Human and Sheep-Hydatidosis : Hemato-Comparative Study

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Abstract:

A study of 61 patients suffering from acute and chronic hydatid cyst disease, were selected randomly, was done in Hilla Teaching Hospital, three private hospitals and in private clinics in Hilla City during the period from September 2011 to June 2012. The patients ranged from (15-≥64) years old of both sex (49 females and 12 males) , Also, there are 15 confirmed hydatid cyst – free individuals were employed as a control group in the present study.In addition, the present study was applied on 15 hydatid cyst-infected sheep and on 5 hydatid cyst-free sheep as a control group in Hilla abattoir station.

Two mil. of blood sample was taken from each infected subject (human and sheep) , and from their control group too. Then transported to the hematological laboratories for counting total and differential white blood cells.

The study results revealed that there were significant differences in both TWBCs and DWBCs in both studied subjects(infected human and infected sheep) when be compared with their control groups:

Since ,there are different percentages of lymphocytes , neutrophils and eosinophils (which were differed significantly) , were presented in both studied groups. While , the present study did not show any significance difference within both studied groups in relation to their bloods percentages of monocytes and basophils respectively.

Key words: Hydatidosis, Blood , *E. granulosus*, Hilla Teaching and Private Hospitals, Abattoir station.

الخلاصة:

اشتملت الدراسة على 61 مريض يعانون من الإصابة الحادة والمزمنة بمرض الأكياس العدرية ، تم أختيارهم عشوائيا، في مستشفى الحلة التعليمي العام وثلاثة مستشفيات أهلية أضافة للعيادات الطبية الخاصة / محافظة بابل ضمن المدة الزمنية من أيلول٢٠١١ الى حزيران ٢٠١٢. تراوحت أعمارهم من ١٥ الى ٦٤ سنة فأكثر ومن كلا الجنسين(٥ ذكور و ٤٩ أنثى) ، كذالك أشتملت الدراسة على ١٥ شخص سليم لغرض المقارنة.أيضا أشتملت الدراسة الحالية على ١٥ شاة مصابة مع مجموعة سيطرة غير مصابة في مجزرة الحلة. تم اخذ عينات الدم (٢ مل) من كافة المجاميع المدروسة لغرض أجراء فحوصات الدم للعدد الكلي والتفريقي لخلايا الدم البيض. أظهرت نتائج الدراسة أختلافات معنوية في العدد الكلي والتفريقي لخلايا الدم البيض لكلا مجموعتي الدراسة بعد المقارنة مع مجاميع السيطرة لها ، حيث ظهرت أختلافات معنوية في النسب المئوية لكل من الخلايا اللمفاوية والعدلة والحمضة على التوالي، بينما لم تظهر نتائج الدراسة أية أختلافات معنوية بالنسب المئوية لكل من الخلايا القعدة وأحادية النواة.

Introduction:

Human cystic echinococcosis (HCE) / Hydatidosis is a major world zoonosis affecting humans as well as domestic animals caused by infection with the taeniidae metacestode (protoscolices) as larval stage of *Echinococcus granulosus* (Thompson,1995; Teggi and Divico, 2002 ; Mathis *et al*.,2005). Tapeworm eggs are passed with the feces of infected carnivores(cannis) and may subsequently infect humans who inadvertently ingest them (Schantz,1991 ; Andersen, 1997).

The life cycle of *Echinococcus* is indirect and involves two hosts, one definitive carnivore host and the other intermediate herbivore host (McManus *et al*.,2003 ; Zhang *et al*.,2008). The problem arises when humans act as an accidental intermediate host and ingest viable oncosphere-containing eggs, which have been shed in the faeces of the definitive host(Menezes and Silva,2003 ;Yang *et al*.,2006).

The oncospheres invade the intestines, enter the vasculature and develop into hydatid cysts in any organ or tissue, where a variety of symptoms can be produced. However,the liver acts as the first filter for hydatid larvae, making it the most commonly affected organ followed by lung(Kir and Baran ,1995 ; Kismet *et al*.,2008).

It has been described that the humans host tissue response to hydatid cysts, which began with the formation of small aggregates of round cells in the fibrous capsule. These aggregates are gradually enlarges. Ultimately the parasite dies and an epithelial cell reaction and the formation of multinucleated giant cells herald its death (Ammann *et al*.,1996 ; Eckert *et al*., 2001).

Allergic reactions occur from leakage of cyst fluid that contains antigens. Itching, fever and rashes are frequent, and fatal allergic reactions (anaphylaxis) have been reported, eosinophils which are blood cells involved in allergic reactions, are increased in many patients(Novak,1990 ; Andersen,1997;Jablawi,1999).

Hydatid cyst infection may be associated with a significant cellular inflammatory responses (Lioyd,1987) that may cause pathology (Finkelman *et al*.,1991; Allen and Maizels, 1996) as there is an increased leukocytes mainly of eosinophilis, lymphocytes and macrophages with oncosphere, necrosis of surrounding cells is followed by infiltration of neutrophils and macrophages 3-5 day after infection in the intermediate hosts (Waller,1992 ; Zarzosa *et al*.,1999).

Eosinophilia and the production of high levels of IgE are the common consequences of infection by helminthes (Capron and Dessaint,1992 ; Bell,1996). It has been suggested that the eosinophil has evolved especially as a defense against the tissue stages of parasites that are too large to be phagocytosed (Haynes and Fletcher,1990; Sreter *et al.*,2003)). Eosinophils are less phagocytic than neutrophils but like neutrophils they can kill larval stages of parasites (Rainbird *et al*.,1998) such as *Echinococcus* by both dependent and independent mechanisms(Meeusem and Balic,2000).

Aim of the study:

From hemato-comparatival view, the present study aimed to estimate some hematological parameters (TWBCs and DWBCs) in both humans- and sheeps-hydatid cyst infected individuals and in a comparison with their both control groups too .

Patients and methods:

1-Patients:

The present study was carried out in Hilla City for the period from mid of September / 2011 until end of June 2012. The study included 61 individuals: (radiology confirmed as a hydatid cyst- infected individuals).

The admitted hospital-based study was carried out in : Government Hilla Teaching Hospital ; Private Hospitals (Al-Hayaat , Al-Sheifaa, Al-Fayhaa) and private Clinics , on all outpatients with inpatients and follow-up patients whom were confirmed as a hydatid cysts-infected individuals. All hydatid cyst infected individuals in the field of present study were chosen randomly (by chance) from both sexes and from different ages too.

In addition to hospitals laboratories, the appointed hematological tests were done in private laboratories(Al-Hayaat and Tabaarak laboratories) for completion the present study work fluently.

A-Hydatid cyst-infected human.

Precisely, 45 hydatid cyst-infected individual (confirmed radiology) out of 61 confirmed individual ,were included in the present study .

B-Hydatid cyst –free human.

The are 15 Casoni test confirmed hydatid cyst-free individual were included in the present study and regarded as a control group.

C-Hydatid cyst-infected sheep.

There are 15 confirmed hydatid cyst- infested sheep were included in the present study (especially the oldest ones and after slaughtering it in Hilla abattoir station).

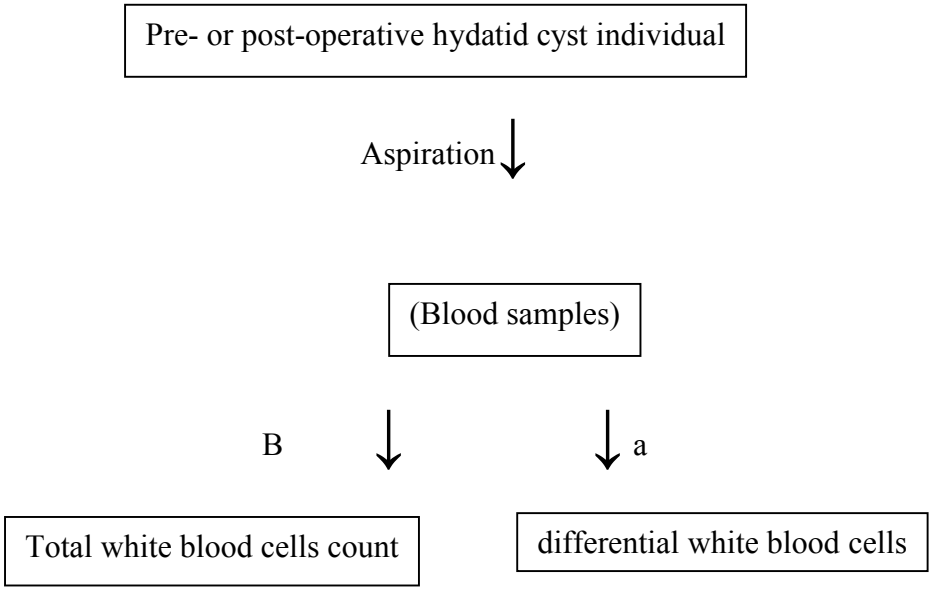
D-Hydatid cyst-free sheep.

Exactly, there are 5 confirmed hydatid cyst-free sheep(after slaughtering it), were included in the present study and regarded as a control group for those sheeps who were infected.

2-Collection of specimens:

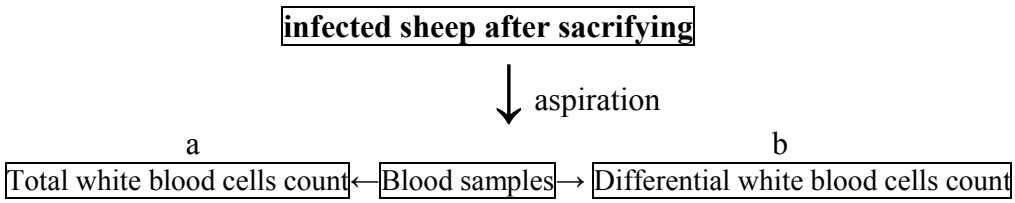
A-Human blood samples.

Blood samples were collected from all hydatid cyst infected individuals before or after their undergoing surgical operation , syringed blood samples (2 milliliter) were put in EDTA tubes for hematological investigations which be tested directly , as shown in the following diagram:



B-Sheep blood samples.

Blood samples were collected from all hydatid cyst infected sheeps, after scarifying its in abattoir station in Hilla City , the obtained blood samples (2 ml.) were put in EDTA tubes for hematological investigations which be tested directly , as shown in the following diagram:



3- Laboratory examinations:

The specimen examination include the following:

A- Parasitic examination:

In human, it was accomplished by diagnosis of hydatid cysts (figure:1), on the based of patient's case history, clinical findings. Also, the diagnosis of hydatid cyst in each individual patient is based on the identification of cyst structures by imaging techniques, predominantly computed tomography, X-ray examinations, and confirmationnal Casoni test (Craig, 1997; Grimm *et al*., 1998; Gottstein, 2000; Pawlowski *et al*., 2001 ;Teggi and Divico, 2002).

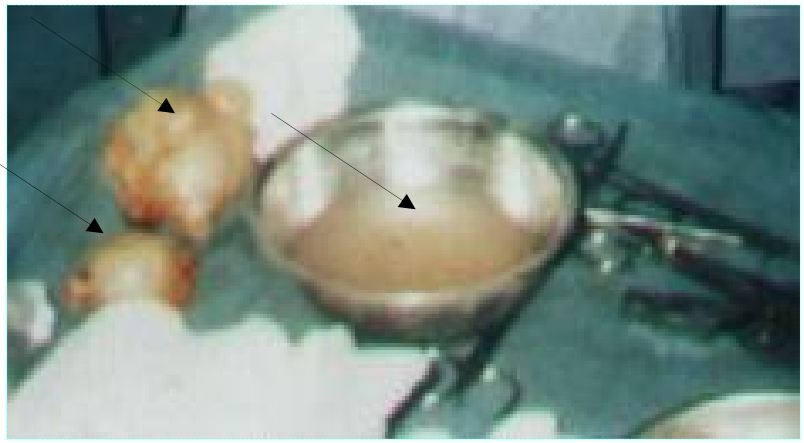


Figure (1): Hydatid cysts from human beings inside operation ward.

In sheep , it was accomplished by diagnosis the hydatid cysts (figure:2), after slaughtering the infected sheep(especially the oldest ones) at abattoir station.

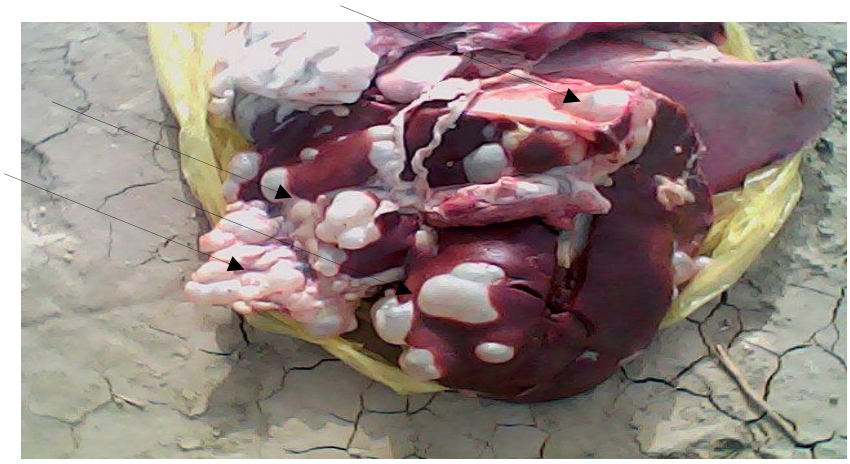


Figure-2: Sheeps hydatid cysts immediately obtained from slaughtered sheep

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B- Hematological examinations:

About (2 ml.) of blood was collected from each hydatid cyst infected individual (human and sheep), and put in tubes with EDTA – K^+ (AFMA – DISPO / Jordan) for hematological estimation. Virtually, all studied hematological tests of present study were done in Hospitals and in private laboratories .
The following hematological tests were performed in the present study: -

1- Total White Blood Cells Count:

The numbers of white blood cells counts were counted under light microscope (10x and 40x) using Neubauer counting chamber (Dacie and Lewis,1995). The W.B.C. were estimated by diluting (20µl) of whole blood in Turk's solution (Glacial acetic acid 1% with 1-2 drop methyl blue to give purple color).

2- Differential White Blood Cells Counts: -

Blood film was made immediately as follows: -

After careful mixing of the blood, an appropriate drop was delivered by a capillary glass and placed in center line of clean microscope slide about one 1cm. from one end. A spreader was placed in front of the drop at an angle of about 30º to the slide and moved it back to make contact with the drop, and then the drop was spread out quickly along the line of contact (Dacie and Lewis,1995).

The slide was flooded with the Leishman's stain. After two minutes, double the volume of buffered water was added for 10 minutes. Then washed in a stream of buffered water and back of the slide was wiped and set it upright to dry. For differential white blood cells count , blood smears were made from bloods of both hydatid cyst infected human and sheeps . The smears were stained with Leishman's stain and 200 leukocytes were counted from each smear. These were differentiated into neutrophils ,eosinophils, basophils, lymphocytes and monocytes and the percentage of each was calculated using oil lens microscope (Cheesbrough,2006).

4-Statistical analysis:

The obtained results were analyzed statistically as percentages ; means ; standard deviation and t- test was done to compare between each two groups. The difference at $p<0.05$, $p<0.01$ was regarded significant (Walker and Shostak , 2010).

Results:

Table-1: Estimation of mean total and differential white blood cells count in 45 human confirmed with hydatid cyst infection.

studied groups	Number	TWBCs (×10)3 (×±SD)	DWBCs (×10)3 (×±SD)				
			N	L	M	E	B
H C patients	45	* 7.58±2.91	* 4.932±2.31	* 2.138±0.13	ns 0.294±0.14	* 0.203±0.08	ns 0.013± 0.024
Control	15	5.979±0.69	3.513±1.1	2.066±0.082	0.22±0.10	0.15±0.011	0.03±0.045

ns=non significant *(p<0.05)

Table-2: Estimation of mean total and differential white blood cells count in 15 sheep confirmed with hydatid cyst infection.

studied groups	Number	TWBCs (×10)3 x±SD	DWBCs(×10)3 x±SD				
			N	L	M	E	B
HC sheep	15	** 9.15±0.37	* 2.37±0.55	** 5.9±0.30	ns 0.27±0.028	* 0.5±0.14	ns 0.11±0.192
Control	5	7.2±0.62	2.01±0.1	4.52±0.93	0.28±0.01	0.33±0.2	0.06±0.13

ns= non significant * (p<0.05) **(p<0.01)

Discussion:

Hematological study:

1- Estimation of total white blood cells counts(TWBCs).

The TWBCs (as a hematological parameter) was estimated in two different intermediate hosts (human being and sheep) to the parasite *Echinococcus granulosus* , and so in their control groups for comparison .

In infected human , the study results showed that the mean of total white blood cells was higher and differed significantly from that in control group 7.58±2.91 and 5.979±0.69 , respectively. This result is in agreement with that recorded by other studies (Das *et al*., 1995 ; Raghavachar *et al.*, 1997 ; Oehler *et al.*, 1998 ;Al-Omoran, 2000 ; Rigano *et al.*, 2001) , and in disagreement with other studies too, (Del-Cacho *et al*.,1996 ; Horton, 1997).

The explanation for such obtained results (increased TWBCs) might be due to increased levels of inflammatory cells during the time of acute infection

as a defense mechanism, against growing parasite, that represented by increased leukocytes mainly of eosinophilis, lymphocytes and macrophages with oncosphere, necrosis of surrounding cells is followed by infiltration of neutrophils and macrophages after infection in the intermediate hosts (Michael *et al*.1985 ; Lioyd,1987 ; Waller,1992 ; Kamhawi *et al*.,1995 ; Zarzosa *et al*.,1999 ; Zhang and McManus,2003).

In the second situation (decrease TWBCs) , the explanation might be due to patients administered medications that can bring about damping with attenuating the severity of infection and then causing low level of host elevated inflammatory cells (Das *et al*.,1995 ; Laurence *et al*.,1997 ; Al-Ghariry,2000 ; Mori *et al*.,2000).

In addition , it was shown in this study that although there were statistical significant differences in both studied hematological parameters(TWBCs and DWBCs) between both studied groups of human "infected against control"(7.58±2.91 : 5.979±0.69) and sheep "infected against control" (9.15±0.37 : 7.2 ±0.62) too, but their values were within normal levels (Coles, 1986 ; Tizard, 1987 ; Dacie and Lewis ,1995 ; Jawasreh *et al*., 2010).

2-Estimation of differential white blood cells counts(DWBCs).

The DWBCs did show obvious significant differences between both studied groups when be compared with their control groups .

In human, significant difference was shown in neutrophil cells(4.932±2.31 : 3.513±1.1) , lymphocytes(2.138±0.13 : 2.066±0.082) , eosinophil cells (0.203±0.08 : 0.15±0.011) , but it was not shown in monocytes(0.294±0.14 : 0.22±0.10)) and basophils(0.013±0.024 : 0.03±0.045) , too. Many studies were reported similar results(Waller,1992; Allen and Maizels, 1996 ; Zarzosa *et al*.,1999 ; Sreter *et al*.,2003).

In infected sheep, the present study was showm significance difference in DWBCs between studied groups , especially in the lymphocytes (5.9±0.30 : 4.52±0.93) , neutrophils(2.37±0.55 : 2.01±0.1) and eosinophils(0.5±0.14 : 0.33±0.2) , respectively. In addition, it was shown no significant difference in monocytes(0.27±0.028 : 0.28±0.01) and in basophils(0.11±0.19 : 0.06±0.13) , too. Several studies noted similar results (Dessaint,1992 ; Waller,1992 ; Bell,1996 ; Zarzosa *et al*.,1999) , and other studies were not (Yarsan *et al*.,1999 ; Craig, 1997; Gottstein, 2000) .

The interpretation for such obtained results of different DWBCs might be due to several reasons such as: The eosinophilic cells are so sensitive to parasite- antigens than other leukocytes within the same infected individual, many studies were issued the fact for this document (Rodak *et al*., 1995 ; Roitt *et al*., 1998 ; Oehler *et al*.,1998 ; Wardlaw and Kay, 2001) .

Other reason which mediated by providing of appropriate antigens from infected tissues with stimulate production of immunoglobulin epsilon(IgE) which in turn stimulate production of eosinophils then to be attracted in peripheral blood lately (Mclaren and Boros, 1983 ; Yarsan *et al.*, 1999 ; Wardlaw and Kay, 2001).

Furthermore, not all types of white blood cells with the same sense against parasitic infection and each cell with its own function in the circulatory system (Dacie and Lewis ,1995 ; Yarsan *et al.*, 1999 ; Gottstein, 2000 ; Meeusen and Balic**,** 2000. ; Jawasreh *et al*., 2010).

Conclusions:

1- *E. granulosus* parasite is considered as main and major pathogenic parasite in causing hydatid cyst disease.
2- Hydatid cyst disease can bring about detectable influences in the infested organs and tissues(as in blood tissue).
3- Hydatidosis can elicit significant difference in both counts of TWBCs and DWBCs in human being and in sheep .
4- Hydatidosis can **not** elicit significant difference in DWBCs(in relation to monocytes and basophils percentages only) in human being and in sheep .

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