Research Article

Leishman stain in detection of hydatid hooklets in liver space occupying lesions

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ABSTRACT

Background: Hydatid disease (HD) is endemic in India. Human beings are usually infected as intermediate host when they ingest contaminated food or water by the egg of *Echinococcus granulosus*. HD usually presents as cysts in various organs of the body. Often the disease presents as solid lesions in liver and ultrasonographically confused with hepatic tumors. These solid lesions can be easily diagnosed by ultrasound guided fine needle aspiration cytology (FNAC) with a demonstration of the presence of hooklets of hydatid. **Objective:** To study the utility of Leishman stain for detection of hydatid hooklets. **Materials and Methods:** Ultrasonographically (USG) diagnosed 340 patients with liver SOL were included in the study. Ultrasound guided FNAC was done and smears from the aspirate were stained with Papanicolaou (Pap), hematoxylin-eosin (HE) and Leishman Stain. **Results:** Out of 340 patients with hepatic SOL subjected to USG-FNAC six cases were diagnosed as HD. Aspirates were stained with Pap, HE, and Leishman stain. Identification of hooklets was regarded as pathognomonic. The hooklets were not clearly visible under lower magnification, and the typical glistening hooklets were visible under high power objective with Pap and HE stained smears, whereas in Leishman stained smears the hooklets were clearly identified under both low power and high power objective. **Conclusion:** Leishman stain, a common stain of all laboratories, is a useful stain for cytodiagnosis and can be used successfully to detect the hooklets of hydatid under the microscope.

KEY WORDS: Hydatid Disease; Hooklets; Leishman Stain

INTRODUCTION

Hydatid disease (HD) caused by *Echinococcus granulosus* is endemic in the cattle and sheep-raising regions of the underdeveloped and developed world.[1] It is endemic in India, as well as other parts of the world, including the Middle East, Africa, South America, New Zealand, Australia, Turkey, and Southern Europe.[2]

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Human beings are usually infected as intermediate hosts when they ingest egg contaminated food or water. Infestation by HD in humans most commonly occurs in the liver (70%), but also in the lungs (20%), and 10% of cysts can occur almost anywhere in the body.[2]

Hydatid cysts in imaging studies can be confused with hepatic tumors, abscesses, cystadenomas, liver cysts, or other lesions. Three-quarters of the infected individuals develop one or more hepatic cysts; the majority occur in the right lobe.[2] Guided aspiration is a simple, safe and cost-effective screening test for deep-seated lesions with a significant role in the detection of clinically unsuspected malignancy making it an investigation of choice for early confirmation or exclusion of pathology.[3]

Fine needle aspiration cytology (FNAC) has been used as a diagnostic tool for human echinococcosis since the late

1980s. This technique is usually performed under ultrasound guidance and is useful in the pre-operative diagnosis of HD by demonstrating the diagnostic hooklets and scolices in the aspiration fluid, which are pathognomonic.[4] Conventionally Direct Air dried smears from the aspirated material are stained with May-Grunwald Giemsa (MGG) stain and alcohol fixed smears are stained with the Papanicolaou (Pap) and hematoxylin-eosin (HE) stain for cytodiagnosis.[1]

Leishman stain is a simple, quick and inexpensive staining technique easily available in both urban and rural laboratories which can be used as an efficacious tool to interpret cytosmears. We have been using Leishman stain to interpret cytosmears for more than 30 years.[5-8]

With this background, this study was aimed to assess the utility of Leishman stain for diagnosis of HD.

MATERIALS AND METHODS

A retrospective analysis of records of consecutive patients attending a pathology laboratory for ultrasound-guided FNAC was performed for 4 years from August 2012 to July 2016.

All the ultrasound-guided FNAC smears were stained with Pap stain, HE stain, and Leishman stain. Stained smears were examined under the microscope. Histopathology confirmation was done wherever required.

HE Stain**:** Air dried FNAC smears were fixed with ethanol followed by cleaning under running water. The slides were stained by 10 dips in freshly prepared hematoxyline. Extra stain was removed by running water following counter staining with Eosin. The slides were cleaned, dried and mounted before examination under the microscope.

Leishman stain: Air dried FNAC smears were kept on the staining tray, and Leishman stain was poured in a manner similar for blood smear staining. After 30 seconds, an equal amount of distilled water was added and kept for 5-7 minutes. The slides were cleaned with running water, dried and examined under the microscope.

We reviewed Pap, HE, and Leishman stained smears from hepatic space occupying lesions (SOLs) of patients which were diagnosed as hydatid cysts. The presence of hooklets on a necrotic and degenerated background was considered as pathognomonic for diagnosis of HD (Figure 1).

RESULTS

The study included 340 patients during a period of 4 years from August 2012 to July 2016. All the cases who presented with hepatic SOL were subjected to ultrasound-guided ultrasonography (USG)-FNAC. Out of 340 cases, 80 cases (24.11%) were diagnosed as hepatocellular carcinoma, hepatoblastoma was diagnosed in eight cases (2.35%), and metastatic carcinoma of the liver was diagnosed in 226 cases (66.47%). 24 cases of benign lesions were identified out of which six cases were diagnosed as HD (Table 1).

The patients of HD were in the age range of 29-60 years and all were male. All the patients were non-vegetarian and from rural background. In all the cases, the right lobe of liver was affected with well-defined hypoechoic lesions. The diameter of the lesions varied from 1.5 to 5.2 cms. Four cases were diagnosed as hepatoma and two cases as HD under USG. Aspirates from all the cases were necrotic in naked eye examination (Table 2).

Hemogram and biochemical parameters of all the cases were within normal limits. However, serum was positive for IgG anti-Echinococcus antibody in all six cases of HD.

In all the cases, the microscopic examination revealed the presence of hooklets scattered on a degenerated necrotic background. Few lymphocytes were, however, present too.

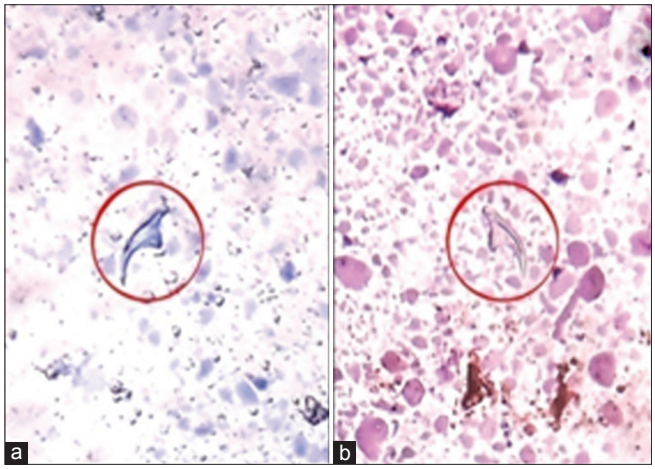

Figure 1: (a) Photomicrograph is showing hooklet of hydatid. (Leishman, ×400); (b) photomicrograph showing hooklet of hydatid (hematoxylin-eosin, ×400)

Table 1: Cytodiagnosis profile of liver SOL (*n*=340)

Types of lesions	Number of cases (%)
Neoplastic lesions	
Hepatocellular carcinoma	82 (24.11)
Hepatoblastoma	08 (2.35)
Metastatic carcinoma	226 (66.47)
Total	316 (92.94)
Nonneoplastic lesions	
Liver abscess	15 (4.41)
Hydatid cyst	06 (1.76)
Hemangioma	03 (0.88)
Total	24 (7.06)

Table 2: Clinical presentation of six cases of HD

Age (years)	Symptoms	Diameter of SOL (in cm)	USG diagnosis
39	Nil	5.0	Hepatoma
60	Nil	2.5	HD
52	Nil	5.2	Hepatoma
29	Abdominal pain	1.5	HD
39	Nil	5.0	Hepatoma
49	Abdominal pain	3.8	Hepatoma

SOL: Space occupying lesion, HD: Hydatid disease, USG: Ultrasonography

The hooklets were better visualized in Leishman stained smears than Pap and HE stained smears.

The hooklets on the Leishman stained smears were visible under lower magnification, whereas in the Pap and HE stained smears it was difficult to locate the hooklets under lower magnification. However, hooklets were clearly identified under higher magnification in all the smears stained with Leishman, Pap, and HE stains.

DISCUSSION

In this study, the age of the patients ranged from 29 to 60 years, and in none of the cases, there was any clinical suspicion of HD. Our observations are on par with two previous studies in which the age of patients ranged from 3rd to 6 decades, and none of the cases was diagnosed clinically as HD.[9,10]

All the six cases encountered in this study had lesions in the right lobe of the liver. All the SOLs presented as uniform solid lesions on USG, and four of the six lesions were in fact diagnosed as hepatoma. In agreement to our observation, other workers stated that imaging studies of hydatid cyst confused with hepatic tumors and majority were in the right lobe.[11]

Ultrasound guided FNAC has been found to be safe, inexpensive and useful in the diagnosis of hydatid cysts in our study. Jha et al.[3] have also documented that guided aspiration is a simple, safe, and cost-effective screening test for deep-seated lesions with a significant role in the detection of clinically suspected malignancy making it an investigation of choice for early confirmation or exclusion of pathology.

In our study, we have stained the dry air dried cytosmears with Leishman and HE stain and wet-fixed cytosmears with Pap stains. Earlier direct air dried smears from the aspirated material were stained with MGG, and alcohol-fixed smears were stained with the Pap and HE stain.[1]

With Pap and HE stained smears, the hydatid hooklets were not clearly visible under lower magnification, the typical glistening hooklets were, however, visible under high-power objective. In contrast, in Leishman stained smears, the hooklets could be clearly identified under lower power objective and still better with higher magnification. Thus, Leishman stain was found to be very useful to identify the hooklets, and was superior in comparison to the Pap and HE stain for this purpose.

CONCLUSION

Leishman staining which is an easy, inexpensive, quick, and ubiquitous technique is ideally suited to diagnose the hooklets of HD. This study shows that it is superior to the conventionally employed HE and Pap stains for diagnosis of hydatid hooklets.

REFERENCES

1. Paksoy N, Ozer D, Tuneli IO. Diagnosis of pulmonary hydatid disease presenting with solid nodule and mimicking malignancy by fine needle aspiration cytology. Cytol J. 2012;9:13.
2. Gole S, Gole G, Satyanarayana V. Unusual presentation of hydatid cyst: A case series with review of literature. Internet J Parasit Dis. 2013;6(1):1-11.
3. Jha BM, Shah R, Patel J. Effectiveness of image guided fine needle aspiration cytology in cases of deep seated lesions. Int J Med Sci Public Health. 2013;2:439-42.
4. Safioleas M, Safioleas C, Misiakos EP. Why fine-needle aspiration cytology is not an adequate diagnostic method for liver hydatid cyst reply. Arch Surg. 2007;142(7):690-1.
5. Rout N. Efficacy of Leishman stain in cytology - A 30 year's experience. Int J Pharm Sci Invent. 2016;5(6):1-3.
6. Rout N, Acharya GS, Nanda BK. Efficacy of Leishman Stain in Cytology (Abstract No. 99). 33rd National Conference of Indian Association of Pathologists and Microbiologists, Manipal (Karnatak), 20-22, December; 1984.
7. Rout N, Singh SP, Satpathy BK, Nanda BK. Rapid cytodiagnosis of endoscopic biopsy specimens in gastro-oesophageal malignancy. Trop Gastroenterol. 1993;14:99-103.
8. Swain M, Rout N, Samantray S, Nanda BK. Is Leishman stain useful for community cytology? Acta Cytol. 2010;54 Suppl 3:397.
9. Das DK, Bhambhani S, Pant CS. Ultrasound guided fine-needle aspiration cytology: Diagnosis of hydatid disease of the abdomen and thorax. Diagn Cytopathol. 1995;12(2):173-6.
10. Singh A, Singh Y, Sharma VK, Agarwal AK, Bist D. Diagnosis of hydatid disease of abdomen and thorax by ultrasound guided fine needle aspiration cytology. Indian J Pathol Microbiol. 1999;42(2):155-6.
11. Kim AR, Park SJ, Gu MJ, Choi JH, Kim HJ. Fine needle aspiration cytology of hepatic hydatid cyst: A case study. Korean J Pathol. 2013;47(4):395-8.

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