Current Status of Newborn Screening for the early Diagnosis of Congenital Hypothyroidism in Bangladesh

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ABSTRACT

Background: Newborn screening programs signify early and presymptomatic detection of treatable disorders which authorize commencement of prompt medication to alleviate notable fatality. Newborn screening (NBS) for congenital hypothyroidism (CH) has been a successful public health initiative in preventive medicine over the last few decades. Previous pilot (1999-2006) and phase 1 (2006-2011) NBS program in Bangladesh reported an increased incidence compared to global data which urged to initiate a second phase NBS program for the detection of CH.

Materials & Methods: Neonates' blood was collected from the umbilical cord or by heel prick and drawn on the Guthrie filter paper card. Thyroid-stimulating hormone (TSH) was measured from this filter paper by Immunoradiometric assay (IRMA) or Dissociation Enhanced Lanthanide Fluorescence Immunoassay (DELFIA) method.

Results: 123 out of 2,61,550 neonates have already been diagnosed with congenital hypothyroidism from September 2018 to August 2020, which represents an incidence of 1:2126.

Conclusion: Ongoing phase-2 project data is already showing some optimistic outcomes. Hence, a time-worthy sustainable policy should be implemented to mark this program as an effective one.

Key words: **Newborn Screening, Congenital hypothyroidism, TSH, IRMA, DELFIA.**

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INTRODUCTION

Congenital hypothyroidism (CH), previously known as cretinism, is a severe deficiency of thyroid hormone in newborns due to inborn errors of thyroid hormone biosynthesis (dyshormonogenesis) or improper development and/or abnormal position of the thyroid gland (dysgenesis). CH is one of the most common preventable neonatal endocrine disorders which causes mental retardation and has a reported incidence of 1 in 2000 to 4000 births regardless of region and ethnicity (1-3). Thyroid dysgenesis accounts for 80 to 85 percent of cases (4). Increased global CH incidence has been reported recently based on newborn screening programs of many countries including USA, Canada, New Zealand, Greece, Italy, and Ireland (5-7). The number of increased cases during past few decades might be due to advancement of detection methods, awareness through screening programs, changes of cutoff values, and diversity in clinical practice and ethnicity (8-11).

The screening program for congenital hypothyroidism has been demonstrated as a tremendous advancement particularly in preventive medicine since its establishment in 1974 in Quebec, Canada as the program has already been launched both in the developed and more and more in developing countries (12,13). This has greatly alleviated the economic burden of intellectual retardation owing to CH. The incidence of CH has drastically declined from the range of 1:7000-1:10000 to range of 1:3000 to 1:4000 globally after the commencement of newborn screening program (14,15). These reports had shed light on the significance of the CH screening program particularly for densely populated countries like Bangladesh.

A phenomenal advancement in the field of molecular technology and research has been observed during last few decades, however, molecular landscape for CH is still poorly convinced. Thyroid dyshormonogenesis (TDH) which accounts for 10 to 20% cases of CH has significant associations with several mutations of TPO (thyroid peroxidase), Tg (thyroglobulin), IYD (iodotyrosine deiodinase, DEHAL1), NIS (sodium iodide symporter, SLC5A5), PDS (Pendrin or SLC26A4), DUOX2, and DUOXA2 (dual oxidase) genes (16,17).

In case of thyroid dysgenesis, low frequency of mutation has been observed. Therefore, a selective category of patients either with a presumptive clinical manifestation or with a familial incidence of thyroid dysgenesis are suggested to have their genetic analysis on FOXE1, NKX2-1 and NKX2-5 gene mutations or PAX8 and TSHR gene mutations accordingly (18).

The newborn screening movement for CH was introduced in Bangladesh in 1999 by Bangladesh Atomic Energy Commission (BAEC) and as a regional project of the International Atomic Energy Agency (IAEA). During this study period of 1999 to 2006, a total of 31,802 newborns were screened where 16 babies were confirmed as CH positive with an incidence rate of 1:1987. Later, an ADP project was executed by BAEC from July 2006 to December 2011 and approximately 2,20,000 newborns were screened and 96 babies were diagnosed as positive cases of CH. Since the incidence of CH was found higher (1:2300) in comparison to global incidence (1:4000), BAEC had launched a new ADP project "Screening of Congenital Hypothyroidism in newborn Babies (Phase-2)" for the period of September 2018 to June 2022. The objectives of the project are to decrease child mortality rate by screening newborns at a very early age and provide treatment accordingly to achieve better demographic data of CH for Bangladesh.

MATERIALS AND METHODS

Study Subjects

A hospital-based case study of CH was performed consisting of 2,61,550 neonates. Blood samples were collected from 669 hospitals and clinics covering a total of 51 districts of Bangladesh. All participants' parents were elucidated about the objective of the study and a structured questionnaire including the information of birth date, gender, name of the neonate, name of the mother, contact address, gestational period, weight of the neonate, sample collection date, sample collection location, phlebotomist's name and contact information were filled up on the specific filter paper card (Guthrie card).

Sample Collection

Umbilical cord blood is collected immediately after birth or heel pricked blood is collected between two to five days after birth to avoid false positive results as physiological surge of TSH occurs during the first 48 hours after delivery (19,20). Blood was collected dropwise as dried blood spot on special filter paper. The blood drop should be ample to spread over the designed area, penetrating the filter paper from one side to another. The blood spots were dried at room temperature in a horizontal position for 2 to 6 hours. During the drying process, the spots should not be subjected to heat or direct light, and neither be stacked nor be touched with other surfaces (21). DBS should not be packaged in air-tight containers for long term storage as air exchange facility enables to maintain the ambient temperature (25°C) and moisture (<50%) (22). Collected filter papers from the entire are being received at a regular basis at in-vitro laboratory of National Institute of Nuclear Medicine and Allied Sciences (NINMAS) and stored at 2-8°C away from direct light and moisture.

Analytical Techniques

Thyroid Stimulating Hormone (TSH) was measured from dried blood spot samples by IRMA or DELFIA method immediately after collection.

- **IRMA:** Immuno-radiometric assay is a non-competitive assay detecting antigen (unknown or standard) by excess labeled antibody (^{125}I labeled antibody).
- **DELFIA:** Dissociation Enhanced Lanthanide Fluorescence Immunoassay is a fast fluorescence intensity detection technology in which lanthanide chelate labeled reagents like Europium, Samarium, and Terbium are used to identify the presence of a particular targeted compound or biomolecule.

AutoDELFIA neonatal hTSH kit (Perkin Elmer) was used for analysis purpose along with AutoDELFIA Plate processor 1235 automatic immunoassay system, specimen gate software, and Panthera Puncher.

During TSH measurement, a cut-off value of 20mIU/L was considered for primarily detected positive CH following the guideline of UK Newborn Screening Programme Centre (UKNSPC) (23). Babies having TSH value higher than the cut-off are contacted immediately and their serum TSH and T4 (Thyroxine) are re-analyzed for confirmation of true positive cases. The confirmed cases are then treated with levothyroxine (10-12 µg/kg/day) and followed up at monthly interval to monitor the neuro-developmental outcome along with blood TSH level (24,25).

Data Analysis

The odds ratios (ORs), as a measure of relative risk, at 95% confidence intervals (95% CI) were calculated using logistic regression models to assess the relative association between gender and gestation period. TSH values were expressed as mean ± SEM (Standard Error of Mean). Fisher's exact test, and t-test were performed using Graphpad Prism version-8. A p-value of less than 0.05 was considered as a level of significance.

RESULT

Previous pilot (1999-2006) and phase 1 (2006-2011) studies reported an increased incidence rate compared to global data (26), which urged to initiate a phase 2 study. 123 out of 2,61,550 neonates have been already diagnosed with congenital hypothyroidism from September 2018 to August 2020. Therefore, the initial incidence rate of this ongoing phase 2 study is 1:2115 which resembles the previous study data.

Table 1: Statistics of Newborn Screening Program in Bangladesh

Time Period	No. of newborn screened	No. of high TSH detected	No. of confirmed cases	Incidence rate
1999-2006	31,802	438	16	1:1987
2006-2011	2,20,000	-	96	1:2300
September 2018 - August 2020	2,61,550	-	123	1:2126

Among the 64 districts, 51 have already been covered. Dhaka, Faridpur, Mymensingh, Gazipur, and Tangail rank topmost sample collected regions and the percentages are 20.83%, 4.58%, 4.51%, 4.16%, and 4.14% respectively.

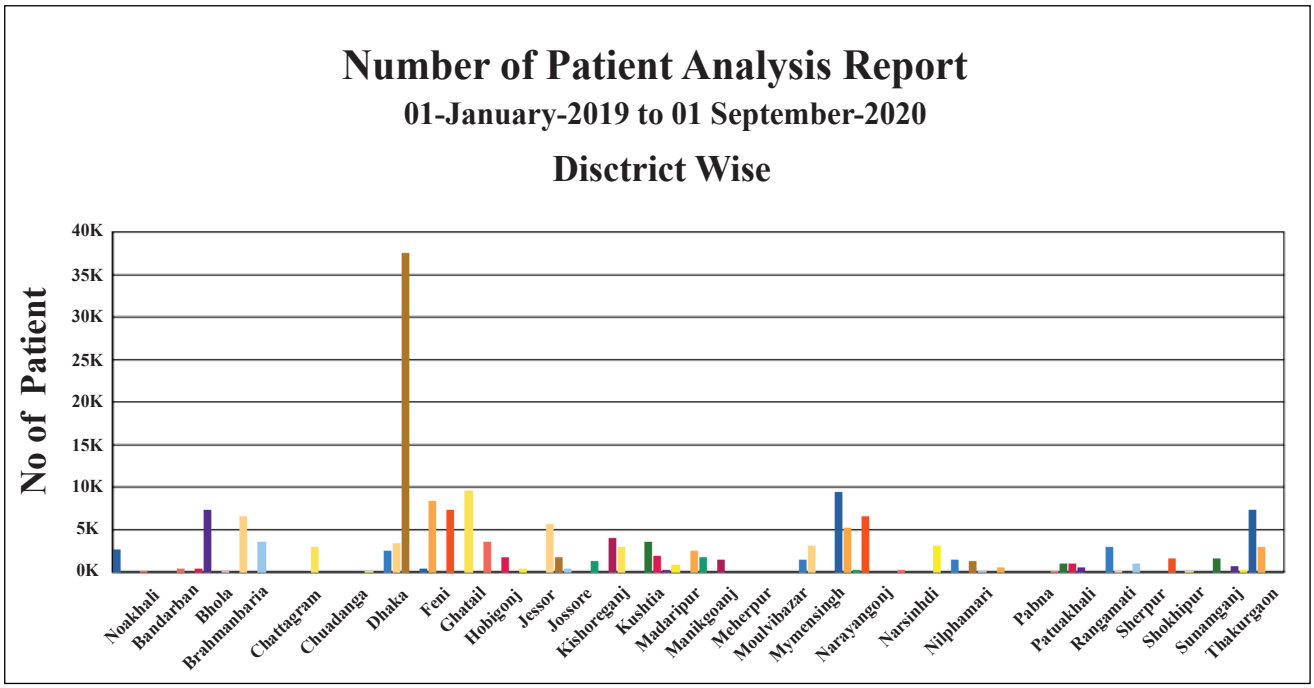

Figure 1: District wise frequency of analyzed samples

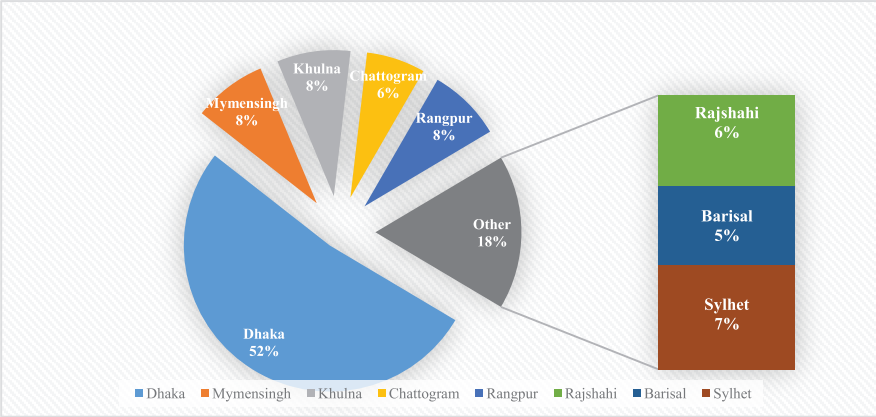

Figure 2: Division wise frequency of positive sample (Ongoing Phase 2 project study)

Table 2: Division wise frequency of analyzed sample and incidence rate of positive CH

Division Name	Sample Analyzed (n=2,61,550) n (%)	High TSH Sample (n=123) n (%)	Incidence Rate
Dhaka	1,28,150(49.0)	64(52.03)	1:2,002.34
Rangpur	26,576(10.16)	10(8.13)	1:2,657.6
Rajshahi	25,226(9.64)	7(5.69)	1:3,603.71
Chattogram	24,807(9.48)	8(6.5)	1:3,100.86
Khulna	19,370(7.41)	10(8.13)	1:1,937.0
Barisal	14,771(5.65)	6(4.88)	1:2,461.83
Mymensingh	14,400(5.51)	10(8.13)	1:1,440.0
Sylhet	8,250(3.15)	8(6.5)	1:1,031.25

Among the screened positive neonates, 64 are from the Dhaka division which covers almost 52% of total positive cases. Again, 24% positive cases are covered by Mymensingh, Khulna, and Rangpur division. Furthermore, the Barisal division has the least number of positive cases. As showed in table 2, Sylhet followed by Mymensingh, Khulna and Dhaka rank the topmost division having the highest incidence rate of CH although the number of samples collected is not uniform.

Correlation between TSH level with gender, gestation period and body weight

Figure 3 represents the correlation between TSH level and gender of the positive cases. TSH level in male and female positive cases was 66.92±9.07 mIU/L and 66.68±17.74 mIU/L respectively. As shown in figure 2, no significant difference was found between the TSH levels of positive males and females.

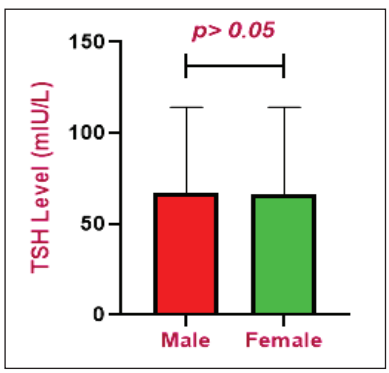

Figure 3: Correlation between TSH level with gender

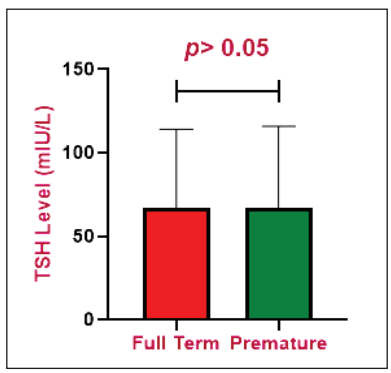

Figure 4: Correlation between TSH level with gestation period

As presented in figure 4, TSH levels in full term and premature positive cases was 66.92±4.765 mIU/Land 67.01±14.13 mIU/L respectively which showed non-significant correlation between TSH level and gestation period. As shown in Table 3, number of male premature CH babies are insignificantly higher than female full-term CH babies. On the other hand, number of premature newborn babies are significantly higher in CH positive cases compared to full term normal newborns shown in Table 4.

Table 3: Gender wise frequency of positive sample in accordance with gestation period (Ongoing Phase 2 project study)

Gender	Full Term n(%)	Premature n(%)	OR (95% CI)	*p* value
Female	65(59.09)	7(53.85)	1 (Ref.)	-
Male	45(40.91)	6(46.15)	1.238(0.3901-3.93)	0.7711

** Results are expressed as number (percentage). Fisher's test was performed to calculate the statistical significance. p<0.05 was taken as level of significance.*

Table 4: Frequency distribution of CH neonates according to gestation period

Gestation Period	Normal Neonate (n=200) n (%)	CH Neonate (n=200) n (%)	OR (95% CI)	*p* value
Full Term	2,57,882(98.61)	110(89.43)	1 (Ref.)	-
Premature	3,645(1.39)	13(10.57)	8.361(4.701-14.87)	<0.0001

** Results are expressed as number (percentage). Chi-square test was performed to calculate the statistical significance. p<0.05 was taken as level of significance.*

The correlation between TSH level and body weight of the positive cases has been shown in figure 5. Pearson correlation coefficient between TSH level and body weight was r=-0.284 indicating low degree of positive correlation which was statistically significant (p= 0.0025).

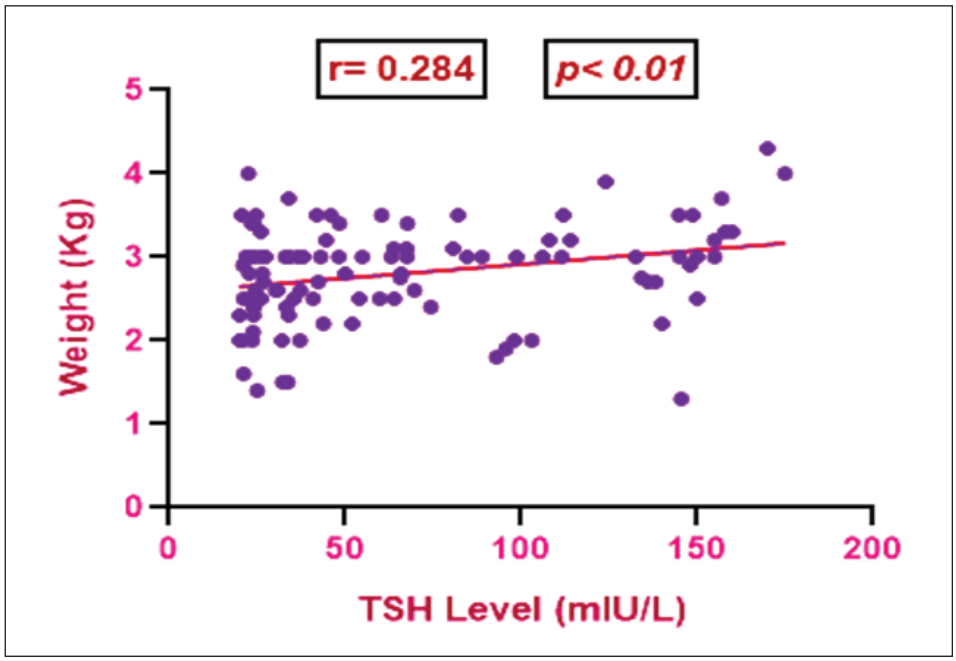

Figure 5: Correlation between TSH level with body weight

Among the diagnosed positive cases, 52.85% cases had the TSH level between 20 to 50mIU/L with a mean± SEM of 30.03 ± 1.009 mIU/L whereas 21.14% and 26.01% cases had the TSH level between 50 to 100 mIU/L and greater than 100 mIU/L accordingly (Table 5).

Table 5: Frequency distribution of TSH level among the diagnosed positive CH newborns (Phase 2 project study)

TSH level (mIU/L)	No. of cases	Percentage (%)	Mean ± SEM(mIU/L)
>20-50	65	52.85	30.03 ± 1.009
>50-100	26	21.14	72.39 ± 2.946
>100	32	26.01	141.1 ± 3.819

Values are presented as Mean ±SEM (Standard Error of Mean)

DISCUSSION

A phenomenal achievement has been obtained in public health by the newborn screening programs through the diagnosis of congenital disorders worldwide, particularly for CH. Ongoing project data compiled from September 2018 to August 2020 already showed optimistic outcomes, though we are in the pipeline of collecting more samples and analyze next few months.

Due to some impediments like high mobility, insensibility, false addresses, cultural beliefs and taboos, umbilical cord blood was our source of sampling for TSH measurement which was supposed to be a good screening approach by Walfish and Fuse et al., although Majeed-Saidan et al. opposed (27-29). Still, we are endeavoring on heel prick sampling because of its higher sensitivity and response rate to recall rate.

2,61,550 umbilical DBS were already tested which covers almost 80% districts of Bangladesh. Dhaka division had the greatest number of screened positive CH neonates as well as the greatest collection of DBS. The incidence rate (1:1,031.25) was found surprisingly high in Sylhet division located at the northeastern side of Bangladesh (Table 2). Since the number of samples collected from this region was the lowest among the divisions, special attention needs to be paid to the collection of more samples to be more confident in our study data.

Among the 123 positive CH neonates, the number of females is almost 1.5 times higher than males with a percentage of 41.9% (M) and 58.1% (F) respectively, which is compatible with the findings of Gaddam et al. (19). In addition, no significant association was found between TSH level and gender as almost similar mean ± SEM values for both male and female CH neonates were obtained (Figure 2). This suggests that CH has an association with gender to some extent, but not with any particular level of higher TSH. Furthermore, no association was found between TSH level and the gestational period for CH neonates although studies by Abdelmoktader and Medda et al. identified a significant association between prolonged gestational age (≥41 weeks) and CH (Figure 3) (30,31). Hence, the frequency of premature newborn babies were found more than 8 times higher in CH positive cases compared to full-term normal newborns (Table 4) which correlated with the finding of Büyükgebiz 2013 (32). The number of premature male CH neonates is slightly higher (1.2 times) than full-term female CH neonates (Table 3). Again, a low degree of positive correlation ($p<0.01$) was observed between the weight and TSH levels of CH infants (Figure 4). As we found that the TSH level of 52.85% neonates is between 20 to 50 mIU/L with a mean ± SEM of 30.03±1.009 mIU/L (Table 5). No association could be established between the weight of the newborns and the risk of CH which is consistent with the study conducted by Anjum et al. (33).

CONCLUSION

The study indicates that the incidence rate of CH in Bangladesh is higher than the global incidence and the rate is not declining with the advancement of current screening programs. As the study is still ongoing, more explicit data are expected to be obtained which will assist in proper diagnosis, prognosis, management and follow

up of CH in newborns and lessen the socio-economic burden subsequently. Based on the current data, it may be proposed that the government needs to run this sort of screening project constantly and have a sustainable policy to combat congenital diseases.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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