ORIGINAL RESEARCH

Comparison of the efficacy of cord blood T3, T4, TSH levels with day 3 fresh blood sample as a screening method for congenital hypothyroidism

[1]Dr. Vani K T, [2]Dr. Prakash S S

[1]Department of Paediatrics, JJM Medical College, Davanagere, Karnataka, India
[2]Professor, Department of Paediatrics, JJM Medical College, Davanagere, Karnataka, India

Corresponding Author
Dr. Vani K T
Department of Paediatrics, JJM Medical College, Davanagere, Karnataka, India

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ABSTRACT
Serum measurements of thyroid hormones (T_4-TSH) in cord blood could detect hypothyroidism in the neonatal period.Traditionally, screening strategies for the detection of CH were either a primary TSH / back up T4 method or a primary T4 / backup TSH method. A third strategy uses TSH plus T4 as the primary test. Changes in TSH levels in response to T3 and T4 blood levels forms the basis of screening for CH. Blood samples were collected in a sterile container drawn from a 15 - 20 cm length of the umbilical cord incised while severing it at the time of birth of the baby. Thus, a mixed cord blood sample (1.5 ml) including both from the umbilical artery and vein was obtained. T3, T4 and TSH were estimated by competitive immunoassay by using VITROS Total T3 and T4 Reagent Pack and the VITROS Immunodiagnostic Products TSH30 Reagent Pack. It was observed that mean value of T3, T4 and TSH was 72.69±13.12 ng/dl, 9.01±2.227 µg/dl and 8.64±6.195 mIU/l respectively in cord blood.Mean value of T3, T4 and TSH was 82.40±11.76 (ng/dl), 11.55±3.596 (µg/dl), and 2.98±2.191 (mIU/l) respectively in day 3 venous blood sample.Comparison and co-relation between T3, T4 and TSH levels between cord blood and day 3 venous sample was statistically significant, with p-value ≤0.001 as very highly significant..

Key words: Cord blood, T3, T4, TSH Levels, congenital hypothyroidism

INTRODUCTION

One of the most common preventable causes of mental retardation in children is Congenital Hypothyroidism (CH). It is a thyroid hormone deficiency present at birth.[1]The disorder is permanent in most cases and is caused by a problem associated with thyroid dysgenesis or a disorder of thyroid dyshormonogenesis. These disorders result in primary hypothyroidism. Secondary or central hypothyroidism at birth results from a deficiency of thyroid stimulating hormone (TSH). Some may be familial, usually caused by one of the inborn errors of thyroid hormone synthesis and may be associated with goitre. Less commonly, the altered neonatal thyroid function is transient, attributable to the trans-placental passage of maternal medication, maternal blocking antibodies, or iodine deficiency or iodine excess in rare cases. CH also may result from a pituitary or hypothalamic abnormality (central or secondary/tertiary hypothyroidism).[2]Over the past three decades neonatal screening for CH has become an integral component of the health care delivery system in most industrialized and affluent nations of the world. The American Office of Technology Assessment concludes that screening for CH is one of the few programs in preventive medicine that has an impact on public health with a positive cost to benefit ratio (10: l).[3]Many newborn infants with CH remain undiagnosed at birth and clinical features are often subtle.[4] This can be due to trans-placental passage of maternalthyroid hormone or due to the moderately functioning thyroid tissue.[5] The slow development of obvious clinical symptoms of CH, coupled with the early importance of treatment led to the implementation of widespread newborn screening. Prior to the onset of newborn screening programs, the incidence of CH, as diagnosed after the manifestation of symptoms, was in the range of 1:7,000 to

1:10,000.[6]With the advent of screening of newborn populations, the incidence were reported to be in the range of 1:3,000 to 1:4,000.[7]With data from state, regional, and national screening programs, it has become apparent that the incidence varies by geographic location. The incidence in India is found to be 1 in 1,700.[8]However, newborn screening for hypothyroidism is not done in many third world countries. Only an estimated 1/3 of the worldwide birth population is screened. It is therefore important that clinicians are able to recognize and treat the disorder.

In the past 10 years, knowledge of the condition has advanced rapidly. Screening and treatment improvements, including regimens that more aggressively target early correction of TSH levels, have led to improved intellectual and neurologic prognosis. Newborn screening and thyroid therapy started within 2 weeks of life can normalize cognitive development. Initial dosage of 10 to 15 µg/kg levothyroxine is recommended. The goals of thyroid hormone therapy should be to maintain total thyroxine (T4) or free thyroxine (FT4) in the upper half of the reference range during the first 3 years of life and to normalize the serum TSH concentration to ensure optimal thyroid hormone dosage and compliance.[9]

Serum measurements of thyroid hormones (T_4-TSH) in cord blood could detect hypothyroidism in the neonatal period.[10] Traditionally, screening strategies for the detection of CH were either a primary TSH/back up T4 method or a primary T4/backup TSH method. A third strategy uses TSH plus T4 as the primary test. Changes in TSH levels in response to T3 and T4 blood levels forms the basis of screening for CH.

Mixed cord blood is a good sampling technique for screening CH. Neonatal screening methods measure thyroid function tests in either cord blood sample or that obtained from blood sample taken at 3 to 4 days of life.The cord blood samples for TSH values had compared well with blood samples taken in the first few days of life.Cord blood for sampling is preferred for its ease of collection of sample, lower rates of follow up losses, more practical for mothers with short hospital stay following delivery.

METHODOLOGY

STUDY DESIGN: Prospective comparative study conducted over a period of 20 months.

SOURCE OF DATA

1550 neonates born in hospitals attached to JJM Medical College, Davngere.

INCLUSION CRITERIA

- Neonates whose parents gave consent for the blood sampling.
- Term neonates with birth weight more than 2kg.

EXCLUSION CRITERIA

- Neonates discharged before day 3 of life.
- Neonates born to hypothyroid mother.
- Neonates admitted to NICU.
- Preterm and low birth weight neonates.

METHOD OF COLLECTION OF BLOOD SAMPLE

Blood samples were collected in a sterile container drawn from a 15-20 cm length of the umbilical cord incised while severing it at the time of birth of the baby.

Thus, a mixed cord blood sample (1.5 ml) including both from the umbilical artery and vein was obtained.

T3, T4 and TSH were estimated by competitive immunoassay by using VITROS Total T3 and T4 Reagent Pack and the VITROS Immunodiagnostic Products TSH30 Reagent Pack.

The following details of the neonates included in the study were recorded on the proforma.

- Maternal address, maternal age, parity, thyroid status of the mothers.
- At birth, sex and weight of the neonates.
- Gestational age (was assessed by new modified Ballard scoring system).
- Informed oral and written consent were taken from the parents.

CATEGORISATION OF THYROID FUNCTION STATUS TO SUSPECT CH IN CORD BLOOD AND ON DAY 3 OF LIFE

- LowT4
- High TSH
- Normal T4 High TSH
- Low T4 High TSH
- Low T4 Normal TSH

A level <6.2 µg/dl was considered as the cut-off value for T4 in cord blood and day 3 venous blood samples for screening CH.

A level of >20 mlU/L was considered as the cut-off value for High TSH in cord blood and day 3 venous blood sample for screening CH.

The thyroid hormone T3 is not taken into account as a screening tool for screening CH, because of variation in values due to peripheral conversion of T4 to T3.

STATISTICAL ANALYSIS

The data was entered in Microsoft excel sheet and was analyzed using SPSS version 22 software. The categorical data was represented in the form of frequency and percentage. Continuous data was represented as mean and standard deviation. Paired t test was used to test the significance for quantitative data. p value <0.05 was considered as significant.

Comparison of cord blood thyroid function tests and day 3 thyroid function tests with gold standard was done using kappa statistics and sensitivity, specificity, negative predictive value and positive predictive valuewas calculated and compared. Kappa value of

>0.2 is considered as good for the measure of agreement of the tests.

RESULTS

Table 1:Comparison of Thyroid Function Test (TFT) Valuesin Cord Blood and Day 3 Venous Sample of the Neonates (n=1550) (mean ± SD)

Variable	Cord blood value	Day 3 value	T value	P value
T3 (ng/dl)	72.69 ± 13.12	82.40 ± 11.76	-56.153	<0.001 (VHS)
T4 (µg/dl)	9.01 ± 2.227	11.55 ± 3.596	-31.618	<0.001 (VHS)
TSH (mIU/l)	8.64 ± 6.195	2.98 ± 2.191	-41.926	<0.001 (VHS)

Statistical test: Paired 't' test. Level of significance: p-value <0.05 as significant,p-value ≤0.01 as highly significant, p-value ≤0.001 as very highly significant.

It was observed that mean value of T3, T4 and TSH was 72.69±13.12 ng/dl, 9.01±2.227 µg/dl and 8.64±6.195 mIU/l respectively in cord blood.Mean value of T3, T4 and TSH was 82.40±11.76 (ng/dl), 11.55±3.596 (µg/dl), and 2.98±2.191 (mIU/l) respectively in day 3 venous blood sample.Comparison and co-relation between T3, T4 and TSH levels between cord blood and day 3 venous sample was statistically significant, withp-value ≤0.001 as very highly significant.

Table 2:Distribution of the Cord Blood and Day 3 Venous Blood Samples Into Low, Normal and High Categories Based on Thyroid Hormones Level

	Thyroid hormones (n=1550)																	
	T3 (ng/dl)						T4 (µg/dl)						TSH (mIU/l)					
	Low		Normal		High		Low		Normal		High		Low		Normal		High	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
CB	0	0	633	40.8	917	59.2	0	0	1550	100.0	0	0	0	0	1419	91.5	131	8.5
D3	482	31.3	1068	68.7	0	0	0	0	1509	97.4	41	2.6	57	3.7	1490	96.1	3	0.2

Low T3: <30 (ng/dl) in cord blood and <75 (ng/dl on day 3).
Normal T3: 30-70 (ng/dl) and 75-260 (ng/dl).
Low T4: <6.2 (µg/dl), Normal T4: 6.2 – 22 (µg/dl).
High TSH: >20 (mIU/l), Normal TSH: <20 (mIU/l).
In the cord blood of the neonates of this study (n=1550) the level of thyroid hormone was as follows.
T3 = Low – 0, Normal – 633 and High 917.
T4 = Low – 0, Normal – 1550.
TSH = Normal – 1419, High – 131.
In day 3 venous blood sample (n=1550).
T3 = Low – 482, Normal – 1068 and High – 0.
T4 = Low – 0, Normal – 1509 and High – 41.
TSH = Low – 57, Normal – 1490 and High – 3.

Table 3: Reliability of TFT for Screening of CH in neonates of the Present Study (n=1550)

Parameters in cord blood (suspected CH)	Parameter in Day 3 venous Blood (CH)	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy	Percent Agreement
Low T4	Normal T4 High TSH	0.00	100.00	0.000	99.8	99.0	99.0
High TSH		100.0	91.7	2.3	100.0	91.0	91.0
Low T4 High TSH		0.00	100.00	0.000	99.8	99.0	99.0
Normal T4 High TSH		100.0	91.7	2.3	100.0	91.0	91.0
Low T4 Normal TSH		0.00	100.00	0.000	99.8	99.0	99.0

- Cord blood low T4 0.0% sensitivity, 100% specificity, 0.0% PPV, 99.8% NPV99% diagnostic accuracy and 99% agreement.
- Cord blood high TSHhas 100% sensitivity 91.7% specificity, 2.3% PPV, 100% NPV, 91% diagnostic accuracy, 91% agreement.
- Low T4 high TSH has 0.0% sensitivity, 100% specificity, 0.0% PPV, 99.8% NPV, 99% diagnostic accuracy, 99% agreement.
- Normal T4 high TSH has 100% sensitivity, 91.7% specificity, 2.3% PPV, 100% NPV, 91% diagnostic accuracy, 91% agreement.
- Low T4 normal TSH has 0.0% sensitivity, 100% specificity, 0.0% PPV, 99.8% NPV, 99% diagnostic accuracy, 99% agreement.

Assessment of TSH alone in cord blood has sensitivity of 100% and specificity of 91.7%, hence screening for CH with cord blood TSH alone is a reliable method.
Assessment of T4 alone in cord blood has 0% sensitivity, 100% specificity.Hence screening for CH with cord blood T4 alone is not a reliable method.
Assessing both TSH and T4 together in cord blood is the ideal method for screening CH in newborns.
Although low T4 with high TSH and low T4 with normal TSH have also to be considered for ruling out CH, in the present study these parameters were not seen in the 1550 sample studied.

DISCUSSION

In the present study statistically significant difference was seen in values of T3, T4, TSH on day 3 compared to the cord blood. The concentration of T4 and T3 increased on day 3. In a study conducted by Sara *et al*., (2014), to establish the regional reference values for Thyroid Function Test during the neonatal period found that the serum TSH, total and free T4 concentration peaked in 5^{TH}-7^{TH}days of life which continued over 2 weeks and then decreased to adult reference range. A significantinverse correlation was found between Age and serum concentration of TSH.[11]

The analysis of the present study showed that serum TSH is inversely correlated with the age and the level decreased in significantly on day 3.This result is in line with other studies. Mutule*et al*., (2012), reported an inverse correlation between TFT levels and age after 3rdday.[12] Najam*et al*., (2003), mentioned that decline in TSH and T4 levels was more marked in the first week, and this downward trend was sharper in TSH compared to T4.[13]

Zurakwoski*et al*., (1999), also showed the association between age and TFT in the study on 50,817 patients aged 1 month to 20 years.[14]

In the present study, the newborns were suspected to have hypothyroidismifT4levels were low <6.2 µg/dl or TSH levels were high >20 mlU/L or combination of TSH level normal <20 mlU/L with T4 levels <6.2 jug/dl or combination of TSH level >20 with T4 low <6.2 µg/dl or combination of T4 normal TSH level high >20mIU/L in cord blood. The persistence of the same on day 3 venous blood were considered as CH. Detecting CH on day 3 venous blood samples are not confirmatory. Since a repeat of the tests is required at the 2nd week of life before starting treatment for CH. In the present study of the 1550neonates screened with cord blood and day 3 venous blood sample, 3 neonate was detected to be hypothyroid on day 3 venous blood sample with persistence of TSH >20 mlU/L with normal T4 level. This 3 neonates is taken as gold standard to find the diagnostic accuracy of thyroid function hormones in screening CH.

The present study used a low cut off range for screening CH compared to higher range in other studies. Corbett *et al*., (2009), suggested that the use of low TSH cut off allowed the detection of an unsuspected number of children with neonatal hypothyroidism, evolving in mild permanent thyroid dysfunction in their later life.[15] In the present study TSH value >20 mlU/L in cord blood was considered as high. Mangli*et al*., (2005), also had taken same comparison of TSH in their study.[49] Devi *et al*., (2004), in their study had also taken TSH value >20 mlU/L as abnormal.[8] Ruth *et al*., (1998), from Estonia have taken a TSH cut-off value of 12 µU/ml for neonatal screening, which is a still lower than the cut-off of this study.[16]

In the present study of the 1550 neonates screened, none of the neonate had low T4 <6.2 µg/dl value. The sensitivity of screening hypothyroid with T4 alone was 0.0% with specificity 100% and diagnostic accuracy of only 99%. In contrast to the present study Abduljabbar (2009), in his study with large sample sizeobserved 9.9 % neonates with low T4 with a cut - off value of T4 <6.6 µg/dl in cord blood. This required further retesting of TSH in same cord blood sample to detect primary CH. Assessing cord blood T4 with back up TSH is necessary.[17] If the T4 is low with high TSH in cord blood we should suspect CH, similarly if T4 is normal with high TSH we should suspect CH and if T4 is low with normal TSH also we should suspect CH in cord blood.

In the present study of the 1550 neonates screened, 131 neonates had TSH value high >20 mlU/L. TSH estimation of the same neonates on day 3 venous blood sample showed out of these 131 neonates having cord blood TSH high in cord blood, only 3 neonates was detected to be having persistent high TSH. Statistically the comparison showed 100 % sensitivity and2.3% PPV and 91 % diagnostic accuracy for cord blood TSH estimation. The present study is comparable with Raj *et al*., (2014), who observed 125 of the 430 neonates (29.06%) were found to have elevated cord blood TSH levels. Repeated TSH estimation done on 3rd postnatal day in the 125 babies who had cord blood TSH levels revealed that only 5 (3.94%) babies hadabnormal TSH levels. Serum T4 levels of the same samples on day 3 showed abnormal values in 3 (2.67%) babies.[18]

Mekonnen*et al*., (2003), observed 18.2% of the 1207 neonates were detected to have Cord TSH level between 10-20 mlU/L and 0.3 % neonates had raised TSH values which was >20 mlU/L and concluded that cord blood is practical and apparently simple to collect. It can be put into practice for large scalescreening programmes in situations where hospital discharges are within 24 hours of delivery.Amit*et al*., (2014), observed 11.45% of the neonates had cord TSH >20 mlU/L.Dussault*et al*., (1983), in the Quebec study found 2 cases of permanent CH (of 93 000 infants screened) had been missed by the primary TSH approach and were detected by the primary T4 approach. Hence it is better to estimate TSH and T4 simultaneously in cord blood.

In the present study of the 1550 neonates, 131 (8.5%) neonates had normal T4 with elevated TSH in cord blood. Out of these 131 (8.5%) neonates 3 (0.2%) neonate showed persistent elevation of TSH with normal T4 on day 3 venous blood sample. Statistically the reliability of samples showing normal T4 with elevated TSH in screening CH had 100% sensitivity, 91.7% specificityand diagnostic accuracy of 91%. Buyukgebiz, (2013), reported elevated TSH despite a normal or low T4 indicates inadequate hormone production. [19]Calacicura *et al*., (2002), observed the etiology is probably heterogenous and can be either a transient or permanent thyroid abnormality or delayed maturation of hypothalamic-pituitary axis.[20] In early

discharged babies (in the first day or second), TSH values are found to be elevated because of the cold induced TSH surge. It could be a transient finding due to goitrogens, iodine deficiency, or medications. Genetic defects of hormone biosynthesis and also dysgenesis, especially ectopia, could be the cause. Elevated TSH levels with normal T4 levels could persist for years. Iodine excess, especially when iodine-containing antiseptics are used, may cause transient hypothyroxinemia in preterm babies.[19]

None of the neonates in the present study had low T4 with normal TSH in cord blood and day 3 venous blood samples. Hence reliability of samples showing low T4 with normal TSH had 0.0% sensitivity and 100% specificity. It has got diagnostic accuracy of 99%. This condition occurs most commonly in premature infants and is found in 50% of babies born less than 30 weeks of gestation. Hanna *et al*. (1986), observed low T4 but normal TSH results are also observed during illness, with protein-binding disturbances such as TBG deficiency (1 in 5000), in central hypothyroidism (1 in 25000 to 1 in 50000 newborn infants). Screening programs that employ primary TSH analysis will miss these infants because of normal TSH levels.

None of the neonates in the present study had low T4 with high TSH in cord blood and on day 3. Hence reliability of samples showing low T4 with normal TSH had 0.0% sensitivity and 100%) specificity. It has got diagnostic accuracy of 99%. Primary CH is the most common cause of this condition. However, transient cases, which may be caused by maternal antithyroid medication, exposure to topical iodine, maternal iodine deficiency or excess, maternal TSH receptor blocking antibodies, medications (dopamines, steroids), or prematurity (<30 weeks), may also occur and are not rare.[19]

T3 has not been taken as a tool for screening CH due to its high variability in its values in neonatal blood due to peripheral conversion of T4 to T3. The major secreted hormone of the thyroid gland is T4 while T3 is secreted only in small amounts, which is derived mainly by the peripheral deiodination of T4. One third of circulating T4 is converted to T3 in peripheral tissues. Both hormones are present in serum either bound to proteins or in the free state. T3 is less tightly bound to plasma proteins than T4 and is therefore more readily available for cellular uptake.

The present study emphasizes that simultaneous assessment of cord blood T4 and TSH is a sensitive method for CH screening in newborns. The diagnosis may be confirmed if the TSH level continue to be elevated in the day 3 venous blood sample of these neonates.

CONCLUSION

- Screening of new borns for detection of CH with cord blood is a reliable method.
- Screening for CH with cord blood T4 alone is not a reliable method.
- Screening for CH with cord blood TSH alone is a reliable method. It has greater sensitivity and specificity compared to cord blood T4 and day 3 venous blood T4 levels.
- But the ideal method is screening for CH with cord blood T4 along with TSH levels.
- The present study also showed that by detecting of neonates with high TSH in cord blood, the number of neonates to be pricked on day 3 of life can be limited.
- This study also reveals that day 3 venous blood T3 and T4 values are increased compared to cord blood values and day 3 TSH value decreased compared to cord blood.

Limitations:

- The present study is a single centric study, but for the sake of comparison, multicentric study is more appropriate
- Although low T4 in cord blood is suggestive of congenital hypothyroidism, none of the screened neonates had low T4 values in the present study

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