

Erythropoietic Protoporphyrin

The Problem of a Suitable Screening Test

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Erythropoietic protoporphyria (EPP) is characterized by increased red cell protoporphyrins and is included in the differential diagnosis of children presenting with photosensitivity. In the past 20 years, using the traditional solvent extraction qualitative screening test for blood porphyrins, the diagnosis of EPP had been missed in 9 out of 10 patients but recently, using fluorescence microscopy of erythrocytes, no patients with EPP have been missed. All 14

patients in Northern Ireland known to have EPP were recalled and it was found that fluorescence microscopic determination was positive in all cases. We recommend fluorescence microscopy as the screening test of choice for the detection of increased red cell porphyrins. *Key words: Photosensitivity; Fluorescence microscopy.*

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Erythropoietic protoporphyria (EPP) is an inherited inborn error of porphyrin metabolism characterized clinically by a number of photocutaneous manifestations commencing in childhood, and chemically by excessive amounts of red cell protoporphyrins (1).

Although EPP is a rare condition, it is one of the two most common porphyrias, along with porphyria cutanea tarda (2). The diagnosis is usually made by dermatologists. The photosensitivity is often described as an intense burning pain occurring within 10–30 min of exposure to sunlight, and persisting for some hours. It is possible that this may not be followed by an objective sign such as redness, swelling or scarring, so that children with EPP may be regarded as 'neurotic', 'nervous' or 'hysterical' (3, 4). The problem is compounded when the diagnosis is suspected clinically and then dismissed after an initial negative qualitative (screening) test. We have experienced this sequence of events in a number of cases.

We have found that one of the traditional qualitative tests for blood porphyrins is insufficiently sensitive to screen for EPP, and have therefore compared this test with another qualitative test, namely fluorescence microscopy of erythrocytes.

MATERIALS AND METHODS

Subjects

By contacting every Dermatology Department in Northern Ireland, all patients known to have EPP were traced. These numbered 14, and all were asked to attend the Dermatology Clinic for blood tests, together with their parents and sibs.

Methods

A 5 ml heparinized specimen of blood, shielded from light, was sent to the laboratory for porphyrin analysis. Three methods of analysis were used:

(a) *Qualitative estimation – solvent extraction (traditional)*
Qualitative estimation of blood porphyrins was carried out according to the traditional procedure described in the Association of Clinical Pathologists Broadsheet No. 109 (5). Porphyrins were extracted with ether/glacial acetic acid and detected as fluorescence under UV light.

(b) *Qualitative estimation – fluorescence microscopy*

A blood film was observed under a fluorescence microscope (Leitz Ortholux II) with mercury light source and excitation wavelength 390–490 nm. Note: Protoporphyrin fluorescence fades quickly due to destruction of the molecule by UV light. If available, an iodine tungsten lamp instead of a mercury-vapour lamp will permit colour photography of the erythrocytes (11). The fluorescence was graded subjectively as O, trace, +, ++ or +++.

(c) *Quantitative measurement – solvent extraction*

Quantitative measurement of blood porphyrins was carried out according to the method of Heller et al. (6).

RESULTS

Brief clinical details of the 14 patients, together with their biochemical investigations are listed in Table I.

The age of onset of photosensitivity in the patients ranged from 3 months to 10 years, and the period from onset of photosensitivity until presentation to a dermatologist ranged from 1 month to 25 years. Qualitative (screening) tests for red cell porphyrins were carried out on all patients at initial presentation to the dermatologist, indicating that EPP was considered as a possible diagnosis.

The traditional qualitative test was used in Cases 1–9 and 13 and fluorescence microscopy in Cases 10–12 and 14. The traditional qualitative test proved negative in Cases 1–9 and this led to a delay in diagnosis of from 2 weeks to 8 years. In contrast, fluorescence microscopy did not miss any case of EPP and consequently there was no delay in diagnosis. In Cases 1–4 the diagnosis of EPP was dismissed on receiving a negative screening test, and made only some years later when the children were re-referred and quantitative biochemical analysis for blood porphyrins was specifically requested. In Cases 5–9, clinical suspicion of EPP was so great that, despite a negative screening test, quantitative biochemical analysis or fluorescence microscopy was requested and the diagnosis was then confirmed. Only in Case 13 was an initial biochemistry screening test positive. This test was carried out in another laboratory using a modification of the traditional screening method (1.0 ml whole blood instead of the recommended 0.1 ml, personal communication).

When the patients were recalled and their blood porphyrins checked, fluorescence microscopy was found to be positive in all 14 cases and the intensity of fluorescence correlated quite closely with the levels of red cell protoporphyrins obtained by quantitative biochemical analysis. The traditional qualitative

Table 1. Clinical and biochemical features of 14 cases of erythropoietic protoporphyria

Case No. /Sex	Age at onset of photo- sensitivity	Time from onset to presentation to dermatologist	Year of presentation	Initial screen- ing test (T or F) ^a	Delay from screening test to diagnosis	Quantitative porphyrin analysis (nmol/l)	Red cell fluorescence
1/M	4 months	4 years	1968	T -ve	8 years	12410	+++
2/F	1 year	10 years	1971	T -ve (x3)	15 years	11250	++
3/M	2 years	25 years	1971	T -ve	15 years	3920	+
4/M	2 years	2 years	1972	T -ve	2 years	12880	+++
5/M	8 years	5 years	1973	T -ve	1 month	6940	++
6/F	4 years	3 years	1976	T -ve	1 month	7640	++
7/F	2 years	1 year	1976	T -ve	4 months	3685	++
8/M	9 years	4 years	1986	T -ve	3 months	4270	++
9/F	3 months	6 years	1987	T -ve (x2)	2 weeks	5570	++
10/F	10 years	1 year	1988	F +ve	None	1870	+
11/M	6 years	1 month	1988	F +ve	None	2050	+
12/F	8 years	5 years	1988	F +ve	None	5390	+
13/F	4 years	12 years	1988	T +ve	None	8600	++
14/F	6 years	11 years	1988	F +ve	None	12600	++

^aT = Traditional screening test, F = Fluorescence microscopy.
(Sibs: Cases 2 and 3; Cases 5 and 6; Cases 8, 10 and 11).

screening tests were also repeated and found to be negative in all cases.

Red cell fluorescence and quantitative measurements for blood porphyrins were determined in 19 symptom-free family members (parents and sibs). Three of these subjects were found to have a trace of red cell fluorescence and the rest were negative in this screening test. The quantitative method for blood porphyrins found that those 3 members with a trace of red cell fluorescence had total blood porphyrins above the upper limit of the reference range (90–800 nmol/l), these results being 1193, 979 and 2029 nmol/l, respectively. One additional sib had total blood porphyrins just above the upper limit of the reference range at 890 nmol/l and had a negative result for red cell fluorescence.

DISCUSSION

Erythropoietic protoporphyria is a rare condition which causes much distress to patients. It is important not to miss the diagnosis, for several reasons. Firstly, lack of objective signs of the disease in some children can lead to their being called 'hysterical' with much emotional trauma in consequence (4). Secondly, treatment is available in the form of reassurance and an explanation about the condition, together with antihistamines, topical sunscreens and

oral beta-carotene (2, 7). Thirdly, a small number of patients develop fatal liver disease, and there is some evidence that early intervention can improve outcome (8). Finally, oral iron therapy has been known to exacerbate some cases of EPP (9). This happened in Case 14 of the present series. We were therefore disturbed to find that we had missed cases of EPP by using the solvent extraction qualitative screening method for blood porphyrins.

The quantitative method of measuring blood porphyrins is complex and time consuming, so most laboratories initiate investigations by using a simple qualitative screening test. In many laboratories it has been customary for quantitative porphyrin measurements to be made in a suspected case of EPP only if a qualitative screening test has proved positive.

Erythropoietic protoporphyria was not clearly defined until 1961 (10) and our first 7 cases presented between 1968 and 1976. Initially we and others had confidence in the qualitative biochemical test, so that the possible diagnosis of EPP was dismissed on the basis of a negative result. Towards the end of this period, when a diagnosis of EPP was suspected, we requested quantitative analysis for porphyrins, even if the traditional screening test was negative. Our last 7 cases were seen in the years 1986–88, and by this stage we were aware of the problems with the traditional screening test. We therefore used either erythrocyte fluorescence microscopy as the screen-

ing test of choice, or always followed a negative traditional screening test by a quantitative analysis. In this way there was no significant delay in diagnosing EPP. Our clinical experience has therefore been that a positive fluorescence microscopy screening followed by quantitative measurement of blood porphyrins is the most effective way of diagnosing EPP. This has been confirmed by recalling all our patients and carrying out fluorescence microscopy on heparinized samples of blood.

In this series we have had no false-positives, although this is possible with such conditions as Gunther's Disease, iron deficiency anaemia and lead poisoning. However, these conditions are easily distinguished clinically or biochemically from EPP (11). A trace of red cell fluorescence was detected in 3 family members of this group of patients and their blood porphyrins were significantly elevated, demonstrating that these members are asymptomatic carriers of the gene for EPP (1, 12).

Recently Deacon has looked at the performance of the traditional biochemical screening tests for various porphyrias including EPP, and noted that among 11 cases of EPP, 7 were missed by the initial screening test (13). In one of 3 cases reported by De Selys, the diagnosis was delayed by inconclusive biochemical investigations, and early biopsy of the hand was recommended (14). Deacon stated that there was no satisfactory alternative screening procedure for red cell porphyrins. We would suggest that fluorescence microscopy is a satisfactory alternative in that it is more sensitive and simpler to perform than the traditional qualitative test, and recommend it as the initial screening test for the detection of red cell porphyrins, provided it is always followed by a quantitative test.

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