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Lactate dehydrogenase isoenzyme patterns in vaginal injury of a 4-year-old girl

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Summary. Lactate dehydrogenase isoenzyme patterns indicative of vaginal injury were identified in extracts of a bloodstain on a pair of knickers belonging to a 4-year-old girl. No evidence of semen or saliva was detected in the stain. The result corroborated the clinical findings and provided strong evidence of child sexual abuse.

Key words: Lactate-Dehydrogenase – Vaginal injury – Blood

Zusammenfassung. In einem Blutspurenextrakt, welcher von Blutspuren auf einem Slip gewonnen wurde, der einem 4jährigen Mädchen gehörte, wurden Isoenzym-Muster der Laktat-Dehydrogenas gefunden, welche auf eine Vaginalverletzung hinwiesen. Kein Beweis für Samen oder Speichel wurde in den Spuren gefunden. Das Resultat der Untersuchung bestätigte die klinischen Befunde und sorgte für einen starken Hinweis auf einen sexuellen Mißbrauch des Kindes.

Schlüsselwörter: Laktat-Dehydrogenase – Vaginale Verletzung – Blut

Introduction

Over a period of several months the victim, a 4-year-old girl, had complained of a sore vagina for which medical treatment was sought. Subsequently the girl's mother found an apparently bloodstained pair of knickers belonging to her daughter. The child was referred to a doctor with experience in child sexual assault who performed a further examination. The medical findings indicated a non-acute abraison of the fossa navicularis probably caused by posteriorly and internally directed trauma. The conclusion of the doctor was that sexual assault had almost certainly occurred. The bloodstained knickers and a vulval swab were submitted to the laboratory for examination.

Materials and methods

The vulval swab and knickers were screened for the presence of seminal fluid with the reagent O-dianisidine [0.5 g/100 ml in 0.1 *M* citrate buffer containing 0.2% w/v 1-naphthyl disodium orthophosphate (Kind 1964). Screening for saliva was carried out using procion red amylopectin as described by Whitehead and Kipps (1975). Determination of species of origin was determined by Ouchterlony radial diffusion (Ouchterlony 1970).

The analysis of lactate dehydrogenase isoenzymes was performed based on the method of Asano et al. (1972). Cellogel (Chemetron Ltd, Milan), stored in methanol, was immersed in veronal buffer pH 8.6 (75 m*M* sodium barbitione, 2.6 m*M* calcium lactate pH 8.6). A central area and an area close to the periphery of the bloodstain were extracted in minimal volumes of water. Also extracted was an area adjacent to the stained area, a bloodstained vaginal swab from a menstruating woman and a 1/5 diluted fresh peripheral blood sample. Extracts were applied centrally to the Cellogel at an equal distance from each electrode. Extracts were applied so that the colour of each was approximately equivalent. Electorphoresis was carried out for 1 h at 60 V. After electrophoresis the cellogel was rinsed briefly in water then incubated in 56 ml 0.05 *M* TrisHCl pH 7.4, 7.7 ml 70% 1.2 *M* sodium lactate solution containing 1 mg *N*-methyl phenazonium methosulphate (PMS), 40 mg NAD and 30 mg nitroblue tetrazolium for 40 min at 37°C. Location of LDH activity appeared as purplish bands on a pale background. After brief washing in distilled water, the colour reaction was fixed by 2 washes in a solution of 10 ml acetic acid, 100 ml methanol and 45 ml water.

Results and discussion

The stain in the crotch of the knickers was found to be human in origin. No seminal fluid was found on the vulval swab or on the knickers. No saliva was detected in the extracts of the knickers for which LDH patterns were determined.

LDH (EC 1.1.1.27) isoenzyme patterns indicative of vaginal/menstrual blood or vaginal secretions were found in extracts from bloodstained areas of the child's knickers (Fig. 1, tracks 2 and 3). Elevated levels of isoenzymes LDH-4 and in particular LDH-5 were found.

LDH activity is widely distributed throughout mammalian tissues. In all cases it catalyses the interconver-

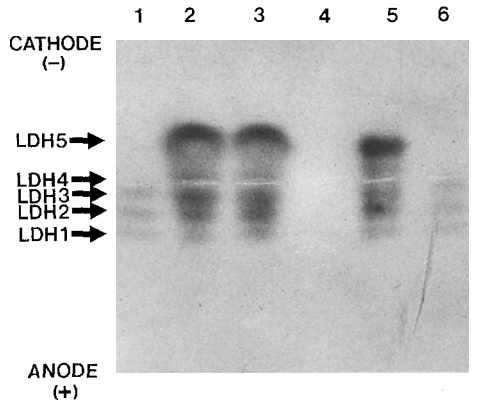

Fig. 1. LDH isoenzyme forms in: (1) and (6) peripheral blood; (2) extract from the edge of the stain in the knickers; (3) extract from the centre of the bloodstain; (4) extract from non-bloodstained area of the crotch of the knickers; (5) extract of vaginal swab from a menstruating woman.

sion of pyruvate and lactate and the enzyme has certain properties characteristic of the tissue from which it is derived. Five bands of LDH have been identified in mammalian tissues by starch gel electrophoresis. Increased LDH isoenzyme band activity is usually associated with tissue damage from specific organ sites, for example increased acitivity of LDH-1 and LDH-2 is usually associated with myocardial infarction (Latner and Skillen 1961). Increased LDH-4 and -5 activity is usually associated with liver damage or with blood of menstrual or vaginal origin (Wieme and Van Maercke 1961; Asano et al. 1972). The use of LDH isoenzyme band patterns for forensic purposes has been descirbed (Asano et al. 1972; Stombaugh and Kearney 1978; Divall and Ismail 1983) and should be treated with caution. Peripheral blood mixed with semen, saliva or vaginal secretions can give LDH isoenzyme patterns that could be interpreted erroneously as having come from menstrual/vaginal blood.

Samples of staining from the central area of the stain on the knickers and from near the edge of the stain were compared with an area of cloth immediately adjacent to the stain in an attempt to show coincidence of the bloodstain with any vaginal secretions present. No LDH activity was found in the non-bloodstained portion of the knickers sampled next to the stain. This result shows that the bloodstain was caused either by vaginal blood alone (resulting from the vaginal injury described by the doctor) or by a mixture of vaginal blood mixed with vaginal secretions that had been applied as a single stain.

As it is virtually impossible to separate menstrual and/or vaginal blood from other vaginal secretions, it is difficult to assess the LDH forms in menstrual/vaginal blood alone. It is most likely that the band pattern observed was composed of a mixture of LDH 1, 2 and 3 from the blood in conjunction with LDH 4 and 5 from vaginal secretions (Divall and Ismail 1983).

It is virtually impossible for a 4-year-old girl to be postmenarchial and no mention of this was made by the doctor in this case. The bloodstaining present in this instance is therefore of vaginal origin. The inability to differentiate between vaginal blood mixed with vaginal secretions from vaginal blood alone does not compromise the evidence obtained in this case. Either way the result can be taken as indicative of vaginal injury, corroborating the evidence of the examining doctor.

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