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Methods

PK assay implementation

The PK assay, based on Beutler’s method1 with a few minor modifications, was implemented in the Red Cell Membrane Unit, Department of Molecular Medicine and Haematology at the University of the Witwatersrand National Health Laboratory Service (NHLS) in Johannesburg. Qualitative (screening) and quantitative (confirmatory) assays were implemented, and the screening assay may be added to the routine tests offered by the NHLS. Guidelines for physicians are available from the authors.

The screening and quantitative assays use the same test principle: Plasma and leucocytes are removed from whole blood and the packed erythrocytes resuspended in 0.9% saline. A 1:20 haemolysate is made of the red cells and used to determine PK enzyme activity by the conversion of PEP to pyruvate. This reaction is coupled to a second reaction, which uses lactate dehydrogenase to convert pyruvate to lactate with nicotinamide adenine dinucleotide (reduced form) (NADH) as a co-factor. The oxidation of NADH is determined by a loss of fluorescence (screening assay) or spectrophotometrically at 340 nm (quantitative assay), and is used as a measure of PK activity.

Ethics clearance was obtained from the Human Research Ethics Committee (Medical), of the University of the

References


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Results and discussion

PK assay implementation

The screening assay detected a decreased PK activity if the sample had an enzyme activity of <50% of the mean. This is considered adequate, since clinically relevant PK deficiency develops when an individual has an enzyme activity of <25% of normal.

There was no difference between the means of the white (13.41 U/g haemoglobin (Hb)) and black (12.64 U/g Hb) groups (p=0.29). The combined mean (± significant deviation (SD)) of this study was 13.01±2 U/g Hb and falls within 1 SD of the published reference range. The coefficient of variation for the precision and reproducibility of the quantitative assay was 5%. The PK enzyme is stable in EDTA whole blood for 5 days if stored at 4℃, and for 3 days if stored at room temperature. The minimum volume of whole blood required to perform the assay is 0.5 ml, which makes it suitable for samples taken from paediatric patients. The assay was performed on a further 10 samples received from laboratories around the country as part of a ‘haemolytic work-up’ to demonstrate the applicability and feasibility of the assay in a routine setting.

PK-deficient patient

A 24-year-old white woman with suspected PK deficiency had suffered a haemolytic episode immediately after the prophylactic use of antiretrovirals (3TC, AZT and Crixivan) a year earlier. A referral letter reported haemolytic episodes as an infant but nothing subsequently. The only medication she used was Sibelium for migraines, and she was otherwise well.

An infant was jaundiced and had no clinically evident splenomegaly. The quantitative PK assay confirmed the enzyme deficiency. The patient’s mean PK activity was 15% of normal (N=4). One parent was identified as an asymptomatic carrier and had an enzyme activity of 58% of normal (N=3). The second parent was unavailable.

The association between haemytic crises in PK deficiency and ARVs has not been reported in the literature previously, and this finding is important in South Africa.

Mutation analysis

The patient was homozygous for the 1529A mutation in the PK-LR gene and heterozygous parent. (a) Sequence data for the PK-deficient patient who is homozygous for the 1529A mutation in the PK-LR gene and heterozygous parent. (b) A model of the mutant protein was generated with SWISS-PDB Viewer (http://www.expasy.org/spdbv). The Arg510Gln mutation is not adjacent to the catalytic site, but the mutation decreases the activity and thermal stability (data not shown) of the mutant enzyme.

Fig. 1. Mutation analysis of the PK-deficient patient and parent. (a) Sequence data for the PK-deficient patient who is homozygous for the 1529A mutation in the PK-LR gene and heterozygous parent. (b) A model of the mutant protein was generated with SWISS-PDB Viewer (http://www.expasy.org/spdbv). The Arg510Gln mutation is not adjacent to the catalytic site, but the mutation decreases the activity and thermal stability (data not shown) of the mutant enzyme.

References


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