Biochemical Study of Germinal and Laminated Layers of Hydatid Cyst of *Echinococcus granulosus* and Surrounding Host Tissues Isolated from Different Intermediate Hosts

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***Abstract*—**This study included biochemical analysis of germinal and laminated layers of hydatid cysts and the surrounding tissue for different hosts (sheep, goats and cattle) during the period from Jon. 2012 to Mar. 2013. The chemical analysis involved the determination of cholesterol, triglyceride and nitrogenous compound.

The laminated layer showed the high content of cholesterol in hydatid cysts of all hosts with the highest being in germinal layer of hydatid cysts in cattle liver (5.82±0.81 mg/100g). Whereas, in host tissues, the highest level was observed in infected sheep liver tissues (28.44±0.66 mg/100g).

The triglyceride in laminated layer of hydatid cysts was higher than that in germinal layer in hydatid cysts of all hosts, with the highest being in the laminated layer of hydatid cysts in cattle lungs (13.04±0.35 mg/100g). While in host tissues, the infected tissues showed the high level of triglyceride with the highest being in cow lungs tissue (38.52±1.18 mg/100g).

The laminated layer of hydatid cysts in sheep lungs contained the highest level of urea and uric acid which were (4.90±1.00 and 1.88±0.85 mg/100g), respectively as compared with the cyst in other hosts. The infected sheep lung tissues, showed the highest level of urea (33.03±0.56 mg/100g), while the highest level of uric acid was observed in goat lung tissues which was (1.51±0.50 mg/100g).

Creatinine contents of laminated and germinal layers of HCs in sheep liver along with host tissue were the highest among the studied hosts (0.13±0.01, 0.19±0.01 and 1.55±0.16 mg/100g), respectively. The bilirubine content of laminated and germinal layers of HC in sheep lungs along with the host tissues which were in direct contact showed the highest level among all studied hosts (0.29±0.03, 0.38±0.04 and 0.67±0.08 mg/100g), respectively.

***Keywords*—**Hydatid cyst, Echinococcus granulosus, layers, Biochemical analysis, Cholesterol, triglyceride, Urea, Uric acid, Creatinine, Bilirubine**.**

I. Introduction

HYDATID disease (HD) or Cystic echinococcosis (CE) is a major parasitic disease of veterinary and public health importance throughout the world. This disease has a great economic and zoonotic importance because it affects almost all the domestic animals and human [1]. It is caused by the larval stage stage (metacestode) of the dog tapeworm *Echinococcus granulosus* by ingesting eggs, which passed with the feces of the definitive hosts, usually feral dogs [2].

The larval form of *E. granulosus* in the intermediate host is characterized by its cystic aspect and behaves as a benign tumor in most of the cases [3]. There are seven species of *Echinococcus*; four of them are infectious to human, namely *E. granulosus*, *E. multilocularis*, *E. oligartharus*and *E. vogeli*. However, *E. granulosus*, is the most prevalent species in all continents, causing considerable public health problems in many regions of the world [4]. Furthermore, it is also common in Iraq [5], [6] and [7].

Epidemiological situation of this parasite is complicated due to the fact that several strains have been identified in most area where infection is endemic. These strains exhibit different degrees of infectivity for certain intermediate hosts [8].

The aim of this study was to perform some chemical analyses of laminated and germinal layers as well as surrounding host tissues to get some information about the nature of these layers and also how close they are to the structure of the host's tissues. This study can form the basis for further studies in this direction which will help to find the weaknesses in these layers and it may lead for a specific chemotherapeutic measures in the near future by specialists.

II. Materials and Methods

This study included a total of 30 HCs along with the tissues of infected organs which were collected from each infected sheep, goats, and cattle.

The cysts were removed from the infected organs, washed with distilled water, and then the germinal layer and laminated layer were removed with scissors. Then 0.5g of each one separately was homogenized with 5mls of Tris-HCl buffer (pH 7.4) by using a glass homogenizer connected to a variable speed stirrer and placed in a beaker containing crushed ice [9]. The extract was centrifuged at 5000 rpm for 15 minutes and the supernatants were kept in labeled sample tubes and stored in a deep freezer at -40°C until used. In the same processes, 0.5 gm of liver and lung of each one of host infected tissues were homogenized by using glass homogenizer, centrifuged and the supernatants were kept in labeled sample tubes and stored in a deep freezer at -40 °C until used. The following parameters were analyzed:

1- Triglyceride [10].

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2- Cholesterol, Uric Acid and Urea [11].
3- Bilirubine [12].
4- Creatinine [13].

III. Results

A. The Chemical Composition of Germinal and Laminated Layers of HCs Isolated from Sheep Along with the Infected Liver and Lung tissues

Table (I) show the cholesterol, triglyceride and nitrogenous compound of the germinal and laminated layer of HCs, infected tissue of sheep liver and lungs.

It is obvious from the results that the cholesterol contents of germinal layer of sheep cysts isolated from both liver and lung was higher than that of the laminated layer of the same cysts, but this difference was statistically non-significant (P> 0.05). Furthermore, the cholesterol content in laminated and germinal layers of HCs in sheep liver was higher than that in the laminated and germinal layers of HC in sheep lung. Regarding the triglyceride in laminated layer of HCs isolated from sheep liver and lungs was higher than that in the germinal layer of the same cysts (10.47±0.49 and 10.96±0.36 mg/100g, respectively). On other hand, the triglyceride content in laminated and germinal layers of HC isolated from sheep lung were higher than that in the laminated and germinal layers of HC isolated from sheep liver, but these differences were statistically non-significant (P> 0.05).

The laminated layer of HC from sheep liver and lung contain more urea than that in germinal layer, and these differences were statistically non-significant (P> 0.05). On the other hand, the liver and lung tissues contain much higher urea, and the highest level of urea was found in infected tissue of liver and lung which were 31.89±0.61 and 33.03±0.56 mg/100g, respectively, and these values were decreased in the infected tissues in contact to the HC.

TABLE I
Cholesterol, Triglyceride and Nitrogenous Compounds of Hydatid Cyst's Wall and Infected Sheep Liver and Lung Tissues

Parameters	Organ	Laminated Layer	Germinal Layer	Surround Tissue
Cholesterol	Liver	3.65±0.48	4.66±0.76	28.44±0.66
	Lung	3.39±0.78	4.25±0.45	21.95±0.81
Triglyceride	Liver	10.47±0.49	9.42±0.25	30.32±0.79
	Lung	10.96±0.36	9.85±0.63	31.94±1.18
Urea	Liver	3.74±0.26	2.93±0.25	31.89±0.61
	Lung	4.90±1.00	4.07±1.16	33.03±0.56
Uric Acid	Liver	1.83±0.28	0.79±0.14	1.01±0.08
	Lung	1.88±0.85	1.05±0.14	1.46±0.50
Creatinine	Liver	0.13±0.01	0.19±0.01	1.55±0.16
	Lung	0.11±0.03	0.17±0.03	1.00±0.05
Bilirubine	Liver	0.25±0.09	0.37±0.10	0.65±0.08
	Lung	0.29±0.03	0.38±0.04	0.67±0.08

In general the uric acid did not show high variation in the infected sheep liver and lung tissues as compared with HC layers. The uric acid in laminated layers of HC isolated from sheep liver and lung was higher than that in germinal layers.

The highest creatinine content was observed in germinal layer of HCs isolated from sheep liver and lungs, which was 0.19±0.01 and 0.17±0.03 mg/100g, respectively. Regarding the organ involved, the creatinine contents of liver was higher than that in lung.

The bilirubine content in germinal layer of HCs in sheep liver and lung were higher than that in laminated layer (Table I).

B. The Chemical Composition of Germinal and Laminated Layers of HCs Isolated from Goats Along with the Infected Liver and lung tissues

The cholesterol, triglyceride and nitrogenous compound of the germinal and laminated layer of HC, infected tissue both in direct contact and those which are at a distance of five centimeters from the cyst, and uninfected tissue of goats liver and lung are shown in Table (II).

It is obvious from the results that the cholesterol content of germinal layer of goats cysts isolated from both liver and lung was higher than that of the laminated layer of the same cyst, but this different was statistically non-significant (P> 0.05). Furthermore, the cholesterol content in laminated and germinal layers of HC in goats' liver was slightly higher than that in the laminated and germinal layers of HCs in goats' lungs.

Regarding the triglyceride, it was higher in the laminated layer of HCs isolated from goats liver and lungs than that in the germinal layer of the same cysts (Table II).

The laminated layer of HCs from goats liver and lungs contain more urea as compared with the germinal layer; however, these differences were statistically non-significant (P> 0.05).

TABLE II
Cholesterol, Triglyceride and Nitrogenous Compounds of Hydatid Cyst's Wall and Infected Goats Liver and Lung Tissues

Parameters	Organ	Laminated Layer	Germinal Layer	Surround Tissue
Cholesterol	Liver	4.02±0.40	4.80±0.70	25.06±0.93
	Lung	3.58±0.18	3.78±0.29	23.16±1.16
Triglyceride	Liver	11.12±0.83	10.05±0.38	34.96±0.75
	Lung	12.03±0.43	10.54±0.33	36.43±0.68
Urea	Liver	2.78±0.63	1.35±0.25	18.61±1.19
	Lung	3.48±0.68	1.61±0.36	20.09±1.48
Uric Acid	Liver	1.31±0.85	0.84±0.11	0.90±0.08
	Lung	1.46±0.65	1.13±0.50	1.51±0.50
Creatinine	Liver	0.12±0.01	0.18±0.03	1.52±0.63
	Lung	0.11±0.01	0.14±0.01	0.91±0.05
Bilirubine	Liver	0.23±0.03	0.28±0.08	0.63±0.01
	Lung	0.26±0.11	0.31±0.08	0.65±0.08

Highest Uric acid content (1.46±0.65 mg/100g) was observed in the laminated layer of HCs isolated from goats' lungs. While the highest creatinine content in HC was observed in germinal layer of HCs isolated from goats liver and lungs, which were 0.18±0.03 and 0.14±0.01 mg/100g, respectively.

Regarding the organ involved, the creatinine content of liver was higher than that in lung.

The bilirubine content in germinal layer of HC in goats' liver and lung were slightly higher than that in laminated layer (Table II).

C. The Chemical Composition of Germinal and Laminated and Layers of HCs Isolated from Cattle Liver and Lungs Along with the Infected Liver and Lung Tissues

The cholesterol, triglyceride and nitrogenous compound of the germinal and laminated layer of HC and infected tissue of cattle liver and lung are shown in table (III).

It is obvious from these results that the cholesterol contents of germinal layer of cattle cysts isolated from both liver and lungs were higher than that of the laminated layer of the same cysts, but this difference was statistically non-significant (P> 0.05). Furthermore the cholesterol contents in laminated and germinal layers of HCs in cattle liver were higher than that in the laminated and germinal layers of HCs in cattle lung.

Regarding the triglyceride, the laminated layer of HCs isolated from cattle liver showed slightly higher triglyceride content than that in the germinal layer, which were 13.04±0.35 and 11.53±0.75 mg/100g, respectively. Similar pattern was observed in cysts isolated from cattle lung, (Table III).

The laminated layer of HC from cattle liver and lung contained more urea than the germinal layer, but these differences were statistically non-significant (P> 0.05). On the other hand, the liver and lung tissues contained much higher urea, with the highest level being found in infected tissue of liver and lung which were 22.07±1.88 and 22.4±1.50 mg/100g, respectively.

TABLE III
CHOLESTEROL, TRIGLYCERIDE AND NITROGENOUS COMPOUNDS OF HYDATID CYST'S WALL AND INFECTED CATTLE LIVER AND LUNG TISSUES

Parameters	Organ	Laminated Layer	Germinal Layer	Surround Tissue
Cholesterol	Liver	4.54±0.04	5.82±0.81	26.63±1.38
	Lung	3.77±1.13	5.19±0.13	26.65±1.51
Triglyceride	Liver	11.53±0.75	10.98±0.25	35.82±0.83
	Lung	13.04±0.35	11.34±0.50	38.52±1.18
Urea	Liver	2.37±0.18	2.27±0.09	22.07±1.88
	Lung	3.60±0.43	2.55±0.30	22.4±1.50
Uric Acid	Liver	1.44±0.20	0.79±0.63	1.05±0.10
	Lung	1.51±0.93	0.90±0.23	1.38±0.50
Creatinine	Liver	0.13±0.06	0.17±0.11	1.36±0.14
	Lung	0.12±0.08	0.13±0.01	0.99±0.08
Bilirubine	Liver	0.24±0.05	0.30±0.04	0.66±0.06
	Lung	0.28±0.04	0.33±0.03	0.67±0.06

The highest uric acid level in HC was observed in the laminated layer of HC isolated from cattle lungs (1.51±0.93 mg/100g). Regarding creatinine, both laminated and germinal layer of HC isolated from cattle liver and lungs showed somewhat similar levels. With respect to host's tissues, the highest creatinine content was found in liver infected tissue (1.36±0.14 mg/100g).

The bilirubine content in germinal layer of HC in cattle lung was the highest (Table III). Regarding the host tissues, the values of bilirubine in cattle liver and lung tissues were somewhat similar (Table III).

IV. DISCUSSION

The biochemical compositions of HCs play an important role in the metabolism, physiology and immunology of the disease [14]. The quantitative difference in the metabolism of *E. granulosus* and variation in the biochemical composition of HC reflect strain variation in different intermediate hosts [14]-[15].

A. Cholesterol

The cholesterol concentration in the germinal layer was higher than that of the laminated layer of HC from infected liver and lungs in all hosts, with the highest being in the germinal layer of HC from cattle liver. However there is no any previous study in this direction in order to compare these results, the only available work is that of reference [16] who worked on germinal layer and reference [17] who worked on laminated layer, and if we compare their results, regarding cholesterol content, their results also showed that the germinal layer contains higher content of cholesterol. On the other hand, the high cholesterol content of infected tissues which is close to HC is in agreement with what has been recorded by reference [18] where he also found high cholesterol content in infected tissues which are close to HC. He attributed it to aggregation of cholesterol in tissue which is near the cyst to be absorbed by HC, since HC cannot produce cholesterol as mentioned by reference [19], while reference [20] stated that the protoscolices of HCs have the ability to oxidize the acetate to produce lipids except cholesterol which was absorbed from host tissue. Reference [21] and reference [22] found that the cholesterol is an important component in germinal and laminated layer and the rises in its level in HC from cattle may be due to the nature of these layers in cattle as compared with its counterpart in other intermediate hosts.

The increasing in the level of cholesterol in the liver as compared with the lung may be related with its function as a structural component [23].

B. Triglyceride

The laminated layer of HCs from liver and lung showed higher triglyceride content as compared to the germinal layer; also this content was higher in the laminated layer of cyst isolated from lung tissues. Reference [24] and reference [7] found high triglyceride content in protoscolices and fluid of HC isolated from host lungs. With respect to triglyceride in host tissue, the highest level was found in uninfected tissue. This agrees with what have been observed by reference [7].

C. Urea and Uric Acid

In the present study, also the laminated layer of lung cysts showed the higher contents of urea and uric acid, but these values are very low as compared with urea and uric acid levels in infected and uninfected host's tissue. This could be a prophylactic measure to avoid deleterious effects on some vital processes [25]. However there is no data to compare

these results, the only available data are those of reference [26] and reference [7] who found low levels of urea and uric acid in the protoscolecies of HCs. These results imply to the accumulation of uric acid in tissues surrounding the HCs. The presence of urea and uric acid indicate the occurrence of urea cycle which is essential to eliminate the toxic level of ammonia produced from amino acid and nucleotide metabolism [27].

D. Creatinine

The highest creatinine level was found in germinal layer of HCs from sheep liver. Reference [27] and reference [28] reported that the creatinine level reflects the ammonia metabolism and energy production. Therefore, this increase of creatinine in germinal layer may be attributed to the vitality effect of germinal layer as compared with laminated layer. On the other hand, these values are very low as compared with creatinine level in host tissue. The same difference was observed by reference [25] and reference [7].

E. Bilirubine

Bilirubine concentration in germinal layer of HCs was higher than that in laminated layer. With respect to host tissues, the bilirubine content in tissue which is in direct contact with HCs was higher than that at 5cm distance and uninfected tissue. This may be attributed to the toxic effect of crude HF or filtered HF reference [18], there is no other previous studies in order to compare the results.

REFERENCES

[1] Baswaid, S. H. "Prevalence of hydatid cysts in slaughtterred sheep and goats in Hadhramout", Yemen. Ass. Univ. Bull. Environ. Res. 10 (2), 2007, pp. 145-146.

[2] Rahimi, H.; Sadjadi, S. M., and Sarkari, B. "Performance of antigen B isolated from different hosts and cyst locations in diagnosis of cystic Echinococcosis", Iranian J. Parasitol., 6 (1), 2011, ppt: 12-19.

[3] Amman, R.W., and Eckert, J. "Cestodes. Echinococcus. In: Parasitic diseases of the liver and intestines". Gastroenterol. Clin. North America, 25, 1996, pp. 655-689.

[4] World Health Organization Office International des Epizooties. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. World Organization for Animal Health, Paris, France, 2001, pp. 100-101.

[5] Al-Nakeeb, R. A. "Seroepidemiological and therapeutic study on hydatid cyst infection in Kirkuk and Tikrit provinces", M.Sc. Thesis, College of Medicine, Tikrit University, Iraq, 2004.

[6] Abdullah, A. M. "Epidemiological, comparative enzymatic and total protein content of hydatid cyst of *Ecchinococcus granulosus* isolated from sheep and goats in Duhok province, Kurdistan region of Iraq", M.Sc. Thesis, College of Education, University of Duhok, Iraq, 2010.

[7] Meerkhan, A. A. "Biochemical studies on hydatid cysts of Echinococcus granulosus isolated from different intermediate host (sheep, goat, cows and human)", M.Sc. Thesis, College of Education, University of Zakho, Iraq, 2011.

[8] Thompson, R.C.A., and McManus, D.P. "Etiology: Parasite and life-cycles. In: WHO/OIE manual on echinococcosis in humans and animals: A public health problem of global concern". (Eckert, J.; Gemmell, M.A,;Meslin, F.X. and Pawlowski, Z.S. ed.) World Organization for animal health, Paris, France, 2001, pp. 1-19.

[9] Mero, W.M.; Al-Zako, S.S., and Zakaria, S.J. "Comparative study on some enzyme activity in Fasciola hepatica, Fasciola gigantia and Bovine liver". J. Edu. And Sci. Vol. 8. Dep. of Biology, College of Science, University of Mosul- Iraq, 1988, pp. 62-67.

[10] Fossati, P. and Prencipe, L. "Serum Triglycerides Determined Calorimetrically With An Enzyme That Produces Hydrogen Peroxide". Clinical Chemistry, 28(10), 1982, pp. 2077- 2080.

[11] Tietz, N. W.; Burtis, C.A.; Ashwood, E. R. and Saunders, W. B. Text book of clinical chemistry 3rd Ed., 1999, pp. 826-835 and 1245-1250.

[12] Malloy, H. T. and Evelyn, K. A. "The determination of bilirubine with the photoelectric colorimeter". J. biol. Chem., 119, 1937, pp. 481-490.

[13] Labbe´, D.; Vassault, A.; Cherruau, B.; Baltassat, P.; Bone`te, R.; Carroger, G.; Costantini, A.; Gue´rin, S.; Houot, O.; Lacour, B.; Nicolas, A.; Thioulouse, E., and Tre´po, D. "Method selected for the determination of creatinine in plasma or serum. Choice of optimal conditions of measurement (in French)". Ann. Biol. Clin., 54, 1996, pp. 285–298.

[14] Thompson, R. C. A. and Lymbery, A. J. "Echinococcus and hydatid disease", 1st ed. Wallingford, CAB International, 1995. (Cited: Radfar, M. H. and Iranyar, N. "Biochemical profiles of hydatid cyst fluids of Echinococcus granulosus of human and animal origin in Iran". J. Vet., 4 (6), 2004, pp. 435-442).

[15] Shaafie, I.A.; Khan, A., and Rambabu, K. "Biochemical profiles of hydatid cyst fluids of E. granulosus of human and animal origin in Libya". J. Helminthol., 73, 1999, pp. 253-258.

[16] Hammodi. H. M. "Chemical composition of protoscolices and germinal layer of Echinococcus granulosus from man and some other intermediate hosts in Iraq". M.Sc. Thesis, College of Science, University of Mosul, Iraq, 1989.

[17] Al-Zobaidy, A. B. "Chemical composition of hudatid fluid and laminated layer of Echinococcusgranulosus from man and some other intermediate hosts in Iraq". M.Sc. Thesis, College of Science, University of Mosul, Iraq, 1989.

[18] Anwar, Z. "The effect of Echinococcosis on Rabbit and Sheep along with its control by indigenous plants of Pakistan". Ph.D. Thesis, Department of Zoology, University of Punjab. Pakistan, 1997.

[19] Frayha, G. j "Comparative metabolism of acetate in the taeeniid tapeworms Echinicoccusgranulosus, Echioncoccus multilocularis and Taenia hydatigena". Comp. Biochem. Physiol., 39, 1971, pp. 167-170.

[20] Vercelli-Retta, J.; Reissenweber, N. J.; Lozano, W., and Siri, A. M. "Histochemistry and histoenzymology of the hydatid cyst of Echinococcus granulosus". Part; 1. The germinal membrane. Z. Parasitkd., 48, 1975, p. 15-23.

[21] Čmelik, S. "Ein Antigene spolysacchridaus den Ecinococcuscysten. Biochem. Ztseh, 322, 1952, pp. 456-462 (English abstract).

[22] Richards, K. S. "Echinococcusgranulosusequinus: The histochemistry of the laminated layer of the hydatid cyst". Fol. Histochem. Et. Cytobiol., 22 (1), 1984, pp. 21-32.

[23] Al-Bayati, S. M.; Aziz, O. H.; Abdulla, A. M., and Abed, S. A. "Biochemical profile of hydatid cyst fluids of Echinococcus granulosus of sheep in Duhok area". Iraq Journal of Veterinary Medicine. 34(1), 2010, pp. 185-191.

[24] Refik, M.; Mehmet, N.; Durmaz, B., and Egri, M. "Determination of some biochemical parameters in hydatid cyst fluids". Erciye Medical Journal, 24(1), 2002, pp. 10-13.

[25] Frayha, G. J. and Haddad, R. "Comparative chemical composition of protoscolices and hydatid cyst fluid of Echinococcusgranulosus (Cestoda)". Int. J. Parasitol., 10, 1980, pp. 359-364.

[26] Rahdar M. H.; Maraghil, S.; Rafei, A., and Razijalali, M. "Comparison of some electrolytes in hydatid cyst fluid and serum of liver hydatidosis of sheep". Jundishapur Journal of Microbiology, 1(1), 2008, pp. 10-14.

[27] Dow, J.; Lindsay, G., and Morrison, J. "Biochemistry molecules cells and body". Addison-Wesleg Publishing Company Inc. Wokingham England, 1996.

[28] Kahn, C. M. The Merck veterinary Manual 9th ed. Merck and Co. Inc., White house station, NJ. USA, 2005.