# PART I

# Introduction

**Dermatophytes and their characteristics:** Dermatophytes was a group of keratinophilic fungi that caused infections to skin, hair and nails. Most of these fungi were originally belonging to the hyphomycetes but several were known to have prefect states in the family Gymnoascaceae of the order Eurotiales **(Rippon,1974 and Emmons *et al*., 1977 & Abdel-Hafez and El-Shrouny , 1990 ).**

The dermatophytes were a group of septate fungi which occurred world-wide and invade superficial keratinized structures such as skin, hair and claws.

More than 30 species of dermatophytes were recognized. Most of them belong to the imperfect fungi, which were classified into three anamorphic genera: *Microsporum, Trichophyton* and *Epidermophyton* **(Knight, 1972).** A few species had been placed in the teleomorphic genus *Arthroderma* in the phylum Ascomycota **(Emmons, 1934 & Miguens *et al*., 1991 and Maruyama *et al*., 1998).**

Dermatophytes can be grouped on the basis of their habitats and host preferences as geophilic, zoophilic or anthrophilic **(Stanley *et al*., 1994 & Christofidou *et al*., 1996 and Elewski 1996)**.The dermatophytes had a soil, animal or human reservoir **(Balajee *et al,* 1997).**

## Methods of Examination:-

**Wood's light examination**: in 50% to 70% of cases, hairs and skin scales infected with *M. canis* or *M. audouinii* may emit a bright greenish-yellow flourescence under ultraviolet light ($\lambda$ = 366nm).

**Direct Microscopic Examination:**

Skin scrapings and hair were examined microscopically for the presence of hyphae and arthroconidia.

Skin scales, nail scrapings, hair and other materials that were thick in consistency or opaque should first be emulsified in a drop of 10% potassium hydroxide on a glass slide **(Germain and Sumerbell 1996).**

**Culture characteristics (**routinely on Sabouraud`s agar)**:**

**The colonial morphology of Dermatophytes** according to **Rebell and Taplin, 1970:**

a) **Macroconidial morphology** was assessed under low or high dry magnification in preparations a transparent adhesive lap mounts of colony samples stained with lactophenol cotton blue. Other structures such as spiral hyphae, microconidia or chlamydospores can be used for differentiation.

b) **Special growth requirements** can be determined using commercially - available trichophyton agar. Control medium, designated trichophyton agar 1 (Tl), was a casein basal agar. Other media, produced by adding growth factors to the basal agar, were thiamine and inositol, T4 containing only thiamine, and T5 containing nicotinic acid.

- *Trichophyton verrucosum*, which had a requirement for thiamine and sometimes for inositol, usually grew on T3 or T4 media.
- *Trichophyton equinum* required nicotinic acid for growth whereas *T. equinum* var. autotrophicum did not. Culture on T1and T5 media can be used to differentiate these variants.
- *Trichophyton mentagrophytes* hydrolyzed urea when grew on Christensen urea agar **(Kan and Fisher, 1971).**

**c) Humidity** was more important environmental factor for penetration of dermatophytes into the stratum corneum than temperature **(Paveia, 1975).** At least 90% humidity was necessary for penetration of dermatophytes into the stratum corneum within a few days **(Junya *et al*., 1998).**

Many dermatophytes characteristically perforated hair *in vitro* by means of special hyphae appendages called perforating organs.

c) **Dermatophyte test medium (DTM)** had been formulated to differentiate dermatophytes from contaminating fungi. Phenol red was used as a pH indicator in this medium. Growth of dermatophytes results in alkaline metabolic products and the color of the medium changes to yellow.

## Common species of Dermatophytes:

### [A] Genus*: Trichophyton*

**(1*) Trichophyton violaceum*:**

Colonies of *T. violaceum* were very slow-growing, beginning as cream-colored, glabrous, cone-shaped, and later becoming heaped up, verrucous (warty), the reverse color is violet to purple, and waxy in consistency. Colonies may often be described as being "port wine". The reverse side of the colony was purple or non- pigmented. Older cultures may developed a velvety area of mycelium and sometimes lose their pigmentation.

Microscopically, microconidia and macroconidia were generally not present; only sterile, distorted hyphae and chlamydospores were found. In some instances, however, swollen hyphae containing cytoplasmic granules may be seen. The growth of *T. violaceum* was enhanced on media containing thiamine **(Rippon, 1988).**

**(2) *Trichophyton mentagrophytes*:**

Two distinct forms recognized. The zoophilic subspecies var. granular produced a fast-growing colony with a flat powdery or granular surface (on indication of abundant sporulation) and an irregular edge. The anthrophilic subspecies var. interdigitale was often undistinguishable from *T. rubrum* on primary isolation since it produced a white fluffy aerial mycelium which may become yellowish or tan with age. The under side of both colony types may be pink, red, yellow or rose brown. Sporulation was much more pronounced in the granular form which also showed the presence of spiral hyphae and macroconidia that were up to six-celled and had smooth thick walls. Macroconidia were rare in the anthrophilic form. Both forms produced microconidia either borne along the sides of vegetative hyphae or in grape-like clusters – especially common in zoophilic isolates **(Georglk, 1954 & Yu, *et al*., 1969).**

**(3) *Trichophyton rubrum*:**

*T. rubrum* was a slow-growing organism that produced a flat or heaped-up colony that was generally white to reddish-pink with a cottony or velvety surface. The characteristic cherry-red color was best observed on the reverse side of the colony; however this was produced only after 3 or 4 weeks of incubation. Occasional strains may lack the deep-red pigmentation on first isolation. Colonies may be of two types: fluffy and granular. Microconidia were uncommon in most of the fluffy strains but were more common in the granular strains and occur as small, tear-drop-shaped conidia often borne laterally along the sides of hyphae **(Rebell and Taplin, 1970).**

Macroconidia were seen uncommonly, although they were sometimes found in the granular strains, where they appeared as thin-

walled, smooth-walled, multi-celled, pencil-shaped conidia with three to eight septa.

*T. rubrum* and *T. mentagrophytes* were the most common species recovered in the clinical laboratory **(Roberts, 1995).**

Other trichophyton species were; *T. equinum*, *T. schoenleinii T. tonsurans*, *T. megninii*, *T. verrucosum*, *T. ajelloi*, *T. soudanese*, etc. **(Ajello, 1968).**

### [B] Genus: *Epidermophyton*

***"Epidermophyton floccosum"***

Colony was almost cerebriform at the center with irregular radial folds and furrows extending towards the periphery. Surface was suede-like and yellow-brown to greenish-yellow in color. Reverse was colorless to yellow-brown.

Macroaleuriospores were abundant. Broadly clavate with thin smooth walls, broad bases and round distal ends; 1-4 celled micro-aleuriospores were absent. Chlamydospores were numerous in older cultures **(Frey *et al*., 1979).**

### [C] Genus: *Microsporum*

***"Microsporum canis"***

Colonies developed rapidly with a cottony white aerial mycelium and a yellow-colored margin. The reverse was characteristically yellow.

Macroconidia were sometimes numerous and sometimes scarce, spindle-shaped, thick-walled, rough and have 6-12 septa.

Microconidia were few, single-celled, clavate and grew directly on the hyphae, mostly without a stalk. Racket and pectinate hyphae as well as chlamydospores were sometimes present **(Thomas *et al*., 1994).**

Other species of *Microsporum* were: *Microsporum audouinii*, *M. cookei, M. boullardii, M. distortum, M. ferrugineum, M. nanum, M. gallinae, M. gypseum, M. persicolor, and M. vanbreuseghemii* **(Ajello, 1968).**

*** Histopathology:-**

Microscopically, epidermal changes usually consist of hyperkeratosis and varying degrees of acanthosis. Spongiosis, intracellular edema and vesicle formation were also occasionally seen. These changes may or may not be accompanied by an inflammatory infiltrate of varying severity in the dermis. When present, inflammation was usually mild and predominantly mononuclear. .

The Periodic-Acid-Schiff stain of Hotchkiss-Mc-Manus was of great value and was widely used.

The cell walls of fungi being rich in polysaccharides stain deep red. Histological fungi were present as hyphae and arthrospores.

On rare occasions, dermatophytes infected the deeper, non-keratinized layers of the skin where they may produce an acute purulent reaction with abscess formation or a granulomatous reaction that was usually of the nodular type.

*** Pathogenicity and body defence:**

Dermatophytes invade keratinized structures such as the stratum corneum of the epidermis, hair follicles and hair shafts. Lesion development was influenced by the virulence of the dermatophytes and the immunological competence of the host. Infective arthrospores adhere to keratinized structures and germinate within 6 hours **(Yu *et al*., 1969 & Cutler, 1991).**

Minor trauma such as gentle rubbing of the skin or bites from arthropods may facilitate infection. Damp skin surface and warmth favour germination of spores. Metabolic products of hyphal growth may provoke a local inflammatory response. Hyphae grew centrifugally from the initial lesion towards normal skin, producing typical ring worm lesion **(Caprilli *et al*., 1980).**

The major antigens associated with dermatophyte infections were keratins (elicit cell-mediated responses) and glycoproteins (carbohydrate moieties stimulate antibody; protein moieties stimulate cell-mediated responses).

## Clinical diseases caused by dermatophytes:-

### "Dermatophytosis"

The term *dermatophytosis* was used to describe infections of the skin, hair and nails due to a group of related filamentous fungi, the dermatophytes, which were also known as the ring worm fungi **(Stanely *et al*., 1994 & Ninomiya *et al*, 1998).**

Dermatophytosis, particularly of the scalp, was a common disease in children and may occur in epidemic form; dermatophytosis of skin and nails were more common in adults. Males were affected more than females, in a ratio of about 3:1. Dermatophytosis was primarily a disease of the poor among whom relatively lower standards of hygiene prevail.

By convention, dermatophyte infections were usually described by using the Latin term, *Tinea,* followed by the appropriate Latin description of the site **(Maghoub 1983, Hay, 1983 & Richardson and Wornock, 1997).**

**[A] Tinea Capitis (Scalp ring worm):-**

Infections of the scalp hair caused by dermatophyte were mainly confined to children below the age of 14; they were not common in later

life and in this age group were most frequently seen in females **(Hay, 1983 & Bargman *et al*., 1995).**

There were a wide variety of clinical presentations, including the asymptomatic carrier state; dry, scaly ring worm of alopecia, black dot alopecia, and kerion or inflammatory tinea capitis **(Maslen and Anderew 1997).**

Endothrix tinea capitis was caused by a variety of fungi; in the United States, most infections were due *to T. tonsurans*. In Europe, *T. violaceum* was more common **(Hay, 1983, Hay; 1996 & Anstey *et al*., 1996)**.

**[B] Tinea Corporis:-**

Tinea Corporis was an infection of the glabrous skin. The term tinea criminate may be used to describe such a lesion. The most common causes of tinea corporis were *T. rubrum, T. violaceum, T. verrucosum* and *Microsporum canis* **(Hay; 1983 & Shah *et al*., 1988)**.

**[C] Tinea Cruris:-**

The term *tinea cruris* was used to refer to dermatophytes infections of the groin and pubic region.

More adults and men were affected than children and women. The three dermatophytes most commonly isolated were *T. rubrum, T. mentagrophytes* and *E. floccosum* **(Mahgoub; 1983)**

**[D] Tinea pedis:**

Tinea pedis was infection of the feet or toes with a species of dermatophyte. Three dermatophytes, *T. rubrum, T. interdigital* and *E. floccosum* caused most cases of ringworm of the feet but in some instances more than one fungus may be present **(Al-Sogair *et al*., 1990)**.

**[E] Onychomycosis:-**

Onychomycosis was common fungal infection of one or more components of the nail plate. In more than 30% of cases, onychomycosis was caused by the dermatophytes *Trichophyton rubrum* and *T. mentagrophytes* and was then referred to as tinea unguium **(Bergus and Johnson, 1993)**.

**[F] Tinea Manuum:-**

Tinea manuum was a dermatophyte infection of the hand, most commonly the palm. The anthrophilic dermatophytes *E. floccosum, T. mentagrophytes* var. interdigital and *T. rubrum* were the most common causes of tinea manuum.

**[G] Tinea Barbae:-**

Ringworm of the beard and moustache which was generally, although not invariably derived form animal sources. The most common causes of tinea barbae are: *T. verrucosum, T. mentographyte, T. violaceum, T. schoenleinii*. Rarely, infections may be caused by *M. canis, T. megninii* or *T. rubrum*. **(Sabota *et al,* 1996)**

## Treatment and Control of dermatophytes:-

Combined topical and systemic treatments was often preferable of two systemic agents, grisofulvin and ketoconazole were more costly and less proven **(Lenhart, 1970 & Egorov, 1995)**.

**Grisofulvin:** was a dermatophyte antibiotic derived from a number of *Pencillium* species. It was the first oral drug for treatment of dermatophytosis. It had a limited spectrum of action which was almost restricted to the dermatophytes: *Epidermophyton floccosum, Microsporum species* and *Trichophyton species*. Its clinical use was limited to these infections **(Williams and Sarkany, 1959).**

**Ketoconazole:** In the 1970s, the first orally effective broad-spectrum azole antifungal, Ketoconazole was introduced offering the first systemic alternative to grisofulvin in the treatment of dermatophyte infection **(Wishart, 1994)**.

Ketoconazole was a synthetic dioxolane compound. It had proved to be effective in many dermatophytic infections, particularly recurrent or chronic ones, generalized *T. rubrum* infections that had failed to respond to other treatment, and nail infections. In all cases, response to treatment with ketoconazole was faster than with other requirements.

In the past, two new oral antifungal agents, Itraconazole and Terbinafine, had been approved for the treatment of distal subfungal onychomycosis (DSO), which was also know as tinea unguium **(Rands;1996)**.

Terbinafine was a member of the allylamine class of antifungal agents. It exerts its antifungal effects at an earlier phase in fungal cell membrane ergosterol formation than do the azoles **(Hay, R.J 1992)**.

Terbinafine was effective against the dermatophytes (*Epidermophyton floccosum*, *Microsporum* species and *Trichophyton* species) and *Malassezia furfur* **(Lackner and clissold, 1989)**.

ltraconazole was a triazole antifungal agent. The 3 nitrogen atoms in the 5-member triazole ring may be responsible for Itraconzoles' broad spectrum of activity, which includes dermatophytes, yeasts and non-dermatophyte molds; improved tissue penetration; and lower toxicity compared with ketoconazole **(Gupta C. and Tandon, R.N, 1997)**.

Fluconazole was an oral antifungal agent with activity against dermatophytes, *candida* and some dermatophyte molds. It had only recently been studied as a potential therapy for onychomycosis. Fluconazole was a bi-triazole, having 2 triazole groups, each containing 3 nitrogen atoms **(Gupta *et al*., 1994).**

Although fluconazole did not yet been approved by the Food and Drug Administration for the treatment of dermatophytosis, reports suggest that it was effective and can be used in weekly doses for infections of the nails **(Wishart, 1994)**.