

# Hydatid Cyst in Children and Youth : Patients characters with parasitological, and biochemical Study of hydatid fluid.

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## Abstract

A descriptive, parasitological, and biochemical study done on three patients with hydatid disease aged 9, 11 and 13 years attending Tikrit Teaching Hospital for the period from first of January to the 20th of September 2007. Two of the study cases undergo surgery and the sample of hydatid cyst fluid were examined parasitologically and biochemically. All the parameters above were compared with that of the adults cattle and sheeps with hydatid disease. Two are females and one male. All the patient from the urban area. Two patients have cough and shoerness of breath as the main complaint while the other pateint have no complaint. Two patients have both hepatic and lung cysts and the other one has single hepatic cyst. All the hepatic cysts were in the right lobe. There is no relation between the age of the patient and the size of hydatid cyst. The highest infectivity was in the hydatid fluid from the sheep (90%) while the lowest was from the cattle (20%). Infectivity in human both children and adults was 30%. The results of biochemical parameters of cyst fluids from various hosts shows that he quantity of uric acid was found to be significantly more in human cyst fluids compared with those from other species. The level of proteins, glucose, triglyceride and creatinin are lower in cysts of human (children and adults) compared to that of sheeps and cattle.

## Introduction:

its shape and size depends on the organ in which it grows(7), the wall differentiated into outer friable, laminated , non nucleated layer and is 1mm thick and an inner germinal layer is nucleated germinative layer of cells supported by a cellular membrane. Various daughter cyst ( brood capsules) bud off from the inner germinal layers and main remains attached or flood free in the interior of the fluid filled cyst. Daughter cysts may grow through the wall of the mother cyst, particularly in bone disease(8,9).

The brood capsules are between 250μ-500 μ and develop internally from the germinative layer and produce multiple protoscolices by asexual budding. Some brood capsules will be free inside the cyst fluid with their scolices and because of their appearance, they are called (Hydatid sand) (7,8).

The size of cyst in the human body is highly variable and usually ranges between 1-15cm, but much larger cysts 20cm in diameter may also occur. The exact time required for the development of protoscolices within cysts in the human host is not known, but it thought it to be more than 10 months post infection. Protoscolices can already be formed in the cysts of 5 to 20 mm in diameter(6).

Hydatid cyst is slowly enlarged to produce pressure symptoms cyst grow to 45 mm in about 3 months(9).

## Hydatid Fluid:

Cyst fluid is clear or pale yellow, has a neutral pH(7 to 7.2) and contains sodium chloride, proteins, glucose, ions, lipids, and polysaccharides. The fluid is antigenic and may also contain scolices and hooklets. Also it contains albumin, creatinine, lecithin, urea. The chief enzymes are diastatic, but there are also proteolyses enzymes, lipase, protease and oxidase enzymes(4).

The inorganic substances that present in the parasite (*Echinococcus granulosus*) are K, Na, Mg, Ca, Fe, Zn, Cu, Sr, P, SI, Co, and Mn, while that present in the cyst fluid are K, Na, Mg, Ca, Fe, Ci, S, P, Si. The reducing sugar present in the hydatid cyst fluid is variable, it is about .0-0.04%. The percentage of glycogen in E.

Unilocular hydatidosis is a disease caused by infection with the metacestode larval stage of the dog tape worm *Echinococcus granulosus*. This is recognized as one of the world relevant zoonoses, affecting both humans and their domestic animals <sup>(1)</sup>.

Hydatidosis is the most frequent cause of liver cyst in the world. The ingested ova burrow through the mesenteric vein. A few ova may pass the liver and get trapped in the lungs, synchronous pulmonary and hepatic hydatid disease may occur in 4% to 25% of cases. Hydatid cysts can be seen in other organs for example heart, pancreas, spleen, CNS and etc. hydatid diseases can invade the inferior vena cava. Hydatidosis is also the most frequent parasitic lung diseases. In a large study the most common symptoms was chest pain 79.1% and in 86% of patients the cysts were unilateral <sup>(2)</sup>.

Man becomes infected accidentally by ingestion the eggs of the parasite through hand, foods, drink or other materials contaminated with dogs faeces. The liver and lungs are the most common sites for cyst formation in humans, although any organ can be involved. Symptoms of presentation are varied and nonspecific <sup>(1, 2)</sup>.

The diagnosis of non-complicated Hydatid cyst depend on clinical suspicion supplemented by ultrasonography or computed tomography which are the most important diagnostic tools helpful in determining the complications and planning treatment <sup>(3)</sup>.

The clinical suspicion of the disease is most often arrived at when radiographic studies show space-occupying lesions of internal organs, bone, or brain<sup>(4)</sup>. Other tests that are helpful in the diagnosis of Haydatid disease include microscopic examination of the cyst demonstrating the presence of brood capsules containing multiple protoscolices or serologic test e.g. the indirect haemagglutination test <sup>(4,5)</sup>.

There are many studies of hydatidosis in adults, but only few articles regarding rate and pattern of involvement have been published <sup>(6)</sup>.

## The morphology of hydatid cyst:

The haydatid cyst is unilocular, subspherical in shape but

cysts from livers of eight infected sheeps from the slaughterhouse of the national for marketing of the meat. Adult human, sheep, and cattle samples are taken from the infected liver from which the infective percent were calculated and the results are compared with the infective percent of that of the children samples.

### **Biochemical study:**

Each sample of hydatid fluid is examined Biochemical for protein, glucose, uric acid, cholesterol, triglyceride and creatinin.

All these Biochemical parameters were compared with hydatid fluid from adults patients, sheep's, and cattle.

All cyst fluid were centrifuged at 15000rpm at 4C for 30 min and the supernant analyzed for various biochemical parameters.(4)

### **Biochemical parameters**

#### **1 Glucose Estimation: (7)-**

Glucose is determined by enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts under catalysis of Peroxidase, with phenol and 4-aminophenazone to form a violet quinoneimine dye as indicator.

#### **2 Serum Total Protein Estimation:- (8)**

Serum protein level was measured by performing serum total protein kit (CAM Tech Medical). Using bromocresol green method and spectrophotometer at a wave length 546. It was expressed as g/dl. Protein, in alkaline condition, forms with copper ions complex blue-violet.

#### **3- Triglycerides: [9]**

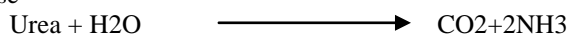
The triglycerides are determined after enzymatic hydrolysis with lipase. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of Peroxidase.

#### **4- Cholesterol [10].**

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine are formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and Peroxidase.

#### **5. Blood Urea(11)**

Enzymatic method was used for measuring urea in serum. Urea is hydrolyzed into ammonia and CO<sub>2</sub>. Ammonia reacts with salicylate and hypochlorite to form a green idophenol. The color intensity is proportional to the concentration of urea.



#### **6. Determination of Serum Creatinine(12)**

Creatinine reacts with picric acid in alkaline conditions to form a salt of yellow-red color; the rate of formation of color is proportional to creatinine concentration in the sample.

#### **7. Determination of Serum Uric Acid(13)**

Uric acid is oxidized by uricase to allantoin forming hydrogen peroxide. This latter, according to the action of peroxide, reacts with 4-aminoantipyrine and TOOS forming a red-colored compound which strength is proportional to the concentration of uric acid. <sup>(99)</sup>

### **Staistical analysis :**

granulosus larva is 2.8% of fresh weight, while it is 19% of dry weight in the liver(10).

The scolices of *Echinococcus granulosus* contain a polysaccharide fraction yielding on acid hydrolysis galactose and glucosamine, but no uronic acid. Similar compounds present in the hydatid cyst membrane which includes (in addition to galactose, galagtosamine) N-acetyl galagtosamine which are identified as hydrolysis product(4,5).

Regarding lipid composition, lipids constitute 2% of fresh tissue and 13.6 of dry tissue of *E. granulosus* (11). The types of lipid present in *E. granulosus* are cholesterol and fatty acids proteins which constitute 61% of *E. granulosus* larva dry tissue(12).

Regarding nucleic acids, the parasite contain the same basis as those of free living animal and therefore no unusual bases has been encountered(6).

In addition hydatid fluid contains the following water soluble vitamins, thiamine, nicotinic acid, and ascorbic acid(4,6).

### **Aim and objectives:**

To describe the character of hydatid diseases in children with identification of parasitological features and some biochemical parameters of the hydatid fluid sample in children and youth and compare it with samples from human adults, and some other animals .

### **Materials and Methods:**

A descriptive, parasitological and biochemical study done on three children with hydatid disease attending Tikrit teaching hospital for the period from first of January to the 20<sup>th</sup> of September 2007.

### **Patient character:**

A descriptive study is done on 3 patients with hydatid diseases aged 9, 11, and 13 years. Each patient was assessed by a prepared questionnaire which includes name, age, sex, residence, symptom of complaint. Physical examination is done looking for general examination, chest and abdominal examination.

The diagnosis of hydatid cyst is based on characteristic picture of hydatid cyst on abdominal ultrasound examination and/or chest X-ray.

Each cyst was examined for site (lungs, liver, or both) and size (by cm) and whether single or multiple.

Two of the study cases undergo surgery (the 9 and the 11 years age child) and the sample of hydatid cyst fluid were examined parasitologically and biochemically .

All the parameters above were compared with that of three adults with hydatid disease.

### **Parasitological study:**

Parasitological study was done on hydatid fluid samples taken from two patient undergo surgery looking for the presence of protoscolices and measuring the infective percentages (number of the live protoscolices over the number of the died scolices in the field multiplied by 100). This technique is achieved by put small drop of hydatid fluid on slide and a drop of the eosin stain where the dead protoscolices take the pink color and the live one stays without color but recognized by the flame movement. The mean of each results are calculated.(4)

For comparative study , four hydatid cysts were obtained from livers of four human patients from surgical theatre of the Tikrit teaching hospital in Tikrit city, and eight

were one. The size of cysts were variable. All the adults have both hepatic and lung cysts. The mean number were 2 in the liver and one in the lung. Regarding the mean size in the liver were 15.7 cm and in the lungs were 25.2 cm.

**Table(2)** shows the relation between the age of the patient and the size of the cyst. It is clearly shown that there is no relation between the age of the patient and the size of hydatid cyst.

#### Parasitological study:

**Table(3)** shows the infectivity percent of the protoscolices among different samples from human, Sheeps and cattle. It is clearly shown that the highest infective was in the hydatid fluid from the sheeps (90%) while the lowest was from the cattle (20%). Infectivity in human both children and adults was 30%.

#### Biochemical study:

The results of biochemical parameters of cyst fluids from the various hosts are shown in table (4). The quantity of uric acid was found to be significantly higher ( $P < 0.05$ ) in human cyst fluids compared with those from other species.

The results are put in tables and the statistical analysis were done using the t-test. P value less than 0.05 considered to be significant.

#### Results

A descriptive, parasitological and biochemical study on three children with hydatid disease attending Tikrit teaching hospital for the period from first of January to the 20<sup>th</sup> of September 2007.

#### Patients characters:

Three children with hydatid disease have been included in this study aged 9, 11, and 13 years 9 (mean age 11 years), two are females (66.6%) and one male (33.3%). All the patient from the urban area.

Regarding symptom of presentation, two presented with cough and shortness of breath while the other was asymptomatic. All the patients have normal physical examination.

Regarding the three adults studied, all of them were males, they are from urban areas, and all were asymptomatics.

**Table(1)** shows that two patient have both hepatic and lung cysts and the other one has single hepatic cyst. All the hepatic cysts were in the right lobe. Regarding the cysts, the mean number in both hepatic and lung cyst

**Table(1): The site and the size of the hydatid cyst among the study groups.**

	Adults (means)		First patient		Second patient		Third patient
	Liver	lungs	liver	lung	Liver	lung	Liver
Number	2	1	1	1	1	1	1
Size(cm)	15.7	25.2	6	9	11	22	6
site	Right lobe		Right lobe		Right lobe		Right lobe

**Table(2): The relation between the size of the cyst and the age of the child.**

Age(years)	Size(cm)	
	Liver	Lungs
9	6	9
11	11	22
13	6	-

**Table(3): The results of infective percent of hydatid cyst in all the taken.**

Infectivity percentage	Children	sheep	cattle	Human adults
	30%	90%	20%	30%

**Table(4): Comparison of some of the biochemical parameters of different hydatid fluid samples.**

Biochemical profile	Children	sheep	cattle	Human adults
Protein	0.049±0.05	0.112±0.05	0.36±0.05*	0.058±0.05
Glucose	1.21±0.2	2.83±1.67*	1.33±0.4	1.31±0.3
Uric acid	0.17±0.1*	0.02±0.01	0.017±0.0078	0.15±0.1
Cholesterol	0.14±0.05	0.14±0.05	0.1±0.07	0.13±0.03
Triglycerides	0.06±0.03	0.078±0.03	0.14±0.07*	0.062±0.03
Creatinin	45±13	47±11	58.08 ±31*	46±12

\* $P < 0.05$  (significant)

Despite equal distribution of Hydatid disease in both sexes in the adults(13), this study shows that most cases were females. This does not go also with other study by Abdel-Hafez *et al.*(2) which shows that it is more common in males. This may be due to difference in the

#### Discussion:

Hydatid disease is generally caused by the larval stage of infestation of the dog tapeworm, *Echinococcus granulosus* (4).

#### Patient character:

parasite) from the sheep strain, then the cattle strain while the infectivity of the human strain is very low so the infection from the human is very rare. Also the human bodies are buried after death so the definitive host don't reach the fluid of the cyst (dead end of the life cycle). This goes with study done in Libya(12), and other study done in Iran (10) which show that the difference in morphology and development of the *E. granulosus* in different intermediate hosts reflected the difference in the infectivity percentage.

### **Biochemical study:**

Biochemical substances within hydatid cysts play a definitive role in the metabolism, physiology and immunology of cystic Echinococcosis(10). The quantitative differences in the metabolism of *E. granulosus* and variation in the biochemical composition of hydatid fluids reflect strain variation in different intermediate hosts. Moreover, the development of the same strain or species of *Echinococcus* in different species of intermediate hosts may also cause shifts in the metabolism essential for parasite survival in different environments (16).

Clearly, biochemical analysis can provide much valuable information on the identification of strains of *E. granulosus* from different host origins which may relate to their possible infectivity to man (15).

In the present study, we compared biochemical parameters of hydatid cyst fluids in the natural intermediate hosts (sheep, cattle) and humans, which may assist in the identification and characterization of the strain of *E. granulosus* prevailing in Iraq.

The level of glucose in sheeps and creatinine in cattle were found to be significantly higher in the cyst fluids than that of humans indicating that the level of these parameters are not influenced by the former hosts. In support of this, Sheriff *et al.*(11) have demonstrated no marked quantitative differences in protein, total lipid, phospholipids, cholesterol and glycerides in hydatid cyst fluids collected from cysts obtained from the lungs and liver of sheep and humans. However, Sheriff *et al.* (11) found no significant difference between biochemical parameters of hydatid fluids from camels and other intermediate hosts.

The concentration of uric acid was found to be significantly higher in human hydatid fluids compared with other cyst fluids.

Sheriff *et al.*(11) have demonstrated higher concentration in uric acid in human hydatid fluids compared with other animal cyst fluids. This increased

of uric acid in human hydatid fluids may be due to a normally high uric acid level in humans compared with domestic animals and/or may also indicate degeneration changes in human hydatid cysts (10).

### **Conclusion :**

Hydatid disease is an important disease in children as well as in adults. Most of the affected children have both hepatic and lung cysts. The infectivity percent of protoscolices in children are less than that of sheeps but is similar to that of adults. Uric acid level in human hydatid fluid ( adults and children) was higher than that of other species while the other biochemical parameters were low compared with sheep and cattle samples

sample size among each study group.

Contrary to what is expected(14), all the study cases were from the urban areas. This does not go with other studies done in children and the adults which shows the Hydatid disease is more prevalent in the rural areas. This should throw a light on the proper sanitation and food handling at the urban areas and may be due to the increasing use of indoor dogs as part of security process. This is also should be warning for the health organization to imply a firm policies to stop the cycle of the parasite in the cities.

Two patient presented with chest pain and shortness of breath. This is goes with other study by Anadol *et al*, who states that hydatid disease of lungs may present as asthma like symptoms(6).

This is does not go with other study on adults which shows that the most presenting symptoms in the adults with Hydatid disease was chest pain (57%)(3), it is also shown that one patient was asymptomatic in spite of having cyst diameter of 6cm. This does not go with the fact that Hydatid disease is asymptomatic when it is 5cm and above(3).

It is clearly shown that combined lung and liver hydatid cyst is more frequent than solitary hepatic cyst. This goes with Abdel-Hafez(2) study who shows the same results that combined lung and liver cyst is more frequent among children(16%) than adults (4%) .

These results does not go with adults studied(3) which shows that liver was the most frequent organ of involvement(70%) than combined lung and liver cysts (5%) or single lung cyst (25%).

It seems that in children scolices more ability to pass from the liver barrier than adults. Since multiple organs involvement is more common in children. Once a cyst is detected in one organ, one has to search and rule out other organs for involvement before surgery.

Lung cyst were clearly shown to be larger than the hepatic cysts. This goes with other studies(15) which shows the same results. This may be due to the less visceral density in the lungs than in the liver so that cyst may become larger within the same period of time.

It is clearly shown the study shows that the mean number of hydatid cyst in adults was more than that of children. This is due to the fact that adults are more prone to expose to the parasite and for a longer period of time so that they have larger number than children. The mean size of hydatid cysts in adults is larger than that of children. This due to the fact that hydatid cyst is growing about 1cm/year so that as the patients go older they have larger size of cysts (3) .

This study also shows that there is no correlation between the age of the patient and the size of the cyst. This is also contradicted with the theory that hydatid cyst is grow 1 cm per year (3).

### **Parasitological study:**

It is clearly that infectivity percent of the protoscolices in human whether children or adults are less than that of sheeps.

The infectivity percentage is important parameter for identification the level of the infectivity of the strain or species, in present study the infectivity of the strain of *E. granulosus* in the cattle so the main cause of infection to the definitive hosts (to complete the life cycle of the

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## مرض الأكياس المائية لدى ثلاثة مرضى من الأطفال و اليافعين : دراسة وصفية للمرضى مع دراسة طفيلية و كيميائية لسائل الأكياس المائية.

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( تاريخ الاستلام: ٢٨ / ١ / ٢٠٠٨ ، تاريخ القبول: ١٥ / ١٢ / ٢٠٠٨ )

### الملخص

دراسة وصفية ،طفيلية و كيميائية أجريت على ثلاث مرضى مصابين بمرض الأكياس المائية (أعمارهم ٩ ، ١١ و ١٣ سنة) المراجعين لمستشفى تكريت التعليمي خلال الفترة من الأول من كانون الثاني إلى العشرون من أيلول ٢٠٠٧. اثنان من المرضى تم إجراء عملية جراحية لهم وتم اخذ النموذج من سائل الأكياس المائية وتم فحصه طفيليا وكيميائيا. جميع المقاييس التي درست تم مقارنتها مع نماذج من سائل الأكياس المائية المأخوذ من المرضى البالغين والخراف والثيران. اثنان من المرضى كانوا إناث والأخر كان ذكرا. جميع المرضى كانوا من المدينة. اثنان من المرض كانت الشكوى الرئيسية لهم هي ضيق النفس مع سعال وأما الآخر فلم يعاني من أعراض مرضية. اثنان من المرضى كانوا مصابين بأكياس الكبد والرئة وأما الآخر كان مصابا بأكياس الكبد فقط. جميع مرضى أكياس الكبد كانت الإصابة في الفص الأيمن. لقد لوحظ من الدراسة عدم وجود علاقة بين حجم الأكياس المائية وعمر المريض. لوحظ إن نسبة فعالية الإصابة كانت أكثر في سائل الأكياس المائية المأخوذ من الخراف (٩٠%) والأقل كان من السائل المأخوذ من الثيران (٢٠%). نسبة فعالية الإصابة في الإنسان (الأطفال والكبار) كانت ٣٠%. لوحظ في الدراسة زيادة في نسبة الحامض البولي في سائل الأكياس المائية من الإنسان مقارنة مع النماذج الأخرى. أما بالنسبة لنسبة البروتين، الكلوكوز ، الكولسترول، الشحوم الثلاثية والكرياتين كانت اقل في نماذج الإنسان مقارنة مع النماذج الأخرى.