



## Estimation of immunoglobulins levels in the sera of patients infected with liver hydatid cysts

Ahlam J. Taher<sup>1</sup>, Nahla G. Abdul-Majed<sup>2</sup> and Ihsan M. Al-Saqr<sup>3</sup>

<sup>1</sup>College of Education (Ibn Al-Hatham), University of Baghdad, <sup>2</sup>Educational laboratories, Medicine City Hospital and <sup>3</sup>Tropical Research Unit, College of Science, University of Baghdad, Iraq.

### Abstract

This study included 46 patients with liver hydatid cyst diagnosed clinically and surgically, control group consist of 22 were naïve from infection had been confirmed by specialist. The patients were divided according to the size of the cysts into more and less than 5 cm diameter size, were 33 and 13 respectively. Also it divided into primary and secondary hydatid cyst infection which were 30 and 16 respectively. The role of immunological response against hydatid cyst parasite, showed a significant increased in humoral immunoglobulins (IgG, IgA, IgM and IgE) which were significantly higher in the hydatid cyst infection than control. Also significant increased in immunoglobulins in secondary infection than primary infection, beside significant increased in the levels of immunoglobulins IgG and IgE in patients with > 5 cm hydatid cyst diameter compared with < 5 cm hydatid cyst diameter, while the increased in the levels of IgA and IgM were not significant.

Keywords: Hydatid cyst, Liver parasite, Infection type, Cysts size, Patient, Immunoglobulins levels.

### Introduction

The hydatid cyst disease known by many names, including hydatidosis, hydatid disease, hydatid cysts, echinococcosis (Goldsmith, 2006; Kakkos *et al.*, 2001; Khuroo, 2002) and the terms hydatid, hydatidosis refers to the infection by Metacestode stage of the parasite in the intermediate hosts, while the term echinococcosis refer to the infection by adult stage of the parasite in the final host (Thompson, 2001). larval stage (Metacestode) of tapeworm belong to the genus *Echinococcus*, family Taeniidae was considered to be the causative agent of the hydatid disease in the intermediate hosts including human (Casarvilla *et al.*, 2003; Breijo *et al.*, 2008; Ramose *et al.*, 2006) and these stages known by Hydatid cysts (Zhang *et al.*, 2003; Ronyany *et al.*, 2006; Abbasi *et al.*, 2003). This kind of parasite have indirect life cycle (Dvorak *et al.*, 2008; Kassai, 1999), as it requires two hosts of mammals to complete life cycle, final host represented by carnivorous and intermediate host represented by herbivorous, while humans are accidental host does not contributed to sustaining the life cycle (McManus *et al.*, 2003).

### Materials and Methods

Study Groups: The study included 82 peoples who were divided into two groups as follows :

A- Patients group: This group contains 60 patients have been confirmed diagnosed clinically and surgically by a specialist doctor with hydatid cysts in liver (46 patients), lung (8 patients), spleen (4 patients) and in the liver and lung together (2 patients) aged between 10-70 years, they reviewed the general surgery consultant at Digestive and Liver Disease Hospital and Kadhimiya Teaching Hospital for the period from December 2007 to September 2008 . The liver patients has been divided depending on the size of the cysts to the patients with cysts larger than 5 cm and less than 5 cm, and their number were 33 and 13 respectively, were divided according to the infection type to patients with primary and secondary infected and their number were 30 and 16 respectively.

B- Control group: These group include 22 healthy people do not suffer from hydatid cysts disease or any other medical condition was examined by consultants above.

Collection of blood samples: Blood samples were withdrawn from the vein by disposable syringe 10 ml size for each individual of study groups. The blood samples were divided according to the study tests, 8 ml of blood was put in plastic test tubes with a lid and leave the blood at room temperature until clotting (15 minutes), then it centrifuged for 10 min at 3000 rpm, pull the serum by pasteur pipette, then put in the eppendorf tubes (0.5 ml) and saved in frozen (-20 °C) until used.

Quantitative determination of immune-globulin's IgM, IgA and IgG :

Single radial immunodiffusion (SRID) method were used, this method which relies on measuring the ring diameter sedimentation immune system consisting of proliferation of antigen-antibody toward located within the gel in the ready-made plate. This method has been used to measure the serum levels of antibodies IgG, IgM and IgA using ready-made dishes from ALT test was conducted as follows:

- 1- Opened up the dishes and left at room temperature for 5 min.
- 2- Added 5 µl of serum of patients and healthy controls under study in each hole.
- 3- Left the dishes for 15 min at room temperature without moving.
- 4- The dishes were putted in a humid place for 48 hrs in the case of IgG, IgA, and for 72 hrs in the case of IgM
- 5- Ring diameter of sedimentation formed around each hole was measured by ocular lens fitted with a ruler.
- 6- After measurements, values extracted by comparing the diameter loop sedimentation with values that are installed with the attached table with dishes processed by the manufacturer and they were reading with (mg / dl).

Quantitative determination of immunoglobulin IgE :

The level of total IgE in the serum was measured by enzyme linked immunosorbant assay method (ELISA) according to the manufacturer's instructions (DRG US) as follows :

- 1 - The digging of special plate were labeling with standard solution and sera of patients and control.
- 2 - Added 20 µl of the standard solution to the digging allocated to them.
- 3 - Added 20 µl of the serum samples of the study (patients and control) to their Special digging.
- 4 - Added 100 µl of zero buffer to each hole.

5- Blending the mixture homogeneously using a vibrator for 30 sec .

6 - Incubate the mixture at room temperature (18-25 °C) for 30 min .

7- Removing the incubation mixture, then washing the dish 5 times with distilled water and remove the remaining water droplets by beating the dish strongly on absorbent paper or paper towels.

8 - Added 150 µl of enzyme conjugate reagent for each hole and mix gently for 10 sec.

9- Removing the incubation mixture, then washing the dish 5 times with distilled water and remove the remaining water droplets by beating the dish strongly on Absorbent paper or paper towels.

10- Added 100 µl of TMB reagent for each hole and mix gently for 10 sec, then the dish was incubate in the dark at room temperature for 20 min.

11- Interaction stopped for 30 sec to make sure the color changes from blue to yellow color completely.

12- Optical density was measured using a plate reader at wavelength 450 nm in 15 sec.

13- Extracted total IgE globulin concentration in study samples sera by drawing standard curve by putting the absorbance values of each standard solution on the vertical axis (y) and its concentrations on the horizontal axis (X) and extract concentrations of IgE by dropping absorbance values reading by ELISA reader on this curve units and unit global /ml (IU / ml) (Figure 1).

### Results and Discussion

The infection of human with hydatid cysts stimulating humoral and cellular immune responses characterized by a high level of antibodies, as well as the simultaneous overlapping cellular kinetics produced by Th1 and Th2 cells , the balance between the products of those cells reflects the final proceeds of the infection (Cardozo *et al.*, 2002). In the human the infection of hydatid cysts associated with humoral response characterized by high levels of immunoglobulins IgG, IgA, IgE and its secondary units (Zhang *et al.*, 2003). The results in Table (1) have shown high levels of IgG In the serum of patients (63.38 ±1519.38) mg / dl compared with control group (46.02 ±577.89) mg / dl and a high level of immune globulin IgA in the patients serum (12.72 ±248.04) mg / dl compared with control group (6.45 ±151.95) mg / dl, as well as for the IgM (10.32 ±279.05) mg / dl compared with control

(1.39 ±86.14) mg / dl, and high level of immune globulin IgE (55.54 ±442.73) mg / dl compared with control (2.49 ±72.45) mg / dl, where these differences are considered significant level of probability (0.05>) for the immunoglobulins IgG, IgA and IgM, and the level of probability (0.01>) for the immune globulin IgE.

The high levels of these immunoglobulins in the hydatid cysts patients serum compared with control group happened as a response to parasite antigens and these results are considered to be similar to other studies (Al-Qauod and Abdel-Hafez, 2005; Kakkos *et al.*, 2001).

The results in Table (2) considered with immunoglobulins levels in the serum of liver hydatid cysts according to the cysts size showed a significant difference in the level of probability (0.05>) when comparing the immune globulin IgG in patients with cysts less than 5 cm size (84.41 ±1266.48) mg / dl with the patients more than 5 cm cysts size (75.66 ±1619) mg / dl, also the differences were significant (0.05>) when compared the level of immune globulin IgE in patients with cysts less than 5 cm size (21.13 ±165.25) mg / dl compared with the patients more than 5 cm cysts size (67.39 ±561.64) mg / dl. While the differences were not significant for the IgA and IgM immunoglobulins levels when comparison between the patients more and less than 5 cm cysts size as shown in the Table (2).

The decline was recorded in the immunoglobulins levels in patients with cysts less than 5 cm compared with patients more than 5 cm cysts may be due to the lack of parasite antigens relies and the dominant of Th1 type immune response (Rogan and Craig, 1997) where the level of immune globulin IgG was higher than the level of IgA and IgM interpreted by Dixon *et al.* (1982) in the interest of the parasite, as it is this kind of

globulin closing and acts as a insulator that prevents lymphocytes from contact with the parasite and thus works to protect it from the impact of these cells and this result agree with Liu *et al.* (1992) who registered a decline in the levels of IgA in the same time IgM increased in IgG level when mice injected experimentally with initial heads, where this has continued to rise for 68 weeks after injection.

The comparing of immunoglobulins levels according to the type of infection (Table 3), significant differences in the levels of IgG between primary infection patients (80 ±1409.67) mg / dl and secondary infection patients (81.66 ±1725.08) mg / dl in the level of probability (0.05>) as well as when compared to the level of IgA in the patients of primary infection (15.52 ±222.34) mg / dl to the patients of secondary infection (16.92 ±296.22) mg / dl, and level of IgM ( 13.09 ±263.07) mg / dl in primary infection patients compared with secondary infection patients (14.34 ±309) mg / dl. The difference was significant (0.01>) when comparing immunoglobulin IgE levels in the patients of primary infection (60.89 ±325.52) mg / dl with secondary infection patients (82.71 ±686.15) mg / dl.

The increasing of immunoglobulins levels in secondary infection patients compared with primary infection patients may be due to the dominant of the immune response type Th2 and presence of high levels of cellular kinetics IL-4 and IL-5 which enhances the differentiation of B cells into plasma cells and its production of immunoglobulins, as well as the fact that the high level of immunoglobulins especially IgE was a characteristics of parasitic worm infection, where these results are similar to the results of several studies (Al-Qauod and Abbel-Hafez, 2008; Khabiri *et al.*, 2006; Zhang and McManus, 2006).

Table (1): Immunoglobulins levels in the liver hydatid cysts patients serum and control group (Means ±Standard Error).

| Study groups | Number | Immunoglobulins   |                   |                   |                    |
|--------------|--------|-------------------|-------------------|-------------------|--------------------|
|              |        | IgG<br>(mg / dl)* | IgA<br>(mg / dl)* | IgM<br>(mg / dl)* | IgE<br>(IU / ml)** |
| Patients     | 46     | 63.38<br>±1519.38 | 12.72<br>±248.04  | 10.32<br>±279.05  | 55.54<br>±442.73   |
| Control      | 22     | 46.02<br>±577.89  | 6.45<br>±151.95   | 1.39<br>±86.14    | 2.49<br>±72.45     |

\* Significant difference at level of probability ( 0.0 5 > ), \*\* Significant difference at level of probability ( 0.0 1 > ).

Table (2): Immunoglobulins levels in the liver hydatid cysts patients serum according to cysts size (Means ±Standard Error).

| Cysts size | Number | Immunoglobulins   |                  |                  |                  |
|------------|--------|-------------------|------------------|------------------|------------------|
|            |        | IgG (mg / dl)*    | IgA (mg / dl)*   | IgM (mg / dl)    | IgE (IU / ml)**  |
| Less 5 cm  | 13     | 84.41<br>±1266.48 | 28.12<br>±213.35 | 23.07<br>±261.79 | 21.13<br>±165.25 |
| Cysts size | 33     | 75.66<br>±1619.00 | 13.42<br>±261.70 | 11.19<br>±285.85 | 67.39<br>±561.64 |

\* Significant difference at level of probability (0.05 >), \*\* Significant difference at level of probability (0.01 >).

Table (3): Immunoglobulins levels in the liver hydatid cysts patients serum according to infection type (Means ±Standard Error).

| Infection type      | Number | Immunoglobulins   |                  |                  |                  |
|---------------------|--------|-------------------|------------------|------------------|------------------|
|                     |        | IgG (mg / dl)*    | IgA (mg / dl)*   | IgM (mg / dl)    | IgE (IU / ml)**  |
| Primary infection   | 30     | 80.78<br>±1409.67 | 15.52<br>±222.34 | 13.09<br>±263.07 | 60.89<br>±325.52 |
| Secondary infection | 16     | 81.66<br>±1725.08 | 16.92<br>±296.22 | 14.34<br>±309    | 82.71<br>±686.15 |

\* Significant difference at level of probability (0.05 >), \*\* Significant difference at level of probability (0.01 >).

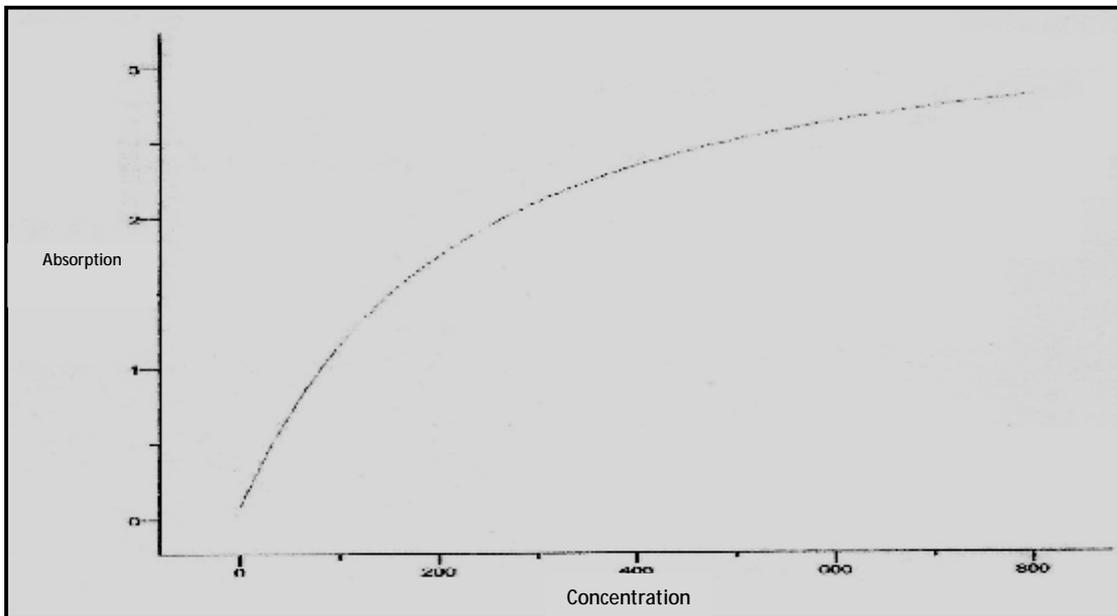


Figure (1): Standard Curve of immunoglobulin IgE.

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