

Diagnostic Modalities in Trichology - An Update

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Abstract

Hair and scalp examination has earned a deserved place in dermatology, the techniques for which can be divided into 3 categories: Non-invasive methods (e.g: questionnaire, general examination, inspection and palpation of the scalp, daily hair counts, standardized wash test, 60-s hair count, global photographs, hair weight, trichoscopy, phototrichogram, trichoscan and polarizing and surface electron microscopy), semi-invasive methods (e.g., trichogram and unit area trichogram) and invasive methods (e.g., scalp biopsy). This article reviews an update on all these modalities.

Keywords: Diagnostic modalities; Trichology; Trichoscopy

Diagnostic Modalities in Trichology

Hair and scalp examination has earned a deserved place in dermatology, the techniques for which can be divided into 3 categories: Non-invasive methods (e.g., questionnaire, general examination, inspection and palpation of the scalp, daily hair counts, standardized wash test, 60-s hair count, global photographs, hair weight, trichoscopy, phototrichogram, trichoscan and polarizing and surface electron microscopy), semi-invasive methods (e.g., trichogram and unit area trichogram) and invasive methods (e.g., scalp biopsy) [1,2].

The questionnaire consists of a set of questions for patient self-assessment, developed by Merck Research Laboratories for male androgenetic alopecia and the four-item Women's Hair Growth Questionnaire for perceived hair growth for females [3]. Mainly ask about age of onset, duration, is hair coming out by the roots or is it breaking, increased shedding or increased thinning, medications, past health family history, diet: is there adequate protein or iron intake, menses, pregnancies, menopause, hair care/hair cosmetics, occupation and hobbies.

General physical examination includes signs of systemic disease, anemia, thyroid disorders, virilization and body mass index. Inspection of the hair includes colour, hair loss patterns and density. Hair loss patterns in males are classified by two systems- Hamilton Norwood into 7 stages and the Ebling system, which describes 5 stages. Female-pattern hair loss is classified using the 3-point Ludwig Scale or the 5-point Sinclair Scale. Another pattern, described by Olsen, is diffuse thinning in the central area with more accentuated loss in the inter-parietal region, forming a triangular pattern [4].

Palpation includes Fold or Jaquet's sign, Sabouraud's sign or Tug test, Pull test and Card test [5]. The fold sign consists of folding an area of the scalp between the two thumbs. If several folds are formed easily, the test is positive and indicates an absence of hair fibers in some the follicles as in cicatricial alopecia. Sabouraud's sign is elicited after removing a group of hairs for trichogram using a rubber-

sheathed Kocher forceps. Increasing traction is applied to the hair while it is still held between the blades of the forceps. It is used to measure resistance of the hair to traction and is positive if the hair breaks easily as in alterations of hair shaft. In Hair pull test, approximately 20-60 hairs are grasped between the thumb, index and middle fingers from the base of the hairs near the scalp and firmly, but not forcefully, tugged away from the scalp. If more than 10% hairs are pulled away from the scalp, this constitutes a positive pull test and implies active hair shedding [6].

The hair card test is a simple examination technique that can be performed using a small white card for pigmented hair and a black card for white hair

. Newly growing hair can be easily differentiated from broken hair using the hair card [7].

In Hair Growth Window test, a 1 cm² area of hair is shaven or clipped as close to the scalp as possible using a razor or thin scissors and a perforated ruler. The length of the hair that regrows in this area is measured after a week. The normal hair growth rate is 2.5 mm a week (1 cm a month). This test is used to measure hair growth, and more importantly, to convince patients that their hair is growing well [5].

For daily scalp hair count, patients are instructed to collect hairs shed in one day, count them and place them in plastic bags. All shed hairs in the shower or sink or on the brush are collected. Daily hair counts for 7 days are maintained. If the patient is losing more than 100 hairs per day, the hair should be examined microscopically to detect the pathology in hair bulb and hair shaft abnormalities. Appearance of the hair bulb can distinguish between telogen effluvium, anagen effluvium and active diffuse alopecia areata [8].

In the standardized wash test, the patient refrains from shampooing for 5 days and then he/she shampoos and rinses the hair in the basin with the hole covered by gauze. The hairs remaining in the water and the gauze are collected and sent for examination. Hairs must be counted and divided into ≤ 3 cm and ≥ 5 cm in length. This is an important technique to differentiate telogen effluvium from female-pattern hair loss. The modified hair wash test' demonstrates that in FAGA 58.9% of hair is vellus, whereas in Chronic Telogen Effluvium (CTE), there are only 3.5% [9].

In 60-S hair count, before shampooing, comb your hair for 60 s and repeat the procedure before three consecutive shampooing and always use the same comb or brush. Count the number of hairs in the comb or brush and repeat the above procedure monthly [10].

In Global Photography use of a stereotactic positioning device on which the patient's chin and forehead are fixed, and on which a given camera and flash device are mounted, assures that the view, magnification and lighting are the same at consecutive study visits. It is important to ask the patients to keep the same hair style and color and the coordinators attempt to duplicate baseline hair parting and combing in subsequent follow-up visits. Four standard views (vertex, midline, frontal and temporal) are advocated [1]. The photographs should therefore always be taken using consistent lighting, camera-to-patient distance, patient position, and hair style. Digital photography is also used for trichoscopy (dermoscopy applied to the hair and scalp) and the trichogram [11].

Hair weight determination requires that the hairs in a given target area be clipped close to the scalp at baseline, the hairs are allowed to grow for a fixed period of time and then the target-area hairs clipped again close to the scalp, collected and subsequently weighed. This method has been used only in clinical trials [12].

Trichoscopy, the dermatoscopy of the hair and scalp, is a quick, noninvasive, cost effective, bedside technique performed with a handheld dermatoscope or a digital video dermatoscope system that provides key physical diagnostic information to assist in the accurate diagnosis of alopecia. It allows for the magnified observation of the hair shafts, hair follicular openings, the perifollicular epidermis and blood vessels. It can be used to evaluate hair disorders in all body areas which include keratosis pilaris, trichostasis spinulosa, pili multigemini, circle hairs, rolled hairs, eruptive vellus hair cyst and ingrown hairs [13]. Trichoscopy allows distinguishing between normal terminal hairs and vellus hairs, which by definition are 0.03 mm or less in thickness and less than 3 mm in length. It analyses the structure and size of growing hair shafts, providing diagnostic clues for inherited and acquired causes of hair loss. Types of hair shaft abnormalities observed include exclamation mark hairs (alopecia areata, trichotillomania, chemotherapy induced alopecia), Pohl-pinkus constrictions (alopecia areata, blood loss, malnutrition), comma and cork screw hairs (tinea capitis), coiled hairs, flame hairs, hook hairs, hair powder and tulip hairs (trichotillomania). The number of hairs in one pilosebaceous unit may be assessed. In healthy individuals the usual number of hair per one pilosebaceous unit is 2. Three to four hairs per unit are observed occasionally. A lower number of hairs is characteristic for hair loss (i.e. telogen effluvium, androgenic alopecia), an abnormally high number is characteristic for tufted folliculitis. In trichoscopy it may be distinguished whether hair follicles are normal, empty, fibrotic ("white dots") filled with hyperkeratotic plugs ("yellow dots") or containing hair fragments ("black dots"). Red dots (in discoid lupus erythematosus) and dirty dots (in healthy children playing in the ground) were recently described. Analysis of perifollicular epidermis and blood vessels may provide additional information allowing trichoscopic diagnosis of most common hair and scalp diseases [14,15]. All genetic hair shaft defects except trichothiodystrophy may be diagnosed by trichoscopy. Trichoscopy may also be used to identify the best area from which to obtain a biopsy specimen. Trichoscopy-guided biopsy can rapidly identify individually affected follicles and allow accurate pathologic assessment [16,17].

Trichometry is a promising technology for hair quantity measurements and has multiple clinical and research applications.

Cross section Trichometry (CST) by Cohen [18], is a simple modality for the quantification of hair mass, and may be used as a convenient and useful tool to clinically assess changes in hair mass caused by thinning, shedding, breakage, or growth in males and females with progressive alopecia or those receiving alopecia treatments. It measures the hair mass index (HMI) which is a ratio of the cross-sectional area of an isolated bundle of hair and the premeasured area of skin from which it was taken using the trichometer device [19].

Light Microscopy of the hair forms an important bedside clinical tool for the diagnosis of various disorders affecting the hair.

Trichogram is a simple, minimally invasive, rapid and economic technique for measuring hair follicle activity. It involves the microscopic examination of hairs plucked from the scalp and provides information about the state of the proximal end of the hair shaft (the root) and the distal end (the tip). The trichogram is a useful complementary tool for clinical evaluation, diagnosis, and the monitoring of treatment response.

The surrounding hairs are fixed with clips and 60-80 hairs are grasped with a hemostat covered with rubber. The hairs are plucked, twisting and lifted rapidly in the direction of emergence from the scalp. Hair shafts are then cut off 1 cm above the root sheaths and roots are arranged side by side on a slide and then taped. The sample is examined using a 4x objective, although a 10x or 40 x objectives can be used if higher magnification is needed. The anagen hair bulbs are seen as darkly pigmented triangular or delta-shaped bulbs with an angle to the hair shaft and there is presence of inner root sheath. The telogen hair is seen as less-pigmented hair with club-shaped hair bulb and there is absence of inner root sheath. Anagen hairs are distinguished from telogen hairs and anagen to telogen ratios are calculated. In Unit area Trichogram, a fixed area is marked on the scalp through a template with a uniform fiber tip pen. All hairs within and on the scribed line are epilated individually with forceps/tweezers in the direction of the hair growth to minimize damage to the hair bulb. UAT is more accurate than the regular trichogram as it takes into account not only anagen/telogen ratios but also hair density and diameter.

The phototrichogram, PTG (combined analysis of two photographs taken at 48 h interval) has undoubtedly become an examination of primary importance in trichology because of its simplicity and sensitivity. It is a non-invasive technique that allows *in vivo* study of physiology of the hair cycle and measurement of various hair growth variables like hair density (number of hairs per cm²), thickness (micrometers), length (millimeter) and linear growth rate (millimeter per day) [20].

Contrast-enhanced phototrichogram (CE-PTG) is a further improvement of the PTG using contrast enhancement together with the scalp immersion proxigraphy method [21]. The contrast-enhanced phototrichogram procedure involves coloring hair with black-colored dye immediately before starting the procedure. These temporarily colored hairs give a better contrast against the white scalp, making this method more sensitive for less-pigmented and thin hairs.

A number of recent techniques in light microscopy are included.

Transmitted Light Microscopy: Due to the difference in refractive index between dry hair, air, glass combined with the thickness of the sample, it is difficult to see beyond the hair surface. Using a liquid like distilled water or immersion oil, between coverslip and slide removes

most of the refractive index differences and provides more detail of the cortex.

Polarized Light Microscopy shows abnormalities in different colours making them more obvious to the observer and the typical tiger tail appearance in Trichothiodystrophy.

Fluorescent light transmitted microscopy used for the fungal infections of the hair shaft.

Reflected light Microscopy can be used to confirm conditions such as pili annulati but has limited use at higher magnifications.

Scanning Electron Microscopy (SEM) provides insight into exactly what is happening to the surface topography of hair as it becomes damaged by different treatments or by routine weathering. With a detailed knowledge of hair cosmetics and the materials designed to deposit on hair, SEM can show product distribution, fiber-to-fiber interactions, and over-deposition and product interactions.

Transmission Electron Microscopy is really of value for research purposes and in understanding the fine structure and organization of the hair and exact changes in the fiber resulting from genetic diseases.

Atomic Force microscopy is an excellent research tool and provides very high quality images.

Confocal scanning microscopy is a novel and interesting non-invasive, high resolution technique for imaging skin lesions and subsurface skin lesions that are not visible to the naked eye or dermatoscopy. Two different imaging modalities are available- The reflectance mode (RCM) and the fluorescent mode (FCM). *in vivo*, reflectance mode is most routinely used as a clinical diagnostic tool. It is useful for choosing the correct biopsy site in patients with inflammatory skin diseases and obtaining microscopic diagnostic criteria. RCM can be considered as an intermediate step between trichoscopy and horizontal histology. Contrast is provided by differences in the refractive index due to molecules and organelle size present in the cell cytoplasm, as well as the extracellular microstructures within the tissue [22]. RCM criteria for primary scarring alopecia : epidermal disarray, spongiosis, exocytosis of inflammatory cells in epidermis, interface dermatitis, peri and intra adnexal infiltration of inflammatory cells, dilated vessels in the dermis, dermal infiltration of inflammatory cells and melanophages and dermal sclerosis [23].

Trichoscan: This technique is automated, based on software that is developed for image evaluation, and dermoscopy photographs are used instead of conventional photography. To enhance visibility, the inspected area is dyed with a coloring solution. This method has similar indications as a classic trichogram. It has the advantage of being automated but has the disadvantage of not being sensitive to hair shaft abnormalities, thin regrowing hair in the telogen effluvium [24,25].

Scalp biopsy: Histopathology of scalp is an essential tool in distinguishing CA from non-CA and in diagnosing the different PCA depending upon the inflammatory infiltrate. For an accurate histopathological diagnosis of PCA, multiple biopsy samples are obtained from active sites and carefully sectioned both vertically and transversely. Unlike in other skin diseases, the information obtained by vertical sections is limited in hair disorders. Transverse sections enable both qualitative (e.g., inflammatory change, fibrosis) and quantitative (e.g., hair follicle numbers, size, phase of hair cycle) examination of scalp biopsy samples. Recently, the "HoVert"

technique, a novel processing technique that produces transverse (horizontal) and vertical sections from a single biopsy has been described. This overcomes the limitation of multiple scalp biopsies [26]. Direct immunofluorescence study is an important diagnostic tool differentiating between PCA due to LPP or CCLE. In CCLE, it shows granular deposits of immunoglobulin (IgG) and complement (C3) at the dermoepidermal junction, while in LPP there are globular deposits of IgM adjacent to the hair follicles or at the dermoepidermal junction [27].

Microarray analysis represents the global gene expression profile, as a diagnostic tool for clinically or pathologically indistinguishable PCA. Recently, a report of microarray analysis generated from total RNA isolates from active lesions of LPP and PPB, distinguished the two conditions, which were thought to be related [28]. However, further studies in this field may elucidate the genetic and molecular markers of various PCAs.

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