Comparative Study on Copper, Zinc, Magnesium and Iron in Hydatid Cyst Fluid ( Supernatant and Residue) in Sheep and Camel in Egypt

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ABSTRACT
Aim: this study included comparative biochemical composition of micro-minerals, including Cu, Zn, Mg and Fe in hydatid cyst fluid (supernatant and residue) from liver of infected sheep and lung of infected camels. Materials and Methods: Organs with hydatid cysts were collected from El-Basateen abattoirs, Cairo, Egypt, during the period from Nov. 2014 to Dec. 2015. Flame ionization by Atomic absorption spectroscopy was used for measuring the micro-minerals concentration. Results: the current study showed: significant difference in Mg, Cu and Fe micro-minerals between cyst’ supernatant (S Sh) and residue (R Sh) of infected sheep (p<0.001, p<0.05); significant difference in only Cu micro-mineral between S Sh and cyst residue (R C) of camels (p<0.001); significant difference in both Fe and Zn micro-mineral between cyst supernatant of infected camels (S C) and S Sh (p<0.05); significant difference in both Mg and Fe between R Sh and R C (p<0.01); significant difference in Mg, Fe and Zn between R Sh and S C (p<0.01, p<0.001) and significant difference in Fe and Zn between S C and R C (p<0.001, p<0.01). Cu was the highest while Mg was the lowest concentration of all tested minerals in both S Sh and S C but Fe was the highest and Zn was the lowest concentration of all tested minerals in both R Sh and R C. Conclusions: in the current work, the parasite that was located in both sheep and camel is characterized by having high levels of Copper in the cyst fluid and of iron in the scolecies. Binding a scolecidal drug with either copper or iron may enhance its efficacy.

Keywords: copper, zinc, magnesium, iron, hydatid cyst, sheep, camel, Egypt.

INTRODUCTION
Echinococcus, also called hydatid disease (Hydatidosis), is a zoonotic disease caused by the larval stage of Echinococcus. Echinococcus affects humans and other mammals, such as sheep, dogs, rodents and camels. Hydatidosis causes harmful, slowly enlarged cysts in the liver, lungs, and other organs that often grow unnoticed and neglected for years. The annual economic losses from echinococcosis are estimated to be approximately four billion US dollars on a global scale. These losses are due to disability and costs of treatment of infected humans as well as animal production losses due to death of infected animals, condemnation of internal organs of slaughtered life stock, reduction in carcass weight, decrease in milk production, fecundity and other production-based losses. Therefore, considering the importance of a problem, the World Health Organization and International Epizootic Bureau included echinococcosis in the list of the diseases which are the subject to radical eradication. The cyst can implant in various and all organs of the body including liver (70%), lung (22%), and other organs such as heart, brain, kidney, spleen, muscle, skeletal system, skin as well as many parts of the body (8%) . There are essential and vital elements in the cyst fluid that are very important in the biology of parasite. The composition of cyst content may differ in various area and strains. The composition of hydatid cyst fluid is nearly 90%, the same as host serum. The various electrolyte, enzymes, proteins, lipids, vitamins and hydrocarbons were seen in hydatid cyst fluid. The relationship between parasite and host is very important. Understanding how parasites can grow in the body and what the requirements of parasites are, can be useful in understanding the ways for prevention of the parasite. Mazzocco4 and Von brand et al.6 identified the various electrolytes in hydatid cyst fluid. McManus and Smyth7 detected large amounts of hydrocarbon molecules such as glycogen and polysaccharides in hydatid cyst fluid. Other researchers also detected urea and uric acid; total protein and nucleic acid; globulin like host, lipids compounds such as phospholipid...

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derivatives, monoglycerides, diglycerides, triglycerides and cholesterol as well as Ca, P, Mg, Na and K

The present study showed comparative biochemical composition of micro-minerals including: Cu, Zn, Mg and Fe in hydatid cyst fluid (supernatant and residue) of infected sheep and camels in Egypt. Identification of cyst fluid micro-mineral composition can help us to recognize different strains of parasite in one endemic area and to determine biological materials that can promote distribution of drug to the cyst through inoperative treatment.

MATERIALS AND METHODS
The present work includes comparative biochemical study on five liver and six lungs hydatid cysts that were collected from infected sheep and camel in El- Basateen abattoir, Cairo, Egypt. The hydatid cysts were identified according to the descriptions of the veterinarians in the slaughtered animals and were examined for degeneration and calcification. Generally, the cysts were recovered from the liver and lungs, illustrated in Table 1 for comparison of the levels of Mg, Zn, Cu and Fe in hydatid cyst fluid (supernatant and residue). Samples were collected carefully and kept in cool box containing crushed ice and transported to the Regional Center for Mycology and Biotechnology (RCMB) in Al-Azhar University, Cairo, Egypt. The length, width and diameter of each cyst were recorded (Table 1).

The cyst fluid was aspirated by sterile needle in aseptic condition and was centrifuged at 15000 rpm for five minutes then supernatant fluid was stored in -20°C until use for various biochemical parameters’ assessment. After collecting all samples, level of Cu, Mg, Zn and Fe in hydatid cyst fluid (supernatant and residue) of infected sheep and camels were measured using flame ionization by Atomic Absorption Spectrophotometer (model: GBC932AA). The study was approved by the Ethics Board of Al-Azhar University.

Statistical analyses were performed using analysis of variance (ANOVA) according to Woolson9. Significant difference between treatment’s means was determined by student-test. Data were presented as mean ±SD and P≤0.05 were considered statically significant.

RESULTS
The volume of hydatid cyst fluid has varied from 1-9 ml and cyst dimensions have also varied from 2-5 cm length and 2-3 cm width (Table 1). The level of the micro-minerals in hydatid cyst fluid isolated from infected sheep and camels are shown in Table 2 and figure 1. The micro-minerals have included Cu, Zn, Mg and Fe in hydrated cyst fluid (supernatant and residue) of these infected animals.

Level of Magnesium
In the present study, a very highly significant difference for level of Mg micro-mineral (P≤ 0.001) has been found between sheep’s cyst supernatant (S Sh) and residue (R Sh). Highly significant difference has also been found (P≤0.01) between R Sh in one hand and camels’ cyst supernatant (S C) and camels’ cyst residue (R C) on the other hand.

In addition, non-significant difference (P≥0.05), has been measured between S Sh and S C, between S Sh and R C and between S C and R C. (Table2; Fig1)

Level of Iron
Very highly significant difference for level of Fe micro-mineral (P≤ 0.001) has been found between R Sh and S C. Highly significant difference (P≤0.01) has also been recorded between R Sh and R C. Significant difference (P≤0.05) has been found between S Sh in one hand and R Sh and S C on the other hand.

Moreover, non-significant difference (P≥0.05) has been found between R C and S C. At the same time, non-significant difference has also measured between R C and S Sh. (Table 2, Fig 1)

Level of Zinc
Regarding Zn micro-mineral level, very highly significant difference (P≤ 0.001) has been recorded between S C in one hand and R Sh and R C on the other hand. Significant difference (P≤0.05) has also been detected between S C and S Sh.

On the other hand, non-significant difference (P≥0.05) has been found in case of Zn micro-minerals between S Sh and R C, between S Sh and R Sh and between R Sh and R C. (Table 2 and Fig 1)

Level of Copper
Regarding Cu micro-mineral level, very highly significant difference (P≤ 0.001) has been measured between S Sh in one hand and R C and R Sh on the other hand. Highly significant difference (P≤ 0.01) has been recorded between S C and R C.

Non-significant difference (P≥0.05) has been found when comparing R Sh in one hand with R C and S C on the other hand. Non-significant difference has also been found when comparing S Sh with S C. (Table 2, Fig 1)
Sorting the concentrations of all tested minerals in hydatid cyst fluid

Levels of Mg and Zn micro-elements are higher in hydatid cyst fluid of camel’s lung than its levels in sheep’s liver while Fe and Cu are higher in cyst of infected sheep’s liver than its levels in camel’s lung (Fig 1).

In supernatant of hydatid cyst fluid of both sheep and camels, the concentration of copper was the highest while the concentration of magnesium was the lowest. Iron was also the highest while zinc was the lowest in the residue of hydatid cyst fluid of both animals (Table 3).

DISCUSSION

In the present study, the naturally infection with hydatid cyst was recorded in the lung of camels and liver of sheep. This result was coincided with that obtained by Haridy et al. who found that the infection rate of hydatidosis was 4.8% in sheep, 1.2% in goats, 0.95% in cattle, 0.46% in buffalo, 18.9% in camels and human. These results were also coincided with that obtained by Hussaini et al. and Elmajdoub and Rahman who showed that the most commonly infected organ in sheep and cattle was the liver (46.03%; 52.9%, respectively). However, in the case of camel, the lung was the most commonly infected organ (55.2%).

Hydatid cyst fluid is clear or pale yellow, has a neutral pH and contains sodium chloride, protein, glucose, ions, lipids and polysaccharides. The fluid is antigenic and may also contain scolices and hooklets.

In the present study, variations in levels of micro-minerals (Cu, Zn, Mg and Fe) of hydatid cyst fluid were significant with regard to the genus of the host as well as the supernatant and residue of hydatid cyst fluid. The amount and proportion of mineral elements in all animal tissues vary widely. The biological functions of minerals (structural, physiological, catalytic and regulatory) are important. Minerals have been found to regulate cell replication and differentiation, for example, calcium influences signal transduction and zinc influences transcription adding to long-established regulatory roles, such as that of the element iodine as a constituent of thyroxin. Moreover, Minerals can act as catalysts in enzymatic and hormonal systems, as integral and specific components of the structure of metalloenzymes or as less specific activators within these systems.

In the current work, Mg level was found significant in cyst residue (R C) and cyst supernatant (S C) of infected camel’s lung compared to those of sheep’s liver residue (R Sh). The mean level of Mg in S C and R C were higher than in R Sh. Mg level was found highly significant higher in cyst supernatant of sheep’s liver (S Sh) than those of R Sh. However, Mg was the lowest of these studied elements in both S Sh and S C. Frayha et al. indicated that the amounts of Ca and Mg in protoscoleces of hydatid cyst were more than hydatid cyst fluid.

The mean level of Zn is significantly higher in S C than in S Sh. Also the mean level of Zn is highly significant in S C in one hand than in both R Sh and R C on the other hand. These differences in the micro-minerals levels may be because parasite uses electrolyte for production of calcareous body in cyst. However, Zn was the lowest of these studied elements in both R Sh and R C. Ozen et al. recorded that Cu and Zn levels in hydatid cyst fluid were significantly lower than serum levels.

The mean level of Fe is high significantly higher in R Sh than both S C and R C. The mean level of Fe is significantly higher in R Sh than S Sh but lower compared to S C. However Fe was the highest of these studied elements in both R Sh and R C. Sultan Sheriff et al. have showed the concentration of zinc in hydatid cyst fluid is higher than Sr, Cu, Fe, Cd, Ni, Cr and Co. The serum and hydatid cyst fluid of selenium, zinc and copper in patient and sheep with hydatid cyst were investigated earlier. They reported that there is a decrease in Se and Zn and a rise in Cu in sera of patient with hydatid disease.

The mean level of Cu is very higher in S C than R C. Also, the mean level of Cu is higher in S Sh than R Sh or R C. However, Cu was the highest of these studied elements in both S Sh and S C. Kojouri and Moshtaghi showed a negative effect of hydatidosis on hepatic copper concentration, which is a major site for copper reserving. Due to this hydatidosis could increase the copper concentration of serum via liver copper depletion and may interfere with determining the normal blood copper level in epidemic area. On the other hand, gravid cyst needs more copper for growth and binding the drug (Scolecidal) with copper may enhance its efficacy.

Moreover, differences in the metabolism of hydatid cyst from different intermediate hosts such as sheep, goats, cattle, camels and human is most probably due to complex geographical strains as well as their biochemical and physiological differences. In the current work, however, the micro-elements Fe and Mg were not significant regarding R C compared to
S C. Non-significant difference has also been found between R Sh and S C for the microelement Cu. Moreover, non-significant difference has been recorded between R Sh and R C or between S Sh and S C for the micro-elements Cu and Zn. Non-significant difference has been detected between S Sh in one hand and R C for Mg, Fe and Zn, R Sh for Zn and S C for Mg and Cu on the other hand. Ozen, et al. confirmed that Cu, Zn and Se levels were insignificant with regard to the genus of the host.

Some studies showed that mean levels of Ca, P, Na and K of infected sheep serum are less than the normal range. It may be because parasite uses electrolyte for production of calcareous body in cyst. Moreover, the analysis of liquid aspiration of suspended cyst and identification of minerals can be useful for hydatid cyst diagnosis from other non-parasitic cyst in humans.

Identification of cyst fluid micro-mineral composition can help us to recognize different strains of parasite in one endemic area and to determine biological materials that can promote distribution of drug to the cyst through inoperative treatment. In the current work, the parasite that was located in both sheep and camel is characterized by having high levels of Copper in the cyst fluid and of iron in the scolecies. Binding a scolecidal drug with either copper or iron may enhance its efficacy.

REFERENCES


Table 1: Shows volume of cyst fluid and cyst dimensions

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Cyst Volume (ml)</th>
<th>Cyst Dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Length (cm.)</td>
</tr>
<tr>
<td>S1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>S2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>S3</td>
<td>4.5</td>
<td>4</td>
</tr>
<tr>
<td>S4</td>
<td>5.5</td>
<td>3</td>
</tr>
<tr>
<td>S5</td>
<td>3</td>
<td>3.5</td>
</tr>
<tr>
<td>C1</td>
<td>4</td>
<td>4.5</td>
</tr>
<tr>
<td>C2</td>
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<td>C3</td>
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<td>C4</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>C5</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>C6</td>
<td>9</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*S1-5 are cysts from sheep liver and C1-6 are from camels’ lung.

Table 2: Showing compared levels of the Mg, Zn, Cu and Fe micro-elements in hydatid cyst of infected sheep’s liver and camel’s lung

<table>
<thead>
<tr>
<th>Metals (ppm)</th>
<th>S Sh</th>
<th>R Sh</th>
<th>S C</th>
<th>R C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>1.11±0.15</td>
<td>0.14±0.01***</td>
<td>1.43±0.27**</td>
<td>1.08±0.27***</td>
</tr>
<tr>
<td>Fe</td>
<td>3.98±0.47</td>
<td>6.07±0.58**</td>
<td>2.95±0.32**</td>
<td>2.20±0.34***</td>
</tr>
<tr>
<td>Zn</td>
<td>1.30±0.67</td>
<td>0.10±0.01</td>
<td>0.10±0.03</td>
<td>3.32±0.14***</td>
</tr>
<tr>
<td>Cu</td>
<td>3.60±0.70</td>
<td>2.05±0.39***</td>
<td>1.28±0.28</td>
<td>3.60±0.70***</td>
</tr>
</tbody>
</table>

R= residue, S= supernatant, Sh= sheep liver, C= camel lung.

* Significant difference from the control at P≤0.05, ** at P≤ 0.01 and *** at P≤ 0.001

Non-significant difference from the control at P≥0.05
Table 3: show the relation among concentrations of micro-minerals in different samples.

<table>
<thead>
<tr>
<th>animal</th>
<th>Organ</th>
<th>Cyst supernatant (Micro-minerals concentration)</th>
<th>Cyst residue (Micro-minerals concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>Liver</td>
<td>Cu &gt; Fe &gt; Zn &gt; Mg</td>
<td>Fe &gt; Cu &gt; Mg &gt; Zn</td>
</tr>
<tr>
<td>Camel</td>
<td>Lung</td>
<td>Cu &gt; Zn &gt; Fe &gt; Mg</td>
<td>Fe &gt; Mg &gt; Cu &gt; Zn</td>
</tr>
</tbody>
</table>