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The Concentrations of Amino Acids in Hepatic Hydatid Cyst Fluid in Random Samples

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Abstract: Hydatid disease is a common parasitic infection in Iraqi community.
Aim: To evaluate the concentration of amino acids in hydatid cyst fluid.
Materials and method: The study included twenty hepatic hydatid cyst from patients (15 female and 5 male), their ages ranged from 39 to 50 years, and inhabitants of the urban and rural areas. Liver hydatid cyst fluid drown by surgeon and used for microscopic and amino acids analyses by using automatic analyzer.
Results: The concentration of alanine, valine, glycine and threonine in fertile cyst was significantly higher than in sterile cyst studied . In contrast, the concentration of lysine was significantly higher in sterile cyst than in fertile cyst. Additionally, alpha amino butyric was sterile cyst and not in fertile cyst.
Conclusion: The study indicated a significant differences in amino acids concentration between fertile and sterile cyst and this could affect the natural history of the disease and outcomes.
Keywords : Protoscolices, amino acids, alanine, valine, glycine, threonine, hydatid cyst

Introduction

Hydatid disease is a parasitic infestation by a tapeworm of the genus *Echinococcus granulosus* with wide world distribution of the disease, the epidemiological situation of this parasite is complicated with endemic area which exhibit different degrees of infectivity to certain intermediate hosts [1]. The clinical signs and symptoms of the disease depend on the location of the cyst and were not specific [2]. The cyst of *Echinococcus* can undetected for many years due to the slow growing and development and the response of the host immune system [3-5].

Depending on the size and location, cysts can eventually exert pressure on nearby structure, producing abdominal discomfort and pain for example, cysts in the liver can compress bile ducts, causing obstruction that can be manifested as obstructive jaundice, abdominal pain, portal hypertension. While the cyst in the brain or spinal cord can provoke acute inflammatory responses and numerous neurological sequelae, including epilepsy and blindness [3,5].

Fluid of the cyst is an important component of the internal environment of the entire cyst, composed of many organic and nonorganic materials, which were essential for the larvae growth and protection from the host immune system [6]. The component of cyst content may

differ in various area and strains, and may include various amino acids, enzymes, proteins', lipids, vitamins and hydrocarbon [7].

Materials and methods

Study group

The retrospective study was conducted during the period from June 2018 to September 2018 on 40 subjects attending General Surgery Department, Baghdad Teaching Hospital at Medical City , Baghdad. The study included 20 hepatic hydatid cyst fluids which collected after cyst surgical removal from patients (10 female, 10 male) with ages ranged from 39 to 50 years, and resident in urban and rural areas. The demographic and clinical characteristics information's were gathered using preformed questionnaire form through direct patients interview.

Collection of hydatid cyst fluid

Ten ml of liver hydatid cyst fluid was drown by surgeon in operative room, separated by centrifuge at 3000 rpm for 3 minutes for microscopic examination (light microscope under 40 x) and amino acid analysis by using automatic analyzer.

Macroscopically examination

This procedure was performed as described before [8] and stained by eosin stain.

Amino acids analyses

1. Hydatid cyst fluid was diluted 1:9 using phosphate buffer saline.
2. The sample sucked by the sample needle
3. Measured sample from transfer capillary installed, which hold volume until injection.
4. The sample needle was lowered into the inject port and pushing the sample volume into sample loop without analysis.
5. After injection, the eluent segment fills completely the capillary between the injection port and sample loop.
6. The injection draws 20 microliters from diluents sample and after five minutes the results appear in digit form [6].

Statistical analysis

Statistical analysis was carried out using SPSS software (Version 20). Student t-test was used to determine the significance of differences in amino acids concentrations between fertile and sterile hydatid cysts. P value of <0.05 considered significant.

Results

The microscopic examination was indicated the presence of protoscolices microscopically under 40X after staining with eosin**, Fig.1.**

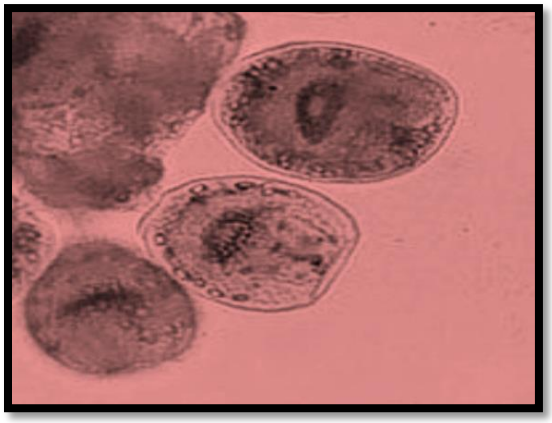

Fig.1. Protoscolices of hepatic hydatid cyst on 40X

The present study indicated a significant (P<0.005) higher concentrations of alanine in fertile cysts (825.67±41.51) as compared to sterile hydatid cysts (330.00±48.48). In contrast, lysine concentration was significantly (P< 0.02) lower in fertile cysts (139.83±12.76) than that in sterile hydatid cysts (198.00 ±15.56), Table.1. Additionally, valine concentration was significantly (P<0.005) higher in fertile hydatid cysts (447.83±27.49) as compared to sterile cysts (218.25±13.57). Also, threonine concentration mean value was significantly (P<0.005) higher in fertile cyst ((171.67±10.25) than in sterile (58.25± 1.38) hydatid cyst, Table.1.

While glycine hydatid cyst fluid concentration mean value was with higher significance difference (P<0.001) than that demonstrated for alanine, valine and threonine. Glycine concentration was (574.83± 35.45) in fertile cyst, while it was (323.50± 24.96) in sterile cyst, Table.1. Alpha amino butyric acid was not detected in fertile cyst, while its concentration was (21.67± 3.47) in sterile hydatid cysts.

Other amino acids, such as arginine, serine, glutamic acid, cysteine, methionine, tryptophan, leucine, glutamine, asparagine and aspartic acid were with higher mean levels in sterile cyst that in fertile cyst, however, the differences not reach significant level. In contrast, tyrosine and histidine were with lower mean concentrations in sterile as compared to fertile, but the difference was not significant. The non significant differences could be attributed to sample size.

Table.1. Amino acids concentrations in fertile and sterile liver hydatid cyst fluid.

Amino acid type	Cyst				P Value
	Fertile		Sterile		
	Mean	SE	Mean	SE	
Alanine	825.67	41.51	330.00	48.48	0.005
Valine	447.83	27.49	218.25	13.57	0.005
Glycine	574.83	35.45	323.50	24.96	0.001
Argenine	66.00	9.53	69.75	13.63	0.821
Serine	87.83	10.76	91.00	11.14	0.849
Glutamic acid	52.33	15.25	58.00	15.16	0.808
Threonine	171.67	10.25	58.25	1.38	0.005
Cysteine	35.00	4.56	38.75	5.31	0.611
Methionine	20.00	2.77	22.75	2.81	0.523
Tyrosine	53.33	7.72	44.25	2.78	0.386
Alpha amino butyric acid	0.00	0.00	21.67	3.47	0.001
Tryptophan	37.17	3.07	41.75	3.90	0.379
Leucine	91.33	3.60	111.00	9.21	0.119
Lysine	139.83	12.76	198.00	15.56	0.020
Histidine	77.33	7.45	71.00	6.67	0.570
Glutamine	465.50	27.21	473.75	39.49	0.836
Asparagine	51.83	3.64	54.50	4.29	0.651
Aspartic acid	0.19	0.13	0.25	0.12	0.742

Discussion

The Echinococcosis is one of the major zoonotic parasitic diseases in the Middle East and Arabic North Africa from Morocco to Egypt [9]. Morphology of the protoscolices cyst were detected in the present study in fertile hepatic hydatid at 40X, this observation were

agree with the findings of others [10]. Hydatid cyst fluid is a clear or yellow liquid with high specific gravity (1.005-1.009), it provides needed nutrition for larval growth, playing an important role in life cycle of *Echinococcus.* The fluid contains essential and vital elements which are very important in the biology of the parasite [6,11].

Eslami [7] documented that the composition of cyst content may differ in various area and strains, and is nearly 90% the same as host serum, the various electrolyte, enzymes, proteins', lipids, vitamins and hydrocarbon were seen in hydatid cyst fluid

Juyi et al [6] and Thompson and McManus [12] suggested that hydatid cyst fluid is an important component of the internal environment and fills the entire cyst, it's composed of many organic and nonorganic materials, and the cyst forms a relatively stable internal environment to avoid damage to the larvae from the host immune system. The present study showed a high concentration of alanine, glycin and valine in the fertile than sterile cyst fluid, a finding that was in agreement with others [13]. Aspartic and butyric acid shown to be absent in fertile cyst fluid suggested that the membrane of the fertile cyst does not contain aspartic acid receptors [14,15]. Alanine is enriched in cyst fluid compared to other amino acids. High levels of alanine were also reported in cyst fluid in other studies [6].

Non-organic substances in hydatid fluid concentrated in protoscolices as compared to cyst fluid [16-18]. The relationship between parasite and host is very important for understanding how parasites can grow in the body and what are the requirements of parasite can assist in understanding the ways for prevention of the parasitic infection [19,20].

In conclusion, the present study indicated a significant differences in amino acids concentration between fertile and sterile cyst and this could affect the natural history of the disease and outcomes. In addition, the present study finding warranted performance of a large scale, well designed study to clarify the role of amino acids presence in hydatid cyst fluid in the natural history and pathogenesis of hydatid disease.

ETHICAL APPROVAL: College of Medical and Health Technology Ethical Committee
CONSENT TO PARTICIPATE: Informed consent was taken from each subject before their enrolment in the study.
HUMAN AND ANIMAL RIGHTS: The study conducted in adherence with Helsinki Ethical standards.
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