

Histopathological Approach in Diagnosis of Mycetoma Causative Agents: A Mini Review

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Abstract

Mycetoma is a unique neglected tropical disease which distributed worldwide and endemic in tropical and subtropical regions. This disease is caused by a large number of micro-organisms of fungal (eumycetoma) or bacterial (Actinomycetoma) origins. The disease is characterized by a swelling in the cutaneous and subcutaneous tissues, with the formation of sinuses tract in some patients with numerous deformities and disabilities. Mycetoma infection affects all ages. The diagnosis of the mycetoma is based on identification of the causative organism and the disease extension which in turn considered as the first steps in the management of the affected patients and eventually predicting disease treatment outcome and prognosis. Different diagnostic tools were developed that aimed to identify the causatives agent. These include direct microscopy, Fine needle aspiration, Imprint cytology, histopathological, culturing techniques and molecular identification. Since histopathology is continues to be a rapid diagnostic tool as well as cost effective means of providing a presumptive or/and in some instances a definitive diagnosis of an invasive mycetoma infection, as well as it able to detect the causative agents invasion of tissues and vessels as well as host tissue reaction to the causative agents, however no way to replace the culture and molecular diagnosis that remain the most powerful tools for the definitive diagnosis of the causative agents. In our clinical setting at Mycetoma Research center the histopathology report, considered as a powerful tool as it include a comment stating the morphological appearance of the causative agents and the type of the host tissue reactions as well as it stated clearly the possible differential diagnosis. In this review we will discuss varies histopathological appearance of mycetoma causative agents as well as the common used histochemical stains that can aid in the diagnosis and the host tissue reaction to varies causative agents.

Keywords: Mycetoma; Histopathology; Diagnosis

Introduction

Mycetoma is a chronic subcutaneous granulomatous inflammatory disease caused by several microorganisms including true fungi (eumycetoma) and bacteria (actinomycetoma) [1,2]. The disease is characterized by numerous deformations and disabilities. Mycetoma is endemic in the so called the Mycetoma belt that includes various countries across the world, but it is reported extensively from Sudan, Mexico and India [3,4]. The disease is characterized by a triad of subcutaneous swelling, multiple sinuses and discharge and painless mass [1]. Most of the patients were come at late stage of the disease and this attributed to the silence onset of the disease that characterized with the painless lesion, and the low socioeconomic status of the affected populations [3,5-7]. The proper treatment of mycetoma is based on the identification of the causative agents. The Histopathological identification of the causatives agent of mycetoma was established earlier in 1950s [8]. The causative agents can be identified using hematoxylin and eosin (H&E) stained sections, but special stains are crucial for comprehensive identification of the causative organisms.

Histopathological appearance of grains using H and E staining technique

Eumycetoma: *Madurella mycetomatis* grains tend to be large, light to dark brown in color, irregular outlines and tend to be fracture (Figure 1). Interestingly there were three types of the grains filamentous one, vesicular and admixed type [9]. The filamentous type, is the most common type, consists of brown septate and branched hyphae that may be slightly more swollen towards the edges (Figure 2).

In the cortex, the filaments are arranged radially while in the medulla they tend to have multi-directional route. The second type of grain is the vesicular one this type is composed of unusually large cells that look like vesicles (Figure 3).

Interestingly, an excellent clue that can aid the pathologist for better diagnosis is looking at the grains, for the brown granular cement material and pigmented materials, for example the grains of *Falciformispora senegalensis* and *Trematosphaeria grisea* have a black color but when careful examination to the center of the grains there is no pigmentation and cement matrix were observed, but interestingly, a

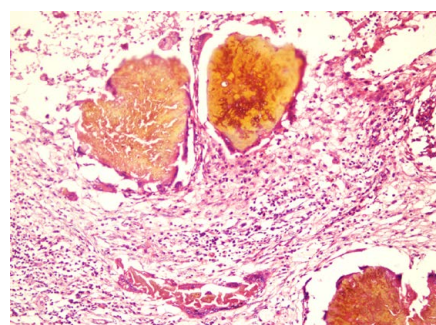


Figure 1: *Madurella mycetomatis* grains that tend to be large, light to dark brown in color, irregular outlines and tend to be fracture (H and E, X20).

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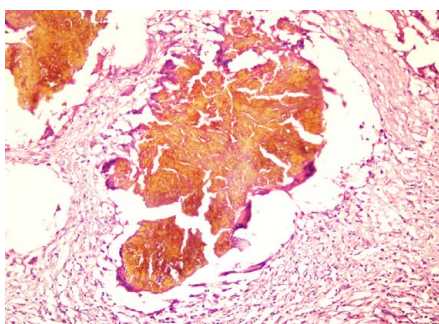


Figure 2: The filamentous Pattern, consists of brown septate and branched hyphae that may be slightly more swollen towards the periphery (H and E, X20).

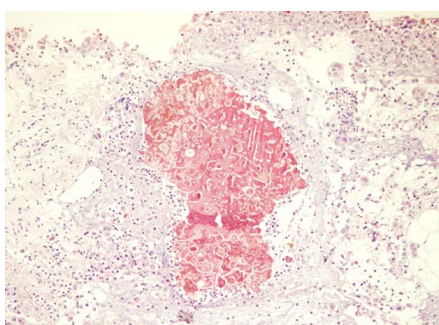


Figure 3: The vesicular Pattern of the grains which is composed of unusually large cells that look like vesicles (H and E, X20).

dark color observed at the periphery of the grains [8]. Histopathology alone failed to discriminate between *F. senegalensis* and *T. grisea*. Another pitfall in histopathology diagnosis of mycetoma were seen when examined histopathological sections for Pale grain eumycetoma that can be caused by *Pseudallescheria boydii*, *Acremonium* spp. and *Fusarium* spp. The histopathological appearance look and therefore it is almost impossible to distinguish from each other [9,10].

Actinomycetoma

***Nocardia brasiliensis*:** The grains of *Nocardia brasiliensis* is failed to be stained with routine H and E staining technique, they are none pigmented. Its shape is varying from small clump like a ball to large irregular clumps with irregular outlines. The grains can easily be differentiated from that of other actinomycete in that it is Ziehl neelson (ZN) positive.

***Streptomyces somaliensis*:** The grains are rounded to oval in shape, with homogenous appearance in tissue section, they appear as faint yellow in unstained section, and the grains are not well stained with H and E. Moreover, as a result of sectioning they may show longitudinal cracks, the hyphae are fine (measured between 0.5 - 2 µm in diameter), closely package and embedded in cement matrix.

***Actinomadura pelletieri*:** the grains are smalls round to oval and semicircular and sickle like where been observed, the filamentous structure are pretty difficult to be detected however a careful and meticulous examination to the periphery of the grains may show some of them, this type of the grains have a characteristic of deep violet stain using H and E (Figure 4) which allow the definitive diagnosis without a need for culturing techniques.

Actinomadura madurae

The grains colors were range from yellow to white therefore in

grossing it is difficult to discriminate it from the surrounding fat. Histologically the grain size can be range from small to large one, the large grains have a characteristic variegated pattern. The periphery of the grain is opaque, homogenous and deep purple when stained with H and E stain, while the center is less dense (Figure 5). Additionally, the periphery of the grains shows an eosinophilic material, This, material contains immunoglobulin's (Figure 6). Smaller grains are more homogeneous and are difficult to distinguish from *A. pelletieri*. However, even the small grains of *A. madurae* have a more deeply stained purple fringe, which is not seen in *A. pelletieri*.

Special Histochemical Stains that can Aid in Diagnosis of Mycetoma Causatives Agent

Several histochemical stains are essential for in-depth identification

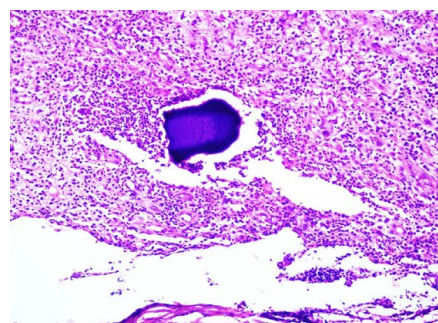


Figure 4: *Actinomadura pelletieri* grains which are smalls round to oval, the filamentous structure are pretty difficult to be detected however a careful and meticulous examination to the periphery of the grains may show some of them, this type of the grains have a characteristic of deep violet stain (H and E).

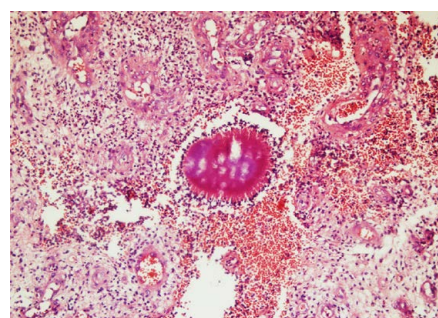


Figure 5: *Actinomadura madurae* grains, as we can see the periphery of the grain is dense, homogenous and deep purple while the center is less dense or even appears hollow (H and E, X10).

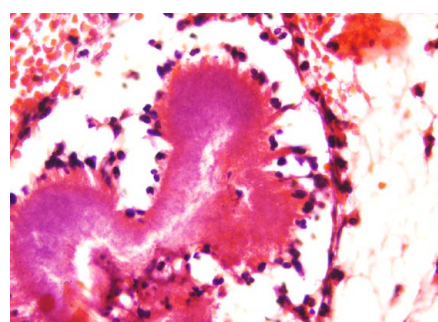


Figure 6: *Actinomadura madurae* grains with the periphery shows a brightly eosinophilic material forming clubs (H and E, X40).

of the causative organisms [11-14]. Gram stains, such as Gram Weigert, play a critical role in differentiating between bacterial and fungal agents of mycetoma the fine filamentous agent with in the grains of actinomycetoma were gram positive whereas eumycetoma are gram negative (Figure 7).

Zeilh Neelsen stain is off value in differentiating *Nocardia* spp. From other actinomycotic agents, *Nocardia* stained positive with ZN technique unlike other actinomycotic agents which were stained negative (Figure 8). [13].

Other special stains like Periodic acid-Schiff (PAS), Gomori methanamine silver, and Gridley are the most useful stains for detecting hyphae and chlamydospores in eumycetoma grains [12-14].

Host Tissue Reaction toward Mycetoma

Three types of host tissue reaction against the organism were described and they are identical in all types of mycetoma [15].

In Type I tissue reaction, there is a zone of polymorphonuclear cells that surrounded the grains, sometimes the grains were intermingled with neutrophils and in this instance the grains shows cracking and fragmentation. Beyond the Neutrophilic zone, there is granulation tissue containing macrophages, lymphocytes, plasma cells. The mononuclear cells increase in number towards the periphery of the lesion. The outermost zone of the lesion consists of fibrous tissue.

In Type II tissue reaction, the neutrophils largely disappear and are replaced by macrophages and multinucleated giant cells. The latter engulf grain material. This consists largely of pigmented cement substance although hyphae are sometimes identified. Other inflammatory cells and histological changes are the same as in type I reaction.

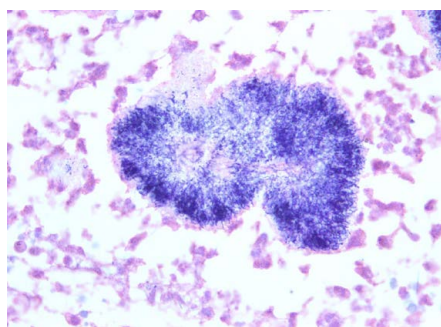


Figure 7: *Nocardia brasiliensis* stained positive with Gram stain technique (Gram stain, X40).

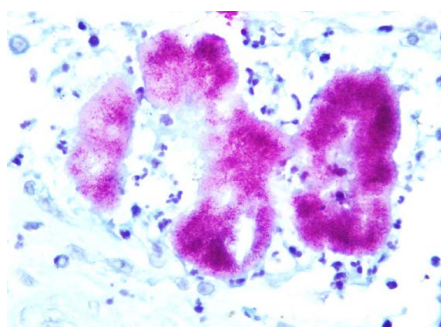


Figure 8: *Nocardia brasiliensis* stained positive with ZN staining technique (ZN, X40).

Type III reaction, is characterized by the formation of a well-organized epithelioid granuloma with Langhan's type of giant cells. The center of the granuloma sometimes contains remnants of fungal material but in some no fungal elements could be identified. Inflammatory and histological changes are the same as described for both type I and II reactions.

Conclusion

In summary, Histopathological report can be considered as a diagnostic tool that can aid in the diagnosis of the causative agent by the mean off it can discriminate whether it is eumycetoma or actinomycetoma, however to discriminate between the actinomycetoma agents some special stains like gram and ZN stain can be used however for the definitive diagnosis regarding the species level its self the histopathology have a limited role in this area and we should seek for another modalities like doing Molecular testing.

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