

## Scaly Scalp in Different Dermatological Diseases: A Scanning and Transmission Electron Microscopical study.

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**Abstract:** Scanning electron microscope (SEM) and Transmission electron microscope (TEM) examination of the scales in different scaly scalp disorders reveal the importance of alteration in the stratum corneum in the diagnosis of these disorders. Each disease has its own characteristic scale features that are distinguished from other scaly disorders. Specimens from 20 patients with different scaly scalp conditions were randomly taken and examined by both SEM and TEM. Each scaly scalp disorder expressed its characteristic SEM and TEM findings. These findings may pave the way for further understanding of the differences between scaly scalp disorders that may look alike or slightly different in their clinical presentation. [Nature and Science 2010;8(12):61-69] (ISSN: 1545-0740).

**Key words:** scanning EM – transmission EM – epidermis – corneum – psoriasis – seborrheic dermatitis – tinea capitis – dandruff – pityriasis rubra pilaris.

### 1. Introduction:

Scales are dry or greasy laminated masses of keratin. The body is constantly shedding imperceptible tiny thin fragments of stratum corneum. When the rate of formation of the epidermal cells is rapid or the process of normal keratinization is interfered with, pathologic exfoliation results and scales are produced (Odom et al., 2000).

The skin of the scalp has several unique features. First, the high follicular density that provides thermal isolation conducting to parasitic infestation. Second, the high rate of sebum production together with desquamated skin cells can provide a food source for microorganisms. Finally, scalp skin is subjected to brushing that can cause friction injury and may introduce microorganisms (Elweski, 2005).

Scaly scalp is a common complaint associated with psoriasis, seborrheic dermatitis, dandruff, pityriasis amiantacea, pityriasis rubra pilaris, scaly tinea capitis and other less commonly encountered disorders. These disease processes of the scalp can have significant overlap in their clinical symptomatology and pathology (Elweski, 2005).

A clear understanding of each disease process and its unique clinical manifestations with the aid of modern equipments such as electron microscopy is a key to developing an accurate differential diagnosis and makes a correlation between morphological data

and pathogenic hypotheses in the dermatoses of the scalp.

In scalp psoriasis, lesions are often discrete nummular plaques that may be thickened and pruritic. Using SEM the corneocytes appear irregular in size with predominantly a triangular outline (Amer et al., 1996) with flattened cell margins with the surfaces showing pronounced villous pattern (Barbareschi et al., 1994). The TEM examination shows widening in the intercellular spaces, in which, accumulations of pathologically structured lipid material are seen, with neutrophils and electron-dense desmosomal remnants denoting a pathologic interaction with the pathological intercellular lipid structures (Ghadially et al., 1996). The size and number of keratohyaline granules are greatly reduced or occasionally absent, while there is a high number of parakeratotic corneocytes (Fartasch, 1997).

In dandruff, there is a diffuse, moderate, fine white or greasy scaling of the scalp without significant irritation, while in seborrheic dermatitis, there is inflammation and pruritis (Warner et al., 2001), which may be irritant in nature owing to the production of toxic metabolites, lipase and reactive oxygen species by *Malassezia furfur* (Nenoff et al., 2001). SEM examination of the stratum corneum reveals diagonal-shaped cells with thin and regular trabeculae (Amer et al., 1996). Also, yeasts are present inside the stratified layers of corneocytes (Pierard et al., 2006) in the form of spotty clumps (Pierard-Franchimont et al., 1995).

Werner and Smola (2001) reported that TEM examination shows the presence of lipid droplets within corneocytes, few desmosomes, corneocyte membrane interdigitations and excessive disorganized intercellular lipids.

In cases of Pityriasis amiantacea the scales have asbestos-like appearance, overlapped and adherent to the hair and encasing them like a sheath (Tracy, 1994).

The cells in pityriasis rubra pilaris cases appear in SEM examination with fusiform outlines, numerous depression pits and few villi. The cell trabeculae are mostly regular (Amer et al., 1996). Kanerva et al. (1983) stated that TEM examination reveals the presence of frequent parakeratotic cells with pyknotic nuclei. The lowermost horny layers are compact exhibiting a keratin pattern with low density filaments set in an amorphous substance. Lipid droplet-like vacuoles are frequent in the corneocytes.

Tinea capitis is a common dermatophyte infection of the scalp in children, which is mainly caused by *Microsporum Audaunii* (Bologna et al., 2003). Langely (1997) reported that the dermis demonstrates a perifollicular infiltrate of mixed lymphocytes, histiocytes, plasma cells and eosinophils. Follicular disruption leads to an adjacent foreign-body giant cell reaction.

## 2. Material and methods:

20 patients with different scaly scalp conditions were randomly chosen from outpatient clinic of Dermatology Department, Kasr El-Aini Hospital, Cairo University, from June to October.

Cases were clinically diagnosed and confirmed histopathologically with no limitations for ages, sex or associated medical conditions. Patients were grouped according to the disease into six groups.

**Group 1:** included six patients with scalp psoriasis.

**Group 2:** included five patients with seborrheic dermatitis.

**Group 3:** included two patients suffering from dandruff.

**Group 4:** included three patients with pityriasis amiantacea.

**Group 5:** included two patients with pityriasis rubra pilaris.

**Group 6:** included two patients with tinea capitis, scaly type.

All cases had not received any topical or systemic treatment for at least two weeks before the trial period whether for skin or other systemic disorders.

Two samples were obtained from each case, one for scanning electron microscopy (SEM) and another for transmission electron microscopy (TEM).

Samples for (SEM) were taken by a skin surface biopsy which allows detailed examination of squamous cells and preserves their in-vivo interrelationships (Marks and Dawber, 1971). A drop of the cyano-acrylate adhesive was placed on SEM "stubs" and placed on the part of the scaly scalp to be studied with the surface bearing the adhesive of the stub to the scales. This was removed after 20 to 30 seconds with the attached sheet of stratum corneum, (Barbareschi et al., 1994) coated with a layer of gold using Sputter Coater S150A Edwards-England, and then transferred to the (SEM) section in the Electron Microscope Unit in the National Research Center for viewing and photography.

Samples for (TEM) were taken by conventional punch skin biopsies under local anesthesia, from affected scaly area of the scalp, immediately fixed by immersion in 4% gluteraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3 and then carried out in 1% osmium tetroxide (OsO<sub>4</sub>) in the same buffer. They were dehydrated in ascending grades of ethanol at 0 – 4 °C and then impregnated by immersion in a mixture of equal parts of the epoxy resin and propylene oxide. Finally, the specimens were embedded in pure resin, cut on LKB ultramicrotome and stained with uranyl acetate and lead citrate. The stained ultrathin sections were examined under an EM 10 Zeiss West Germany transmission electron microscope at 80 KV accelerating voltage.

## 3. Results:

### Results of SEM:

#### 1) Psoriasis:

At low magnifications the corneocytes appeared as clusters of desquamated overlapped cells that showed alterations in size and shape. Some of them showed prominent folded cell margins. Others exhibited many grooves on their surfaces that represented the outline of cells in the next layer (Fig. 1,a). At a higher magnification prominent villi appeared covering the surface of the corneocytes except at the areas of the grooves, among them depression pits were seen. The villi were equal in size and height (Fig. 2,a).

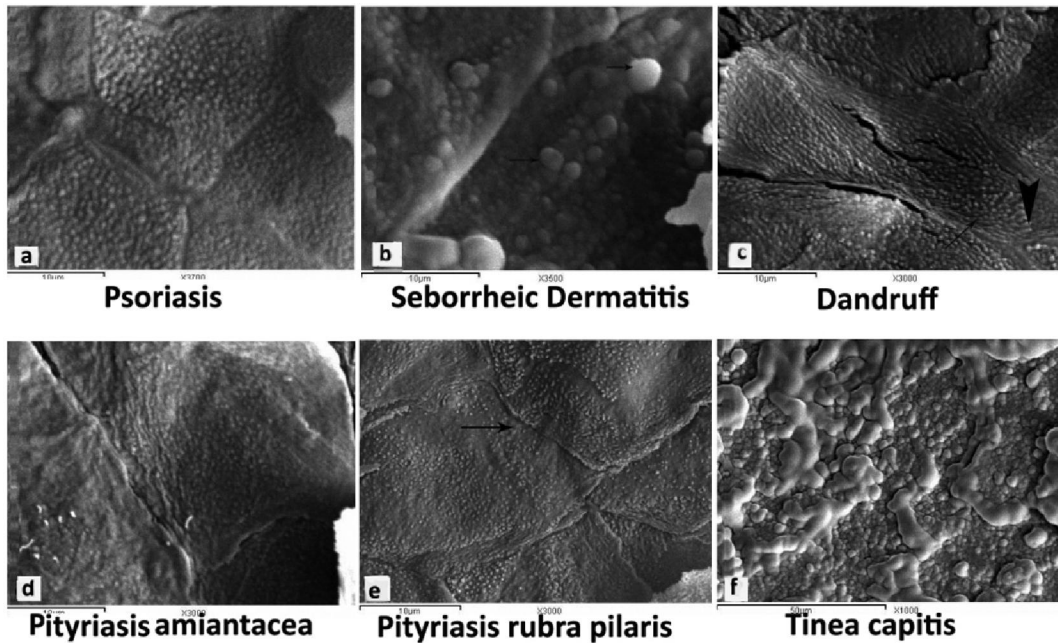


Figure 1. At low magnifications the corneocytes appeared as clusters of desquamated overlapped cells that showed alterations in size and shape. Some of them showed prominent folded cell margins. Others exhibited many grooves on their surfaces that represented the outline of cells in the next layer.

## 2) Seborrheic dermatitis:

At low magnification the corneocytes appeared with variable sizes and heart-shaped appearance. Their margins were thin and regular. At high magnification, villous structures on the outer surfaces of some corneocytes were observed exhibiting uneven distribution, but the characteristic feature was the presence of *Malassezia* yeasts, whose density varied greatly from one corneocyte to another (Fig. 1, b). At higher magnifications, variable bulbous structures of yeast infection were seen (Fig. 2, b).

## 3) Dandruff:

Heavy desquamated corneal cells were observed around the hair shaft, they were variable in size and shape. By examination micronudular-like villi of equal size and uniform shape were observed distributed unevenly on the surface of the corneocytes (Fig. 1, c). Some cells exhibited mild *Malassezia* yeast infection (Fig. 2, c).

## 4) Pityriasis Amiantacea:

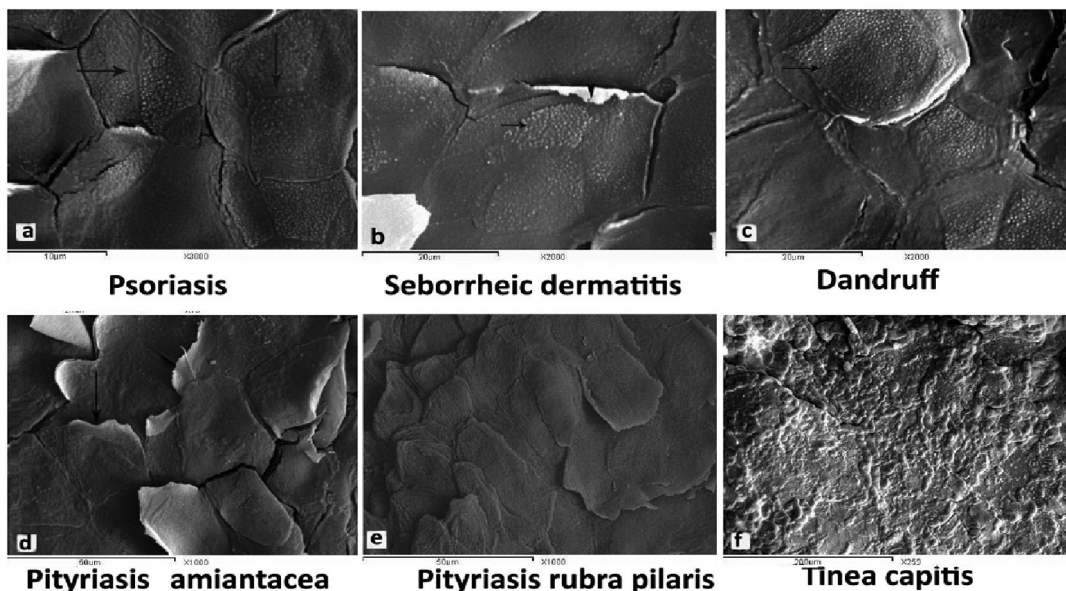
At low magnifications the corneocytes were adherent to the shaft of hair near the scalp. They appeared polyhedral in shape with rough surface. Their margins were thin and showed some irregularities (Fig. 1 d). At higher magnifications villous structures were seen with uneven distribution (Fig. 2, d).

## 5) Pityriasis rubra pilaris (RPR):

At low magnifications clusters of desquamated corneocytes that were overlapping each other were observed. The corneocytes were of variable sizes giving rock-like appearance. Their margins were thin and regular. Villous structures and depression pits were observed with uneven distribution (Fig. 1, e). These villi were variable in size and shape and gathered in groups separated by grooves (Fig. 2, e).

## 6) Tinea Capitis:

At low magnifications corneocytes appeared shed with adherent hair (Fig. 1, f), while by high magnification the margins of the corneocytes were obscured by colonies of fungal infection (Fig. 2, f).



**Fig. 2:** (a) A scanning electron micrograph of psoriatic plaque showing villi of equal sizes and height that are evenly distributed over the surface of cells. (b) Is a case of seborrheic dermatitis showing a mild degree of *Malassezia* colonization with variable bulbous structures of the yeast infection (arrows). (c) A case of dandruff showing shows the villous structures on the outer surface of some corneocytes (arrow) with some yeast infection (arrow head). (d) A case of psoriasis amiantacea shows villous structures on the outer surface of corneocytes that are not evenly distributed. (e) a case of pityriasis rubra pilaris showing the villous structures that are gathered in groups separated by grooves (arrow). (f) A case of tinea capitis shows numerous variable sizes and shapes of bulbous structures of fungal infection that obscure the margins of the corneocytes.

### **Results of TEM:**

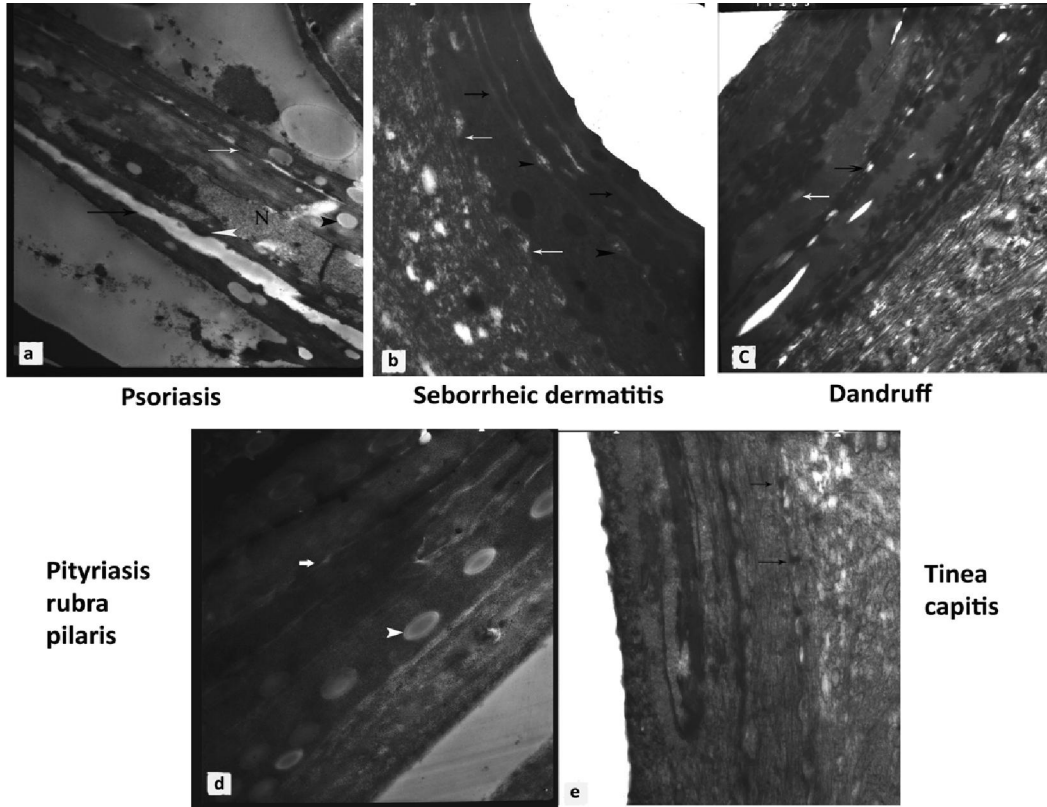
#### **1) Psoriasis:**

A high number of parakeratotic corneocytes (with remnants of nuclei) were present with lipid droplets in their cytoplasm (Fig. 3, a). There was narrowing of the intercellular spaces, which were devoid of lamellar bilayer, except for some focal dilatations with pathologically structured lipid materials. Some of the desmosomes were dilated denoting disintegration. Increased number of epidermal lamellar bodies was observed denoting that the corneocytes display retained lamellar bodies which appeared in high layers of stratum corneum as electron-dense materials. Transitional zone between stratum granulosum and stratum corneum showed remarkable irregularity (Fig. 4, a).

#### **2) Seborrheic dermatitis:**

The cytoplasm of corneocytes contained lipid droplets with variable densities and some of them exhibited linear flowing structures (Fig. 3, b). There was infiltration by *Malassezia* spores and widening of ICS, which were filled with granular material resembling the ground substance and numerous darkly-stained particulate deposits. Some corneocytes had remnants of nuclei (Fig. 4, b).

**3) Dandruff:** Dandruff scales showed multiple lipid droplets of variable electron density ranging from transparent to medium-electron density (Fig. 3, c). There were dilated ICS and wide separations between the corneocytes that were filled with transparent electron materials and finger-like projections. These transparent electron materials were pathologically accumulated lipids. Some corneocytes exhibited remnants of nuclei indicating parakeratosis (Fig. 4, c).



**Fig. 3:** (a) A transmission electron micrograph of stratum corneum of a psoriatic case shows remnants of nuclei (N), multiple lipid droplets (black arrow head), normal desmosomes (white arrow) and disintegrated ones (white arrow head). (b) a seborrheic dermatitis case showing irregularity of the SG-SC interface (white arrow), while the intercellular spaces between corneocytes are focally widened and filled with granular material (arrow head). The rest of the ICS are slightly widened (black arrow). The cytoplasm of the corneocytes show numerous lipid droplets. (c) A dandruff case shows multiple corneocyte lipid inclusions of variable electron densities, from transparent (black arrow) to medium-electron dense inclusions (white arrow). (d) A pityriasis rubra pilaris case shows multiple large lipid vacuoles (arrowhead) in the cytoplasm of the corneocytes. The ICS are normally widened (arrow). (e) Tinea capitis case shows multiple desmosomes in the SG-SC interface (arrow) with remnants of nuclei.

#### 4) Pityriasis amiantacea:

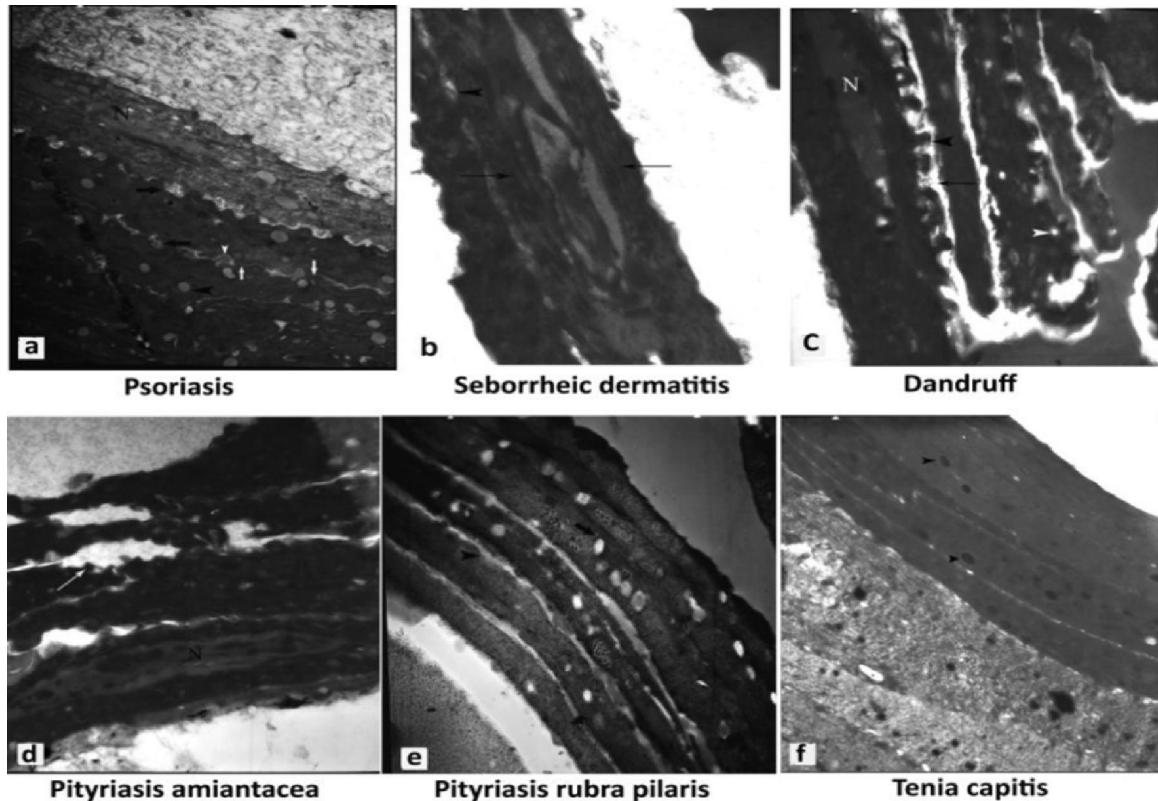
Multiple small lipid droplets were observed in the cytoplasm of corneocytes with disintegration of desmosomes. The intercellular spaces were widened with focal separations forming gaps between corneocytes. Parakeratosis was also observed (Fig. 4, d).

#### 5) Pityriasis rubra pilaris:

Multiple large lipid vacuoles were seen within the cytoplasm with remnants of nuclei (Fig. 3, d). The ICS showed some abnormalities in width. Focal dilatations of ICS containing intercellular lamellar bodies that appeared as medium electron dense materials were observed (Fig. 4, e).

#### 6) Tinea capitis:

TEM study of Tinea capitis revealed normal stratum corneum structure with massive invasion of fungal spores (Fig. 3, e & 4, f).



**Fig. 4:** (a) A transmission electron micrograph of stratum corneum of a psoriatic case shows remnants of nuclei appear in a transitional zone between stratum granulosum and stratum corneum. The SG-SC and the SC-SC interfaces show remarkable irregularity (black arrows) with few areas of dilatations (white arrow head). Numerous lipid droplets (black arrow head) are seen in the cytoplasm of the corneocytes. (b) a seborrheic dermatitis case showing a great amount of lipid material in the form of linear structures filling the cytoplasm of the corneocytes (arrow). The intercellular spaces are occasionally widened (arrow head). (c) A dandruff case shows observable separation between corneocytes (black arrow) in which finger-like projections (black arrowhead) are seen. The cytosol of the corneocytes shows multiple lipid inclusions of light density (white arrowhead) and remnants of nuclei (N). (d) a pityriasis amiantacea case reveals remnants of nuclei (N) and small lipid droplets in the cytoplasm of corneocytes. The ICS are markedly widened at certain areas forming large gaps (arrow). (e) A pityriasis rubra pilaris case shows numerous lipid vacuoles (arrow) and slightly dilated ICS with deposition of a medium-electron dense material in these spaces (arrow head). (f) Tinea capitis case shows multiple fungal spores in the cytoplasm of corneocytes (arrow head). Neither parakeratosis nor abnormalities in the ICS are observed.

#### 4. Discussion:

Removal of corneocytes from the epidermis by skin surface biopsy allows a detailed examination of the cell with SEM examination.

The morphological criteria of the scales taken from the scalp in this study were found to be similar to those taken from the body as described by Amer et al. (1997) and Tring and Jolly (1973) in cases of psoriasis and by Amer et al. (1997) in cases of seborrheic dermatitis and pityriasis rubra pilaris.

In the present study, it was noticed that the surface morphology of individual corneocytes

revealed a specific surface appearance for each disease: hexagonal scales in psoriasis, heart-shaped scales in seborrheic dermatitis, polyhedral scales in dandruff and pityriasis amiantacea and rock-like scales in PRP, which means that the different underlying pathologic process of every disease has its own print.

The presence of villous projections on the outer surface of corneocytes was a common finding in scaly disorders of the scalp with variable degrees, being marked in psoriasis and PRP, moderate in dandruff and pityriasis amiantacea, mild in seborrheic

dermatitis and absent in *Tinea capitis*. This was explained by Menton and Eisin (1971) to be due to the presence of parakeratotic cells, which was a common finding in all these disorders, as their studies on the granular layer cells in normal epidermis showed prominent surface interdigitating folds that become less obvious in stratum corneum layer. So, the villi seen may be a reflection of the normal granular layer since parakeratotic cells are only "recent arrival" in stratum corneum.

Sim (1970) considered these villous elevations to be attachment sites as they are specialized areas on opposing cells essential for maintenance of cell contact and adhesion. This is in agreement with results of present study that revealed the presence of villous structures on the surfaces of overlapped cells.

Only in psoriasis and PRP, the results of the present work revealed the presence of depression pits on the surface of corneocytes. We suggested that the prominent surface villi and depressions could contribute to more rapid transfer of substances through the stratum corneum because of the greater area available at the cell surface.

In our study, all the scaly scalp disorders except *Tinea capitis* exhibited a pronounced thinning of cell margins. The margins of these cells showed a proclivity to folding in Psoriasis. This observation is broadly in agreement with those of Tring and Jolly (1973) who suggested some alterations in intercellular adhesion as the cause of the thinning and folding margins of corneocytes in psoriasis.

The present study revealed the presence of colonies of fungal spores on the surface of corneocytes in dandruff, seborrheic dermatitis and *tinea capitis*. The severity of it varied, being marked in *tinea capitis*, moderate in seborrheic dermatitis and mild in dandruff. In the latter two cases the yeast density varies greatly from one corneocyte to another.

These observations are broadly in agreement with those of Pierard-Franchimont et al. (1995) who reported that dandruff and seborrheic dermatitis may be primarily viewed as focal disorders of the control of *Malassezia* biocence.

In (2006) Pierard et al. mentioned that there were two major mechanisms concerned in up regulation of the amount of *Malassezia* spores. On one hand, the density of high-affinity adhesion sites for the yeasts at the corneocyte surfaces could be increased. On the other hand, the natural antimicrobial peptides that are synthesized by keratinocytes could fail to be operative on certain clumps of corneocytes.

The TEM results of the present work revealed a common finding, which was the presence of lipid droplets and remnants of nuclei in the cytoplasm of

corneocytes that was correlated with villous pattern seen by SEM.

Large number of parakeratotic cells was seen in psoriasis and PRP, focal parakeratotic cells were observed in dandruff, seborrheic dermatitis and pityriasis amiantacea and only few parakeratotic cells were seen in *tinea capitis*. This result is in coincidence with those of Warner et al. (2001) in dandruff and with those of Fartasch (1997) and Barbareschi et al. (1994) in psoriasis, who suggested that parakeratosis in psoriasis may be due to pronounced hyperproliferation of keratinocytes.

On the other hand, Warner et al. (2001) suggested that the focal nature of parakeratosis in dandruff might be due to microbial populations. This explanation is in correlation with our SEM results which revealed that *Malassezia* yeasts were uniformly dispersed among the affected stratum corneum cells.

However, by TEM we observed marked infiltration by fungal spores in *tinea capitis*, moderate infiltration in seborrheic dermatitis, while this infiltration was absent in dandruff in spite of the presence of yeast colonies on the surface of corneocytes by SEM. This finding may be due to a different pathogenic mechanism for dandruff than seborrheic dermatitis, or due to focal (spotty) distribution and mild density of yeast colonization that we could not observe the infiltration of the spores in the field we examined but this does not mean it does not exist.

The results of the present study revealed that there were lipid droplets within corneocytes and intercellular lipid structures in all scaly scalp disorders with varying degrees, being more dense in the scalp dermatoses (observed in the present work) as compared with those in altered stratum corneum structure in same disorders affecting other areas in the body as reported by Fartasch (1997) in psoriasis and by Kanerva et al. (2003) in PRP.

Pathological intercellular lipid structures and lipid droplets within corneocytes were marked in dandruff, seborrheic dermatitis and pityriasis amiantacea, moderate in psoriasis and PRP and minimal in *tinea capitis*.

Another common TEM finding in scaly scalp disorders was the presence of frequent separation of corneocytes, as they were not tightly apposed but separated by dilated intercellular spaces filled by accumulations of intercellular lipids in seborrheic dermatitis and dandruff. Wide corneocyte separations with finger-like projections were seen in dandruff and pityriasis amiantacea. Less frequent separations were noticed in psoriasis and PRP while almost compact cells were seen in *tinea capitis*.

Corneocytes separation is accompanied by functional reduction, disintegration or absence of desmosomes (Warner et al. 2001). This finding is in agreement with the results of our work, where we observed intact desmosomes in tinea capitis, some normal desmosomes and disintegrated others in psoriasis, while we didn't observe normal desmosomes in other dermatoses showed wide separations between corneocytes.

In psoriasis, in particular, we observed a clear presence of retained lamellar bodies that seem to be due to alteration in epidermal barrier structures. Many of the lamellar body lipids failed to be secreted and retained within the cytoplasm of corneocytes. In some cases we observed areas of psoriatic stratum corneum which were devoid of the lipid bilayer with presence of epidermal lamellar bodies in occasional dilations of the intercellular space.

The results of the present work are broadly in agreement with those of Fartasch (1997) and Barbareschi et al. (1994), as they reported that there was a precise relationship between structural changes in lipid architecture and faulty desquamation in psoriasis. The abnormal cohesiveness of stratum corneum in psoriasis which leads to excessive stacking of psoriatic scales and thickening of the stratum corneum might be partly due to altered structural lipid-desmosome interaction (Fartasch, 1997).

Finally, we can summarize causes of abnormal desquamation to: increased epidermal cell proliferation, abnormal lamellar body lipid content and decreased secretion of lamellar bodies, decreased corneocytes hydration and disintegration of desmosomes.

Dandruff and seborrheic dermatitis had different electron microscopy characteristics in spite of the fact that, by many authors, the terms of seborrheic dermatitis and dandruff are commonly used as synonyms.

Cases suspected of being tinea capitis may be diagnosed by light microscopy, by fungal culture and by biopsy with PAS stain, yet electron microscopy though being more sophisticated and more expensive has no fallacies in selected cases.

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