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ASPECTS OF LABORATORY DIAGNOSIS IN THE INFECTION CAUSED BY MICROSPORUM CANIS. A SERIES OF THREE PEDIATRIC CASES

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Abstract: *Among dermatophytes, Tinea Corporis is a more frequent affection in children. It is fundamentally a diagnosis supported by lab tests. Using a microscope still provides the quickest and easiest means of diagnosis. Using a culture is more specific than using a microscope: this is the gold standard of diagnosis even in cases where direct usage of a microscope yields a negative result. The article presents aspects of the laboratory diagnosis in three pediatric patients.*

INTRODUCTION

Techniques for the diagnosis of dermatophytes support the role of the laboratory in the quick and precise diagnosis of micotic agents which cause *tinea corporis*.

In recent years, many new and modern diagnosis methods in the case of dermatophytes have been developed, such as PCR-RLB (reverse line blott), RT-PCR, Maldi Tof (Matrix Assisted Laser Desorption Ionisation), Multiplex PCR which enables simultaneous detection of multiple pathogens, etc. These methods are costly and not many laboratories are equipped with such systems.

On the other hand, many laboratories do not perform mycology examinations for the diagnosis of dermatophytes since some specialists still believe that the techniques and procedures required for this diagnosis are difficult.

Dermatophytes or dermatophyte fungi are keratinophilic filamentous micromycetes taxonomically classified in the order Onygenales, family Arthrodermataceae and are potential human and animal pathogens.

From a morphological point of view, the most important defining feature of dermatophytes is the presence of two types of conidia: microconidia (with a single cell) and macroconidia (with several nested cells). Their imperfect (asexual) stages, also called anamorphic forms, are grouped into three genera: Epidermophyton, Microsporum and Trichophyton.(1)

Under natural conditions, dermatophytes have the ability to break down keratin. This ability allows them to survive in the environment and sometimes produce superficial diseases with integumentary, hairy or nail localization in humans or animals. M. canis is a zoophilic species that can be transmitted to humans from asymptomatic carrier cats, rarely from other pets (dog, hamster) and exceptionally from patients with lesions.

AIM

This article draws attention upon the fact that even through conventional classical methods a diagnosis is possible, especially given that many small laboratories do not benefit from the resources required for a modern diagnosis.

MATERIALS AND METHODS

The cases in this study involved pathological products collected from three pediatric patients aged between 5 and 13 years, at a private laboratory in Sibiu. The examination was requested because children in contact with animals (cats, dogs) had tinea corporis, round, erythematous-scaly plaques, clearly delimited by an active inflammatory border (figureS no.1,2,3).

Cutaneous scales were collected for each child. They were harvested by rinsing the peripheral, active area of the lesion with a sterile curette or blunt scalpel and wiping with a sterile swab moistened with sterile saline. If the harvest was also done with the swab, it was immediately seeded on the isolation media.

All patients had multiple injuries, especially on the hands, forearms, chin etc. The most recent lesions (scales closest to the healthy area) were chosen for collection, because scraping collection from old lesions may not be satisfactory for diagnosis.

Figure no. 1. Skin lesions. Photos from personal collection

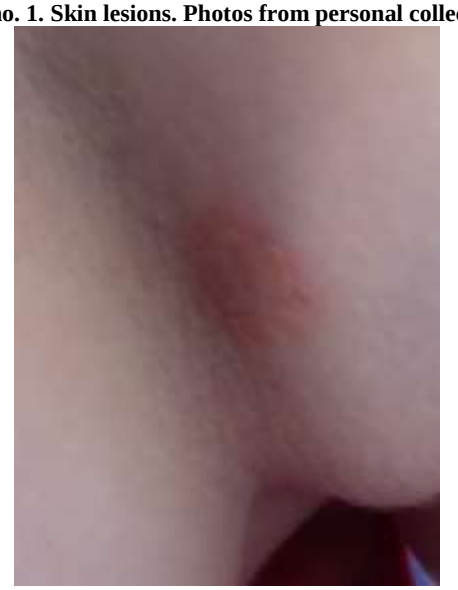

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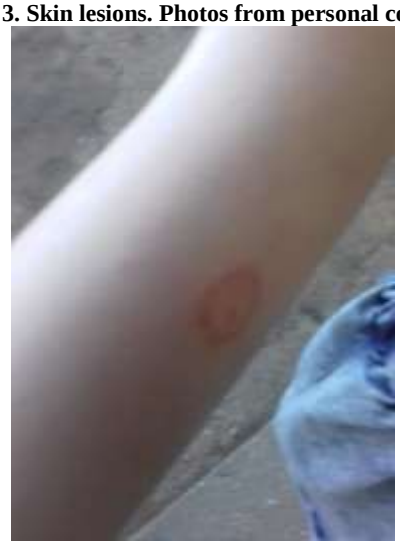
Figure no. 2. Skin lesions. Photos from personal collection

Figure no. 3. Skin lesions. Photos from personal collection.



For each case, a complete microbiological examination was carried out as far as the laboratory's equipment allowed, striving to observe the quality standards in all the essential stages of the diagnosis. All successive and complementary stages were observed, with the aim of confirming or denying the suspicion of dermatophytes and identification of the species involved.

Sabouraud agar with chloramphenicol, Mycosel agar, mycological broth were used for cultivation. KOH solution and lactophenol blue were used for the direct examination of the scales, respectively for the examination of the fungal structures in the cultures.(2)

RESULTS

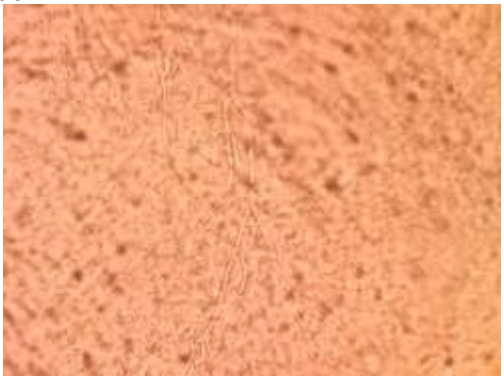
The direct examination of pathological products (scales) is part of the minimum tests required for diagnosis. It is mandatory and has great diagnostic value because it allows the rapid confirmation of the suspicion of mycotic infection and offers the dermatologist a quick orientation, long before the appearance of the culture.(2)

Extemporaneous preparations were made between the slide and the slide, with 20% KOH solution for scales. The examination was done with the usual optical microscope (objectives x10, x20, x40).

Although the sensitivity of the method is relatively low, fluorescent substances with affinity for the cell wall of the fungi under examination (Calcofluor) could not be used, because the laboratory did not have a fluorescence microscope. The results were good, as can be seen in the photo so that a diagnosis of orientation towards a dermatophyte infection could be made quite quickly (figure no. 4).

In all three cases, fungal elements were observed on direct examination.

Figure no. 4. Bright field, ob.x40, direct examination of scales with KOH. Fungal elements visible. Personal collection



Cultivation was done in Petri dishes on Sabouraud agar (SDA) with chloramphenicol and Mycosel agar, and sowing was done by the usual technique of embedding in the melted medium cooled to 45-47°C.

The SDA environment was controlled from the point of view of sterility but also of nutritional and selective efficiency with reference strains, e.g. Candida albicans ATCC 10231. The scales were seeded in several points to increase the probability of isolating any dermatophytes. Incubation was carried out at 25°C, for approx. 30 days.

Cultures were examined daily, looking for the following aspects of the colonies: size, shape, surface, relief, colour (obverse and reverse of colonies), presence of pigmentation, consistency and speed of growth.

On SDA, after 3-5 days, white, star-shaped colonies developed; the central area had a velvety appearance surrounded by hyphae emerging from the agar. After 10-12 days the colonies kept their velvety texture and radial streaks, but the outline became fringed (figure no. 5).

Figure no. 5. Colonies of M. canis on SDA, obverse and reverse. Photo personal collection

The differential diagnosis was made with Microsporum audouinii but in this species the colonies are flat and cantered by a raised button, with Microsporum gypseum but the colonies are flat with a granular or powdery appearance.

In addition to the macroscopic examination of the colonies, the microscopic examination was also done using the adhesive tape technique. The extemporaneous preparation with adhesive tape and lactophenol blue helps a lot to capture all aspects useful for taxonomic classification (hyphae appearance, mycelium appearance, chlamydospores, macro- and

microconidia).(3,4)

Macroconidia were present in large numbers, fusiform, with a thick wall, obviously echinulate. Apical extremity slightly curved. The macroconidia are divided into 6-8 lobules by several well-marked septa. Microconidia were not observed (figure no. 6).

Figure no. 6. Bright field ob. x40 ex. with lactophenol. Photo personal collection

The differential diagnosis was made again with Microsporum audouinii, but in this species the macroconidia rarely appear or are usually absent in subcultures and have a bisac appearance due to a strangulation in the middle portion. In M.gypseum the macroconidia are elliptical in shape and have a thin wall.

DISCUSSIONS

Dermatophytes cause infections that affect the epidermis and fascia. Although they are most often considered ordinary, common diseases, the morbidity rate and local but also psychological consequences can sometimes be important.(5)

Skin, hair, and nail infections with dermatophyte fungi are common in both developed and developing countries.(6)

Tinea corporis si capitis is reaching epidemic proportions in some parts of UK cities. Prompt diagnosis is necessary to stop its spread from child to child, but on the other hand it is observed that the varied clinical aspects facilitate missed diagnosis.(7)

Tinea is contagious, patients, especially children, can self-seed the infection in several anatomical areas, but they can also transmit it to other people.

In microbiological diagnosis, direct examination with potassium hydroxide is a very sensitive technique to determine whether fungal elements are present in pathological products, but it cannot discriminate between living and dead cells and cannot determine certain species. Other disadvantages of this technique diagnosis are: it requires mycological equipment and highly experienced staff.

Culture is an important means of diagnosis, especially in patients with negative direct microscopy.(8)

Clinical features of dermatophytoses such as itching, maceration, pain, desquamation, blistering or scaling, and the rate of erythema are variable from mild to moderate and may be confused with other dermatological diseases such as psoriasis, seborrheic dermatitis, contact dermatitis, atopic dermatitis, impetigo, candidiasis etc.(9)

Treatment of dermatomycoses is long, expensive and potentially toxic. It is necessary to first establish the diagnosis with certainty on the basis of mycological examination.

Advances in laboratory technology offer a number of diagnostic methods that save time and costs and ensure high safety. However, the identification of dermatophyte species still remains a challenge.

It seems that limited access, lack of qualified personnel and high costs are the main factors that oppose the introduction of high-precision techniques as methods to be routinely used on a large scale.

The growing prevalence of dermatophytoses and the problems of correct diagnosis continue to remain a health problem, which could be solved through the international collaboration of mycology specialists for the uniform definition of identification methods and algorithms, but also of the current limits in diagnosis and develop strategies to improve diagnosis.(9)

CONCLUSIONS

A complete mycological examination requires the correlation of the result obtained in the microscopic examination with that in the culture. Although many new and modern methods of diagnosis have been developed, in the case of dermatophytes the classical conventional methods can make an accurate diagnosis, with much lower costs.

Microscopy still provides the fastest and easiest means of diagnosis. Culture is more specific than microscopy, remaining the gold standard method of diagnosis even in cases with negative direct microscopy

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