

Neonatal screening for hypothyroidism by time - Resolved fluoroimmunoassay in Jharkhand

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ABSTRACT

Objective: To screen newborns for congenital hypothyroidism by time resolved fluoroimmunoassay (TRFIA) (TRFIA meter by Perkin Elmer). **Methods:** Participants were randomly selected from the Department of Paediatrics/Obstetrics and Gynaecology, Rajendra Institute of Medical Sciences and Maternity Hospitals around 20 km of Rajendra Institute of Medical Sciences, Ranchi. Blood samples were obtained by heel prick for thyroid-stimulating hormone (TSH) estimation from newborns selected from above-mentioned hospitals. Samples were collected from 3rd day of life to 7th day of life on a filter paper card and analyzed by TRFIA-based method. **Results:** 60% of the newborns were male and 40% were female among 150 newborns selected for study. Out of them, 65.33% were term, 28% were pre-term, and 6.67% were post-term. 92% newborns had TSH level <10 mIU/L, 8% had TSH between 10 and 20 mIU/L and none had TSH value >20 mIU/L. **Conclusion:** The prevalence of borderline TSH assay was 80/1000 newborns, which is significant. Screening studies for such conditions need larger sample size, stronger logistics to cover a larger area.

Key words: Congenital hypothyroidism, Newborn, Thyroid-stimulating hormone, Time resolved fluoroimmunoassay

The aim of any neonatal screening program is to detect affected neonates and provide early intervention for preventable and correctable congenital conditions. Congenital hypothyroidism (CH) being one of the major correctable disease, if detected earlier can help in improving neurodevelopmental outcome of child and ultimately, leading to completely normal life for him [1-3]. Hypothyroidism occurs due to decreased production of thyroid hormones or a defect in thyroid hormone transport/or receptor activity. Causes of CH are thyroid dysgenesis, dyshormonogenesis, and transient and hypothalamic-pituitary defects. Early diagnosis and adequate treatment of neonatal hypothyroidism from 1st week of life result in normal growth and development of the affected child [4,5]. Most of the infants with CH are asymptomatic at birth because of transplacental passage of moderate amounts (1/3rd) of maternal T4. Transplacental passage of thyroid-stimulating hormone (TSH) does not occur [6]. Due to low level of serum T4 and concomitantly high levels of TSH in these newborns, it is possible to screen neonates to detect CH.

Heel prick TSH assay has been found to be more sensitive than cord blood TSH assay in identifying CH [7]. Neonatal screening for CH is a routine in most of the developed countries. Incidence of CH in developed countries is in the range of 1:3600-1:5000 newborns [8] while in India, it was reported to be as high as 2.1/1000 [9]. District-wise surveys

conducted by Indian Council of Medical Research/Director General of Health Services in Jharkhand state found that 8 of the 9 districts surveyed are endemic for iodine deficiency disorder. Consumption of adequately iodized salt was only 64.2% in Jharkhand [10]. We could not find any study on the prevalence of CH in children in Jharkhand; therefore, the current study was planned to fill this gap. Current random sample survey and screening for the presence of CH was carried out among children born in tertiary care hospitals of Jharkhand. Pregnant women from all over state report for delivery in these hospitals and other nearby hospitals.

METHODS

In this cross-sectional study was conducted over a period of 1 year after getting approval from the Institutional Ethics Committee. Written consent from parents of the newborns was taken before sampling. Babies were included in the study using sampling by convenience. In the current study, hospitals were selected around 20 km radius of Rajendra Institute of Medical Sciences to facilitate logistics. The nursing staffs, laboratory technicians, and residents were trained for the collection and storage of blood samples in a proper manner in newborns from 2nd to 7th day of life. The various screening programs recommend collection of blood sample between 2 and 7 days of life. The best time for screening

of neonates is 3-5 days of life when the post-natal physiological TSH surge subsides.

Blood was collected by heel prick method from the most medial or lateral portion of the plantar surface of the heel after taking universal precaution. The blood was collected on filter paper by gently touching it against the large blood drop and in one step a sufficient quantity of blood was allowed to soak through and completely fill three circles of about half inch diameter. Blood was applied to only one side of the filter paper. The samples collected were transported to processing laboratory.

Analysis of samples was done in the laboratory of Gen-next at Ranchi. The samples were tested for the determination of TSH level by time-resolved fluorometry-based dissociation-enhanced lanthanide fluorescent assay (DELFI A). The technology is based on the use of lanthanide chelate labels with unique fluorescence properties. The usefulness of a time-resolved fluoroimmunoassay (TRFIA) kit (DELFI A neonatal TSH) for the determination of thyrotropin TSH in dried blood spots in routine newborn screening for CH had been evaluated [11]. The new method fulfills the criteria for precision and sensitivity of a screening assay. Following cutoff values of TSH were considered: TSH <10 mIU/L - normal, TSH between 10 and 20 mIU/L - Borderline cases and TSH >20 mIU/L - hypothyroid.

RESULTS

A total of 150 newborns were included in the study. The mean birth weight was 2500 g (1160-3400 g) and mean gestational age was 38.3 weeks (28-43 weeks). Total 104 newborns were delivered vaginally and 46 were delivered by lower segment cesarean section (LSCS). In this study, there were 42 pre-term, 98 term and 10 post-term neonates. The gestational age of newborns was determined according to the obstetric history, antenatal ultrasound, and new Ballard scoring system. There were 14 (9.33%) very low birth weight babies, 82 (54.67%) low birth weight and 54 (36%) normal birth weight babies. 70 newborns had uneventful post-natal period, 36 newborns had perinatal asphyxia, 16 had neonatal jaundice, 20 had early onset sepsis, 6 had late onset sepsis, and 2 newborns had dehydration fever. National Neonatal Perinatal Database (2002-2003) definitions for above-mentioned neonatal morbidities have been used for the classification.

In this study, 40% of the sample was taken at 3rd day of life, 32% on 2nd day, 16% on 4th day, and 5.33% samples were taken on 5th day of life. Table 1 show that 92% of the newborns had TSH level <10 mIU/L and 8% had TSH between 10 and 20 mIU/L while no newborn had TSH >20 mIU/L. TSH level ranged from 1.16 to 15.74 mIU/L with a mean value of 5.94±2.9 mIU/L.

DISCUSSION

The prevalence of borderline TSH assay cases was 80/1000 newborns. We did not find any confirmed case of CH. 8% of the newborns in our series had values between 10 and 20 mIU/L. This is the group which needs further evaluation to confirm the thyroxine deficiency either acquired or congenital.

Table 1: Value of TSH among newborns (n=150) measured by heel – prick on 2nd-7th day of life

Mean±SD	5.94±2.9 mIU/L
Median range	5.23 (1.16-15.74) mIU/L
Mean TSH in newborns delivered vaginally	5.90±2.7 mIU/L
Mean TSH in newborns delivered by LSCS	5.63±3.28 mIU/L
Mean TSH in pre-term	5.92 mIU/L
Mean TSH in term	5.90 mIU/L
Mean TSH in post-term	5.48 mIU/L
Number of newborns with TSH values	
<10 mIU/L	138 (92)
10-20 mIU/L	8 (8)
>20 mIU/L	0 (0)

TSH: Thyroid-stimulating hormone, LSCS: Lower segment cesarean section, SD: Standard deviation

Mean TSH of newborns born vaginally were similar to mean TSH of newborns born by LSCS. We also did not find any significant difference in TSH level between pre-term, term, and post-term newborns. The average prevalence of CH worldwide is about 1 in 4000 (range of 1:3600-1:5000) newborns. Data on incidence of CH in India are emerging and these data point toward much higher burden of the disease in India.

Desai et al. in 1987 screened 12,407 newborns for CH using cord blood TSH measurements and found the incidence of 1:264 [12]. Sanghvi et al. reported an incidence of 2.1 in 1000 [9]. Newborn screening conducted in Hyderabad reported a prevalence of 1 in 1700 live births [13]. A study from Chandigarh reported prevalence of 1:3400 [14]. Medda et al. in a population case-control study found that there was high risk for CH in low birth weight and high birth weight newborns. They also reported that female gender, twin, additional birth defects, and post-term were significantly associated with permanent CH [15]. Gopalakrishnan et al. found significantly higher TSH level in males, low birth weight and vaginally delivered newborns [16]. We did not find such correlation probably due to small sample size. Some researchers have suggested lower TSH cutoff level for screening as a cutoff level of 20 mIU/ml would miss as much as 45% newborns with CH including cases of thyroid dysgenesis [17]. A recent study from Bengaluru used same method for TSH assessment (TRFIA - DELFI A) and lower cutoff value of 12 mIU/ml reporting an incidence of 1:825 for CH [18].

Limitation of study were small sample size and inability to conduct follow-up and do further confirmatory tests for the borderline cases. Collection of samples from other centers and motivating staffs to collect samples were the major hurdles in this study. Collection of neonatal blood sample demands strong operational and logistic efforts with a team approach. Our study highlighted that this project needs to be undertaken in areas like Jharkhand with a large tribal population and known iodine deficiency. Large state program requires large resources. However, this experience of small project gave us immense guidance and confidence to start program in Jharkhand whenever

government or any large non-governmental organizations is willing to undertake the responsibility.

CONCLUSION

Lower cutoff for TSH may have more sensitivity for detecting CH. Large-scale population-based study on CH is needed in Iodine deficiency prone state like Jharkhand.

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