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Comparative Evaluation of Scalp Hair by Phototrichogram and Unit Area Trichogram Analysis within the Same Subjects

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Quantitative evaluation of scalp hair requires techniques that are reproducible. The unit area trichogram is such a method but is unsuitable for large-scale clinical trials. An alternative may be the phototrichogram – a non-plucking, non-invasive method. Hair variables were evaluated in 12 Caucasian subjects employing both methods. The mean value for total hair density was significantly underestimated by the phototrichogram (181 versus 237 hairs/cm^2); however, no significant difference was found between this phototrichogram value and the number of non-vellus hairs/cm^2. Estimates for the percentage of anagen hairs were similar with both methods. Hair diameters from the phototrichogram were too unreliable to be of any practical use. Analysis of the individual hair data revealed that light hair was much more difficult to evaluate than dark hair. Consequently, Caucasian subjects with light hair or dark skin subjects with dark hair should be excluded from studies employing phototrichograms.

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Attempts to obtain quantitative measurements for scalp hair growth, or lack of it, have focused upon three principal approaches. This has involved the use of invasive (biopsies (1, 2)), semi-invasive (epilations (3–6)), and non-invasive techniques (visual counts (7–9); phototrichograms (10–13); hair weight (14)). With the almost concurrent development of small versatile video cameras, powerful desk-top computers, and the desire to find more effective molecules capable of influencing hair growth, pressure for a quantitative, reproducible, yet patient-friendly hair evaluation method has lead researchers to focus upon a technique known as the phototrichogram. At least in theory, this technique is capable of evaluating all four hair variables (hair density, hair/cm^2; fibre thickness, μm; per cent of follicles in the active growth phase, anagen %; and the rate of linear hair growth, mm per day (15, 16)). The phototrichogram would, if sufficiently reproducible, be suitable for large-scale clinical trials; however, to our knowledge no comparative or reproducibility studies have ever been published.

The unit area trichogram is considered by many as the standard by which other hair growth evaluating methods should be compared. The technique estimates three of the four fundamental hair variables (productive follicular density, proportion of anagen fibres, fibre diameter) with a mean sampling error of <5% (17–22); in addition hair length can also be measured. This method has been used to follow scalp hair changes during oral anti-androgen therapy in women (21–23), topical minoxidil treatment in men (18) and the natural progression of male pattern baldness (20). We were interested therefore in fully evaluating the phototrichogram against a proven quantitative method, for a range of hair variables, within the same subjects. The results of this comparative study are presented.

MATERIALS AND METHODS

Selection of subjects

Twelve healthy Caucasian subjects (1 male and 11 females) aged between 23 and 67 years (mean 43), with a range of hair densities (83–342 hair/cm^2) and hair colours (see Table I), were evaluated for predefined hair variables with the unit area trichogram and phototrichogram techniques. In subjects in whom the non-vellus hair density was reduced (values <212 non-vellus hair/cm^2), all were clinically assessed to have diffuse androgen-dependent alopecia, also known as androgenetic alopecia, androgenic alopecia, diffuse hair loss, common baldness or female genetic hair loss (19). Since we had quantitative hair density values from the unit area trichogram, individuals were also classified by the subjective global grading system as proposed by Ludwig (24). All were fully aware of the nature of this study and all gave their oral, witnessed, informed consent. All followed the same standardized procedure one month prior to sampling, which involved shampooing the hair daily or on alternate days, and on the morning of sampling. Combing or brushing was allowed during this time, but not

Table I. *Values for total hair density and non-vellus hair density obtained with the unit area trichogram, compared to total hair density values from the phototrichogram in the same subjects, with respect to hair colour, and Ludwig classification*

Subject	(Hair colour)	Unit area trichogram		Phototrichogram	
		Total Hair/cm^2	Non-vellus Hair/cm^2	Total Hair/cm^2	Ludwig Type
(1)	Grey	231	174	96	0-I
(2)	Grey	265	239	150	I-II
(3)	Grey	342	300	157	0-I
(4)	Light brown	83	51	122	III
(5)	Light brown	248	229	225	0-I
(6)	Light brown	259	232	219	II-III
(7)	Brown	204	189	193	I-II
(8)	Brown	206	186	140	I-II
(9)	Brown	232	183	184	II
(10)	Dark brown	193	148	201	I-II
(11)	Dark brown	276	257	214	0-I
(12)	Dark brown	307	175	266	II
Mean		237	197	181	
± SD		65.1	62.9	48.8	
Significance level		——	($p < 0.01$)	——	
Student's *t*-test (paired samples)		——	(NS)	——	

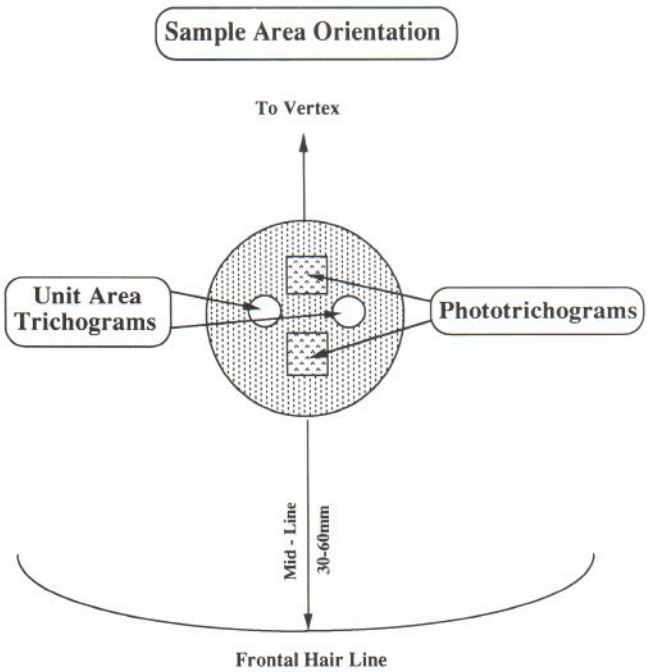

Fig. 1. Relationship between the unit area trichogram and phototrichogram sample sites. No visible difference in hair density between the sample sites was a criterion for sampling.

less than 4 times and no more than 10 times a day. Unit area trichogram and phototrichogram investigations were undertaken on the same day, and the entire group was sampled over a 2-day period in July.

The unit area trichogram (Fig. 1)

Two unit area trichograms were performed within the frontal region, as detailed previously (5, 17, 19). The distance between the two sample centres was ≤25 mm and the orientation of the sample sites within the frontal region, with respect to the phototrichogram sites, is given in Fig. 1. Values obtained from both sites were pooled to provide an average, and the difference (as a percentage) between this pooled value and one site was calculated for each variable, and for each subject. Grouping these values for all 12 subjects provided a mean estimate of the prevailing within subject sampling error (biological and experimental variability) for each variable and each method.

Hairs were classified according to their growth phase: anagen, catagen or telogen. Hair in the catagen phase was assigned to the telogen population for data analysis. Hair diameters were calculated microscopically by measuring the major and minor axes as detailed previously (5). Dysplastic or dystrophic hairs, i.e. hairs that could not be readily recognised as either anagen or telogen, were assigned to the anagen population if the root end exhibited bending, or to the telogen population if not. Broken hairs arising from the epilation procedure were matched with their respective anagen or telogen root sections. When this was not possible, broken hairs were assigned to the telogen population if the proximal end exhibited tapering and/or loss of pigment; otherwise the fibre was classified as anagen. However, the occurrence of such hairs in this study was <5%.

The phototrichogram (Fig. 1)

Phototrichograms were undertaken basally and again 2 days later, as described previously (11, 12). From a pilot study comparing ×1, ×2, and ×3 enlargement factors we have concluded that a ×3 enlargement is the most appropriate magnification to use. Consequently, a ×3 enlarged photographic slide transparency of the sample site was obtained after the initial hair clipping (*t0*)and again after 2 days (*t2*). Phototrichograms were undertaken in two sites separated by ≤25 mm and at right angles to those of the unit area trichogram (Fig. 1). As with the unit area trichogram, no visible difference in hair density between the sample sites was a criterion for sampling. Values obtained from both sites were pooled and the data grouped for analysis as described above. All visible hairs at *t0* were drawn manually onto acetate transparent overlays taped to an Agfa Diamator slide projector screen. This procedure was repeated on the *t2* slide taken from the same scalp site. Comparing the overlays allowed the identification of growing hairs. Both slides and overlays from *t0* and *t2* were scanned at a constant magnification with a video camera connected to a IBAS II Kontron image analyser, and the generated image data were handled by customized IBAS II software.

Hair variables determined in the assessment of hair quality

Hair variables determined with the unit area trichogram were: total hair density (total hair/cm^2), non-vellus hair density (non-vellus hair/cm^2), per cent of hair in the anagen growth phase, per cent of non-vellus hair in the anagen growth phase, per cent of vellus hair (hair ≤40 μm in diameter, ≤30 mm in length) (17), fibre diameter, and fibre length.

From phototrichogram images, hair growth as a % change in hairs within the reference frame (11) and fibre diameter were evaluated with computer-assisted image analysis. From analysing the acetate overlays, total hair density (total hair/cm^2), and per cent of hair follicles in the anagen growth phase were also determined with computer-assisted image analysis.

Statistical analysis

Group mean differences were compared statistically by Student's *t*-test or the Wilcoxon signed rank test, as appropriate. All analyses were undertaken on an Apple Macintosh™ computer, using the statistical programs STATWORKS™ & STATVIEW 512™.

RESULTS

The distance between unit area trichogram centres was ≤25 mm and the mean area sampled was 36 mm^2 ± 2.4 standard deviation (sd), range 32 mm^2 to 41 mm^2, providing a mean of 83 ± 21 sd hairs per site (range 33 to 112). The distance between phototrichogram centres was also ≤25 mm and the mean area sampled was 34 mm^2 ± 3.1 sd, range 29 mm^2 to 39 mm^2, providing 62 ± 20 hairs per site (range 24 to 95).

Estimates for total hair density and non-vellus hair density (Table I)

Values obtained with the unit area trichogram and phototrichogram for total hair density and non-vellus hair density are presented in Table I together with their assigned Ludwig grade. The phototrichogram had great difficulty in estimating the actual hair density of our (Caucasian) subjects with grey or light-coloured hair. Non-vellus hair density best reflected the global grading system proposed by Ludwig.

Comparisons between unit area trichogram- and phototrichogram-generated values for the percentages of hair in the anagen growth phase and non-vellus hair in the anagen growth phase were also performed. No significant difference between mean values could be found; however, the phototrichogram-derived values were consistently lower in all subjects with grey and light-brown hair ($n = 6$), but higher in 4 of the 6 subjects with mid-brown or darker hair compared to unit area trichogram values.

Hair diameter measurements

Hair diameters measured microscopically from samples ob-

tained with the unit area trichogram were significantly more reliable than those determined by computer-assisted image analysis from the phototrichogram. This would suggest that diameter related variables such as vellus hair (hair ≤40 μm in diameter, ≤30 mm in length (17) cannot be estimated with any certainty by phototrichograms employing magnifications of ×3 or less when obtaining the primary image.

DISCUSSION

From our studies we have been able to assess the reproducibility of the phototrichogram by comparing values generated for several hair variables with those obtained from the unit area trichogram, a proven quantitative plucking method (5, 17). The phototrichogram appears capable of providing acceptable grouped mean estimates for hair density and the per cent of hair follicles in the active growth phase, although the actual total number of hairs calculated was underestimated. However, this technique (unlike the unit area trichogram) can only be used reliably in subjects in whom there is a contrast between hair and skin tone, for example Caucasians with brown or darker hair. The determination of hair diameter also presented problems for the phototrichogram and, unless highly magnified (×20 to ×40) images are generated, phototrichograms employing magnifications of ×3 or less should not be used to estimate this variable. However, using highly magnified images to resolve this problem creates other distortions.

Sampling two adjacent sites (<30 mm apart) allows the within subject variation to be estimated, from which a correction factor can be applied, significantly increasing the confidence of any reported estimate (17). However, where this criterion is not fulfilled, reporting only the grouped data ($n \geq 10$) should be considered. In subjects with diffuse androgen-dependent alopecia samples obtained in a left to right orientation, rather than in a frontal to vertex direction, reduced the inherent biological variation. Eight of the 11 subjects studied had less hair density in the sample area nearest the frontal hair-line, 30 to 60 mm from the frontal margin, compared to the second site <25 mm towards the vertex (Fig. 1). This phenomenon has also been observed during unit area trichogram comparisons, where sampling in a frontal to vertex orientation has previously been performed. These findings would suggest that the progression of diffuse androgen-dependent alopecia in females emanates from the frontal hair-line, or starts from just behind the conserved frontal margin. Interestingly, these changes between sites remained unnoticed by both clinical observers (DHR & DVN).

The phototrichogram, like the unit area trichogram, is a time-consuming technique, and although computer-assisted image analysis has eliminated the tedium of counting hairs, manual reprocessing is still required when detailed data are needed. Establishing the reproducibility of a method prior to its use in a clinical trial is essential. As yet, no single approach can determine all four fundamental hair variables in a standardized, reproducible, and relatively simple manner. Further work is required before the phototrichogram can be accepted as the method of choice for evaluating scalp hair growth.

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