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Comparison of Biochemical Compounds of Fertile and Infertile Hydatid Cyst Fluid of Animaland Human Origin

Original article

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Abstract

Background and objectives: Hydatidosis is an important zoonotic disease with widespread distribution. For unknown reasons, some cysts are unable to produce protoscoleces, and little is known about the mechanisms involved in infertile cyst production. Therefore, characterization of hydatid cyst fluid (HCF) components could help clarify the host-parasite relationship and the fertility process of cyst. The aim of this study was to identify and quantify biochemical components of HCF of fertile and infertile cysts from different hosts.

Methods: A total of 35 HCF samples were obtained from the liver and lung of 16 cattle, 16 sheep and three humans. Fertility of cysts was determined by examination of cysts' content. Then, total fluid was aspirated aseptically from each cyst. The samples were centrifuged at 10000×g for 15 min at 4°C, and then concentrated and dialyzed against phosphate buffer saline using an Amicon Ultra-15 5000 MWCO centrifugal filter device (Millipore, USA). All biochemical components were quantified by an automatic analyzer.

Results: The value of lactate dehydrogenase, potassium, calcium, cholesterol, glucose, urea and uric acid differed significantly in cysts collected from different hosts ($P<0.001$). There was also a significant difference in the amount of lactate dehydrogenase, aspartate aminotransferase and cholesterol between fertile and infertile cysts ($P<0.039$).

Conclusion: Due to the differences in biochemical composition of HCF of different host origin, it is possible that the host plays a crucial role in determining the type of biochemistry in hydatid cyst as well as in hydatid cyst fertility.

Keywords: Echinococcus granulosus, fertile and infertile hydatid cyst, biochemical compounds.

INTRODUCTION

Echinococcus granulosus causes one of the most serious tapeworm larval infections in men. It is called hydatid worm because it forms hydatid cysts filled by water-like fluid in various organs, especially in the liver, lung, spleen, kidney, brain or even bone (1). The cyst is filled with hydatid cyst fluid (HCF), a complex mixture of host serum components and parasite antigens (2). The fluid provides nutrients that are necessary for larval development and plays an important role in the life cycle of *Echinococcus* (3). Although the mechanisms involved in the development of fertile cysts have not yet been elucidated, increased apoptosis and presence of proteins in the protoscolex might reflect differences between fertile and cysts (4). Important biochemical constituents of HCF include amino acids (glycine, leucine, methionine, tyrosine, histidine, valine, arginine, glutamine, serine and proline), organic elements (calcium, potassium, magnesium, copper, cadmium, zinc, selenium and sodium), biochemical substances (urea, creatinine, uric acid, cholesterol, triglyceride, glucose) and enzymes (glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase, alkaline phosphatase, transglutaminase, lactic dehydrogenase, creatine kinase isoenzyme of creatinekinase) (5, 6). From the abovementioned components, some elements such as Na, Zn, and Se as well as some amino acids such as glycine, arginine, glutamine and serine are thought to trigger hydatid cyst fertility. On the other hand, enzymes such as glutamic-pyruvic transamianse, transglutaminase and lactic dehydrogenase are thought to be involved in hydatid cyst infertility (7).

Host immunoglobulin G (IgG) molecules have been found in hydatid cysts and as many as 2 million protoscoleces may be present in a large cyst that may contain 2 liters of fluid. From the inner layer of cyst, cell masses are budded out into the cystic cavity that become vacuolated and later stalked. These vacuolated buds are named brood capsules. From the inner wall of these capsules, the protoscoleces develop and invaginate as they become fully mature. Some cysts are sterile and never produce brood capsules; in other cysts the brood capsules never produce protoscoleces; hence they are called acephalocysts (7).These sterile cysts are important epidemiologically because they could not infect intermediated hosts and has no role in secondary disseminated hydatidosis. This characteristic is very important for surgeons because the operation will be without risk of protoscoleces spillage and secondary cysts formation (8).

Finding biochemical compounds and elements in the HCF that are involved in cyst fertility or infertility can be useful for controlling the parasite by interrupting the transmission cycle and limiting surgery complications. Because HCF exchanges substances with the host for the survival and reproduction of protoscoleces, an understanding of the larval environment will aid in identifying the essential components for parasite growth and production of protoscoleces (fertility). Therefore, the main objective of this study was to identify the biochemical components of HCF and probable difference of these components between fertile and infertile cysts.

MATERIALS AND METHODS

The study protocol was approved by the Research Council of Hamadan University of Medical Sciences (Project code: 940728405). A total of 32 liver and lung samples (16 cattle, 16 sheep and 3 human samples) infected with apparently healthy, non-calcified hydatid cysts of different sizes, were collected from the abattoir of Hamadan (Iran) and transported to the Parasitology Research Laboratory of Hamadan University of Medical Sciences. The cysts were transported to a germ-free environment. The cysts' surface was washed with phosphate buffer saline (PBS) and sterilized with alcohol. Then, contents of the cysts were collected into a tube using a 10-cc sterile syringe. A piece of the germinal layer and one drop of precipitant of HCF were examined under a light microscope for the presence or absence of protoscoleces and fertility determination .

Human hydatid cysts were obtained from operating rooms of hospitals affiliated to the university. After determining cysts fertility status, total hydatid fluid of each cyst was aspirated and poured into a tube. The fluid was centrifuged at 10000×g for 15 min at 4°C, and concentrated and dialyzed against PBS using an Amicon Ultra-15 5000 MWCO centrifugal filter device (Millipore, USA).The resulting supernatant was stored at -20 until biochemical analysis. Biochemical components were quantified by an automatic biochemistry

analyzer apparatus (Hitachi, Japan) and commercial kits (Man Co., Iran). Finally, the components of fertile and infertile cysts of different origin were compared.

Data were presented as mean ± standard deviation (SD).

All analyses were carried out in SPSS 16 statistical software (Chicago, IL, USA) and at statistical significance of 0.05.

RESULTS

The mean level of components and elements present in the hydatid cysts are presented in table 1.

Table 1- Mean level of biochemical indices and mineral elements in hydatid fluid of the studied cysts

Component/element	Mean ± SD	Measurement unit	Min.	Max.
Lactate dehydrogenase	92.7±2.49	IU/L	0	482
Alkaline phosphatase	10±22.35	IU/L	4	32
Aspartate aminotransferase	4.15±9.68	mg/dl	0	46
Sodium	136.18±342.2	mEq/l	95.6	169.1
Potassium	9.08±9.36	mEq/l	3.52	14.66
Calcium	11.48±31.88	mg/dl	0.7	27.70
Triglyceride	18.48±159.27	mg/dl	5	73
Cholesterol	4.62±4.41	mg/dl	1	9
Glucose	30.65±67.29	mg/dl	6	120
Uric acid	1.16±0.252	mg/dl	1	3.5
Creatinine	0.34±0.018	mg/dl	0.1	0.7
Albumin	0.14±0.37	g/dl	0.1	1.2
Total protein	0.754±6.96	g/dl	0.1	15.90
Urea	12.40±44.18	mg/dl	1	28

Table 2-Mean level of biochemical elements and components in small and large HCF

Component/element	Small cysts	Large cysts	Measurement unit	P-value
Lactate dehydrogenase	96.76	96.81	IU/l	0.99
Alkaline phosphatase	8.70	11.70	IU/l	0.66
Aspartate aminotransferase	5.88	2.31	mg/dl	0.30
Sodium	134.37	139.06	mEq/l	0.46
Potassium	9.40	8.95	mEq/l	0.67
Calcium	11.66	11.10	mg/dl	0.77
Triglyceride	20.11	17.47	mg/dl	0.55
Cholesterol	4.94	4.05	mg/dl	0.20
Glucose	26.52	33.88	mg/dl	0.42
Urea	12	12.94	mg/dl	0.69
Uric acid	1.06	1.24	mg/dl	0.30
Creatinine	0.31	0.37	mg/dl	0.26
Albumin	0.10	0.18	g/dl	0.22
Total protein	1.13	0.34	g/dl	0.38

Table 2 compares the amount of biochemical components and various elements in lung and liver HCF.

Based on the results, the mean level of lactate dehydrogenase, potassium, calcium, cholesterol, glucose, urea, uric acid and LDH differed significantly among difference hosts. However, there was no significant difference in the mean level of other parameters between different hosts (Table 3).

There was no significant difference in the studied parameters between lung and liver cysts (Table 4).

Table 3- Mean level of biochemical elements and components of HCF in different hosts

Component/element	Sheep	Cattle	Human	P-value
Lactate dehydrogenase	0	50	200	0.01
Alkaline phosphatase	10.2	5	15.3	0.14
Aspartate aminotransferase	2.80	5.3	3.75	0.77
Sodium	134	139	130	0.62
Potassium	7	4.7	11.5	0.000
Calcium	14.2	9	7.8	0.019
Triglyceride	15	22.5	7.8	0.08
Cholesterol	3.30	5.51	6	0.001
Glucose	40.8	14	23	0.001
Uric acid	1	1.25	2.25	0.000
Creatinine	0.386	0.288	0.360	0.67
Albumin	0.168	0.100	0.200	0.52
Total protein	1.253	0.25	0.38	0.56
Urea	16.8	8	12	0.000

Table 4- Mean level of biochemical elements and components of liver and lung HCF

Component/element	Liver	Lung	Measurement unit	P-value
Lactate dehydrogenase	96.76	96.81	IU/l	0.99
Alkaline phosphatase	8.70	11.70	IU/l	0.66
Aspartate aminotransferase	5.88	2.31	mg/dl	0.30
Sodium	134.37	139.06	mEq/l	0.46
Potassium	9.40	8.95	mEq/l	0.67
Calcium	11.66	11.10	mg/dl	0.77
Triglyceride	20.11	17.47	mg/dl	0.55
Cholesterol	4.94	4.05	mg/dl	0.20
Glucose	26.52	33.88	m/dl	0.42
Urea	12	12.94	mg/dl	0.69
Uric acid	1.06	1.24	mg/dl	0.30
Creatinine	0.31	0.37	mg/dl	0.26
Albumin	0.10	0.18	g/dl	0.22
Total protein	1.13	0.34	g/dl	0.38

DISCUSSION

The present study showed that the the amounts of some biochemical compounds and mineral elements differed significantly in different hosts but not in fertile and infertile cysts. The level of lactate dehydrogenase and aspartate aminotransferase only was significantly higher in infertile cysts, while cholesterol level was significantly higher in infertile cysts. There was no significant difference between the fertile and infertile cysts for other elements and compounds. Our results suggest that the host type plays a more significant role in determine the composition of HFC compared the fertility status of the cyst. In fact, cyst fertility might be determined by genetics and has nothing to do with environmental factors.

Given that intact human HCF samples are hard to collect, we could obtain only three intact and proper cysts from operating rooms. Therefore, comparing the HCF components between human and animal cysts may not produce reliable findings.

In recent decades, much attention has been given to the biological role of hydatid fluid constituents and the parasite-host interactions (9, 10). Limited studies have been performed on the amount of these substances in fertile and sterile cysts. The aim of the present study was to determine the amount of these substances and compounds in fertile and sterile cysts in order to determine the cause of hydatid cyst infertility.

Minerals and organic materials play an important role in the metabolism, physiology and immunogenicity of hydatid cysts (11, 12). These compounds also regulate function of the membrane enzyme gamma glutamyl transpeptidase, which plays a key role in the transport of amino acids and peptides from the cell membrane (13). In the study of Sharif et al., there was a significant difference between the amount of potassium and calcium in different cysts, whereas the amount of sodium in different host cysts was not statistically significant (7).

The level of organic compounds such as triglyceride, albumin, cholesterol, uric acid, glucose, creatinine and total protein have also been reported to be variable in cysts depending on fertility status and host type (13).

Albumin levels in sheep cysts were higher than in other hosts, and cholesterol levels were different in different hosts. It seems that the amount of cholesterol in fertile and sterile cysts is significantly different and it is more in fertile cysts than sterile cysts. It may be involved in the metabolism of protoscoleces or their development (14). The amount of uric acid in human cysts is reported to be higher than in other hosts, which may be due to the total elevation of this substance in humans or degenerative changes in the cysts. The lowest amount of uric acid was found in sheep cysts, which are mostly fertile. However, we found no significant difference in the uric acid content of fertile and sterile cysts.

Several studies have been performed to identify and quantify the enzymes present in hydatid fluid (9, 16). The amount of lactate dehydrogenase in fertile hepatic cysts of sheep is lower than in pulmonary cysts and sterile cysts. In the present study, the amount of this enzyme was higher in cyst samples collected from cattle compared to samples collected from other hosts. This finding is in line with previous findings which suggested a relationship between the amount of lactate dehydrogenase and cyst infertility (17).

In fact, higher lactate dehydrogenase levels may prevent fertilization. However, ALP levels were higher in hepatic and fertile cysts than in pulmonary and infertile cysts. This enzyme appears to be physiologically relevant to parasite metabolism and survival. Similar to lactate dehydrogenase, aspartate aminotransferase level has been reported to be higher in infertile cysts. In the present study, the level of aspartate aminotransferase did not differ significantly in samples collected from different hosts; however, aspartate aminotransferase level was significantly higher in sterile cysts than in fertile cysts. There was no significant difference in the amount of creatinine, total protein and albumin in different hosts and cysts. This might suggest that these organic compounds do not play a major role in the biology and physiology of hydatid cysts (18-20).

CONCLUSION

Our findings indicate that the determining the composition of HCF alone cannot determinant of hydatid cysts fertility.

Performing genetic studies is recommended to clarify the role of some intrinsic factors that may play a role in cyst fertility.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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