The Touch Imprint Cytology: A simple method for the diagnosis of mycetoma causative agents.

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Abstract:

Accurate diagnosis of mycetoma is mandatory for the proper treatment. The current diagnostic tools to confirm mycetoma are invasive, expensive, time consuming and not field friendly. With this background, the current study was set-out to determine the efficacy of touch imprint cytology as simple and non-invasive method for the identification of mycetoma causative agents.

The study included 50 patients with mycetoma seen at the Mycetoma Research Centre, University of Khartoum, Khartoum, Sudan. Material for cytological smears was collected from the active open discharging sinuses seen in these patients. From each Patient three smears were prepared and stained by Diff Quick, Hematoxylin and Eosin. The results obtained were compared to the histopathological findings of the surgical biopsy.

The findings observed in the imprint cytological smears were comparable to that seen in the histopathological slides. It can identify the different types of mycetoma causative organisms based on grains morphological features. The sensitivity of the technique was84% and its specificity was 100%.

This technique proved to be simple, non-invasive, rapid and sensitive. It can be used in the routine diagnosis of mycetoma and for field diagnosis.

Introduction:

Mycetoma is a chronic, specific, granulomatous, progressive and disfiguring inflammatory disease. It is caused by true fungi or by certain bacteria, and hence it is usually classified into eumycetoma and actinomycetoma respectively[1,2]. Madurella mycetomatis is the commonest eumycetoma causative agent, while Streptomyces somaliensis and Nocardia brasiliensis are the common causative organisms for actinomycetoma[3,4]. The triad of a painless subcutaneous mass, sinuses formation and purulent or seropurulent discharge that contains grains is pathognomonic of mycetom [5,6]. The true incidence and prevalence of mycetoma world-wide is not precisely known[7-9]. It is interesting to note that most of the reported mycetoma data are related in most cases to hospital patients with advanced disease[10,11]. This is attributed to the nature of mycetoma which is usually painless, slowly progressive and the late presentation of the majority of patients due to the poor health education, lack of health facilities and financial constraints[12,13].

The current diagnostic tools for mycetoma are various and that include: imaging techniques such as conventional radiography, ultrasonography, computed tomography scan, MRI[14-17] and molecular techniques such as Polymerase Chain Reaction, serodiagnosis as ELISA, CIE as well the classical grain culture and histopathological diagnosis and fine needle aspirations[18-22]. It is intriguing to note most of these techniques are not available in majority of mycetoma endemic regions as well as some of these techniques like excisional biopsy and fine needle aspiration are painful for patients.The aim of this current study is to evaluate the role of imprint cytology as simple, cost effective and rapid diagnostic tool in the diagnosis of mycetoma with draining sinuses.

Materials and Methods:

This is an observational descriptive, cross-sectional hospital-based study which was conducted at the Mycetoma Research Centre, University of Khartoum, Khartoum, Sudan. Using convenient sampling technique, 50 patients with mycetoma were included after informed written consent was obtained. Ethical clearance was obtained from Soba Hospital Ethical Committee. The demographic characteristics of the patients' population were collected in a pre-designed data collection sheet.

From each patient three cytological smears were obtained by gentle pressing of the glass slide on the draining sinuses. The smears were allowed to air dry and then stained using Diff Quick (two slides), Hematoxylin and Eosin(one slide) as described previously[23].

From each patient surgical biopsy was obtained, fixed in 10% formal-saline for 24 hours and paraffin blocks were prepared. The sections were stained with Haematoxylin and Eosin (H&E), Grocott's hexamine, and Gram stain methods.

Statistical Analysis:

Data were managed using the Statistical Package for the Social Sciences computer programme (SPSS version 16). The technique sensitivity and specificity rates were calculated compared to the histopathological results.

Results:

Most of the patients were males 36(72%). Their ages ranged between 10 to 65 years with a mean age of 32.14±13.01. The majority of the patients 30(60%) were less than 40 years old at presentation. All the patients had active draining sinuses. Most of the discharge contained grains and their colour was black 35(70%),and yellow in colour 2(4%).In 13(26%)patients no grains were observed.

Examination of cytological smears showed evidence of Madurella mycetomatis in 39 (78%) smears, *Streptomyces somaliensis* in 2 (4%), Actinomadura pelletierii in 1(2%) and in 8 (16%) of the smears no grains were identified. Multi-inflammatory cells were seen including macrophages, lymphocytes, polymorphonuclear cells and plasma cells in a necrotic background, (Figs. 1-6).

The histopathological examinations of surgical biopsies revealed evidence of M. mycetomatis in 43 patients (86%), Streptomyces somaliensis in five patients (10%) and Actinomadura pelletierii in two patients (4%).

Comparing the imprint cytology technique to the gold standard method, i.e histopathological examination of the surgical biopsy revealed sensitivity of 84%and 100% specificity for the diagnosis of mycetoma with discharging sinuses .

Table 1.Comparison between the results obtained by the imprint cytology and histopathological examination of the surgical biopsies.

Organism	Imprint Cytology	Histopathology
Madurella mycetomatis	39 (78%)	43 (86%)
Streptomyces somaliensis	2 (4%)	5 (10%)
Actinomadura pelletierii	1 (2%)	2 (4%)
None Specific Inflammation	8 (16%)	0(0%)

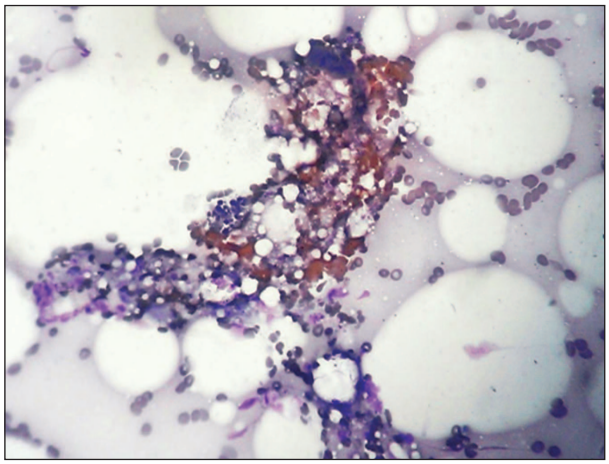

Fig.1.Photomicrograph showing ***M. mycetomatis*** surrounded by multi-inflammatory cell (Diff Quick X200).

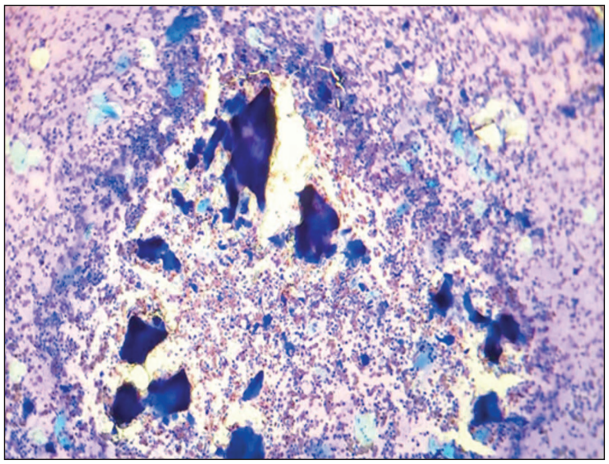

Fig.2.Photomicrograph showing ***Actinomadura pelletierii*** surrounded by multi-inflammatory cell (Diff Quick X200).

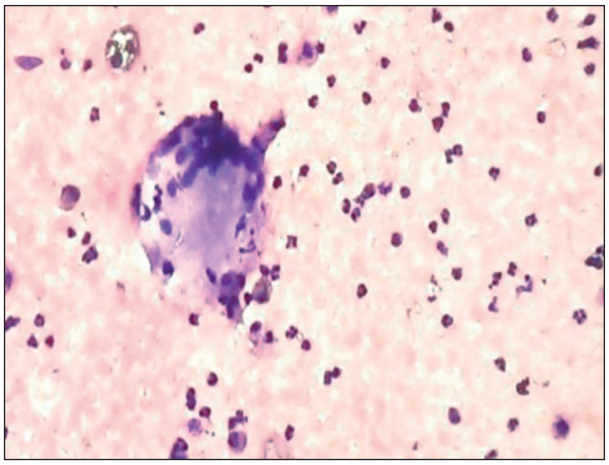

Fig.3.Photomicrograph showing type II Tissue Reaction; smear consists of multinucleated giant cells, neutrophils, plasma cells and small lymphocytes (H&E X10).

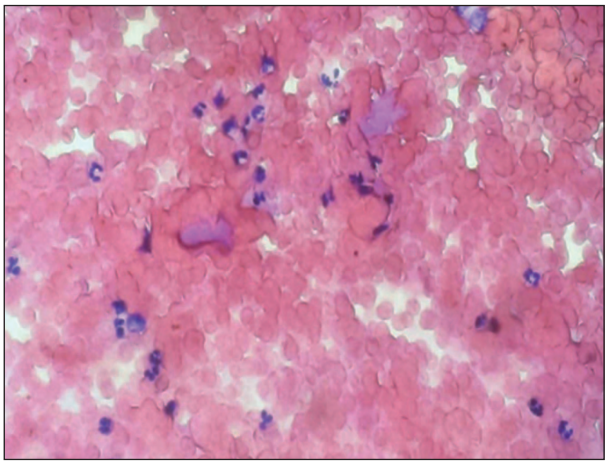

Fig.4.Photomicrograph showing type I Tissue Reaction; smear consists of neutrophils, small lymphocytes scattered near to ***Streptomyces somaliensis*** pointed by arrow (H&E X10).

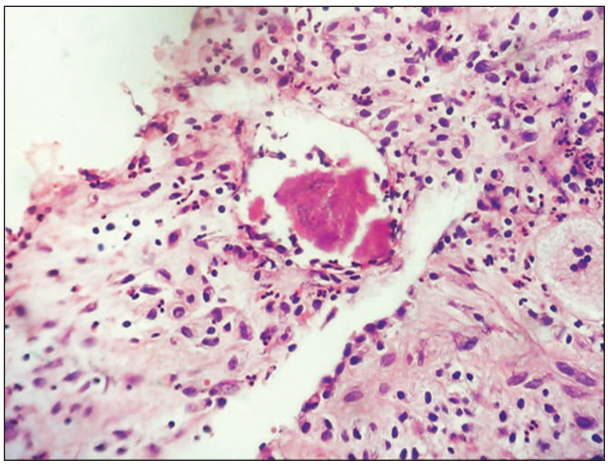

Fig.5. Photomicrograph showing ***Streptomyces somaliensis*** in tissue section surrounded by inflammatory cells (H&E X10).

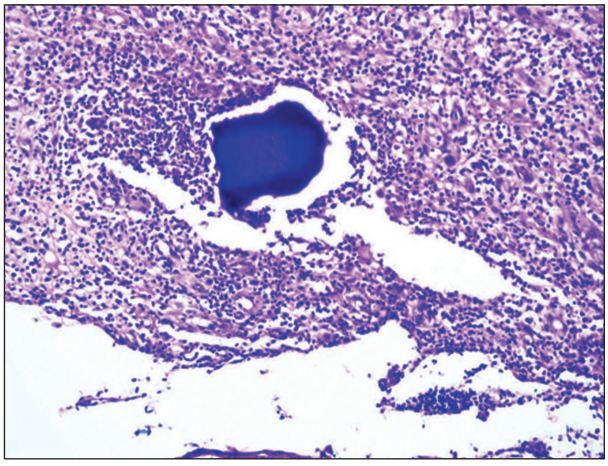

Fig. 6. Photomicrograph showing ***Actinomadura pelletierii*** in tissue section (H&E X10).

Discussion:

The proper treatment of mycetoma depends mainly on accurate diagnosis. The diagnosis of mycetoma and its type and extent are based on meticulous clinical interview and examinations and a battery of investigations including various imaging techniques, organism identification using grains culture, phenotypic morphological identification, molecular techniques and cyto-histopathological identification[17,22].

The available diagnostic tools and techniques have many shortcomings and limitations. Most of them are invasive, time consuming, of low specificity and sensitivity[24].

This study was conducted to explore the role of the imprint cytology in the identification of mycetoma causative organisms .It is a simple, rapid, non-invasive and field friendly diagnostic tool. Imprint cytology was first introduced by Dudegoen and Patrick, as a powerful tool for intraoperative identification of histological patterns and types of the tumors[25-33].

Recently, cytopathological techniques gained attention for the diagnosis of many lesions due to their simplicity, accuracy and cost effectiveness. In mycetoma, fine needle aspiration for cytology and cell block technique are integral parts of the diagnostic protocol. It proved to be simple, rapid and of reasonable sensitivity (84%).(22) However, at least three injections and aspiration are required to obtain sufficient material for cytological examination and the procedure may be painful in some patients and furthermore in patients with secondary bacterial infection the chance of cellulitis and sepsis is considerable.

In the current study, with the imprint technique it was possible to identify the causative organism in 92% of the studied smears. In the smears, the causative organisms were seen, infiltrated and surrounded by multi-inflammatory cells. The cells which included polymorphonuclear cells, macrophages, lymphocytes, plasma cells and giant cells scattered in necrotic background. This appearance is in line with that documented in cytological smears and histopathological sections. It was possible to differentiate between eumycetoma and actinomycetoma causative organisms.

In the cytological smears,Madurella mycetomatis grains tended to be large to small in size, light to dark brown in color with irregular outlines and crushing artifact using H&E technique.

Streptomyces somaliensis grains did not stain well by H&E. It required a careful examination as it can be stained bright pink to hazy pink in color, but it exhibited round, oval to irregular granular shapes when found as aggregates.

Whereas Actinomadura pelletieri grains were seen as small-rounded to oval and they stained deep blue with H&E. Furthermore, in addition to the ability of imprint cytology to discriminate between eumycetoma from actinomycetoma; it can easily detect the type of host tissue reaction based on the type of inflammatory cells that are encountered on the cytological smear and histopathological sections previously described[29].

One of the limitations of this study is that the number of the studied patients is rather small. A further study with a large number of patients is recommended. The presence of active open sinuses is a pre-requisite to perform the test.

The present study showed that, the imprint cytology technique has a role in the diagnosis of mycetoma. The technique proved to be simple, non- invasive, inexpensive, accurate and field friendly.

References:

1. Hay RJ, Fahal AH. Mycetoma: an old and still neglected tropical disease. *Trans R Soc Trop Med Hyg*. 2015; 109:169-70.

2. Nenoff P, van de Sande WW, Fahal AH, Reinel D, Schöfer H. Eumycetoma and Actinomycetoma - an update on causative agents, epidemiology, pathogenesis, diagnostics and therapy. *J Eur Acad Dermatol Venereol*. 2015; 29:1873-83.

3. Fahal A, Mahgoub el S, El Hassan AM, Jacoub AO, Hassan D. Head and Neck Mycetoma: The Mycetoma Research Centre Experience. *PLoS Negl Trop Dis*. 2015; 9: e0003587. doi:10.1371/journal.pntd.0003587

4. Fahal AH, EL Hassan AM, Mahgoub ES, Abdel-Rahman ME. Mycetoma in the Sudan: The Mycetoma Research Centre Update.*PLoS Negl Trop Dis*. 2015; 9:e0003679. doi: 10.1371/journal.pntd.0003679. eCollection.

5. van de Sande WW. Global burden of human mycetoma: a systematic review and meta-analysis. *PLoS Negl Trop Dis*. 2013; 7: e2550.

6. Hala Taha, Ahmed Fahal, Wendy WJ van de Sande. Mycetoma: Epidemiology, Treatment Challenges and Progress. Mycetoma: epidemiology, treatment challenges and progress. *Res Rep Trop Med*. 2015; 6; 31-36.

7. Fahal AH. Mycetoma. Review article, *Khartoum Med J*. 2011; 4: 514-523.

8. Fahal AH .Mycetoma thorn on the flesh. Review article. *Trans R Soc Trop Med* Hyg. 2004; 98: 3-11.

9. Ahmed AO, van Leeuwen W, Fahal A, van de Sande W, Verbrugh H, van Belkum A. Mycetoma caused by Madurella mycetomatis: a neglected infectious burden. *Lancet Infect Dis*. 2004; 4: 566-74.

10. Fahal AH, Hassan MA, Mycetoma. *Br J Surg*. 1992; 79:1138–1141.

11. Ahmed AA, van de Sande WW, Fahal A, Bakker-Woudenberg I, Verbrugh H, van Belkum A. Management of mycetoma: major challenge in tropical mycoses with limited international recognition. *Curr Opin Infect Dis*. 2007; 20: 146–51.

12. Fahal AH. Mycetoma in Williams, Bulstrode, O'Connell, Bailey and Love's Short Practice of Surgery 26E: 26th Edition, Oxford University Press, 2013, pp 84-88.

13. López Martínez R, Méndez Tovar LJ, Lavalle P, Welsh O, Saúl A, Macotela Ruíz E. Epidemiology of mycetoma in Mexico: study of 2105 cases. *Gac Med Mex*. 1992; 128: 477-481.

14. El Shamy ME, Fahal AH, Shakir MY, Homeida MM. New MRI Grading System for the Diagnosis and Management of Mycetoma. *Trans R Soc Trop Med Hyg*. 2012; 106:738–42.

15. Fahal AH, Sheik HE, Homeida MM, Arabi YE, Mahgoub ES. Ultrasonographic imaging of mycetoma. *Br J Surg*. 1997; 84:1120–1122.

16. Abd El Bagi ME. New radiographic classification of bone involvement in pedal mycetoma. *Am J Roentgenol*. 2003; 180: 665–668.

17. Abd El-Bagi MEB, Fahal AH. Mycetoma revisited. Incidence of various radiographic signs. *Saudi Med J*. 2009; 30:529-33.

18. Ahmed AO, Mukhtar MM, Kools-Sijmons M, et al. Development of a species-specific PCR-restriction fragment length polymorphism analysis procedure for identification of Madurella mycetomatis. *J ClinMicrobiol*. 1999; 37: 3175–8.

19. EL Hassan AM, Fahal AH, EL Hag IA, et al. The pathology of mycetoma: Light microscopic and ultrastructural features. *Sudan Med J*. 1994; 32: 23–45.

20. EL Hag IA, Fahal AH, Khalil EAG. Fine needle aspiration cytology of mycetoma. *Acta Cytologica*. 1996; 40: 461-46.

21. Fahal AH, El Toum EA, El Hassan AM, Mahgoub ES, Gumaa SA. Host tissue reaction to Madurella mycetomatis: New classification. *J Med Vet Mycol*. 1995; 33: 15-17.

22. Yousif BM, Fahal AH, Yahia MY. The Cell Block Technique: A New Diagnostic Tool for Mycetoma. *Trans R Soc Trop Med Hyg*. 2010; 104:6-9.

23. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 5th ed. New York,

NY: Churchill Livingstone, 2002.

24. Van de Sande WW, Fahal AH, Goodfellow M, Mahgoub el S, Welsh O, Zijlstra EE. The merits and pitfalls of the currently used diagnostic tools in mycetoma. *PLoSNegl Trop Dis*. 2014, July 03, DOI: 10.1371/journal.pntd.0002918.

25. Dudgeon LS, Patrick CV. A new method for the rapid microscopical diagnosis of tumours, with an account of 200 cases so examined. *Br J Surg*. 1927; 15: 250–61.

26. Lee TK. The value of imprint cytology in tumor diagnosis: a retrospective study of 522 cases in northern China. *Acta Cytol*. 1982; 26:169-71.

27. Silverberg S, Nochomovitz L, Jannotta SF, et al. A Guide to Smears, Imprints and Frozen Sections. 1st ed. Washington DC: ASCP; Intraoperative Consultation, 1989.

28. Kim K, Phillips ER, Paolino M. Intraoperative imprint cytology: Its significance as a diagnostic adjunct. *DiagnCytopathol.* 1990; 6: 304–7.

29. Manafis K. An intraoperative consultation. Usefulness, reason and accuracy of the method. *Arch Hell Pathol*. 1997; 11:472–7.

30. Chehrei A, Ahmadinejad M, Tabatabaee SA, et al. Touch imprint and crash preparation intra operative cytology versus frozen section in thyroid nodule. *J Res Med Sci.* 2012; 17: 475–480.

31. Kolte SS, Satarkar RN. Role of scrape cytology in the intraoperative diagnosis of tumor. *J Cytol*. 2010; 27: 86-90.

32. Khan N, Afroz N, Haider A, Hassan MJ, Hashmi SH, Hasan SA.. Role of fine needle aspiration, imprint and scrape cytology in the evaluation of intraoral lesions. *J Cytol*. 2013; 30: 263–269.

33. Taneri F, Poyraz A, Salman B, et al. Using imprint and frozen sections in determining the surgical strategies for thyroid pathologies. *Endocr Regul*. 2001; 35:71-4.