

A Study to Determine the Normative Data for Cord Blood Free Thyroxine and Thyroid Stimulating Hormone in Indian Babies

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ABSTRACT

A study was conducted to determine the normative data for free thyroxine and thyroid stimulating hormone in cord blood. It was a prospective observational study with total of 1000 samples from umbilical cord were collected in a sterile container and free thyroxine(FT4) and thyroid stimulating hormone(TSH) estimation was done. The norms for TSH and FT4 were determined by calculating the descriptive statistics of TSH and FT4 in terms of minimum, maximum range, mean and S.D norms and standard error of mean. The percentiles were calculated as 1st, 3rd, 10th, 25th, 50th, 70th, 90th, 95th, 97th and 99th percentiles for deriving the norms for TSH and FT4. The level of statistical significance was taken as $p \leq 0.05$. The data was analyzed by using SPSS statistical software version 16.0.

Key words: Newborn, cord blood, FT4, TSH, newborn screening, normative data.

INTRODUCTION

Congenital hypothyroidism (CH) is a major preventable cause of mental retardation. It has an incidence of 1 in 2000 to 1 in 4000 births in various neonatal screening programs. [1] The exact incidence of congenital hypothyroidism in India is not known. It appears from the review of literature that incidence of CH in India is much higher (ranging from 1.6:1000 to 1:600 in various studies) than described in western literature of 1 in 4000 live births. With large number of babies being discharged early, cord blood samples are being used. [2-7] In our country, it is often very difficult to follow up babies once discharged because of various reasons. So cord blood remains a very practical alternative for screening purposes, and is also being practiced in some Asian countries like Malaysia and Singapore. Mixed cord blood samples for TSH values have been

compared well with filter paper samples taken in the first few days of life. [8,9] Use of cord blood as a screening tool is an attractive proposition because it is easily available and accessible at the time of birth of baby. Very few reports of cord blood values of only TSH or T4 exist in Indian literature [10,11] and use of free thyroxine (FT4) and TSH in cord blood for hypothyroid screening is an attractive proposition but normative data on cord blood FT4 and TSH is lacking in our Indian babies, hence this study was conducted.

MATERIALS AND METHODS

The present study was conducted in the department of Pediatrics and Obstetrics and Gynaecology, Tirathram Shah Hospital, Delhi. The study was a prospective observational study, which was carried out over a period of 12 months w.e.f. June 2013 to May 2014. The study population

consisted of all newborns delivered during the study period with >32 weeks of gestation, not meeting any of the exclusion criteria. These babies were included in the study after taking written and informed consent from parents. Cord blood from the placental end was collected in a plain vial and FT4 and TSH were analyzed in NABL accredited laboratory in the hospital

Study population: All deliveries, with period of gestation >32 weeks, conducted at this hospital during the study period were part of the study.

Inclusion criteria: All consecutive deliveries, with >32 weeks of gestation, conducted in this hospital during study period.

Exclusion criteria

1. Babies born less than completed <32 weeks of gestation.
2. Babies with moderate to severe birth asphyxia and with intracranial and severe gross congenital malformations.
3. Critically ill babies, babies with shock requiring inotropic support, steroids or blood transfusions.

Sample size: study was carried on a total number of 1000 cases.

Sample and data collection: Umbilical cord mixed blood samples (2 ml) were collected in a sterile container. The sample was sent to NABL accredited laboratory of hospital, where sample was processed and FT4 and TSH estimation was done on the same day; else sample was stored in freezer at 2-8 degree Celsius and analyzed within 24 hours

Statistical analysis: The norms for TSH and FT4 were determined by calculating the descriptive statistics of TSH and FT4 in terms of minimum, maximum range, mean and S.D norms and standard error of mean. The percentiles were calculated as 1st, 3rd, 10th, 25th, 50th, 70th, 90th, 95th, 97th and 99th percentiles for deriving the norms for TSH and FT4. The level of statistical significance was taken as $p \leq 0.05$. The data was analyzed by using SPSS statistical software version 16.0.

OBSERVATIONS AND RESULTS

The observation and results recorded in the study were evaluated under following headings:

Gender Distribution of Neonates

Of total 1000 newborns, 517(52%) were males and 483(48%) were females. The male female ratio was 1: 0.93.

Table 1: Showing Gender Distribution of Neonates

Gender	No. of Neonates	%
Female	484	48.4%
Male	516	51.6%
Total	1000	100%

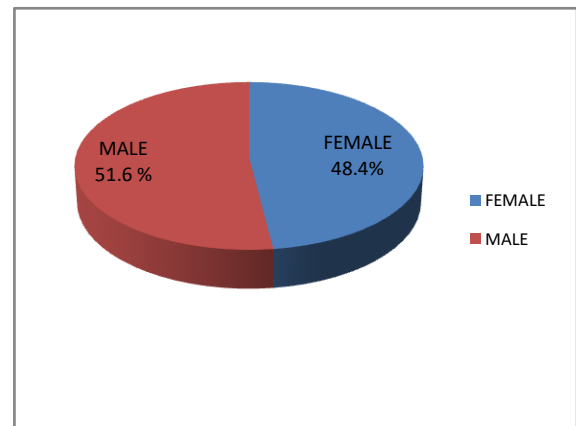


Figure 1: Showing Gender Distribution of Neonates

Table 2: Maturity Distribution of Neonates:

Maturity	No. of Neonates	%
TERM	847	85%
PRE-TERM	153	15%
TOTAL	1000	100%

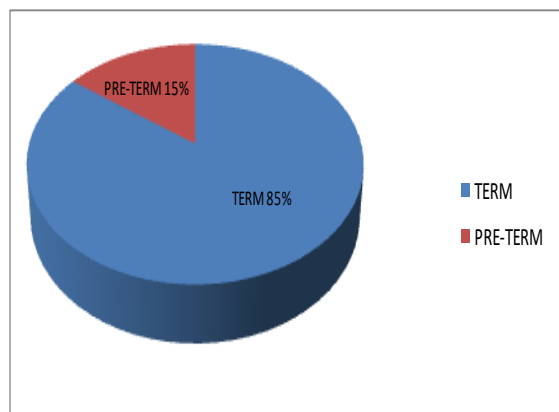


Figure 2: Showing Maturity Distribution of Neonates

Of the total 1000 neonates, 847 (85%) were term, 153(15%) were preterm.

Table 3: Gender Distribution of Term and Preterm Babies

Gender	Pre-term	Term
Male	74 (48.4%)	443(52.3%)
Female	79 (51.6%)	404(47.7%)
Total	153	847

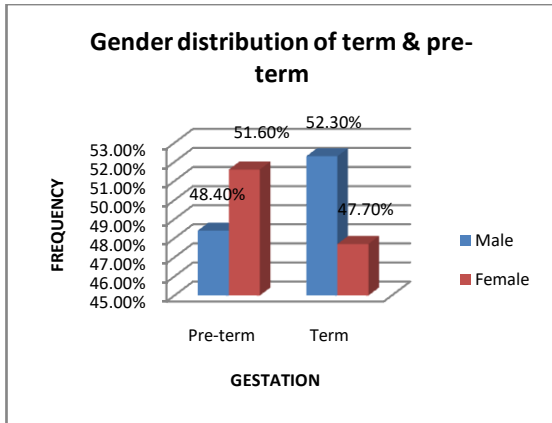


Figure 3: Showing Gender Distribution of Term and Preterm Neonates

Of total term neonates 443(52.3%) were males and 404(47.7%) were females and among preterm 74(48.4%) were male and 79(51.6%) babies were females. Thus in both the groups male and female distribution was statistically similar.

Table 4: Showing Cord Blood TSH Distribution Of 1000 Neonates:

Cord blood TSH(μ IU/mL)	No. of neonates	Frequency
0--4.99	123	12.3%
5--9.99	589	58.9%
10--14.99	203	20.3%
15--19.99	36	3.6%
20--24.99	8	0.8%
25--29.99	10	1.0%
30--34.99	9	0.9%
35--39.99	4	0.4%
40--45.99	6	0.6%
46--49.99	4	0.4%
50--99.99	5	0.5%
>100	3	0.3%
Total	1000	100.0%

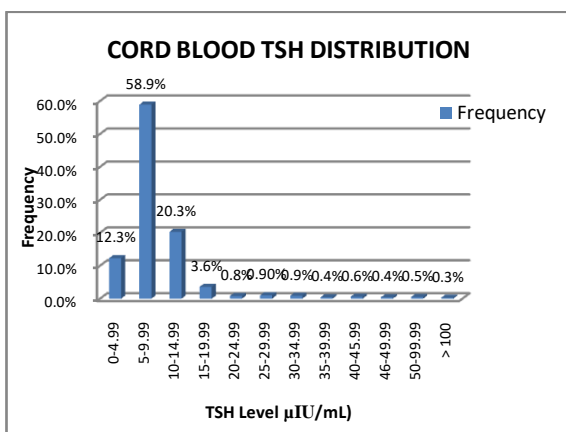


Figure 4: Showing Cord Blood TSH Distribution Of 1000 Neonates

Of total 1000 umbilical cord blood samples taken, majority of values(58.9%) were below 10 μ IU/ml (i.e., between 5-9.99 μ IU/ml). While 4.8% values were above 20

μ IU/ml. And only 0.3% of cord TSH values were above 100 μ IU/ml.

Table 5: Showing Cord Blood Ft4 Distribution Of 1000 Neonates

S.No	CORD BLOOD FT4(ng/dL)	Number	Frequency
1	0.2-0.39	1	0.1%
2	0.4-0.59	0	0.0%
3	0.6-0.79	3	0.3%
4	0.8-0.99	98	9.8%
5	1-1.39	688	68.8%
6	1.4-1.79	188	18.8%
7	1.8-1.99	15	1.5%
8	2-2.19	6	0.6%
9	2.2-2.39	1	0.1%
10	Total	1000	100.0%

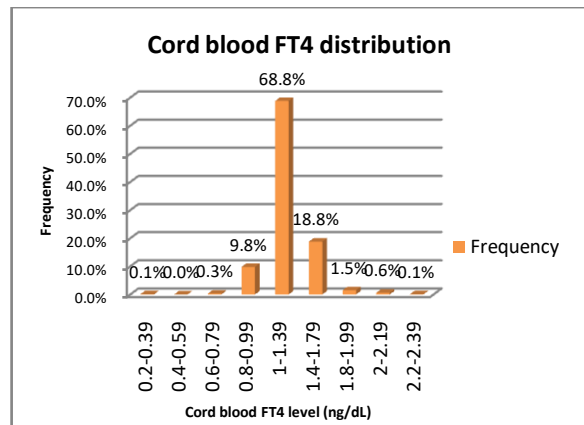


Figure 5: Showing Cord Blood Ft4 Distribution Of 1000 Neonates

In the study group of 1000 neonates, majority of cord blood FT4 values 688(68.8%) were noted in the range of 1.0-1.39 ng/dl. Only 10% values were noted to be below 1.0 ng/dl and 0.1% value was between 0.2-0.39 ng/dl.

Table 6: Showing Normative Data of Cord Blood Tsh And Ft4 For 1000 Neonates

	Cord TSH(μ IU/mL)	Cord FT4(ng/dL)
N	1000	1000
Min	1.1	0.2
Max	702	2.2
Range	700.9	2.0
Mean	10.905	1.204
S.D	28.00	0.22
Median	7.900	1.200
S.E of Mean	0.885	0.007
Percentile		
1st	2.301	0.800
3rd	3.400	0.900
10th	4.600	0.900
25th	6.500	1.100
50th	7.900	1.200
70th	9.900	1.300
75th	10.677	1.300
90th	14.190	1.500
95th	19.595	1.600
97th	30.194	1.700
99th	49.000	1.900

The mean TSH level in cord blood of 1000 neonates was 10.905 ± 28.0 μ IU/ml with range of 1.1 to 702 μ IU/ml. The median value was 7.90 μ IU/ml. The cord TSH values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles in total neonates were 2.30, 3.40, 4.60, 6.50, 7.90, 9.90, 10.68, 14.19, 19.595, 30.19, and 49.00 μ IU/ml respectively. The mean cord blood FT4 was 1.204 ± 0.22 with range of 0.2-2.2ng/dl. The median value was 1.20 ng/dL. FT4 values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles in total neonates were 0.80, 0.90, 0.90, 1.10, 1.20, 1.30, 1.30, 1.50, 1.60, 1.70, and 1.90 ng/dl respectively.

Table 7: Showing Normative Data Of Cord Blood TSH And Ft4 For Total Male Neonates (N=516)

	Cord TSH(μ IU/mL)	Cord FT4(ng/dL)
N	516	516
Minimum	2.2	0.6
Maximum	500	2.2
Range	497.8	1.6
Mean	10.637	1.192
S.D	22.732	0.213
Median	7.900	1.200
S.E of Mean	1.0007	0.0094
Percentile		
1st	2.825	0.800
3rd	3.451	0.800
10th	4.500	1.000
25th	6.500	1.100
50th	7.900	1.200
70th	9.900	1.300
75th	10.900	1.300
90th	14.130	1.500
95th	23.735	1.600
97th	31.629	1.649
99th	48.167	1.883

The mean TSH level in cord blood of male neonates was 10.64 ± 22.73 μ IU/ml with range of 2.2 to 500 μ IU/ml. The median value was 7.90 μ IU/ml. The cord TSH values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles in male neonates were 2.83, 3.45, 4.50, 6.50, 7.90, 9.90, 10.90, 14.13, 23.74, 31.63, and 48.17 μ IU/ml respectively. The mean cord blood FT4 was 1.19 ± 0.21 with range of 0.6-2.2ng/dl. The median value was 1.20 ng/dL. FT4 values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles in male neonates were 0.80, 0.80, 1.00, 1.10,

1.20, 1.30, 1.30, 1.50, 1.60, 1.65, and 1.88 ng/dl respectively.

Table 8: Showing Normative Data Of Cord Blood TSH And Ft4 For Total Female Neonates (N=484)

	Cord TSH(μ IU/mL)	Cord FT4(ng/dL)
N	484	484
Minimum	1.1	0.2
Maximum	702	2.1
Range	700.9	1.9
Mean	11.19	1.22
S.D	32.717	0.23
Median	8.00	1.2
S.E of Mean	1.487	0.011
Percentile		
1st	2.085	0.800
3rd	3.155	0.900
10th	4.700	0.900
25th	6.500	1.100
50th	8.000	1.200
70th	9.900	1.300
75th	10.500	1.300
90th	14.200	1.500
95th	18.265	1.600
97th	27.735	1.700
99th	51.495	2.000

The mean TSH level in cord blood of female neonates was 11.19 ± 32.72 with range of 1.1 to 702 μ IU/ml. The median value was 8.00 μ IU/ml. The cord TSH values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles in female neonates were 2.09, 3.16, 4.70, 6.50, 8.00, 9.90, 10.50, 14.20, 18.27, 27.74, and 51.50 μ IU/ml respectively. The mean cord blood FT4 was 1.22 ± 0.23 with range of 0.2-2.1ng/dl. The median value was 1.20 ng/dL. FT4 values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles in female neonates were 0.80, 0.90, 0.90, 1.10, 1.20, 1.30, 1.30, 1.50, 1.60, 1.70, and 2.00 ng/dl respectively.

Mean TSH level in cord blood of term neonates was 11.035 ± 30.23 with range of 1.7 to 702 μ IU/ml. The TSH values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles in term neonates were 2.54, 3.40, 4.60, 6.50, 7.90, 9.90, 10.60, 13.80, 19.40, 28.82, and 49.10 mIU/L, respectively. The mean cord blood FT4 was 1.205 ± 0.22 with range of 0.2-2.1 ng/dl. The FT4 values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles in term neonates were 0.80, 0.90, 1.00, 1.10, 1.20,

1.30, 1.30, 1.50, 1.60, 1.70, and 1.90 ng/dL, respectively

1.30, 1.30, 1.50, 1.60, 1.64, and 2.09 ng/dl respectively

Table 9: Showing Normative Data Of Cord Blood TSH And Ft4 For Total Term Neonates (N=847)

	Cord TSH(μ IU/mL)	Cord FT4(ng/dL)
N	847	847
Minimum	1.7	2.1
Maximum	702	2.1
Range	700.3	1.9
Mean	11.035	1.205
S.D	30.23	0.22
Median	7.900	1.20
S.E of Mean	1.039	0.0076
Percentile		
1st	2.54	0.800
3rd	3.40	0.900
10th	4.60	1.00
25th	6.50	1.100
50th	7.90	1.200
70th	9.90	1.300
75th	10.60	1.300
90th	13.80	1.500
95th	19.41	1.600
97th	28.82	1.700
99th	49.10	1.900

Table 10: Showing Normative Data Of Cord Blood TSH And Ft4 For Total Preterm Neonates (> 32-37 Weeks) (N=153)

	Cord TSH(μ IU/mL)	Cord FT4(ng/dL)
N	153	153
Minimum	1.1	0.80
Maximum	61.0	2.20
Range	59.90	1.40
Mean	10.19	1.199
S.D	8.22	0.225
Median	7.90	1.20
S.E of Mean	0.6643	0.0181
Percentile		
1st	1.478	0.800
3rd	2.834	0.800
10th	4.500	0.900
25th	6.800	1.100
50th	7.900	1.200
70th	10.168	1.300
75th	10.900	1.300
90th	16.692	1.500
95th	27.690	1.600
97th	35.628	1.638
99th	54.520	2.092

Mean TSH level in cord blood of preterm neonates was 10.19 μ IU/ml \pm 8.22 with range of 1.1 to 61 μ IU/ml. The cord TSH values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles in preterm neonates were 1.48, 2.83, 4.50, 6.80, 7.90, 10.17, 10.90, 16.69, 27.69, 35.63, and 54.52 μ IU/ml, respectively. The mean cord blood FT4 was 1.20 ng/dl \pm 0.23 with range of 0.8-2.2ng/dl. FT4 values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles were 0.80, 0.80, 0.90, 1.10, 1.20,

DISCUSSION

In our study, majority (71.1%) of cord TSH values were less than 10 mIU/L, this was similar to study done by Manglik et al, [7] on a cohort of 1200 neonates. In our study, 4.8% values were above 20 mIU/L, while Manglik et al had found 1.58% values above 20 mIU/L. In a recent study conducted by Gupta et al, [12] 11.5% of babies were found to have cord TSH more > 20 mIU/L, which was significantly higher higher than values found in our study. Only 0.3% of cord TSH values were above 100 mIU/L in present study while it was 0.08% in study by Manglik et al.

In the present study, the mean cord TSH value of entire cohort (1000 neonates) was found to be 10.90 \pm 28.00 mIU/L with range 1.1-702 mIU/L, which was somewhat higher than found by Manglik et al, whose mean value was 6.13 \pm 5.29 mIU/L with range of 0.8- 101.2 mIU/L. Our mean value was slightly higher than mean value obtained by Singh RA et al, [13] a mean cord TSH value of 8.833 mIU/L was found in a cohort of 1000 neonates.

In our study, the values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles were 2.54, 3.40, 4.60, 6.50, 7.90, 9.90, 10.6, 13.8, 19.41, 28.82, 49.10 mIU/L, respectively. While in Manglik et al study, the values corresponding to 3rd, 10th, 25th, 50th, 75th, 90th, 97th, and 99th percentiles were 1.6, 2.5, 3.3, 5.8, 7.0, 9.0, 14.9, and 25.8 mIU/L, respectively. Thus our TSH percentiles were higher than shown by Manglik et al study.

Raj S et al [14] in their study on 430 neonate, found cord blood mean TSH level as 12.88 mIU/L, which is slightly higher than the mean value in our study.

Basarir T et al [15] gave normative values in a cohort of 56 neonates and mean cord blood TSH value was 7.83 \pm 4.49 mU/ml in their study while in our study the mean cord TSH value was somewhat higher

than in this study. In a recent study, Gupta et al studied the effect of perinatal factors on cord blood TSH levels, the median TSH value was 8.75 mIU/L with range of 1.01-63.74 mIU/L, while in our study median value was somewhat similar (7.90 mIU/L) with range of 1.1-702 mIU/L, although the lower limit (minimum value) was similar but upper limit was significantly higher.

In present study, majority (68.8%) of cord FT4 values were between 1.0- 1.39 ng/dL, 0.1% values were above 2.20 ng/dL and only 0.4% values were below 0.8 ng/dL. The mean cord FT4 value of entire cohort of 1000 babies in our study was 1.204 ± 0.22 ng/dL with range 0.2-2.2 ng/dL and median value was found to be 1.2 ng/dL. In our study values corresponding to 1st, 3rd, 10th, 25th, 50th, 70th, 75th, 90th, 95th, 97th, and 99th percentiles were 0.80, 0.90, 0.90, 1.10, 1.20, 1.30, 1.30, 1.50, 1.60, 1.70, and 1.90 ng/dL, respectively.

Basarir et al [15] gave cord blood FT4 value of 18.66 ± 4.18 pmol/L (1.45 ± 0.33 ng/dL), which was a bit higher than the present study. Clemantine YF et al [16] found cord blood FT4 levels ranging from 0.97-2.22 ng/dL, the upper range is similar to present study while lower range is higher than in our study.

Henry JG et al [17] determined the FT4 values in cord blood of neonates chosen without any consideration to health of neonates, gestation, birth weight or any neonatal parameters. In their study, the mean cord FT4 value was 1.15 ng/dL with range 0.93-1.52 ng/dL, while in our study the mean cord FT4 value was similar but different range was found (0.2-2.2 ng/dL) in cord blood of 1000 neonates.

In our study, the mean (\pm S.D) cord TSH values in Males and females neonates were 10.64 ± 22.73 mIU/L and 11.19 ± 32.72 mIU/L, respectively. These mean values in our study was found to be higher than the values in Manglik et al study, in whom the mean (\pm S.D) cord blood TSH values in male and female babies were 6.48 ± 5.2 mIU/L and 5.75 ± 4.16 mIU/L, respectively. The median cord TSH value in

male neonates was 7.90 mIU/L while in female it was 8.00 mIU/L.

The mean (\pm S.D) cord TSH values in term and preterm neonates were 11.04 ± 30.23 mIU/L and 10.19 ± 8.22 mIU/L, respectively. Basarir T et al derived the normative values in term neonates and mean cord (\pm S.D) blood TSH level was 7.83 ± 4.49 mIU/ml, which is lower than the mean values found in our study of term neonates. The median cord TSH value in both term and preterm neonates was 7.9 mIU/L.

In present study, the mean (\pm S.D) cord FT4 values in term and preterm neonates were 1.21 ± 0.22 ng/dL (range 0.2-2.1 ng/dL) and 1.20 ± 0.23 ng/dL (range 0.8-2.2), respectively. The median value in both term and preterm neonates were same i.e., 1.20 ng/dL. Thus similar mean (\pm S.D) and median levels were found in term and preterm neonates.

Mean (\pm S.D) cord FT4 levels in males and females were 1.19 ± 0.21 ng/dL (range 0.6-2.2) and 1.22 ± 0.23 (range 0.2-2.1) ng/dL, respectively. Similar mean (\pm S.D) cord FT4 levels were also found in males and females. The median levels in male and female subjects were similar i.e., 1.20 ng/dL. Thus somewhat similar FT4 distribution was seen in male and female neonates in our study. Although different lower limit of range was found, the upper limit of range was almost similar in both groups males vs females and term vs preterm neonates.

CONCLUSION

This study tried to render normative data, for the cord blood TSH and FT4 in Indian babies, which is scanty in our country and worldwide. As now a day's cord blood is being used for screening purposes for congenital hypothyroidism, presence of normative data will help in formulating guidelines for screening for Indian population. Although the study was conducted on fair numbers of babies still it seems inadequate and requires study on a

large scale to derive normative data for general population.

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