

## Investigating some Immunological Parameters for Patients Infected With *E. Granulosus* in Central and Northern Iraq

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### ABSTRACT

The study included 45 samples of hydatid cysts isolated from humans in the period from April 2021 to November 2021 from hospitals in central and northern Iraq, and 3 mL of blood was collected from the same people with hydatid cysts. The primary study was conducted in private laboratories near the hospitals. As for the immunological study, it was conducted in the Sama Dubai laboratory for pathological analyzes / Al-Harithiya, the study aimed to investigate some immunological indicators in patients with hydatid cysts and compare them with the same indicators for healthy people. These indicators included interleukin-6, interleukin-10, tumor necrosis factor-alpha, IgE, and IgG of *Echinococcus granulosus*. The results of the study showed an increase in the levels of interleukin-6, interleukin-10, tumor necrosis factor-alpha, IgE and IgG to *Echinococcus granulosus* in the blood serum of patients with hydatid cysts with a very significant difference from the healthy ones, as well as a high level of the studied immune indicators in the blood serum of patients who had isolated hydatid cysts From the liver, about the level of the immunological indicators studied in the serum of patients who were isolated from the lungs by hydatid cysts, except for the IgG to *Echinococcus granulosus*.

**Keywords:** Interleukin-6, Interleukin-10, Tumor necrosis factor-alpha, IgE and IgG to *Echinococcus granulosus*

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### INTRODUCTION

Hydatidosis is one of the most important zoonotic diseases in the world, as well as one of the most important animal diseases that infect people and animals. It is caused by the larval stage of the parasite *Echinococcus granulosus* and several other medically important species such as *Echinococcus multilocularis* [1]. This disease has caused several medical, economic, and veterinary problems. It's also a public health problem that affects rural and poor communities, where livestock breeders interact with dogs, which are the parasite's final hosts, as well as domestic animals like sheep, cows, and buffaloes, which are intermediate hosts, resulting in economic losses. As a result of the injury to important organs, especially the liver, which results in significant economic losses, the clinical symptoms in humans are caused by the hydatid cyst's pressure on the organs within it,

affecting their functions and inhibiting their growth due to the explosion of the shock-causing sac, fatal injuries have been reported, and some important organs, such as the brain, are difficult to surgically interfere with, therefore life may be risked or fatal [2]. Hydatid disease is distributed worldwide, especially in agricultural countries where people have grown sheep and cows, such as Southeast Asia and the Middle East, including the Arabian Gulf, North Africa, and South America, where large numbers of dogs are primarily carriers of the parasite [3]. The nature of the immune response that occurs during infection has been discovered through a new application of the immunological approach, which improves the accuracy of the diagnosis and monitoring the disease during treatment, The hydatid cyst parasite induces a cellular and humoral immune response in the blood serum, which is shown by increased levels of antibodies, complement proteins, and cytokines [4].

### MATERIALS AND METHODS

#### Groups Study

This study included 75 healthy patients and people infected with hydatid cyst disease *E. granulosus*, and the study groups were divided into three groups that included 30 healthy people, 38 people with hydatid cyst

disease in the liver and seven people with hydatid cyst disease in the lungs. The process of collecting samples began from April of 2021 to October of 2021 from central and northern governorates Iraq.

### Collecting Blood Samples

A total of 3 ml of blood was withdrawn from each of the three study groups using a disposable syringe. The blood samples were placed in test tubes and left to clot for 10 minutes, and then the test tubes were placed in a centrifuge at a speed of 4000 RPM for a period of 10 minutes. Then, the blood serum was divided into three sections, the serum was placed in Eppendorf tubes, and the samples were kept in the freezer at -20°C until the subsequent examinations were performed.

### Assay Procedure

The examination kit was prepared by the Chinese company Sun long and included both the Human Interleukin 6 ELISA KIT, Human Interleukin 10 ELISA KIT, Human TNF  $\alpha$  ELISA KIT, and Human *Echinococcus granulosus* IgG ELISA KIT, except for the Immunoglobulin E ELISA KIT, which was prepared by the monobind company (USA). The solutions for all the kits and blood serum samples were placed at room temperature (17–26°C) to stabilize before starting to measure the levels of the immune indicators under study, as the tests were carried out in the Sama Dubai laboratory for pathological analysis in Al-Harithiya city, Baghdad.

### PRINCIPLE

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to TNF-  $\alpha$ . Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody. Specific for TNF- $\alpha$  is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain TNF- $\alpha$  and HRP conjugated TNF- $\alpha$  antibody will appear blue in color and then turn yellow after the addition of the stop solution. The Optical Density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of TNF- $\alpha$ . We calculate the concentration of TNF- $\alpha$  in the samples by comparing the OD of the samples to the standard curve. The other tests same this principle except the Immunoglobulin E ELISA KIT that shows some difference.

## RESULTS AND DISCUSSION

### Measuring the Level of Tumor Necrosis Factor Alpha

The current study in table 1 found a significant increase in the level of TNF- $\alpha$  in the blood serum of people infected with *Echinococcus granulosus* compared to healthy people, Aside from the significant difference in average TNF-  $\alpha$  levels between the hydatid cyst parasite-infected group and the uninfected group, serum blood between hydatid cysts in the liver and hydatid cysts in the lungs.

The findings of this study agree with the findings of [5] and [6], which showed an elevated level of TNF-  $\alpha$  in patients with hydatid cysts due to a high level of TNF-  $\alpha$  in patients' serum, Hydatid cyst indicates the presence of antigen B and antigen-5 on the surface of the parasite's protoscolexes, which stimulates CD4 T cells to produce TNF- $\alpha$ . The reason for the significant difference in average TNF- $\alpha$  level between patients with hydatid cysts in the liver and patients with hydatid cysts in the lungs is attributed to several factors, including the size of the hydatid cysts removed from the liver, which is larger than the average size of the lungs. as well as the number of hydatid cysts in the liver, is greater than what is present in the lungs and the vitality of the protoscolexes, Some of the hydatid cysts removed from the liver appeared to contain live protoscolexes, while all the cysts removed from the lungs were either sterile or contained dead protoscolexes, This is the reason for the high level of the tumor necrosis factor alpha to extracellular vesicle proteins found in hydatid fluid such as 110k that stimulate TNF- $\alpha$  production [7].

### Measurement of *E. granulosus* IgG level

The results of the current study showed an increase in the level of *E. granulosus* IgG antibody in the blood serum of people infected with the *E. granulosus* parasite with a very significant difference from healthy people Table 2, no significant difference was observed in the rate of concentration of anti-G-antibody to *Echinococcus granulosus* in the blood serum of those infected with hydatid cysts in the liver and those with hydatid cysts in the lungs.

The results of this study agree with the findings of [8] and [9] when they showed an elevated level of IgG antibody in patients with hydatid cysts. The reason for the high level of IgG antibody in the serum of patients with hydatid cysts is the presence of the antigen B on the surface of the protoscolexes of the parasite, which stimulates B cells to differentiate into plasma cells, which in turn produce IgG antibody, The reason for the absence of significant differences in the average levels of *Echinococcus granulosus* antibodies in the group of

**Table 1: Tumor Necrosis Factor-Alpha Level in Serum of Hydatid and Healthy People.**

Groups study	Number of samples	Mean ng/L	StDev	P-value
Liver	38	279.52 a	12.27	0.000004
Lung	7	235.39 b	6.15	
Healthy	30	20.27 c	3.78	

patients with hydatid cysts in the liver and the average levels of IgG antibodies to *Echinococcus granulosus* in the group infected with hydatid cysts in the lungs is due to the fact that this antibody has a specificity for this parasite, as it increases. The level of this antibody in the blood serum increases in the presence of antigens specific to the parasite *Echinococcus granulosus*.

### Interleukin-6 Level Measurement

The results of the current study showed an increase in the level of interleukin-6 in the blood serum of people infected with *Echinococcus granulosus*, with a very significant difference from healthy people. The results table 3 of the study also showed that there were significant differences in the average concentration of interleukin-6 in the blood serum between those infected with hydatid cysts in the liver and those with hydatid cysts in the lungs.

The results of this study are in agreement with the findings of [10], which showed an elevated interleukin-6 level in patients with hydatid cysts. on the surface of the parasite's protoscolexes which stimulates CD4 T cells to produce interleukin-6, The reason for the significant difference in average interleukin-6 levels between patients with hydatid cysts in the liver and patients with hydatid cysts in the lungs is attributed to several factors, including the size of the hydatid cysts removed from the liver, which is larger than the average size of the lungs. as well as the number of hydatid cysts in the liver, is greater than what is present in the lungs and the vitality of the protoscolexes, Some of the hydatid cysts removed from the liver appeared to contain live protoscolexes, while all the cysts removed from the lungs were either sterile or contained dead protoscolexes, Also, the reason for the increase in interleukin-6 in infected people can be attributed to another reason related to one of the most important evasion methods that the parasite takes to protect itself from the immune response by directing the immune system to activate T-helper cells type II and inhibit T-helper cells type I, thus making the disease chronic.

### Interleukin-10 Level Measurement

The results of the current study showed an increase in

the level of interleukin-10 in the blood serum of people infected with *Echinococcus granulosus*, with a very significant difference from healthy people. The results of the table 4 study also showed that there were significant differences in the average concentration of interleukin-6 in the blood serum between those infected with hydatid cysts in the liver and those with hydatid cysts in the lungs.

The results of this study are in agreement with the findings of [10], which showed an elevated interleukin-10 level in patients with hydatid cysts. on the surface of the parasite's protoscolexes which stimulates CD4 T cells to produce interleukin-10, The reason for the significant difference in average interleukin-10 levels between patients with hydatid cysts in the liver and patients with hydatid cysts in the lungs is attributed to several factors, including the size of the hydatid cysts removed from the liver, which is larger than the average size of the lungs, as well as the number of hydatid cysts in the liver, is greater than what is present in the lungs and the vitality of the protoscolexes, Some of the hydatid cysts removed from the liver appeared to contain live protoscolexes, while all the cysts removed from the lungs were either sterile or contained dead protoscolexes, And there are many studies that suggest increasing the production of interleukin-10 as a way to survive for a long time as well as to evade the immune response by activating the immune system [11].

### Immunoglobulin-E antibody level measurement

The results of the current study table 5 showed an increase in the level of IgE antibody in the blood serum of people infected with *Echinococcus granulosus*, with a very significant difference from healthy people. As well as healthy people, in addition to the significant difference in the average level of IgE-antibody between the groups infected with the hydatid cyst parasite and the group of non-infected people hydatid cysts in the lungs.

The results of this study agree with what was found by [12] and [13] who showed the elevated level of anti-E

**Table 2: The level of IgG to *Echinococcus granulosus* in the serum of healthy and hydatid people.**

Groups study	Number of samples	Mean IU/ml	StDev	P-value
Liver	38	58.41 a	3.53	0.000006
Lung	7	57.66 a	3.26	
Healthy	30	7.25 b	3.17	

**Table 3: The Level of Interleukin-6 in the Serum of Healthy and Hydatid People.**

Groups study	Number of samples	Mean ng/L	StDev	P-value
Liver	38	74.16 a	5.9	0.000003
Lung	7	55.65 b	4.3	
Healthy	30	8.14 c	3.36	

**Table 4: The level of interleukin-10 in the serum of healthy and hydatid people.**

Groups study	Number of samples	Mean pg/ml	StDev	P-value
Liver	38	35.88 a	4.77	0.000002
Lung	7	22.43 b	2.88	
Healthy	30	4.72 c	1.74	

Table 5: The level of IgE in the serum of healthy and hydatid people.

Groups study	Number of samples	Mean U/L	StDev	P-value
Liver	38	333.8 a	23.9	0.00004
Lung	7	225.7 b	24.9	
Healthy	30	91.3 c	16.88	

antibody in patients with hydatid cysts, and the reason for the high level of IgE antibody in the serum of patients with hydatid cysts is the presence of antigen B, On the surface of the parasite's protoscolexes, which stimulates B cells to differentiate into plasma cells, which in turn produces the IgE-antibody. The reason for the significant differences in the average level of anti-E antibody in the group with hydatid cysts in the liver from the average level of IgE antibody in the group with hydatid cysts in the lungs is due to several reasons, including the size of the hydatid cysts removed from the liver, which are larger than the ones in the lungs, as well as the number of The hydatid cysts in the liver are larger than those in the lungs and the vitality of the protoscolexes, as some hydatid cysts removed from the liver appear to contain live protoscolexes, while all cysts removed from the lungs are either sterile or contain dead protoscolexes.

### CONCLUSION

The high level of immunoglobulins type IgE, IgG, interleukin-6, interleukin-10, and tumor necrosis factor alpha of people infected with *Echinococcus granulosus* has a very significant difference compared to non-infected people.

### REFERENCES

- Lundstrom-Stadelmann B, Rufener R, Hemphill A. Drug repurposing applied: Activity of the anti-malarial mefloquine against *Echinococcus multilocularis*. *Int J Parasitol Drugs Drug Resist* 2020; 13:121-9.
- Tamarozzi F, Deplazes P, Casulli A. Reinventing the wheel of *Echinococcus granulosus* sensu lato transmission to humans. *Trends Parasitol* 2020; 36:427-34.
- Casulli A. Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis. *Lancet Glob Health* 2020; 8:e470-1.
- Zhang W, Ross AG, McManus DP. Mechanisms of immunity in hydatid disease: Implications for vaccine development. *J Immunol* 2008; 181:6679-85.
- Khelifi L, Soufli I, Labsi M, et al. Immune-protective effect of echinococcosis on colitis experimental model is dependent of down regulation of TNF- $\alpha$  and NO production. *Acta Tropica* 2017; 166:7-15.
- Casaravilla C, Pittini A, Ruckerl D, et al. Unconventional maturation of dendritic cells induced by particles from the laminated layer of larval *Echinococcus granulosus*. *Infect Immun* 2014; 82:3164-76.
- Zhang W, Ross AG, McManus DP. Mechanisms of immunity in hydatid disease: Implications for vaccine development. *J Immunol* 2008; 181:6679-85.
- Omran VF, Rouhani S, Kazemi B, et al. Seroprevalence of IgG antibodies against *Echinococcus granulosus* by ELISA method using recombinant Agb in Lorestan Province, Western Iran. *Iran J Public Health* 2017; 46:1132.
- Kordafshari S, Hosseini SH, Jalousian F, et al. Evaluation of dot immunogold filtration assay (DIGFA) by recombinant protein EPC1 for anti-*Echinococcus granulosus* IgG antibody. *Iran J Parasitol* 2015; 10:30.
- Lin K, Zhou D, Li M, et al. *Echinococcus granulosus* cyst fluid suppresses inflammatory responses by inhibiting TRAF6 signalling in macrophages. *Parasitology* 2021; 148:887-94.
- Amri M, Mezioug D, Touil-Boukoffa C. Involvement of IL-10 and IL-4 in evasion strategies of *Echinococcus granulosus* to host immune response. *Eur Cytokine Netw* 2009; 20:63-8.
- Hameed AT, Hussein AB, Abdullah SS. Study of some immunological manifestations in patients with *Leishmania tropica* and the effect of *Achillea fragrantissima* and *Capsella bursa-pastoris* plant extracts on the speed of the healing of experimentally infected Balb/c mice. *Biochem Cell Arch* 2019; 19.
- Khabiri AR, Bagheri F, Assmar M, et al. Analysis of specific IgE and IgG subclass antibodies for diagnosis of *Echinococcus granulosus*. *Parasite Immunol* 2006; 28:357-62.