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Research Article

Neonatal Screening for Aminoaciduria: Can TLC be used as an Affordable Method in Developing Countries?

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Abstract: In the view of high birth rate, a large burden of preventable mental retardation in the form of aminoaciduria occurs in developing country like India. While neonatal screening by tandem mass spectrometry (TMS) and HPLC (High Pressure Liquid Chromatography) is almost universal in developed countries but scarce in India due to high cost. Hence, this study was planned to asses thin layer chromatography (TLC), a simple, economic and easily available technique as a possible tool for mass neonatal screening. The study was conducted in departments of Biochemistry and Pediatrics, Christian Medical College and Hospital, Ludhiana. All the newborns born/ admitted at the institution over a period of 18 months were subjected to urine examination for aminoaciduria by thin layer chromatography (TLC). HPLC for aminoaciduria was done in both TLC positive and clinically suspicious cases. Aminoaciduria (homocystinuria) was detected in one case among 1530 neonates screened by TLC and confirmed by HPLC. In 58 clinically suspected neonates, both TLC and HPLC were negative for aminoaciduria. TLC can be an effective and cheaper alternative to TMS to begin mass screening for aminoacidurias. Besides reducing morbidity it can also be a stepping stone for future introduction of TMS based universal screening. The incidence and pattern of aminoacidurias in north India seems to be different from other countries as well as south India; homocystinuria appears to be the commonest.
Keywords: Aminoaciduria, Neonatal Screening, Thin layer chromatography.

INTRODUCTION

Almost half a million infants with congenital malformation and/or inborn errors of metabolism are born every year in India because of high birth rate and even an apparently healthy newborn has a significant risk of being maimed or killed by inborn error of metabolism. Being a developing country, the health policies in India have typically targeted mortality and infectious morbidities but not disabilities. These policies have been successful in lowering infant mortality rates, but the net effect of these gains have been somewhat offset by an increase in disability [1].

Aminoacidurias are the most common cause of preventable mental retardation after congenital hypothyroidism among neonates [2]. Thus, mass screening is needed to prevent disability and death by early intervention, follow-up and counseling. The increasing level of literacy and wealth is making parents aware of metabolic disorders but the rarity of testing facilities and very high cost of detection discourage the implementation of universal screening.

Aminoaciduria is defined as increased amount of one or more amino acids in urine [3]. It can be diagnosed with bio-assay, chromatography [4], tandem mass spectrometry [5] or HVE (High Voltage Electrophoresis) [6]. The worldwide incidence of aminoaciduria ranges from 1:200 in Saudi Arabia to 1:6250 in Australia [7]. No nation-wide study is available in India, though one study in south India reports the incidence to be 1:3660 [8]. While from North India, no incidence study is available.

In this study thin layer chromatography (TLC) was used to screen neonates as it is a reliable method for qualitative as well as semi quantitative estimation of amino acids in urine. In addition, it is economical and more readily available than HPLC (High performance liquid chromatography) & TMS (Tandem mass spectrometry), while being superior and faster than paper chromatography in estimation.

Thus, keeping in view the high cost of diagnosis, this study was designed to find the prevalence of aminoaciduria in this part of globe and to evaluate TLC as an effective and economic alternate of TMS, which has been widely used in US and other developed countries where newborn screening is universal [5].

MATERIAL AND METHODS

This prospective study was conducted in the departments of Biochemistry & Pediatrics, Christian Medical College and Hospital, Ludhiana for one and half year. All the live births and admitted newborns at this institution during this period were subjected to screening for aminoaciduria.

An informed consent was taken from parents of all the newborns, who were to be subjected to screening after obtaining approval from institutional ethical committee. A careful family history was taken for any of the inborn errors of metabolism in the form of mental retardation, frequent abortions, still-birth and early neonatal death. Urine samples were collected using sterile precautions from neonates, before discharge. The prerequisite of the study was that the baby should have received at least 3-4 milk feeds before the sample was collected [1, 9].

Thin Layer Chromatography was used as a screening technique, using butanol: acetic Acid: water as solvent (mobile phase) and ninhydrin as staining agent [8]. TLC plates were coated with cellulose with the help of commercially available template and applicator from Accumax, India.

The urine was examined for cystine, ornithine, lysine, arginine, phenylalanine, tyrosine, threonine, homocystine, valine, isoleucine, leucine, methionine and hydroxyproline to screen following diseases:

Cystinuria, arginosuccinuria, phenylketonuria, alkaptonuria, threoninemia, homocystinuria, maple syrup urine disease and cystathionuria. Ferric chloride test (for phenylketonuria, MSUD), dinitrophenol test (for maple syrup urine disease and phenylketonuria) and cyanide nitroprusside test (for homocystinuria) were performed on all urine samples [4].

The case was also analyzed for serum homocysteine level with sandwich ELISA assay by kit from Axis/ Shield diagnostic limited, U.K.; serum vitamin B12 and folic acid level with Electrochemilluminiscence Assay (ECLIA) by kit from Roche diagnostics using sandwich assay.

The diagnosis was confirmed by reverse-phase HPLC following the pre-column derivatisation with phenylisothiocyanate (PITC) in dried blood spot collected on filter paper [8]. 58 other cases with strong clinical suspicion but TLC negative were also confirmed to be negative with the same HPLC method.

The incidence of aminoaciduria has been calculated with standard formula of dividing the number of case by total of live births and admitted neonates.

RESULTS AND DISCUSSION

The incidence of aminoaciduria was found to be 1 per 1530 live births and the only case detected was a male baby suffering from homocystinuria. On follow up at the age of 6 months, the case identified with homocystinuria had complaints of epiphora and glaucoma and had development quotient of 0.5 for fine motor reflexes. The affected baby had history of death of elder male sibling with respiratory cause (bronchopneumonia) at 10 months of age.

Glycine (81.3%), histidine (79.5%) and alanine (69.3%) were found to be the most commonly present aminoacids in the urine of neonates, while glutamic acid, threonine, lysine, arginine, aspartic acid, serine were also frequently found in the urine of normal newborns. Valine, isoleucine, leucine, phenylalanine, methionine, proline, hydroxyproline were not detected in urine of any of the newborn.

The diseased newborn was found to have serum vitamin B12 level 78 pg/ml (reference range 160-970 pg/ml), serum folic acid 1050 ng/ml (reference range 8.6- 37.7 ng/ml) and serum homocysteine levels of 70 µmol/L (reference range 2.9-16 µmol/L) in serum sample. The urine of the case was positive for cyanide-nitroprusside test while all other urine samples were found negative for the same. Ferric chloride test, dinitrophenol test were found to be negative for all the urine samples.

The HPLC test on blood spot was also done on 58 neonates with high clinical suspicion (15 with hyperammonemia, 18 with hypoglycemia, 11 with seizure and 14 with metabolic acidosis), but no case of aminoaciduria was detected out of them. All these cases were being negative for aminoaciduria in TLC screening.

In this study the incidence of aminoaciduria was found to be higher than that observed in a study in four major government hospitals in Hyderabad, India, where it was reported to be 1 per 3660 [8].

Phenylketonuria has not been found to be the commonest aminoaciduria in North India in a study conducted at AIIMS by Manjit Kaur and Verma on 2560 cases with mental retardation. They reported that the commonest amino acids disorders were homocystinuria, hyperglycinemia, alkaptonuria and maple syrup urine disease in this high risk North Indian population group. However, the data on the frequency of various metabolic disorders among subjects with mental retardation cannot be used to provide the frequency of these disorders at birth. This study

indicates that homocystinuria is more common than phenylketonuria in north India [4]. In a different publication on clinical and biochemical studies in homocystinuria, the same authors mentioned that 10 out of 15 cases positive for homocystinuria were from Punjab. The same study in north India reported that the all 15 cases positive for homocystinuria had presented with some ophthalmic lesion. Though glaucoma was found in 2 (13.3%) out of 15 cases positive for homocystinuria [10]. In the present study also the case identified for homocystinuria had complaints of epiphora and glaucoma on follow up at 6 months of age. As the study was conducted on newborns, the relation with mental retardation could not be established. However, on follow up at the age of 6 months, the case identified with aminoaciduria had development quotient of 0.5 for fine motor reflexes.

The TLC has been found to be fairly acceptable for detection of amino acids in urine. The cases negative by TLC has remained in agreement with the results of ferric chloride test, DNP test and cyanide-nitroprusside test. Moreover, the case detected as positive for homocystinuria was also confirmed as case by HPLC on blood spot and its serum homocysteine levels (by ELISA) was also elevated. Many workers have used TLC successfully and reported it as useful and affordable tool in their studies on detection of aminoaciduria [4, 11, 12]. In our study, TLC was found to be 100% specific and even in cases with strong clinical suspicion, TLC has been found to be in agreement (negative for aminoaciduria) with reverse phase HPLC.

QNUSP (Québec Newborn Urine Screening Program) reports that studies on MS/MS indicate sensitivity of TLC generally exceeds 90% when screening for several of the diseases targeted in their screening program [13]. Though, false positive reports are reported to be more with TLC in comparison to TMS by Levy HL [14], we did not find any false positive case in our study.

The collection of a urine specimen is noninvasive, unlikeblood sampling, which being invasive may lead to parental refusal. It also has the advantage of permitting the detection of inborn errors of metabolism associated with metabolite transport [13].

In our study, use of TLC is found to be quite economic. The requirement include just a plastic template, an applicator and a small glass tank along with simple reagents like butanol, acetic acid, distilled water (as mobile phase), cellulose (as coating) and ninhydrin (for staining). With this simple in house plate coating apparatus, the cost was very nominal per sample which can even be brought down if used for larger number of samples. Its cost is very less when compared with HPLC and TMS, which are mostly unavailable in our country. So, it can be very easy to implement the use of TLC for mass screening. Even in developed countries, the neonatal screening was started with simple tests like Guthrie's test before the availability of TMS.

The study was hospital-based and time bound with collection of limited number of samples. HPLC could not be done on all the negative samples due to cost limitation. Larger studies or screening programs are required to address this unmet need of society.

CONCLUSION

With availability of such economic, reliable and time tested procedure, the routine screening must not be overlooked especially in the background of high birth rate and preventable morbidities in our country. TLC can be used for mass screening of aminoaciduria in developing countries. There is need to increase the awarenessin health care staff, administration and general public especially in developing country like India regarding the availability of such affordable and reliable test.

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