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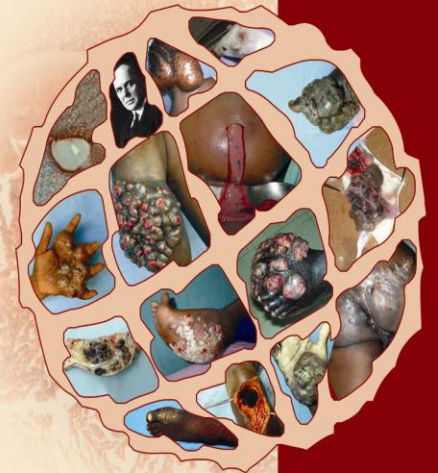
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Mycetoma

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ANTIGENICITY of MADURELLA MYCETOMATIS

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About forty years ago antibodies against the fungus *Madurella mycetomatis* were first demonstrated to be present in eumycetoma patients. To date nothing is known about the individual immunoreactive antigens present in this fungus. We here identify the first immunogenic antigens: galactomannan and a protein homologous to the translationally controlled tumour protein (TCTP).

By using the platelia Aspergillus assay it was demonstrated that *M. mycetomatis* secreted significant amounts of galactomannan-like compounds into the culture medium. However, these compounds were not detected in patient serum.

TCTP, a well-conserved histamine release factor in a range of eukaryotes, was identified from a newly developed cDNA expression library. The gene for this antigen was demonstrated to be present in two variants in *M. mycetomatis*, with 13% amino acid difference between the two proteins encoded. *In vitro*, TCTP was secreted into the culture medium. *In vivo*, it was found to be expressed on hyphae present in developing stages of the eumycetoma-characteristic black grain. Significant IgG and IgM immune responses, against the whole protein and selected *M. mycetomatis*-specific peptides, were determined. The antibody levels correlated with lesion size and disease duration. Overall, the patients with the largest lesions had the highest antibody level, which lowered with decreasing size of the lesion. After 6-15 years of disease duration the antibody levels were the highest. TCTP is the first well-characterized immunogenic antigen, simultaneously the first monomolecular vaccine candidate, identified for the fungus *M. mycetomatis*.

Melanin Biosynthesis in *Madurella Mycetomatis*: Implications for Rational Therapy.

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Mycetoma is a chronic, subcutaneous infection which is characterized by discharge of grains and purulent material through sinuses. One of the most common causative organisms is the fungus *M. mycetomatis*. Treatment of the disease still relies on surgery and prolonged antifungal treatment with either itraconazole or ketoconazole.

To estimate the success of antifungal treatment MICs should be determined for the causative agent, and breakpoints should be established. To develop an in vitro susceptibility test for *M. mycetomatis* is troublesome, since this fungus does not normally sporulate. The tests developed so far all have been based on usage of a hyphal inoculum. The first test introduced in the literature was a test based on the CLSI criteria. To facilitate endpoint reading MICs were determined after adding 2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT). Later, a commercial assay, the Sensititre-assay, was adjusted to determine *M. mycetomatis* MICs for various antifungal agents. Both tests seemed very reproducible.

From our in vitro susceptibility tests we learned that *M. mycetomatis* is highly susceptible to the antifungal agents itraconazole and ketoconazole. Unfortunately therapy success rates vary considerable. What could be the cause of this discrepancy?

Mycetoma caused by *M. mycetomatis* is characterized by the discharge of its black grains. The black colour of these grains was shown to be due to DHN-melanin. After isolating *M. mycetomatis* melanin and adding it to the culture medium, it appeared that MICs of ketoconazole and itraconazole were 16 to 32 times elevated in comparison to MICs determined in the absence of melanin.

Although *M. mycetomatis* appeared to be highly susceptible in vitro for itraconazole and ketoconazole under normal test conditions, caution should be taken when translating such results into clinical practice. Melanin, for instance, seemed to influence the in vitro susceptibility to these agents. To evaluate the full use of antifungal susceptibility testing for this fungus in vitro results should be coupled to either therapy success rates or results from therapy trials in animal models of mycetoma.

Host Susceptibility in Mycetoma: The Role of Wound-Healing

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Madurella mycetomatis is the main causative agent of mycetoma, a tumorous fungal infection characterized by the infiltration of large numbers of neutrophils at the site of infection. In endemic areas the majority of inhabitants have antibodies to certain antigens of *M. mycetomatis*, although only a small proportion of individuals actually develop mycetomal disease. Furthermore, men are more often infected than women are. It was therefore hypothesised that host factors could predispose towards mycetoma susceptibility. Answers were sought in factors involved in wound healing. Both sex hormones and neutrophils are important factors in wound healing. To test this hypothesis, single nucleotide polymorphisms (SNP) in genes involved in sex hormone synthesis and neutrophil function were studied in a population of Sudanese mycetoma patients versus geographically and ethnically matched controls. Sex hormones are synthesised from cholesterol by a number of gene products, for all of which genetic single nucleotide polymorphisms (SNPs) are described which influence sex hormone synthesis. Three of these polymorphisms, encountered in the genes cytochrome P4501B1 (CYP1B1), cytochrome P450 19A (CYP19A) and COMT were differently distributed between patients and healthy controls. The CYP19A polymorphism was associated with a higher 17 β -estradiol (E2) production, while the CYP1B1 and COMT polymorphisms found were associated with a higher conversion from E2 via 4-OH-estradiol to 4-methoxy estradiol. The higher estradiol levels in male patients were confirmed by enzyme amplified sensitivity immunoassay (EASIA). In women no significant difference in E2 levels was found, which could be due to the high variation of E2 concentrations during the menstrual cycle. Furthermore for both male and female patients lower levels of dehydroepiandrosterone (DHEA) were found. The COMT polymorphism was also clearly associated with lesion size. Although for 17 β -estradiol, both anti-inflammatory and pro-inflammatory effects have been demonstrated, overall it is thought that 17 β -estradiol enhances the inflammatory response in infections, while testosterone and DHEA repress it. Apparently, in patients there is an enhanced inflammatory response. To determine if there was also a genetic predisposition found in genes encoding for neutrophil function, SNPs were also determined in those genes. It appeared that five polymorphisms, encountered in the genes complement receptor 1 (CR1), nitric oxide synthase 2 (NOS2), interleukin 8 (CXCL8), receptor for interleukin 8 (CXCR2) and thrombospondin 4 (TSP4) were differently distributed between patients and healthy controls. The genotypes obtained for

CXCL8, its receptor CXCR2, and TSP-4 all predisposed to a higher CXCL8 expression in patients, which was supported by the detection of significantly elevated levels of CXCL8 in patient serum ($p < 0.0001$) and the in situ demonstration of CXCL8 producing cells at the site of infection. The NOS2 genotype observed in healthy controls was correlated with an increase in NOS2 expression and higher concentrations of nitrate and nitrite in control serum ($p < 0.0001$). Interestingly, higher CXCL8 production and lower nitric oxide production are both implicated in less efficient wound healing, which could be a significant risk factor for developing mycetoma. In conclusion, we present the first evidence of human genetic predisposition toward susceptibility to mycetoma, a neglected infection of the poor.

Mycetoma revisited: Incidence of the various radiographic signs

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The purpose of this study is to define and quantitate the various radiographic signs of mycetoma infection to improve detection rate and maximize the benefit of simple radiographs.

A retrospective study of the radiologic records of randomly selected 516 patients seen between December 1996 and 2004 at the Mycetoma Research Center of the University of Khartoum in the Sudan. All cases were previously confirmed by clinical examination, initial radiographic findings and histopathological examination of aspirates, transcutaneous or post-surgical specimen biopsies. Review was limited to the initial radiographs on first presentation before the start of medical or surgical treatment.

The commonest abnormalities in this cohort of 516 patients were soft tissue swelling 93%, bone sclerosis 56%, bone cavities 32% and periosteal reaction 27%. The incidence of bone expansion 22%, extrinsic cortical scalloping 22% and fanning of the rays in 10% were reported for the first time in this communication. Osteoporosis was seen in 19%. Only 7% of all 516 patients had normal radiographs. This was achieved by careful scrutiny of the radiographs.

This is the largest radiographic review of bone mycetoma. Some new signs are reported for the first time. Maximal scrutiny of plain radiographs alone or over-reading of radiographs by experienced radiologists is vital for diagnosis, staging or classification because other imaging techniques are not available where mycetoma is prevalent.

The pathology of *Madurella mycetomatis*

Prof. A M EL Hassan.

Emeritus Professor, Institute of Endemic Diseases, University of Khartoum

M. mycetomatis is the commonest cause of mycetoma due to true fungi in Sudan. This presentation describes the morphology of the grains, the inflammatory cellular reaction, the mechanism of recruitment of the inflammatory cells and their role in attempting to contain the infection. A classification of the inflammatory response is presented. The study involved light microscopy, immunohistochemistry and electron microscopy.

Therapeutics for Eumycetoma

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Fungal mycetoma (Eumycetoma) is a chronic subcutaneous infection, mostly of the lower extremities that is prevalent in tropical and subtropical regions. Accurate estimates of incidence or prevalence are lacking. The disease is caused by a wide range of fungal species including hyaline and dematiaceous fungi. *Madurella mycetomatis* remains the most common cause of eumycetoma in Africa. Clinical pharmacological research on therapeutics for eumycetoma is generally limited. This is mainly due to limited market for antifungals in typically lower-income societies. Therefore all the drugs that have been used for the treatment of fungal mycetoma were developed and approved for other fungal infections. Data on efficacy of the antifungals against eumycetoma have come mainly from anecdotes and case series. Comparative studies are lacking. The data are scares on the relatively newly marketed antifungal agents. The clue towards possible efficacy of such agents can only be suggested from a few in vitro studies or other clinical forms of disease caused by the fungi that also cause eumycetoma. The typical example is activity of Voriconazole and posaconazole against *Fusarium* species and *Scedosporium apiospermum*.

Success in therapy is dependent on several factors: these include the extent of local destruction, especially bone involvement, the causative organism and host immune status. Surgery remains a mainstay treatment of patient with eumycetoma with adjuvant antifungal therapy. Identification of the causative organism is critical in selecting the best antifungal agent. Broad-spectrum triazoles such as voriconazole and posaconazole have shown activity against several fungi that can cause eumycetoma in vitro and in few patients. However, strong data is not available on such agents, and still most of the experience has been obtained with the older imidazoles and triazoles like ketoconazole and itraconazole. Terbinafine has been shown to have synergy with azoles and may have a future a role in treatment of eumycetoma. Parenteral agents like amphotericin B or echinocandins are inconvenient for therapy because of the need for prolonged therapy. Amphotericin B is less active and more toxic than azoles against many fungi that cause mycetoma. Duration of therapy is arbitrary and depends on the extent of disease, patient's response and drug toxicity. Most cases require several months or longer courses of antifungal therapy. Amputation would be the last option after failure of debulking surgery and appropriate antifungal therapy.

Developing Molecular Tools to Study Nocardia

Dr. Paul A. Hoskisson

**Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde,
Royal College.**

Many species of Nocardia cause the disease Nocardiosis in humans which can manifest itself in a number of ways including debilitating infections of the lung, cutaneous tissue, central nervous system and brain. There are few studies on the infection biology of Nocardia and our understanding of their virulence mechanisms is limited. Often infections are complicated by high levels of intrinsic antibiotic resistance, which interferes with chemotherapy. Currently we are developing methods for the genetic manipulation of these organisms to facilitate understanding of their molecular pathogenicity. We are also developing methods for rapid identification and typing of strains to build an epidemiological picture of isolates in both humans and animals. We are also screening a library of 5000 natural products to identify novel compounds that inhibit Nocardia, which may also have implications for drug discovery in TB.

The Relation between *Madurella Mycetomatis* Pigmentation and the Melanin and Metal Contents

Mrs. Anahaed Azziz EL Din, Sudan

Master Student, Mycetoma Research Centre, University of Khartoum

Eumycetoma caused by *Madurella mycetomatis* is the commonest type of mycetoma in the Sudan. It is characterized by the of formation of black grains embedded in black-brown cement-like materials, which probably impedes the penetration of systemically administered antifungal drugs. The black colour and the hardness of the grains are mainly due to the presence of fungal melanin pigment, metals and tissue debris.

In this study, the fungal melanin pigment was identified by using special melanin detecting techniques and that included Masson-Fontana, Schmol's reaction, Bleaching technique and formalin inducing fluorescence.

Atomic Absorption Spectrophotometer (AAS) was used for the detection of Zinc, Copper, Calcium and Ferric ions in the grains and surrounding tissues. Copper, Zinc and Calcium was found in concentrations four, six and sixteen fold higher than normal tissue respectively. Other special stains to detect some metals in grains were used and that included Von-Kossa's stain for calcium ions and Perl's Prussian blue and Lillie's method for Iron (ferric and ferrous). Iron was absent in the grains but was found in surrounded tissue while calcium was detected in grains, especially, in the wall of mycelia and surrounded tissues. The concentrations of these metals increased with the severity of disease. Further studies are needed to determine contribution of melanin and metals in the disease pathogenesis.

Mycetoma: An Overview.

Professor Elsheikh Mahgoub

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Mycetoma, to many of us, is still a fascinating, somewhat mysterious disease in spite of the voluminous literature written about it. Why is this?

First of all why do some persons get it while others exposed to the same risk factors and livings in the same environment do not get it! The same clinical picture of tumefaction, painlessness, in the majority of cases, sinus formation and discharge of coloured grains in spite of the variety of the different twenty four causal organisms! These, however, belong to different genera and different species!

Though, over a period of forty years, we have come to a reasonable stage of management, the outcome is still endangered with a varying degree of recurrence! This multifaceted disease avails opportunities for all types of research: Basic, Clinical, Health System or Social, as you will see in the different presentations of this seminar.

I have been taking care of mycetoma patients and doing research on the subject for the last forty six years and still each new patient excites interest in us as much as the first patients we used to see. Similarly does each new research finding.

We have witnessed progress in imaging radiology to determine the extent of spread of the disease. Serology has become useful in both diagnosis and monitoring progress of medical management. Molecular biology studies have exposed fascinating results in classification of causal organisms. With the advent of the AIDS epidemic drug companies developed interest in antifungal drugs particularly the azoles which proved to be beneficial in the treatment of eumycetoma

I think we can humbly conclude by mentioning three outstanding achievements:

- Establishment of a Mycetoma Clinic where patients have regularly been seen free of charge the last fifty years.
- Holding a Mycetoma International Conference every two to three years; the 5th in the series will take place next May in Barquisimeto in Venezuela.
- The establishment of this Mycetoma Research Centre in the country that witnessed the longest history of research in a disease which constitutes a major health problem.

Fungi Causing Eumycotic Mycetoma

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Mycetoma Research Centre, University of Khartoum

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Eumycetoma begins with the inoculation of soil or plant saprophytes into the subcutaneous tissues. Eumycetoma, like other subcutaneous mycoses, starts passively after minor trauma such as thorn-pricks. The environment in endemic areas, is rich with numerous fungi that have pronounced ability to grow and survive in vertebrate tissues, and can easily cause mycetoma with co-incidentally inoculated in the subcutaneous tissues. The large number of soil or plant fungi that can cause mycetoma, which can be easily recognized by mycologist and plant pathologist, usually present problems for the clinical microbiologist who often has no formal training in the identification of fungi. Fungal identification can be challenging and sometimes frustrating because of the importance placed on morphological characteristics of the organisms and the need to become familiar with a range of different structures and terms. In this presentation, the mycology of fungi that commonly cause eumycetoma will be discussed. The fungi, which will covered include established filamentous ascomycetes representing different orders such as: Pleosporales (*Curvularia* spp, *Leptosphaeria senegalensis*, *Madurella grisea* and *Pyrenochaeta romeroi*), Chaetothyriales (*Exophiala jeanselmei*), Diaporthales (*Phaeoacremonium* spp), Hypocreales (*Fusarium* spp), Microascales (*Pseudallescheria boydii*) and Sordariales (*Madurella mycetomatis* and *Neotestudina rosatii*).

Mycetoma in Children

Prof. A.H. Fahal, Professor of Surgery

Mycetoma Research Centre, Khartoum- Sudan

In this longitudinal prospective study, we report on 926 patients with histological and ultrasonic confirmed mycetoma. **There were male (71.6%),** their ages ranged between 4-15 years and most of them were students. The majority of the patients were from the Central and Western Sudan. The disease duration ranged between 6 months and 12 years. Most of the patients had eumycetoma (73%) caused by *Madurella mycetomatis*. The clinical course of disease was typical in the majority of the patients; pain and history of local trauma was reported by 22.5% and 22.6% of them respectively. Surgical recurrence was reported in 47.2% which is high and unaccepted rate. Family history of mycetoma was reported in 13% of patients and this can be genetic or environmental. The left foot was affected most, that is followed by knee and hand. Rare sites were involved and that included the head and neck, chest wall and buttock. Most of the patients had calcaneum involvement radiologically and the cause of this is unclear. Cytological, histological and ultrasonic examinations of the lesion were the corner stones in the diagnosis of mycetoma in these patients. Combined medical treatment and surgical excision are the important treatment modalities. Actinomycetoma was more amenable to medical treatment. The morbidity in this group of patients was high, with high school dropout and a number of socio-economic impacts.

Clinical Presentation of Mycetoma

Professor Ahmed Hassan Fahal, Professor of Surgery, Mycetoma Research Centre, Sudan

Male predominance is a constant finding in mycetoma with a sex ratio of 3.7:1. This is commonly attributed to the greater risk of exposure to organisms in the soil during the outdoor activities. No age is exempted but mycetoma commonly affects adults between 20-40 years of age. However, in endemic regions children and elderly people may also be affected. Mycetoma is seen more conventionally in cultivators, field laborers and in herdsmen who come in contact with the land in endemic areas people of other occupations are affected.

The clinical presentation of mycetoma is almost identical irrespective of the causal organism. However, the rate of progress is more rapid with actinomycetoma than with eumycetoma. The characteristic triad of a subcutaneous mass, sinus and the presence of discharge containing grains are pathognomic of mycetoma. The discharge is usually serous, serosanguinous or purulent. During the active phase of the disease the sinuses discharge grains, the colour of which depends on the causative organism.

Mycetoma is usually painless in nature. As the mycetoma granuloma increases in size skin changes take place. In some of the mycetoma patients there may be areas of local hyperhidrosis confined only to the mycetoma lesion and the skin around it. Mycetoma eventually invades the subcutaneous tissue, muscles and bone. This is usually gradual and delayed in eumycetoma while in Actinomycetoma, it rapid and progressive especially in infections induced by *A. pelletierii*. For unknown reasons, the tendons and the nerves are curiously spared until very late in the disease process, this may explain the rarity of neurological and trophic changes even in patients with long standing mycetoma. The absence of trophic changes may also be explained by the adequate blood supply in the mycetoma area.

In the majority of patients the regional lymph nodes are small and shotty. An enlarged regional lymph node is not uncommon and this may be due to secondary bacterial infection, genuine lymphatic spread of mycetoma or it may be due to immune complex deposition as part of a local immune response to mycetoma infection.

The commonest site for mycetoma is the foot (79.2%), most of the lesions are seen on the dorsal aspect of the forefoot and for unexplainable reasons the left foot is affected more. The hand ranks as the second commonest site (6.6%), the right hand is more affected. In endemic areas other parts of the body may be involved but less frequently and these include the knee, arm, leg, head and neck, thigh and the perineum. Rare sites such as the chest and abdominal walls, fascial bones, mandible, paranasal sinuses, eyelid, vulva, orbit, scrotum and surgical incisions may be affected.

Streptomyces as agents of mycetoma in humans and fistulous withers in donkeys

Professor Michael Goodfellow, School of Biology, The Ridley Building, Newcastle University, Newcastle upon Tyne,

The genus *Streptomyces* contains over 500 validly described species nearly all of which are considered to be saprophytes. An exception, *Streptomyces somaliensis*, causes mycetoma in humans. Relatively little is known about the causal agents of a related disease, fistulous withers in animals. The present study was designed to clarify the taxonomy of actinomycetes isolated from mycetoma patients in the Sudan and from lesions of fistulous withers in donkeys. All of the isolates had chemotaxonomic and morphological properties consistent with their assignment to the genus *Streptomyces*. It was evident from 16S rRNA gene sequencing studies that the isolates formed distinct phyletic lines in the *Streptomyces* gene tree. The isolates were distinguished from one another and from their nearest phylogenetic neighbours based on phenotypic and DNA:DNA relatedness data. It is evident that streptomyces causing mycetoma in humans and fistulous withers in donkeys are grossly underspeciated, a fact that has implications for the treatment of these diseases.

Aspiration Biopsy Cytology of Mycetoma

Dr. Badreldin Mirgani Yousif.

Consultant Histopathologist, Soba University Hospital

Aspiration biopsy cytology (ABC) is not a recent innovation. Since the mid- 19th century, reports of aspirated specimens have described the findings not only of neoplastic cells but also of specific microorganisms. Targeted for examination by ABC are lesions from all areas of the body, which may be visible, palpable, or deep seated; viewed by imaging modalities.

ABC is also known as needle aspiration biopsy (NAB), or Fine needle aspiration (FNA). The basic tools for FNA consist of a needle and a syringe. The needle used is a disposable one with an outer diameter varying from 0.6 to 1.2 mm (23 to 18 gauge). The length of the needle varies from 3 to 20 cm depending on the location of the lesion. The syringe varies from 3 to 20 ml. The sample aspirated can be smeared directly on glass slide, centrifuged, or used as a cell block.

Mycetoma is a chronic progressive subcutaneous granulomatous infection caused by true fungi (eumycetoma) or aerobic bacteria (actinomycetoma). In the Sudan *Madurella Mycetomatis*, which is a true fungus, is the most common causative organism encountered. *Streptomyces somaliensis*, *Actinomadura madurae*, and *Actinomadura pelletierii* are the most common agents cause actinomycetoma.

The diagnosis of Mycetoma can be made clinically, radiologically, serologically, histopathologically, and/or by culture.

The disease presents as a tumor like mass with or without sinuses. Since soft tissue masses are now routinely aspirated for cytodiagnosis, Mycetoma lesions may be encountered, and they may pose diagnostic difficulty since their cytological features are not well established.

Fine needle aspiration (FNA) of Mycetoma was described for the first time by El hag, Fahal and Gasim in a paper published in (*Acta Cytologica* 1996; 40:461-464). They studied 14 patients with different types of Mycetoma lesions which were aspirated with 23 gauge needle attached to 10 ml syringe. The aspirate is directly smeared on glass slides. Smears were reviewed without knowing the type of Mycetoma. Their results demonstrate that Mycetoma can be accurately diagnosed by FNA, which is simple, inexpensive, rapid, and sensitive technique. They showed that it can be used in routine diagnosis of Mycetoma, in epidemiologic surveys, and in material collection.

Mycetoma lesions have a distinct appearance in a cytology smear characterized by the presence of polymorphous inflammatory cells consisting of admixture of neutrophils, lymphocytes, macrophages, and foreign body giant cells with presence or absence of aggregates of epithelioid macrophages. The diagnostic feature of Mycetoma is the presence of the grains which could be identified in most of the cases. The grains are surrounded and infiltrated by neutrophils. FNA allows morphological identification of Mycetoma and its classification into Eumycetoma and

Actinomycetoma. Examination of direct smears is very informative regarding the inflammatory background as well as presence or absence of the grains. Identification of the type of the grains in the smears may be difficult due to presence of many artifactual changes.

In January 2007 we adopted a new technique for the diagnosis of Mycetoma by using a cell block as well as direct smears.

The cell block is obtained by aspiration of the lesion with 21 gauge needle attached to 10 ml syringe. The aspirated material, which is usually bloody, is left to clot. 10% formalin is added for fixation. After fixation the block is processed according to the routine technique for histopathologic examination. Sections from the cell block are equivalent to histopathology sections. Grains which are present in sections of cell block are morphologically identical to that of histopathology tissue sections. Morphological identification of Mycetoma and its classification into Eumycetoma and Actinomycetoma is the rule. Even Actinomycetoma can be subclassified according to the causative agent.

The indications of ABC of Mycetoma are diagnosis of lesions of unknown nature, lesions without discharging sinuses, early lesions, lesions of Actinomycetoma, to confirm recurrence, obtaining fresh samples for culture, and for epidemiological studies.

Evaluation of specimen adequacy is critical to the diagnosis.

Complications of FNA are very rare, which may include uncontrolled hemorrhage in patients with bleeding disorders, and extension of infection.

Disadvantages and Pitfalls of diagnosis include pain, inexperience, bad collection, sampling errors, extensive fibrosis, presence of striated muscle fibers similar to grains, presence of few grains, and too small lesions not suitable for FNA.

Impact of Mycetoma on the Patients Life Quality

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Mycetoma is a chronic, progressive, destructive, relatively painless subcutaneous infection characterized by extensive masses, usually with sinuses draining pus, blood, and fungal or higher bacterial grains. Mycetoma is a worldwide disease that frequently occurs in the tropics with the highest prevalence being in Africa. It affects individuals of all ages, although disability is most severe in adults who work outdoors. Compared with major diseases like TB, malaria, and HIV, mycetoma is underestimated but has a strong socio-economic impact. Mycetoma studies have concentrated on clinical outcomes; no studies have examined the impact of mycetoma on patients' quality of life (QoL). The QoL of patients diagnosed with mycetoma who attended mycetoma clinics in the Mycetoma Research Center, Khartoum, Sudan in the period November 10th – December 29th, 2008 was studied using a modified Glasgow Health Status Inventory (GHSI) questionnaire completed in one-to-one interviews as a cross-sectional study. The study showed the strong disease negative impacts on patients' daily physical activities, jobs and psychological and social aspects of their livies. It also shed the light on the importance of health education, psychological support and occupational rehabilitation in the multi-disciplinary management of mycetoma.

Recognition & Management of Mycetoma Wound Infections

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Mycetoma infection is caused by a number of different fungi and actinomycetes characterized by draining sinuses, granules and tumerifaction. The disease results from the traumatic implantation of the aetiologic agent and usually involves the cutaneous and subcutaneous tissue, fascia and bone of different parts of the body. Sinuses discharge serosanguinous fluid containing grains. The disease is commonly associated with secondary bacterial infection; merger knowledge is available regarding the aetiology of mycetoma secondary infections and their susceptibility to antimicrobial agents. Therefore, this study was undertaken to study the pattern of secondary infection in mycetoma.

This is prospective, cross sectional, observational, descriptive study. It was carried out over a two months period starting from October 2008 through December 2008 at Mycetoma Research centre. It included 70 mycetoma patients with open sinuses. Specimens were collected using dry sterile swabs. All specimens were inoculated on blood agar and MacConkey agar. Fifty eight of the 70 mycetoma patients were males and twelve were females. The study showed no significant relationship between infection incidence and gender (p value=0.2), and age groups (P value =0.8). There was significant relationship between the infection incidence and with the disease duration and the frequency of surgical procedures (P value = 0.01).

The microbial isolates from 70 patients were as follows: 33(33%) *Staphylococcus aureus*, 3(3%) Coagulase - negative staphylococci, 4(4%) *Streptococcus pyogenes*, 1(1%) *Enterococcus faecalis*, 2(2%) *Escherisia coli*, 9(9%) *Proteus mirabilis*, 1(1%) anaerobic Gram - positive cocci and in 17 patients (17%) cultures revealed no growth.

Most Gram – positive cocci were susceptible to Vancomycin, all isolates of Enterobacteriaceae were susceptible to Amikacin and Ciprofloxacin. The frequency of different prophylactic antibiotics used was analysis and there was no significant relationship between the infection and the type of antibiotics used (P value =0.9).

Further work is needed to determine the microbial aetiology of mycetoma wound infections and their antimicrobial susceptibility testing.

Amikacin with Trimethoprim-Sulfamethoxazole as first line therapy for Actinomycetoma.

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Actinomycetoma is a chronic specific granulomatous infection caused by higher bacteria (Actinomycetes). The infection is characterized by painless swelling involving the subcutaneous tissues with formation of sinuses discharging grains. Combined drug therapy is the preferred treatment to avoid drug resistance and to eradicate residual infection. Amikacin was previously used after failure of first line treatment and when there was danger of dissemination to adjacent organs. This is a prospective interventional study, it included 193 patients with actinomycetoma presenting to the Mycetoma Research Centre. Amikacin sulphate 15 mg/Kg was administered IM bd in combination with trimethoprim-sulphamethoxazole tablets 14mg /Kg twice daily. Treatment was in the form of cycles each cycle consisting of 5 weeks or more. Amikacin was administered over the first three weeks of each cycle.

Patients showed excellent response to treatment judged by regression in the size of the swelling, decrease of the number of active sinuses and the amount of grains discharged. Patients were followed up clinically and radiologically. Renal function tests and crude tests of hearing were done after each cycle.

This study reports the excellent response to the combination of Amikacin and trimethoprim-sulfamethoxazole as first line therapy for actinomycetoma. Only few patients showed side effects that hindered treatment.

Sensitivity and specificity of tissue investigation in the diagnosis of mycetoma

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Mycetoma is an endemic disease affecting wide regions in Sudan. Diagnosis of mycetoma is based on a combination of clinical history and examination, imaging (x-ray and ultra sound) and tissue diagnosis (histopathology and fine needle aspiration cytology (FNAC)).

This is prospective descriptive study which was carried out at the Mycetoma Research Centre and included 501 patients. Fine needle aspiration cytology results were compared with the clinical and histopathological diagnosis for the same patients.

Fine needle aspiration cytology results were in line with the clinical and histopathological results in 496 patients and not in accordance in 105 patients (p value =58.9, DF =15). Data of those 105 patients showed that those patients were mainly adults males, from Central Sudan, had lesion duration of more than 2 year, had lesion size of more than 5 cm, their lesion situated mainly on the feet and most of them had no grains at the presentation.

Role of hypoxia-inducible factor-1 in the induction of angiogenesis in mycetoma lesion

Authors

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Eumycetoma lesion is well vascularized but the mechanisms involved in the control of angiogenesis in the lesion are poorly understood. We have investigated the role of hypoxia-inducible factor 1 (HIF-1), which regulates the transcription of a number of hypoxia-inducible genes, including those encoding vascular endothelial growth factor (VEGF) and adrenomedullin (ADM), both are well known proangiogenic factors.

HIF-1 α protein expression in the lesion was assessed by counting positively immunocytochemically stained cells, using a monoclonal antibody to the α subunit (HIF-1 α). In vivo expression of VEGF and ADM mRNA was assessed by quantitative real time reverse transcriptase- polymerase chain reaction (qRT-PCR).

Expression of HIF-1 α protein was found to be upregulated in the fungal infested tissue ($p = 0.003$, $n=23$). The expression of HIF-1 α , VEGF and ADM mRNA was evident in the fungal infested tissue (HIF-1 α : $p = 0.008$; VEGF: $p = 0.001$, $n=23$; ADM: $p = 0.000$, $n=23$).

These findings denote an important role of HIF-1 in the vascularization of eumycetoma lesion caused by *Madurella mycetomatis* which is upregulated at least in part by an oxygen-independent mechanism in the lesion.