Abstract

Introduction: The pathognomonic trichodystrophy in Netherton’s syndrome is trichorrhexis invaginata. This hair shaft anomaly is not constantly present and it can be associated with other anomalies like trichorrhexis nodosa or pili torti.

Methods: We retrospectively analyzed hair samples from patients diagnosed with NS over the past 10 years in the Dermatology Clinic Timisoara by using scanning electron microscopy. The samples were of scalp hair, eyebrows, eyelashes and pubic hair. We also evaluated some of these samples with trichoscopy and confocal microscopy.

Results: The scanning electron microscopy results showed that trichorrhexis invaginata was evident in all cases, followed by trichorrhexis nodosa and pili torti respectively. In these patients there was more than one type of trichodystrophy present at the same time. All of these modifications were perceptible with the confocal scanning microscope and by trichoscopy.

Discussion: The electron microscopy helps by supplying three-dimensional images of the hair shaft, thus enabling the observation of the hair samples with a greater clarity and sharpness than through classical methods. Also reflectance confocal microscopy and trichoscopy have proven to be very useful in the diagnosis of hair shaft anomalies.
Introduction

Netherton syndrome (NS) is a rare autosomal recessive genodermatoses caused by loss-of-function mutations in a SPINK5 gene (serine protease inhibitor of kazal type 5) situated on chromosome 5q32. This gene encodes LEKTI-1 (lympho-epithelial kazal type related inhibitor) expressed in stratified epithelia(1). The loss of this inhibitor results in an early activation of stratum corneum trypsic/chymotryptic protease, resulting in proteolysis of adhesion molecules(2).

From the clinical point of view, it is characterized by severe skin inflammation and scaling (ichthyosiform erythroderma in newborns or ichthyosis linearis circumflexa), a specific hair shaft defect and atopic diathesis. The atopic manifestations are present in most cases, represented by: atopic dermatitis, asthma, angioedema, urticaria, high levels of IgE and hypereosinophilia(3,4).

The most characteristic trichodystrophy is trichorrhexis invaginata (“bamboo hair”) in which the distal hair segment (fully keratinized and hard) is intussuscepted into the proximal one (soft due to abnormal keratinization). This anomaly is due to the abnormal cornification of the internal root sheaf. Other hair shaft defects seen in NS are trichorrhexis nodosa and pili torti. The trichorrhexis nodosa is caused by the appearance of a breach in the hair cuticle with separation associated with fraying of the exposed cortical fibers leading to a node-like swelling. The exposed fibers fracture and the shaft breaks taking the appearance of a splayed paint brush. The other trichodystrophy seen in patients with NS is pili torti characterized by the flattening and twisting of the hair shaft on its own axis(5,6). These hair shaft abnormalities can be seen at different intervals along an otherwise straight hair shaft.

Nowadays, there are a lot of tools which can be used to identify hair shaft defects ranging from trichoscope, light microscope to scanning electron microscope and even reflectance confocal microscope. In our study we used scanning electron microscopy to identify the hair shaft anomalies and on some hair samples trichoscopy associated with reflectance confocal microscopy (RCM).

Material and methods

In this study we included 8 patients diagnosed with NS at the Timisoara Dermatology Clinic during a period of ten years. Their diagnosis was based on the pathognomonic triad represented by congenital ichthyosis, atopic manifestations and hair shaft anomalies and was confirmed by the presence of trichorrhexis invaginata in optical microscopy. The plucked hairs were first observed through light microscopy to confirm the presence of an abnormality and to select abnormal hairs; these hairs were then analyzed by scanning electron microscopy (SEM). The studies were performed on samples fixed on copper supports. The surface was examined by using an Environmental Scanning Electron Microscope (ESEM) type Quanta 200, operating at 20kV with secondary electrons in Low vacuum mode.

For the reflectance confocal microscopic (RCM) analysis we used the VivaScope 3000 hand held device and for trichoscopy we use microDERM dermatoscopic camera. At first we identified and isolated the affected hairs from the preexisting...
samples and then we used the confocal microscope to analyze the hair which presented trichodystrophies.

**Results**

In scanning electron microscopy we found that trichorrhexis invaginata was evident in all cases. Trichorrhexis nodosa was seen in 62.5% of patients and pili torti in 50% of cases. Patients with combined hair dystrophies represented 87.5%, of which 25% had findings consistent with trichorrhexis invaginata, trichorrhexis nodosa and pili torti, 37.5% had evidence of trichorrhexis invaginata and trichorrhexis nodosa and 25% presented trichorrhexis invaginata and pili torti. (Table 1) In the samples selected for trichoscopy we were able to confirm the presence of these trichodystrophies. In addition to this, by using RCM, we were

<table>
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<th>Hair shaft anomalies</th>
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<td>L.A., F., 0-6 months</td>
<td>Trichorrhexis invaginata, trichorrhexis nodosa</td>
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<td>I.V., F., 9 years</td>
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<td>G.S., M., 0-4 months</td>
<td>Trichorrhexis invaginata, pili torti</td>
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**Table 1. Hair shaft anomalies found in our patients**

![Figure 1](image1.png)

**Figure 1.**

a) Trichoscopy trichorrhexis invaginata: “bamboo hair” (30x magnification); b) Scanning electron microscopy: Trichorrhexis invaginata: intussusception of the distal hair shaft into the proximal hair shaft results in a distinctive ball-and-socket hair shaft deformity; in the areas around the intussuscepted hair shaft, there is disorganized keratinization; c) RCM the ball in socket deformity associated with abnormal keratinization on the surface of the hair shaft; d) RCM same area we but inside the shaft - the lack of continuity
able to analyze the modifications present inside the hair shaft. Results of the SEM, RCM and trichoscopic evaluation of the hair shafts are shown in the figures below (photos from personal archive of the Emergency City Hospital Timisoara, Dermatology, Center for morphologic study of the skin). (Figs 1, 2, 3)
The major hair dystrophies (trichorrhexis invaginata, trichorrhexis nodosa, pili torti) are accompanied by minor modifications such as longitudinal fractures of the hair shaft (trichoptilosis), transversal fractures (trichoschisis), breaks, fissures and splits, as a result of the fragility of intrinsically abnormal hair.

Conclusions and discussions
Our cases presented all the manifestations of NS described in literature. Although in literature trich-
orrhexis nodosa is more frequent than trichorrhexis invaginata. In our group the incidence was reversed, the latter being present in all the patients while trichorrhexis nodosa was seen in 62.5% of cases. The explanation for this difference might be the fact that our study investigated a small group of patients and the analyzed samples were obtained at different stages in their evolution.

At first we focused on using optical microscopy or electron microscopy to diagnose the hair shaft anomalies because these were the proven methods. But then by using trichoscopy and confocal microscopy we demonstrated that these diagnostic tools seem to be very useful in the identification and analysis of trichodystrophies.

We used SEM because it was proven that it offers the possibility of a more precise examination of cuticular scales of hair, it allows detailed observations of scale patterns and the possibility of considerable magnification. In addition to this, we used RCM. This choice was made because we believed that confocal microscopy could be used to identify these hair shaft anomalies and could offer additional information regarding what happens underneath the surface of the hair. Seeing how with the VivaScope 3000 we could only analyze a limited area (500 m/500 m), we needed a tool in order to identify the affected hairs. Therefore, we used trichoscopy. In conclusion, electron microscopy helps by supplying three-dimensional images of the hair shaft, thus enabling the observation of the hair samples with a greater clarity and sharpness than through classical methods. Also reflectance confocal microscopy and trichoscopy have proven to be very useful in the diagnosis of hair shaft anomalies offering additional information.

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**Bibliography**


