ORIGINAL ARTICLE

Ultrasound-guided fine-needle aspiration cytology significantly improved mycetoma diagnosis

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Abstract

Background Ultrasound (US)-guided fine-needle aspiration cytology (US-FNAC) has improved the diagnosis of many malignancies, infections and other diseases as it is safe, simple, quick and accurate. In mycetoma, it is assumed that this technique may have a better diagnostic yield than the conventional FNAC as it can accurately identify the optimal site for the aspiration.

Objective To compare the diagnostic yield of conventional FNAC with US-FNAC.

Methods This descriptive cross-sectional hospital-based study included 80 patients with clinically suspected mycetoma.

Results Of the 80 patients included, 35 proved to have actinomycetoma, and 37 had eumycetoma based on surgical biopsies, histopathological examination and the culture of grains. Eight patients appeared to have no mycetoma. For actinomycetoma diagnosis, the US-guided FNAC improved sensitivity to 97% and negative predictive value (NPV) to 83% compared to the conventional FNAC, which had 63% sensitivity; and NPV of 28%. No improvement was found for specificity. For eumycetoma, the conventional FNAC had 86.5% sensitivity, 100% specificity, 100% PPV and 37.5% NPV. The US-FNAC for the diagnosis of eumycetoma had 100% sensitivity and specificity.

Conclusions and relevance The obtained results showed that US-FNAC is better than the conventional FNAC with lower false-negative results. It can accurately distinguish between the two types of mycetoma, allowing rapid initiation of proper treatment. The technique can be used in rural areas with low resources and for epidemiological surveys as a quick screening tool for patients suspected of mycetoma.

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Conflicts of interest

The authors declare no conflict of interests.

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Introduction

Mycetoma is a subcutaneous skin neglected tropical disease endemic in tropical and subtropical regions.[1,2] Clinically, the disease is characterized by the triad of painless swellings, multiple sinuses and sero-purulent discharge containing grains.[3–5] There are two types of mycetoma: actinomycetoma caused by eumycetoma of fungal origin.[3] Clinically, it is difficult to differentiate between the two types.[6] It is vital to distinguish between the two types to initiate proper therapy as actinomycetoma is treated with a combination of antibiotics and eumycetoma by a combination of antifungal agents and surgery.[7] Currently, the recommended techniques for the identification of mycetoma causative agents are culturing of grains and histopathological examination of the deep surgical biopsies.[8,9] More recently, in certain specialized centres, molecular identification by PCR is in use.[10,11] However, all these techniques are invasive, require good and safe anaesthesia, are time-consuming and are associated with certain complications such as bleeding, sepsis and local

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disease spread.[12] Additionally, these procedures are not suitable for epidemiological surveys or field-friendly; and hence, patients need to travel to more specialized centres for disease confirmation and treatment.

Fine-needle aspiration cytology (FNAC) for mycetoma diagnosis is less invasive than surgical biopsies and can be used in remote rural settings. However, its sensitivity and specificity are greatly operator-dependent.[13] Mycetoma grains are located in deep-seated cavities surrounded by thick capsules and fibrous tissue and can easily be missed with the conventional FNAC, leading to false-negative results.[13] It is assumed that ultrasound (US) guided-FNAC (US-FNAC) for the diagnosis of mycetoma can localize the grains accurately and thus differentiate between the different types of mycetoma and non-mycetoma lesions.[14] With this background, this descriptive cross-sectional study was conducted at the Mycetoma Research Centre (MRC), Khartoum, Sudan, and compared the accuracy of the FNAC technique with and without ultrasound guidance.

Methods

Ethical statement

Ethical approval was obtained from the Mycetoma Research Centre Institutional Review Board (IRB). Written informed consent was obtained from each adult patient and parents or guardians of those patients under 18 years old.

Inclusion and exclusion criteria

In this study, patients were included who had a newly diagnosed mycetoma and did not undergo prior surgery for this. The patients were included when they were willing to participate and willing to undergo conventional FNAC and US-FNAC, as well as an excisional biopsy which is part of standard care in our Mycetoma Research Centre. Any patients who had been previously diagnosed with mycetoma were excluded from this study as well as patients who refused to undergo all three procedures including FNAC, US-FNAC and deep excisional biopsy.

Study cohort

This is a cross-sectional, prospective, hospital-based study conducted at Mycetoma research centre, a World Health Organization (WHO) collaborating centre and Mycetoma specialized centre, University of Khartoum, Khartoum Sudan between 01 March 2021 and September 2021. Eighty mycetoma suspected patients were enrolled in this study. Written informed consent was obtained from each patient. All had a thorough clinical review and examination. Each participant had conventional FNAC, US-FNAC and deep excisional biopsy at the same session. The material obtained from the excisional biopsy was used for histopathology, culturing of the grains and in case of black grains for *M. mycetomatis* specific PCR.

Fine-needle aspiration cytology

Conventional FNAC was performed by inserting a 22-gauge needle attached to a 10-mL syringe into the lesion and moved back and forth within the lesion. Suction was applied when the needle was advanced into the lesion and halted before the needle was removed from the lesion. Two aspirations were conducted in different directions. The obtained specimen was placed on clean glass slides and allowed to air-dry.

US-FNAC was performed using the Toshiba Sol, EUB-25M, real-time ultrasound linear scanner equipped with a high-resolution probe (a 3-75-MHz transducer) by an experienced radiologist. Using US, grains are visualized as sharp echoes in the case of eumycetoma grains.[15] Furthermore, a thick wall and no acoustic enhancement is also observed in the eumycetoma lesion. In actinomycetoma lesions, the hyper-reflective echoes are fine, closely aggregated and commonly settle at the bottom of the cavities. A thinner cavity wall is observed compared to eumycetoma.[15] After visualizing the pockets, US-FNAC was performed, See Figure 1.

Cytology from FNA smears (FNAC)

The air-dried smears obtained from the two FNA procedures were fixed in ethanol. The spots were stained with H&E and May Grunwald-Giemsa (MGG) stains. The species we expected to encounter were *Madurella mycetomatis, Streptomyces somaliensis, Actinomadura madurae* and *Actinomadura pelletieri*. In order to identify those species based on histopathology and cytology, the slides were examined independently by an expert histopathologist and identified based on the criteria as previously described.[13] In short, according to these criteria, *M. mycetomatis* grains usually have different sizes ranging from small, medium to large grains with irregular margins. Grains stain light to dark brown in colour when stained with H&E and MGG.[13] *A. madurae* grains are usually well stained and stained violet in H&E staining. *A. pelletieri* grains are usually small and round to oval shape, often fractured and stained violet in H&E staining.[13] *S. somaliensis* grains are usually more difficult to identify cytological.[13] They stain pale pink, irregular in shape and often had a thread-like appearance at the periphery of the grains.[13] They can be confused with fibrin (Fig. 2).

Deep-seated biopsy

After FNAC, each patient had a deep excisional surgical biopsy obtained under local anaesthesia using 2% W/V Lidocaine from the same site where the needle was inserted for the aspiration. The biopsy was split into two parts. One part was fixed in 10% neutral buffered formalin and processed for histopathological examination. 3–5-µm sections were prepared and stained with H&E as previously described.[16] The second part was washed three times in normal saline and inoculated in a suitable culture medium. When eumycetoma was suspected, the grains were inoculated into Sabouraud Dextrose Agar (SDA) supplemented

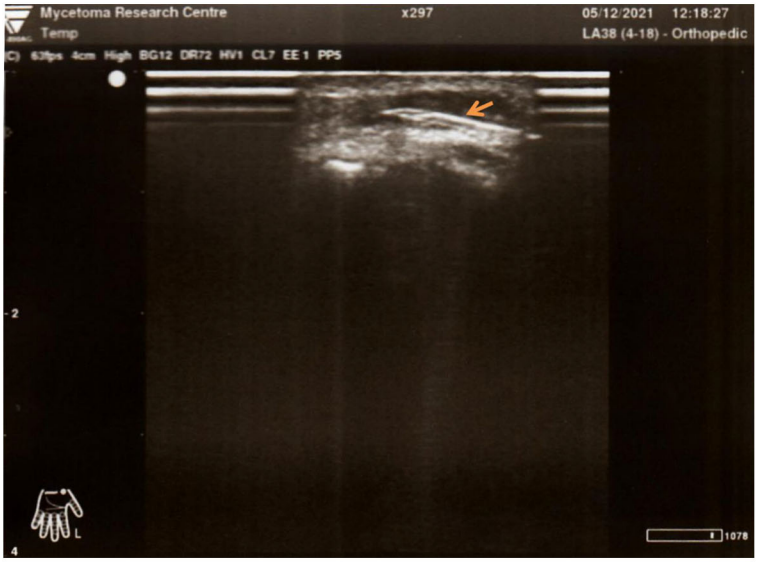

Figure 1 Showing the US-FNAC technique with the needle (arrow) inside the pocket of mycetoma lesion.

with gentamicin sulphate (400 μg/mL) and incubated at 37°C for 1–6 weeks. When actinomycetoma was expected based on clinical data and the ultrasound report, grains were inoculated into blood agar and yeast extract agar and incubated at 37°C for 7–14 days. The final identification of the causative agent was based on the data obtained from the deep-seated biopsy histopathological examination, morphology obtained after culturing the grains and in the case of *M. mycetomatis* on a positive PCR result with *M. mycetomatis* specific primers.

Genomic DNA extraction from deep excisional biopsy for eumycetoma patients

For DNA extraction, the grains were isolated from the infected tissue using a sterile surgical blade and transferred to a sterile Eppendorf tube (1.5 mL), containing 10 metal beads and 700 μL Bashingbead buffer (Zymo DNAfungal/bacteria extraction kits, Irvine, CA, USA). The mixture was then lysed using a Tissuelyser II (Qiagen, Germany) for 3 min at 30 hertz. The supernatant was removed and added onto a Zymo-Spin III-F filter column and spun down for 1 min at 8000 g. From then onwards, DNA was isolated *via* the protocol of the manufacturer. The concentration and purity of the extracted DNA were measured using a NanoDrop spectrophotometer (Implen, Germany). The obtained DNA was stored at −20°C until further use.

Polymerase chain reaction

The extracted genomic DNA was amplified using pan fungal primers ITS4 and ITS5 and *M. mycetomatis* specific primers 26.1a (5′-AATGAGTTGGGCTTTAACGG-3′) and 28.3a (5′-TCCCG GTAGTGTAGTGTCCCT-3′)[17] and Mmy-Fw (5′-TCTCCTGTC CTACGACATCTGTGG-3′) and Mmy-Rv (5′-TTCCTCACCTC CCAGCCCTTT-3′).[10] In each PCR reaction, 1 μL genomic DNA; 1 μL forward primer (100 pmol/μL); 1 μL reverse primer (100 pmol/μl) and 20 μL distilled water were added to freeze-dried Maxime™ PCR PreMix (i-Taq) (Intron, South Korea). The PCR reaction for the pan-fungal PCR and the *M. mycetomatis* specific PCR using primers 26.1a and 28.3a was performed in the Aeris PCR system (Esco scientific, Singapore) with a 4 min primary denaturation step at 95°C followed by 40 cycles consisting of a 30 s denaturation step at 95°C, a 30 s annealing step at 55°C and a 1 min extension step at 72°C. After a final extension step of 7 min at 72°C, the mixture was cooled down to 4°C. The *M. mycetomatis* specific PCR with primers Mmy-Fw and Mmy-Rv consisted of a 2 min primary denaturation step at 95°C followed by 30 cycles consisting of a 30 s denaturation step at 95°C, a 30 s annealing step at 62.8°C and a 40 s extension step at 72°C. After a final extension step of 5 min at 72°C, the mixture was cooled down to 4°C. PCR products were visualized on a 1.3% agarose gel. The pan-fungal amplicon for *M. mycetomatis* was 600 bp long, while the *M. mycetomatis* specific amplicons were 420 bp when using primers 26.1a and 28.3a and 470 bp when using primers Mmy-Fw and Mmy-Rv.

Statistical analysis

The mycetoma causative organisms' identification based on conventional FNAC and US-guided FNAC were compared to the golden standard methods. The number of true-positive (TP), false-positive (FP), true-negative (TN) and false-negative (FN) test results were calculated using the 2 × 2 Chi-squared test

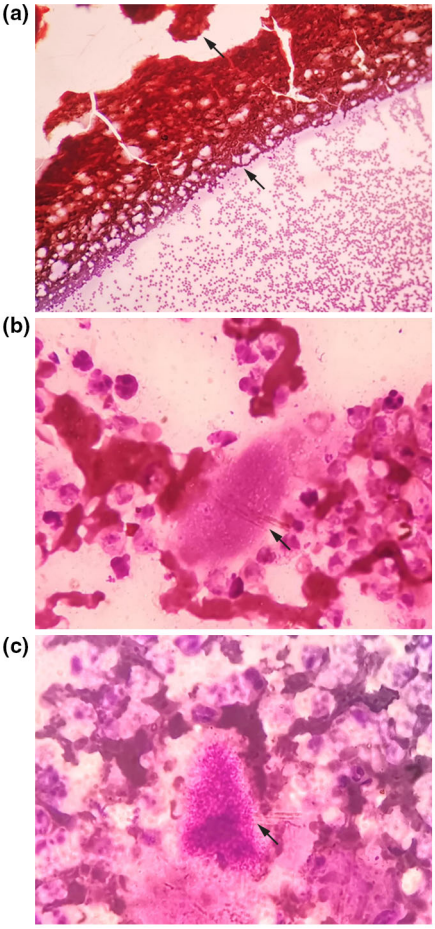

Figure 2 Showing cytological smears obtained with US-FNAC and stained with H&E stain ×40. (a) eumycetoma grains stained bright to dark brown in colour (arrowhead); (b and c) *S. somaliensis* grains staining light pink in colour and demonstrating the ball of threads like appearance common actinomycetoma cytological feature.

using SPSS version 16.0. According to these results, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated.

Results

Eighty patients were included, 19 patients (23.8%) were female, and 61 patients (76.2%) were male. This is in line with the normal mycetoma gender distribution reported from our centre.[18] The patients were between 16 and 59 years old with a mean age of 32.8 ± 0.83 SD years. The mean duration of the disease was 30.8 ± 2.6 months. Most of the patients were clinically diagnosed with foot mycetoma (n = 70, 87.5%); 56 patients had left foot mycetoma, and 14 had a right foot lesion. Six patients (7.5%) were clinically diagnosed with left-hand mycetoma, and four patients (5%) with right-hand mycetoma (Table 1).

Table 1 Showing the characteristics of the 80 patients suspected for mycetoma

Variable	No. (%)
Age (Mean ± SD) years	32.8 ± 0.83 years
Gender	
Female	19 (23.8%)
Male	61 (76.2%)
Duration in months (Mean ± SD)	30.8 ± 2.6 months
Site of the lesion	
Left foot	56 (70%)
Right foot	14 (17.5%)
Left hand	06 (7.5%)
Right hand	04 (5%)
Conventional FNAC	
Positive	54 (67.5%)
Negative	26 (32.5%)
US-guided FNAC	
Positive	71 (88.8%)
Negative	9 (11.3%)
Histopathological examination results	
Positive mycetoma	72 (90%)
Negative mycetoma	
Osteomyelitis	1 (1.25%)
Fibroma	1 (1.25%)
Foreign body granuloma	6 (7.5%)

Diagnosis of the causative agents using the current golden standard procedures

The histopathological examination and culture results of the deep-seated surgical biopsies confirmed the diagnosis of actinomycetoma in 35 patients with *S. somaliensis* actinomycetoma. For the patients suspected for eumycetoma by *M. mycetomatis*, also two *M. mycetomatis*-specific PCRs were performed. In total, 37 patients had *M. mycetomatis* eumycetoma. In eight mycetoma suspected patients, the diagnosis was not confirmed by culture or histopathological examination or PCR in the case of eumycetoma (Tables 1 and 2). Of the eight patients, who had negative results for FNAC and US-FNAC, six patients were

Table 2 Showing the diagnostic yield of two techniques compared to the histological examination results

		Conventional FNAC		US-FNAC	
		Positive	**Negative**	**Positive**	**Negative**
Overall	**Positive**	54	18	71	1
	Negative	0	8	0	8
Actinomycetoma	**Positive**	22	13	34	1
	Negative	0	5	0	5
Eumycetoma	**Positive**	32	5	37	0
	Negative	0	3	0	3

found to have a foreign body granuloma by histology of the deep-seated biopsy; one had a fibroma and the last patient had osteomyelitis (Table 1).

Diagnosis of the causative agents using fine-needle aspiration cytology

All patients underwent both conventional FNAC and US-FNAC. Using conventional FNAC, *S. somaliensis* grains were noted in smears obtained from 22 of the 40 suspected actinomycetoma patients. No grains were noted in the smears of the other 18 patients. This resulted in a 62.9% sensitivity, 100% specificity, 100% PPV and 27.8% NPV and 66.57% accuracy. When US-FNAC was used, grains were visible in the smears obtained from 34 actinomycetoma patients, and six patients were reported to have a negative smear. For US-FNAC, the sensitivity was 97.1%, specificity was 100%, PPV was 100%, NPV was 83.3% and accuracy was 97.4% (Table 2).

Using non-US guided FNAC, *M. mycetomatis* grains were visible in smears obtained from 32 of the 40 eumycetoma suspected patients. In the other eight suspected patients, no grains were noted. This resulted in 86.5% sensitivity, 100% specificity, 100% PPV and 37.5% NPV and 87.8% accuracy. With the US-FNAC in the eumycetoma suspected cases, sensitivity increased, and smears showed *M. mycetomatis* grains in 37 patients. Only in three patients, no grains were noted (Table 2). In these three patients, there was also no sign of *M. mycetomatis* in the deep-seated biopsy by histopathology, culture and *M. mycetomatis*-specific PCRs. This resulted in a 100% sensitivity, specificity, PPV and NPV and accuracy.

Discussion

This study demonstrated that US-FNAC reduced the number of false-negative results for both actinomycetoma caused by *S. somaliensis* and eumycetoma caused by *M. mycetomatis* considerably. For actinomycetoma, the number of false-negative results decreased from 13 to one and for eumycetoma, from five to zero, demonstrating that ultrasound-guided FNAC is superior to the non-US guided FNAC.

One of the limitations of our study is that we were only able to focus on the causative agents prevalent in Sudan and of those *A. madurae* and *A. pelletieri* were not encountered in this study. Furthermore, other common causative agents such as *Nocardia brasiliensis,* the most prevalent cause of actinomycetoma in Mexico, are not endemic in Sudan.[3] Therefore, the sensitivity and specificity of US-FNAC could only be determined for *M. mycetomatis* and *S. somaliensis*. Furthermore, the identification of *S. somaliensis* was based on histopathology and culture, while it is known that *S. somaliensis* probably represents at least two different *Streptomyces* species.[19] Others have demonstrated that US-FNAC for mycetoma caused by *Nocardia spp*. is also very useful and could be used to identify *Nocardia spp*. from a mycetoma lesion.[20] However, a more extensive study is needed to determine the sensitivity and specificity for other mycetoma causative agents as well.

A second limitation is that we could not include molecular identification for the *Streptomyces* causative agents as there is currently no reliable PCR for that. We relied on histopathology and culture for the final identification of these species. In many of the endemic regions, histopathology is used as the sole diagnostic procedure for identifying mycetoma causative agents.[13] It can accurately distinguish between bacterial and fungal mycetoma and according to some publications it can be accurately used to identify at least to the genus level.[13] However, histopathology cannot readily identify the causative agent to the species level. Unfortunately, in most of the endemic regions, histopathology is the only technique available, and using FNAC instead of deep-seated biopsy to obtain the infected tissue would be a big step forward. In remote field settings, FNAC is superior to histology since it requires only a simple setting with limited equipment and can be performed under local anaesthesia. FNAC is rapid and cost-effective compared with histopathological examination.[21] This allows for prompt patient management. Furthermore, for FNAC, only H&E stain is required to confirm the diagnosis; however, special stains such as the Gram stain, the Periodic acid Schiff reaction (PAS) and calcofluor white are not routinely used. They are used for difficult cases to differentiate bacteria from fungi and other structures such as degenerated collagen fragments, haemolysed blood, fibrin deposits and calcified tissue from the causative agents under the light microscope or fluorescent microscope.[13,22–24]

In our study, we reported relatively low sensitivity of the non-US guided FNAC. The reported sensitivity was 62.9% for suspected actinomycetoma patients and 86.5% for eumycetoma patients. This sensitivity was lower than reported in 1996 by El-Hag and his colleagues but comparable to that reported by Siddig and his associates on 991 suspected mycetoma patients.[13,25] In the El-Hag study, only 14 patients were included, and a sensitivity of 100% (four of four) was reported for actinomycetoma patients and 90% (9 of 10) for eumycetoma patients.[25] In the study reported by Siddig and his associates, sensitivity of 37.5%, 60.5% and 68.7% were reported for actinomycetoma caused by *S. somaliensis*, *A. madurae* and *A. pelletieri,* respectively, and of 80.5% for *M. mycetomatis* eumycetoma.[13] These data indicate that the evaluation of new techniques needs sufficient numbers of patients. The type of the causative microorganism should be taken into account in the interpretation of the test results. The difference in the sensitivity of conventional FNAC reported in these studies may also indicate that the test is operator dependent. The grains are usually small, scanty and enclosed in deep-seated cavities encapsulated with thick fibrous tissue aspirating needle can miss them.

This study demonstrated that US-FNAC had greatly improved the technique's sensitivity as the grains within the cavities can be identified and aspirated accurately. That was clearly seen in actinomycetoma suspected patients. As previously reported,

ultrasound is considered essential for patient care in the low-resource health system by the WHO,[14] and portable ultrasound machines are available. Hence, the technique can greatly help patients in remote endemic regions.

In conclusion, using US-FNAC in the field will increase the diagnostic yield and ensure grains are obtained in the aspirate for further tests. That will reduce the need for deep-seated surgical biopsies for histopathological and culture examinations.[13,23]

Data availability statement

All original data presented in the study are included in the article material, for further inquiries please contact the corresponding authors.

References

1 Fahal AH, Hassan MA. Mycetoma. *Br J Surg* 1992; **79**: 1138–1141.
2 Hay RJ, Asiedu KB, Fahal AH. Mycetoma – a long journey out of the shadows. *Trans R Soc Trop Med Hyg* 2021; **115**: 281–282.
3 van de Sande WW. Global burden of human mycetoma: a systematic review and meta-analysis. *PLoS Negl Trop Dis* 2013; **7**: e2550.
4 Emery D, Denning DW. The global distribution of actinomycetoma and eumycetoma. *PLoS Negl Trop Dis* 2020; **14**: e0008397.
5 Oladele RO, Ly F, Sow D *et al*. Mycetoma in West Africa. *Trans R Soc Trop Med Hyg* 2021; **115**: 328–336.
6 Fahal AH, Suliman SH, Hay R. Mycetoma: the spectrum of clinical presentation. *Trop Med Infect Dis* 2018; **3**: 97.
7 Siddig EE, Verbon A, Bakhiet S, Fahal AH, van de Sande WWJ. The advanced molecular biological identification tools for mycetoma causative agents: an update. *Acta Trop* 2021; **225**: 106205.
8 Ahmed AA, van de Sande W, Fahal AH. Mycetoma laboratory diagnosis: review article. *PLoS Negl Trop Dis* 2017; **11**: e0005638.
9 Siddig EE, van de Sande WWJ, Fahal AH. Actinomycetoma laboratory-based diagnosis: a mini-review. *Trans R Soc Trop Med Hyg* 2021; **115**: 355–363.
10 Lim W, Siddig E, Eadie K *et al*. The development of a novel diagnostic PCR for Madurella mycetomatis using a comparative genome approach. *PLoS Negl Trop Dis* 2020; **14**: e0008897.
11 Emmanuel P, Dumre SP, John S, Karbwang J, Hirayama K. Mycetoma: a clinical dilemma in resource limited settings. *Ann Clin Microbiol Antimicrob* 2018; **17**: 35.
12 Abhishek K, Khunger N. Complications of skin biopsy. *J Cutan Aesthet Surg* 2015; **8**: 239–241.
13 Siddig EE, Mhmoud NA, Bakhiet SM *et al*. The accuracy of histopathological and Cytopathological techniques in the identification of the Mycetoma causative agents. *PLoS Negl Trop Dis* 2019; **13**: e0007056.
14 Aide memoire for Diagnostic Imaging Services. Geneva: World Health Organization. 2009. URL http://whqlibdoc.who.int/aidememoire/a71903.pdf [accessed on 12 November 2020].
15 Gooptu S, Ali I, Singh G, Mishra RN. Mycetoma foot. *J Family Community Med* 2013; **20**: 136–138.
16 Hematoxylin and Eosin (H&E) Staining Protocol. Roy Ellis. URL http://www.ihcworld.com/_protocols/special_stains/h&e_ellis.htm [accessed on Jan 2019].
17 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 Clin Microbiol* 1999; **37**: 3175–3178.
18 Fahal A, Mahgoub el S, El Hassan AM, Abdel-Rahman ME. Mycetoma in The Sudan: an update from the Mycetoma Research Centre, University of Khartoum, Sudan. *PLoS Negl Trop Dis* 2015; **9**: e0003679.
19 Quintana ET, Wierzbicka K, Mackiewicz P *et al*. Streptomyces sudanensis sp. nov., a new pathogen isolated from patients with actinomycetoma. *Antonie Van Leeuwenhoek* 2008; **93**: 305–313.
20 Kauser N, Sri AS, Kumari KS, Triveni B. Cytological diagnosis of actinomycotic mycetoma- a case report. *Ann Int Med Den Res* 2020; **6**: PT04–PT06.
21 Florentine BD, Staymates B, Rabadi M, Sarda N, Barstis J, Black A. Cancer Committee of the Henry Mayo Newhall Memorial Hospital. Cost savings associated with the use of fine-needle aspiration biopsy (FNAB) for the diagnosis of palpable masses in a community hospital-based FNAB clinic. *Cancer* 2006; **107**: 2270–2281.
22 Crist H, Hennessy M, Hodos J *et al*. Acute invasive fungal Rhinosinusitis: frozen section histomorphology and diagnosis with PAS stain. *Head Neck Pathol* 2019; **13**: 318–326.
23 van de Sande WW, Fahal AH, Goodfellow M, Mahgoub el S, Welsh O, Zijlstra EE. Merits and pitfalls of currently used diagnostic tools in mycetoma. *PLoS Negl Trop Dis* 2014; **8**: e2918.
24 Bakhiet SM, Siddig EE, Abdallah OB, Mohamed ESW, Fahal AH. Fine needle aspiration cytology utility in the identification of Mycetoma causative agents: The Mycetoma Research Center experience. *Khartoum Med J* 2018; **11**:1499-1506
25 IA ELH, Fahal AH, Gasim ET. Fine needle aspiration cytology of mycetoma. *Acta Cytol* 1996; **40**: 461–464.