Fine needle aspiration cytology utility in the identification of Mycetoma causative agents: The Mycetoma Research Center experience.

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Abstract:

Mycetoma is a serious neglectedtropical disease, characterised by various disabilities and high morbidity. Numerous causative organisms of fungal or bacterial origin are implicated in causing mycetoma. Accurate identification of the causative organism is mandatory for proper treatment and management. Currently, the available diagnostic tests for mycetoma are invasive, tedious, of low sensitivity and specificity and not available in the mycetoma endemic areas.

In this communication, we report the Mycetoma Research Centre (MRC), Khartoum, Sudan experience on the fine needle aspiration for cytology (FNAC) technique for the identification of the mycetoma causative organisms based on the cytological reports at the MRC Biobank in the period between 1991-2017. The sensitivity and specificity of the technique for the identification of *Madurella mycetomatis* was 88.7% and 57.3 %, respectively. For *Actinomadura madurae* it was 28.7% and 96.6%, respectively. Furthermore, it was 74.1% and 88.9%, respectively for *Streptomyces somaliensis* and 46.7% and 99.5%, respectively for *Actinomadura pelletieri*. From this study, it can be concluded that the technique is reasonably accurate in the identification of mycetoma causative organisms, rapid, minimally invasive, and cost-effective procedure. It can be used for the diagnosis of mycetoma in rural areas. The use of the ultrasound-guided aspiration may improve its accuracy.

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Introduction:

Mycetoma is a distinctive form of the chronic granulomatous inflammatory diseases characterised by the presence of the causative organisms in the form of grains in the affected tissues [1, 2]. It is caused by the inoculation of the causative agents that can be either fungi (eumycetoma) or bacteria (actinomycetoma) into the subcutaneous tissue [3 – 5]. The infection then spreads to affect the skin, deep structures and bones leading to devastating distortions, disabilities and high morbidity. It can be fatal if not managed appropriately [6 – 8]. This disease mostly affects the young individuals in the age group 15-30 years; however, in endemic areas, all age groups are affected. In some series, 30% of the affected patients were children [9, 10]. The triad of a painless subcutaneous mass, multiple sinuses and purulent or sero-purulent discharge that contains grains is pathognomonic of mycetoma [11 -13]. The extremities are affected most (80%). However, other sites such as chest and abdominal walls, perineum, gluteal areas can be affected [14 – 19]. The differential diagnosis of mycetoma includes many soft tissue benign and malignant neoplasms, foreign body granulomas, chronic osteomyelitis and others [20– 23]. The appropriate treatment of mycetoma is governed by the precise identification of the causative organisms and how the disease spreads along the tissues planes.

Various mycological and molecular tests are required to identify the causative organisms in surgical biopsies, and that include grain microscopy, culture, histopathological examinations and PCR

identification [24 – 31]. Grains can be obtained by fine needle aspiration for cytology (FNAC) and can be examined by cytological and histopathological using cell block technique. The disease extent can be determined by several imaging techniques such as conventional X-ray, ultrasound, MRI or CT scans [32 – 45]. Most of these tests and techniques have low specificity and sensitivity; require surgical biopsies; invasive; and proved to be expensive for patients and health authorities. Furthermore, they are not available in mycetoma endemic areas and require good experience [39].

Presently, there is no point of care diagnostic test for mycetoma. To establish the diagnosis of mycetoma, patients usually travel for long distances to tertiary health facilities in big cities. However, their poor socio-economic status, low health education, and roadblocks in particular during the rainy season always hinder them from achieving that [46 – 48]. With this background, this study was carried out to determine the accuracy of FNAC in the identification of the common mycetoma causative agents encountered at the MRC. The study included both the cytological and histopathological records of 1032 patients who presented to the MRC in the period of January 1991-July 2017 and we believe this is the largest ever reported series and, hence, it is worth sharing the experience.

Materials and Methods:

Data collection:

In this study, the surgical biopsies for histopathological examinations and the FNA cytological examination reports of 1032 patients seen at the MRC stored at the (MRC) Biobank during the last 25 years were carefully reviewed. The FNA technique was performed in patients with palpable masses. They were aspirated using 20 ml disposable syringe attached to 22-27 gauge needles with one inch needle length. A 25-gauge needle was used first and if the yield was not satisfactory then a wider needle 23 gauge was used. A 27 gauge needle was used when excessive bleeding from the lesions was encountered. The smears were allowed to air dry and stained using Diff-Quick staining techniques. Finally, the smears were examined by an expert histopathologist for the presence of the following cytomorphological features: cellularity of smears; the host inflammatory tissue reaction; the presence and types of the causative organisms' grains. All patients had surgical biopsies and that were fixed in 10% formal saline or formalin solutions. Paraffin processed blocks were prepared, the tissue blocks were cut using rotary microtome, and subsequently 3-5-ìm sections were obtained. Then, the sections were stained using Hematoxylin and Eosin stain (H&E) [49].

Statistical analysis:

FNAC-based diagnosis was compared to the histological examination of the same specimens. All data were entered, verified and analysed using SPSS version 16.0. Data were summarised as percentages for the categorical variables. Sensitivity of FNA was measured as the proportion of positive histopathological examination results. The specificity was measured as the proportion of negative histopathological examination results.

Ethical Statement:

Study ethical clearance was obtained from Soba University Hospital Ethical Committee. Individual patient's informed consent proved to be not necessary for this study as these tests were part of the routine diagnosis of the patients.

Results:

The studied population included 1032 patients with confirmed mycetoma. Their age ranged between 5-75 years, with a mean age of 27.9. There were 771 males (74.7%) and 261 females (25.3%). The study included 339 (32.8%) students, 174 (16.9%) workers, 172 (16.7%) farmers and (12.4%) housewives, (Table 1).

The accuracy of fine-needle aspiration cytology (FNAC):

The data included four mycetoma causative organisms, and these were *Madurella mycetomatis, Actinomadura madurae, Streptomyces somaliensis* and *Actinomadura pelletieri* (Figure 1). The

Madurella mycetomatis FNAC sensitivity and specificity rates, positive and negative predictive values were 88.7%, 57.2%, 77.6% and 75.2%, respectively, (Table 2). For *Actinomadura madurae* the statistical analysis of FNAC showed sensitivity, and specificity rates, positive and negative predictive values of 28.7%, 96.6%, 47.5% and 92.6%, respectively (Table 2). For the identification of *Streptomyces somaliensis* the statistical analysis of FNAC showed sensitivity and specificity rates, positive and negative predictive values of 74.1%, 88.9%, 28.5% and 98.30%, respectively (Table 2). For Actinomadura *pelletieri* the statistical analysis of FNAC showed sensitivity, and specificity rates, positive and negative predictive values of 46.7%, 99.5% 58.3% and 99.2 %, respectively (Table 2).

Table 1. The demographic characteristics of the studied population

Demographic characteristics	No. (%)
Sex	
Male	771 (74.7%)
Female	261 (25.3%)
Age in years	
< 20	259 (25.1%)
21-39	585 (6.7%)
>39	187 (18.1%)
Occupation	
Students	339 (32.8%)
Workers	174 (16.9%)
Farmers	172 (16.7%)
Housewife	128 (12.4%)
Unemployed	87 (8.4%)
Clerk	10 (1%)
Employee	16 (1.6%)
Others	98 (9.5%)

Table 2. Showing the sensitivity and specificity of the FNA in the identification of the different causative organisms.

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Madurella Mycetomatis		Cytological report		
Histological report	Positive	Negative	Total	%
Positive	573	165	738	71.5
Negative	73	221	294	28.5
Total	646	386	1032	100
Actinomadura Madurae		Cytological report		
Histological report	Positive	Negative	Total	%
Positive	29	32	61	5.9
Negative	72	899	971	94
Total	101	931	1032	100
Streptomyces somaliensis		Cytological report		
Histological report	Positive	Negative	Total	%
Positive	43	108	151	14.6
Negative	15	866	881	85.3
Total	58	974	1032	100

Actinomadura pelletieri	Cytological report			
Histological report	Positive	Negative	Total	%
Positive	7	5	12	1.16
Negative	8	1012	1020	98.8.
Total	15	1017	1032	100

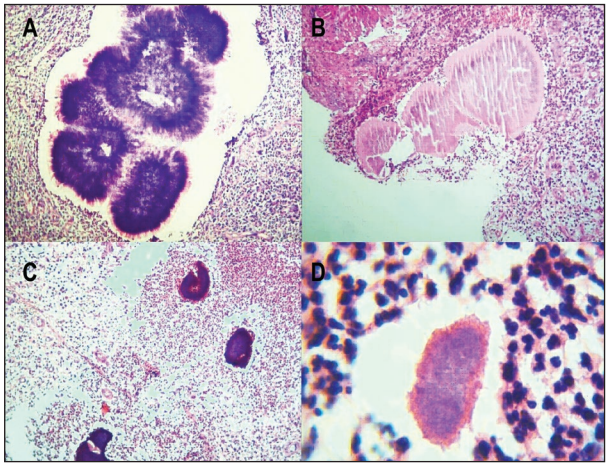

Fig 1. (A) Showing H&E stained histological sections of (A) *Actinomadura madurae* (B) *Streptomyces* somaliensis (C) *Actinomadura pelletieri and (*D) Nocardia Spp. (X40).

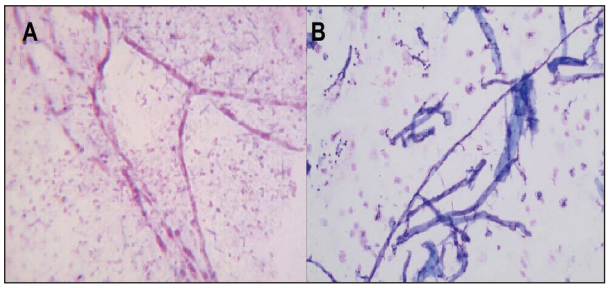

Fig 2. Showing cytological smears of (A) *Madurella mycetomatis* hyphae stained with Giemsa stain, (B) Synthetic Fibers stained Wright-Giemsa stain. (Giemsa stain, X40).

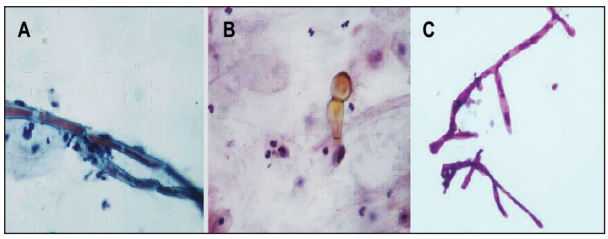

Fig 3. (A) Smear showing elongated structures of the Oedogoniales order. The chloroplasts form a chain interrupted by clear zones (Papanicolaou stain, ×400). (B) Smear showing fragment with swollen, bulbous segments of an algae of the order Oedocladium (Papanicolaou stain, ×400). (C) Smear showing hyphae of *Madurella mycetomatis*.

Discussion:

Fine needle aspiration for cytology technique is used for the diagnosis of many solid tumours, body fluid abnormalities and the identification of many infectious diseases' causative agents. Grieg and Gray in 1904 used the FNAC for the diagnosis of African trypanosomiasis for the first time [50 – 52]. It is interesting to note that, body fluids cytological examination was known prior to that, in particular, the urine and sputum cytological examinations which were performed in the 1800s. In 1930, a new era in the use of FNAC began due to rapid development in the needles design and industry that enabled obtaining sufficient and adequate cytological samples collection to make an accurate diagnosis [53] .

Regarding mycetoma, Bapat and Pandit in 1991 were the first to use the FNA for the diagnosis of actinomycetoma [54, 55]. Then El Hag and associates in 1996 used the technique for the diagnosis of mycetoma in 14 patients caused by different causative agents [56]. Yousif and colleagues in 2010 reported on the use of FNAC and cell blocks techniques to increase the accuracy of the diagnosis of mycetoma. Currently, the FNA is a routine test for the diagnosis of mycetoma at the MRC as it is a simple test to perform, not time-consuming and minimally invasive. However, its accuracy needs to be determined. Hence, the objective of this study is to determine the accuracy of the test.

The study had included 1032 cytological reports that were compared to the surgical biopsies histopathological examination which is the standard test in many centres. In the current study, the sensitivity and specificity of FNAC for the diagnosis of *Madurella mycetomatis* was 88.7%, and that is in line with Yousif and associates report

(87.5%). However, the low specificity (57.25%) reported here is not in line with their report. The small sample size (168 patients) included in their study may be the explanation of this different result. The low specificity can be attributed to the fact that many structures can mimic *Madurella mycetomatis* and that can induce false positive results. The most commonly encountered mimicry structures are vegetables or synthetic fibers [57, 58] as well as contaminating threads which can be encountered on the cytological slides and can resemble portion of fungal hyphae especially when these are stained by Romanowsky stains (Fig 2). Another possible mimicry structures are the *Algae* which can be encountered in the cytological smear as a contaminant, and they may be confused with the fungus, Fig 3, [59]. Furthermore, calcification may sometime resemble fungal yeasts, when it is fragmented, for the linear or branched hyphae. Also in cytological smears with scanty or fragmented *Madurella mycetomatis* hyphae, it is difficult, if not impossible, to make a diagnosis. Moreover, in cytological smears with extensive necrosis, the hyphae may appear more swollen and distorted and the resultant atypical morphological features may generate many errors. Therefore, careful examination of the aspirated materials and recognising these potent mimics that look like the grains of *Madurella mycetomatis* can help in reducing the false positive results.

This study revealed low sensitivity of the FNAC for the diagnosis of *Actinomadura madurae (*28.71%) *and Actinomadura pelletieri (*46.67%). That can be attributed to morphological similarities between the actinomycetes, and difficulties in obtaining adequate grains in the aspirates. To improve the aspirate yield and to obtain an adequate amount of grains, ultrasound-guided aspiration may be useful to target the grains within the cavities. Furthermore, good correlation between the patients' clinical presentation, grains culture, histological and cytological examinations is mandatory to reach a proper diagnosis. This technique is an operator-dependent and good training is a prerequisite.

In conclusion, to improve the accuracy of the FNAC, great caution must be exercised in obtaining, processing and interpretation of cytological smears. The technique can be used as the first line of investigation for the identification of the mycetoma causative agents. It is useful in epidemiological field studies as well as the clinical settings with meagre and scanty resources. To improve its accuracy, sensitivity and specificity, ultrasound-guided aspirations may be helpful to aspirate the grains within the suspicious lesions. The technique is rapid, minimally invasive, and cost-effective particularly in mycetoma endemic areas. Close correlation between the clinical presentation, grains culture and histological examination is mandatory to reach a correct diagnosis.

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