Analysis of chemical and biochemical composition of fertile and sterile hydatid cyst fluids of human origin

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Received: 3/11/2014
Accepted: 14/8/2016

Summary

The present study was made for the analysis of major chemical and biochemical components of twenty hydatid cysts fluids including two groups (10 fertile cysts and 10 sterile cysts) obtained from the lung and liver of 29 infected patients admitted to Medicine City Hospital for operation during a period of 6 month. These components were measured by colorimetric assay kits (Biolabo®, Human). Results revealed marked ($P<0.05$).differences in the mean values, particularly among enzymes (Glutanicoxaloacetic transaminase, Glutanic pyruvic transaminase and Alkaline phosphatase), between fertile and sterile fluids while the Lactate dehydrogenase level exhibited high activities in fertile and sterile isolates. These values were less than the normal values in serum. The mean values of Iron, Zinc Sodium Phosphorus and potassium were found higher in fertile isolates, while a marked decrease accured in sterile isolates. Furthermore, the Copper revealed quantitative differences ($P<0.05$) between the two isolates. Manganese and chloride constituted the major volume in the fluid anions, in fertile and reached the maximum in sterile fluid. Differences were detected in the levels of glucose of fertile which increased significantly ($P<0.05$). The data detected no significant difference ($P>0.05$) in total protein which exhibited high in fertile and sterile fluid. Also Calcium and Magnesium mean values revealed the same in both isolates. Trace elements like Urea, Uric acid, Bilirubin Creatinine and Lipid increase in liver diseases such as the Cholesterol, and Triglycerides level which was found higher in fertile and sterile isolates. In comparison with previous studies the present investigations reflected quantitative difference in mean values of biochemical parameters of fertile and sterile of hydatid cyst fluids contributed to fertility of cyst and viability of protoscoles. Fertile cyst which contained broad capsule and protoscoles responsible of higher concentration in these elements than sterile fluid which lack the broad capsule and protoscoles, which may point to some variable in parasite metabolism endurance, growth rate, intermediate host specificity location of cyst among lungs or livers or even strain variation.

Keywords: Haydatid cyst fluid, Fertile, Sterile, Biochemical analysis, Human.

Introduction

Cystic echinococcosis (CE) is a disease caused by the metacestode hydatid cyst of the adults tapeworm *Echinococcus granulosus*. The disease caused considerable economic and public health importance (1). In farm animals, the disease caused economic loss include decreased in fecundity, meat and milk production, reduced hide and fleece value due to condemnation of edible organs. The incidence of human hydatid disease in any country is closely related to the prevalence of the disease in domestic animals and is highest where there is a large dog population and high sheep production (2). Antigens associated with mature hydatid cyst have been investigated extensively, particularly cyst fluid, which is in essence an internal a repository of somatic and functional antigens. Hydatid fluid is rich antigenically, and immuno electrphoresis revealed the presence of more than 10 distinct antigens of parasite origin together with variable amount of host components (3). While (4) demonstrated that hydatid cyst fluid antigens (HCF. Ag) are variable in their antigenicity according to the host, localization of cyst and fertility or development stage of the cyst. The metabolic products of the protoscolices which accumlate in the fluid are responsible for the potential antigenicity, this explains the poor antigenicity of cysts lacking protoscolices. Sheep and human HF are considered to be highly antigenic among other intermediate species. Liver HCF antigens considered to be better than lung HCF antigens due to the high concentration of protein

content. And (5) reported that 17 to 200 mg of protein per 100 ml, were found in hydatid cyst fluid. HF known to contain enzymes such as GOT, GPT, ALP and LDH. Traces of elements like urea, uric acid, bile pigment, creatine and bilirubin were found occasionally in hydatid fluid, according to the cyst location (6 and 7). The non-organic substances detected in hydatid fluid are potassium, magnesium, calcium concentrated in protoscolices as compared to cyst fluid. HCF is also known to contain Lipids and complement activitating factors which upon interaction with complement contribute to the shock reaction (8 and 9). The aim of this study was to investigate variation in the chemical and biochemical components in fertile and sterile hydatid fluid of human.

Materials and Methods

Twenty hydatid cysts were collected from the lung and liver of (35) patients admitted to Medical City Hospital in Baghdad suspected with hydatid cyst and confirmed for operation during a period of 6 month. Hydatid cysts include 10 fertile cysts and 10 sterile cysts. Each cysts collected was transferred in a cold box to the laboratory of parasitology in the college of Veterinary Medicine of Baghdad University. After several washing with normal saline, aspiration of the fluid from cysts were done by sterile syringes G18 and transferred in the sterile test tube and centrifuged at 3000 rpm for 5 min. The supernatant fluid was collected in new sterile test tubes and stored at -20˚C.

The stored fluids were transferred in cold container to Monthar lab in Al Saadon area in Baghdad for chemical and biochemical components analysis, using colorimetric assay kits (Biolabo®, Human) and (Biolabo®) company France, and according to the manufacturer's instructions the components were measured including magnesium (mg/dl), copper, potassium, Sodium chloride, Zinc and Calcium (mmol/l). Manganese (mg/dl), Phosphorus and Iron (mmol/l). Lipids (cholesterol, triglyceride), Glucose (mg/dl) and total protein (g/l). Urea (BUN), uric acid. Creatinine (mg/dl) and Bilirubin (mmol/l) were measured according to (10 and 11). The activation of the enzymes Glutanic oxaloacetic transaminase (GOT), Glutanic pyruvic transaminase (GPT), ALkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) were measured by machine (KEnza 240 TX)and method of (12 and 13).Values given are of 10 samples of two reciprocals.

Means were compared using Unpaired *t*. test. $P<0.05$ considered significant. (14).

Results and Discussion

In the present work chemical and biochemical analysis of fertile and sterile hydatid cyst fluid (HCF) of human origin was conducted to detect various components between them. Hydatid cyst fluid is not a simple substance; it is composed of many organic and nonorganic materials (15 and 16). Biochemical analysis of hydatid cyst fluid was done by many Investigators (17-19). The present data for biochemical parameters revealed significant ($P<0.05$) differences between fertile and sterile hydatid cyst fluids of human isolates, among enzymes, the mean values of GOT, GPT and ALP were (21.33±18.10), (6.33±4.41) and (4.44±5.68) respectively, in fertile isolates and reached the maximum in sterile isolates (52.16±23.5), (17.0±114.18) and (58.83 ±43.26) respectively. While LDH level exhibited high activities in fertile (123.0 ±93.1) and sterile isolates (103.16±49.23). These values were less than normal values in serum (Table, 1 and 2). These findings were similar to have been reported by (16 and 20).

Iron, Zinc Sodium Phosphorus potassium, mean values was found higher in fertile isolates of human (160.04±29.76), (122.87 ±15.41), (122.30±0.64) (34.37±6.40) and (14.2±0.21) respectively. While marked decrease in sterile isolates (9.94±0.06) (8.54±0.02) (19.05±1.22), (0.054±0.02) and (7.55±0.13) respectively, in comparable to normal values. Copper level was lower (16.63±5.21) in fertile and reach maximum in sterile (124.0±92). The data for fertile and sterile showed significant difference ($P<0.05$). Similar findings in mean levels of calcium and zinc were recorded by (21), these values were different from those reported by (22).

Calcium and Magnesium mean values

revealed the same in fertile (9.98±0.28) and (9.3±0.23), and in sterile isolates (8.43±0.26) and (6.63±0.21) respectively. Manganese and chloride constituted the major volume in the fluid anions. Chloride values were found higher in fertile (99.64±0.72) and (97.43±0.32) and reached the maximum level in sterile isolates (104.48±0.52) and (100.11±0.32) respectively. Total protein was found in fertile fluid (94.12±0.78) and (91.04±1.6) in sterile. These values were different from those reported by (22 and 23). Also the mean values of glucose values exhibited in fertile isolates (100.1±0.02), while in sterile isolates were less (82.3±0.76). The data showed no significant difference (P>0.05) and were different as compared to (23).

Trace elements like Urea, Uric acid, Bilirubin Creatinine mean levels were (50.15±0.47), (12.01±0.36), (1.53±0.81)and (0.20±0.01) respectively in fertile isolates and (46.72±0.28), (9.93±0.05), (2.53±0.62) and (0.98±0.01) in sterile isolates respectively. The data for fertile and sterile showed no significant difference (P>0.05). By quantitative analysis of HCF of liver it was found that values of glucose, urea, and sodium were not statistically significant by (1). Similar elevated in level in uric acid urea, iron, phosrphus, manganese, chloride, sodium, total protein, glucose, bilirubine, creatinine, potassium, magnesium, copper and insignificant differences (P>0.05) were found in human fertile and sterile isolates, was reported by (5 and 22) and in contrast to those reported by (20).

Lipid increase in liver diseases such as Cholesterol and triglycerides level was found higher in fertile isolates (199.9±2.18), (180.40 ±1.01) and in sterile isolates (188.02±2.14) (178.32±2.54) respectively. The data for fertile and sterile showed no significant difference (P>0.05). (Table, 1 and 2). These values were different from those reported by (21 and 22). While (16, 23 and 24) were found significant differences in the following parametric values of triglyceride, cholesterol also in potassium, sodium, calcium, uric acid, glutamyl transferase, aspartate, aminotransferase and creatinine. Values of (25) for glucose, triglycerides, calcium varied from our work but sodium, potassium, chloride were nearly similar in all sterile samples of human also reported that mean glucose contents in hydatid cyst fluids of sheep's lungs were higher than that in livers (44.3 versus 35.8 mg/dl). Mean micrototal proteins were 26.1 and 21.4 mg/dl and mean triglycerides were 2.8 and 2.9 mg/dl in hydatid cyst fluids of sheep's lung and livers, respectively. There was no a statistically significant difference from corresponding values of sheep (P>0.05).

In comparison with previous studies the present investigations reflected some differences for many values and also among lungs or livers. Guantitative difference in mean values of biochemical parameters of fertile and sterile in the hydatid cyst fluids contrbuted to fertility of cyst and viability of protoscoles. Fertile cyst which contains broad capsule and protoscoles the fluid is thought to have high concentration in the chemical and biochemical elements than sterile fluid which lacked the broad capsule and protoscoles. It may acquire changes in metabolism for parasite endurance or may be related to intermediate host specificity (15 and 19) which may point to some variables in parasite metabolism, growth rate or even strain variation. It is concluded that the cysts of human represent different strains of *Echinococcus granulosus*. Variation in composition of hydatid cyst fluids in different species of animals and human may be due to multiple strains of *E. granulosus* (26 and 27). Similarities in the biochemical composition in hydatid cyst fluids from other hosts like sheep proposed the existence of sheep strains of E. granulosus the focal strain which causes infection in human is sheep strain which is the widely distributed strain in the world that infected both human and sheep (15 and 26).

Analysis will be further useful for immunological studies, diagnostic tests and may be helpful to find out various strains of *E. granulosus* in Iraq. Advanced study is required for describing better criteria for strain identification among different species. Determination of the chemical and biochemical parameters in hydatid cyst fluids of infected sheep and cattle may help to identify the source of human infection.

Table, 1: Biochemical components (mean±SE) of fertile and sterile hydatid cyst fluid values given of 10 samples of two reciprocals.

Biochemical profile	Unit	Type of hydatid cyst	
		Fertile	Sterile
GOT	mmol/l	21.33 ± 18.10	52.16 ± 23.5
GPT	mmol/l	6.33 ± 4.41	17.0 ± 114.18
ALP	mmol/l	14.44 ± 5.68	58.83 ± 34.26
LDH	mmol/l	123.0 ± 93.1	103.16 ± 49.23
Iron	mmol/l	160.04± 29.76	9.94 ± 0.06
Zinc	mmol/l	122.87± 15.41	8.54 ± 0.02
Phosphorus	mmol/l	34.37 ± 6.40	0.054 ± 0.02
Calcium	mmol/l	9.98 ± 0.28	8.43 ± 0.26
Magnessium	mg/dl	9.3 ± 0.23	6.63 ± 0.21
Manganeze	mg/dl	99.64 ± 0.72	104.48 ± 0.52
Potassium	mmol/l	14.2 ± 0.21	7.55 ± 0.13
Chloride	mmol/l	97.43 ± 0.32	100.11 ± 0.32
Copper	mmol/l	16.63 ± 5.21	124.0 ± 92
Sodium	mmol/l	35.3 ± 1.23	34.02 ± 1.32
Total protein	g/l	94.12 ± 0.78	91.01 ± 1.6
Glucose	mg/dl	100.1 ± 0.02	82.3 ± 0.76
Urea	mg/dl	50.15 ± 0.47	46.72 ± 0.28
Uric acid	mg/dl	12.01 ± 0.36	9.93 ± 0.05
Bilirubin	mmol/l	1.53 ± 0.81	2.53 ± 0.62
Creatinine	mg/dl	0.20 ± 0.01	0.098 ± 0.01
Cholestrol	mg/dl	199.9 ± 2.18	188.02 ± 2.14
Triglyceride	g/l	180.41± 1.01	178.32 ± 2.54

The statistical analysis has been determined by applying multivariate analysis of variance and for the comparison of mean, T-test applied to compare the means of fertile and sterile isolates of human, all have significant difference (P<0.05).

Table, 2: The normal values of enzymes and ions of human body.

Enzymes and ions	Normal values
GOT	10-34 ul
GPT	10-44 ul
ALP	11-50 ul
LDH	240-480 ul
Iron	Acima 50 mg/dl
Zinc	2-4 g
Phosphorus	2.5-4.5 mg/dl
Calcium	8.8-10.5 mg/dl
Magnessium	1.7-2.2 mg/dl
Manganeze	10-20 mg/dl
Potassium	3.5-5.0 mmol/l
Chloride	98-108 mmol/l
Copper	1-2 mmol/l
Sodium	135-145 mmol/l
Total protein	6.0-8.3gm/dl
Glucose	70-110 mg/dl
Urea	10-45 mg/dl
Uric acid	3.4-7.0 mg/dl
Bilirubin	0.64-1 mmol/l
Creatinine	0.6-104 mg/dl
Cholestrol	200 mg/dl
Triglyceride	150 mg/dl

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تحليل للمكونات الكيموحيوية للسائل العدري الخصب والعقيم المعزول من الإنسان

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الخلاصة

هدفت الدراسة الحالية إلى تحليل القيم الكيموحيوية لسائل الكيس العدري الخصب والعقيم لطفيلي المشوكات الحبيبية (20 كيساً عدرياً من الرئة والكبد تضمن 10 أكياس خصبة و10 أكياس عقيمة) والمعزول من الإنسان خلال مدة الدراسة (6 اشهر) وباستعمال الطرائق الطيفية اعتماداً على العدد القياسية الجاهزة. أظهرت النتائج فروقات واضحة في القيم لهذه المكونات الكيموحيوية خصوصا بين الانزيمات (كلوتانيك أوكسالو استك ترانسمينيز وكلوتانيك بايروفك ترانسمينيز وألكلاين فوسفتيز) للسائل الخصب والعقيم واظهر انزيم (لاكتيت ديهايدروجينيز) نشاطاً عالياً فى كلا السائلين مقارنة مع القيم الطبيعية. وكانت معدلات قيم الحديد والزنك الصوديوم والفسفور والبوتاسيوم عالية في السائل الخصب وانخفضت فى السائل العقيم، علاوة على ذلك أظهر النحاس فروقات بين السائلين وعد المنغنيسوم والكلورايد المكون الرئيس لسائل الخصب إذ وصل إلى الحد الأعلى في العقيم. كُشفَ عن فروقات معنوية ($P\leq 00.5$) لمستوى الكلكوز بالنسبه لسائل الخصب. كشفت البيانات عدم وجود فروقات معنوية في كمية البروتين بين السائل الخصب والعقيم كذلك في ايوني الكالسيوم والمغنيسيوم واليوريا وحامض اليوريك والكرياتنين والبيلروبين لم نجد فروقات إحصائية. وأظهرت الدهون تبايناً واضحاً في الكلستيرول والكليسترول الثلاثي في الكمية كانت القيم الكيموحيوية متباينة بالكبد عنها بالرئة. وأظهرت النتائج لجميع ما ذكر أعلاه لهذه التحاليل الكيموحيوية فروقا إحصائية واضحة بعضها اختلفت وبعضها تطابقت مع نظيراتها السابقة من الدراسات. وأظهرت فروقاً إحصائية متباينة بين السائل الخصب والعقيم اذ تعزى خصوبة السائل لاحتوائه على محافظ الفقسة وحيوية الروئيسات الاولية المسؤلة عن تركيز هده المكونات فى السائل الخصب وانعدامها فى السائل العقيم أو إلى اختلاف في نسبة نمو الطفيلي والعمليات الأيضية للطفيلي أو خصوصية المضيف الوسطي ومكان تواجد الكيس فى الرئة أو الكبد أو حتى الإختلاف في العتر.

الكلمات المفتاحية: السائل العدري، خصب، عقيم، تحاليل كيموحيوية، الإنسان.