

Lipoprotein Electrophoresis in Recessive X-linked Ichthyosis

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Recessive X-linked ichthyosis (RXLI) is consistently associated with steroid sulphatase deficiency, and a definite diagnosis can be made by measurement of the activity of this enzyme, e.g. in cultured skin fibroblasts and leucocytes. Demonstrating an increased electrophoretic mobility of plasma low-density lipoprotein in RXLI patients has been proposed as a simpler method for the diagnosis of this condition. Our findings in 7 RXLI patients and 7 normal controls confirmed that a discrimination between patients and controls can be obtained by routine lipoprotein electrophoresis. However, due to variation in the results of repetitive performances further studies are needed to evaluate the overall reliability of this diagnostic approach. *Key words: Recessive X-linked ichthyosis; Electrophoretic mobility; Low-density lipoproteins.* (Received July 23, 1985.)

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An exact diagnosis of recessive X-linked ichthyosis (RXLI) can be obtained by proving a deficiency of the steroid sulphatase (STS) enzyme (1). However, assays to determine the activity of this enzyme, e.g. in cultured skin fibroblasts or leucocytes (2, 3) have so far been limited to a few specialized laboratories.

In RXLI patients the plasma level of cholesterol sulphate, one of the natural substrates of the STS enzyme, has been shown to be at least ten-fold above normal (4). Epstein et al. (4) seconded by Traupe et al. (5) demonstrated that low-density lipoprotein (beta-lipoprotein) from patients with RXLI has abnormally rapid anodic electrophoretic mobility, possibly caused by enhanced electronegativity imparted by increased binding of cholesterol sulphate. Thus, Epstein et al. (4) assumed that a definite diagnosis of RXLI can be made by routine lipoprotein electrophoresis in a local hospital clinical laboratory.

In order to evaluate the reliability of routine lipoprotein electrophoretic findings for the diagnosis of RXLI, we have investigated seven RXLI patients with biochemically proven STS deficiency.

MATERIAL AND METHODS

Seven male patients with RXLI were included in the study. In all patients the diagnosis was confirmed by the absence of STS-activity in peripheral leucocytes as previously described (3).

Blood samples were obtained in the morning after 12 hours' fasting. Plasma lipoprotein electrophoresis was performed on agarose according to Johansson (6) in the hospital laboratory routine programme. The relative electrophoretic migration of low-density lipoproteins (LDL) was expressed as the distance from the origin to the peak of the beta-band divided by the distance from the origin to the end of the alpha- (high-density) lipoprotein band (Fig. 1) as described by Epstein et al. (4). The index beta/alpha was calculated for each run.

The studies were performed in two steps:

Study 1. Plasma lipoprotein electrophoresis of three patients and three controls in parallel runnings on the same agarose gel. This procedure was repeated four times (twice a day for two days) with the same patients and controls included.

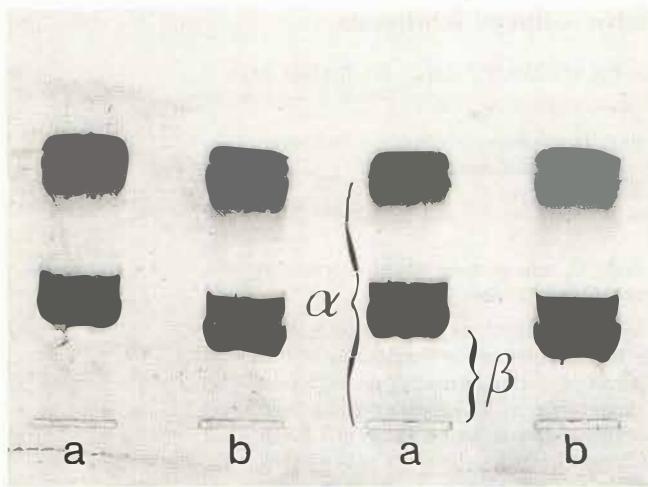


Fig. 1. Lipoprotein electrophoretic pattern in RXLI patient and control illustrating the reading of the index beta/alpha. Two serum samples from patient (a) and control (b) are alternately run in parallel. Anode (top).

Study 2. Each patient was compared with only one control; 3 or 4 replicates from both patient and control were run in parallel on the same agarose gel plate.

The reading of the electrophoresis and calculation of the indices were performed by a biochemist blinded to patients and controls.

RESULTS

In all patients and controls the total amounts of cholesterol and triglycerides in serum were within normal limits.

Study 1

Beta/alpha indices obtained in patients and controls by electrophoresis of the same series of plasma samples on four different gel plates are shown in Fig. 2. As seen, indices varied considerably. A one-way analysis of variance showed within-day variance equal to or higher than between-day variance.

Study 2

Beta/alpha indices obtained from electrophoresis of plasma samples from seven patients, each running in parallel with one control, are shown in Fig. 3. Three or four alternating replicates from patient and control were run on the same gel. As seen from Fig. 3, only one index is overlapping if each plate is evaluated separately. Nevertheless, the between-plate/between-person variation precludes ascertainment of a cut-off value between patients and controls.

DISCUSSION

Both Epstein et al. (4) and Traupe et al. (5) found lipoprotein electrophoresis to be a reliable diagnostic test in the separation of RXLI from other types of ichthyosis when biochemical verification of STS-deficiency was not available. However, the cited reports left out some essential information. Thus, Epstein et al. (4) made no comment on the reproducibility of their results, and Traupe et al. (5) reduced this matter to the statement

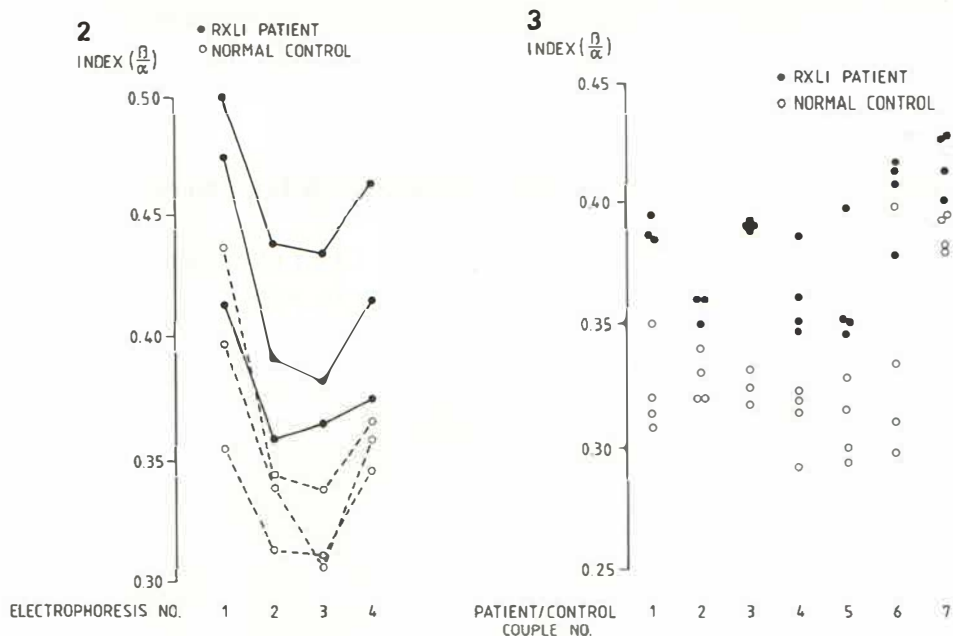


Fig. 2. Beta/alpha indices for 3 RXLI patients and 3 controls. Values obtained at different runnings in single patients connected by unbroken lines, values of single controls connected by dotted lines. For further explanation: see text.

Fig. 3. Beta/alpha indices for RXLI patients and controls. For further explanation: see text.

that an increased electrophoretic mobility of beta- and pre-beta lipoproteins was a constant finding in their 8 patients with RXLI. In our study, considerable variation in beta/alpha indices was found when using a well-known Scandinavian method for routine lipoprotein electrophoresis (6). This might be due to the fact that a standardized mobility is difficult to achieve, as the mobility of low-density lipoproteins is susceptible to minor variations in buffer composition, field strength, etc. (6). The variation in the individual beta/alpha indices precluded the ascertainment of a cut-off value between RXLI patients and controls (Fig. 3). However, calculated separately for runnings on the same gel plate (Study 2) the mean values of beta/alpha indices for patients were constantly higher than those of controls (range of patient/control ratios: 1.07–1.21). The discrimination was narrow, and a study covering several more patients and controls is needed to establish whether routine lipoprotein electrophoresis always discriminates well enough to allow a diagnosis of RXLI to be made without a confirmatory STS determination.

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New Parameters for Evaluation of Blood Flow in Patients with Leg Ulcers

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Kristensen JK, Karlsmark T, Bisgaard H, Søndergaard J. New parameters for evaluation of blood flow in patients with leg ulcers. *Acta Derm Venereol (Stockh)* 1986; 65: 62-65.

Three new parameters have been introduced to provide data for quantitative evaluation of peripheral cutaneous blood flow in patients with leg ulcers. Reactive hyperemia was induced by occlusion of the blood flow for four minutes at the thigh-level. Blood flow was subsequently measured by laser-Doppler velocimetry in an unselected group of 14 patients with leg ulcers and a matched control group. The parameters used for evaluation was: 1) "peak flow" $f(p)$, 2) divided by time to "peak flow" $t(p)$, rendering a rate constant k ($k=f(p)/t(p)$) expressing the ability to increase blood flow abruptly in case of need. These parameters were all significantly reduced in the patients with leg ulcers, indicating that this simple and atraumatic technique was useful for discriminating blood flow values that may be relevant for healing time and with a sensitivity comparable to the measurements of distal systolic blood pressure. Distal systolic blood pressure measurements can still be considered to be of value when screening for arterial insufficiency in patients with leg ulcers. The values obtained were significantly lower in 36 patients with leg ulcers compared with 9 age-matched control persons. (Received May 20, 1985.)

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Leg ulcers are usually caused by a vascular disease, where venous insufficiency has been regarded the most common etiological factor, but in the individual patient several etiological factors are often operating (1). In the management of patients with leg ulcers, direct measurement of arterial function is a common procedure. Usually the preferred method only comprises measurement of the distal systolic blood pressure. However, this may be insufficient, as diastolic blood pressure was recently shown to be the determining factor governing blood flow (2). The present work was undertaken in an effort to provide data for quantitative evaluation of peripheral cutaneous blood flow in patients with leg ulcers. In addition, data have been obtained by conventional measurement of distal systolic blood pressure for comparison and for evaluation of the sensitivity of this technique.

MATERIAL AND METHODS

Thirty-six consecutive patients with ulcers of the leg (aged 58 to 89 years) and 9 age and sex matched controls (40 to 79 years) were investigated. In 14 unselected cases from the group of 36 patients and in the 9 normal persons reactive hyperemia (5) was registered after 4 min of occlusion of the circulation to the leg using a blood pressure cuff around the thigh inflated to suprasystolic level. Reactive hyperemia curves (Fig. 1) was interpreted as the peak flow value $f(p)$, the time to peak flow $t(p)$ or as the peak flow value divided by the time to peak flow

(k) (i.e. $k=f(p)/t(p)$).

Systolic toe blood pressure was measured as previously described (3, 4) using a laser-Doppler flowmeter and a miniature blood pressure cuff applied around the base of the big toe. Systolic blood