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A Retrospective Study Of The Histopathological Spectrum Of Mycetoma: A Quest For Species Identification.

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Abstract

Mycetoma is a neglected tropical disease. In endemic areas, only a few diagnostic tests and techniques are available that have badly affected the patient's proper diagnosis and management. To study the histopathology of different grain patterns on hematoxylin-eosin stains for species identification. Materials and Methods: This retrospective study was conducted in 25 cases diagnosed as mycetoma. Records of the biopsies of the last five-year period were reviewed. Out of twenty-five cases in eighteen cases, nine Actinomycetoma and nine Eumycetoma grains were seen in this study. Out of nine Actinomycetoma cases, four cases showed the morphology of Nocardia species. Three cases showed the morphology resembling the Actinomadura madurae species. One case showed a morphology similar to Actinomadura pelletierii species. One case showed the morphology of Streptomyces somaliensis. Out of nine Eumycetoma cases, six showed a vesicular and hyphae appearance without a brown matrix, of which three cases showed morphology resembling the Petriellidium boydii. Two cases showed a morphology similar to Acremonium. However, one out of six was tricky, and exact species histopathological identification was not possible. However, it fulfilled the criteria of Petriellidium boydii. Out of nine Eumycetoma cases, three cases gave vesicular and hyphae appearance with a brown matrix, of which two cases resembled the Madurella mycetomatis, and one showed brown matrix dense at the periphery, suggesting either Madurella grisea or Madurella mycetomatis species. Morphology, size, the color of the matrix material, density in periphery and center, crackling, and fracture of grain; these parameters help to narrow down the mycetoma species if correlated with each other and with clinical-epidemiological parameters.

Keywords: Actinomycetoma, Eumycetoma, Grain, Species, Morphology

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INTRODUCTION

Mycetoma is a neglected tropical disease caused by several microorganisms, including fungi (Eumycetoma) and bacteria (Actinomycetoma). Correct diagnosis is mandatory for proper treatment. In endemic areas, the medical and health settings are commonly suboptimal, and only a few diagnostic tests and techniques are available that have badly affected the patient's proper diagnosis and management. Various diagnostic tests and techniques are used for specific diagnoses and species identification.

Currently, culturing the grains is considered to be the golden standard for species identification in many institutes. However, this technique is tedious and time-consuming due to the slow growth rate, and it needs expert microbiologists to identify the causative agents based on the macroscopic appearance of the isolates. Furthermore, contamination is common. Patients on medical treatment may have non-viable gains, so identifying the causative organism is difficult. In a histopathological examination, it is easy to discriminate between fungal and bacterial causative agents. Though identification at the species level is more challenging, each species shows a unique appearance on histopathology, which can be used as a parameter to diagnose the species without culture reports or repeated negativity of these reports. In conclusion, histopathological examination of grain morphology for species identification is a more practical technique for rapid species identification than grain culture in many endemic regions.

MATERIALS AND METHODS

It was a single-center retrospective observational study of hematoxylin and eosin-stained slides of all cases of mycetoma, proven on biopsy, showing grain (including Eumycetoma and Actinomycetoma) and on clinicopathological correlation (in hematoxylin and eosin-stained slides without grain) in last five years. Twenty-five patients of mycetoma were included.

The study was carried out after obtaining the requisite ethics committee permission. Records of biopsies conducted over the last five-year period were reviewed. The cases showing grain (18 out of 25) were selected for in-depth morphological analysis. Records of available clinical data at the time of biopsy were documented. The biopsy specimen was preserved as paraffin blocks, and hematoxylin and eosin-stained slides were reviewed for three months. Quantitative data is represented as their mean ± SD. Categorical and nominal data are expressed in percentages. The t-test is used for analyzing quantitative data, and categorical data is analyzed using the chi-square test. The significance threshold of the p-value was set at <0.05. All analyses are carried out using MS Excel and GraphPad software.

RESULTS

Age

The patients included in this study showed the age range between 17 to 70 years, with 92 percent male dominance and a male-to-female ratio of (11.5:1). The mean age was 43.60 years.

Demography

The most cases were from Maharashtra state, 15 cases (60%), with Uttar Pradesh 4 cases (16%), Gujrat and Rajasthan each showing 2 cases (8%) and 1 case each from West Bengal and Madhya Pradesh (4%). Out of nine Actinomycetoma cases, seven cases belonged to the Maharashtra region, whereas two cases (one from Madhya Pradesh and one from Gujrat) were outside Maharashtra. In nine cases of Eumycetoma only three hailed from Maharashtra, whereas six cases (Northside- Two from Rajasthan and Four from Uttar Pradesh) were from outside Maharashtra. This geographic distribution correlates with most of the studies.

Occupation of patient

Our study also shows that the highest percentage of patients (16 cases; 64%) affected belonged to the category of heavy outdoor work, which included farmers and field workers.

History of Trauma

The majority of patients (64%) had a history of local trauma; (36%) patients had no history of local trauma.

Duration of lesion

A clinical lesion of mycetoma in terms of duration showed that 40 % of patients were reported to have a duration of >1 year to 9 years, with 36 % of cases showing > 9 years to 15 years. 16 % of cases showed the duration of the lesion for less than one year, and 8 % of cases were present for more than 15 years.

Site of Lesion

The Lower foot was affected in 22 cases (88%). Twelve cases (48%) occurred on the left foot.

History of discharge of grain

The majority of the patients (64 %) gave a history of discharge containing grains, and the majority of these grains were white (11; 44%), followed by black (3; 12%), yellow (2; 8%) and no discharge of grain in (9; 36%) cases.

Histopathological statistics

In this study, the most frequent epidermal reaction pattern was irregular epidermal hyperplasia in 17 cases (68%). Trans-epidermal elimination of grain was observed in 9 cases (36%). The most common dermal reaction pattern observed was a type 1 reaction pattern (15 cases; 60%) followed by a type 2 reaction pattern (10 cases; 40%). Fibrosis was the most consistent finding in almost every case (100%). Deposition of fibrin in vessel walls was observed in 22 cases (88%), with absence in 2 cases (8%).

After evaluation of grain on histopathology, in 18 cases, Actinomycetoma and Eumycetoma were seen in 9 cases each (36%). No grain was seen in 7 cases (28%).

On histopathological examination with hematoxylin and eosin-stained sections, nine cases were Actinomycetomas, all showing blue/ basophilic grain color. Whereas out of nine Eumycetoma cases, 5 cases showed pink/eosinophilic color, three showed brown color, and only one showed blue/basophilic color.

In this study, out of 9 Actinomycetoma cases, 5 cases showed < 0.5mm size, and 4 cases showed a size of >0.5mm to 2mm. Out of 9 Eumycetoma cases, 7 cases showed a size of >0.5mm to 2mm, one showed a size of >2mm to 4 mm, and one size >4mm. Most of the morphologically identified or narrowed down species showed approximately similar size variation of that specific species as mentioned in the standard textbooks except that of morphologically identified Streptomyces somaliensis and Actinomadura pelletierii.

In this study, the Pale grain Eumycetoma species was more likely to show the invasion of grain by neutrophils.

The Eumycetoma showing crackling in grain in this study belonged to the Madurella mycetomatis species. Out of nine cases of Actinomycetoma, only one showed the crackling of grain; the one that showed the crackling morphologically resembled the grain of Streptomyces somaliensis.

In this study, fracturing was seen in the Actinomadura madurae species. However, these grains retained the characteristic peripheral deep, dense basophilic staining, whereas Actinomadura pelletierii grain maintained the peripheral and central densely packed filamentous morphology with uniformly dense purple-blue basophilic staining similar to the mother grain. Out of nine Actinomycetomas, only one case showed the pale blue compact matrix. Out of nine Eumycetoma cases, six cases showed no brown matrix; two cases showed the presence of brown matrix throughout the grain resembling the Madurella

mycetomatis, and one showed brown matrix dense at the periphery, suggesting either Madurella grisea or Madurella mycetomatis species.

DISCUSSION

Each species of mycetoma, Eumycetoma, or Actinomycetoma showed characteristic morphological findings, which were evaluated with the studies that correlated their findings with culture reports. In this study, most of the morphologically identified or narrowed down species showed approximately similar size variation along with histopathological morphology of that specific species as mentioned in the standard textbooks.

We will discuss the detailed histopathological appearance of grains below:

Out of nine Actinomycetoma cases, four showed the morphology of very small grain <1mm, closely packed as granular grain, not easily discernible interlacing filaments [Figure 1] resembling Nocardia species.

Grains of Nocardia, as per standard description, show the filamentous appearance of the grains with positive ZN staining. Its shape varies from small clumps like a ball to large irregular clumps with irregular outlines. The grain is lightly basophilic and well-stained, but the individual filaments are not discernible. The grains can easily be differentiated from other actinomycetes because they are Ziehl neelson (ZN) positive [1].

Out of nine Actinomycetoma cases, three cases showed a small size grain (>1mm-2mm) with a multi-lobulated irregular and oval shape and morphology of fine branching with easily discernible interlacing filaments [Figure 2]. The periphery of the grain appeared densely packed filaments with dense basophilic staining, whereas the center of the grain appeared less dense with rarefaction and lightly basophilic staining [Figure 3], resembling the Actinomadura madurae species.

Grains of Actinomadura madurae, as per standard description, show two types identified as large and small. The large type is multi-lobed with a deeply stained basophilic outer border and a slightly amorphous paler center. The small type is either rounded or oval with basophilic homogeneous staining, with a wide pink border and wide eosinophilic club-like projections.

Smaller grains are more homogeneous and are difficult to distinguish from A. pelletierii. However, even the small grains of Actinomadura madurae have a more deeply stained purple fringe, which is not seen in Actinomadura pelletierii [1].

Out of nine Actinomycetoma cases, one case showed the morphology of densely packed interlacing filaments with small size grain (>0.5mm-2mm). The periphery and center of the grain appeared equivocal, showing densely packed filaments with dense homogenous purple-blue basophilic staining [Figure 4] resembling the Actinomadura pelletierii species.

Grains of Actinomadura pelletierii, as per standard description, show small grains compared with those of the other two species of actinomycetes. They are either deep violet or basophilic in color. The grains are rounded or oval, semicircular, and sickle-like [1] and have an angulated border. The filamentous structures are difficult to detect; however, a careful examination of the periphery of the grains may show some of them.

Out of nine Actinomycetoma cases, one showed the morphology of a homogenous pale blue/ basophilic grain appearance with crackling [Figure 5]—a very small size grain (<0.5mm) with regular smooth outlines resembling the Streptomyces somaliensis.

Grains of Streptomyces somaliensis, as per standard description, show a rounded and homogeneous appearance in sections. They are eosinophilic or basophilic and often show longitudinal cracks due to sectioning [1].

For further simplification, the observations and references of Actinomycetoma are provided.

In this study, out of nine Eumycetoma cases, six cases showed Vesicular and hyphae appearance without brown matrix, and 3 cases gave Vesicular and hyphae appearance with brown matrix.

Out of Six cases of Eumycetoma grain with vesicular and hyphae morphology and without brown matrix, Three cases showed the morphology of vesicles and hyphae with pink/eosinophilic staining. All three belonged to the small-size grain category (>0.5mm-2mm) with irregular outlines.

The periphery of the grain showed large, prominent, variable-sized vesicles, whereas the center of the grain showed less dense [Figure 6], few hyphae, and small-sized vesicles resembling the Petriellidium boydii.

Grains of Petriellidium boydii, as per standard description, are more likely to contain numerous vesicles or swollen hyphae and be surrounded by a prominent eosinophilic fringe. The vesicles are large and more prominent at the periphery. Vesicles were the largest in grains, with a thick eosinophilic fringe. In cases of Petriellidium boydii infection, where the eosinophilic fringe was absent or minimal, large vesicles could be seen scattered throughout the grains [2].

Out of Six cases of Eumycetoma grain with vesicular and hyphae morphology and without brown matrix, Two cases showed the morphology of vesicles and hyphae of small size grain (>0.5mm-2mm) with the periphery of grain showing small vesicles of the same size [Figure 7]. In contrast, the center of the grain showed an intricate network of hyaline mycelium and hyphae in interweaving skeins with swirling patterns [Figure 7] resembling the Acremonium.

As per the standard description, grains of Fusarium and Acremonium usually have a minimal fringe and contain a dense mass of intermeshing hyphae, forming a swirling arrangement of hyphae. However, Fusarium rarely shows vesicles even if a swirling pattern is present, separating it from acremonium species [2].

However, the features separating Petriellidium boydii, Fusarium, and Acremonium grains are not invariable and can only be used as an approximate guide to the correct identity of the organism [2].

However, one out of six was tricky, and exact species histopathological identification was not possible. However, it fulfilled the criteria of Petriellidium boydii.

Out of nine cases of Eumycetoma, three cases showed vesicular and hyphae morphology with brown matrix; out of these 3 cases, two cases showed the morphology of vesicles and hyphae with brown staining. The periphery of the grain showed homogenous vesicular spores with radially arranged hyphae embedded in a dense brown [Figure 8] black matrix. The center of the grain showed the homogenous vesicular spores and hyphae embedded in a less dense brown matrix [Figure 8], resembling the Madurella mycetomatis.

Grains of Madurella mycetomatis, as per standard description, show three structural forms, namely filamentous, vesicular, and mixed variety, in which both filamentous and vesicular structures are seen. They are rounded, oval, or irregular due to crushing. The hyphae of the filamentous grains are large and segmented. The whole grain is embedded in a hard brown material called cement secreted by the organism [1].

Of the three cases, vesicular and hyphae morphology with brown matrix, one showed the morphology of vesicles and hyphae with brown staining with small grain (<0.5mm). The grain periphery showed vesicles and hyphae distributed randomly with the dense collection and embedded in a dense brown-black matrix with rarefaction in the center resembling [Figure 9] either Madurella grisea or Madurella mycetomatis species.

The presence of an amorphous matrix narrows the diagnosis to consideration of only three eumycotic agents: Madurella mycetomatis, Madurella grisea, and Leptosphaeria. If this amorphous matrix is present throughout the colony, imparting a grainy appearance, a provisional diagnosis of Madurella mycetomatis can be given. If a peripheral amorphous matrix is present, the etiologic agent can be any of the three. The absence of the amorphous matrix rules out the above three and indicates other eumycotic agents [3, 4].

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Figure 1: Multiple closely packed appearing as granular grain, not easily discernible interlacing filaments. 40x (H & E)

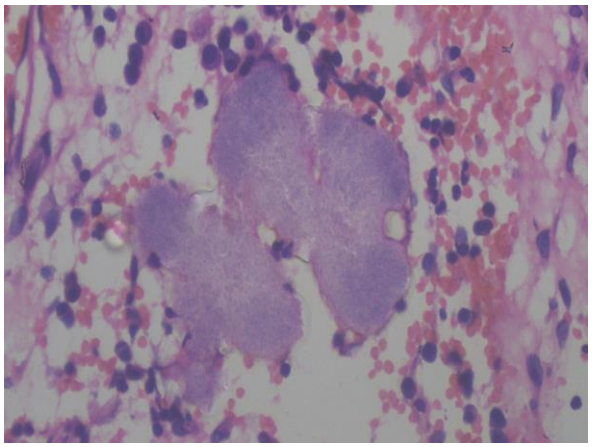

Figure 2: Grain with bluish appearance and dense peripheral staining. 10x (H & E)

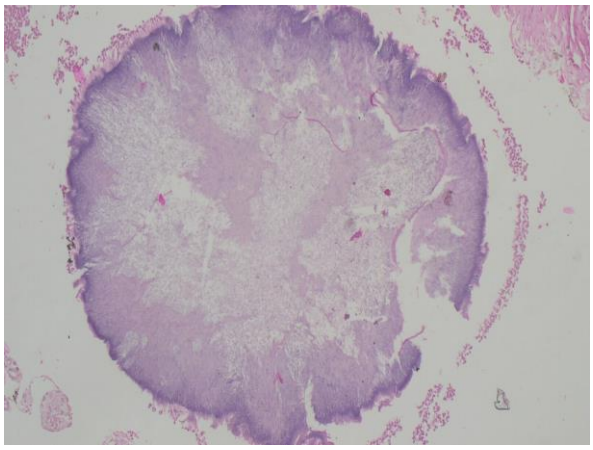

Figure 3: Peripheral dense basophilic staining, whereas the grain center appeared less dense with rarefaction and was lightly basophilic. (20x) (H & E)

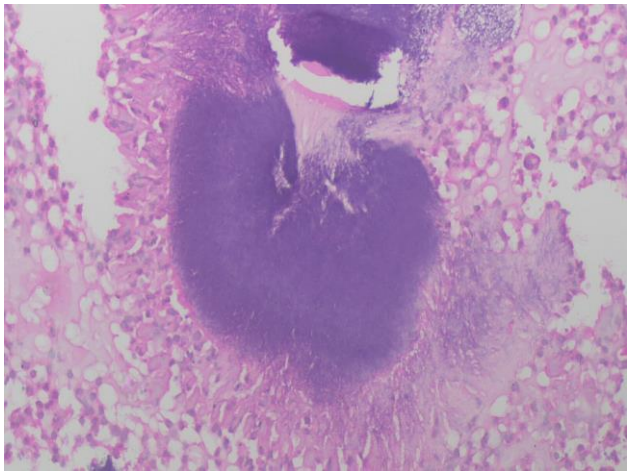

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Figure 4: Dense dark purple-blue basophilic stained homogenous appearance. 20x (H & E)

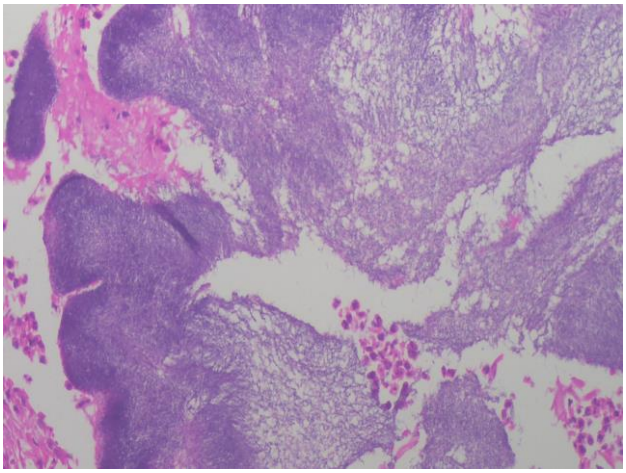

Figure 5: Homogenously compact pale blue grain with smooth outlines and crackling. 40x (H & E)

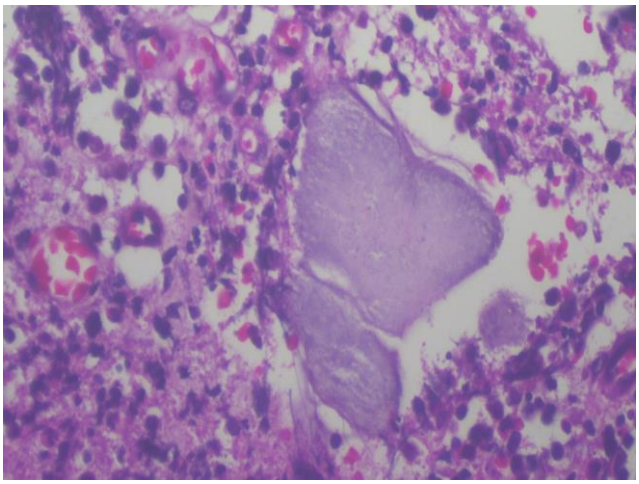

Figure 6: Vesicles and hyphae with large prominent vesicles of irregular shape at the periphery and club-shaped dense spledore-hoeppli phenomenon. 40x (H & E)

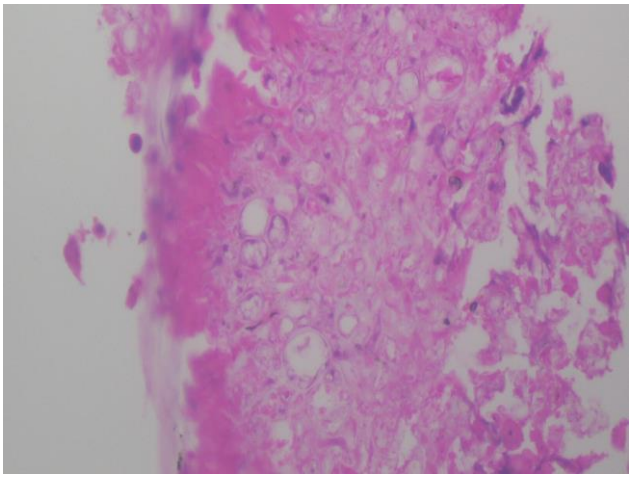

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Figure 7: The periphery of the grain with small vesicles of the same size, whereas the center of the grain shows an intricate network with a swirling pattern and dense club-shaped splendor- hoeppli phenomenon. 40x (H & E)

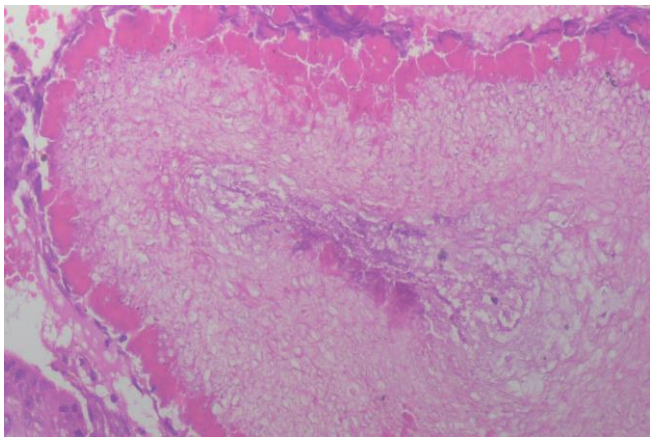


Figure 8: Large grain with irregular outlines and compact brown matrix. 10x (H & E)

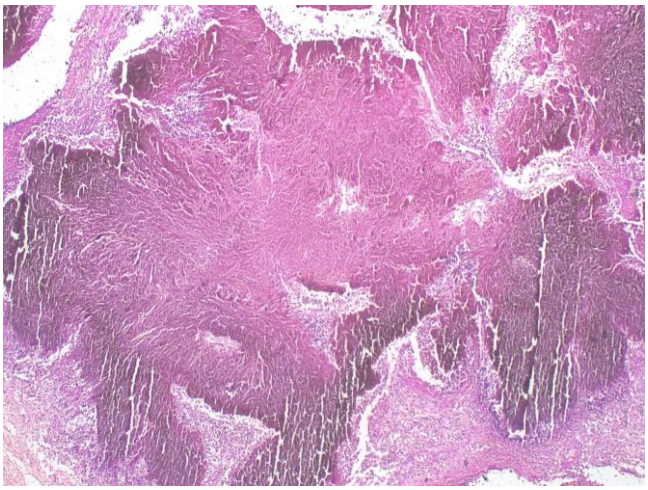
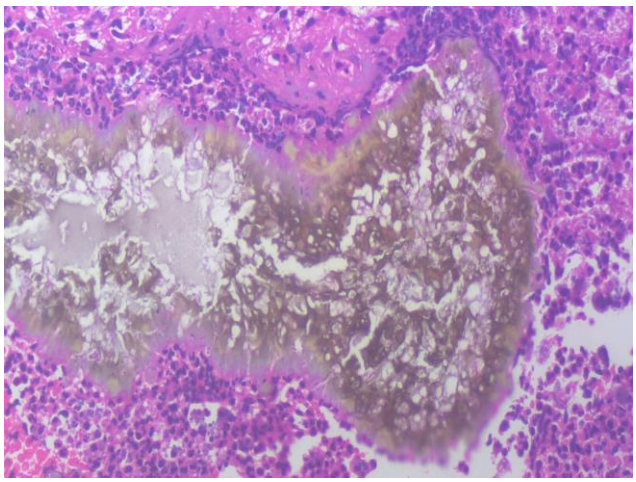


Figure 9: Vesicles and hyphae with dense compact brown matrix at periphery and rarefaction in the center. 20x (H & E)



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CONCLUSION

The cumbersome procedure of grain culture in mycetoma showed repeated culture negativity; diagnosing these cases on histopathological characteristics is practicable. Each species of mycetoma, Eumycetoma, or Actinomycetoma showed characteristic morphological findings, as discussed, which were evaluated with the studies that correlated their findings with culture reports. In this study, most of the morphologically identified or narrowed down species showed approximately similar size variation of that specific species as mentioned in the standard textbooks except that of morphologically identified Streptomyces somaliensis and Actinomadura pelletierii. Each species showed a unique appearance on histopathology, which can be used as a parameter to diagnose the species in the absence of culture reports or repeated negativity of these reports. Morphology of grain on hematoxylin and eosin-stained slides, size of grain on histopathology, the color of the matrix material, crackling of grain, fracture of grain, geographic location of the patient, and color of discharged grain, these parameters help to narrow down the species if correlated with each other. In conclusion, histopathological examination of grain morphology for species identification is a more practical technique for rapid species identification than grain culture in endemic regions.

REFERENCES

[1] Siddig, Emmanuel & Fahal, Ahmed. Histopathological Approach in Diagnosis of Mycetoma Causative Agents: A Mini Review. J Cytol Histol 2017;8: 466.
[2] Hay RJ, Mackenzie DW. The histopathological features of pale grain Eumycetoma. Trans R Soc Trop Med Hyg 1982;76: 839-844.
[3] Ameen M, Arenas R. Emerging therapeutic regimens for the management of mycetomas. Expert Opin Pharmacother 2008;9: 2077-2085.
[4] Reiss E, Shadomy HJ, Lyon GM (2011) Fundamental Medical Mycology. Reiss E, Shadomy HJ, Lyon GM. 1st ed. New Jersey: John Wiley and Sons; 2011. p. 521-522.

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