

■ **Original Article**

Levels of T4 and TSH in mother's blood and in cord blood at the time of delivery

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Abstract

Background: In conditions of maternal iodine deficiency, the frequency distribution of neonatal thyroid stimulating hormone (TSH) is shifted towards elevated values. Elevated serum TSH in the neonates indicates insufficient supply of thyroid hormones to the neonates, a major complication of iodine deficiency. **Objectives:** To determine the cord blood serum T4 and TSH levels and interrelationships with maternal thyroid hormones. **Method:** cord blood serum T₄ and TSH levels of 45 consecutive newborns born at BPKIHS, Dharan, Nepal and maternal T₄ and TSH levels were analyzed using ELISA based kits. **Results:** The mean age of mothers was 23.73± 3.86 years and average weight of the neonates was 3.038±0.45 Kg. The median levels of maternal and neonatal T₄ were 1.09 ng/dl and 1.26 ng/dl respectively. The corresponding median TSH levels were 3.71 mIU/L and 11.9 mIU/L. The maternal and neonatal levels of T₄ were positively correlated. In 36 mothers who had TSH level within euthyroid range (0.3-6.2 mIU/L, Thyroid lab of BPKIHS), 22 neonates (61.11%) had TSH levels above 10 mIU/L. Among 9 mothers having TSH levels above 6.2 mIU/L, 7 (77.77%) neonates had TSH levels above 10 mIU/L. **Conclusion:** Overall 29 (64.44%) neonates had TSH level above 10 mIU/L which indicates mild degree of iodine deficiency. Iodine supplementation is required before pregnancy in majority of women of reproductive age.

Keywords: cord blood, serum T4 and TSH levels, neonatal hypothyroidism, iodine deficiency

Introduction

Infantile hypothyroidism, either caused by iodine deficiency disorder or congenital hypothyroidism, is the world's leading cause of preventable mental retardation.¹ Hypothyroidism in the newborn is almost overlooked and delayed diagnosis leads to the most severe outcome of congenital hypothyroidism, mental retardation, emphasizing the importance of neonatal screening.² In iodine deficiency, the levels of neonatal thyroid stimulating hormone (TSH) is shifted towards elevated values.³ In iodine deficiency, both maternal and fetal thyroid functions are affected together and

therefore it is primarily the degree and precocity of the maternal hypothyroxinemia due to iodine deficiency during pregnancy that will drive the potential repercussions for fetal neurological development.⁴

During pregnancy complex changes of maternal thyroid function occur and they are influenced by the maternal iodine supply. With decreasing iodine intake, maternal goiter and hypothyroxinaemia as well as fetal and neonatal hypothyroidism become more prevalent. The severity of iodine deficiency and hypothyroidism in mother during early and mid gestation is related to the severity of the neural damage in her fetus.⁵

The adequate functioning of both the maternal and fetal thyroid gland play important role to ensure that

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the fetal neuropsychointellectual development progresses normally.⁶ The fact that maternal thyroid status influences the fetal brain development has been established.⁷ Early diagnosis and treatment are essential to prevent irreversible and permanent nervous system damage and developmental delay.⁸ Indian Academy of Pediatrics recommends the use of cord blood samples for screening for congenital hypothyroidism.⁹ In most screening programs, blood samples are collected at 5-6 days of birth, but with large number of babies being discharged early, cord blood samples are being used as well.^{10,11}

Sub- Himalayan region has been described in literature as iodine deficient region with high prevalence of endemic goiter and cretinism.^{12,13} Dharan falls in this region hence this study was planned as a study to explore the possible existence of maternal and neonatal hypothyroidism in Dharan and surrounding areas.

Methods

This hospital-based cross sectional study was carried out in the Department of Basic and Clinical Physiology and Biochemistry in collaboration with the Department of Obstetrics & Gynaecology from May 2007 to May 2008. In this study, 45 pregnant mothers and their 45 newborns were enrolled. Blood samples of pregnant mothers and cord blood samples were taken. Informed consent was taken from all subjects. All pregnant mothers were admitted in the antenatal ward of Obstetrics & Gynaecology Department of BPKIHS were included in the study for safe confinement. Pregnant mothers already diagnosed as hypothyroid, family history of hypothyroidism, twin pregnancy, with any complications like eclampsia, pre-eclampsia, any other systemic diseases like diabetes, heart disease, liver disease, renal disease and other endocrine disorders were excluded from the study. About 5 ml of each, maternal blood from normal pregnant mothers and umbilical cord blood were collected in a plain vial. The samples were stored at 4^o C for biochemical analysis. FT₄, TSH (mothers), FT₄ and TSH (cord blood).

Thyroid profile (fT4 and TSH) were measured by using commercially available ELISA based kits (Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany). Quality control serum was run with each batch of samples to be tested.

The fT4 ELISA based on the principle of competitive binding between fT4 in a test specimen and T4 peroxide conjugate for a limited number of binding sites on the anti-T4 (sheep) coated well was used. Thus the amount of T4 peroxide conjugate bound to the well was inversely proportional to the concentration of fT4 in the specimen. After incubation of specimen and T4-peroxidase conjugate unbound enzyme conjugate was removed in the equilibrium state by washing. TMB/Substrate solution was added and blue color developed. The intensity of this color, which changed to yellow after stopping the reaction, was inversely proportional to the amount of the T4 in the specimen.

The TSH-ELISA based on the classical sandwich ELISA technique. With highly specific monoclonal anti-TSH antibody coated on the surface of the microtitre wells were used. In the first incubation step, specimens, calibrators or controls and enzyme conjugate (peroxidase-labeled anti-TSH) were mixed to form the sandwich complex and bound to the surface of the wells by the interaction with the immobilized antibody. At the end of the incubation, excess enzyme conjugate was washed out. Substrate reagent was added and the resulting color, which turns into yellow after stopping the reaction with the stop solution, is measured photometrically. The absorbance increases directly proportional to the TSH concentration in the sample. The concentration was evaluated by means of calibration curve, established from the calibrators supplied with the kit. The analytical sensitivity was determined from the variability of the zero calibrator and resulted in <0.10 mIU/L. The cross reactivity with other compound occurring in the sample (HCG, FSH and LH) was less than 0.01 %. The normal level of TSH in human serum is 0.3-6.2 mIU/L.

The data were analyzed using SPSS. Median and interquartile range of cord blood T4 and TSH and maternal T4 and TSH values were considered to report the result.

Results

Forty five pregnant women and their neonates were enrolled in this study. General characteristics of mothers and neonates are presented in Table. 1.

Table 1: General characteristics of mothers and neonates

General characteristics (n=45)	Mean± SD (n=45)
Mother's Age	23.73 ±3.8
Mother's weight	57.71± 5.95
Mother's height	1.53 ± .049
Neonate's weight	3.038 ± .45

Table 2: Median and interquartile range of TSH and T₄ level in cord blood and maternal serum

Parameters	Neonates (n=45)	Mothers (n=45)
T ₄ (ng/dl)	1.26 (1.04-1.52)	1.09 (1.0-1.49)
TSH (mIU/L)	11.9 (7.58-19.31)	3.71 (2.46-5.34)

Median and interquartile range of maternal T₄ and TSH levels were 1.09 (1.0-1.49) and 3.71(2.46-5.34) respectively. The reference value of maternal T₄ and TSH were 0.8-2.2 and 0.3-6.2 respectively. The median and interquartile range of cord blood T₄ and TSH levels were 1.26 (1.04-1.52) and 11.9 (7.58-19.31) respectively. The frequency distribution of neonates according to thyroid stimulating hormone levels are presented in Table 3. 10 (22%) of neonates had TSH levels above 20 mIU/L.

Table 3: Frequency of neonates with different cord blood TSH levels

Cord blood TSH range (mIU/L)	Frequency (n)	Percentage (%)
<10 mIU/L	16	35.56 %
>10 mIU/L	29	64.44 %

Mothers are classified into two groups A and B according to their TSH levels (Table 4). Group A comprises 36 mothers who had TSH levels within normal euthyroid reference range of 0.3-6.2 mIU/L. Their neonates exhibited heterogeneous pattern of TSH which were not consistent with their mothers. T₄ levels were within normal euthyroid range. In group B mothers who had TSH >6.2 mIU/L; out of 9 neonates seven had TSH levels above 10 mIU/L. Overall 64.44% of neonates had TSH levels above 10 mIU/L.

Table 4: Median TSH and T₄ levels in two groups of mothers and their neonates

Reference TSH levels of maternal blood (mIU/L)	Median maternal TSH (mIU/L)	Maternal median T ₄ (ng/dl)	Median TSH at Cord (mIU/L)	Median T ₄ at cord (ng/dl)
A. 0.3-6.2 mIU/L (n=36)	2.78	1.075	11.91 (22=had TSH >10)	1.26
B. >6.2 mIU/L (n=9)	8.05	1.12	10.59 (7= had TSH >10)	1.28

There was positive correlation found between neonatal cord T₄ and maternal T₄ levels as presented in Table 5.

Table 5: Correlation coefficient (r) between serum T₄ and TSH levels of mothers at delivery and neonates at cord

Neonatal cord sample	Mothers at delivery	
	T ₄	TSH
T ₄	.373 (r) 0.012 (p value)	
TSH		.191(r) .209 (p value)

Discussion

Many studies have been conducted around the globe for neonatal thyroid screening. Different workers have given different cut-off values of TSH in cord serum to consider them as iodine deficient and hypothyroid. There has been no known report till date on thyroid status of pregnant mothers and their neonates from Nepal. Hence this study was planned in sub-himalayan region to assess the thyroid status of pregnant mothers and their neonates considering the fact that iodine deficiency remains a mild to moderate public health problem among pregnant and lactating women despite the availability of iodized salt.

This cross-sectional study included 45 normal pregnant mothers and their 45 newborns. Cord blood samples and maternal blood samples were taken to determine T₄ and TSH. The result demonstrated that 29 neonates had TSH levels above 10 mIU/L. Out of these 10 neonates had TSH above 20 mIU/L. These findings were closely comparable to the report of Abbas et al; they also used the cut-off point as 10 mIU/L TSH values for cord serum indicating mild degree of iodine deficiency disorder.¹⁴ Assuming the

numerous iodine deficient areas in Nepal, it may be reasonable to take a lower cut-off value of TSH for neonates for considering them as hypothyroid. Some investigators consider the threshold value for a significant TSH elevation to be 20-25 mIU/L for blood samples taken from heel pricks.¹⁵ These figures were comparable to figures from India.¹⁶ Elevated serum TSH in the neonates indicates insufficient supply of thyroid hormones to the developing brain. International organizations like WHO/UNICEF/ICCIDD have included the neonatal TSH as one of the indicators for assessing iodine deficiency disorders and their control in a population. Also according to the criteria set by WHO/UNICEF/ICCIDD in 1994; to assess the severity of iodine deficiency based on the frequency of elevated TSH concentration in newborn screening program;¹⁷ our sample is said to be iodine deficient. Neonatal TSH has the major advantage of being the single indicator allowing prediction of possible impairment of mental development at a population level.³ In the present study, 9 out of 45 mothers had TSH above 6.2 mIU/L which indicates hypothyroid state. Seven neonates of these mothers had TSH above 10 mIU/L. Among 36 mothers who had TSH within euthyroid range; 22 neonates had TSH above 10 mIU/L. Human fetal thyroid tissue continues to rely on the maternal supply of thyroxin during pregnancy such that maternal fetal transfer of thyroxin accounts for up to about 50% of fetal serum thyroxin at term.¹⁸ In normal pregnant women, the thyroid gland maintains euthyroidism with only minor fluctuation in the serum T4 (transient decrease) and TSH (transient increase). However, in women with limited thyroid reserve, due to iodine deficiency hypothyroidism can develop. Fluctuation in thyroxin metabolism that occur during pregnancy may further impair maternal fetal transfer of thyroxin despite apparently optimal maternal thyroid status.¹⁹ Pregnancy is also often accompanied by a decrease in the availability of maternal thyroid iodide, resulting in a relative iodine deficient state.²⁰

This study also demonstrated that in the neonates, level of cord serum T4 was independent of TSH level; reflects the relative immaturity of interactions among different components of the hypothalamus-pituitary-thyroid axis at the time of birth.^{21,22} Another reason as proposed in other studies^{21, 22 23} may be virtual absence of T3 in the fetal circulation and fetus does not actively convert T4 into T3 in the

peripheral tissues; and T4, rather T3 is the principal hormone accounting for feedback control of pituitary TSH secretion. In moderate iodine deficiency, fetal thyroid stimulation is increased in order to have the required amount of T4, which is reflected by hyperthyrotropinemia.

The major limitation of this study was limited sample size which was due to limited resources and we could not do the follow up for those who had TSH above 10 mIU/L. Because patients were discharged on the next day of delivery and also analysis was done at a later date.

Conclusion

It can be concluded that level of cord serum TSH was independent of maternal TSH levels. Overall, in 64% of the neonates TSH levels were above 10 mIU/L corresponding to some degree of iodine deficiency.

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