Biochemical profiles of hydatid cyst fluids of *Echinococcus granulosus* of human and animal origin in Iran

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ABSTRACT

A comparative study on the biochemical parameters in hydatid cyst fluids of sheep, goat, camel, cattle and human cystic forms of *Echinococcus granulosus* has been made in Iran. Quantitative variations in the levels of glucose, calcium and creatinine in the cystic fluids of camels were found with hydatid fluids of sheep, goat, cattle and humans. These differences were statistically significant (P<0.05). Similarities in the biochemical composition in hydatid cyst fluids of sheep, goat, cattle and humans suggest the existence of sheep strains of *E. granulosus* and differences in the biochemical composition in hydatid cyst fluids of camel with other domestic animals and humans suggest the existence of camel strains of *E. granulosus* in Iran.

Key words: hydatid fluid, biochemical analysis, E. granulosus, strain

Introduction

Unilocular hydatid disease (hydatidosis, echinococcosis) caused by the cystic larval stage of *Echinococcus granulosus*, is recognized as being one of the world's major zoonoses. Its distribution is normally associated with underdeveloped countries, especially in rural communities, where man maintains close contact with the dog definitive host and the various domestic animals which may act as intermediate hosts. Hydatidosis is endemic in Iran where many domestic animals including sheep, goat, camel, and cattle act as intermediate hosts, while dog, coyote

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and wolf act as final host of *E. granulosus* (ESLAMI and HOSSEINI, 1998; ESLAMI and MOHEBALI, 1988; HOGHOUGHI and JALAYER, 1967; MOBEDI et al., 1970; SADIGHIAN, 1969).

In Iran, the average national surgical incidence of cystic hydatid disease is 1.2 per 100000 but varies between 0.1 and 4.5 per 100000 in different provinces (THOMPSON and LYMBERY, 1995). Because of the epidemiological significance of the variation exhibited between populations of *E. granulosus*, such variant populations are currently designated as being different strains and there is strong evidence which suggests the existence of at least nine host–adapted strains, the majority of which are geographically widely distributed (THOMPSON and LYMBERY, 1995). Taxonomic consideration of *E. granulosus* based on morphological criteria in differentiating between intraspecific strains has been questioned (BOWLES and MCMANUS, 1993a) and is a matter of controversy and some confusion (BOWLES and MCMANUS, 1993b; THOMPSON et al., 1994).

However, biochemical studies are useful in differentiating strain variations of *E. granulosus* in different countries (SHAAFIE et al., 1999; KUMARATILAKE et al., 1979; MCMANUS and MACPHERSON, 1984).

The strain characterization is particularly important in regions where more than one species of livestock intermediate host exists and where there is the possibility of different cycles of transmission and sources of infection for humans (THOMPSON and LYMBERY, 1995).

There has been no work on the biochemical aspects of cystic echinococcosis in Iran and biochemical studies on hydatid cysts from different host origins (animals and humans) can provide valuable information for characterizing and determining of strains of *E. granulosus* in this country. The present study is designed to evaluate the biochemical profiles of hydatid cyst fluids from different hosts (sheep, goats, camels, cattle and humans) for identification of strain variations of *E. granulosus*.

Materials and methods

Hydatid cyst fluids. Five samples of hydatid fluids were collected from the lung cysts of each host, i.e. sheep, goats, camels, cattle and humans.

Hydatid cyst fluids originating from humans were obtained after surgical removal of cysts from patients at Afzali Surgical Hospital, Kerman, whereas the

cyst fluids derived from domestic animals were collected from the official abattoir of Kerman. All cyst fluids were centrifuged at 15000 rpm at 4 °C for 30 min and the supernatants analysed for various biochemical parameters.

Biochemical analysis. Glucose, urea, uric acid, triglycerides and cholesterol were determined by the Enzymatic method, creatinine by Jaffe's method, and calcium by the O-kersolphthaleine-Komplexon method. These parameters were estimated by a commercially available diagnostic Kit from Man and Parsazemon Company on the Technicon RA 1000. Protein was determined by the colorimetric method with use of trichloroacetic acid (TCA), sodium and potassium by the flame photometry method, copper and magnesium by the spectrophotometry method. These parameters were estimated by a commercially available diagnostic Kit from the Randox Company.

Results

The results of biochemical analyses of the cyst fluids from the various hosts are shown in Table 1. Camel hydatid cyst fluids contain significantly more glucose,

Table 1. Biochemical profiles of hydatid fluids collected from different hosts infected with cystic echinococcosis (mean \pm SE, n = 5)

Biochemical profiles	Units	Host origin				
		Sheep	Goat	Cattle	Camel	Human
Total protein	gL ⁻¹	0.13 ± 0.05	0.068 ± 0.02	0.34 ± 0.058	0.58 ± 0.07	0.06 ± 0.05
Glucose	mmolL ⁻¹	2.81 ± 1.73	2.45 ± 1.24	1.35 ± 0.45	4.77 ± 1.03*	1.35 ± 0.3
Urea	mmolL ⁻¹	9.49 ± 1.78	5.44 ± 1.41	6.67 ± 5.11	6.53 ± 2.96	5.57 ± 0.74
Uric acid	mmolL ⁻¹	0.019 ± 0.01	0.012 ± 0.005	0.018 ± 0.008	0.01 ± 0	$0.14 \pm 0.1*$
Cholesterol	mmolL ⁻¹	0.13 ± 0.06	0.14 ± 0.03	0.13 ± 0.08	0.094 ± 0.05	0.12 ± 0.05
Triglycerides	mmolL ⁻¹	0.081 ± 0.04	0.1 ± 0.07	0.13 ± 0.08	0.06 ± 0.03	0.06 ± 0.04
Creatinine	μmolL ⁻¹	60.09 ± 32.1	49.49 ± 10.1	49.5 ± 11.58	91.92 ± 25.4*	48.62 ± 13.26
Calcium	mmolL ⁻¹	2.75 ± 1.15	2.64 ± 0.9	1.25 ± 0.45	4.47 ± 1.9*	1.27 ± 0.35
Sodium	mmolL ⁻¹	134.8 ± 8.92	136.2 ± 10.1	135 ± 12.6	148.6 ± 10.03	145 ± 15
Potassium	mmolL ⁻¹	5.72 ± 0.78	6.4 ± 0.9	6.5 ± 1.8	6.6 ± 1.6	5.83 ± 1.7
Magnesium	mmolL ⁻¹	1.34 ± 0.34	1.18 ± 0.2	1.22 ± 0.5	1.36 ± 0.4	1.32 ± 0.04
Copper	μmolL ⁻¹	1.58 ± 0.65	2.17 ± 0.38	1.79 ± 0.3	2.23 ± 0.5	2.21 ± 0.64

^{*} P<0.05

calcium and creatinine (P<0.05) than those in other hosts. However, the quantity of uric acid was found to be significantly more (P<0.05) in human cyst fluids compared with those from other species.

Discussion

Biochemical substances within hydatid cysts play a definitive role in the metabolism, physiology and immunology of cystic echinococcosis (THOMPSON and LYMBERY, 1995; SHAAFIE et al., 1999). 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 (SHAAFIE et al., 1999; THOMPSON and LYMBERY, 1995; MCMANUS and MACPHERSON, 1984). 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 (THOMPSON, 1991; THOMPSON and LYMBERY, 1995).

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 (MCMANUS, 1981).

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

The level of glucose, creatinine and calcium were found to be significantly lower in the cyst fluids of sheep, goats, cattle and humans compared with camels, indicating that the level of these parameters are not influenced by the former hosts. In support of this, SHERIFF et al. (1989) 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, SHAAFIE et al. (1999) 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.

SHAAFIE et al. (1999) have demonstrated higher concentration in uric acid in human hydatid fluids compared with other animal cyst fluids. This increased level

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.

In the present study, quantitative similarities in the biochemical profiles of hydatid fluids in cystic echinococcosis from sheep, goats, cattle and humans, and quantitative variation in the biochemical profiles of hydatid fluids of camels from other domestic animal intermediate hosts and humans, suggest the existence of sheep and camels strains of *E. granulosus* in Iran.

Morphological and mitochondrial DNA marker studies of *E. granulosus* in Iran also report the existence of two distinct strains of *E. granulosus* in Iran, with the sheep strain occurring in sheep, goats, cattle and humans and the camel strain occurring in camel (HOSSEINI and ESLAMI, 1998; LIHUA et al., 1998).

The sheep strain is the main cause of infection in humans (THOMPSON and LYMBERY, 1995).

Until very recently all surgically obtained human isolates of *E. granulosus* examined by isoenzyme (MACPHERSON and MCMANUS, 1982) and DNA (MCMANUS and RISHI, 1989; BOWLES et al., 1992; BOWLES and MCMANUS, 1993a; WACHIRA et al., 1993) analysis conformed to the common domestic sheep strain. Camels are commonly infected in the Middle East and Africa, yet opinions differ with regard to the infectivity of *E. granulosus* of camel origin to humans (MCMANUS et al., 1987; ECKERT et al., 1989; WACHIRA et al., 1993).

Important domestic intermediate hosts in the Middle East include the sheep and camel, with cattle and goats also harbouring hydatid infections (AL-ABBASSY et al., 1980; EL-YAMAN et al., 1985; ABDEL-HAFEZ et al., 1986; FARAH, 1987; GUSBI et al., 1987; ABDUL-SALAM and FARAH, 1988; KAMHAWI and HIJJAWI, 1992). In the endemic Mediterranean area sheep and dromedaries are the intermediate hosts (PATRICE, 2001).

BOWLES et al. (1992), BOWLES and MCMANUS (1993b) and MCMANUS et al. (1987) identified two distinct strains of *E. granulosus* in Kenya, with the sheep strain occurring in sheep, cattle, goats and humans, and the camel strain occurring in camels and, occasionally, in goats.

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References

- ABDEL-HAFEZ, S. K., F. M. AL-YAMAN, I. M. SAID (1986): Further studies on prevalence of hydatidosis in slaughtered animal from North Jordan. Z. Parasitenkd. 72, 89-96.
- ABDUL-SALAM, H. M., M. A. FARAH (1988): Hydatidosis in camels in Kuwait. Parasitol. Res. 74, 267-270.
- AL-ABBASSY, S. N., K. I. ALTAIF, A. K. JAWAD, I. M. AL-SAQUR (1980): The prevalence of hydatid cyst in slaughtered animal in Iraq. Ann. Trop. Med. Parasitol. 74, 185-187.
- BOWLES, J., D. P. MCMANUS (1993a): Rapid discrimination of *Echinococcus* species and strain using a PCR-based RFLP method. Mol. Biochem. Parasitol. 57, 231-239.
- BOWLES, J., D. P. MCMANUS (1993b): Molecular variation in *Echinococcus*. Acta. Trop. 53, 291-305.
- BOWLES, J., D. BLAIR, D. P. MCMANUS (1992): Genetic variation within the genus *Echinococcus* identified by mitochondrial sequencing. Mol. Biochem. Parasitol. 54, 167-174.
- ECKERT, J., R. C. A. THOMPSON, S. A. MICHAEL, L. M. KUMARAYILAKE, H. M. EL-SAWAH (1989): *Echinococcus granulosus* of camel origin: development in dogs and parasite morphology. Parasitol. Res. 75, 536-542.
- EL-YAMAN, F. M., L. ASSAD, N. HAILAT, S. K. ABDEL-HAFEZ (1985): Prevalence of hydatidosis in slaughtered animal from North Jordan. Ann. Trop. Med. Parasitol. 84, 473-475.
- ESLAMI, A., S. H. HOSSEINI (1998): *Echinococcus granulosus* infection of farm dogs of Iran. Parasitol. Res. 84, 205-207.
- ESLAMI, A., M. MOHEBALI (1988): Parasitism des chiens de begerset implication en sante publique en Iran. Bull. Soc. Path. Exot. 81, 94-96.
- FARAH, M. O. (1987): Infection rates, cyst fertility and larval viability of hydatid disease in camels, sheep and cattle in Gassim, Suadi Arabia. Vet. Res. 11, 493-495.
- GUSBI, A. M., M. A. Q. AWAN, W. N. BEESLEY (1987): Echinococcosis in Libya, IV. Prevalence of hydatidosis (*Echinococcus granulosus*) in goat, cattle and camels. Ann. Trop. Med. Parasitol. 84, 477-482.
- HOGHOUGHI, N., T. JALAYER (1967): The prevalence of *Echinococcus granulosus* in dog in Shiraz, Iran. Ann. Trop. Med. Parasitol. 61, 437-439.
- HOSSEINI, S. H., A. ESLAMI (1998): Morphological and development characteristic of *E. granulosus* derived from sheep, cattle and camels in Iran. J. Helminthol. 72, 337-341.
- KAMHAWI, S., N. HIJJAWI (1992): Current studies on the epidemiology of unilocular hydatidosis in Jordan and its social implication. Report on Parasitic Diseases of the Middle East. National Institutes of Health, Bethesda, Maryland, p. 8.
- KUMARATILAKE, L. M., R. C. A. THOMPSON, J. D. DUNSMORE (1979): Intraspecific variation in *Echinococcus*: a biochemical approach. Z. Parasitenkd. 60, 291-294.

- M. H. Radfar and N. Iranyar: Biochemical profiles of hydatid cyst fluids of *Echinococcus granulosus* of human and animal origin in Iran
- LIHUA, Z., A. ESLAMI, S. H. HOSSEINI, D. P. MCMANUS (1998): Indication of the presence of two distinct strain of *Echinococcus granulosus* in Iran by mitochondrial DNA marker. Am. J. Trop. Med. Hyg. 59, 171-174.
- MACPHERSON, C. N. L., D. P. MCMANUS (1982): A comparative study of *Echinococcus granulosus* from human and animal hosts in Kenya using isoelectric focusing and isoenzyme analysis. Inter. J. Parasitol. 15, 137-140.
- MCMANUS, D. P. (1981): A biochemical study of adult and gystic stage of human and animal origin from Kenya. J. Helminthol. 55, 21-25.
- MCMANUS, D. P., C. N. L. MACPHERSON (1984): Strain characterization in the Hydatid organism, *Echinococcus granulosus*. Current status and new perspective. Ann. Trop. Med. Parasitol. 78, 193-198.
- MCMANUS, D. P., A. K. RISHI (1989): Genetic heterogeneity within *Echinococcus granulosus*: isolated from different hosts and geographical area characterized with DNA probe. J. Parasitol. 99, 17-29.
- MCMANUS, D. P., A. J. G. SIMPSON, A. K. RISHI (1987): Characterization of the hydatid disease organism *Echinococcus granulosus* from Kenya using DNA marker. In: Helminth zoonoses. (Geerts, S., V. Kumar, J. Brandt, Eds.), Dordrecht, Nijhoff, pp. 29-36.
- MOBEDI, I., H. MADADI, F. ARFAA (1970): Camel, *Camelus dromedarius*, as intermediate host of *Echinococcus granulosus* in Iran. J. Parasitol. 57, 493-495.
- PATRICE, B. (2001): Hydatidosis: Dynamics of transmission. World. J. Surg. 25, 4-9.
- SADIGHIAN, A. (1969): Helminth parasite of stray dogs and jackals in Shahsavar area, Caspian region, Iran. J. Parasitol. 55, 272-275.
- SHAAFIE, I. A., A. H. KHAN, K. RAMBABU (1999): Biochemical profiles of hydatid cyst fluids of *E. granulosus* of human and animal origin in Libya. J. Helminthol. 73, 253-258.
- SHERIFF, D. S., M. FAKHRI, S. A. KIDWAI (1989): Lipid in hydatid fluid collected from lung and liver of sheep and man. J. Helminthol. 63, 266-268.
- THOMPSON, R. C. A. (1991): *Echinococcus and Giardia*: variation on a theme. Inter. J. Parasitol. 77, 75-82.
- THOMPSON, R. C. A., A. J. LYMBERY (1995): *Echinococcus* and hydatid disease, 1st ed. Wallingford, CAB International.
- THOMPSON, R. C. A., A. J. LYMBERY, C. C. CONSTANTINE (1994): Variation in *Echinococcus* toward a taxonomic revision of the genus. Advan. Parasitol. 35, 145-176.

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SAŽETAK

Provedeno je komparativno istraživanje biokemijskih pokazatelja hidatidne tekućine iz cista *Echinococcus granulosus* u ovaca, koza, deva, goveda i čovjeka u Iranu. Dokazana su kvantitativna kolebanja u nalazima glukoze, kalcija i kreatinina u cističnoj tekućini kod deva u odnosu na ovce, koze, govedo i čovjeka. Razlike su bile statistički značajne (P<0,05). Sličnosti u biokemijskom sastavu hidatidne tekućine u ovaca, koza, goveda i čovjeka ukazuju na prisutnost cista *E. granulosus* podrijetlom iz ovce dok razlike u biokemijskom sastavu hidatidnih tekućina deve i ostalih domaćih životinja i čovjeka ukazuju na prisutnost cista *E. granulosus* podrijetlom iz deve.

Ključne riječi: hidatidna tekućina, biokemijska analiza, E. granulosus, soj