A CASE STUDY OF HUMAN HEPATIC HYDATIDOSIS AND THE BIOCHEMICAL PROFILE OF CYST WALL AND FLUID.

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ABSTRACT:

The non specific haematological changes and negative Casoni's test suggest that ultrasonography is more reliable for the diagnosis and epidemiological studies of human hydatidosis. The major biochemical components like glycogen, proteins, nucleic acids, total lipids, triglycerides, cholesterol, free fatty acids and phospholipid fractions were analysed and compared with the other host's cysts. The differences were discussed in the light of strain variations in Echinococcus granulosus.

Key Words: Ultrasonography, Hydatid cyst, Detection, Biochemical composition, Strain.

INTRODUCTION:

Hydatidosis is a cyclozoonotic disease of cosmopolitan distribution. In India, a rising trend in the prevalence of human hydatidosis has been observed due to the increasingly close association with street dogs[1]. The sensitivity and specificity of various immunodiagnostic tests for hydatidosis have been comprehensively reviewed by many workers[2,3]. Feasibility of serological and ultrasonographic detection of hydatid cysts has been compared in human population, and it has been suggested that ultrasonography is superior to serological tests[4].Casoni's intradermal test is still being used for clinical diagnosis, however, its specificity and sensitivity is doubtful[5].

To date, a large number of intraspecific variants or strains have been reported from different parts of the world, involving different intermediate hosts by using various parameters like infectivity, biochemical composition and antigenicity[6,7,8]. But on human hydatidosis, such informations are scanty. The present communication deals with the suitability of diagnostic methods and the biochemical composition of human hydatid cysts / fluid.

MATERIAL AND METHODS:

A patient aged 18 years was admitted to the JNMC hospital with the complaints of abdominal pain, a lump on the right side, nausea, loss of appetite and weight. This uneducated villager belonging to low income group was an agricultural field worker, and had close association with dogs.

INVESTIGATIONS DONE ON THE PATIENTS:

(a) **Haematological:** Routine haematological investigations like total leucocyte count (TLC), differential leucocyte count (DLC), erythrocyte sedimentation rate (ESR), haemoglobin, blood urea, blood sugar, and serum creatinine were estimated[9,10].

(b) **Casoni's test:** The intradermal test for diagnosis of hydatid disease was employed by injecting 0.1 ml of cyst fluid. The reaction was considered positive as the formation of wheal was more than 5 cm in diameter in about 30 min. (Span Diagnostics Pvt. Ltd., Surat, India).

(c) **Ultrasonography:** Liquid paraffin or jelly was applied on the abdomen and the upper abdominal cavity was scanned by General Electric RT 3000 Scanner (Japan). Sonogramme was taken by multiformat camera and the cysts were identified in the liver.

(d) **Hydatid cyst handling:** Surgically removed cysts were brought to the laboratory and fluid was aseptically withdrawn with the help of a hypodermic syringe. The cyst mass was isolated and washed with HBSS before being used for biochemical assays. The fluid was centrifuged at 5,000 rpm to remove the debris.

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Biochemical assays: Various biochemical components of the hydatid cyst wall and fluid were estimated by spectrophotometric method. The glycogen, protein, RNA, DNA, total lipids, cholesterol, triglycerides, free fatty acids and phospholipids were extracted and estimated according to the methods described earlier[11]. Phospholipids were further fractionated by ascending thin layer chromatography[12] using chloroform: methanol: water (65:25:4 v/v) as solvent system. The fractions were identified after comparing their Rf values with standard phospholipids (V.P. Chest Institute, New Delhi), applied on the same plate. The data was subjected to statistical analysis[13].

RESULTS:

(a) **Haematological investigations:** The results of haematological investigations are given in Table I. The total and differential leucocyte counts were normal except for the increased level of eosinophils. Further, no detectable variation was noticed in creatinine and blood urea. Blood sugar and ESR levels were found to be considerably increased while haemoglobin was decreased.

(b) **The Intradermal Casoni's test** was found to be negative.

(c) **Ultrasonographic investigation:** The ultrasonographic images revealed a big echo-free area (shadow) on the right side of the abdomen containing several well defined rounded echo-free shadows (Fig. I). These observations suggest the cyst to be of polycystic type but the nature was not ascertained.

On surgery the cyst was removed from the liver which contained 22 daughter hydatid cysts. Out of these only one was found fertile containing about two hundred protoscoleces

(d) **Biochemical composition of cyst wall and fluid:** The results of biochemical estimations are presented in Table II. The total proteins followed by lipids were found maximum in the cyst wall and fluid. Among the known lipid fractions, triglyceride was found predominant, followed by phospholipids both in cyst wall and fluid (fig. II a). The levels of known phospholipid fractions were in the following order: Phosphatidylethanolamine > Phosphatidylcholine> sphingomyelin > Lysophosphatidylcholine >Lysophosphatidylethanolamine in cyst wall and Sphingomyelin>Lysophosphatidylethanolamine > Phosphatidylethanolamine > Phosphatidylcholine > Lysophosphatidylcholine in the cyst fluid (Fig - IIb).

DISCUSSION:

It is evident from the investigations of the patient that haematological changes can not be used for diagnosis, they being non-specific in nature. Increased number of eosinophils is a normal feature in most of the parasitic infections[14]. The increased ESR might be a consequence of binding of the immunoglobulins/complexes to RBCs[15].

The negative response of the Casoni's test further confirms the earlier assumptions that it does not always give positive response and all the positive cases were not confirmed by operation[5,16]. Hence, ultrasound detection can be used for epidemiological studies. Macpherson and coworkers[4], also suggested that ultrasonography is superior to and more reliable than the serological tests. However, the nature (fertile or sterile) of the cysts can not be ascertained. An early diagnosis of hydatid disease is essential in order to check the growth of cysts and to prevent the anaphylactic shock due to rupture of the cysts[17].

The biochemical analysis of the cyst wall and fluid revealed a diverse molecular heterogeneity. Among the major biochemical components, protein was found maximum in the cyst wall which may be due to accumulation of host's proteins including immunoglobulins[18,19]. Both DNA and RNA were found in the cyst wall due to presence of different types of nuclei and cytoplasm in the germinal epithelium[20].Comparatively higher level of RNA was seen because of the fact that RNA constitutes a marker for overall metabolic activities and asexual multiplication of protoscoleces in the germinal membrane. The high level of triglyc-

erides in the cyst wall may be involved, to fulfill the energy requirements for asexual multiplication[21]. The lipids were specially accumulated in the differentiated metabolic zones related to brood capsule insertions and the lipids from host origin could be hydrolyzed for use by the germinal layer and protoscoleces[22]. The occurrence of phospholipids indicates their possible involvement in membrane synthesis during growth of cysts.

The various biochemical components in the cyst fluid may be secretory/excretory exudate, formed by the lysis of the host tissue, as germinal membrane has been reported to be involved in controlling permeability and osmoregulation of the cyst and thus act as a filter[23,24]. In the present study, the protein concentration in the fluid was found more, compared to the levels reported from buffalo and goat[25,26], and less than the sheep hydatid fluid[27].Thus, it is possible that the human hydatid fluid may show better antigenic response than the hydatid fluid of goat and buffalo origin, as a relationship between the protien concentration of fluid and its binding capacity with antibodies has been reported[28].The higher amount of proteins in the fluid may be due to presence of immunoglobulins as their penetration through laminated layer into the fluid has been reported[29]. Further, quantitative and qualitative aspect of the antigenic polypeptides should be investigated from the cyst fluid of various hosts which might be useful in the development of a potential immunodiagnostic test.

The differences in the biochemical composition between human hydatid cyst and the reports available from other animals are probably related to the strain characteristics[7]. Therefore, biochemical investigations of the cyst wall and fluid from different hosts and habitats should be analysed and compared in order in ascertain the strain variations, antigenicity and infectivity to human population.

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TABLE - I

HAEMATOLOGICAL PROFILE OF A PATIENT OF HYDATID DISEASE.

Clinical investigations	Normal Value	Observed Value
Total W.B.C.	5,000-10,000 cells/cumm	10,000 cells/cumm
DLC		
Polymorphs	40-75%	46%
Lymphocytes	20-45%	30%
Eosinophils	1-6%	24%
Monocytes	2-10%	Nil
Basophils	0-1%	Nil
Erythrocyte Sedimentation rate (ESR)	3-15 mm/h	50mm/h
Creatinine	0.7 - 1.4 mg%	1.2 mg%
Blood urea	15-40 mg%	36mg%
Haemoglobin	13-18mg%	9.5 gm%
Blood sugar(Random)	80-`120 mg%	105 mg%
Casoni's Test	-	-ve

TABLE-II

BIOCHEMICAL COMPOSITION OF HUMAN HEPATIC HYDATID CYST WALL AND FLUID.

Biochemical components	Cyst wall+	Hydatid fluid++
Glycogen	0.14±0.02	0.01±0.00
Proteins	74.58±4.38	1.76±0.06
Lipids	2.83±0.13	0.52±0.09
RNA	1.92±0.23	0.18±0.00
DNA	0.05±0.00	-

Values are expressed as mg/g wet weight of tissue^{+}, and mg/ml of fluid^{++} ± SEM of three replicates. - Not detected.

Fig - I

Ultrasound images of unilocular hepatic hydatid cysts.

A. Ultrasonogram (sector scan) taken at 5.0 MHZ, scale 17: 34:34 cms, showing hydatid cyst (C) in liver (L) with multiple daughter cysts (arrow).

B. Ultrasonogram (Linear scan) of the same patient, showing hydatid cysts (C) in liver (L) with multiple daughter cysts (arrow).

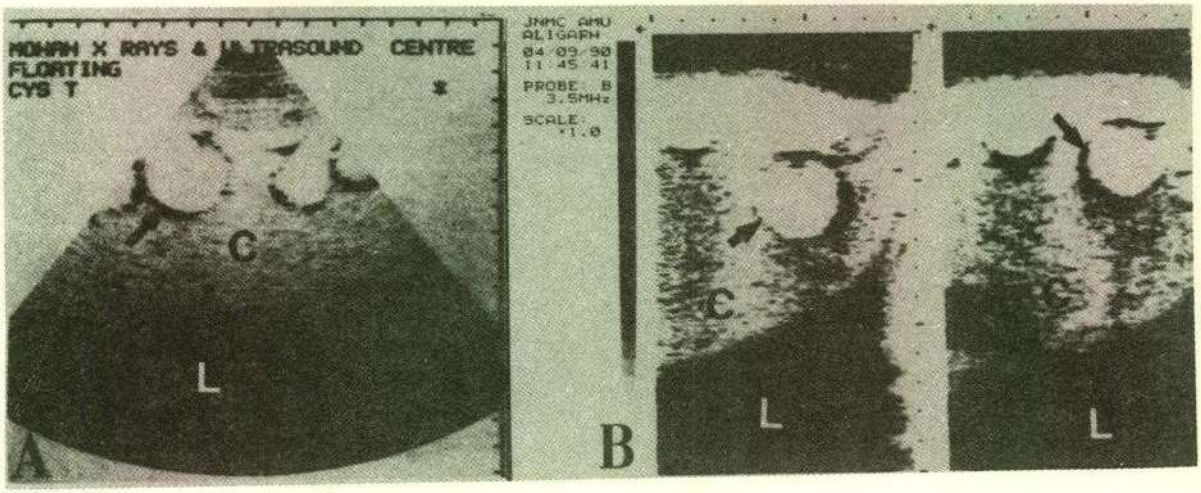

Fig - II

Lipid (a) and phospholipid fractions (b) of the cyst wall and cyst fluid from human hydatid cyst. A. Triglycerides, B. Phospholipids, C, cholesterol, D. Free fatty acids, E. Unidentified lipids, F. Lysophosphatidylcholine, G. Sphingomyelin, H. Phosphatidylcholine, I. Lysophosphatidylethanolamine, J. Phosphatidylethanolamine, K. Unidentified fraction.