ATOMIC FORCE MICROSCOPY APPLIED TO ATOPIC DERMATITIS STUDY

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Rezumat

Dermatita atopică (DA) este cea mai frecventă boală inflamatorie a pielii, care afectează până la 25% dintre copii și 2% până la 5% dintre adulți. Metodele de diagnostic oferă recomandări extinse bazate pe dovezile disponibile. Evaluarea morfologică rămâne o caracteristică principală a investigației clinice și principalul criteriu de diagnostic.

Metodă. Am colectat pielea normală și afectată de la un pacient cu vârsta de 6 luni care a fost diagnosticat prin examen dermatologic. Caracteristicile clinice și diagnosticul de dermatită atopică au fost în conformitate cu criteriile Hanifin și Rajka. Morfologia și integritatea structurală au fost investigate prin microscopie de forță atomică.

Rezultate. Imaginile optice și topografice indică faptul că, în cazul DA leziunilor cutanate, structura cuticulei a fost grav deteriorată și distorsionată odată cu aplatizarea și încrețirea plăcilor, care au un aspect neregulat. Din morfologiile de suprafață ale probelor, demonstrăm că forma corneocitelor, cu aspect granular și alungit, specifică pielii normale este transformată în plăci rupte și deteriorate cu aspect discontinuu în DA.

Concluzii: În diagnosticul inițial al DA, modificările proprietăților pielii pot fi un indicator. Criteriile Hanifin și Rajka împreună cu microscopia de forță atomică pot fi o tehnică utilă și necesară diagnosticării cazurilor de dermatită atopică.

Cuvinte cheie: dermatită atopică, microscopie de forță atomică.

Abstract

Atopic dermatitis (AD)-the commonest inflammatory skin disease affects up to 25% of children and 2% to 5% of adults. Methods of the diagnostic provide expanded recommendations founded on available evidence. Morphological evaluation remains a principal feature of clinical investigation and the main criteria of diagnosis.

Methods. We collected normal and affected skin from a 6-month child patient who was diagnosed through dermatologic examination. Clinical characteristics and the diagnosis of

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atopic dermatitis were in accordance with Hanifin and Rajka criteria. Morphology and structural integrity were investigated by Atomic Force Microscopy.

Results. *Optical and topography images indicate that in the case of AD skin lesions the cuticle structure was severely damaged and distorted with the flattening and grading of the plates, which have an irregular appearance. From the surface morphologies of the samples, we demonstrate that the shape of the corneocytes, with granular and elongated appearance, specific to normal skin is transformed by AD into broken and collapsed plates with discontinuous appearance.*

Conclusions. *In the initial diagnosis of AD changes of the skin properties can be an indicator. Hanifin and Rajka criteria together with Atomic Force Microscopy can be a useful and necessary technique diagnosing cases of atopic dermatitis.*

Keywords: *atopic dermatitis, atomic force microscopy.*

Introduction

Atopic dermatitis (AD) is a chronic inflammatory or recurrent skin disease, and plays an important role in the quality of life among affected persons and their families becoming a major issue in health care spending^(1,2,3).

Recent studies indicate an increase in the prevalence of AD and allergic diseases in industrialized countries⁽⁴⁾. Prevalence ranges from 2.1% / 1.5% in Japan to 4.9% / 3.9% in the US and 3.5% in Europe^(1, 5). A percent of 10-15% in the case of children with age before school age is usually met. Children with AD are at high risk of developing asthma and allergic rhinitis. Of those with AD before

2 years of age, 50% will develop asthma later^(2,6,7).

AD is one of the first indicators for subsequent allergic diseases; imposing the idea, that quality control of AD may prevent the development of respiratory allergy or reduce the severity of asthma. Numerous studies have shown that AD and asthma have a common genetic and pathogenic basis, providing evidence for the atopic walk from AD to allergic rhinitis and asthma⁽⁴⁾.

Clinical features of atopic dermatitis-eczematous lesions, intensely itchy, which become excoriated and lichenified, vary depending on the stage (acute or chronic) of the disease and the age of the patients^(2, 8). Infants may have gray plaques or white scabs

on the scalp, associated with damage to the face, neck, trunk, ankles and extension and flexural areas of the extremities⁽⁹⁾.

Patient history and clinical manifestations are the main clinical criteria for the diagnosis of AD, while specific paraclinical diagnostic tests do not exist^(2,10).

Skin changes in AD are complex processes that involve a modification in cell structure, chemistry, and function^(11,12).

Atomic Force Microscopy is a new technique commonly used in surface imaging. Length and height measurements are performed to generate topographic images at the nanometric and micrometric scale, due to its very high resolution which is more than 1,000 times better than optical microscopy, with several orders of magnitude above the normal detection of optical microscopes. An Atomic Force Microscopy is based on the interaction forces between a sharp tip attached to the extremity of a flexible cantilever and the sample surface that determines the cantilever's deflection.

The small deviations of the cantilever are detected by an optical lever system exploiting the reflection of a fixed laser beam over a small mirror attached to the body and the position of the point of light is also measured and used as a basis to reconstruct a pseudo-3D image of the sample surface^(13,14).

Atomic force microscopy was used to analyze the individual corneocytes that are the outermost layer of the epidermis. Kashibuchi et al. performed the detailed study on 3D morphology of corneocytes isolated from the upper arm and cheek of volunteers of different ages⁽¹⁵⁾.

The main objective of our work was to investigate the microstructures and the properties at the nanometric scale of skin affected by atopic dermatitis. Using AFM we verify and confirm this hypothesis and

correlated with the Hanifin and Rajka criteria which are the basis for the diagnosis of AD. The diagnosis of AD was made clinically and is based on historical features, morphology and distribution of skin lesions, and associated clinical signs. In this study, the patient presents 3 major Hanifin and Rajka criteria including pruritus, typical morphology and distribution, facial and extensor eruptions, and personal or family history of atopy (asthma, allergic rhinitis, atopic dermatitis). Other minor criteria which include early age of onset, anterior neck folds, perifollicular accentuation, and a tendency toward non-specific hand or foot dermatitis.

Clinical characteristics^(16, 17, 18, 19, 20) and the diagnosis of atopic dermatitis were in accordance with Hanifin and Rajka criteria.

Materials and Methods

We present the case of a 6 month-old male infant who was evaluated in Allergy Department for skin lesions who is characterized by a lively red infiltrate, crusting, excoriations and squamous erythematous plaques ; eczema is localized at the flexures of the elbows and legs and to the face.

Figure 1. (a) Clinical images of a 6-month-old female of the forearm; (b) face

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Figure 2. Atomic Force Microscopy, Park XE-100 equipment

Squamous erythematous plaques were removed from the patient skin with lesion (Figure 1 - a and b). The samples were taken directly from the cheek by scraped, afterward the skin morphology was evaluated using AFM. Normal skin samples were taken and examined in the same way and used for comparison. All skin samples were fixed in same mode on a glass slide and the probe was measured directly without extra treatment.

To investigate the surface topography the skin samples were collected with a scalpel blade and are superficial. Initially, the microscope slides were cleaned and left in the ultrasonic bath with ethanol to avoid contamination. Tissue samples were

transferred onto a glass slide immediately after collection to prevent tissue autolysis and decomposition. Small wax bites melted, which is very close to the room temperature, has been dripped onto the surface of the blades so that the corners of the skin sample are glued to the blade. Gluing at the center of the sample was avoided to eliminate the risks of deforming the samples⁽¹¹⁾.

In this study, for samples were removed only the outermost layers of skin with a special razor blade or scalpel. It was only necessary to use the tools to detach the outer portion of the affected skin and to place it on the glass slide so that the integrity of the surface was as little affected as possible, not for deep cutting.

No antiseptic, preoperative or hygiene treatments were applied on the specimens, in case of their use there is a risk of alteration or hydration of the samples, which would have led to false images and affected morphological characteristics. In order to obtain the necessary skin samples in this study, a biopsy was not required, as in the case of cancer therapies, this obtaining procedure described in the article produces a minimum of both mental and cosmetic discomfort and does not require the use of local anesthesia.

In addition, Atomic Force Microscopy does not require thick pieces of skin, (specimens a few microns thick can be scanned) and therefore only skin surface specimens were involved in

Figure 3. (a) Optical images of control skin; (b) AD skin, scanned area $40 \times 40 \mu\text{m}^2$

Figure 4. (a) 2D surface topography for the control skin; (b) 3D surface topography for the control skin, scanned area, $40 \times 40 \mu\text{m}^2$. The blue circle indicates form of corneocyte. Height is given in colour brightness - lateral dimension by scale bar (1 μm). The graphs under figures indicate a cross-section that of samples

Figure 5. (a) 2D surface topography collected from lesion regions of AD; (b) 3D surface topography collected from lesion regions of AD, scanned area, $40 \times 40 \mu\text{m}^2$. The blue circle indicates form of corneocyte. Height is given in colour brightness - lateral dimension by scale bar (1 μm). The graphs under figures indicate a cross-section that of samples. Height fluctuations in the graph indicate increased cell surface roughness

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the study. The scanning area is 40×40 microns so it is not important that the edges of the sample are well delimited. Before capturing the topography images, some photos were scanned and evaluated. The scanned area was initially evaluated in terms of microscopic characteristics and the edges are generally avoided in these evaluations because it is tried to avoid breaking the cantilever of the Atomic Force Microscope.

The surface topographies were recorded using AFM, Park XE-100 equipment (silicon tip with conical shape) in the standard imaging Non-contact atomic force microscopy, with cantilever nominal length of 125 mm, nominal force constant of 40 N/m, oscillation frequencies in the range of 275–373 kHz (Figure 2). In the non-contact mode the cantilever is never in contact with the sample, and the forces between tip and the sample are attractive. The surface topographies were recorded five to seven times for each sample over a scan area of $40 \times 40 \mu\text{m}^2$. We used horizontal line by line flattening as planarization method.

Results

Morphological differences at nanometric scale between affected and healthy skin may be helpful in diagnosing AD.

In the present study, the morphology of the surface was investigated. Initially, optical

images of the samples of the skin affected by AD were scanned and recorded and compared with the control (healthy skin). (Figure 3) a and 3b show representative AFM optical images of AD and control skins.

Figures 4a and 4b show the 2D and 3D surface topography taken in an area of $40 \times 40 \mu\text{m}^2$ for the control skin and figure 5a and 5b shows the surface topography collected from lesion regions of AD, respectively.

Discussion

A significant difference in the surface morphology was observed between the AD skins and control skins. In AD skin lesions the cuticle structure was severely damaged and distorted.

The difference in topography of the cuticles between the AD and control skin is more clearly seen in three-dimensional images of the surface and the line profiles, which were measured in the position as indicated with red and green lines in AFM images (Figure 6 - a and b).

The surface architecture, the shape of the corneocytes, the granular and elongated appearance, is transformed into broken and collapsed plates with discontinuous appearance. In the case of AD samples, the cuticles can be damaged and deformed, together with the flattening and grading of the plates, which have an irregular

Figure 6. (a) Surface morphology and line profiles of the control skins; (b) Surface morphology and line profiles of the AD skins. (a) Blue arrows indicate the profile of corneocyte; (b) The destruction of the corneocytes is confirmed by the steep profile in the blue circle. Height is given in colour brightness – lateral dimension by scale bar (1 μm). Red lines represent horizontal line profiles and green lines represent vertical line profiles. The blue lines indicate a flat surface along two profile lines and in the blue circle in figure b is presented the destruction of the shape of the corneocytes

appearance. These differences can be more easily observed with the help of the profile lines, where differences in both flatness and severe damage to the cuticle edge are observed (Figure 6 - a and b).

Conclusions

The results proved that depending on the presence of AD, morphological parameters of corneocytes were different. The 3D characteristics of corneocytes in the diseased skin varied significantly from those of healthy skin. The volume of corneocytes was larger in the case of unhealthy skin. We observed AD effects on cuticle structure and scale by AFM. The cuticle was damaged severely by AD; specifically, the edge of the cuticle was torn and collapsed.

In conclusion, we can say that the purpose of our work has been achieved. A case confirmed with the Hanifin and Rajka criteria was subjected to further investigations to

confirm the hypotheses of nanometric destruction of the skin structure using the atomic force microscopy technique. Structural changes of the skin can be an indicator in the initial diagnosis of atopic dermatitis, and together with other criteria such as Hanifin and Rajka can form the complete picture of the diagnosis. Atomic force microscopy can be a useful and necessary technique in diagnosing cases of atopic dermatitis.

Conflict of interest

On behalf of all authors the corresponding author states that there is no conflict of interest.

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