

CASE REPORT

Enhancing Cytological Detection: Evaluating Various Staining Techniques for Demonstrating Hydatid Hooklets

Yamini P M¹, Shobini Vishali¹, Volga Harikrishnan¹

¹ Department of Pathology, Saveetha Medical College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Thandalam - 602 105, Tamil Nadu, India.

ABSTRACT

Echinococcosis, a zoonotic disease caused by *Echinococcus granulosus*, commonly affects the liver and lungs, presenting as solid lesions often mistaken for tumours. A middle aged female with abdominal pain and loose stools was diagnosed via MRI with a hydatid cyst, confirmed by aspiration cytology, revealing protoscolices and hooklets. Surgery followed by histopathological examination confirmed the diagnosis, employing stains like Leishman, Ziehl Neelsen (ZN), Papanicolaou (PAP), Grocott Methamine Silver (GMS) stain, and Hematoxylin and Eosin (H&E) stains to visualize hydatid hooklets and *Echinococcus granulosus* morphology. Smears displayed occasional scolices and hooklets in a dirty background. Ziehl Neelsen stain showed acid-fast positive hooklets. Histopathological specimens exhibited acellular laminated membrane, detached hooklets, and calcified debris. Leishman stain proved effective for hooklet detection under low power, suggesting its utility in routine diagnostics. This highlights the importance of specialized staining techniques for accurate diagnosis and underscores Leishman stain's efficiency in detecting hydatid hooklets.

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Corresponding Author:

PM Yamini, MD Pathology
Email: pmyamini17@gmail.com
Tel : +91 8220954820

INTRODUCTION

Hydatid disease is caused by *Echinococcus granulosus* commonly called the dog tapeworm, which belongs to cestodes of family Taeniidae. The adult worm was discovered by Hartmann (1695) and the larval form by Goeze (1782). Echinococcosis has a worldwide distribution with a global annual incidence rate of 1–200 per 100000. (1)

Hydatid cysts may occur in any organ of the body with the liver being the predominant site of occurrence (60-70%), followed by the lung (20-25%). Unusual locations of involvement include musculoskeletal, renal, splenic, neurological and orbital region (2). The clinical manifestations depend upon the local signs like visible swelling if the cyst is located superficially. In most cases, the disease remains latent for many years which are then detected only during autopsy.

Understanding of its quantifiable characteristics,

identification and treatment is necessary. Usually, intervention with needle for cytology aspiration is not done in view of probable type 1 allergic reaction (3) which makes it challenging for cellular pathologists to gather expertise in cases where there is a suspicion of hydatid cyst. Cytologically, hydatid cysts frequently contain juvenile scolices, rostellar hooklets, and cuticular layer fragments. (2)

Though the Leishman stain is the routinely used one, we studied the cytological smears under various stains to study the morphology of the organism and to know the utility of various stains for a better and quicker diagnosis.

CASE REPORT

A woman in her late forties was suffering from symptoms of pain in the abdomen and loose stools for 4 days. Her hemogram, urine analysis and liver parameters were all normal except for a mild increase in alkaline phosphatase levels. CT abdomen revealed a hydatid cyst (Fig.1) following which guided FNAC was done. Fluid analysis showed few protoscolices with hooklets of hydatiform larvae. Various stains were used for a better appreciation of hooklets and protoscolices. After confirming the diagnosis, open hydatid cyst deroofing

with marsupialization and omentoplasty was done and the histopathological specimen shown in Fig.2.

Materials and methods: FNAC sample obtained was looked for organisms under various techniques like wet mount, Leishman’s stain, Ziehl Neelsen stain (ZN stain), PAP stain (Papanicolaou stain), GMS stain (Grocott methamine silver stain) and using Hematoxylin and Eosin stain (H&E stain) to study the utility of various stains for better visualization of hydatid hooklet and the morphology of Echinococcus. Conventionally air-dried smears were used for Leishman’s stain and alcohol-fixed smears were used for H&E and Pap stains. Air-dried and heat-fixed smears are used for ZN stain.

Results: Fig.1a showing a CT scan of the patient revealed a well-defined cystic lesion that is unilocular with a hyperdense matrix and multiple small vesicular cysts (daughter cysts) resembling a spoke-wheel pattern. Fig.1b demonstrates a cyst with multiple daughter cysts within in a post-operative specimen. Fig.1c and 1d is a Wet mount demonstration of protoscolices and hooklets. Leishman’s stain (Fig.2a) shows refractile hooklets in a dirty background. Hooklets appear black and pink-red under Grocott Methamine Silver (GMS) stain (Fig.2b). Papanicolaou (PAP) stain (Fig.2c) demonstrates hydatid hooklets and laminated membrane (Fig.2d). Hooklets appear pink-red in Ziehl Neelsen (ZN) stain (Fig. 3a). Hematoxylin and Eosin stain (Fig.3b) in histopathological specimen shows acellular laminated membrane and refractile hooklets in a background of fine calcified debris and necrosis.

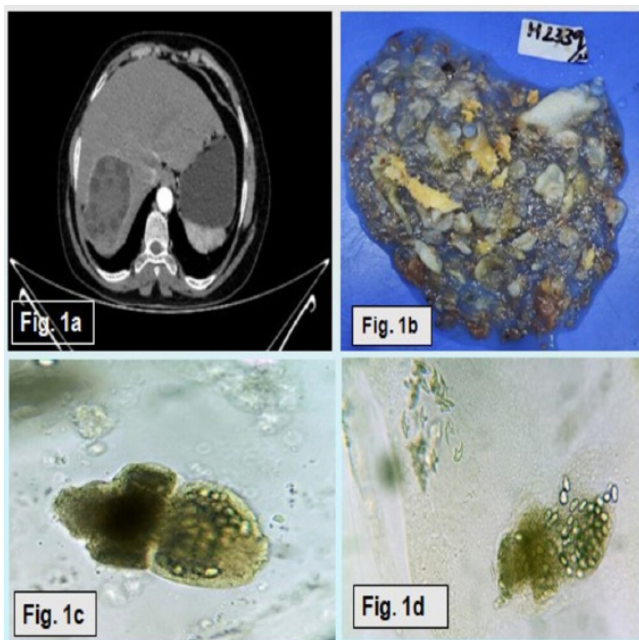


Fig 1: a) CT scan showing an unilocular cystic lesion with hyperdense matrix with multiple tiny cysts within b) Histopathologic specimen showing a cyst with multiple daughter cysts within c,d) wet mount demonstration of protoscolices and hooklets (10x)

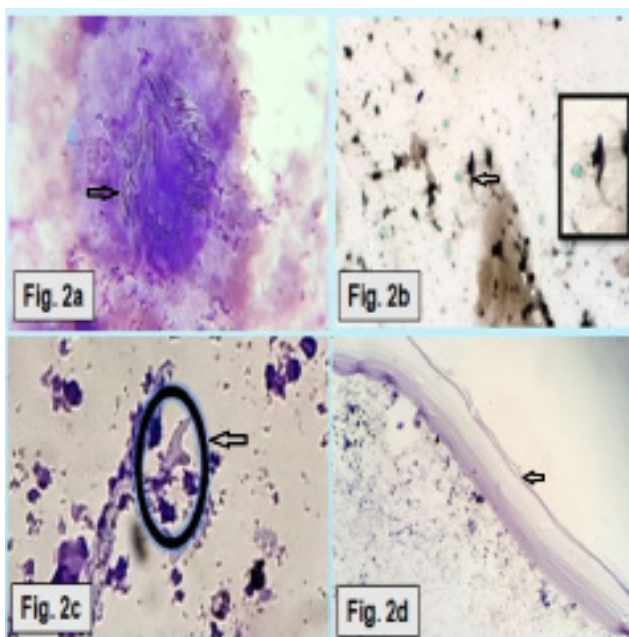


Fig 2: a) Leishman stain (20x) showing refractile hooklets in a dirty background. b) GMS stain (40x) showing hooklets in black. c,d) PAP stain showing hydatid hooklets and laminated membrane under 40x

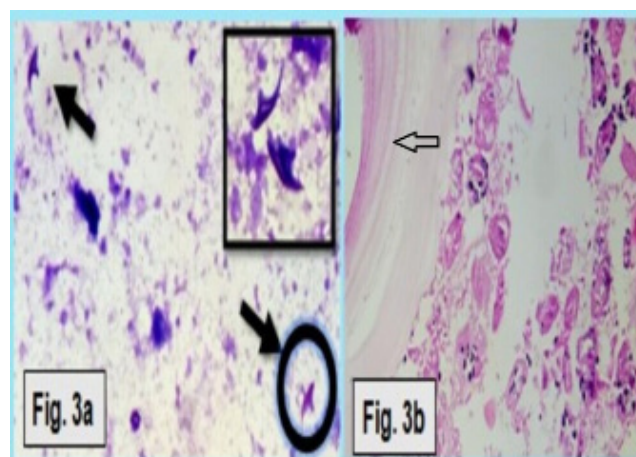


Fig 3: a) Ziehl Neelsen stain (40x) showing hooklets in pink-red color b) Hematoxylin and Eosin stain showing laminated membrane and detached hooklets under 40x in histopathological specimen

DISCUSSION

The adult worms of *E. granulosus* are found in large numbers in the mucous membranes and small intestines of infected dogs, whereas in humans, the larval form enters the liver through the portal vein and causes an unilocular hydatid cyst. Although hydatid disease has a worldwide distribution, it is more commonly found in cattle and sheep-raising countries. The endemic regions include the Mediterranean region, Africa, South America, Australia, the Middle East and India (4). The definitive host is dog and other canine animals and the sheep appears to be the optimum intermediate host. Man being an accidental intermediate host, is infected by ingestion of food contaminated with eggs of *Echinococcus* from dog’s faeces.

Hepatic hydatid cysts can cause organomegaly, conjugated hyper bilirubinemia following obstruction, and inflammation of biliary tract. The diagnosis on CT and MRI may be suggested by the presence of an undulating membrane and several daughter cysts inside a mother cyst. Serum albumin and alp levels were significantly increased in a study done by Zhijia et al (5), who also presented a remarkable correlation and significant increase in inflammatory parameters like platelet distribution width, eosinophil count, neutrophil-lymphocyte ratio (NLR ratio), alkaline phosphatase to platelet ratio (APPR). They have also found that elevated Platelet Distribution Width (PDW) and APPR returned to normal levels after treatment with surgery. Eosinophilia is a significant marker for parasitic infections in general, but in cases of hydatid cysts, only complicated cases showed an increase in eosinophil count. In our case, eosinophil levels were found to be normal.

The identification of hydatid cysts using ultrasound-guided FNAC has been demonstrated to be simple, affordable, and safe. It is also helpful as a screening test for deep-seated lesions in the diagnosis of clinically suspected malignancy (4). Imaging studies, serology and cytology help diagnose the hydatid cyst and preliminary blood investigations like complete blood count might reveal eosinophilia which prompts some parasitic infestations.

Cytological studies show the presence of hooklets, scolices and laminated membranes all of which point towards diagnosis of hydatid disease. Gram stain and AFB staining helps identifying the hooklets which are distinctive in nature but not much common, so that it saves the need for better lighting conditions to facilitate the search. The cuticular layer fragments, mostly missed or misinterpreted as mucus or artefacts can be recognized by PAS, AFB and GMS and orients towards the correct diagnosis (2). The refractile hooklets were visible only under higher power of PAP and H&E smears.

Hydatid cysts of the liver can also mimic liver abscesses, both of which have non-specific clinical manifestations like fever, abdominal pain, nausea and vomiting which can simulate either neoplasms or cysticercosis. If left untreated, hydatid cysts can get complicated as a septic foci or rupture followed by peritonitis which can lead to potentially fatal anaphylaxis.

The chances of secondary complications like intraoperative seeding of parasite and recurrence of the disease can be reduced by complete excision of the hydatid cyst along with preoperative albendazole, which is the main stay of definitive treatment.

CONCLUSION

Serology and imaging examinations can be used to identify hydatid disease, however, they are not conclusive because of their low sensitivity and specificity. By showcasing distinctive characteristics such as hyaline fragments and laminated cyst wall membrane, as well as confirmed findings like scolices or hooklets in the smears, cyst aspiration can aid in diagnosing the hydatid cyst perioperatively. Postoperative diagnosis is made by histopathological examination. Leishman stain being the most commonly used one in all laboratories, is very useful to identify the refractile hooklets compared to other stains.

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