Laboratory Evaluation of Serum TSH Levels in Neonates Born in Tertiary Care Hospital of Northern India

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ABSTRACT

Objective: *To study the serum levels of Thyroid Stimulating Hormone (TSH) in neonates. Also to assess the sex differences in thyroid function and to compare our results to previously published reference data.*

TSH was estimated in 91 neonates using enhanced Chemiluminescence technique using vitros ECi-Ortho Clinical Diagnostics.

Results: *Although the mean serum levels of TSH were higher in females, no significant differences were observed in serum TSH between males and females.*

Conclusion: *The study underlines the importance of new born screening for thyroid dysfunction. Due to high reported prevalence of congenital Hypothyroidism (CH) in our country and the lack of international standardization, performing multi-central studies with more population helps in making a more precise evaluation of thyroid status in neonates. Frequent laboratory monitoring in infancy is essential to ensure optimal neurocognitive outcome. Serum TSH and free T4 should be measured every 1- 2 months in the first 6 months of life and every 3-4 months thereafter. In general, the prognosis of infants detected by screening and started on treatment early is excellent.*

Keywords: *Thyroid stimulating hormone (TSH), congenital hypothyroidism (CH), chemiluminescence*

INTRODUCTION

In recent years, enhanced Chemiluminescence has become very popular in Clinical Biochemistry due to its high sensitivity, wide dynamic range and complete automation. It is a rapid and simple method without radioactive pollution as a detection principal in immunoassay for determination of molecules (e.g. proteins, hormones, drugs, nucleic acids and environment pollutants).[1,2]

Congenital hypothyroidism (CH) is defined as thyroid hormone deficiency present at birth. Thyroid hormone deficiency at birth is most commonly caused by problem with thyroid gland development (dysgenesis) or a disorder of thyroid hormone biosynthesis (dyshormonogenesis) table 3. Congenital hypothyroidism is classified into permanent and transient CH. Permanent CH refers to a persistent deficiency of thyroid hormone that requires life- long treatment. Transient CH refers to a temporary deficiency of thyroid hormone, discovered at birth, but then recovering to normal thyroid hormone production.

The overall prevalence of congenital hypothyroidism is high. Prior studies suggest that a child with undiagnosed CH will have a 5 to 10 point decline in IQ, delays in speech and language development and decreased attention and memory skills.[3]

The purpose of this study is to evaluate serum TSH levels in neonates using enhanced Chemiluminescence. The study underlines the importance of newborn screening for thyroid dysfunction. Frequent laboratory monitoring in infancy is essential to ensure optimal neurocognitive outcome. Serum TSH should be measured every 1- 2 months in the first 6 months of life and every 3- 4 months thereafter.

More recent studies have shown the potential benefits of screening newborns for congenital hypothyroidism (CH) over the past five decades, improvements in the sensitivity and specificity of thyroid-test methodologies have dramatically impacted the Clinical strategies for detecting and treating thyroid disorders.

In the 1950s, only one thyroid test was available an indirect estimate of the serum total (free + protein bound) thyroxine (TT4) Concentration using the protein bound iodine (PBI) technique. Since 1970 technological advances in radioimmunoassay (RIA) and immunometric assay (IMA) methodologies have progressively improved the specificity and sensitivity of the methods.[3]

METHODS

This study was done in the department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar. The serum TSH estimation was done by enhanced Chemiluminescence technique using vitros Eci- ortho Clinical Diagnostics.

SAMPLE COLLECTION

The blood samples from hospital based population of children aged 1 day – 1 week were

collected in a plain- tube (red top vacutainer) under sterile conditions and sent to the laboratory immediately for serum separation.

The sample size for the evaluation of TSH was 91. The serum TSH levels (3rd Generation assay) were estimated in these subjects using vitros ECi by ortho clinical diagnostics. It is Non- competitive immunoassay- sandwich immunoassay. Enhanced chemiluminescence is the chosen technology for vitros Eci. It uses Horseradish peroxidise (HRP) as the label, Luminol is the substrate together with H_2O_2 and enhancer (acetanilide) act as catalysts. Enhancers speed the oxidation of the luminal by HRP by as much as 1000 times. Enhancer enhances the light intensity of each luminal molecule and sustains light production so that resulting light output is transformed from flash to glow. This result in an increased light production compared to other indirect methods.

On practical terms, the immunoassay procedure with vitros Eci delivers the result for TSH within 37 minutes that meets requirements for same day reporting of the results in an institution. Data were collected routinely according to standard procedures. The Table 1 shows the age-wise distribution of the subjects who were divided into two groups. Group 1 included subject in the age range of 0 – 3 days and there were 48 subjects in this group. Group 2 included neonates in age group of 4-7 days and there were 43 neonates in this group.

Ethical Consideration: The project was approved by the institutional Ethics Committee.

RESULTS

The descriptive data was given as mean ± standard deviation. The chi- square test was used for the statistical analysis. The differences were considered to be statistically insignificant when the P value obtained was greater than 0.05.

The mean ± SD of serum TSH levels in group 1 was 4.048 ± 2.379 mIU/L. The serum TSH was normal in 95.8% neonates. It was decreased in 4.2 % of the neonates in group1.

The mean ±SD of serum TSH in group 2 was 3.779 ± 2.531 mIU/L. The serum TSH Levels were normal in 88.4% and decreased in 11.6 %. The difference in the serum TSH levels in group1 and group2 was not significant (p>0.05).

The Table 2 shows the sex wise distribution of the subjects. There were 53 male and 38 females in the subjects under study. It was seen that males (n=53) were more in number as compared to females (n=38) in the subjects under study.

The table 2 shows the comparison of serum TSH in males and females amongst the subjects under study. The serum TSH was normal in 90.6% males and decreased in 9.4%. The mean ±SD of serum TSH in males was 3.630 ± 2.432 mIU/L. In case of females (n=38) the mean ±SD was 4.325 ± 2.429 mIU/L. Although the mean serum levels of TSH were higher in females, no significant differences were observed in serum TSH between males and females (p>0.05).

Table: 1

TSH Level	Age group		Total
	0 – 3	4 - 7	
Normal	41 (85.4%)	36 (83.7%)	77
Decreased	7 (14.6%)	7 (16.3%)	14
Total	48	43	91
	$x^2 = 0.050$; df =1; p = 0.823; NS		
Mean ± SD	4.363 ± 4.040	3.492 ± 2.785	$p = 0.240^{NS}$

NS: p > 0.05; Not Significant

Table 2:

TSH Level	Male	Female	Total
Normal	46 (86.8%)	31 (81.6%)	77
Decreased	7 (13.2%)	7 (18.4%)	14
Total	53	38	91
	$x^2 = 0.462$; df =1; p = 0.497; NS		
Mean ± SD	3.805 ± 3.099	4.154 ± 4.054	$p = 0.948^{NS}$

NS: p > 0.05; Not Significant

Crosstabs

TSH grp *Sex wise distribution

			Sex		Total
			F	M	
TSH_grp	Normal	Count	31	46	77
		% within Sex	81.6%	86.8%	84.6%
	Decreased	Count	7	7	14
		% within Sex	18.4%	13.2%	15.4%
Total		Count	38	53	91
		% within Sex	100.0%	100.0%	100.0%

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.462[a]	1	.497		
Continuity Correction[b]	.148	1	.700		
Likelihood Ration	.457	1	.499		
Fisher's Exact Test				.563	.347
N of Valid Cases	91				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.85.
b. Computed only for 2x2 table

T-Test

Group Statistics

	Sex	N	Mean	Std. Deviation	Std. Error Mean
Age	M	53	3.6038	1.93490	.26578
	F	38	3.6316	2.05888	.33399
TSH	M	53	3.8052	3.09892	.42567
	F	38	4.1543	4.05361	.65758

Table 3: Genetic Causes of Congenital Hypothyroidism

Defective Gene Protein	Inheritance	Consequences
PROP-1	Autosomal recessive	Combined pituitary hormone deficiencies with preservation of adrenocorticotropic hormone
PIT-1	Autosomal recessive Autosomal dominant	Combined deficiencies of growth hormone, prolactin, thyroid – stimulating hormone(TSH)
TSHβ	Autosomal recessive	TSH deficiency
TTF-1 (TITF-1)	Autosomal dominant	Variable thyroid hypoplasia, choreoathetosis, pulmonary problems.
TTF-2 (FOXE-1)	Autosomal recessive	Thyroid agenesis, choanal atresia, spiky hair
PAX-8	Autosomal dominant	Thyroid dysgenesis
TSH- receptor	Autosomal recessive	Resistance to TSH
Albright hereditary osteodystrophy	Autosomal dominant	Resistance to TSH
Na/I symporter	Autosomal recessive	Inability to transport iodide
THOX2	Autosomal dominant	Organification defect
Thyroid peroxidase	Autosomal recessive	Defective organification of iodide
Thyroglobulin	Autosomal recessive	Defective synthesis of thyroid hormone
Pendrin	Autosomal recessive	Pendred syndrome: sensorineural deafness and partial organification defect in thyroid
Dehalogenase 1	Autosomal recessive	Loss of iodide reutilization
Source: Harrison's Principles of internal Medicine 18th Edition Vol.2;2912.(15)		

Table 4: Clinical features of CH

Lethargy, apathy, and sluggishness	Feeding difficulties
Hypotonia and delayed reflexes, Puffy face and macroglossia	Hoarse cry
Bradycardia and hypothermia	Constipation
Large anterior fontanel and	Umblical hernia and abdominal distension
Persistently open posterior	Cold and mottled skin and
Fontanel	Prolonged neonatal jaundice
Information from reference 20.	

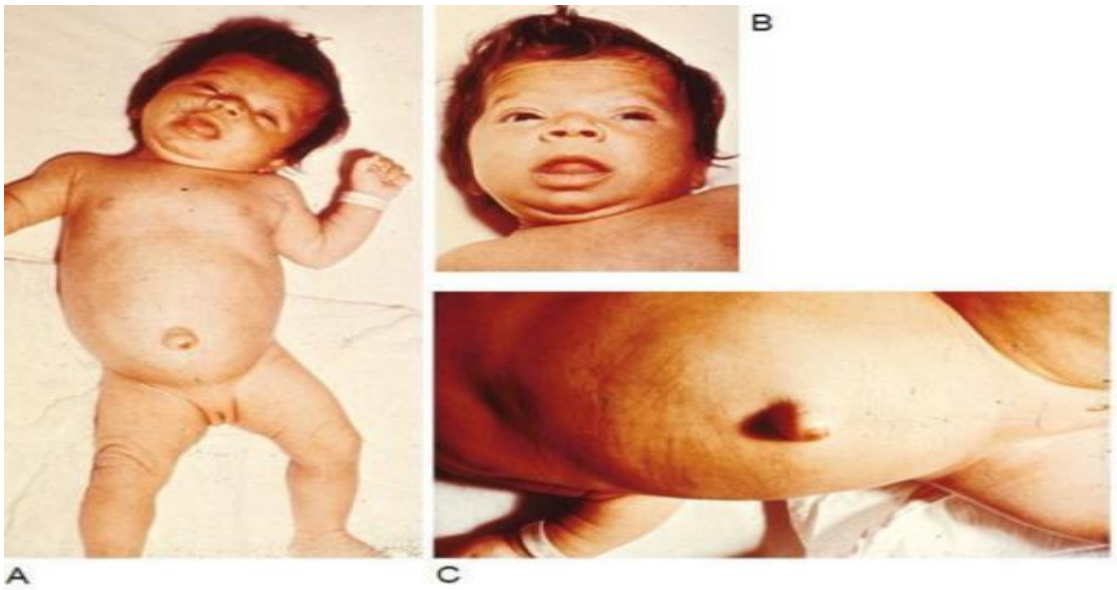

Fig. 1: Infant with hypothyroidism. A- 3 month old infant with untreated CH: picture demonstrates hypotonic posture, myxedematous facies, macroglossia, and umbilical hernia. B- Same infant, close up of face, showing myxedematous facies, macroglossia, and skin mottling. C- Same infant, close up showing abdominal distension and umbilical hernia.

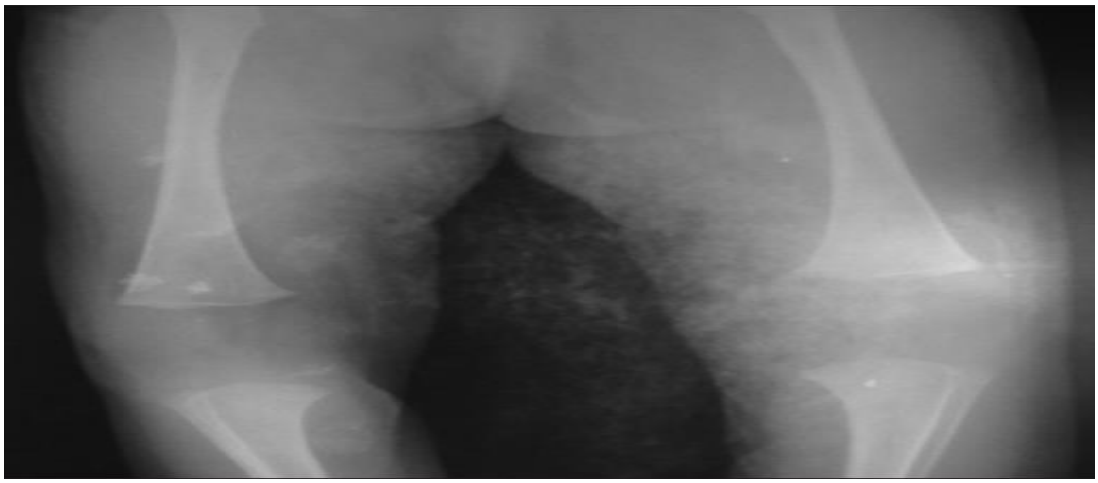

Fig. 2: X-ray of lower limb revealed absent distal femoral epiphyses

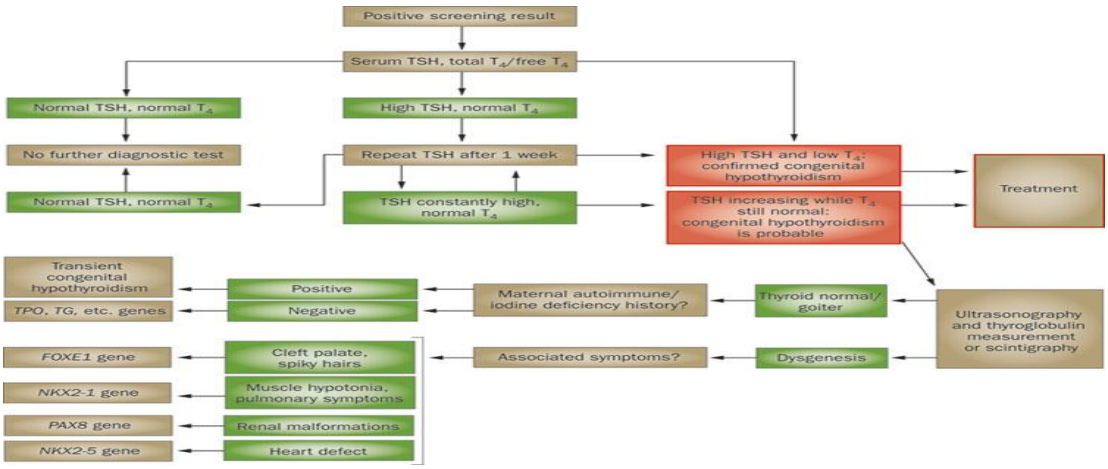

Fig. 3: Algorithm for the management of abnormal newborn screen

DISCUSSION

Congenital hypothyroidism (CH) is one of the most common preventable causes of mental retardation in children. Early diagnosis and treatment prevent the devastating outcome of mental retardation.

The incidence of Congenital Hypothyroidism (CH) in India Varied from 1 in 476 in one study[4] and 1 in 1,700 in another study[5] which is higher than population based incidences reported in Western Countries.[6,7] However, the exact incidence of CH in India is not known; this is largely due to the fact that neonatal screening is still not Universal in India and is only sporadically implemented at local health systems.

Newborn Screening (NS) Universal NS for Congenital Hypothyroidism (CH) has been implemented in the United States, Canada, Western Europe, Japan, Australia, New Zealand, Taiwan, parts of China, parts of Mexico and Israel. However, Universal NS is still under development in other developing countries across Asia and Africa.

NS is done by collecting blood on a filter paper by heel prick on the Second to the fifth day of life, which is then sent to a Central laboratory for testing. Some programs test for total T4 value with follow up TSH if total T4 was abnormal and other Programs test for TSH value. Initial testing of TSH allows for detecting subclinical hypothyroidism in the setting of normal T4 value while initial testing of total T4 allows detection of Central hypothyroidism in the setting of normal or low TSH. If either T4 or TSH were the initial testing and was abnormal, the other value needs to be followed. Most NS programs have switched to initial testing of TSH only.

The usual TSH cut off value at which further serum testing and clinical evaluation is warranted is typically set between 10 mIU/L and 15 mIU/L.[9] If the TSH cut off is lowered, more infants with milder congenital hypothyroidism will be detected.

Eighty five percent of Cases of Primary permanent CH are due to abnormal thyroid gland formation (thyroid dysgenesis).

At birth, newborns with CH do not have obvious Clinical features indicative of CH. This is probably explained by the fact that there is some transplacental transfer of maternal T4 which can be detected in the umblical cord blood at birth yet not in enough amounts to normalize TSH levels.[10] This provides a protective effect, especially to fetal brain.[11] Also the most common form of congenital hypothyroidism has some moderately functioning thyroid tissue.[12] The slow development of obvious clinical symptoms[13], coupled with the importance of early treatment led to the implementation of widespread newborn screening for this condition.[14]

Where NS is not implemented, infants affected by CH present with a variety of Clinical features that appear after the immediate neonatal period.

If CH is left untreated it results in various degrees of neurologic and physical growth impairments. It is imperative for physicians in Countries where newborn screening is not implemented to be familiar with the classical presentation of CH (Table 4) fig. 1, fig. 2.

All infants with a low T4 concentration and a TSH concentration greater than 40 mIU /L are considered to have congenital hypothyroidism and should have immediate confirmatory serum testing. If the TSH concentration is slightly elevated but less than 40mU per L, a second screening test should be performed on a new sample. Results should be interpreted using age- appropriate normative values (the TSH reference range at two to six weeks of age, the most common period of retesting, typically is 1.7 to 9.1mU per L (table 4) . Approximately 10 percent of infants with confirmed congenital hypothyroidism have TSH values between 20 and 40 mIU per L. Hyperthyrotropinemia is characterized by high TSH concentration in the neonatal period with normal concentration of T4 and FT4. It may be caused by transient or permanent thyroid abnormality or delayed hypothalamic- pituitary axis maturation, and it is more common in infants with Down syndrome. The need for therapy is controversial. A Normal TSH level with low T4 values occurs in about 3 to 5 percent of neonates and may indicate thyroid insufficiency. It is more common among preterm or ill infants. Possible causes are hypothalamic immaturity, protein binding disturbances such as TBG deficiency, central hypothyroidism, or primary hypothyroidism with delayed TSH elevation. Treatment with LT4 has no proven benefit except in infants with central hypothyroidism or delayed TSH elevation. Delayed TSH elevation is more common in infants with low birth weight and those who are critically ill. Serum TSH levels in these infants increase in the first few weeks after birth to concentrations characteristic of primary hypothyroidism. Rarely, abnormal screening results may be caused by transient hypothyroidism, and results of follow –up T4 and TSH are normal. Causes of transient hypothyroidism include fetal exposure to maternal antithyroid drugs, prenatal or postnatal exposure to excess iodides, and iodine deficiency. Transplacental passage of maternal thyrotropin receptor- blocking antibodies (TRBAbs) is rare but should be suspected if there is a maternal history of autoimmune thyroid disease or previous affected children. Cord blood can be tested for thyroid abnormalities. Elevated T4 and TSH levels resulting from maternal antithyroid drugs typically return to normal within one to three weeks without treatment.[16]

Once the diagnosis is suspected by either an abnormal NS or by Clinical suspicion then serum testing of Free T4 (FT4) and TSH needs to be done. Algorithm for the management of abnormal newborn screen is shown in fig. 3.

Timely initiation and maintenance of treatment of CH is very important to effect adequate neurocognitive development during the critical first 3 years of life. Not only delaying the start of treatment should be avoided, but also optimal treatment should be ensured to maintain normal thyroid hormone levels.

Oral levothyroxine (L-T4) is the recommended treatment of choice. The American Academy of Pediatrics (AAP) recommends doses of 10 to 15 mcg/kg/d.[17,18] The treatment goals as outlined by the American Academy of Pediatrics (AAP) Update of screening and therapy for congenital hypothyroidism[19] are similar to published European Society for Pediatric Endocrinology (ESPE) guidelines[18] and are as follows:

1. Serum Free T4 or total T4 should be kept in the upper range of normal during the first year of life.
2. Target values during the first year are 130 to 206 nmol/L(10-16 µg/dl) for the serum T4 and 18 to 30 pmol/L(1.4 to 2.3 ng/dl) for free T4.
3. Serum TSH should be kept under 5mU/L.

Clinical evaluation should be performed every few months during the first three years of life along with frequent measurements of serum T4 and TSH.

CONCLUSION

There is need for the routine assay of thyroid hormones in neonates as a child with undiagnosed CH will have low IQ, delay in speech and language development, and decreased attention and memory skills. Moreover, children with CH who are started on supplemental thyroid therapy within the first few weeks of life "have a normal or near-normal neuro developmental outcome". It is therefore important for these patients to receive early treatment and close follow up.

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