

**Case Report****Cytomorphology of Hydatid Cyst: Comparison of Various Staining Techniques**

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**Abstract**

Human echinococcosis (hydatidosis, or hydatid disease) is caused by the larval stages of cestodes (tapeworms) of the genus *Echinococcus*. The disease is extensively distributed worldwide. The diagnosis of hydatid cyst infection is established on clinical grounds along with radiological findings. However, specific diagnosis is based on microscopic examination of the cyst fluid and by demonstrating the different hydatid elements such as protoscolices, hooklets or fragments of laminated membrane. Thus, for better visualisation of these elements, various stains (Papanicolaou, May – Grunwald Geimsa (MGG), Hematoxylin and eosin (H & E), Masson's trichrome (MT), Ziehl-Neelsen (ZN), Grams and PAS stains) were studied at our laboratory. Among these stains, Masson Trichome , Hematoxylin & Eosin, May – Grunwald Geimsa and Ziehl-Neelsen stains were more effective in staining and visualisation of the hydatid hooklets. We describe the cytologic features of a case of a hepatic hydatid cyst in a 70-year-old female patient where we conclude that due to the difficulty of staining and examining filter slides compared to sediment slides, we consider centrifugation preferable to filtration whenever the sample volume to be processed is suitable for the available centrifuge.

**Keywords:** *Echinococcus, Hydatid disease, Hepatic, Stains.*

**Introduction**

Hydatid disease or cystic hydatid disease (CHD) is caused by the larval form of *Echinococcus granulosus* ("*Taenia echinococcus*"). The larval form was first discovered by Goeze in 1782. Although the hydatid disease is world-wide in distribution<sup>[1]</sup>, it is most commonly found in those countries (e.g., Australia, New Zealand, Tasmania, Middle East countries, Turkey, Greece, USSR, Cyprus, Latin America and the Far East etc.)<sup>[2]</sup> where sheep and cattle-raising constitutes

an important industry and consequently, there is a close association between man, sheep and dog. It is more a disease of temperate climates than of tropical areas.<sup>[1]</sup> Cestodes of genus *echinococcus* are also known to exist in India where the highest prevalence is reported in Andhra Pradesh and Tamil Nadu than in other parts of the country.<sup>[2]</sup> The definite diagnosis of hydatid cyst is confirmed by microscopic examination of the cyst fluid and demonstrations of the presence of protoscolices, hooklets, & fragments of the

laminated membrane. The visualization of these elements becomes more important when these are absent or scarce or when there is presence of heavy amorphous debris in the background.<sup>[3]</sup>

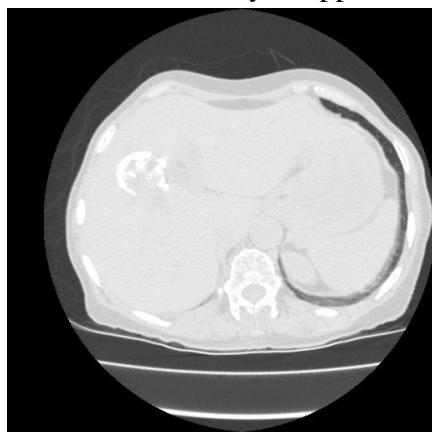
### Case Report

A 70-year-old female patient residing in Navi Mumbai and native of Bihar came with complaints of pain in abdomen (on & off) since one year but had aggravated since a day. The pain was sudden in onset, generalised and non-radiating. No other significant past history noted. Physical examination revealed tenderness in right hypochondriac region and hepatomegaly.

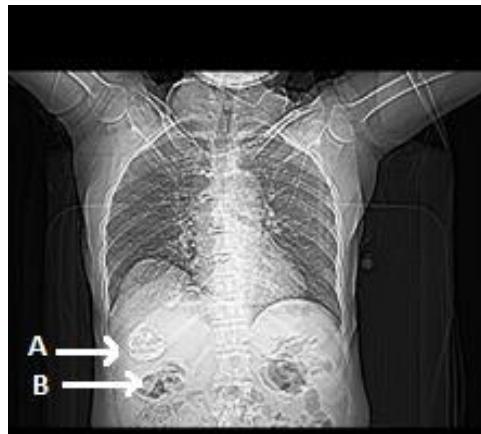
### Investigations

Hematological and biochemical investigations were within normal limits.

Serologic testing for hydatid cysts was not performed. Ultrasound abdomen showed hepatomegaly and two cysts in liver measuring 1) 7x5.9cm hyperechoic area with multiple anechoic cysts within it. 2) 3.5cm calcified cyst. Abdominal computed tomography showed the presence of cysts in segment VII and VIII of liver with first cyst in active stage and second in inactive stage along with calcification (Figure 1 & 2). A right lobe PAIR<sup>[4]</sup> (puncture, aspiration, injection, re-aspiration) along with surgery was performed, the resected specimen was received in histopathology & cyst fluid for cytological examination. The cyst fluid was whitish and hazy in appearance.

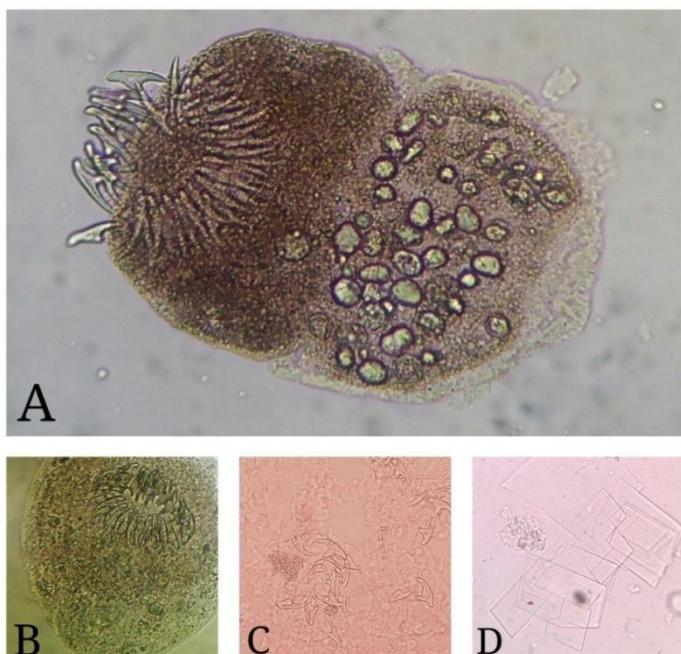


**Figure 1:** Abdominal CT scan showing calcified hepatic hydatid cyst in segment VII.



**Figure 2:** A scout film reveals two hepatic hydatid cysts; A. In inactive stage with calcification, B. In active stage

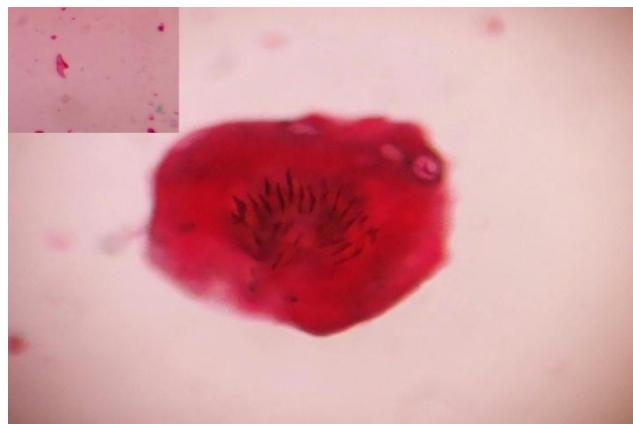
The wet mount showed multiple protoscolices in the shape of mushroom with hooklets & calcareous corpuscles. Amorphous debris, occasional inflammatory cells, detached hooklets & cholesterol crystals were also seen.<sup>[3,5,6]</sup> (Figure 3-A to D)



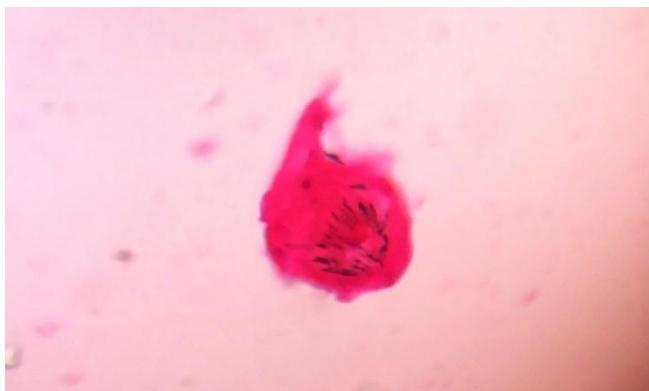
**Figure 3:** WET MOUNT – A. Protoscolex in the shape of a mushroom with radially arranged hooklets and calcareous corpuscles (evaginated hooklets from the vesicle);  
B. Protoscolex with invaginated hooklets within the vesicle;  
C. Dispersed refractile hooklets;  
D. Cholesterol crystals.

The fluid was centrifuged, smears were prepared & stained with Papanicolaou, May – Grunwald Geimsa (MGG), Hematoxylin and eosin (H & E), Masson's trichrome (MT), Ziehl-Neelsen (ZN), Grams and PAS stains.

Microscopic examination revealed scolices which were round to oval structures with radially arranged hooklets along with plenty of detached hooklets & amorphous debris.<sup>7</sup> The various stains were performed and the microscopic features are as follows:



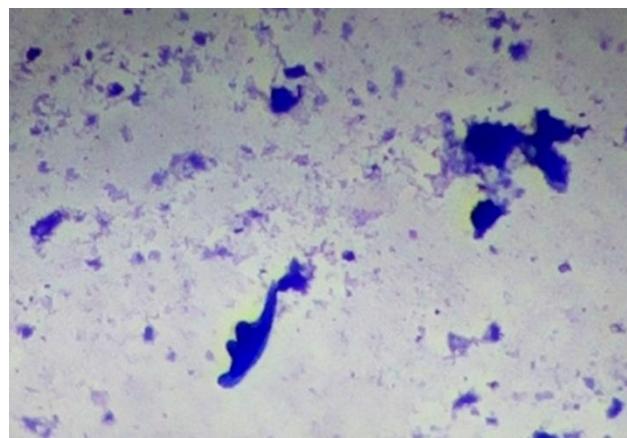
**Figure 4:** Protoscolex with purple-blue hooklets; inset shows a better visualised hooklet; (Masson trichrome stain, x40)



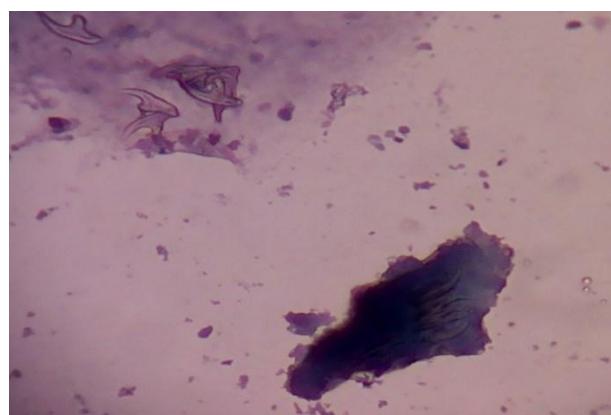
**Figure 5:** Protoscolex with purple-blue hooklets; (Hematoxylin & eosin, x40)



**Figure 6:** shows single light pink colour hooklet (Ziehl - Neelsen Stain, x40)

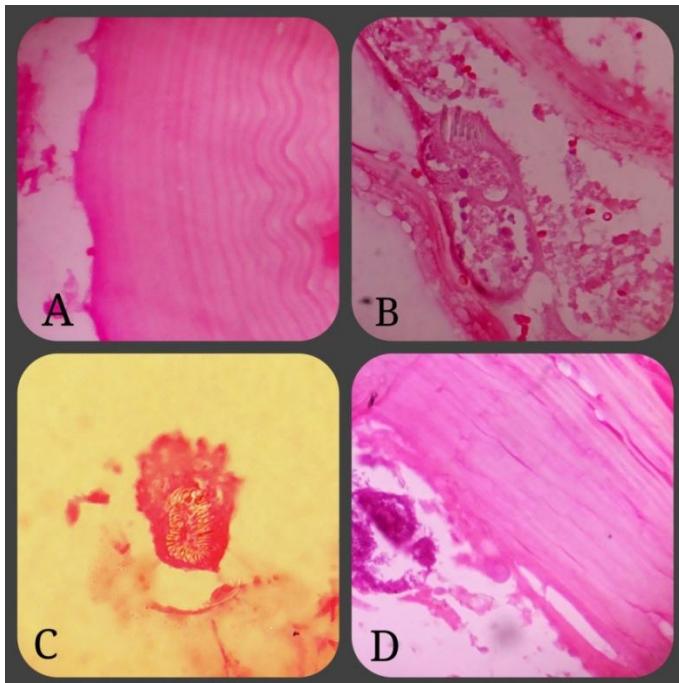


**Figure 7:** Single hooklet stained blue in colour along with a cellular laminated membrane on a background of amorphous debris. (MGG, x 40)



**Figure 8:** Protoscolex with hooklets (unstained) and scattered sickle-shaped hooklets. (Pap, x40)

We also received specimen labelled as cyst wall containing multiple greyish white gelatinous tissue bits aggregating to 4cms. Histopathological examination showed laminated chitinous membrane along with protoscolices & hooklets.<sup>[7]</sup>



**Figure 9: Histopathology:**

- A. Laminated membrane with parallel striations;
- B. Protoscolex with hooklets;
- C. Protoscolex with unstained hooklets;
- D. Cyst wall composed of acellular chitinous membrane with dystrophic calcification. (H & E stain, x40).

## Discussion

Human echinococcosis is a zoonotic disease (a disease that is transmitted to humans from animals) that is caused by parasites, namely tapeworms of the genus *Echinococcus*. Echinococcosis occurs in 4 forms:

- cystic echinococcosis, also known as cystic hydatid disease (CHD) or hydatidosis, caused by infection with *Echinococcus granulosus*;
- alveolar echinococcosis or alveolar hydatid disease (AHD), caused by infection with *E. multilocularis*;
- polycystic echinococcosis or polycystic hydatid disease (PHD), caused by infection with *E. vogeli*;
- unicystic echinococcosis, caused by infection with *E. oligarthrus*.

The two most important forms, which are of medical and public health relevance in humans, are cystic echinococcosis and alveolar

echinococcosis.<sup>[8]</sup> The most commonly affected organs are the liver and the lung. Most cysts remain clinically silent and are diagnosed incidentally or when complications occur.<sup>[8,9]</sup>

*Echinococcus granulosus* is a small, hermaphroditic tapeworm about 3 - 5 mm in length. The tapeworm comprises 3 - 4 segments and lives in the upper small intestine of the definitive canine host. The eggs produced by the mature tapeworm contain an embryo that has 3 pairs of lancet-shaped hooklets. The contaminated faeces are ingested by the intermediate host (humans are accidental intermediate hosts), in which the eggs hatch and the embryo migrates through the intestinal wall into the portal system. Most embryos lodge in the liver, mainly the right lobe due to preferential portal flow, where they either die or develop into hydatid cysts within months to years. Approximately two-thirds of patients develop liver cysts. The embryos may escape this first filter and lodge in the capillaries of the lung where they develop. A small percentage of embryos may find their way into the systemic circulation, where they may involve brain, bone or any other site. Most patients (approximately 80%) have single-organ involvement. The life cycle is complete when the definitive host ingests infected offal containing hydatid cysts.<sup>[4]</sup>

## Pathology

The developing hydatid cyst has three layers. The outer pericyst is composed of host fibroblasts, eosinophils, giant cells and modified hepatocytes. The middle laminated membrane is acellular and impermeable to bacteria, and the innermost layer, the germinal layer or brood capsule, is translucent and is the origin of scolices and daughter cysts within the primary cyst. The cyst usually contains crystal-clear fluid which is strongly antigenic and may cause anaphylaxis if released into the circulation of the host. Most cysts remain silent when small and present only when complications such as rupture into the biliary tree, bacterial super infection or free intra-abdominal rupture occur.

Owing to the lack of symptoms in the early stages, the actual accurate assessment of the growth rate of these cysts is difficult.<sup>[4]</sup>

### **Signs and Symptoms**

Human infection with *Echinococcus granulosus* leads to the development of one or more hydatids located mainly in the liver (55-70%)<sup>[9]</sup> and lungs (18-35%)<sup>[9]</sup>, and less frequently in the bones, kidneys, spleen, muscles, central nervous system, and eyes. In the majority of cases, the disease remains latent for many years and its presence is only detected at autopsy or by its pressure effects on the surrounding tissues or when the cyst ruptures or suppurates. Non-specific signs include anorexia, weight loss and weakness. Abdominal pain, nausea and vomiting are commonly seen when hydatids occur in the liver. If the lung is affected, clinical signs include chronic cough, chest pain and shortness of breath. Larval metastases may spread either to organs adjacent to the liver (e.g. the spleen) or distant locations (lungs, brain) following dissemination of the parasite via the blood and lymphatic system. If left untreated, alveolar echinococcosis is progressive and fatal. Rupture of a hydatid cyst is associated with anaphylactic symptoms and formation of localised or generalised secondary echinococcosis.<sup>[8]</sup>

The cytomorphological diagnosis is made by identifying the presence of Hydatid elements, especially hooklets. Even when fragments suggestive of a laminated membrane are present on smears without protoscolices, a diagnosis of hydatid cyst should be considered.<sup>[9]</sup> In old lesions, where hydatid elements are difficult to visualise, various stains which are available at the laboratory can be used to identify them. In the present study, various stains (Papanicolaou, May – Grunwald Geimsa (MGG), Hematoxylin and eosin (H & E), Masson's trichrome (MT), Ziehl-Neelsen (ZN), Grams and PAS stains) available at our laboratory were used for identification of hydatid components.

Trichrome stain (Fig 4), H & E (Fig 5) and May Grunwald Giemsa (Fig 7) stains were more effective than Ziehl-Neelsen stain (Fig 6). The hooklets were stained purple-blue by trichrome and H & E stains, blue by Giemsa and light pink in colour by Ziehl-Neelsen stain.<sup>[9]</sup> The cyst membrane took up the periodic Schiff (PAS) stain. The PAP (Fig 8) and Grams stains failed to stain the hooklets. They appear as semi-translucent, refractile, sickle-shaped or triangular in shape.<sup>[10]</sup> The wall of the cyst was grayish white. Histopathological examination revealed laminated membrane along with protoscolices with unstained hooklets. Single hooklets along with dystrophic calcification were also seen.<sup>[7]</sup> (Fig 12-A to D).

Before staining the smears, it should be mandatory to see the wet mount preparations of hydatid fluid. In the present case, many protoscolices with radially arranged hooklets, calcareous corpuscles, dispersed hooklets, cholesterol crystals and amorphous debris were noted.<sup>[5]</sup> G.Adams, D.J.J.Bezuidenhout in their case report of "Hydatid cysts simulating massive ascites" revealed a clear fluid mostly showing cholesterol crystals.

### **Conclusion**

*Echinococcus granulosus* infections remain silent for years before causing its symptoms. It is a clinical problem worldwide. Early diagnosis and referral is of prime importance in the management of the patients. Hence, special emphasis on various stains is made for better visualization and confirmation of Hydatid elements.

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No submission of same work anywhere else.

No conflicts of interest

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