وكذلك لوحظ اتغايرات في مستويات إنزيمات الكبد والبروتين التفاعلي C في مجموعة المرضى مقابل السيطرة. كانت مستويات IL-40 في المرضى اقل مما هيا عليه في المجموعة الضابطة، في حين كانت مستويات IL-41 أعلى بكثير (p<0.05) من الأصحاء.**الاستنتاجات**: تشير الدراسة الحالية إلى أن زيادة مستويات IL-41 وانخفاض مستويات IL-40 في مصل مرضى الاكياس العدارية، والتي يمكن أن ترتبط بشكل إيجابي مع شدة المرض ويمكن استخدام هذه النتائج كمؤشر حيوي محتمل للاصابة بالاكياس العدارية المزمنة.

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INTRODUCTION

Hydatid cystic disease (Echinococcosis), caused by one of the cestodes, *Echinococcus granulosus*, is transmitted from carnivores to herbivores. Herbivores act as the intermediate hosts of the parasite, where the larvae reside [1]. After intermediate hosts ingest them, the eggs transform into active oncospheres that transport through the circulation, generally to the liver but also to other organs. The hexacanth embryo grows into a larger, unilocular hydatid cyst once it reaches its final location in the body. It then forms protoscolices, or daughter cysts, in its inner layer [2]. Two types of hydatid cysts can occur in naturally infected intermediate hosts: fertile cysts, containing protoscolices either freely floating in hydatid fluid or connected to the germinal layer, and non-fertile cysts, which lack protoscolices. It is likely that the host's immune response contributes to the formation of non-fertile *Echinococcus* cysts (EC), although the

exact mechanisms are not fully understood [3,4]. For a parasite to reach this evolutionary stage, it must successfully resist the immune system's first response, including complement-dependent or antibody-mediated death, and progress to the fertile stage. The intermediate host can mount a strong immune response to *E. granulosus* infection, but the parasite has evolved sophisticated mechanisms to evade the host's defenses and thus avoid being eliminated [5]. In fact, it is vital to take intermediate host immunity into account when establishing a host-parasite relationship. The parasite secretes various chemical species that have the ability to affect immunocompetent human cells. Parasites have evolved a variety of survival mechanisms to evade the immune responses of their hosts; these mechanisms eventually lead to chronic infection. One such is cytokines (interleukins), which are secreted by different cells in response to a variety of stimuli and are an essential element in the regulation of the immune system and one of the basic modes of cell-to-cell transmission [6,7]. Interleukins play primary roles in many important functions, such as migration, adhesion, maturation, and proliferation. In addition, they help in the activation and differentiation of immune cells [8] and also play a primary role in pathogeneses [9,10]. To exert their effects, cytokines must bind to specific receptors on their target membranes. As a result, the first step in cytokine initiation is to match the cytokine receptor to its ligand. Elucidating this pathway has allowed us to gain further knowledge about the ways in which cytokines influence the development of immune responses [11]. The immune response against hydatid cysts is characterized by the simultaneity of both Th1 and Th2 responses, which means the involvement of pro- and anti-inflammatory cytokines, respectively [12]. There are two newly discovered cytokines, IL-40 and IL-41. Interleukin 40 (IL-40) is a new pro-inflammatory cytokine produced by the C17orf99 gene [13]. Bone marrow, fetal liver, and peripheral B cells after activation are the main organs responsible for the production of this cytokine [14]. IL-40 is implicated in immunoglobulin A (IgA) production and in interfering with humoral immune responses and the development of the B cell [15]. IL-40 may also play a regulatory role in the pathogenesis of inflammatory diseases such as rheumatoid arthritis [16]. Metrnl/IL-41 is a recently discovered anti-inflammatory cytokine, identified in 2004. It is produced by the METRNL gene, which is located on human chromosome 17 (17q25.3) [17]; indeed, the cells responsible for producing IL-41 and IL-41 target cells are still the subject of significant investigation, where different tissues have shown the ability to express this cytokine, such as the intestines, skin, and respiratory tract. These investigations have further demonstrated that IL-41 is expressed by alternatively activated macrophages and M2-like macrophages, indicating its involvement in both innate and adaptive immunity [18]. Hydatid cysts are a major public health problem worldwide [19]. In Iraq, various research projects have studied the parasite in all its immunological, pathological, diagnostic, and therapeutic aspects, yet the hydatid cyst still represents a serious public health issue in Iraq [20–22]. In this study, serum levels of IL-40 and IL-41 in Iraqi patients with hydatid cysts were analyzed to determine their role in disease severity. Furthermore, the relationship between IL-40 and IL-41 levels in patients according to the clinical baseline and laboratory data was evaluated. This investigation may clarify the functions of IL-40 and IL-41 in the pathophysiology of cystic echinococcosis (CE).

METHODS

Study design and setting

Between November 2022 and March 2023, a total of 35 patients diagnosed with hydatid cysts were referred for surgical excision in different hospitals in Baghdad, Iraq (Ghazi Hariri Surgical Specialties, Gastroenterology and Hepatology Teaching Hospital, Medical City, and Baghdad Teaching Hospital). Imaging modalities, including computed tomography (CT) and ultrasonography (US), are universally recognized as critical diagnostic procedures for identifying infections. Additionally, a control sample of forty healthy volunteers (individuals who showed normal levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)) was assembled and matched with patients according to their age, gender, and ethnicity.

Collection of samples

Five milliliters of venous blood were obtained from each participant (patient and control). The blood was divided into two aliquots; the first was drawn in an EDTA tube for the detection of white blood cells (WBC), hemoglobin (Hb), packed cell volume (PCV), and erythrocyte sedimentation rate (ESR). The second aliquot was dispensed in a plain tube to collect serum; after clotting, blood was centrifuged at 2000 rpm for 15 minutes at room temperature, and the resulting aliquots were transferred to firmly sealed Eppendorf containers and stored at -20^{o}C until being assayed for biochemical liver function (ALT and AST) and assessment of cytokine levels (IL-40 and IL-41).

Fertility cyst examination

After removing the cyst from the infected organ (liver or lung) tissue via surgery, its surface was cleaned with an ethanol solution (70%). The cyst fluid was then extracted using a syringe and examined under a microscope; if the cyst fluid appeared cloudy, this indicated that the cyst was fertile. For more precise results, we also microscopically examined the cystic fluid to identify the presence of protoscolices.

Outcome measurements

Hemoglobin (Hb), white blood cell count (WBC), and erythrocyte sedimentation rate (ESR) assays were performed using conventional laboratory

methods. The serum levels of AST, ALT, and C-reactive protein (CRP) were measured using a Germany automated (Cobas c311, Roche). The levels of IL-40 and IL-41 in the blood of both patients and healthy people were determined using sandwich ELISA kits from BT LAB, China. These kits are intended for quantitative measurement of human IL-40 and IL-41. The findings were derived by interpolating from a standard curve determined in the same test as the specimens, with standard curve fitting equations for the parameters.

Statistical analysis

The data was analyzed statistically using IBM's SPSS v27 software. The results are reported as mean±standard error (SE); after data processing, the Duncan test was performed to establish statistical significance (i.e., when *p*-value was less than 0.05).

RESULTS

Thirty-four patients with the human hydatid cyst were confirmed to have undergone surgery. The age group of the patients ranged from 8 to 63 years old. The rate of infection among females was found to be higher compared to males, at 56% and 44%, respectively. The incidence of infection was higher in rural areas (62%) than in urban areas (38%). Regarding the organs affected by the disease, the study results indicate that cystic echinococcosis (GE) mostly affects the lung, with a prevalence of 59%. The disease also has a significant impact on the liver, with a prevalence of 23%. It should be noted, however, that 18% of cases showed concurrent liver and lung damage. In addition, there were six cases (18%) of secondary hydatid cysts and 28 cases (82%) of primary hydatid cysts. Based on the results of the cyst fertility examination, it was observed that 85% of the patients showed active cysts, while 15% showed non-fertile cysts, as reported in Table 1.

Table 1: The demographics of the groups studied

Characteristic		Patients n=34	Controls n=40
Age (year)		8-63	15-60
Sex	Males	15(44)	16(40)
	Females	19(56)	24(60)
Residence	Urban	13(38)	23(58)
	Rural	21(62)	17 42)
Cyst location	Lung	20(59)	-
	Liver	8(23)	-
	Lung & liver	6(18)	-
Fertilization	Fertile	29(85)	-
	Sterile	5(15)	-
Recurrent infection	Yes	6(18)	
	No	28(82)	

Values were expressed as frequencies and percentages.

Table 2 reports the clinical symptoms of patients with hydatid cysts as distinguished from those of asymptomatic infections by varying clinical symptoms according to the location of the infection. We observed that the majority of infections in the lung were characterized by cough, chest pain, fever, and vomiting but could also be asymptomatic (8, 6, 3, and 5), respectively. In the cases of liver issues, the majority of patients (5 cases) experienced abdominal pain, while 2 cases noted the presence of a mass. There were two cases of injuries that did not exhibit any symptoms.

Table 2: Clinical symptoms of patients with hydatid cysts

Organ infected	Symptoms	Frequency
lung	Cough	8
	Chest pain	6
	Fever and vomiting	3
	Asymptotic	5
	Abdominal pain	5
liver	Mass	2
	Asymptotic	2
	Chest pain	3
Lung & liver	Abdominal pain	2
	Chest pain with fever and vomiting	1

Also, the study revealed a highly significant increase (p<0.001) in the number of white blood cells in patients compared to HC patients. The mean level of white blood cell counts was 11.90±0.41 cells x 10^9/L in the patient group and 7.87±0.40 cells x 10^9/L in the control. It was found that the erythrocyte sedimentation rate (ESR) of the patients was significantly higher than that of the healthy control group (30.30±2.12 mm/h vs. 8.90±1.31 mm/h). The C-reactive protein result was 21.17±3.60 in patients, while in the healthy group it was 3.37 ± 0.56. After infection with the hydatid disease, the levels of AST and ALT in the blood increased. The AST level was 33.0±2.71 and the ALT level was 31.36±1.66 in the patient group compared to 21.19±1.08 and 20.8 ±1.51 in the control group, respectively, as reported in Table 3.

Table 3: The hematological and biochemical changes among study groups

Parameters	Patients	Controls	*p*-value
WBC (x10^9/L)	11.90±0.41	7.87±0.40	< 0.001
Hb (g/dL)	11.99±0.31	13.60±0.23	< 0.001
ESR (mm/hr)	30.30±2.12	8.90±1.31	< 0.001
PCV%	37.30±1.02	42.9±0.69	< 0.001
CRP (mg/mL)	21.17±3.6	3.37±0.56	< 0.001
ALT(U/L)	33.0±2.71	21.19±1.08	< 0.001
AST (U/L)	31.36±1.66	20.86±1.51	< 0.001

The results were expressed as mean±SE.

Moreover, the results showed that the concentration of IL-40 in the serum of patients with CE was significant (p<0.05) compared to that of the healthy controls. The mean IL-40 level in patients was 19.30±1.90 pg/mL, compared with 29.25±4.45 pg/mL in the control group; the amount of IL-41 in the blood of patients was also significantly higher (p<0.05) than that of the healthy controls, at 2.474±0.248 pg/ml and 1.824±0.161 pg/ml, respectively. Also, the results indicated that the average ratio of IL-40/IL-41 was 7.8±0.38 in CE-infected patients, compared to 16.04±1.4 in the control, as reported in Table 4.

Table 4: Serum levels of IL-40 and IL-41 in hydatid cyst patients and controls

Cytokines level (pg/ml)	Patients	Controls	*p*-value
IL-40	19.3±1.9	29.25±4.45	<0.05
IL-41	2.47±0.25	1.82±0.16	<0.05
IL-40/IL-41	7.8±0.38	16.04±1.4	<0.05

The results were expressed as mean±SE.

It was also found that there is no relationship between the concentrations of IL-40 and IL-41 according to site of infection (lung, liver, and both lung and liver) (18.05±1.59, 20.83±6.28, and

20.35±4.63 for IL-40, and 2.27±0.30, 2.10±0.79, and 2.73±0.61 for IL-41, respectively). In addition, it was noted that the concentrations of IL-40 and Il-41 in fertile cysts were higher than those in non-fertile cysts, although this result was non-significant (20.01±2.13 and 15.24±3.86 for IL-40, and 2.47±0.30 and 2.19±0.84 for IL-41, respectively). The results confirm that patients who suffer from recurrent hydatid cysts show a significant increase in concentration of IL-41 compared to the primary infection (2.57±0.61 and 1.78±0.29, respectively), while there was no significant difference in IL-40 with regard to recurrent infection (20.66±4.77 and 18.84±2.08) (Table 5).

Table 5: Serum levels of IL-40 and IL-41 in hydatidosis patients according to cyst location, cyst fertilization, and recurrent infection

Characteristic	Status	IL-40 level (pg/ml)	IL-41 level (pg/ml)
Cyst location	Lung	18.05±1.59a	2.27±0.3a
	Liver	20.83±6.28a	2.10±0.79a
	Lung & liver	20.35±4.63a	2.73±0.61a
Cyst Fertilization	Fertile	20.01±2.13b	2.47±0.3b
	Infertile	15.24±3.86b	2.19±0.84b
Recurrent infection	Yes	20.66±4.77c	2.57±0.61c
	No	18.84±2.08c	1.78±0.29d

The results were expressed as mean±SE. Values with non-identical letters (a,b,c,d) within the same character are significantly different (p<0.05).

DISCUSSION

Hydatid disease is a neglected zoonotic tropical disease. Its annual impact is estimated to be one million disability-adjusted life years. The disease is distinguished by the development of bladder-like larvae that are full of fluid and inhabit internal organs [23]. Variable clinical manifestations of cystic echinococcosis (CE) are influenced by the lesions' location, size, and condition. Variable growth rates of cysts range from one to five centimeters in diameter annually. The gradually proliferating echinococcal cyst is frequently well tolerated until its magnitude induces dysfunction [24]. Hydatid disease affects all age groups; here, the youngest patient was eight years old, while the oldest was 63. Based on the findings, the total occurrence of CE was significantly greater in females compared to males. Some agreed with our findings, as in the results reported by Khan *et al*. [25], while in contrast, Ilbeigi *et al*. (2015) revealed that surveys suggested a greater susceptibility of males in comparison to females to the disease [26]. The results indicate that the geographical distribution showed a higher incidence of infection in rural regions compared to urban areas, in contrast with Uchiumi *et al*. [27], the authors of which conducted a separate study that revealed a higher incidence of infection among individuals residing in urban regions compared to those in rural areas. In fact, numerous factors, including substandard living conditions, inadequate health education in rural regions, economic volatility, and budgetary constraints that affect the management of disease and its prevention, can contribute to the observed outcome. Another observation is that reported by Asghari *et al*. [28] who showed that living in rural regions may be considered a risk factor for CE, but Bait Almal *et al*. [29] found that CE is becoming more urbanized and can no longer just be considered a rural illness. In addition, it was found that the lung is the most susceptible organ to infection by hydatid cysts compared to other organs such as the liver. Ali *et al*. (2020) explained that the reason for this was that the liver tissue surrounding a cyst shows a remarkable level of resistance, hindering or even entirely preventing its spread over a long period of time. In contrast, the lungs have a reduced ability to combat the expansion of the hydatid cyst due to their inherent elasticity, which facilitates the cyst's continued growth [30]. The current study also agreed with many studies regarding the occurrence of hematological variations in white blood cells, hemoglobin levels, and erythrocyte sedimentation rate, as well as variations in liver enzyme levels and C-reactive protein [31,32]. In fact, the clinical symptoms range from asymptomatic to a variety of symptoms, depending on the organ implicated. Moreover, CE symptoms are characterized by abdominal pain and a mass in the abdomen in most liver infections, while patients with lung hydatidosis most frequently present with chest pain and cough, as consistent with that reported by Heikal and El-Lessy [32]. Indeed, the clinical manifestation of the disease lesion may reflect the host's immune responses to the parasite in terms of range and intensity. The presence of *E. granulosus* in humans stimulates both humoral and cellular immune responses. This reaction is characterized by increased levels of antibodies in the bloodstream and the presence of T helper cell 1 (Th1) and T helper cell 2 (Th2) cytokines. To determine the severity of CE disease, this study investigated two types of newly discovered interleukin: IL-40, which is a proinflammatory interleukin produced by Th1 cells, and IL-41, which is an anti-inflammatory interleukin produced by Th-2 cells. The result was clear: the concentration of IL-41 in the patients' sera exceeded the concentration of IL-40. Also, there was no apparent relationship between the concentrations of IL-40 and IL41 with regard to the site of infection or fertility of the cyst. It has been confirmed that patients who suffer from recurrent hydatid cysts have an increased concentration of IL-41 compared to the concentration of IL-40, for the purposes of reaching a clearer understanding of the role of the studied cytokines in the incidence of hydatid cysts. These cytokines were evaluated in terms of the ratio of the concentration of proinflammatory interleukins (IL40) to that of anti-inflammatory interleukins (IL-41). The ratios between the patients with hydatid cysts and the control group were 7.8±0.38 and 16.04±1.4, respectively. Many studies tend to present these concentrations in this way because this could potentially shed light on the polarity of the cells producing these cytokines. This indicates an increase in the anti-inflammatory interleukin (IL-41) compared to the pro-inflammatory interleukin concentration (IL-40). The increased number of Th-2 cells over Th-1 cells suggests that the development of chronic infection typically correlates with the dominant Th2 cell immune response. In fact, both Th1 and Th2 cells are present in the immune

response against *E. granulosus*. Th1 cells activate during early parasite infection, contributing to the host's immune protection, while Th2 cells activate during the parasite's chronic stages [16,33]. Thus, this result is consistent with the many studies that have shown an increase in Th2 cells in parasitic infections. Also, Al-Aouadi *et al*. [34] showed that Th2 cytokines (IL-4, IL-5, and IL-10) had a much higher concentration in late active HC compared to early active HC and the control group (p<0.05). This implies that antigen B enhanced an immune evasion species, potentially causing a shift in the immune response from Th1 to Th2. This species inhibits elastase activity and neutrophil chemotaxis, leading to a non-protective Th2 immune reaction; as a result, the parasite is able to evade the immune response [35]. Little is known about cytokine production during the initial stages of a primary *E. granulosus* infection as a result of oral exposure to eggs. However, Baz *et al*. observed an initial low level of both Th1 and Th2 cytokines during a primary *E. multilocularis* infection, which later increased [7]. Additionally, it is believed that the Th2 cytokines are responsible for hindering the elimination of parasites due to the anti-inflammatory effects of IL-10 [36]. Elevated levels of IL-10, a cytokine commonly associated with immune response control, characterize chronic *Echinococcus* infection [37].

Conclusion

The current study suggests that the severity of the disease was positively correlated with the decrease in serum IL-40 and the increase in IL-41 levels in the serum of patients with hydatid cyst. Furthermore, IL-41 has the potential to function as a biomarker for chronic infection with hydatid cysts and may be essential to the immunopathological mechanism of the hydatid cyst.

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Conflict of interests

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Bhutani N, Kajal P. Hepatic echinococcosis: A review. *Ann Med Surg (Lond)*. 2018;36:99-105. doi: 10.1016/j.amsu.2018.10.032.
2. Moro P, Schantz PM. Echinococcosis: a review. *Int J Infect Dis*. 2009;13(2):125-133. doi: 10.1016/j.ijid.2008.03.037.
3. Hidalgo C, Stoore C, Strull K, Franco C, Corrêa F, Jiménez M, et al. New insights of the local immune response against both fertile and infertile hydatid cysts. *PLoS One*. 2019;14(1):e0211542. doi: 10.1371/journal.pone.0211542.
4. Corrêa F, Hidalgo C, Stoore C, Jiménez M, Hernández M, Paredes R. Cattle co-infection of Echinococcus granulosus and Fasciola hepatica results in a different systemic cytokine profile than single parasite infection. *PLoS One*. 2020;15(9):e0238909. doi: 10.1371/journal.pone.0238909.
5. Díaz Á. Immunology of cystic echinococcosis (hydatid disease). *Br Med Bull*. 2017;124(1):121-133. doi: 10.1093/bmb/ldx033.
6. Siracusano A, Margutti P, Delunardo F, Profumo E, Riganò R, Buttari B, et al. Molecular cross-talk in host-parasite relationships: the intriguing immunomodulatory role of Echinococcus antigen B in cystic echinococcosis. *Int J Parasitol*. 2008;38(12):1371-1376. doi: 10.1016/j.ijpara.2008.06.003.
7. Baz A, Ettlin GM, Dematteis S. Complexity and function of cytokine responses in experimental infection by Echinococcus granulosus. *Immunobiology*. 2006;211:3–9. doi: 10.1016/j.imbio.2005.09.001.
8. Ferreira VL, Borba HHL, Bonetti AF, Leonart LP, Pontarolo R. Cytokines and Interferons: Types and Functions. In: Khan WA, (Ed.), Autoantibodies and Cytokines, In TechOpen, 2018. doi: 10.5772/intechopen.74550.
9. Ahmed ZA, Idan EM, Ardalan NM. Role of IL-37 and Dectin-1 during Toxoplasmosis. *Baghdad Sci J*. 2023;20(3):0746. doi: 10.21123/bsj.2022.7618.
10. Mohammed SM, Saaed HK. Interleukin-6 suppression by different TNF inhibitors in rheumatoid arthritis patients during maintenance therapy. *Al-Rafidain J Med Sci*. 2023;5:184–191. doi: 10.54133/ajms.v5i.230.
11. Lunney JK. Cytokines orchestrating the immune response. *Rev Sci Tech*. 1998;17(1):84-94. doi: 10.20506/rst.17.1.1094.
12. Dorosti Z, Tolouei S, Khanahmad H, Jafari R, Jafaee F, Sharafi SM, et al. IL-4 gene expression in adventitial layer (fibrous layer) of hepatic ovine and bovine hydatid cysts. *J Parasit Dis*. 2016;40(3):855-859. doi: 10.1007/s12639-014-0593-5.
13. Zlotnik A. Perspective: Insights on the nomenclature of cytokines and chemokines. *Front Immunol*. 2020;11:908. doi:10.3389/fimmu.2020.00908.
14. Zingaretti C, Arigò M, Cardaci A, Moro M, Crosti M, Sinisi A, et al. Identification of new autoantigens by protein array indicates a role for IL4 neutralization in autoimmune hepatitis. *Mol Cell Proteomics*. 2012;11(12):1885-1897. doi: 10.1074/mcp.M112.018713.
15. Catalan-Dibene J, McIntyre LL, Zlotnik A. Interleukin 30 to Interleukin 40. *J Interferon Cytokine Res*. 2018;38(10):423-439. doi: 10.1089/jir.2018.0089.
16. Gong L, Zhou Y, Shi S, Ying L, Li Y, Li M. Increased serum IL-41 is associated with disease activity in rheumatoid arthritis. *Clin Chim Acta*. 2023;538:169-174. doi: 10.1016/j.cca.2022.11.021.
17. Zheng SL, Li ZY, Song J, Liu JM, Miao CY. Metrnl: a secreted protein with new emerging functions. *Acta Pharmacol Sin*. 2016;37:571–579, doi: 10.1038/aps.2016.9.
18. Ushach I, Burkhardt AM, Martinez C, Hevezi PA, Gerber PA, Buhren BA, et al. Meteorin-like is a cytokine associated with barrier tissues and alternatively activated macrophages. *Clin Immunol*. 2015;156(2):119-127. doi: 10.1016/j.clim.2014.11.006.
19. Zhang W, Zhang Z, Wu W, Shi B, Li J, Zhou X, et al. Epidemiology and control of echinococcosis in central Asia, with particular reference to the People's Republic of China. *Acta Trop*. 2015;141(Pt B):235-243. doi: 10.1016/j.actatropica.2014.03.014.
20. AL-Asadi SAM, Hansh WJ, Awad AHH. Employing NADH dehydrogenase subunit 1 in the determination of Echinococcus granulosus strain in sheep, cattle and human in Thi-Qar Province, Iraq. *Baghdad Sci J*. 2021;18(2):0238. doi: 10.21123/bsj.2021.18.2.0238.
21. Al-Tikrity IAA, Al-Janabi ZA, Al-jubory AHA. Comparative study of hydatid cysts isolated from livers of different hosts. *Baghdad Sci J*. 2014;11(2):928-933. doi: 10.21123/bsj.2014.11.2.928-933.
22. Edan EM, Ardalan NM. Estimation of humural immune response on the rabbits that immunizing with Hydatid cyst

antigens by using IHAT and ElISA. *J Fac Med Baghdad*. 2009;51(3):332-335. doi: 10.32007/jfacmedbagdad.5131145.
23. Agudelo Higuita NI, Brunetti E, McCloskey C. Cystic Echinococcosis. *J Clin Microbiol*. 2016;54(3):518-523. doi: 10.1128/JCM.02420-15.
24. Kammerer WS, Schantz PM. Echinococcal disease. *Infect Dis Clin North Am*. 1993;7(3):605-618. PMID: 8254162.
25. Khan H, Ahmed H, Afzal MS, Awan UA, Khurram M, Simsek S, et al. Detection of Anti-Echinococcus granulosus antibodies in humans: An update from Pakistan. *Pathogens*. 2021;11(1):29. doi: 10.3390/pathogens11010029.
26. Ilbeigi P, Mohebali M, Kia EB, Saber-Inasab M, Aryaeipour M, Bizhani N, et al. Seroepidemiology of human hydatidosis using AgB-ELISA test in Isfahan City and Suburb Areas, Isfahan Province, Central Iran. *Iran J Public Health*. 2015;44(9):1219-1224. PMID: 26587496.
27. Uchiumi L, Mujica G, Araya D, Salvitti JC, Sobrino M, Moguillansky S, et al. Prevalence of human cystic echinococcosis in the towns of Ñorquinco and Ramos Mexia in Rio Negro Province, Argentina, and direct risk factors for infection. *Parasit Vectors*. 2021;14(1):262. doi: 10.1186/s13071-021-04753-y.
28. Asghari M, Mohebali M, Kia EB, Farahnak A, Aryaeipour M, Asadian S, et al. Seroepidemiology of human hydatidosis using AgB-ELISA test in Arak, Central Iran. *Iranian J Public Health*. 2013;42:391-396.
29. Bait Almal NY, Mekal FH, Ali HM, Ali MS. Prevalence of human cystic echinococcosis: A clinico-epidemiological study in Northeast of Libya. *Int J Pharm Life Sci*. 2020;11(2):6505–6509.
30. Ali R, Khan S, Khan M, Adnan M, Ali I, Khan TA, et al. A systematic review of medicinal plants used against Echinococcus granulosus. *PLoS One*. 2020;15(10):e0240456. doi: 10.1371/journal.pone.0240456.
31. Christodoulidis G, Samara AA, Diamantis A, Floros T, Sgantzou IK, Karakantas KS, et al. Reaching the challenging diagnosis of complicated liver hydatid disease: A single institution's experience from an endemic area. *Medicina*. 2021;57(11):1210. doi: 10.3390/medicina57111210.
32. Heikal E, El-Lessy F. Demographic aspects of human hydatidosis in Egypt. *Egypt J Hospital Med*. 2021;85(2):3706-3711. doi: 10.21608/ejhm.2021.203748.
33. Siracusano A, Delunardo F, Teggi A, Ortona E. Host-parasite relationship in cystic echinococcosis: an evolving story. *Clin Dev Immunol*. 2012;2012:639362. doi: 10.1155/2012/639362.
34. Al-Aouadi RF, Jassim SM, Al-Aouadi F. Study of immunological response to early and late stages of hydatid cyst formation. *J Babylon Univ*. 2016;24: 1433-1446.
35. Rigano R, Buttari B, Profumo E, Ortona E, Delunardo F, Margutti P, et al. Echinococcus granulosus antigen B impairs human dendritic cell differentiation and polarizes immature dendritic cell maturation towards a Th2 cell response. *Infect Immun*. 2007;75(4):1667–1678. doi: 10.1128/IAI.01156-06.
36. Vuitton DA. The ambiguous role of immunity in echinococcosis: protection of the host or of the parasite? *Acta Trop*. 2003;85(2):119-132. doi: 10.1016/s0001-706x(02)00230-9.
37. Zheng Y, Guo X, He W, Shao Z, Zhang X, Yang J, et al. Effects of Echinococcus multilocularis miR-71 mimics on murine macrophage RAW264.7 cells. *Int Immunopharmacol*. 2016;34:259-262. doi: 10.1016/j.intimp.2016.03.015.