ORIGINAL ARTICLE

Diagnostic evaluation of heel prick newborn screening of thyroid stimulating hormone on dissociation-enhanced lanthanide fluorescence immunoassay with the establishment of reference value in Pakistani neonates

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Abstract

Objective: To develop and validate a method on Dissociation Enhanced Lanthanide Fluorescence Immunoassay for neonatal heel prick blood human thyroid stimulating hormone and the establishment of its reference value in the local population.

Methods: The multi-centre cross-sectional validation study was conducted from September 2016 to September 2018 at Zahra Beau Naqvi Foundation Welfare Trust laboratory, Islamabad, Pakistan, and comprised samples related to newborns aged 1 month or less taken from neonatal units of 39 hospitals based in Punjab, Khyber Pakhtunkhuwa, Gilgit-Baltistan and Azad Jammu and Kashmir. Samples were collected after 24 hours of birth using the heel prick test. The samples were dried and sent to the laboratory for assessment where Dissociation Enhanced Lanthanide Fluorescent Immunoassay was used to estimate thyroid stimulating hormone levels. Data recorded included age, gender, and birth detail, like gestational age, mode of delivery etc. Data was analysed using SPSS 21. Method validation and reference value were manually calculated.

Results: Of the 14,147 samples received, 8,207(58%) related to boys and 5,940(42%) to girls. Most samples 4903(34.6%) came from Peshawar. The overall mean age of the newborns was 5.6±4.8 days. Thyroid stimulating hormone data was divided into three groups; positive with median value 27.8±36.6 uIU/ml, negative with median 1.42±1.60 uIU/ml, and borderline with median 11.4±4.12uIU/ml. Prevalence of congenital hypothyroidism in high-risk population in the positive group was 39(0.3%), negative 14,012(99.0%) and borderline 96(0.7%). Reference cut-off was calculated as 7.06uIU/ml for screening of healthy and positive cases of congenital hypothyroidism. Method Validation results showed limit of detection -0.5uU/ml, limit of quantitation LOQ 0.8uU/ml, accuracy 100±5%, precision coefficient of variation at each level of calibrators -4, 8.8, 1.2, 11.3, 7.2 and 4.3% respectively, and linearity from to 0.8uU/ml to 254.1uU/ml.

Conclusion: Neonatal human thyroid stimulating hormone by heel prick blood was found to be an affordable and highly sensitive method of screening for congenital hypothyroidism.

Keywords: DELFIA, Dissociation enhanced fluorescence immunoassay, Newborn screening, Congenital hypothyroidism. (JPMA 71: 191; 2021)

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Introduction

Newborn Screening (NBS) is vital for the early diagnosis of inherited metabolic disorders (IMDs) that cause unnecessary emotional burden and socioeconomic challenges for the affected families. According to Child Mortality Report of 2017, congenital disorders contributed 11% to the deaths in children aged <5 years and Pakistan is one of the five countries that accounted for half of all newborn deaths.[1]

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If IMDs are diagnosed in infancy and treated as prescribed, the disease management can prove life-saving.[2] Congenital hypothyroidism (CH) is one of the most abundant of the IMDs found in Pakistan. It is considered to be the common cause of mental retardation.[3] The worldwide incidence of CH is 1 in 4000.[4] In a recent study conducted in a tertiary care hospital of Lahore, despite its very small sample size (770), high frequency of congenital hypothyroidism was reported to be 1 in 257 patients.[3] In another study done in Islamabad, the incidence was 3 in 1337 babies.[5]

The decrease in thyroid stimulating hormone (TSH) threshold over time with technological advancement has increased the sensitivity and specificity of NBS equipment.[6,7] This has ultimately resulted in an increased reported incidence of CH.[8] Generally, TSH cut-off <15 U/L

is considered good enough to identify both transient as well as permanent hypothyroidism.[9] The International Federation of Clinical Chemistry (IFCC) and the Clinical Laboratory Standards Institute (CLSI) recommend that reference value of any parameter should be population-based, calculated from reference population with a minimum of 120 individuals identified from a reference population through probability sampling and using non-parametric statistical methods without having disease of concern. For screening of neonatal hypothyroidism, the neonate should be defined as any newborn aged 30 days or less.[10,11]

Unfortunately, Pakistan is among the countries where NBS has still not been institutionalised to address the alarming need to prevent IMDs. The current study was planned to develop and validate a method on Dissociation Enhanced Lanthanide Fluorescence Immunoassay (DELFIA) for neonatal heel prick blood human thyroid stimulating hormone (hTSH) and the establishment of its reference value in the local population.

Materials and Methods

The multi-centre cross-sectional validation study was conducted from September 2016 to September 2018 at Zahra Beau Naqvi Foundation (ZBF) Welfare Trust laboratory, Islamabad, Pakistan, and comprised samples related to newborns aged 1 month or less taken from neonatal units of 39 hospitals based in Punjab, Khyber Pakhtunkhuwa, Gilgit-Baltistan and Azad Jammu and Kashmir. After approval from ethics review committee of Izzat Ali Shah Hospital, Wah Cantt, Pakistan, the sample size was calculated at 95% confidence interval (CI), 0.9/1000 acceptable difference. Our sample size for this rare disease was 6145 as calculated per guideline mentioned in literature with prevalence sample size formulae considering prevalence of disease 0.025% (incidence 1/4000) worldwide, precision was half of prevalence of rare disease and as for screening study we even duplicate this sample size.[4,13] Samples taken came from newborns aged 1 month or less. After consent from the parents, the samples were collected after 24 hours of birth using the heel prick test using all due aseptic precautions onto Guthrie cards approved by the United States Food and Drug Administration (FDA). The samples were dried and then sent to the ZBF laboratory for assessment. Replacement samples were requested against the rejected samples by the lab. DELFIA was used to estimate the TSH levels. Infants with TSH level equal to 9 µU/mL and above were declared borderline, and, therefore, repeat samples were requested from the hospital concerned. Infants with TSH level equal and above 18 µU/mL were declared positive, and the parents concerned were advised to opt for the confirmatory test which comprised serum testing of thyroxine (T4) and TSH.[10]

Data noted comprised gender, age, specimen collection date, hospital name, patients' and doctors' contact detail, birth details, like gestational age, mode of delivery, Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) score at birth, and singlet or twin. Data was analysed using SPSS 21. Test of normality Shapiro Wilk was applied on data which showed its nonparametric nature, and, non-parametric descriptive statistics of quantitative variables were done and expressed as median and interquartile range (IQR). Qualitative data was expressed as frequencies and percentages. Non-parametric Mann Whitney t statistic was applied for significant gender difference of heel prick Guthrie card blood TSH considering $p<0.05$ as statistically significant. Reference interval (cut-off) determination was done by using Reference Value Advisor (RVA) v2.1 (National Veterinary School, Toulouse, France)[11]. Reference cut-off 7.06 uIU/ml was calculated which differentiated non-disease from CH suspicion, and then reference intervals were calculated for the three groups using Reference Value Advisor, available as freeware at, http://www.biostat.envt.fr/spip/spip.php?article63).[12] Reference value of heel prick blood TSH was taken.[11,12] TSH heel prick method was evaluated on DELFIA with neonatal hTSH kit (Perkin Elmer Wallac DELFIA catalogue No: A032-310) containing 6 calibrators with concentrations 1.0, 9.8, 23, 48.3, 98.8, and 257 uU/ml and 2 levels of controls: Low 16.1 (12.9-19.3uU/ml) and High 66.4 (53.1- 79.7 uU/ml). Method validation was done against following limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, linearity, roboustness.

Results

Of the 14,147 samples received, 8,207(58%) related to boys and 5,940(42%) to girls. The overall mean age of the newborns was 5.6±4.8 days. Most samples 4903(34.6%) came from Peshawar (Table-1). Data were not normally distributed and was significantly positively skewed ($p<0.000$). The nonparametric TSH data was divided positive 39(0.3%) with median 27.8±36.6 uIU/ml, negative 14012(99.0%) with median 1.42±1.60 uIU/ml, and borderline 96(0.7%)11.4±4.12uIU/ml groups (Figure).

Reference interval was calculated as 15.47-165.21 uIU/ml for positive group, 0.15-6.32 uIU/ml for negative group and 9.15-17.6 uIU/ml for borderline group. The reference values had no significant association with gender ($p>0.784$).

Table-1: Distribution of Guthrie cards samples collection sites.

Cities Names	Frequency and % of Guthrie samples collected	Cities Names	Frequency and % of Guthrie samples collected
Islamabad	4664 (33%)	Gilgit	176 (1.3%)
Peshawar	4903 (34.6%)	Lahore	114 (0.8%)
Wah	2887 (20.4%)	Gujranwala	86 (0.6%)
Sheikhupura	808 (5.7%)	Others	252(1.8%)
Rawalpindi	256 (1.8%)		

Table-2: Showing Results of Method validation of neonatal human thyroid stimulating hormone (hTSH) by Dissociation Enhanced Lanthanide Fluorescence Immunoassay (DELFIA).

Parameter	Method validation calibration						Precision CV%
	Expected conc.(uU/ml)	Recovered conc.(uU/ml)	Accuracy (%)	LOD (uU/ml)	LOQ (uU/ml)	AMR (uU/ml)	
Neonatal heel prick blood hTSH	1.0	1.003	100.3	0.5	0.8	254.1	4.0
	9.8	9.8	100				8.8
	23	22.98	99.9				1.2
	48.3	50.38	104.3				11.3
	98.8	104.0	105.2				7.2
	257	254.1	98.9				4.3

LOD: Limit of detection; LOQ: limit of quantitation; CV Coefficient of variation; AMR: Analytical Measurement Range.

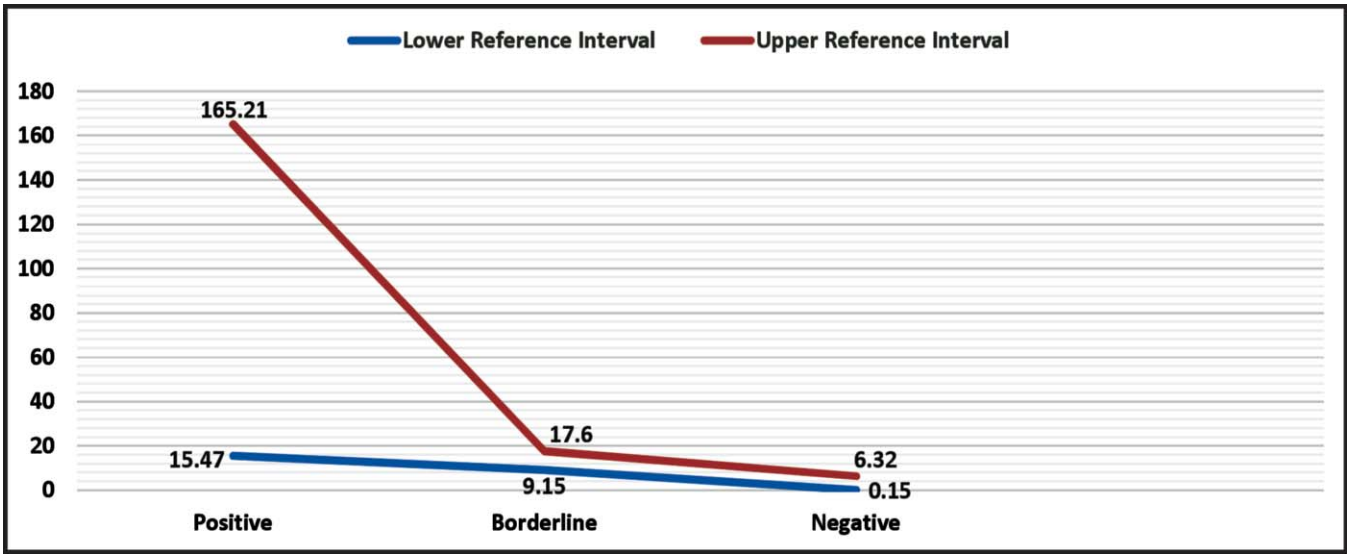

Figure: Showing Reference Intervals of heel prick blood thyroid stimulating hormone (TSH) on Dissociation Enhanced Lanthanide Fluorescence Immunoassay (DELFIA) for Newborn Screening (NBS) for three levels; negative (0.15 to 6.32) uU/ml, borderline congenital hypothyroidism (9.15 to 17.6) uU/ml and Positive congenital hypothyroidism (15.47 to 165.21) uU/ml.

LOD, or analytical sensitivity was found to be 0.5 uU/ml, LOQ 0.8uU/ml, accuracy 100±5%, precision coefficient of variation (CV)% at each level of calibrator was 4, 8.8, 1.2, 11.3, 7.2 and 4.3 % respectively, while linearity was from 0.8uU/ml to 254.1uU/ml, which was analytically specific to TSH and not affected by haemolysis, icteric and lip emic nature of blood and robustness to delayed handling and temperature effects at 2-4C0 and 26-32C0 (Table-2). Precision of control materials showed laboratory-specific, Low control with mean value 15.6±1.55 uU/ml with inter-assay and intra-assay variability CV <9.94%, and High control with mean value 67.9±6.8uU/ml with inter-assay and intra-assay variability CV <10.0%.

Discussion

To the best of our knowledge, the current study is first of its kind in Pakistan which not only showed CH prevalence in infants as screened on DELFIA, but also showed

reference value of heel prick blood on Guthrie cards.

The study showed positivity rate/prevalence of borderline and high-risk categories of CH by TSH-only methodology as it is the most commonly used technique for screening of thyroid diseases. The CH prevalence was found to be 0.3% with male predominance, while in previous studies[6-8,11] worldwide CH incidence has been reported to be 1 in 4,000.[4] Previous local hospital-based studies in different cities of Pakistan showed CH incidence of 1 in 257[3] and 3 in 1337 babies[5] which is higher than the current findings. The difference with studies in different parts of the world was in terms of sample size and high-risk neonates, while the selection of neonate was flexible in the current study.

The current study established the reference cut-off of 7.06 µU/mL which differentiated non-disease from CH suspicion. It was lower than 9 µU/mL and 15 µU/mL reported in previous studies.[9,10] Hence, for more confirmation, reference value against negative, borderline and positive CH cases was established to avoid any false negative (FN) cases due to the gravity of the disease. DELFIA technique was evaluated which showed very low detection and quantitation limit with greater sensitivity and specificity, and, thus, it was found to be a very useful method for CH screening.

NBS was only started to prevent the development of mental retardation in society within minimal resources and owing to the fact that no population-based facility was available. The adoption of this derived reference interval in Pakistan is recommended.

The current study has limitation as it used hospital-based data which always has certain positive biases.

Conclusion

Neonatal hTSH by heel prick blood on Guthrie card was found to be an affordable and highly sensitive method of CH screening at the population level which might prevent many infants from developing mental retardation with early prompt and inexpensive management.

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Conflict of Interest: None.

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