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Mycetoma at a tertiary care hospital in Saudi Arabia: correlation of histopathological and clinical findings

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PEER REVIEW

Peer reviewer

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Comments

This is a paper that gives a brief overview of mycetoma in a particular region of Saudi Arabia, offering insight on the type of condition that occurs in that particular region. It gives very precise information about the mycetoma in Saudi Arabia, and allows us to learn more about the etiology and clinical characteristics of mycetoma in that region.

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ABSTRACT

Objective: To present the histopathological and clinical correlation of mycetoma among patients attending King Abdulaziz University Hospital between 1998-2013.

Methods: The data of all histopathologically diagnosed mycetomas in the period between January 1998 and January 2013 were collected through a computerized database search of the anatomic pathology archives at King Abdulaziz University Hospital. The collected data were analysed. Identification of species were performed for five patients using 16S ribosomal DNA and internal transcribed spacer 2.

Results: There were 19 patients with mycetoma with an average age of 44.26 years and male: female ratio of 4:1. Actinomycetoma were 63.15% and eumycetoma were 36.84%. All patients presented with the classic lesions; presenting as painless subcutaneous mass, sinuses and discharge containing grains. The swellings were of slow evolution, with preferential foot localization. Species specification performed for samples from five patients with active lesions revealed species of *Actinomyces israelii* and *Madurella mycetomatis* in respective cases.

Conclusions: Actinomycetoma is more common than eumycetoma in this region. The fact that one of the patients with eumycetoma was a Saudi national raises the possibility of an indigenous species similar to *Maduraella mycetomatis* to be further explored for characteristics and pathogenesis. The disease has to be prioritized again and more robust and quick molecular diagnostic tools should be made available in order to save patients form disfiguring amputations.

KEYWORDS

Mycetoma, Madura, Cementing matrix, Actinomycetoma, Saudi Arabia, 16S rDNA

1. Introduction

Mycetoma is a chronic, granulomatous disease of the subcutaneous tissue invading the skin, which may involve muscle, bones and neighboring organs[1,2]. It is characterized by the triad of tumefaction, draining sinuses and presence of colonial grains in the exudates[3]. The most common site of occurrence is foot (approximately 70% cases), which explains the synonym "Madura foot"[4]. The term was coined by Gill after Madurai a district of Tamilnadu, India in 1842[5]. It can occur in almost any region of the body] and occasionally involves the hands, back or shoulders[1,6]. Mycetoma usually occurs in farm workers and people habituated to walking bare-footed[1]. The organisms enter the subcutaneous tissue by traumatic inoculation[4]. It is endemic in relatively arid areas such as tropical or sub-tropical regions[2,4].

Mycetoma is caused by variety of organisms that show wide geographic distribution with variable disease course that necessitates different therapies. In order to address this diversity

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mycetoma has been divided into two main groups according to causative agents: actinomycetic mycetoma caused by aerobic bacteria actinomyces, and eumycetoma caused by true fungi, most cases were caused by bacteria (50.8%) and a smaller percentage by fungi (41.7%), although the distribution varies in different countries. In many African countries more eumycetoma cases have been reported than those of actinomycetoma[7,8]. Both forms present as a progressive, subcutaneous swelling, although actinomycetoma has a more rapid course. Multiple nodules develop and may suppurate and drain through sinuses, discharging grains during the active phase of the disease[4]. Therapy of these two groups is dependent on the entity of causative agents. Actinomycetoma is amenable to treatment by antibiotics, either alone or in combination[9]. Eumycetoma is usually treated by aggressive surgical excision combined with antifungal treatment[4].

The diagnosis of mycetoma is often challenging as it gets delayed for a long time. It can lead to deformities and amputation of limbs. Diagnosis of mycetoma in Saudi Arabia requires a high degree of suspicion. The available reports on the frequency and pattern of mycetoma in Saudi Arabia can be dated back to 1999[10]. We present this study to update our understanding of mycetoma in this region.

The aim of this retrospective study was to present the histopathological and clinical correlation of mycetoma among patients attending King Abdulaziz University Hospital between 1998-2013.

2. Materials and methods

2.1. Study setting and population

A retrospective study of all histopathologically diagnosed mycetoma patients in the period between January 1998 and December 2013 was performed through a computerized data base search of the anatomic pathology archives at King Abdulaziz University Hospital (KAUH), Jeddah.

2.2. Data collection

The data was filtered using appropriate morphology Systematized Nomenclature of Medicine codes indicating the following parameters: Date of receiving biopsy, demographics, clinical diagnosis, morphology and radiography. Nineteen cases of mycetomas were retrieved and classified into eumycetoma and actinomycetoma. The diagnosis was based on clinical examination, radiological evidence, histopathological examination and correlation with grain cultures. Sufficient blocks for each specimen were submitted in order to ensure sampling adequacy. All cases were processed as per standard histopathological techniques, which include paraffin embedding, hematoxylin and eosin staining followed by Grams stain for actinomyces, Gomori methenamine silver (GMS) and periodic acid Schiff (PAS) stains for fungi. Microscopic examination was done by two pathologists separately to arrive at consensus. 16S ribosomal DNA (16S rDNA) and internal transcribed spacer 2 (ITS2) sequencing to identify bacteria and fungi to species level were performed in five cases with active lesions. And the treatments for the patients were recorded.

We analyzed the Medline literature search of the reported studies including epidemiological studies and diagnostic articles about mycetomas in the English literature from 2000-2013 through the national library of medicine, Pubmed, and OVID search engines. We used key words “Madura foot”, “mycetoma” “eumycetoma”, “actinomycetoma” and “*Madurella mycetomatis*” (*M. mycetomatis*), “Saudi Arabia” for Medline search.

3. Results

There were 19 patients with mycetoma with an average age of 44.26 years and male: female ratio of 4:1. Five among the nineteen patients (26.31%) studied were of Saudi origin with 80% of these being affected by actinomycetoma. The only Saudi patient with eumycetoma gave no history of farming enterprise. Detailed age and sex, demographic distribution, location of lesion, culture findings and radiological findings of all patients are presented in Table 1. Actinomycetoma were 63.15% and eumycetoma were 36.84%. All patients presented with classic lesions preferentially in the foot (73.6%). All cases in the study group were agriculture and farming workers.

Among twelve cases microscopic examination on hematoxylin and eosin (H & E) stained sections showed small to large actinomycetoma grains. These were ill-defined and multilobated, surrounded by neutrophils, epithelioid cells, plasma cells and multinucleated giant cells at the outer border. Macrophages were also seen at the periphery along with minimal fibrosis. Colonies showed slightly pale central eosinophilia. Outer border was deeply basophilic with ill-defined but delicate, radiating and branching filaments. The basophilia was demarcated by a fringe of hyaline-like material known as Splendore-Hoeppli phenomenon (Figure 1). Gram’s stain showed branching filaments, 1 micron thick, not breaking into bacillary or coccoid forms, in all cases (Figure 2), while special stains such as PAS and GMS were negative. Although grain cultures performed (on eight cases) were negative on two occasions, a histological diagnosis of actinomycetoma was given on the combination of the clinical picture of indurated swelling of soft tissue exhibiting multiple discharging sinuses with macroscopically typical grains, radiological evidence and the characteristic histopathological appearance .

Among the remaining seven cases grain culture was positive for *Madurella* species in two cases. Microscopic examination on H & E) stained sections among all seven cases showed rounded well demarcated colonies of eumycetoma. These were also surrounded by suppurative granulomas in between fibrotic tissue. The colonies had intricate filaments and were embedded in amorphous brownish cementing matrix (Figure 3). The matrix was highlighted by PAS stain imparting a grainy aspect to the colony (Figure 4). PAS (Figure 4) and GMS (Figure 5) stained sections highlighted interlacing septate hyphae 2 to 3 microns thick with rounded polygonal chlamydospores.

Table 1

Demographics, distribution of lesion, grain cultures and radiological findings of mycetomas among patients at KAUH, Jeddah, Saudi Arabia.

Mycetoma	Case no.	Age (years)	Sex	Nationality	Location	Grains culture (n = 12)	Radiology (MRI) (n = 19)	Automated microbiological identification (n = 6)	16S r DNA/ITS2
Actinomycetoma	1	50	M	Chadian	Foot	*	Soft tissue swelling with bony sclerosis	~	~
	2	12	F	Saudi	Maxilla	*	Soft tissue swelling with osteolytic lesions	Positive for actinomyces	*A. israelii*
	3	36	M	Saudi	Foot	*	Soft tissue swelling with periosteal reaction and bony sclerosis	~	~
	4	18	M	Saudi	Nose	*	Soft tissue swelling with osteolytic lesions	~	~
	5	42	M	Sudanese	Foot	*	Soft tissue swelling with osteolytic lesions	Positive for actinomyces	*A. israelii*
	6	44	M	Indian	Foot	*	Periosteal reaction and bony sclerosis	~	~
	7	65	M	Mali	Foot	*	Soft tissue swelling with periosteal reaction and bony sclerosis	~	~
	8	60	M	Saudi	Foot	*	Osteolytic lesions	~	~
	9	27	F	Yemen	Foot	*	Soft tissue swelling with bony sclerosis	Positive for actinomyces	*A. israelii*
	10	44	M	Bangladesh	Foot	*	Osteolytic lesions with periosteal reaction	~	~
	11	55	M	Yemen	Foot	*	Osteolytic lesions	~	~
	12	37	M	Yemen	Abdominal wall	*	Soft tissue swelling with osteolytic lesions	~	~
Eumycetoma	1	35	M	Yemen	Knee	*Madurella* spp.	Soft tissue swelling with multiple osteolytic lesions	Positive for madurella	*M. mycetomatis*
	2	53	M	Sudanese	Pre sacral	*	Soft tissue swelling with multiple osteolytic lesions	Positive for madurella	*M. mycetomatis*
	3	69	F	Somalian	Foot	*Madurella* spp.	Soft tissue swelling with multiple osteolytic lesions	~	~
	4	48	M	Indian	Foot	*	Soft tissue swelling	~	~
	5	43	M	Saudi	Foot	~	Soft tissue swelling with multiple osteolytic lesions	~	~
	6	54	M	Bangladesh	Foot	~	Soft tissue swelling with periosteal reaction	~	~
	7	45	F	Bangladesh	Foot	~	Soft tissue swelling	Positive for madurella	*M. mycetomatis*

*: Non contributory; ~: Not available; *A. israelii*: *Actinomyces israelii.*

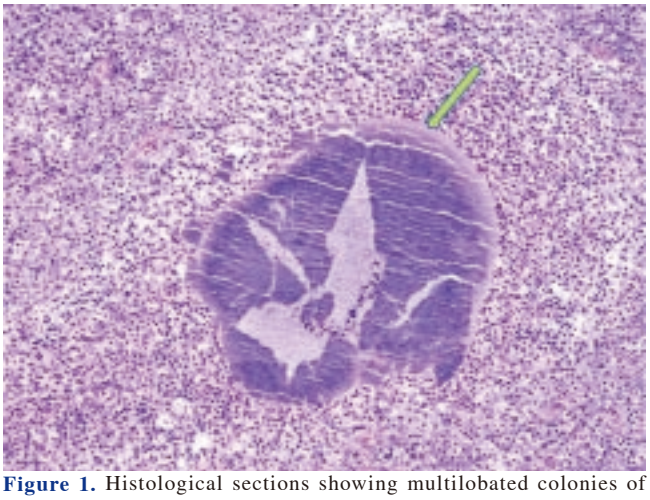

Figure 1. Histological sections showing multilobated colonies of actinomycetoma with peripheral basophilia, and Splendore-Hoeppli phenomenon represented by the arrow (H & E, 10×).

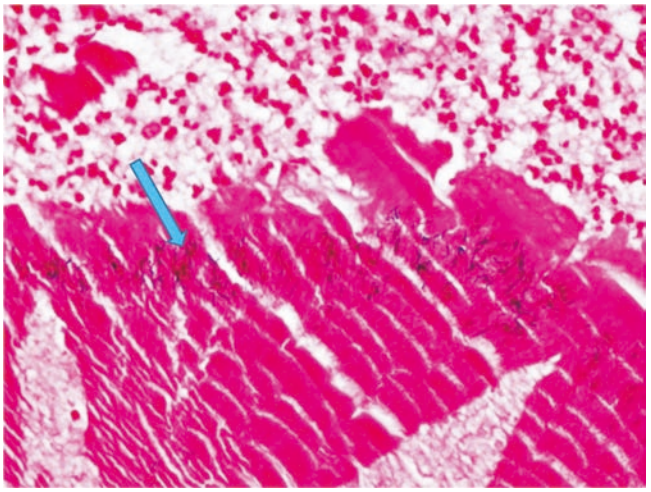

Figure 2. Histological sections showing the positively stained colonies of actinomycetoma with fractures represented by the arrow (Gram's stain, 20×).

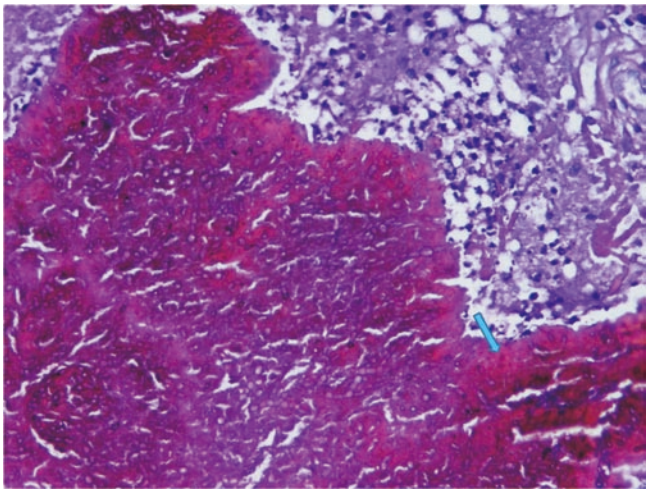

Figure 3. Histological sections showing maduramycetoma colony embedded within brownish cementing matrix (arrow) and surrounded by fibrocollagenous tissue.

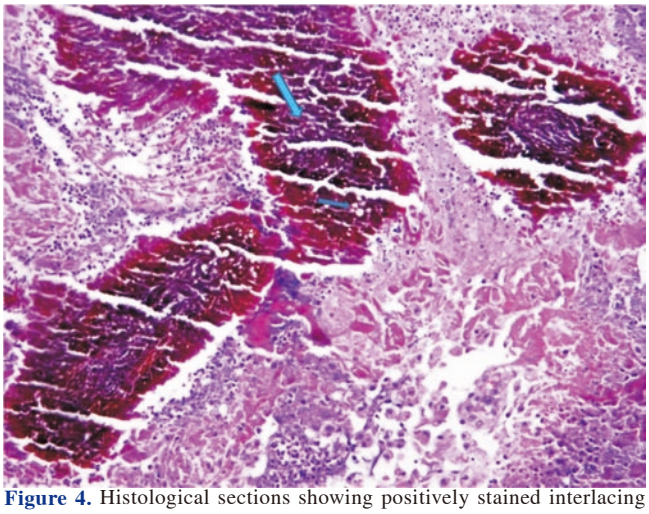

Figure 4. Histological sections showing positively stained interlacing hyphae and oval club shaped ends, chlamydospores represented by arrow. The stain also highlights the matrix in the background of the colony (PAS, 20×).

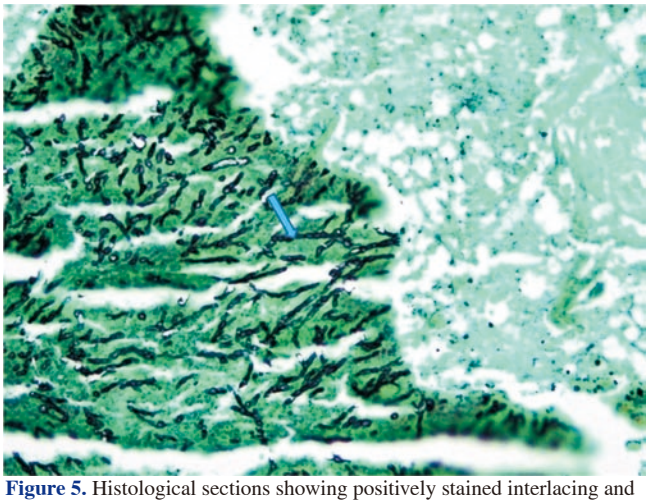

Figure 5. Histological sections showing positively stained interlacing and branching hyphae represented by arrow (GMS, 20×).

16S rDNA and ITS2 sequencing to identify the sepecies of bacteria and fungi were performed in five cases with active lesions. The results of 16S rDNA of three specimens (one each Saudi, Yemeni and Sudanese) amplified by PCR and sequenced revealed bacterial species of *A. israelii*. The ITS2 of two specimens (one each Yemeni, Sudanese and Bangladesh) amplified by PCR and sequenced revealed fungal species of *M. mycetomatis*. The results from molecular sequencing methods correlated well with automated microbiological identification also performed simultaneously for these cases and with cultures and histopathological features previously performed respectively.

Regarding the treatment actinomycetoma patients were treated with surgical debridement and antibiotic therapy while eumycetoma were treated mostly with surgical amputations and antifungal agents. Eight (66%) patients underwent surgical amputation in actinomycetoma group not just due to delayed diagnosis and repeatedly negative cultures but also due to deep soft tissue and bone involvement. All patients in the current study are alive.

4. Discussion

Mycetoma ranks among the most neglected diseases worldwide, to the point that it was omitted even by major neglected tropical disease initiatives across the globe1[11]. Recently, mycetoma was added to the World Health Organization's list of neglected tropical disease priorities[12]. The known geographic distribution of mycetoma etiological agents shows intriguing variation with respect to environmental factors: they occur in arid areas with a short rainy season, and extreme conditions have been suggested as a prerequisite for survival of the causative organisms[13]. In 2013, the World Health Organization recognized mycetoma as one of the neglected tropical conditions due to the efforts of the mycetoma consortium. Knowledge gaps were identified by the same consortium. One of the gaps was that very few data are available on the epidemiology and transmission cycle of the causative agents. Previous work suggested a soil-borne or *Acacia* thorn-prick-mediated origin of mycetoma infections, but no studies have investigated effects of soil type and *Acacia* geographic distribution on mycetoma case distributions. Ecological niche modeling system in Sudan and South Sudan to map risk of mycetoma infection was studied[12]. Mycetoma occurs in the "mycetoma belt" stretching between the latitudes of 15° South and 30° North Sudan, Mexico, Venezuela, India, Pakistan, Senegal, and Somalia[3,4,11-13]. The United States, Asia and other Latin American countries have reported it less frequently[3,4,11-13]. The geographical distribution of causative agents depends on climatic and ecological factors, with eumycetomas predominating in Africa and southern Asia, and actinomycetomas predominating in Latin America. In Mexico, the principal causative agents for actinomycetoma were *Nocardia brasiliensis* (78.21%) and *Actinomadura madurae* (8.7%); meanwhile, for eumycetomas: *M. mycetomatis* and *Scedosporium boydii* (synonym: *Pseudallescheria boydii*)[14]. *M. mycetomatis* is the most prevalent and is responsible for 70% of cases in Sudan[3,4,11-13]. However, sporadic cases have been reported in the United States and Europe especially among the migrant population[2,4]. Malone *et al*.[15] also noted sporadic cases in the United States with isolated cases in Texas, California and Nabraska. Sibiany *et al*.[10] reported

36 cases from three teaching hospitals stretched across the Western regions of Saudi Arabia along with cumulative of 89 cases from other regions of the Saudi Arabia. Mycetoma is an uncommon infection in Saudi Arabia and most of the cases in the present and previous studies were diagnosed too late which reflects the neglected status of mycetoma prevalent in this region, making the identification of causative agents to species level difficult.

The grains are diagnostic for mycetoma as they represent collections of fungal hyphae or bacterial filaments[2,16,17]. The grains of actinomycetoma can be white, yellow or red[18], while those of eumycetoma can be dark (black) or pale and yellow[2,19]. The pigment is melanin[20]. Grains are histologically seen as bacterial filaments or intertwined fungal hyphae surrounded by neutrophils which lead to a purulent tissue reaction containing fibroblasts. This prevents the antibiotic from acting on the micro-organisms warranting debridement in certain cases[9]. Histopathological study of colonies is useful in differentiating actinomycetoma from eumycetoma. By adopting a stepwise approach focusing on the unique features of the colonies (grains/granules) and the use of special stains one can reasonably differentiate between the two. In our laboratory the approach to the diagnosis starts with application of three special stains, *i.e.*, PAS, Gram's stain, and GMS stain. Gram positive colonies exhibiting filamentous bacteria less than 1 micron thick are most likely to be actinomycetes. The actinomycetoma grain is surrounded by homogenous eosinophillic material (Splendore-Hoeppli reaction). In cases of eumycotic mycetoma, thick club-shaped structures (chlamydospores) are seen[21]. PAS and GMS positive colonies showing 2 to 6 microns thick hyphae embedded in amorphous matrix are suggestive of eumycetes. The presence of an amorphous or cementing matrix narrows the diagnosis to consideration of only three eumycotic agents, *M. mycetomatis*, *Madurella grisea* and *Leptosphaeria*. If this matrix imparts a grainy appearance, a provisional diagnosis of *M. mycetomatis* can be considered[22].

However, grains of many species have overlapping morphological features and therefore culture is required for accurate identification of the causal agent and confirmation of diagnosis[3]. Although theoretically more accurate than histology, culture is difficult practically[18]. Differentiation between the two mycetomas is important as the two etiologic agents have a different course of disease progression and treatment[23]. As even multiple cultures can provide no growth at times[2,24], culture-negative cases can be diagnosed and common species can be identified on histopathology if a careful stepwise approach is followed[24].

Molecular approaches have been promising for setting up new tools for the diagnosis of mycetoma agents directly from infected tissues. Analysis of small-subunit rRNA genes and ITS sequences of black grain mycetoma revealed that ITS sequencing is a useful molecular tool for reliable and rapid identification of most black-grain mycetoma agents and can be used for DNA bar coding of this group of fungi[25]. The homogeneity of *M. mycetomatis* has been reported previously, at least in Sudan[26]. By sequencing the ITS region with the ITS4 and ITS5 primers and using large-scale random amplification of polymorphic DNA, those authors suggested that this fungus had a clonal origin. Desnos-Ollivier M *et al.*[27] confirmed that some strains of *M. mycetomatis* were very similar, with identity over ca. 600 bp in the ITS1 and ITS2 regions. However they were unable to demonstrate any correlation between *M. mycetomatis* strains and a specific phenotypic trait in culture or a geographical origin as identical ITS sequences were found in strains recovered from different geographical origins (Sudan, Morocco, and Niger). One explanation given by the authors is the genotype conservation across remote geographic areas. Futher more their inability to amplify the ITS1 regions of four strains of *M. mycetomatis* suggests that either these strains belong to another species of *Madurella* or *M. mycetomatis* is not as homogenous as previously reported[27]. Polymorphisms in genes encoding for chemokine ligand 5 and interleukin-10 are associated with the development of the mycetoma granuloma[28].

Due to slow and relatively pain free progression of the disease, mycetoma is often at an advanced stage when first diagnosed. Actinomycetoma has better prognosis than eumycetoma as the latter has a high rate of recurrence and may require amputation[3]. A high incidence of secondary bacterial infection in mycetoma lesions has been reported, which can cause increased pain and disability as well as fatal septicemia. This emphasizes the need for its correct diagnosis[3].

In conclusion among patients attending King Abdulaziz University hospital for the past 15 years, mycetoma was most common in the age group of 44.26 years and showed male predominance. Actinomycetoma was more frequent than eumycetoma, with preferred foot localization in both instances. The fact that one of the patients with eumycetoma was a Saudi national raises the possibility of an indigenous species similar to *M. mycetomatis* to be further explored in this region. The history of travel to a nearby mycetoma belt such as Sudan could not be excluded in this case, which also remains a consideration. However, in spite of the identification of pathogenic agents, the delay of diagnosis and the osseous infringement imposed amputations and made the medical treatment less effective in our cases. Therefore a high degree of suspicion is warranted. As such the disease has to be prioritized again and more robust and quick molecular diagnostic tools should be made available in order to save patients form disfiguring amputations

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Mycetoma is a chronic granulomatous disease caused by true fungi and actinomycetes, is considered a neglecta disease and occurs in a particular area of the world ("the mycetoma belt"), for the particular ecological conditions.

Research frontiers

The present investigation is a retrospective study of diagnosis of mycetoma by histopathological tests, which describes the characteristics of the grains and their staining affinities.

Related reports

Mycetoma has been diagnosed for years by histopathology, there are so many studies, however, would be significant correlation with the etiologic agent identified and better molecular identification yet.

Innovations and breakthroughs

The study gives very accurate and useful data on the histopathological characteristics of different grains.

Applications

Due to mycetoma affect low-income populations, the histopathology is a good tool for diagnosis.

Peer review

This is a paper that gives a brief overview of mycetoma in a particular region of Saudi Arabia, offering insight on the type of condition that occurs in that particular region.

This report gives very precise information about the mycetoma in Saudi Arabia, and allows us to learn more about the etiology and clinical characteristics of mycetoma in that region.

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