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Trichoscopy and Trichogram

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Abstract

Hair and scalp examination techniques can be classified into three categories: noninvasive methods (clinical history, general examination, photography, hair count, weighing shed hair, pull test, global hair counts, dermoscopy, electron microscopy, laser scanning microscopy, etc.); semi-invasive methods (the trichogram, unit areatrichogram); and invasive methods (biopsies in cicatritial alopecia). Scalp dermoscopy or trichoscopy is one of the noninvasive techniques for the evaluation of patients with hair loss that allows for magnified visualization of the hair and scalp skin. It may be performed with a manual dermoscope (10× magnification) or a videodermoscope (up to 1000× magnification). This method is simple, quick, and easy to perform, is well-accepted by patients, and is useful for monitoring treatment, determining severity of the disease and follow-up. It is a simple, minimally invasive and rapid technique for measuring hair follicle activity. Trichogram represents a semi-invasive technique for the evaluation of patients with hair loss that allows the microscopic examination of hairs plucked from the scalp and provides information about the state of the proximal end of the hair shaft and the distal end. The trichogram is a useful complementary tool for clinical evaluation, diagnosis, and the monitoring of treatment response.

Keywords: trichoscopy, trichogram, scalp dermoscopy, dermoscopy, dermatoscopy, hair loss, cicatritial alopecia, noncicatritial alopecia, alopecia, alopecia areata, androgenetic alopecia, discoid lupus erythematosus, frontal fibrosing alopecia, lichen planopilaris, telogen effluvium

1. Introduction

Hair loss is the most common hair disease and diagnosis of the type of alopecia may sometimes be challenging. Methods commonly used to investigate can be classified as either invasive (e.g., biopsies in scarring alopecia), semi-invasive (trichogram, unit areatrichogram), or noninvasive (e.g., hair count, weighing shed hair, pull test, global hair counts, dermoscopy,



phototrichogram, electron microscopy, laser scanning microscopy) methods. In this chapter, I will try to explain the basics of trichoscopy and trichogram.

2. Trichoscopy

Scalp dermoscopy is a simple and noninvasive instrument for appraising a number of hair and scalp disorders. Scalp dermoscopy, which may be defined as "trichoscopy" [1–3] is widely used in dermatology for the evaluation of pigmented skin lesions, represents a valuable, noninvasive, and rapid technique for the assessment of patients with hair loss that allows for a magnified visualization of the hair and scalp skin [4–7]. Trichoscopy allows for magnified observation of the following: hair shafts, hair follicle openings, the perifollicular epidermis, and blood vessels. In particular, trichoscopy may be useful for the diagnosis, prognosis and follow-up of androgenetic alopecia (AGA), alopecia areata (AA), telogen effluvium (TE), trichotillomania, congenital triangular alopecia, tinea capitis, cicatritial alopecias, and hair shaft disorders [8, 9]. Dermoscopy may be performed with a manual dermoscope (10× magnification) or videodermoscope (up to 1000× magnification) [10]. Both polarized and nonpolarized light may be used, with or without the use of immersion oil (dry dermoscopy).

2.1. Trichoscopy equipment

Both handheld dermatoscope and videodermatoscope is suitable to perform trichoscopy. Handheld dermatoscopes allow for tenfold magnification, while the magnification of digital dermatoscopes is ranging from tenfold to 50-fold and higher. Handheld dermatoscopes have the advantage of being both time and cost-effective. On the other hand, digital dermatoscopes have the superiority of taking easier photography and having higher magnifications [5]. I prefer using videodermatoscope that allows vascular structures more obvious [8, 9]. Water, ultrasound gels, aqueous gels, liquid paraffin, alcohol, and oil can be used to enhance clarity and visualization [11]. The choice of a particular device and fluid immersion is a matter of individual preference.

Trichoscopy images can be used to assess hair shaft thickness by folliscope [12] or analyzing hair growth by trichoscan [13] while trichoscan has been criticized by some dermatologists for the need of shaving and dying hair in the analyzed area [14, 15]. These images may be used to obtain optimal areas for scalp biopsy in cicatricial alopecia [16].

2.2. Trichoscopy structures and patterns

2.2.1. Scalp without any complaint

Normal scalp is characterized by the presence of follicular units containing about 2–4 terminal hairs and 1 or 2 vellus hairsof uniform thickness and color [17–20]. The mean thickness of normal hair was about 0.06 mm [19]; however, up to 10% of hairs are represented by vellus hairs which lack the medulla [6, 21, 22]. A normal terminal hair is uniform in thickness and color throughout its length, however, vellus hairsare lightly pigmented [21, 22]. Simple, fine red loops, which represent capillaries within the dermal papillae, are generally visible among

hairs, and the subpapillary plexus is visible as linear arborizing vessels. In dark-skinned individuals, a perifollicular pigmented network (honeycomb pattern) is usually appreciated over the scalp which is accentuated over sun-exposed areas, and follicular openings and ecrine sweat gland duct openings appear as white dots (WD) [7, 23].

2.2.2. Dots

Trichoscopy may distinguish whether hair follicle openings are normal, empty, fibrotic, or containing biological material, such as hyperkeratotic plugs or hair residues. "Dots" is a common term for small, round hair follicle openings seen by trichoscopy [5, 6, 24].

2.2.2.1. Yellow dots

Previous studies showed that yellow dots (YD) indicates dilated infundibula of follicles with remnants of hair [25–28, 41] and follicular openings filled with keratotic material and/or sebum [24, 29]. They vary in color, shape and size. Regularly distributed YD are present in 60% of patients with AA and are considered a marker of disease severity and less favorable prognosis [24] while there was no relation with disease severity according to our study [30]. Yellow dots are present in AA [24, 30] discoid lupus erythematosus (DLE), TE [28, 30], and AGA [19, 28]. Large, dark yellow to brownish YD (keratotic plugs) are characteristic of DLE and correspond to wide infundibula filled with keratotic material [31, 32] although we saw these brown dots in patients with AGA at the same time [28]. Yellow dots are also seen in patients with patterned hair loss, YD in the frontal area compared to the occipital area favors the diagnosis of female AGA [19], they differ from the YD observed in other diseases by their "oily" appearance that most probably results from the predominance of sebum over keratotic material [9].

Yellow dots imposed over dark hair shaft residues appearing as large "3D" soap bubbles have been described in dissecting cellulitis [31] and in trichotillomania [24]. According to some dermatologists [5], when there is no suspicion of AGA, if you see YD, the diagnosis of AA incognita is obvious to differ AA incognita from TE and trichotillomania. On the other hand, in addition to these diseases, YD may be seen in patients with TE, trichotillomania, psoriasis, and seborrheic dermatitis [28, 30].

2.2.2.2. Brown dots

Yellow dots are firstly described by Ross and colleagues [5] as uniform structures yellow-ish-pink in color, while some of these dots were found to be brown in color in our patients [28, 30]. Brown dots were found with a statistically higher frequency in patients with AGA [30]. Scattered brown areas are seen in actinic keratosis and DLE, and peripilar brown areas are seen in AGA, in TE and in healthy individuals, simply sometimes, we can see brown dots in a distribution similar to YD in dark-skinned patients [8, 28, 30], and therefore, I call them yellow-brown dots instead of YD as a separate category.

2.2.2.3. Black dots

Black dots (formerly "cadaverized hairs") are residues of pigmented hairs that have been broken or destroyed at the level of the scalp [33]. They are considered a marker of high disease activity while there was no relationship during our study [24, 30]. Black dots may be pres-

ent in dissecting cellulitis, tinea capitis, chemotherapy-induced alopecia, trichotillomania, but may be incidentally observed also in other diseases and after laser depilation or trichogram [33, 34]. Black dots are not present in healthy individuals or in patients with patterned hair loss or TE [4, 28, 30, 35].

2.2.2.4. Black dotted pigmentation

Black dots sized smaller than declared black dots (cadaverized hairs) those are seen in AA previously [30] were named as black dotted pigmentation (BDP) by us, and they related positively with disease severity in AA. A biopsy from one of these areas in a patient with AA showed no cellular infiltration but revealed intense demodex colonization in follicular ostia, so it was thought that the trichoscopic appearance might be due to this infestation. In a study [36], dirty dots on scalp represented nonmicrobial environmental particles in healthy children.

2.2.2.5. Red dots

Red dots are widened follicles surrounded by dilated vessels and extravasated erythrocytes were described in DLE and are believed to be a positive prognostic factor [29]. Regularly distributed brown or brown-gray dots are a characteristic finding in the eyebrow area of patients with frontal fibrosing alopecia (FFA). This finding is a favorable prognostic factor for eyebrow regrowth [6]. Additionally, red dots have been described in individuals with vitiligo [37].

2.2.2.6. *Grey dots*

Pink-grey and grey dots have been observed in the eyebrow area of patients with FFA [9].

2.2.2.7. White dots

The classic, big, irregular WD represent areas of perifollicular fibrosis and are observed most commonly in lichen planopilaris (LPP) [6]. Another type of WD, the small, regular pinpoint WD are observed in the sun-exposed scalp of patients with skin phototypes III and IV and in the normal scalp of those with phototypes V and VI [6, 28, 30, 38]. They have been correlated with the acrosyringeal (ecrine sweat duct openings) and empty follicular openings [38–40]. According to us, another type of WD are cumulus like clustered WD that are intersecting WD in a nested form. We think in severe AA the classic, irregular WD is nested together with pinpoint WD, so we call them clustered WD similar to cumulus clouds [30]. In advanced stages of AGA, follicles can be replaced by connective tissues, afterwards causing atrophy. These empty follicular ostia are seen as WD [5, 19, 24, 28, 30, 31, 38, 39]. WD are more common in the late stages of AGA [5, 28]. Kossard and Zagarella [42] observed WD in scarring alopecia and considered these WD as being the melanin pour places in fibrous tracts of scar tissue.

2.2.3. Hair shafts

Abnormalities in hair shaft structure may provide diagnostic clues for multiple acquired and inherited causes of hair loss. Rudnicka et al. recently proposed a classification of hair shaft

abnormalities observed by trichoscopy [18, 43-45]. The features of hair shafts include exclamation mark hair in AA, trichotillomania, and chemotherapy-induced alopecia (also called "tapering hairs ": 1- to 2-mm-long fractured hairs, whose tips are wider than the proximal portion of the shaft), broken hairs (fractured hairs with uniform shaft diameter), vellus hairs in patterned hair loss and in long-lasting AA (less than 0.03 mm in thickness and less than 3mm long, representing miniaturized hairs or regrowing hairs can be differentiated from short, healthy regrowing hairs, which are darkly pigmented and straight with pointed ends), coiled hairs in trichotillomania (broken hairs that curl back), comma hairs (short, c-shaped hairs), and cork screw hairs in tinea capitis (short hairs, spiral in shape), Pohle Pinkus constrictions in AA, chemotherapy-induced alopecia, blood loss, malnutrition, and chronic intoxication, flame hairs (semitransparent, wavy, and cone-shaped highly specific hair residues, resembling a fire flame that remain attached to the scalp after anagen hairs have been pulled out), Vsigns (two or more hairs emerging from one follicular unit and broken at the same length), and sprinkled hairs (only a sprinkled "hair powder", resulting from hair damage, is visible) in trichotillomania, and tulip hairs (diagonally fractured short hair shafts with a tulip leaf-like hyperpigmentation at the distal end] in trichotillomania and AA [43, 44]. Trichoscopy has also been successfully used to diagnose many genetic hair shaft disorders [45].

2.2.4. Perifollicular and interfollicular epidermis

According to the color and structure (scaling, discharge, and surface structure) of the areas, the classification of perifollicular and interfollicular skin surface abnormalities in trichoscopy can be classified as; perifollicular discoloration (hyperpigmentation), predominant in androgenetic alopecia, and perifollicular fibrosis, characteristic for some form of fibrosing alopecia [8, 46].

Due to epidermal and perifollicular inflammation in seborrheic dermatitis and psoriasis [47, 48], proximal hair shaft with macropits [49] may look relatively hidden under a white-grey epidermal diffuse proliferation that we called as hidden hair [50] that differs from perifollicular scaling observed in LPP and in folliculitis decalvans [6, 31].

During our knowledge, honeycomb hyperpigmentation is a normal finding in sun-exposed areas (chronic sun exposure) and in patients with Fitzpatrick skin phototypes IV, V, and VI [5–7], but in our study, when this pattern is observed trichoscopically, the estimated alopecia risk was 3.2 times higher regardless of age that is why, we do not think it is the characteristic of normal aging scalp [28]. Perifollicular brown coloration ("peripilar sign") is believed to correspond to the perifollicular presence of lymphocytic infiltrates [51] and is common in patients with patterned hair loss [2]; however, the peripilar sign may be observed in up to10% of hair follicles in healthy individuals [21]. Scattered brown discoloration is characteristic of DLE [31].

2.2.5. Blood vessels

Appearance of cutaneous microvessels in trichoscopy may vary in type and number depending on disease and activity of the process. The significance of blood vessel abnormalities observed on trichoscopy has not been explored in detail thus far. Common types of vessels

in alopecia includes elongated vessels in LPP, thick arborizing vessels in DLE and seborrheic dermatitis, twisted red loops and comma vessels in seborrheic dermatitis, atypical red vessels, structureless red areas, signet ring vessel, twisted red loops and glomerular or coiled vessels in linear or circular alignment in psoriasis [9, 19, 31, 50, 52].

2.2.6. Other structures

Other common trichoscopy signs include yellow or yellow-red discharge (e.g., folliculitis decalvans, bacterial infections, dissecting cellulitis, and tinea capitis) and structural changes in the skin surface (e.g., starburst pattern hyperplasia in folliculitis decalvans) [9, 31].

2.3. Alopecia areata

The most characteristic trichoscopic findings include the following: black dots, exclamation mark hairs, tapered hairs, broken hairs, coudability hairs (hairs of normal length with a narrowed proximal shaft and are mostly found in the scalp surrounding the alopecic patch), coiled hairs, YD, hypopigmented vellus hairs, trichorrhexis nodosa, monilethrix-like hairs (constrictions in the hair shaft), and Pohle Pinkus constrictions [4–9, 24, 30, 53–57]. Broken hairs are not exclusive to AA, as they may also be observed in trichotillomania [7]. Exclamation mark hairs represent the most specific signs of acute AA [17]; however, they are also observed in chemotherapy alopecia. Black dots may also be observed in trichotillomania, cicatritial alopecia, and tinea capitis. Yellow dots may be observed both in acute and in chronic forms of AA and generally have a regular distribution and in severe forms have a nested formation [5, 7, 24, 30]. Yellow dots are highly sensitive but have low specificity for AA, as they may be seen in other hair disorders, including AGA, congenital hypotrichoses, and DLE [7, 28]. Short, hypopigmented vellus hairs are a common finding in AA and are usually indicative of remitting disease [4, 7].

In our study [30], major risk factors for AA were determined to be black dots, WD, and YD (risk ratios were estimated as 170-fold, 5.9-fold, and 5.3-fold, respectively).

Active (acute) AA can be distinguished from nonactive AA using trichoscopy. Features of disease activity include black dots, exclamation marks, broken hairs, trichoptilosis, pig tail, short vellus hairs, and upright regrowing hair whereas YD, WD, clustered WD, honeycomb pigmentation, black dotted pigmentation, and vellus hairs are markers of disease severity and inactive late stage disease [4, 12, 20, 24, 30, 55, 58]. In addition to this, disease activity showed negative relation with atypical red vessels in our study, we believed that these atypical red vessels indicated rejuvenation after the catabolic process in progressive AA [30]. Early features of hair regrowth include the presence of pigmented, upright, regrowing hairs [24], and pigtail hairs [23]. Recent data show that trichoscopy may also be applied in the evaluation of treatment response in AA patients [59].

2.4. Androgenetic alopecia

Male and female pattern hair loss share similar trichoscopic features. These include hair shaft thickness heterogeneity (anisotrichosis, hair diameter diversity), YD, pinpoint WD, hon-

eycomb pigmentation, focal atrichia, epidermal scaling, arborizing red lines, perifollicular brown, and white discoloration (peripilar sign), an increased proportion of vellus hairs, and an increased proportion of follicular units with only 1 emerging hair shaft instead of 2–4 hair shafts [4, 5, 9, 11, 19, 28, 43, 60–63]. On the other hand, in early AGA, we saw multihair follicular unit more than follicular units with only 1 emerging hair shaft [28].

Trichoscopy was demonstrated to be superior to the classical trichogram for the evaluation of early female AGA [64], showing 75% sensitivity and 61.54% specificity in a recent study [65].

All of the trichoscopic features appear most prominently in the frontal scalp area [19, 21]. An increased ratio of vellus hairs to all hairs in androgen dependent scalp regions is characteristic of AGA [6, 7, 19, 28]. The most important finding of AGA is the hair diameter variability, which reflects hair miniaturization [44]. Hair miniaturization does not equally affect all hair follicles of the same area, resulting in the simultaneous presence of terminal, intermediate, and vellus hairs. When the hair diameter variability of more than 20%, which means that vellus hairs account for more than 20% of all the hairs in the same view, was regarded as a hallmark of AGA in previous reports [6, 7, 60]. In addition to this, three major diagnostic criteria for female AGA have been suggested: more than four yellow dots in four images (70-fold magnification) of the frontal area, a lower than average hair thickness in the frontal area compared to the occipital area, and vellus hairs (below 0.03 mm) comprising more than 10% of hairs in the frontal area [19]. Recently, some authors have suggested that the presence of more than six vellus hairs in the frontal scalp may be indicative of initial female AGA [66].

In more advanced and severe stages of AGA, trichoscopy shows the presence of empty follicular ostia, YD, brown dots, and a honeycomb-like pigmented network in bald, sun-exposed areas [5, 7, 28, 63].

In our study, PFP was detected to be a characteristic trichoscopic finding of AGA that was described as a normal feature of the scalp in healthy persons younger than 25 years [21, 28].

2.4.1. Telogen effluvium

Although no specific trichoscopic criteria of TE have been recognized, the diagnosis may be suspected when empty hair follicles (sometimes appearing as YD), a high percentage of follicular units with only 1 hair, brown perifollicular discoloration (the peripilar sign), and short, dark, multiple upright regrowing hairs of normal thickness are present in the absence of the characteristic features of other scalp disorders [7, 19, 40, 67, 68]. In TE patients, no significant differences are observed in the trichoscopic findings between the frontal and occipital areas; this differentiates TE from patterned hair loss [9].

2.5. Trichotillomania

Trichoscopy shows the presence of broken hair shafts of different lengths with no significant changes in the perifollicular area. The extremities of the hairs have a typical frayed aspect (split ends) [69]. Trichoscopy is also useful to demonstrate the signs of plucking to the parents [70]. Recently, a number of other signs, all variants of broken hairs, have been described, including coiled hairs, flame hairs (semitransparent, wavy, and cone-shaped highly specific

hair residues, resembling a fire flame that remain attached to the scalp after anagen hairs have been pulled out), V-signs (2 or more hairs emerging from one follicular unit and broken at the same length), tulip hairs (diagonally fractured short hair shafts with a tulip leaf-like hyperpigmentation at the distal end), and sprinkled hairs (only a sprinkled "hair powder", resulting from hair damage, is visible) [68]. Black dots and exclamation mark hairs may be sometimes observed [6, 57, 68], and it can be very difficult to distinguish these cases from AA.

2.6. Congenital triangular alopecia

Trichoscopy shows normal follicular openings, highlights the clinical presence of long, thin vellus hairs that are surrounded by normal terminal hairs in the adjacent scalp and allows for differential diagnosis with AA and cicatritial alopecia [7, 71, 72].

2.7. Tinea capitis

Comma hairs are comma-like structures that are associated with both ectothrix and endothrix types of fungal invasion [73–76]. In some patients, hairs are more intensely coiled than typical comma hairs. These hairs have been called "corkscrew hairs" [73, 75, 76]. Corkscrew hairs are also observed in patients of African descent who are infected by *Trichophyton soudanense* [75], *Trichophyton tonsurans* [76], *Trichophyton violaceum* [77], and *Trichophyton verrucosum* [78]. Other less common findings include Morse code hairs (interrupted hairs), bent hairs, zigzag hairs, broken hairs, and black dots [6, 18, 68, 73, 79].

2.8. Anagen effluvium

Trichoscopic images of anagen effluvium are characterized by the presence of black dots, monilethrix-like hairs, and exclamation mark hairs [33, 34].

2.9. Psoriasis and seborrheic dermatitis

Kim et al. reported that red dots and globules, twisted red loops, and glomerular vessels were mostly seen in psoriasis while atypical red vessels, arborizing red lines, and structureless red areas were seen in seborrheic dermatitis [52]. On the other hand, in our study we observed red dots and globules, atypical red vessels, structureless red areas, hidden hair and signet ring vessel mostly in psoriasis while twisted red loops and comma vessels mostly in seborrheic dermatitis [50]. Twisted red loops are thought to be the characteristic videodermatoscopic figure of scalp psoriasis in comparison with seborrheic dermatitis [5]. On the other hand, we considered red dot and globules as the characteristic videodermatoscopic figure of psoriasis and arborizing red lines for seborrheic dermatitis according to our study [50].

2.10. Common cicatricial alopecias

2.10.1. Discoid lupus erythematosus

One of the most typical trichoscopic features of active DLE is the presence of large YD that differ from the YD observed in AA by their larger size and darker, yellow-brownish color [31, 32, 80]. Occasionally, in long-lasting DLE, thin and radial arborizing vessels are observed to

emerge from these dots ("red spider in YD" appearance) which some authors consider a characteristic of DLE [6, 31]. Thick arborizing vessels are commonly present at the periphery of the lesion. Trichoscopic findings for long-lasting, inactive DLE lesions do not differ from those for other types of cicatricial alopecia and are characterized by structureless milky-red or white areas lacking follicular openings [31]. When present, red dots, which are regularly distributed around follicular openings, indicate the expression of active disease and are related to a good prognosis with possible hair regrowth upon prompt treatment [29, 31].

2.10.2. Lichen planopilaris

Trichoscopy reveals the absence of follicular openings and the presence of whitish perifollicular casts that surround the hair shafts at their emergence as the most characteristic feature [4–7, 31, 81, 82]. Scales migrate along the hair shafts and form tubular structures that cover the proximal portions of the emerging hair shafts. This phenomenon is called tubular perifollicular scaling [31]. Some authors have described the presence of blue-gray dots arranged in a target pattern around the hair follicles (due to the presence of melanophages) [80]. In dark-skinned subjects affected by LPP the persistence of a normal pigmented network inside the plaques of hair loss is typical, as the interfollicular epidermis is commonly unaffected by the inflammatory process [83].

Milky-red areas are characteristic for inflammation-mediated fibrosis of recent onset [31]. Small hair tufts, of 5–9 hairs, may be present in late LPP [31]. To sum up, possible differences between DLE and LPP are the presence of blue-gray dots with a diffuse distribution along the patch "speckled" pattern, resulting from interface dermatitis and the subsequent pigment incontinence [80] and the loss of the normal pigmented network in dark-skinned patients due to the involvement of the interfollicular epidermis.

2.10.3. Frontal fibrosing alopecia

Trichoscopic findings in FFA include the lack of follicular openings and minor perifollicular scaling is lower than that of LPP [84–87]. A characteristic finding of FFA is the abrupt interruption of the hairline, with the absence of the vellus hairs that are typically observed in normal scalp. The background in patients with FFA is usually ivory-white to ivory-beige [86, 87]. Pink-grey and grey dots are commonly observed in the lateral eyebrow area of patients with FFA [9]. To sum up, lonely hairs, surrounded by areas of fibrosis [84], and the absence of vellus hairs in the frontal hairline [85] have been discussed as possible clues for the diagnosis of FFA.

Trichoscopy is very helpful in the differential diagnosis with other types of alopecia involving the scalp margin in female patients; in AGA, it shows an increased presence of vellus hairs at the hairline, in FFA, the absence of vellus hairs represented the predominant trichoscopic pattern, followed by perifollicular scaling, and the absence of follicular openings, in ophiasic AA, it shows the typical signs of the disease, while in traction alopecia vellus hairs are preserved [85].

2.10.4. Folliculitis decalvans

Trichoscopy shows severe pustulation, scaling, and crusting that are generally prominent around follicular units. When cicatritial alopecia occurs, trichoscopy shows the absence of

follicular openings and, in cases of tufted folliculitis, the outgrowth of several hairs (hair tufts) from single and dilated residual follicular openings [5, 88, 89] that is the most characteristic trichoscopic feature [82, 90]. Some authors have described the presence of a perifollicular hyperplasia with a typical starburst pattern in such cases. A perifollicular concentration of blood vessels may also be present. In long-standing disease, white and milky red areas lacking follicular openings are predominant [31].

2.10.5. Dissecting cellulitis

In early stages, dissecting cellulitis shows trichoscopic features that may be similar to those observed in AA [39]. In a study [54] including 11 patients with dissecting cellulitis, trichoscopy showed the presence of YD, red dots, empty follicular openings, and black dots and may mimic AA [33, 54]. As the diseases progresses, other trichoscopic features become more prominent, including yellow structureless areas and YD with "3-dimensional" structure imposed over dystrophic hair shafts [31]. Some authors describe these yellow dots with "3D" structure that are imposed over dystrophic hair shafts as the most characteristic feature of dissecting cellulitis [6, 31]. End-stage fibrotic lesions are characterized by confluent ivory-white or white areas lacking follicular openings [31, 91].

2.10.6. Central centrifugal alopecia

One article described the trichoscopic aspect of the disease as reduced hair density with hair shaft variability, pinpoint WD, and peripilar white halos. Moreover, pigmented, asterisk-like macules with sparse terminal and vellus-like hairs may be present. The residual terminal hairs may emerge as a single hair or as a group of two hairs and are generally surrounded by a characteristic, peripilar gray-white halo [7].

2.11. Hair shaft disorders

When using light microscopy, multiple samples may be needed before an abnormal hair shaft is identified therefore trichoscopy may replace light microscopy in the evaluation of genetic hair shaft defects, such as monilethrix [92, 93], trichorrhexis invaginata [94, 95], trichorrhexis nodosa [45], pili annulati [45, 96], pili torti [5, 96], and others [17, 18]. On the other hand, in trichothiodystrophy, the characteristic tiger tail pattern is not visible on trichoscopy, and polarized microscopy remains the criterion standard for diagnosing this condition [9, 45]. When using trichoscopy, different features may be observed in the following disorders:

2.11.1. Monilethrix

Elliptical nodes of normal hair thickness that are regularly separated by dystrophic constrictions in which the hairs have no medulla and that are the sites of fracture; this finding has also been described as the "regularly bended ribbon sign" [10, 45, 92, 93, 96, 97].

2.11.2. Pili torti

Flattened hair shafts with regular twists at irregular intervals along the long axis [10, 92, 96].

2.11.3. Pili trianguli and canaliculi (uncombable hair)

Triangular-shaped shafts with longitudinal grooving or flattening [9].

2.11.4. Pili annulati

Alternating light and dark bands, light bands corresponding to air-filled cavities within the hair shaft [6, 17, 45, 96].

2.11.5. Trichorrexis nodosa

White knots along the distal shafts and brush-pattern fractured ends [17].

2.11.6. Trichorrexis invaginata (bamboo hair)

Multiple ball-shaped nodes along hairs that resemble the ball-in-cup rings of bamboo and are due to invagination of the distal portion of the hair shaft into its proximal portion; the fragile node breaking off results in ragged, cupped proximal hair (golf-tee hairs) [17, 95, 96].

2.11.7. Woolly hair

Hair shafts resembling a crawling snake with short wave cycles [45].

2.11.8. Trichothiodystrophy

Nonhomogeneous structure resembling grains of sand and having a wavy contour [45].

3. Trichogram

In this procedure, 60–80 hairs are plucked with a rubber-armed forceps from a 5-day unwashed hair. Hair bulbs are immediately placed with their roots on a glass slide in an embedding medium, which allows information about the state of the proximal end of the hair shaft (the root), the distal end (the tip) and hair root is necessary [98].

The trichogram is a useful complementary tool for clinical evaluation, diagnosis, and the monitoring of treatment response [99].

It should be noted that the trichogram simply provides a snapshot of the hair follicle at the time of examination and that the condition of follicles can vary within the same patient depending on numerous factors, such as sampling site, previous washing or brushing of the hair, and time of the year [100].

3.1. Trichogram equipment

Appropriate sampling site for male pattern hair loss should be taken from the central interparietal area, while the second sample, if needed, should be taken from the temporal or occipital area. In female pattern hair loss, samples should be taken from the center and the vertex of

the scalp. The sites for telogen hair loss and scarring alopecia are, respectively, the central interparietal area and the advancing border of the alopecic patch. Using a rubber-sheathed Kocher forceps, a tuft of 15–20 hairs must be removed. To do this, you have to place the forceps 1–2 cm from the scalp and pluck out the hairs rapidly and firmly in the direction of the natural growth of the hair. If the hairs are not plucked out firmly, they may appear as pseudo-dystrophic hairs under the microscope, or exhibit frayed or broken roots [99].

The next step is to prepare the hairs for examination under the microscope. They should be parallel to each other and that the roots are aligned. Next, they are covered with clear adhesive tape. To avoid artifacts and obtain a sharper, cleaner image, you may apply several drops of balsam (such as that used to mount histological slides) and cover the hairs with a cover slip. The use of polarized light improves image quality [99].

3.2. Hair examination

The sample is examined using a 4× objective, although a 10× or 40× objective can be used if higher magnification is needed. A higher-quality image can be obtained by fitting 2 polarizers to the microscope: 1 between the condenser and the sample and the other between the sample and the observer [99].

3.2.1. The proximal end

Anagen hair shafts are longer, have a uniform diameter, a rectangular shape, and a slight distal angle. Pigmentation is intense in the bulb area and there are sheaths and membranes.

Telogen hair shafts are shorter and appear higher up in the trichogram, above the roots of anagen hairs; the root is thick and club shaped and there are no distal angles.

Pigmentation is weak or absent; the sheath is also absent or found only at the distal end.

Very few hairs in the catagen phase are observed in the trichogram as they account for a very small percentage of all hair.

The anagen to telogen ratio varies, mainly according to age and sex. Children have the highest percentage of anagen hair (95% anagen vs. 5% telogen), and the ratio decreases with age. The anagen to telogen ratio is 86:11 in women and 83:15 in men. In a normal trichogram, an average of 89% of hairs are in anagen, 10% in telogen, and 1% in catagen. A diagnosis of telogen effluvium is established when over 20% of the hairs examined are in telogen phase.

Dystrophic hairs have a decreased proximal diameter, an irregular contour, no epithelial sheaths, and an angle of over 20. They are common in AGA or in hair that has not been removed correctly from the scalp. Keratotic material may be observed on the tip of the hair in conditions such as seborrheic dermatitis, psoriasis, and folliculitis. A common finding in patients with demodicosis is the presence of Demodicosis folliculorum in contact with the root of the hair, although this condition is usually diagnosed by superficial skin biopsy [99].

3.2.2. The hair shaft

Normal hair is uniform in appearance and structure along the entire length of the hair shaft; this uniformity is also observed between the different hairs in a sample. Hair dysplasias are

malformations of the hair shaft. Although scanning electron microscopy is the diagnostic tool of choice in such cases, certain signs may be observed in the trichogram [101]:

3.2.2.1. *Monilethrix* (beaded hair)

Alternating segments of narrowings and nodosities, giving a characteristic beaded appearance.

3.2.2.2. Pseudomonilethrix

Round hairs with irregular, sporadic rounded nodosities. There are no narrowings.

3.2.2.3. Pili torti

Twists of hairs with bending at different angles and regular intervals.

3.2.2.4. Trichorrhexis invaginata (bamboo hair)

Ball-shaped deformity with cupping at the proximal end of the hair shaft.

3.2.2.5. Trichothiodystrophic hair

Patients with trichothiodystrophy may have hair with ribbon-like flattening, characteristic trichoschisis-like fractures (clean transverse breaks), with an irregular surface, and tiger-tail banding.

3.2.2.6. Trichonodosis

Single or double knots on the hair shaft. Tie knots and other more complex knots are also observed.

3.2.2.7. Trichorrhexis nodosa

Bulging hair characterized by fracture nodes with open splitting of the cortex on both sides of the node. If the hair eventually splits, it will leave a brush-like appearance at both ends.

3.2.2.8. Bubble hair

Short, broken hairs with a wavy surface, and bubbles inside the shaft.

3.2.2.9. Loose anagen hair

Twisted anagen hairs with a ruffled cuticle at the proximal end. Long, pili canaliculi type canals on the shaft are a common finding [102].

3.2.2.10. Pili annulati

Hair shafts with alternating light and dark bands.

3.2.2.11. Woolly hair

Thin curly hair forming small woolly balls.

3.2.2.12. Uncombable hair (pili canaliculi)

Canalicular formation along the entire length of the hair shaft. This formation can be difficult to spot under a microscope but the micrometer can be moved if pili canaliculi is suspected.

3.2.3. The distal end

Three types of hair shaft tips can be observed:

Javelin tip: A very sharp, spear-like tip is seen in hair that is growing well and has never been cut, paintbrush tip: Fractures in the hair shaft (trichoschisis) give the tip a paintbrush-like appearance, seen in hair shaft anomalies such as monilethrix, alopecia areata, or in hair fragility induced by cosmetic products an clean-cut tip: The distal end has been cut and ends in a perfectly straight line. It is typically seen in hair that has been cut and in cases of trichotillomania [99].

3.3. Common alopecia in trichogram

In alopecia areata, hair shaft is with alternating narrow and normal sections. Pseudomonilethrix and/or trichoschisis may be observed in some hairs. Hair shaft diameter variability has been demonstrated in women, with larger diameters seen in higher stages of the Ludwig Scale [103]; the differences were minimal at stage I and maximal at stage III. In TE, the hairs are shorter than normal, have a uniform diameter, a rounded proximal end (club-like appearance) and a lack of pigment and membranes. In anagen effluvium normal anagen hairs that are longer than telogen hairs, pigmented, and having sheaths and membranes whose distal end is angled like a golf club are seen in the trichogram. Nits or lice may be seen in patients with pediculosis capitis [99].

Abbreviations

AA	alopecia areata
AGA	androgenetic alopecia
DLE	discoid lupus erythematosus
FFA	frontal fibrosing alopecia
LPP	lichen planopilaris
TE	telogen effluvium
WD	white dots
YD	yellow dots

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