Serum Protein Profile Of Iraqi Hydatidosis Patients with Different Sites of Infection

Ban N.AL-Qadhi*

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Abstract:

Blood samples of One hundred and twenty patients from different hospitals in Baghdad infected with hydatidosis in different sites of the body (Liver, Lung, multiorgans and kidney) were collected for this study. On the other hand, 30 healthy individuals were included as a control group.

This study was conducted to evaluate the effect of this disease on the serum protein profile of the patients using electrophoresis.

The results revealed four different protein banding patterns with difference in number of bands and their molecular weights in comparison to the control group, and these differences depended on the site of infection. However the data showed a presence of the same band in all patients with different site of infection.

Introduction:

Cystic echinococcosis or hydatidosis is an infectious disease, caused by the larval stage of the cestode species Echinococcus granulosus [1]. In humans it is potentially dangerous depending on location, size and type of the cyst [2] .

The disease is characterized by the steadily growing fluid –filled, unilocular cysts surrounded by a two- layered hydatid cyst wall in the host internal organs, mostly liver followed by the lungs and, less frequently in spleen, kidneys, heart, bones and central nervous system [3].

Live hydatid cysts can rupture into physiologic channels, free body cavities or adjacent organs [4]. Some of these hydatid cysts cause spontaneous cutaneous fistulization which leads to biochemical changes in patients [5].

The human body contains different proteins; the number of distinct proteins within one cell is estimated at 3000 to 5000. More than 3000 different proteins can now be identified in plasma alone. Many of these have a specific biochemical role; organic disease may result when their concentration in plasma is reduced. Other plasma proteins, including most enzymes and tumor markers, have no known function in blood and arise as a result of cell death or tissue damage [6].

Performing serum protein electrophoresis is avaluable screening test since changes in certain bands are clearly associated with particular disorders [7].

Using the standard methods , the serum proteins arrange themselves into five bands : albumin travels farthest to the anode followed by $\propto 1$ –globulins ,$\propto 2$ – globulins , β- globulins and γ - globulins , although a 6th band (B2,C3,complement components) may be visible if the serum is fresh and if a buffer containing Ca+2 ions is used [8] .

Several principle plasma proteins can be grouped as acute- phase reactant (APR).The concentration of these proteins rises significantly during acute inflammation due to causes such as surgery, myocardial infarction, infections and tumors. Presumably, they all play a part in the very complex defensive process of inflammation [9].

*Department of Biology, College of Science, Baghdad University

Standard method separates the protein into 5 distinct zones, which comprise many individual proteins. By modifying the electrophoretic parameters, these fractions may be further resolved into as many as 12-zone. This modification, known as high – resolution electrophoresis (HRE), is accomplished by the use of higher voltage coupled with a cooling system in the electrophoretic apparatus and more concentrated buffer [10].

Thus, in an attempt to explain if there is any serum protein electrophoresis changes associated with the site of hydatidosis disease, this study was carried out.

Materials and Methods:

Subjects:

One hundred and twenty patients infected with hydatidosis from different hospitals in Baghdad were included in this study. Fifty – two patients with liver hydatidosis, 40 with pulmonary hydatidosis, 17 infected with more than one organ (multi-organs) and the last 11 patients were with renal hydatidosis, all patients with ruptured cysts. None of the patients had been pharmacologically treated for at least one year prior to this study. Neither atopic manifestations nor malignant disease were reported among the subjects studied.

On the other hand, thirty healthy volunteers were included as the control group. These had neither personal nor familiar history of hydatid disease and negative specific serology was confirmed by Indirect-heamagglutination test using a commercial kit (bioMerieux , France) .

Blood Samples:

Two ml of venous blood were collected from patients and healthy donors in plain plastic tubes and the serum was obtained by centrifugation at 4 °c, 400 × G for 10 minutes, and used freshly for electrophoresis.

Sera Protein Profile:

The sera were developed after electrophoresis as described by LKB [11], using coomasie brilliant blue staining.

After staining the protein bands, the migration distance of the calibration proteins were measured, the relative mobility (RM) was measured too:

$$RM = \frac{\text{Distance of protein migration}}{\text{Distance of dye migration}}$$

The calibration curve was constructed by plotting RM versus log molecular weight for calibration proteins Fig (1), and the molecular weight of proteins of interest was determined from the position of its RM value on the calibration curve.

Results:

By using conventional electrophoresis on polyacrylamide gel, serum protein profile revealed four different banding patterns, with difference in number of bands and their molecular weights (MW) and most of these differences occurred due to the difference in the site of infection as follows:

1) Control group:

Thirty healthy control (100%) showed nine bands, ranging from (0.015 – 0.476) RM with MW ranging from (794328.2 – 33884.4) da in comparison to the standard MW Pattern 1 Fig. (2).

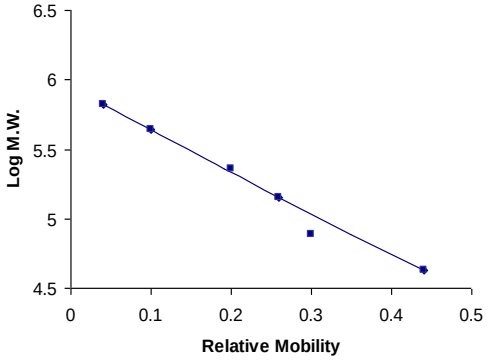

Figure (1): Calibration curve for approximate estimation of M.W. of varies standard proteins using conventional PAGE 7.5%.

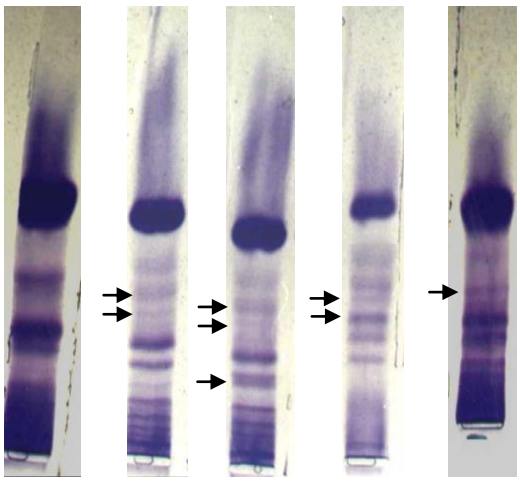

Figure (2): photograph of the electrophoretic patterns obtained with hydatidosis sera for protein profile.
Strips 1 control, 2 liver ,3 multi-organs,4 lung,and5 renal hydatidosis

2) Liver hydatidosis group:

Figure 2 pattern 2 showed 11 bands in 47 patients (90.38%), while the rest of the patients were similar to the control group. The elevation in band number was due to appearance of two faint different bands with MW (190546.07 and 74131.02) da, bands number 8, 9 respectively, in comparison to the control group.

3) Multi – organ hydatidosis group:

All patients (17) of this group showed those different bands that appeared in liver hydatidosis group with MW (190546.07-74131.02) da respectively. Also another different band appeared with MW (245470.87) da in only 14 patients (82.35 %) in comparison to the control group, band number was 6 Fig. (2) Pattern 3.

4) Pulmonary hydatidosis group:

This group showed very different banding patterns in comparison to the control group. Thirty –two patients (80 %) of this group showed disappearance of four normal bounds that appeared in all other subjects that were studied (patients and control) with bands number (2, 3, 4, 5) respectively, while the other eight patients were similar to control group. Also there was the appearance of two other different bands with MW (190546.07-74131.02) da in all patients of this group in comparison to the control group, bands number were (8, 9) respectively. Fig. (2) Pattern 4.

5) Renal hydatidosis group:

Figure 2 pattern 5 showed similar banding patterns to the control group in eleven patients (100 %) with renal hydatidosis. Seven patients (63.63 %) of this group showed another different band with MW (74131.02) da, band number 8.

Discussion:

Patients with simple or uncomplicated multivesicular cysts are usually asymptomatic. The clinical symptoms are related to pressure on adjacent organs or the presence of complications [4]. The hydatid disease can remain symptom-free for years or cause serious complications resulting in death. The main complications are rupture into the peritoneal cavity, infection, compression of the biliary tree, intrabiliary rupture, anaphylaxis and secondary hydatidosis [1].

This study reveals the first picture of serum protein profile of hydatidosis patients with ruptured cysts in different infected organs by electrophoresis.

Conventional electrophoresis on polyacrylamide gel has been used to differentiate between protein patterns in sera of hydatidosis patients and normal control subjects. A variety of dyes have been used to visualize bands and the comassie brilliant blue stain is the most sensitive stain [12].

Hydatidosis patients demonstrated four different patterns in comparison to the control pattern.The differences among hydatidosis patients occurred especially according to the site of infection.

The alternation in the position of protein bands, that happened in hydatidosis sera as the results revealed may reflect the altered gene expression [13], and the production of different protein bands with variable molecular weights in hydatid disease patients more than the normal bands, may have resulted to provide more protection for the host against the presence of the parasite. However the data support an interesting finding of the presence of the same band at 74131.02 daltons in all patients with different site of infection in comparison to the control group. This result enables us to consider this band as a marker for this disease,though such result need more studies

Finally, the electrophoresis patterns can give information about the relative increases and decreases in the protein population, as well as information about the homogenicity of a fraction. Therefore, it could be considered a useful tool to distinguish between hydatidosis patients concerning the site that is infected.

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References:

1. Sayek,I.,Tirnaksiz,MB. and Dogan, R.(2004) Cystic hydatid disease : Current trends in diagnosis and management .Surg.Today,34:987-996.

2. Murray ,R., Drew,W., Koyashi, G. and Thomson,J.(1990) Medical Microbiology. Mosby books Inc. New York(website).

3. Taherkhani,H. and Rogan, M.(2000) General characterization of Laminated layer of *Echinococcus granulosus*. IranJ. Med.Sci., 25: 95 -104.

4. Milicevic, MN.(2000) Hydatid disease. In:Blumgart LH, Fong Y, eds. Surgery of the liver and Biliary tract. London : W.B. Saunders company Ltd.

5. Kismet,K.,Ozcan,A.H.,Sabuncuoglu,M .,Gencay,C.,Kilicoglu,B.,Turan,C.and Akkus,M.(2006)Arare case:Spontaneous cutaneous fistula of infected splenic hydatid cyst.World J Gastroenterol.,12: 2633-2635.

6. Smith,A.,Beckett,G.,Walker,S. and Rae,P.(2000) Lecture notes on clinical biochemistry .6th ed. pp.82-83.Black Well Science , U.K.

7. Jeppson,J.,Laurell,C. and Franzen, B.(1979) Agarose gel electrophoresis. Clin.Chem.,25:629-638.

8. Karcher,R. and Nuttal,K.(1999) Electrophoresis .In:Tietz textbook of clinical chemistry . 3rd ed. pp.150-164. Burtis,C.A. and Ashwood, R.E. Saunders W.B.Co.London.

9. Nagashima,M. and Schreiber, G.(1984) Changes in specific proteins during Cu inflammation. Specific protein laboratory improvement program .Continuing education monograph .Am. Assoc. Clin. Chem.,1:1-8.

10. Bishope,M.,Duben- Engelkirk,J. and Fody,E.(2000) Clinical chemistry. 4thed. pp. 185. Lippincott Williams and Wilkins. Philadelphia. Baltimore .

11. LKB application note 306. (1977) SDS and conventional polyacrylamide gel electrophoresis with LKB 2117 multiphor .

12. Johnson,A., Rohfs, E. and Silverman ,M.(1999) Protein. In : Tietz textbook of clinical chemistry . 3rd ed. pp.477-530. Burtis,C.A. and Ashwood , R.E. Saunders W.B.CO.London .

13. Abe , H., Okuno, N. Takeda, O.(1994) Analysis on heterogeneity of squamous cell carcinoma antigen by two–dimensional electrophoresis. Electrophoresis , 15(7):988-91.

صورة بروتينات مصل المرضى العراقيين المصابين بالاكياس المائية في مواقع مختلفة من الجسم

بان نوري القاضي*

*قسم علوم الحياة/كلية العلوم/جامعة بغداد

الخلاصة :

جمع مصل دم 120مريضا مصابا بمرض الاكيس المائية في مواقع مختلفة من الجسم (كبد،رئة،اصابة اكثر من عضوواحد،كلية)من مستشفيات متعددة في بغداد.من جانب اخرتم اخذ مصل 30 شخصا سليما كمجموعة سيطرة .

تمت هذه الدراسة لمعرفة تاثير هذا المرض في صورة بروتين المصل Serum protein profile باستخدام الهجرة الكهربائية Electrophoresis اظهرت النتائج وجود اربعة انماط بروتينية مختلفة مقارنة بمجموعة السيطرة.وحصلت هذه الفروقات اعتمادا على موقع الاصابة وقد شملت هذه الاختلافات ظهور فروقات في عدد الحزم البروتينية وفي اوزانها الجزيئية.كذلك برزت في هذه الدراسة نتيجة مهمة هي وجود حزمة واحدة متماثلة في جميع المرضى المصابين في مواقع مختلفة من المرض عند الوزن الجزيئي74131.02 دالتون هذه النتيجة مكنتنا من اعتبار هذه الحزمة ربما تعمل كدليل لوجود الاصابة بالمرض.