

THE EFFECT OF PROTEINASES (KERATINASES) IN THE PATHOGENESIS OF DERMATOPHYTE INFECTION USING SCANNING ELECTRON MICROSCOPE

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ABSTRACT:

Objective: To study the inter-relationship between the stratum corneum of host and the fungal microorganisms using scanning electron microscopy for a complete understanding of the host parasite relationship.

Setting: The patients attended the outpatients of Department of Dermatology, King Abdul Aziz Hospital Makkah. The isolation & identification was carried out at the Department of Mycology University Hospital of Wales, Cardiff, U.K.

Material and Methods: Skin surface biopsies were obtained from two patients suffering from tinea cruris infection. One patient was infected with *Trichophyton rubrum* and the other with *Epidermophyton floccosum* strains.

Results: The scanning electron microphotographs obtained from two patients showed a large number of villi in the infected area. The fungal hyphae were seen to be placed intercellularly as well seem to be transversing through the corneocytes in many places.

Conclusion: From the results observed in this study it could be suggested that the secretion of proteinases from the fungal hyphae together with the mechanical force of the invading organisms in vivo might be playing part in the invasion of the organisms.

KEY-WORDS: Scanning electron microscopy, Proteinases, Skin surface biopsy.

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INTRODUCTION

Proteinases are produced by dermatophytes in vitro and may well play an important role

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in the pathogenesis of fungal infections in vivo. Tsuboi et al suggested that the pathogenicity of microorganisms is related to the production of proteinases which enables them to parasitize in tissues such as stratum corneum, nails and hairs¹. This interaction between the fungal hyphae and its products with the host tissue results in the clinical expression of the diseases.

Tsuboi et al isolated proteinases having enzyme activity at acidic pH from the culture filtrates of *Trichophyton mentagrophytes*. The existence of proteinases under acidic conditions suggested that these enzymes might have a pathogenic role in causing dermatophyte infections². Many workers in the past have attempted to assess the role of proteinases and the effects of mechanical forces exerted by the invading microorganism on the host tissue. These researchers believed that the fungal

microorganisms attacked non-fibrillary matrix and fed on non-keratins. They also suggested that the penetration by the organisms was probably mechanical^{3,4}. Mercer et al performed electron microscopy of hairs infected with *Trichophyton mentagrophytes* and were the first to suggest that the invading tips of the hyphae were releasing proteolytic enzymes which might be helping the organisms in the digestion and penetration of hairs⁵. Kunert et al examined the degradation and digestion of human hairs by *Microsporum gypseum* in vitro and suggested it to be due to the action of enzymes, however mechanical action of the mycelia was also observed on the cells of cuticle⁶.

The proteinases secreted could have an important role in the pathogenesis of dermatophyte infections. To understand the significance of the proteolytic enzymes (keratinases) in the pathogenesis of dermatophyte infections we studied the skin surface biopsies obtained from two patients with dermatophyte infections using scanning electron microscope technique. Skin surface biopsies have also been used in the past to study the stratum corneum using scanning electron microscopy⁷. As a part of our study, the relationship between the dermatophyte hyphae and the surrounding stratum corneum was observed employing this technique.

METHODS

Fungal Organisms:

Multiple skin surface biopsies were obtained from a patient having tinea cruris infection with fungal strain of *epidermophyton floccosum*, similarly another set of skin surface biopsies were obtained from patient having tinea cruris infection with *trichycophyton rubrum*. Both patients attended the department of dermatology, King Abdul Aziz Hospital Makkah, Saudi Arabia. The fungi were isolated and identified at the Department of Medical Microbiology, University Hospital of Wales, Cardiff, UK.

Skin Surface Biopsies:

Skin surface biopsy technique is quite a simple method and enables us to study the various microbial invaders of the stratum corneum in vivo arrangement. The procedure involves cleaning the skin and placing a drop of Cyanoacrylate adhesive glue onto the skin. The clean glass slide is then placed on top of the lesion very carefully and then placed lightly onto the skin. The glass slide is then quickly removed after 30-40 seconds with a firm pulling action. The biopsies were taken from each patient, from immediately adjacent sites at the periphery of the lesions. A layer of stratum corneum is removed with the adhesive glue which remains attached to the glass slide. This transparent layer of stratum corneum can be examined with naked eye or directly by microscopy. This procedure has a number of advantages. It is possible to employ some of the staining methods of histology and histochemistry. The fungal organisms stain purple red with PAS Stain and are easy to recognize. It provides us a means to have a permanent record of a patient's infection.

Preparation of skin surface biopsies for performing scanning electron microscopy:

0.2M solution of di sodium hydrogen orthophosphate and 0.2 M solution of potassium dihydrogen orthophosphate were prepared. 16.4mls of di sodium hydrogen orthophosphate and 3.6mls of potassium di hydrogen orthophosphate were mixed thoroughly and adjusted to P.H 7.2. This solution was labeled as solution 1.

1ml of 25% glutaraldehyde was taken in a bottle and to it 4.5mls of distilled water was added. 4.5ml of solution 1 was added to the above mixture to make a final volume of 10mls of 2.5% glutaraldehyde. 30mls of 2.5% glutaraldehyde solution, prepared as mentioned above was put inside a plastic jar. The skin surface biopsies were left inside the jars for a week.

0.1M of phosphate buffer, was prepared for washing the biopsies. After one week the solution in the plastic jar was emptied using syringe, the procedure being carried out under

sterile conditions. The biopsies were then washed twice with 40mls of 0.1M phosphate buffer for duration of 15 minutes each time.

The glass slides with skin surface biopsies on top were placed inside the cabinet and each of them was overlaid with drops of 1% omiom sulphate for one hour. Then each biopsy was given two washes of 10 minutes each, in 70% alcohol followed by one wash of 10 minutes in 90% alcohol and finally two washes of 10 minutes each, in 100% alcohol.

The biopsies were then placed inside dessicator and vacuum dried overnight. The biopsies were then glued on mounts using Cyno-Acrylate. Two mounts were prepared from skin surface biopsy, of each patient. Finally mounts with portion of biopsies were purged twice or thrice with organ sulphate gas, followed by gold plating using "SEM Sputter Coater", EM SCOPE LTD, Ashford, Kent, UK. The films used were Kodak tri-X-Pan (TX 120). Photomicrographs were taken on a Joel JSM - 840 A Scanning Electron Microscope.

All these steps were carried out in the electron microscope unit of Heath Hospital, University of Wales, Cardiff, U.K.

RESULTS

The scanning electron micro photographs presented with some interesting observations Figure-1 demonstrates scanning electron microphotograph of skin surface biopsy from a patient with *Trichophyton rubrum* infection

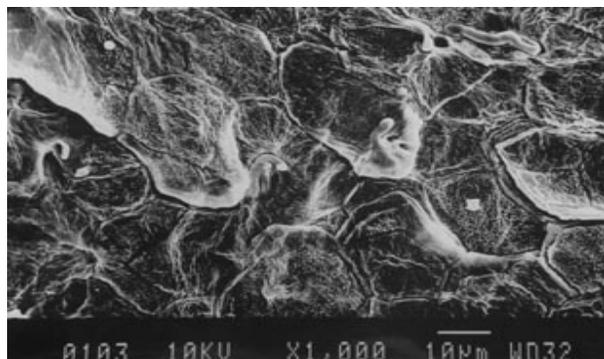


Figure-1: Scanning electron micro-graph of skin surface biopsy from a patient with *Trichophyton rubrum* infection demonstrating prominent villi on the surface of corneocytes (arrow).

showing large number of prominent villi on the surface of corneocytes. A few hyphae can be seen which could be lying between the corneocytes. The presence of such prominent villi on the surface of squames as compared to normal areas have been reported in psoriatic lesions and some other scaly disorders in the past by other workers⁷.

Most of the electron microphotographs taken in this study as shown in Figure-2 demonstrates the hyphae to be running horizontally in between the corneocytes and in some cases weaving in and out of the cytoplasm of the corneocytes themselves. This observation is

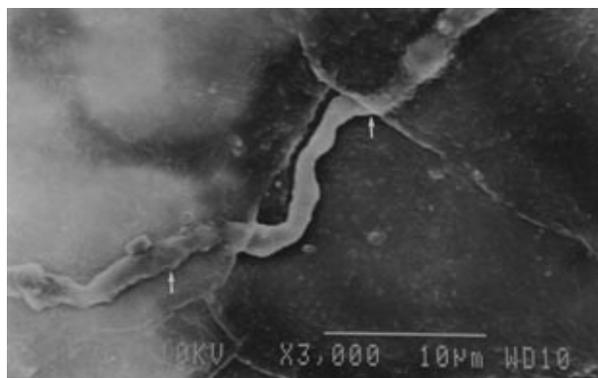


Figure-2: Scanning electron micro-graph shows the hyphae could be lying within the cytoplasm of the corneocytes or underneath it (arrow lower left) and later emerging to reenter in between two corneocytes (arrow upper right).

similar to the clinical findings where the lesions spread radially from the point of inoculation⁸. Farrell reported similar results after performing scanning electron microscopy on candida grown on tissue culture cells obtained from uterine cervix. They reported the hyphae to be piercing the cell and then re-emerging within the same cell or in an adjacent cell⁹.

DISCUSSION

Many workers in the past have attempted to assess the role of proteinases and the effects of mechanical forces exerted by the invading microorganism on the host tissue. Some of them after performing studies on dermatophytes believed that the fungal microorganisms attacked non-fibrillary matrix and fed on non-keratins. They also suggested that the penetration by the organisms was probably

mechanical^{3,4}. Mercer et al as mentioned earlier, performed electron microscopy of hairs infected with *Trichophyton mentagrophytes* and were the first to suggest that the invading tips of the hyphae were releasing proteolytic enzymes which might be helping the organisms in the digestion and penetration of hair⁵. Some other groups of researchers on the other hand were able to isolate enzymes from culture medium in vitro containing hairs. They observed that these enzymes caused degradation and degeneration of human and guinea pig hairs used as substrates in vitro and because of this specific actions by these enzymes they were termed as Proteinases or Keratinases^{1,2,6}.

In this study the spreading hyphae showed no evidence of enzymatic or mechanical damage to the surrounding host tissue. There could be a number of explanations for these observations. There is a possibility that the amount of enzymes released by the hyphae in this particular situation were not quantitatively large enough to cause damage to the surrounding tissues, the other possibility could be that, enough proteinases might have been released and they might have caused damage to the surrounding host tissue, but the area of damage around the hyphae may be too small to be seen, i.e it is below the resolving power of the SEM. This could be due also in part to the nature of the preparation steps involved especially the metal coating/ shadowing which could mask the damage caused to the surrounding tissue by the releases of the enzymes from the hyphae.

Similar findings with evidence of no biochemical or mechanical damage have been reported. Miyazaki et al, observed *Trichophyton mentagrophytes* and *Trichophyton rubrum* in the stratum corneum using transmission electron microscopy and found the fungal elements to be present inter-cellularly as well as hyphae appeared to be running horizontally inside the horny cells¹⁰. Another group of scientists reported intracellular location of the hyphae, within the cells of stratum corneum of guinea pigs, infected experimentally with *Trichophyton mentagrophytes*¹¹.

CONCLUSION

Some workers suggest that the mechanical action of the invading mycelium penetrating the stratum corneum is an important part of the destructive process while others have suggested that proteolytic enzymes are the major contributing factor¹⁻⁶. Scanning electron microphotographs in this study demonstrated the fungal hyphae to be present inter and as well as intracellularly. Although there was no evidence of biochemical or mechanical damage, it could be suggested that the secretion of proteinases together with the mechanical force of the invading organisms in vivo might be playing part in the invasion of the organisms.

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