Newborn screening for classic galactosaemia and primary congenital hypothyroidism in the Nkangala district of Mpumalanga province, South Africa

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Objectives. The main objective of this work was to establish the incidence of classic galactosaemia and primary congenital hypothyroidism in newborns in the Nkangala district of Mpumalanga. In the process a cost-effective protocol for neonatal screening of both diseases was developed.

Study design and setting. Blood spot specimens were collected from a third (1 012 out of 3 297) of newborn infants in the Nkangala district of Mpumalanga province from June to November 2005. The specimens were subsequently screened for classic galactosaemia and hypothyroidism using metabolite quantification assays. Galactose-1-phosphate uridyltransferase (GALT) enzyme activity assays were also performed to confirm the reliability of the total galactose assays. The real-time polymerase chain reaction (PCR) was used to detect commonly occurring mutations in the GALT gene that cause galactosaemia. Thyroid-stimulating hormone (TSH) levels were evaluated as a diagnostic metabolite for primary congenital hypothyroidism.

Subjects and outcome measures. Informed consent was obtained from the babies' parents before commencement of screening. Total galactose levels above 0.9 mg/l and TSH concentrations above 25.1 mU/l were considered to indicate galactosaemia and hypothyroidism, respectively. A decrease in the total financial input on the screening protocol was evaluated for significance in cost reduction.

Results. The prevalence of hypothyroidism was found to be 0.1%, while none of the newborns presented with classic galactosaemia. There was an up to 20% reduction in direct input costs of screening when our protocol was applied.

Conclusion. Cost-effective newborn screening is possible when classic galactosaemia and congenital hypothyroidism are screened for simultaneously. Cumulative disease frequency plots confirm the already established fact that hypothyroidism tends to occur at higher frequencies than classic galactosaemia.

Galactosaemia is the most common name given to a category of clinically heterogeneous, life-threatening metabolic disorders of autosomal recessive inheritance in which an enzyme deficiency affects the normal metabolism of galactose,[1] a food sugar obtained mainly from dairy products.[2] Deficiency of one of the three enzymes of the Leloir pathway (Fig. 1) leads to elevated levels of galactose and its metabolites, resulting in a number of life-threatening complications, including feeding problems, failure to thrive, hepatocellular damage and sepsis, if untreated. The most common form of this disease is caused by galactose-1-phosphate uridyltransferase (GALT) enzyme activity deficiency and is often referred to as classic galactosaemia.[3] Studies evaluating the incidence of classic galactosaemia in South African populations have been few, and the prevalence of this disorder has been estimated to be between 1/14 400 and 1/21 904.[4,5] The most prominent studies in South African populations have been by Manga and colleagues,[4] who evaluated the prevalence of classic galactosaemia in black children, and Henderson *et al.*,[5] who analysed cord blood samples to ascertain the prevalence of classic galactosaemia. Both studies showed higher figures than the global average, although both used small population samples to project the national prevalence. Laboratory diagnosis in newborn screening for galactosaemia involves demonstration of an elevated erythrocyte total galactose (galactose and galactose-1-phosphate) concentration,[6] whereas determination of GALT enzyme activity is used exclusively for confirmation of classic galactosaemia.[5] Measurement of total galactose can be used to screen for deficiency of any of the three Leloir pathway enzymes.[2]

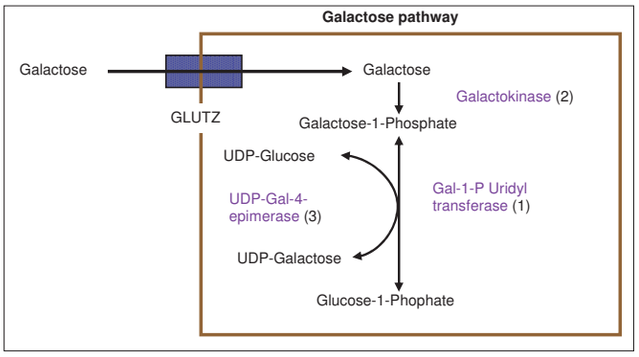

Fig. 1. The Leloir pathway of galactose catabolism with the involved enzymes labelled 1, 2 and 3.

Hypothyroidism is the most common hormonal congenital condition routinely screened for in the USA.[7] Abnormal thyroid-stimulating hormone (TSH) and/or thyroxine (T4) levels indicate a probablility of defective production of the thyroid hormones and hence hypo- or hyperthyroidism.[8] Congenital hypothyroidism is one of the most common causes of preventable mental retardation and affects approximately 1 newborn infant in 3 000 in Canada[8] and 1 in 3 500 - 4 500 in the UK and the USA.[7,9]

There is currently no means of preventing galactosaemia or hypothyroidism pre- or postnatally. The only known treatment for galactosaemia involves a galactose- and lactose-free diet. If such a diet is provided during the first 10 days of life, the presenting symptoms usually quickly resolve. The current treatment for hypothyroidism involves hormonal therapy with intravenous or oral levo-thyroxine.[7,9] However, this treatment is also only effective when instituted early in the neonate's life. In both disorders early and accurate identification of patients is therefore critical. The prevalences of galactosaemia and hypothyroidism in South Africa are still unknown.

Materials and methods

Sample collection and storage

Ethical approval for the study was obtained from North-West University (ref. 04M04) and the Mpumalanga Provincial Department of Health. All neonates born at Middelburg Hospital on Monday to Friday of the week between 1 June and 30 November 2005 were targeted for this study (the dates were chosen so as to accommodate the retired nursing sister who collected the samples). After obtaining the parents' consent, samples were collected in the maternity, paediatric and high-care wards of the hospital by venepuncture of either side of the heel of the newborn babies. After sterilisation with ethanol the heel was punctured and blood was allowed to flow freely and soak onto the Guthrie card. The blood spot was subsequently dried for 2 - 4 hours on a dry clean surface at room temperature. Thereafter the cards were stored at 4°C in a sealed container and transported to the laboratory for analysis within 14 days of collection. All cards were labelled with information indicating the patient's identity and relevant clinical information.

Total galactose assay

Dried blood discs of 2.2 mm diameter from the Guthrie filter papers were punched out and placed in 275 µl capacity conical-bottomed 96-well plates. A neonatal total galactose assay kit was supplied by Bio-Rad (Cat. No. 5326053). The total galactose assay was performed on the Coda EIA analyser (Bio-Rad) using the total galactose kit according to the manufacturer's recommendations.

Cost-effective screening with proper care and follow-up is possible even in the most remote of rural settlements with this study model.

GALT enzyme activity assay

Enzyme activity of the GALT protein was assayed in 20 randomly selected specimens according to the Beutler method as described in the 2nd edition of Grune and Stratton's *Manual of Biochemical Methods*.[10] In this assay, the conversion of NADP+ to NADPH was measured using a Perkin Elmer fluorometer set at excitation and emission wavelengths of 347 nm and 460 nm, respectively. The amount of NADPH produced per unit of time represented the rate of catalysis of galactose-1-phosphate breakdown by the GALT enzyme.

DNA extraction and polymerase chain reaction

Genomic DNA extracts suitable for polymerase chain reaction (PCR) were prepared from punched discs (1.2 mm), which were treated with methanol (200 µl) for 30 minutes and air-dried for 30 minutes. Subsequently, the dried blood spots were boiled in 50 µl of distilled water. Aliquots of 10 µl were used for each PCR reaction, in a total volume of 25 µl. This protocol for DNA extraction was developed in-house. A hot start was required to obtain consistent and reliable amplification. In samples where the amplification protocol yielded poor results an alternative method was followed. Here the DNA was isolated from 1.2 mm diameter dried blood specimens using the FTA (Flinders Technology Association) DNA extraction kit (Cat. No. WB120061) according to the manufacturer's directions (Merck). The FTA-treated 1.2 mm diameter blood card was then used as the DNA template in the final PCR mixture without further DNA extraction in a total volume of 25 µl. The PCR protocol, primers and fluorophore-labelled probes were as described by Dobrowolski *et al*.[11]

Common mutation genotyping

Specimens known to contain the S135L mutation were used as positive controls to validate the PCR cycle and hybridisation probes as described by Dobrowolski *et al*.[11] Genotyping of the two common GALT mutations (S135L and Q188R) were performed on the Bio-Rad icycler.

The TSH assay

The Coda EIA analyser was used together with the radio-immunoassay kit from Bio-Rad (Cat No. 7C2570A) for TSH measurements and the manufacturer's recommended method for TSH assay was applied without any changes. The CDC (Centers for Disease Control and Prevention) validated standards as well as the in-house standards for hypothyroidism were used for calibration as positive and negative controls for TSH levels.

Cost savings

The screening costs were calculated as the total savings from the single screen for classic galactosaemia and congenital hypothyroidism, respectively, versus the dual testing using the same Guthrie card and analysing equipment. The difference was then computed as the total cost savings. The total time taken to perform analysis of a second sample when required and report back to the investigating clinician and the parents of the infant was between 14 and 21 days.

Results and discussion

Sample collection

According to Middelburg Hospital records, an average of 14 babies per day were delivered over the sampling period of 6 months. The highest number of deliveries was 26 babies in a 24-hour period. On average 11 out of 14 parents (79%) gave consent for sampling and testing for both classic galactosaemia and congenital hypothyroidism. Of the mothers who did not participate in the study even though they had initially agreed to the test (totalling 285), half (142) had left the hospital premises before the sampling could take place. This was because resources in the maternity wards at Middelburg Hospital are limited, so mothers are discharged as soon as possible to enable others to use the facilities. The other half (143) either decided that they were unwilling to participate after all, or had given birth prematurely – we did not sample premature babies, since their livers may not have reached full maturity and they may therefore have had false-positive results. The earliest sampling time was 6 hours after birth and the latest 36 hours after birth, and of the babies 52% (526/1 012) were girls and 48% (486/1 012) boys.

Total galactose assay

None of the 1 012 samples had a total galactose level indicating galactosaemia. This finding was supported by the absence of clinical characteristics of galactosaemia in the newborn infants. A repeat sample was collected and re-evaluated for all inconclusive results from the first screen. All repeat samples (second screen) showed values within acceptable limits, confirming the absence of classic galactosaemia in the respective newborns (Fig. 2).

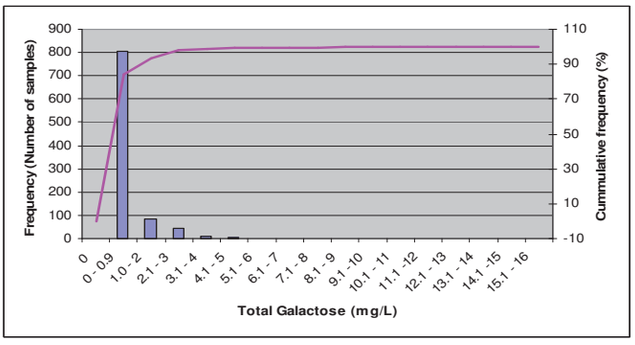

Fig. 2. Graph of total galactose versus sample frequency and cumulative frequency.

GALT enzyme activity assay

Randomly selected samples (20 of every 100 that tested negative for galactosaemia in the total galactose assay) were subjected to a GALT enzyme activity assay according to the method of Beutler.[10] The GALT enzyme activity of all these specimens was within the normal limits of 19.2 - 33.8 µmol/h/g Hb.[12,13] The correlation between the total galactose values and enzyme activity assay was good.

Thyroid-stimulating hormone assays

The cut-off value for the TSH assay for hypothyroidism is 25.1 mU/l. Although 81 samples had TSH values above the cut-off point on initial testing, at the 3-week follow-up assay the TSH value had dropped to less than 25.1 mU/l, indicating normal thyroid gland function. However, in 1 case a relatively high TSH value (initially138 mU/l, Fig. 3) persisted for over a month. The baby was referred for follow-up by the resident paediatrician within 18 days, and L-thyroxine therapy was started after clinical and radiological confirmation of hypothyroidism.

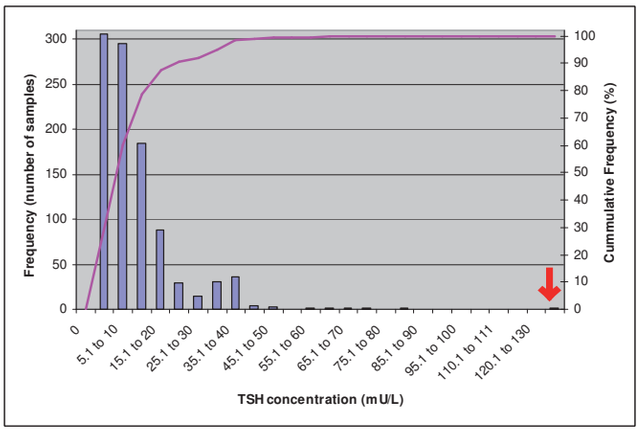

Fig. 3. Graph of thyroid-stimulating hormone versus sample frequency and cumulative frequency. The arrow shows the concentration of the sample that was repeatedly positive for hypothyroidism.

Common mutation genotyping

One thousand and twelve samples were genotyped for the presence or absence of the S135L mutation, and 8 were found to be heterozygotes. This number of heterozygotes is equivalent to a heterozygous allele frequency of 0.0079. Given that the S135L mutation is known to be prevalent in black populations,[14] the 0.79% prevalence is not unexpected considering that 99% of the screened infants were of African origin and descent. However, since there are population groups in the Mpumalanga region that are of mixed ancestry and/or not of African origin, and members of these groups participated in the screening programme, the Q188R mutation common in Caucasian populations was also screened for. Using the real-time protocol, none of the infants presented with heterogeneity for the Q188R mutation. Studies of several ethnic groups around the world suggest that specific sets of mutations tend to segregate with specific populations.[2,4,5]

Cost savings

The total input cost of combined screening was calculated to be up to 20% lower than when classic galactosaemia and congenital hypothyroidism were screened for individually. The actual input costs of screening are set out in Table I. These figures exclude other savings that may be possible through use of community networks such as tracing parents using the established taxi system. Individual taxi drivers generally know the people on the rural parts of their routes, and if a repeat sample is required one informs the taxi driver, who lets the parents know that they should take the baby back to the hospital where he or she was born. This system was employed in at least 8 of our cases in which second samples were required, and all the mothers brought the infants back within 48 hours of the request being logged with the taxi driver. The

TABLE I. COMPARISON OF THE CONVENTIONAL NEWBORN SCREENING COST STRUCTURE AND THE ONE USED IN THIS STUDY BASED ON 12 SAMPLES COLLECTED PER DAY

	Conventional (South African rands)	Our study (South African rands)
Sample collection	160	80
Sample preparation and courier services	150	75
Laboratory analysis (primary and secondary assays)	350	350
Results interpretation and dissemination	95	95
Total	755	600

rest of repeat samples required were co-ordinated through a direct phone call to the mothers of the infants or through a relative who had a mobile phone.

In summary, our programme applies three cost saving strategies: (*i*) one piece of equipment (Coda EIA analyser, Bio-Rad) is used to screen for both diseases, and one specimen per patient is used for the identification of galactosaemia and hypothyroidism; (*ii*) incorporation of a real-time PCR protocol to identify commonly presenting mutations as a second-tier protocol to metabolic screening has the potential to reduce costs further if the presenting population mutation profile is well established; and (*iii*) since both classic galactosaemia and congenital hypothyroidism can cause mental retardation and can be treated with a satisfactory outcome, it is logical that a screening programme for both disorders that integrates disease identification and treatment will be highly cost effective.

Conclusion

This first South African newborn hormone screening study found an incidence of 0.1% for congenital hypothyroidism and no patients with classic galactosaemia. The study also demonstrated the cost effectiveness of simultaneous screening. It is critically important to screen newborns for galactosaemia and hypothyroidism where possible, since the diagnosis of both disorders currently depends mainly on clinical suspicion. Our full protocol includes collation of clinical information, proper handling of samples, biochemical tests, molecular tests, and patient treatment and/or disease management of affected individuals. Overall, cost-effective newborn screening with proper patient care and follow-up is possible even in the most remote of rural settlements with this study model.

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