Establishment of the First Newborn Screening Program in the People’s Democratic Republic of Laos

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Summary

Objectives: The People’s Democratic Republic of Laos belongs to the minority of countries worldwide without an established newborn bloodspot screening (NBS) system.

Methods: In 2008, we initiated a pilot project of a neonatal screening system in the delivery suites of the Laotian capital, Vientiane. Samples were analysed for thyrotropin-stimulating hormone and 17-hydroxyprogesterone.

Results: Altogether 11 362 samples were taken; an initially high recall rate dropped eventually to just above 4%. Two cases of hypothyroidism and one case of congenital adrenal hyperplasia were identified and received timely treatment.

Conclusions: In summary, we have demonstrated the feasibility of establishing an NBS system in a low-resource setting as prevalent in Laos. Obstacles for the establishment of a general NBS covering the whole country include the question of financial cover, treatment costs, and adequate teaching and supervision of technicians and doctors.

Key words: congenital adrenal hyperplasia, hypothyroidism, neonatal screening, newborn bloodspot screening, Laos, Southeast Asia.

Introduction

Newborn bloodspot screening (NBS) is a means of early identification of inborn errors of metabolism and endocrine diseases. Early detection and therapy of these conditions have been shown to reduce infant morbidity and mortality [1]. Whereas NBS was introduced during the early 1960s in Europe and the USA, first efforts to start NBS in the Asia Pacific region date back to the mid-1960s [2]. In the very beginning, only developed countries like New Zealand, Australia and Japan started establishing NBS systems. Also at that time, Singapore began screening for Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency. During a second phase, in the 1980s, countries like Taiwan, Hong Kong, India, Malaysia and the Shanghai area within China initiated their screening activities [2]. In the following decade (1990s) and on the grounds of other countries’ experience, screening programs were established in Korea, Thailand and the Philippines [2]. Since the year 2000, screening for congenital hypothyroidism has been initiated in Indonesia, Mongolia, Sri Lanka, Myanmar and Pakistan [2]. At that stage, there were no screening activities reported from Nepal, Cambodia, Laos and the Pacific Island nations. The lack of an existing screening program became obvious during several neonatal teaching sessions at the University of Laos in Vientiane, Laos, between 2004 and 2006.

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the framework of a master degree course in Obstetrics and Gynaecology organized by a German non-governmental organization, there were teaching sessions on neonatal topics covering also neonatal screening. This workshop and the following on-going debate were the trigger to proceed with applications for funding and organization of a pilot project of neonatal screening in Laos. As opposed to others, we decided to implement the necessary teaching within Laos rather than inviting those concerned with screening to other countries already in possession of established screening programs [3]. There were several declared aims of our pilot study: firstly, to get information about the frequency of hypothyroidism and congenital adrenal hyperplasia (CAH) in the urban area of Vientiane; secondly, to establish a collection system for bloodspot specimens within the city of Vientiane; and thirdly, to show the feasibility of recall and confirmation testing in the management of positive tests. Medium- and long-term aims were the establishment of methods of laboratory determination for a variable number of screening parameters, extension of the screening to other large urban areas in Laos and, in the long run, to the whole country and extension of the number of parameters screened for to those diseases that can be treated successfully and that would be affordable to the majority of the population.

Material and Methods

First bloodspot samples were taken after an initial workshop on neonatal screening in July 2008. The aim was to include every newborn infant in all of the participating hospitals in the urban area of Vientiane. Samples were either taken by capillary heel prick or by venous puncture according to the preference of the attending physician. Neither nurses nor midwives were involved in the procedure of blood sampling. Interestingly, physicians in Laos expected some kind of incentive, and we ultimately agreed to pay the amount of 0.23 USD per sample for blood sampling. If possible, samples were taken on the second day of life, earlier only if discharge was imminent. The aim was that no infant should leave the hospital without having blood taken for NBS, unless the parents refused consent. As can be easily seen (Table 1), participation was unevenly distributed between hospitals and not exclusively related to the number of deliveries. NBS samples were then collected at a central place in Vientiane and sent by air transport to Hamburg, Germany, every Friday. The reason for sending samples on Friday was to ensure that samples would not arrive on the weekend at the screening laboratory so as to avoid unnecessary delays. Screening results were sent by email to the coordinating centre in Duesseldorf and to the study centre in Vientiane. Retests were organized by the local study centre in Vientiane, excessively high screening results were identified in Duesseldorf and recommendations emailed to Vientiane regarding start of therapy or further confirmatory tests. The arrangement of retests was much facilitated by the recent advances in telecommunication. Because in Laos, nowadays, approximately 90% of women in childbearing age are in possession of a mobile phone, the loss to follow-up during NBS was negligible.

Participating hospitals in the urban area of Vientiane were the large delivery hospitals: Mahosot Hospital, Sethathirath Hospital, Mittaphab Friendship Hospital and Mother & Child Health Hospital (for details see Table 1). During the time course of the project, other hospitals in Vientiane and also from other cities joined in and collected specimens: Vientiane Provincial Hospital Maria Theresa, Champasack Provincial Hospital, Borikhamxay Provincial Hospital and Khammouane Provincial Hospital (for geographical details see Figure 1). The last samples during this pilot trial were collected in May 2010. Cut-off levels for thyrotropin-stimulating hormone (TSH) were set at $>20\mu U/ml$ and $>50\text{nmol/l}$ for 17-hydroxyprogesterone. In the beginning, cut-off levels were set as normally used in

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Deliveries per year</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother &amp; Child Health Hospital, Vientiane</td>
<td>3500</td>
<td>6866</td>
</tr>
<tr>
<td>Sethathirath Hospital, Vientiane</td>
<td>2000</td>
<td>1653</td>
</tr>
<tr>
<td>Mahosot Hospital, Vientiane</td>
<td>2500</td>
<td>119</td>
</tr>
<tr>
<td>Mittaphab Friendship Hospital, Vientiane</td>
<td>700</td>
<td>1134</td>
</tr>
<tr>
<td>Vientiane Provincial Hospital Maria Theresa, Vientiane</td>
<td>1600</td>
<td>893</td>
</tr>
<tr>
<td>Champasack Provincial Hospital, Champasack</td>
<td>1600</td>
<td>447</td>
</tr>
<tr>
<td>Borikhamxay Provincial Hospital, Borikhamxay</td>
<td>1000</td>
<td>131</td>
</tr>
<tr>
<td>Khammouane Provincial Hospital, Khammouane</td>
<td>1300</td>
<td>119</td>
</tr>
<tr>
<td>Total</td>
<td>14200</td>
<td>11362</td>
</tr>
</tbody>
</table>

TABLE 1

Participating hospitals with numbers of deliveries per year and number of samples sent
Germany (TSH >15 µU/ml, 17-OHP >30 nmol/l). However, this led to a very high number of retest requests owing to early blood sampling. We obtained a positive vote from the ethics committee of the University of Laos in Vientiane; parents of all participating infants gave informed consent.

**Results**

In total, there were 11,362 samples sent to the German screening laboratory, 633 (5.57%) of which were above the cut-off levels as described previously. The rate of retests was higher between September 2008 and February 2009 (9.90%), and decreased after the change of cut-off levels to 4.32% for the remainder of the pilot study. Although in Germany, samples for newborn bloodspot screening are taken beyond the first 36 h of life, average sampling time in the largest delivery hospital in Vientiane (Mother & Child Health Hospital) was just below 18 h. More than 83% of samples were taken during the first 24 h of life, some of them as early as 1 h after delivery. Early blood sampling led primarily to frequent retests related to TSH and 17-OHP; often both parameters were above the pre-defined cut-off level (for details see Table 2). The total number of recommended retests as communicated by the screening laboratory was 633, 276 (44%) of which ultimately arrived at the Hamburg laboratory. The remainder of 357 samples (56%) was
not initiated either because patients did not return to the hospitals or owing to the fact that the initial level was regarded as being just outside the reference value, and a retest was subsequently regarded as unnecessary by the local paediatrician in charge.

### Discussion

The establishment of an NBS system appears feasible also in a low-resource setting as present in the People’s Democratic Republic of Laos. As before our pilot trial there have been no newborn screening data available from Laos [2], we started initially to screen for TSH and 17-OHP. Owing to the lack of laboratory equipment in Vientiane, after air transport, tests were performed in a German screening laboratory in Hamburg. Despite early discharge policies in Laotian delivery hospitals implying early NBS sampling, longer duration of air transport and early notification of abnormal results by email, we managed the initiation of treatment in cases of confirmed disease in due time. Early blood sampling also had the disadvantage of a higher number of false-positive results particularly affecting measurements of TSH and 17-OHP. Apart from affecting the number of retests, early sampling can also increase the risk of missing cases of hypothyroidism due to delayed rises of serum TSH. To minimize this risk of missing cases with delayed rise of TSH, a repeated screening has been advocated [4]. With two identified cases of hypothyroidism of 11,362 samples, the frequency of the disease corresponds to European and North American numbers. These results should alleviate the concern that the high prevalence of iodine deficiency might imply a higher incidence of hypothyroidism in Southeast Asia [5, 6].

Testing for 17-OHP resulted in a similar number of retests as testing for hypothyroidism (see Table 2). Again early discharge and subsequently early testing are the main responsible factors for this phenomenon. Because gestational age and 17-OHP levels are inversely related, even higher numbers of retests could be expected, unless corrections for low gestational age are made [7]. These corrections are already integrated in the recall system of the Hamburg screening laboratory and should also be used when establishing a Laotian recall system in the future. Within the whole pilot project we identified one case of CAH, which is again within the expected range. It has to be noted that screening results do not differentiate between CAH infants with and without salt loss. Because the majority of infants are at risk for salt loss, all infants identified to have CAH in the developed world are treated with hydrocortisone and a mineralocorticoid. The latter is not readily available in Laos, which should be kept in mind for the refinement of future screening activities.

We limited the screening program to the parameters TSH and 17-OHP being the most frequent and easiest treatable diseases. The main reason not to screen further for Phenylketonuria (PKU) and other inborn errors of metabolism was the lack of available treatment options, e.g. the availability of a standardized phenylalanine-restricted diet in Laos. Even in countries with higher-resource settings like neighbouring Thailand and despite the availability of phenylalanine-restricted diet, moderate to severe psychomotor retardation appears to be frequent [8].

Countries with increased resources have recently shown how efficiently tandem mass spectrometry can be used for NBS [9, 10]. However, in a low-resource setting like Laos, efforts might be better concentrated on establishing a conventional screening system including only one or two parameters initially. The challenge then will be to cover the whole of Laos, including the minorities in the mountainous regions of the country. Because there is no general health insurance system in Laos, the individual family would have to pay for the screening costs. The same is obviously true for treatment costs. L-thyroxine is comparably cheap in Laos, daily treatment costs are in the range of 0.01 USD. Treatment of CAH patients is clearly more expensive, particularly because mineralocorticoids have to be imported from neighbouring Thailand. Apart from the monetary aspect, there will be an on-going need for teaching and supervision accompanying the general introduction of an NBS system.

Non-compliance with the recommendations of the screening laboratory will have to be discussed when NBS will become standard in the future. More than 50% of the recommended retests were not performed, the majority of which were not initiated by the paediatrician in charge because levels were just outside the reference values. Strict adherence to pre-defined cut-off values is pivotal for the successful establishment of any screening system. The obvious risk when increasing cut-off levels is to miss cases of hypothyroidism with a gland in situ and mild permanent thyroid dysfunction later in life [11].

In summary, we have shown the feasibility of establishing a newborn screening system in a low-resource setting like Laos. Despite the time delay caused by air transport, treatment could be initiated sufficiently early in infants diagnosed with hypothyroidism and CAH. A major problem was the non-compliance of local physicians with the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of retests</th>
<th>Confirmed diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>270</td>
<td>2</td>
</tr>
<tr>
<td>17-OHP</td>
<td>269</td>
<td>1</td>
</tr>
</tbody>
</table>
recommendations of the screening laboratory for re-testing. Because of low patient numbers in our pilot project, we cannot really comment on local incidences of the diseases screened for. However, according to our impression, hypothyroidism and CAH appear to be not dramatically more frequent in the urban area of Vientiane compared with the rest of the world.

References