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EVOLUTIONARY CHANGES AND IMMUNODEFICIENCY AFFECTING PATHOGENESIS OF SUPERFICIAL MYCOTIC AGENTS

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ABSTRACT

Dermatophytes colonize the keratinized skin layer attacking hair, nail & skin. In recent years we have experienced that the dermatophytes are gaining access to the deeper sites. As a result, dermatophytes are beginning to adapt a symbiotic relationship with human tissue causing systemic mycosis. The number of clinical cases of dermatophytes causing systemic fungal infections is low. We have come across four dermatophytic agents causing infection beyond non-superficial sites. Three of the four isolates belong to the *Trichophyton* genus and the fourth belongs to *Microsporum* genus. The first isolate identified as *Trichophyton rubrum* (recovered from lung biopsy), the second isolate *Trichophyton tonsurans* (recovered from cornea), the 3rd isolate identified as *Trichophyton violaceum* (recovered from occipital bone) and the 4th isolate *Microsporum gypseum* (recovered from BAL specimen). We assume that more infections caused by dermatophytic agents will be seen in the future, and turning this group of fungi as an opportunistic pathogenic entity capable of causing local, systemic and disseminated fungal infection.

SUPERFICIAL MYCOTIC AGENTS (DERMATOPHYTES)

Dermatophytes are known to cause site-specific mycosis involving dead skin layers such as the stratum corneum and remain confined to the hair, skin and nails[1, 2, 3, 4, 5, 6, 7, 8, 9]. Dermatophytes are made up of three genera; *Microsporum*, *Trichophyton* & *Epidermophyton* infecting superficial skin, hair & nail of normally immunocompetent hosts[1, 2, 5, 7, 8, 9]. Such anatomical sites have the required pH, temperature and nutritional requirements for the dermatophytes [8]**.** As a result Dermatophytes are restricted to invade deeper site.

KEY WORDS: Dermatophytes, hair, skin & nails

1. A lung biopsy specimen from a middle age lung transplant patient in the mid 1990s was processed for C&S, Viral, fungal and mycobacterial analysis. All tests for C&S, TB and Viral studies were negative except for a white mould, isolated from the mycology culture media after 10 days. The isolate was identified as *Trichophyton rubrum*. The recovery of *Trichophyton rubrum* was a total surprise for the microbiology staff and no other fungus was isolated from the specimen. Direct smear results such as Gram and Calcofluor white (CW) showed no bacteria or fungal elements. After much debate, the department decided to ignore the recovery of *Trichophyton rubrum* as a contaminant or unrelated to the lung sample as dermatophytes are known to cause superficial infections only, and not known to cause deep seated fungal infection. As a result the fungal culture report was issued as "No Fungus Isolated" but the information was retained for future reference.

2. In 2005, we received a cornea specimen from the Ophthalmology clinic suspecting *Acanthamoeba* and also requesting C&S and fungal investigation. No *Acanthamoeba* was observed in direct smear and the culture rendered negative for *Acanthamoeba* as well. Initial Gram stain results were reported as NBS. No growth was reported from C&S culture after 48 hours. The fungal smear (CW) done in mycology showed fungal elements under the microscope but were not too clear. The fungal smear was also observed under the bright field and fungal elements were now clearer as compared to CW prep. Septate hyphae were reported from CW (fungal smear). A white mould that turned beige and started to produce pigment was recovered from IMA (inhibitory mould agar) identified as *Trichophyton*

tonsurans. The Gram smear was retrieved and fungal elements were observed[10, 11]. This was an unusual case since cornea is not a preferred environment for dermatophytes.

3. In 2008, a middle aged female cancer patient developed a nodule in the occipital bone. The clinician suspected an opportunistic fungus such as *Aspergillus*. A biopsy specimen was sent to the microbiology lab requesting C&S and fungal analysis. Gram stain showed pus cells but no bacteria were seen. Fungal smear showed very few fungal elements occurring as round cells attached in chain suspicious of a dermatophytic agent. The fungus of mycological media started to grow slowly as smooth colonies with low mycelium. The colonial morphology on the surface started to display pinkish pigmentation followed by intense purple with age. The reverse pigmentation was deep reddish-purplish. The LPAB prep showed arthroconidia in chains and no other microscopic structures were seen. The fungus was identified as *Trichophyton violaceum* and confirmed by the reference laboratory[11].

4. In 2009, we received a BAL specimen from an immunocompromised host for C&S and fungal culture. Two types of fungi were recovered from this specimen; one of the two fungi was a dermatophytic agent. A 57 years old female lung transplant recipient came to the clinic for follow up procedures. A BAL specimen was sent to the microbiology lab for C&S, Viral, TB and fungal culture. Gram smear results were posted as pus cells and commensal flora seen. Bacterial culture recovered no pathogens; initial ZN showed no AFB except that the mycobacterial culture results reported as *Mycobacterium avium*. The fungal smear (CW) showed two distinct types of septate hyphae. A week later, mycology media started to grow two types of fungi. A fungus, initially white that turned beige later on was identified as *Microsporum gypseum* and the second type, a grayish mould that was identified as *Scolecobasidium gallopava*. The recovery of a dermatophyte from the BAL specimen was unexpected and reported out to the clinician without any qualifying statement. The other fungus *Ochroconis* (*Scolecobasidium gallopava*) is known to produce systemic mycosis among immunocompromised hosts. The recovery of two types of fungi from BAL, one of which turned out to be a dark fungus; the Gram smear was retrieved and examined. The two kinds of septate hyphae seen appeared as hyaline and pigmented in nature are often observable in the Gram stained smear[10, 11].

Dermatophytes have uncommonly been isolated from the ear; a site that is usually located in a hidden ear canal and not exclusively a deep seated site. We have isolated *Trichophyton tonsurans* from ear on occasions[11]. The recovery of dermatophytes from the ear canal is infrequent but not unheard of. Therefore isolating dermatophytes from the ear canal is not considered unusual but a part of the superficial site.

Physiology and the Pathogenesis of Dermatophytes

Dermatophytes colonize the keratinized skin layer. As a result, host response is not against the invading organism but to the enzymes released by the organism hence limiting its spread by forming a boundary around the lesion known as "Tinea or Ring-Worm"[1, 2, 3, 4, 11]. It has been indicated that dermatophytes have the ability to cause deeper or systemic mycosis and produce granulomatous inflammatory reaction in the dermis and hypodermis [1, 2]. The histological examinations showed granulomatous inflammatory infiltrates with fungal elements in the dermis including epithelial cells, giant cells, lymphocytes and eosinophils[1, 2]. Dermatophytes are the only fungi that have developed evolutionary dependency on humans and animals for survival that now appear to attack deeper human tissue[8, 11]. In other words, the superficial mycotic agents are learning to adjust in the unfavorable living environment causing systemic infections.

Fungi are usually nonpathogenic and may become opportunistic pathogen based on the nutritive requirements, temperature, host's intact immune system, nosocomial and community base distribution[8, 11]. They begin to cause infections upon depletion of the body's resistance and cooperation in creating a mutual partnership with human hosts in order to survive in different tissue environment as opposed to the natural environment such as soil, decaying matter and dead wood[1, 2, 3, 4, 9, 11]. As the evolution continued to make advances, structural changes in the behavior of fungi also began to affect the physiology and conidiogenesis of the dermatophytes. Dermatophytes readily attack non-viable cells (incapacitated in biological activity). When the

host reduces its natural resistance due to various factors, dermatophytes become more aggressive due to the keratinizing enzymes resulting in added assaults. Delayed hypersensitivity against the antigens plays an important role in clinical manifestation[4]. Unlike with other opportunistic fungi, dermatophytes have selective host and organ specificity. They are usually mildly infectious; however, the use of cortisone and immunosuppressors initiate "steroid-modified tinea"[4, 8]. In other words, the normally nonpathogenic or mildly infectious agents are provoked by the use of immunosuppressant.

DISCUSSION

Factors like innate immune system, fungal proteinase inhibitors and the normal flora of the skin, limit the dermatophytes to epidermis and keratinized tissue. Dermatophytes can invade the deeper sites and cause systemic infections in immunocompromised hosts. The immunocompromised patients are most susceptible to systemic infections as they have a poor innate immunity and lack a proper inflammatory response. The pathogenesis includes spread via hematogenous route, direct spread or lymphatic channel[8].

Systemic mycosis involving multi organ sites due to dissemination occurs due to the suppression of both types of immune cells such as T and B cells that allows superficial mycotic agents to dig deep inside since there is no resistance to keep them at bay[8, 9]. The immunosuppression is usually due to underlying HIV infection, malignancy, diabetes, lupus, organ transplantation or immunosuppressive drugs[8, 9].

Dermatophytes utilize keratin as a source of nitrogen and are incapable of penetrating the deeper subcutaneous tissue. Dermatophytes have been grouped into geophilic, zoophilic and anthropophilic species based on their ecology and host preference[4]. Anthropophilic species of dermatophytes may have evolved from zoophilic species that spread via direct or indirect contact between humans. Human to animal transmission is rare. The saprophytic nature of dermatophytes living in soil and existing as colonizers of keratinized tissue generally correspond to decreased or complete loss of conidiation and loss of sexual sporulation ; however, infections caused by anthropophilic dermatophytes are usually chronic in nature and difficult to resolve spontaneously[3].

CONCLUSION

The very first dermatophyte we isolated from lung tissue in the mid 1990s was not reported due to the fact that it was considered a contaminant; since dermatophytes were known to attack superficial sites only and normally fail to attack healthy tissue beyond the epidermis. Later on more dermatophytes were isolated from the deeper sites causing systemic infections in immunocompromised hosts.

Complete speciation of dermatophytes, the host preference and ecology play an important role in epidemiology and prevention of infection by these agents. Several outbreaks with *T. tonsurans* have been experienced in nurseries and among school children. The fungus survives for extended periods of time in the form of arthroconidia and chlamydoconidia found within desquamated epithelium and hair. Dermatophytic infections occurred in different body sites, in which each site of the focal point has been involved with direct inoculation[3, 8, 9]. Their access to deeper tissue and dependency on humans was facilitated by evolution for their survival. Continuous and spontaneous evolutionary changes at a slow pace have started to bring about additional changes in dermatophytes, not only to promote the existence of dermatophytes in nature but also allowing them to penetrate deeper sites by becoming more aggressive and competitive.

On the basis of primary habitat association, dermatophytes may be grouped as geophilic, zoophilic, and anthropophilic. Adaptation to growth on humans by most geophilic species resulted in diminished loss of sporulation, sexuality, and other soil-associated characteristics[3, 4, 9]. Dermatophytes are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host[9]. The development of cell-mediated immunity correlated with delayed hypersensitivity and an inflammatory response is associated with clinical cure, whereas the lack of or a defective cell-mediated immunity predisposes the host to chronic or recurrent dermatophyte infection[8, 9].

We have noticed three important changes in ecology as well as the pathogenicity of fungi that was almost negligible to non-existent in the

previous centuries and started to change its own behavior by being able to invade human tissue in spite the fact that fungi feed on decaying organic matter and were unable to feed on complex live human tissue. The other change that made fungi acquire pathogenesis is the evolution that brought changes in fungi at a fixed rate until it started to invade deeper parts of the human tissue. The evolutionary change also brought structural changes in fungi in order to exert invasive action and bypassing the three barriers of temperature, nutritional requirements and immunity[10, 11]. As a result, more cases of dermatophytes causing systemic fungal infections will be seen in the future. Dermatophytes have slowly evolved acquiring the mechanism necessary to attack deeper tissue of the immunocompromised hosts. The trend would continue as more and more immunocompromised hosts become available due to disease, medication and organ transplant. Coupled with the evolutionary change, these superficial mycotic agents would be able adapt the deep tissue environment where they would remain viable to cause infections in the same way as all other opportunistic fungi do[10, 11]. We believe that the ability of dermatophytes to penetrate beyond the superficial site has been indicated by two main factors; **1) the evolutionary change, and 2) the increased incidence of immunocompromised patient population.**

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