ORIGINAL ARTICLE

Light microscopic examination of scalp hair samples as an aid in the diagnosis of paediatric disorders: retrospective review of more than 300 cases from a single centre

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Background: Microscopic examination of scalp hair can provide important diagnostic information in a range of paediatric conditions. It is a non-invasive and cost effective investigation, which is not widely performed.

Aims: To examine retrospectively the value of hair examination by light microscopy, including polarising microscopy, in a specialist paediatric pathology department during a 15 year period (1989–2004) and to describe the morphological abnormalities indicative of specific paediatric conditions.

Methods: Three hundred and twenty two hair samples were submitted. Microscopic changes were analysed in the light of clinical information categorised as: (1) erythroderma, (2) neurological impairment, (3) immunological/haematological defect, (4) ectodermal dysplasia, (5) abnormal hair only, and (6) non-specific/absent clinical details.

Results: Abnormalities were evident in 49% of the samples. In 25%, the changes were compatible with specific diagnoses including Menkes disease, Netherton's syndrome, trichothiodystrophy, Griscelli and Chediak-Higashi syndromes, monilethrix, uncombable hair, and loose anagen syndromes. In respect of the clinical presentation groups noted above, diagnostic changes were seen in 41%, 32%, 33%, 0%, 29%, and 0%, respectively.

Conclusions: Morphological light microscopic examination of scalp hair is an inexpensive, rapid, and non-invasive investigation, which can provide valuable diagnostic information in a range of paediatric conditions.

Abnormalities of hair shafts may be congenital or acquired. In the young baby, abnormalities are often congenital as part of a syndrome such as Menkes,¹ Netherton's,² trichothiodystrophy,^{3,4} Chediak-Higashi,⁵ Griscelli,⁶ or uncombable hair syndrome.⁷ Hair shafts may be simply examined using routine light microscopic procedures available in all histopathology departments. Normal hair shafts are cylindrical with a smooth surface, varying diameters, and ovoid or round profiles in cross section. Hair colour is variable but in normal hair the pigmentation within the shaft is usually uniform.

"Normal hair shafts are cylindrical with a smooth surface, varying diameters, and ovoid or round profiles in cross section"

A wide range of morphological abnormalities may be seen, including twists at irregular intervals in the shaft (pili torti), swellings along the shafts, particularly associated with areas of breakages (trichorrhexis nodosa); nodose swellings resembling bamboo shoots (trichorrhexis invaginata); pigment clumping or hypopigmentation; longitudinal canalicular depressions with kidney shaped or triangular cross sections (pili canaliculati et trianguli), which are easier to detect using scanning electron microscopy⁷; and "tiger tail" appearance under polarised light. These abnormalities can be best seen in cut samples of hair and indeed plucking may cause unwanted breakages at the sites of trichorrhexis invaginata in Netherton's syndrome, thus yielding the sample non-diagnostic.⁸ Disorders of the hair follicle such as loose anagen syndrome can only be diagnosed on a sample of plucked hair containing the root. The hairs are misshapen anagen hairs,

which lack the external root sheaths. The bulbs are distorted and the cuticle just above the root has a wrinkled stocking appearance. Above this zone the rest of the hair shaft appears to be normal.^{9,10}

Therefore, there are several important clinical groups of paediatric patients where light microscopic examination of hair may provide valuable diagnostic information. For example, in the clinical setting of an erythrodermic infant with immunodeficiency, the finding of trichorrhexis invaginata is compatible with the diagnosis of Netherton's syndrome. Examination of hair may also be particularly helpful in the assessment of the neurologically impaired child—for example, if the sample demonstrates pili torti or tiger tails, the findings are compatible with diagnoses of Menkes disease or trichothiodystrophy, respectively. Other immunological/haematological disorders and ectodermal dysplasias may also exhibit abnormal hair appearances.

The aim of our study was to examine retrospectively the usefulness of hair examination on light microscopy, including polarising microscopy, in a specialist paediatric pathology department during a 15 year period to describe the morphological abnormalities indicative of specific paediatric conditions, and to promote wider use of this simple and inexpensive test.

MATERIALS AND METHODS

A search of a computerised histopathology database was carried out to identify all hair samples received during a 15 year period (1989–2004) at the department of paediatric pathology, Great Ormond Street Hospital, London, UK. Clinical details provided and findings of microscopic examination were reviewed, in addition to basic demographic data.

Table 1 Clinical indications, demographic data, and morphological findings of 320 adequate hair samples from paediatric patients

Clinical information	Median age, years (range)*	Abnormal	No	%	Statistics†
Erythroderma (n = 56)	1 (0–15)	Netherton	23	41	Z = 0.54, p = 0.57
Neurological symptoms and signs (n = 108)	Z = 2.01, p = 0.04	Abnormal non-diagnostic	7	13	
		Normal	26	46	
	Z = 2.85, p = 0.004	Menkes	16	15	
		Atypical Menkes	5	5	
		Trichothiodystrophy Griscelli	8	7	
Immunological/haematological (n = 12)	Z = 0.34, p = 0.74	Uncombable hair	1	1	Z = –1.1, p = 0.27
		Abnormal non-diagnostic	12	11	
Ectodermal dysplasia (n = 27)	Z = –2.72, p = 0.006	Normal	61	56	Z = –1.1, p = 0.26
		Chediak-Higashi	4	33	
Hair abnormalities only (n = 65)	Z = –3.53, p = 0.0004	Normal	8	67	Z = 0.22, p = 0.70
		Abnormal non-diagnostic	14	52	
	Z = –0.83, p = 0.41	Loose anagen	13	48	Z = 2.88, p < 0.01
		Uncombable hair	9	14	
Non-specific or absent clinical information (n = 52)	Z = –1.8, p = 0.08	Monilethrix	9	14	Z = –1.8, p = 0.08
		Abnormal non-diagnostic	1	2	
	Z = –0.83, p = 0.41	Normal	26	40	Z = –1.8, p = 0.08
		Abnormal non-diagnostic	20	30	
Non-specific or absent clinical information (n = 52)	Z = –0.83, p = 0.41	Normal	19	37	Z = –1.8, p = 0.08
		Abnormal non-diagnostic	33	63	

*Mann-Whitney U test for comparison of age distribution compared with overall group; †comparison of proportions test for prevalence of any abnormalities v overall group.

Our study was approved by the local research ethics committee.

During this period 322 hair samples were submitted. Hairs had been placed on a microscope slide and covered by a sealed coverglass usually without the use of a mounting medium. Routine light microscopy together with polarised light had been used to examine the hairs. All results were anonymised. The morphological findings were related to the clinical information provided using the following classification. (1) erythroderma (n = 56), (2) neurological symptoms and signs (n = 108), (3) immunological/haematological defects (n = 12), (4) ectodermal dysplasia (skin, hair, and nails; n = 27), (5) clinically apparent isolated hair abnormalities (n = 65), and (6) non-specific/absent clinical information (n = 52). Two samples were inadequate for assessment. Comparison of frequencies of abnormalities and distributions between groups was carried out using the comparison of proportions test and Mann-Whitney U test as appropriate.

RESULTS

Of the 322 hair samples submitted, 159 (49.4%) showed morphological abnormalities on light microscopy. The abnormalities present included non-specific changes such as weathering (lifting and damage of cuticular cells) and splits, especially in the distal shaft, twisting (not classical pili torti), and mild flattening or grooving. Trichorrhexis nodosa was noted as a sole finding in 5.6%, but this is non-diagnostic without other changes and only indicates that the hair is easily breakable as a result of injury.¹¹

Of the 159 abnormal hair samples, around half (25.2% of the total), had abnormalities compatible with specific diagnoses, such as Menkes disease (pili torti), Netherton's syndrome (trichorrhexis invaginata), trichothiodystrophy (tiger tail anomaly), Griscelli syndrome (pigment clumping), Chediak-Higashi syndrome (pigment clumping), monilethrix (beading as a result of periodic narrowing of the shaft), uncombable hair syndrome (pili canaliculati et trianguli), and loose anagen syndrome (absent inner or outer root sheath and a wrinkled stocking effect).

Of the various clinical groups, specific diagnoses were seen in 23 of 56 hair samples submitted in the erythroderma group

and in 19 of 65 in the hair only abnormality group. Abnormalities indicating a specific diagnosis were seen in 35 of 108 (32%) neurologically impaired patients and in four of the 12 samples in the immunological/haematological abnormality group. In the clinical group of possible ectodermal dysplasia, half of the samples showed a range of non-specific changes, whereas the other half had no morphological abnormalities. In the group with non-specific or no clinical details provided, 19 of 52 showed only non-specific changes and no abnormality was detected in 33. Therefore, abnormalities in 81 specimens were compatible with a specific diagnosis (tables 1 and 2). Two were inadequate for formal assessment, and no abnormality was detected in 161. Tables 1 and 2 summarise the morphological abnormalities detected in relation to the different clinical groups, in association with basic demographic data.

There was no significant difference in the prevalence of abnormal findings on light microscopy between the various clinical groups compared with the overall frequency, except for the group with apparently isolated hair abnormalities, in which non-specific morphological abnormalities were found more commonly, although most findings in this category of

Table 2 Light microscopic morphological appearances in 322 hair samples submitted during a 15 year period (1989–2004)

Morphological abnormality	N	%	Clinical syndrome
Isolated Trichorrhexis nodosa	18	5.6	Non-specific
Trichorrhexis invaginata	23	7.1	Netherton
Pili torti	21	6.5	Menkes
“Tiger tail”	8	2.5	Trichothiodystrophy
Pili canaliculati et trianguli	10	3.1	Uncombable hair
Pigment clumping	9	2.8	Chediak-Hagashi
Hypopigmentation			Griscelli
Monilethrix	1	0.3	Monilethrix
Absent outer root sheath and wrinkled stocking effect	9	2.8	Loose anagen
Abnormal not diagnostic	60	18.6	Non-specific
No pathological abnormality	161	50	None
Inadequate	2	0.6	N/A

N/A, not available.

Figure 1 Age distribution of 322 hair samples submitted for histological examination.

patients were non-specific (table 1). The median age of the patients was 2 (range, 0–43 years; fig 1), and this varied with clinical indication (table 1; older patients were often parents of affected children). Figure 2 provides examples of the major types of morphological abnormalities detected.⁷

DISCUSSION

Our study has shown that morphological abnormalities may be identified in about half of the hair samples examined for a range of clinical indications, including neurological, immunological, and dermatological presentations, in addition to those cases with clinically apparent abnormal appearing hair. Such examination is simple, cheap, rapid, and non-invasive and should be considered as a first line investigation in many such conditions. The technique is uncomplicated and sampling may be carried out in the clinic. Hair samples may be obtained by plucking; this may be carried out either by gripping less than 10 hairs between a finger and the thumb, or by gripping a couple of rows of hairs with a needle holder at the base, and pulling sharply.^{11 12} Plucked hair is needed if the hair root needs to be examined. However, in obtaining cut hair samples to study hair shaft abnormalities the hairs should be cut close to the scalp because plucking in such cases may lead to breakages of the shaft. The hairs are then dry mounted (without mounting medium), by placing a rectangular frame with double sided sticky tape edges (Frame-Seal incubation chamber; Hybaid, Basingstoke, Hampshire, UK) on a microscope slide. The hairs are lined up in parallel order securing one or both ends to the sticky edge(s) of the frame. Care must be taken not to damage the hairs. A coverglass is then placed over the Frame-Seal containing the strands of hair. It may be advisable in some instances—for example, if Chediak-Higashi or Griscelli syndrome is suspected—to use a mountant (DPX) to give a clearer view of the specimen. It is important to examine as many strands of hair as possible because not every hair may demonstrate morphological abnormalities in the lengths examined. This may not always be easy, because many infants, particularly those with Menkes disease, often have sparse, fine, wispy, blond hair. Similarly, infants with monilethrix and Netherton’s syndrome may also have a paucity of scalp hair, and it has been suggested that the examination of eyebrow hair may improve the likelihood of making the diagnosis in such settings.¹³ Given these caveats, it is remarkable that in our study of 322 samples only two were inadequate for assessment.

The morphological changes described as being compatible or indicating specific diagnoses must be interpreted in the

light of appropriate clinical information, and it should be noted that such specific changes are often also accompanied by other non-specific abnormalities, such as trichorrhexis nodosa, breaks, fissures, and splits, as a result of the fragility of intrinsically abnormal hair.

In our present study, 16 samples showed classic short, pale “kinky hairs” with twists, which were completely rotated through 180° around their long axis at irregular intervals in the shaft, as encountered in Menkes disease. In a further five samples there were also typical kinky hairs but these were pigmented. Clinically, these patients showed all the features of Menkes disease but with a milder phenotype. Molecular genotyping analysis was not available. It is of interest that one patient in the neurologically impaired group showed characteristic features seen in uncombable hair syndrome with mental retardation, calcification of basal ganglia, and very coarse hair after 8 years of age. Uncombable hair may be associated in some syndromes with ectodermal dysplasia, anhidrotism, cleft lip, and cleft palate,¹⁴ or retinal pigmentary dystrophy and dental abnormalities.¹⁵ No neurological association of uncombable hair has been reported to date, but our patient might have a previously undescribed syndrome because the features represent a morphological appearance rather than a specific diagnosis.

“Many conditions associated with morphological abnormalities of hair are linked with a range of specific gene defects or genetic syndromes, and the genotype may correlate with the clinical presentation”

Trichorrhexis invaginata was found in 23 cases in our current series, all in association with Netherton’s syndrome. The pathophysiological mechanism resulting in this morphological abnormality has been examined using electron microscopic and histochemical methods, and is thought to be a consequence of “softening” of the hair cortex, possibly secondary to abnormal composition of cortical fibre proteins.¹⁶

Netherton’s syndrome is an autosomal recessive disease now known to be caused by mutations in the *SPINK5* gene, which encodes a serine protease inhibitor, LEKTI. A wide range of mutations may be found in affected kindreds, many novel, but including nonsense mutations, frameshift insertions or deletions, and splice site defects with reduced *SPINK5* mutant transcript levels. Wide interfamilial and intrafamilial variation in disease severity is seen, with no correlation

Table 3 Details of molecular defects associated with the specific diagnoses where examination of hair may be helpful

Disorder	OMIM	Chromosome	Gene
Netherton	256500	5q32	<i>Spink5</i> ; encodes LEKTI
Menkes	309400	Xq12–q13	<i>ATP7A</i> ; encodes ATPase, Cu ²⁺ transporting α polypeptide
Trichothiodystrophy	601675	19q13.2–q13.3	<i>ERCC2/XPD, ERCC3/XPB</i> (rare); encode the 2 helicase subunits of transcription/repair vector TFIIH
Chediak-Higashi	214500	1q42, 1q42.2	Lysosomal trafficking regulator gene (<i>LYST/CHS</i>)
Griscelli type 1	214450	15q21	<i>MYO5A/RAB27A</i>
Griscelli type 2	607624	15q21	
Uncombable hair	191480	Not known	Not known
Monilethrix	158000	12q13	Keratin genes <i>HB1</i> and <i>HB6</i>
Loose anagen	600628	Not known	Not known

Figure 2 Photomicrographs of hair. (A) Appearance of normal hair shafts on routine light microscopy. (B) Trichorrhexis nodosa under polarised light. There is a fracture of the hair shaft clearly present. This is a non-specific response to injury of the hair but may be associated with underlying defects. (C) Monilethrix demonstrating hair shaft beading without abnormal twisting (polarised light). (D) Pili torti in which the hair is flattened at irregular intervals and twisted around the long axis. (E) Hair from a case of Menkes syndrome, a defect in copper metabolism, which results in abnormal hair twisting and other features, including progressive psychomotor retardation. (F) Hair under polarised light demonstrating "tiger tail" abnormality, with alternate light and dark banding. This is a feature of trichothiodystrophy. (G) Trichorrhexis invaginata in a case of Netherton's syndrome, in which the proximal element overlaps the distal element, resulting in a "bamboo-like" appearance. Skin erythema and scaling are associated clinical features. (H) Light microscopy of trichorrhexis nodosa, which may be seen in a range of conditions.

between the specific gene mutation and phenotype.¹⁷ Recently, the cellular pathogenic mechanism in Netherton's syndrome has been described using studies of *Spink5* knock-out mice, which show the clinical features of human Netherton's syndrome. The homozygous negative mice have LEKTI deficiency with abnormal desmosome cleavage in the granular layer of the epidermis and degradation of desmoglein-1, leading to defective stratum corneum adhesion.¹⁸ Although most cases of trichorrhexis invaginata appear currently to be associated with Netherton's syndrome, other rare genetic syndromes with this feature have also been reported, including siblings with an autosomal recessive condition characterised by progressive neurological deterioration and trichorrhexis invaginata.¹⁹ Increasing use of morphological examination of hair in children with a range of indications will probably lead to further syndromes being reported. Although we have included cases of Griscelli and Chediak-Higashi syndrome separately in our study, it should be noted that the microscopic hair appearances in these

conditions are very similar, both demonstrating abnormal pigment clumping within the shaft. Therefore, to distinguish between the entities it is important to take account of the clinical presentation and also examine a blood film for the presence of abnormal neutrophil granulation, which is present in Chediak-Higashi but not Griscelli syndrome.²⁰ Detection of such specific constellations of findings is becoming increasingly important because many conditions associated with morphological abnormalities of hair are linked with a range of specific gene defects or genetic syndromes, and the genotype may correlate with the clinical presentation (table 3). For example, Griscelli syndrome usually presents with partial albinism and immunodeficiency, often with associated episodes of haemophagocytosis, and it has been suggested that this presentation is associated with *rab27a* gene mutations, whereas Griscelli syndrome presenting with primary neurological features, rather than immunodeficiency, may be more often associated with *MYO5A* mutations.²¹ However, it should also be noted that

Take home messages

- Morphological light microscopical examination of scalp hair is an inexpensive, rapid, and non-invasive investigation, which can provide valuable diagnostic information in a range of paediatric conditions
- These include Menkes disease, Netherton's syndrome, trichothiodystrophy, Griscelli and Chediak-Higashi syndromes, monilethrix, uncombable hair, and loose anagen syndromes
- Hair abnormalities may point to specific genetic conditions for which molecular genetic testing will probably become increasingly available

this view has been challenged, and although the involvement of these two closely related genes on chromosome 15q is now widely recognised, the relevance of any genotype-phenotype relation remains to be determined.^{22, 23} Chediak-Higashi syndrome also affects multiple organ systems, but with hypopigmentation, cytopenias, bleeding disorders, and recurrent infection, rather than primary neurological deterioration. However, although there is no central nervous system involvement, peripheral neuropathy may occur, and may be progressive in late onset cases or after bone marrow transplantation.²⁴ Most patients with Chediak-Higashi syndrome present in childhood and die without bone marrow transplantation, but a minority show a less severe phenotype, often surviving into adulthood, but with increasingly severe neurological abnormalities. It appears that the severe childhood cases are usually associated with null mutant *CHS1* gene mutations, whereas the adult forms usually have missense mutations encoding *CHS1* products with partial function, again suggesting a genotype-phenotype association.²⁵

Our findings have shown that routine light microscopic examination of hair may provide important diagnostic information in a range of paediatric conditions and may point to specific genetic conditions for which molecular genetic testing will probably become increasingly available. It is a non-invasive and cost effective investigation, which should be more widely performed in the appropriate clinical circumstances.

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