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Original Article

SERUM THYROXINE (T_4) AND THYROID STIMULATING HORMONE (TSH) LEVELS IN CORD BLOOD OF NEWBORNS IN LAHORE

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ABSTRACT:

Objective: The primary aim of this study was the early detection and treatment of hypothyroidism in neonates. This paper describes the determination of cord blood serum T_4 and TSH levels, their mean levels, reference ranges and interrelationship.

Design: Retrospective analysis of serum T_4 and TSH levels in 1153 cord blood samples collected and analyzed during July 1998 to June 2000.

Setting: Centre for Nuclear Medicine (CENUM), Mayo Hospital, Lahore.

Patients: Cord blood samples of unselected neonates born at Lady Aitchison Hospital, Lady Willingdon Hospital & Government Mian Munshi Hospital, Lahore, randomly collected immediately after delivery.

Main outcome measures: Cord serum T_4 and TSH levels, their respective means and reference range.

Results: Mean (± SD) T_4 level in cord blood serum was 115 ±36 nmol/L (range: 15-350 nmol/L) and mean TSH level was found to be 5.6 ±5.1 mIU/L (range: 0.05 - 150). The reference range for T_4 and TSH was 49-189 nmol/L and 0.4-17.6 mIU/L respectively. A trend in T_4 and TSH levels distributions towards higher values was noted. Serum T_4 and TSH levels were not correlated significantly. Except at lowest and highest levels no reciprocal relationship between T_4 and TSH levels was found as is observed in adults. T_4 and TSH levels in 90% neonates were between 60-173 nmol/L and 1.0-14.2 mIU/L respectively.

Conclusion: In most neonates, level of cord serum T_4 was independent of TSH level. Mean T_4 and TSH levels and their normal ranges were lower as compared to an iodine-sufficient country like USA. In 11.4% of neonates TSH levels were above 10 mIU/L, corresponding to mild degree of iodine deficiency disorders in Lahore.

KEY WORDS: Cord blood, serum T_4 and TSH levels, neonatal hypothyroidism, iodine deficiency, low-birth weight babies.

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INTRODUCTION

Cord blood is a source for the determination of thyroid related hormones and thyroxine-binding globulins (TBG) in neonates.[1] Many workers have used cord blood as a sample source for:(a) screening of congenital hypothyroidism in newborns,[2,3](b) comparison of mother and neonate thyroid hormones at term,[4,5](c) study of changes in thyroid hormones levels after therapeutic intervention in pregnant women and neonates,[6,7] (d) examining the interrelations of thyroid related hormones during gestation[8,9] and (e) evaluation of the severity of iodine deficiency in an environment.[3] Thyroid hormone play an

important role in normal brain development of fetus and its severe deficiency during gestation is associated with permanent brain damage due to hypothyroidism[3,5,7]. Pakistan is a developing country where socio-economic conditions and illiteracy lead to the late diagnosis of this disorder when the child grows up and presents with symptoms and different complications. Secondly iodine deficiency, autoimmune thyroiditis and Grave's disease are quite common in general population which end up in hypothyroidism. We have recently completed a pilot project for detection of congenital hypothyroidism by measuring T_4 and TSH from cord blood serum. The primary aim of this study was the early detection and treatment of hypothyroidism in neonates. This paper describes the determination of cord blood serum T_4 and TSH levels, their mean levels, reference ranges and interrelationship. The data was also analyzed to determine the degree of iodine deficiency disorders (IDD) in Lahore.

SUBJECTS AND METHODS

The cord blood samples of unselected neonates born at Lady Aitchison Hospital, Lady Willingdon Hospital and Government Mian Munshi Hospital, Lahore were randomly collected immediately after delivery. Four milliliters of cord blood was obtained after ligation of the cord from placental side in a disposable syringe and stored in refrigerator. The serum was separated by low-speed centrifugation (2000×g) for 5 minutes at room temperature. Serum samples were stored at -20°C until analysis. Estimation of T_4 and TSH was carried out using commercial kits of Ortho-clinical Diagnostics (Amersham, UK) and North Eastern Thames Regional Immunoassay (St. Bartholomew's Hospital, London, UK). T_4 was determined by competitive radioimmunoassay (RIA) technique. There was a competition between T_4 present in the sample and ^{125}I labeled T_4 for a limited number of binding sites on a sheep anti- T_4 antibody bound to magnetizable polymer particles. TSH was measured by high sensitive immunoradiometric assay (IRMA) which utilized coated tubes. There was a reaction of TSH present in the sample with a monoclonal mouse antibody coupled to the tube and ^{125}I radiolabelled polyclonal antibody. This assay was highly sensitive even at very low concentration of TSH (detection limit 0.05 mIU/L). Measurement of radioactivity, fitting of the standard curve and analysis of samples was carried out using a computerized gamma counter (Cap-RIA 16, CAPINTEC; Inc. USA). Assay reliability was determined by the use of commercially derived control sera of low, medium and high concentrations which were included in every run. All assays were carried out in duplicate and results were expressed at less than 10% CV of imprecision profile.

The analysis of T_4 and TSH levels distribution was carried out using SPSS program (SPSS Inc., Chicago, IL) on a personal computer. Cord serum T_4 and TSH levels were not normally distributed. Therefore, the results are expressed as medians and percentiles. A non-parametric central 0.95 interfractile interval was used to establish the reference values instead of usual 2.5 and 97.5 percentiles as lower and upper limits (95%), which are used when distributions are normal.[10] Pearson product moment correlation coefficient (r) between T_4 and TSH distributions was calculated after log transformation.

RESULTS

During July 1998 to June 2000, 1153 cord blood samples were collected. Both T_4 and TSH were measured in all samples. Serum T_4

Table-I: Serum T_4 and TSH levels in cord blood

Hormones	*mean (±SD)*	*median*	*observed range*
T_4 (nmol/L)	115±36	113	15 - 350
TSH (mIU/L)	5.6±5.1	4.5	0.05 - 150

Table-II: Interrelation of cord serum T4 and TSH levels

Percentile Range	*Conc. Range*	*No. of neonates(%)*	*Mean Hormone*
TSH (mIU/L)			**Mean T_4±SD**
Upto 5th	Upto 0.9	61(5.3)	120.6 ± 60.3
6th - 50th	1.0 - 4.5	550 (47.7)	115.2 ± 37.8
51st - 95th	4.6 - 14.2	485 (42.1)	114.8 ± 31.3
>95th	>14.2	57 (4.9)	105.0 ± 29.1
T4 (nmol/L)			**Mean TSH±SD**
Upto 5th	Upto 59	58 (5.0)	8.1 ± 20.5
6th-50th	60-113	527 (45.7)	5.9 ± 5.5
51st-95th	114-173	513 (44.5)	5.5 ± 4.3
>95th	>173	55(4.8)	4.0 ± 3.2

Table-III: Mean levels of cord serum T4* and TSH

Country	*T4 (nmol/L)*	*TSH (mIU/L)*	*Ref.No.*
USA	131	9.0	(14)
Belgium	147	8.2	(3)
Zaire	102	69.8	(3)
This Study	115	5.6	

* Values in µg/dl were converted into nmol/L by multiplying with 12.87.

Table-IV: Reference ranges of cord serum T4 and TSH

Country	*T4 (nmol/L)*	*TSH (mIU/L)*	*Ref.No.*
USA	84—225	2.5—17.0	(15)
This Study	49—189	0.4—17.6	

and TSH levels showed a wide scatter or variability. Important characteristics of both T_4 and TSH levels distributions like mean (±S.D), median and observed range are shown in Table-I. The range of T_4 levels was 15-350 nmol/L with 173 nmol/L (95th percentile) and 59 nmol/L (5th percentile). The median of T_4 levels was 113 nmol/L. TSH range was 0.05-150mIU/L with 14.2 mIU/L (95th percentile) and 0.9mIU/L (5th percentile). TSH levels showed a median of 4.5 mIU/L. Both distributions of T_4 and TSH levels were asymmetrical and skewed toward right side (positively skewed) which means their trends towards higher values. TSH levels were more skewed as compared to T_4 levels with coefficient of skewness 1.1 and 0.1 respectively. Correlation coefficient (r) between T_4 and TSH distributions was 0.013 which was statistically non-significant.

In order to study interrelation between cord serum T_4 and TSH more elaborately, their levels were grouped according to 5th, 50th and 95th percentile values and the respective mean level of T_4 or TSH with the relative frequency of each group is shown in Table II. No significant change in both mean T_4 and TSH levels within 6th percentile to 95th percentile range of TSH (1.0-14.2 mIU/L) or T_4 (60-173 nmol/L) respectively was observed. It means there was no reciprocal relation between T_4 and TSH concentrations within their 6th to 95th percentile values. However, on either side of this range, such a relation was observed in some neonates. In 4 neonates with T_4 levels below 5 th percentile, TSH levels were above 95th percentile. This pattern was similar to that observed in hypothyroidism. A pattern similar to hyperthyroidism was observed in 10 neonates with T_4 levels above 95th percentile and TSH levels below 5th percentile level. But in 10 neonates both T_4 and TSH levels were below their 5th percentile levels. Analysis of data shows that 11.4 % neonates had TSH levels above 10 mIU/L.

DISCUSSION

Analysis of cord serum T_4 and TSH data showed that the interaction between T_4 and TSH in fetuses was different as compared to adults i.e. the threshold for negative feedback from thyroid hormones to the pituitary in fetus is higher as compared to adults (Table II). This observation is in accordance with other studies[8,9] and reflects the relative immaturity of interactions among different components of the hypothalamus-pituitary-thyroid axis at the time of birth.[9,11] An apparent reason, as proposed in other studies[8,9,11] may be the virtual absence of T_3 in fetal circulation. Fetus does not actively convert T_4 to T_3 in peripheral tissues and T_4, rather than T_3, is the principle thyroid hormone accounting for negative feedback control of pituitary TSH secretion. Another reason proposed for this phenomenon is the increased secretion of thyrotropin-releasing hormone (TRH) from the hypothalamus which counterbalances the inhibitory action of T_4.[9]

The fetal thyroid gland and pituitary-thyroid axis become functional late in the first trimester. The secretory activity of fetal thyroid gland begins to increase by mid-gestation and total T_4 levels rise progressively in the fetal blood until term.[8,9] Fetal serum T_4 concentrations rise from approximately 25 nmol/L at 12 weeks to 128 nmol/L near term. Similarly serum TSH gradually rises from 4 to 8 mIU/L between 12 weeks and term[12]. Serum T_4 concentration of 30 to 70 nmol/L is essential to protect the fetal brain from permanent damage.[13] The mean level of cord serum T_4 (115 nmol/L) determined in this study was comparable to that of Saeed et al[1] (110 nmol/L) who determined this parameter for 49 cord blood samples in Lahore in 1991. However, mean TSH level was lower (5.4 mIU/L vs. 6.3 mIU/L). This may be due to larger number of samples analyzed in this study. Comparison of mean T_4 and TSH levels with those of USA[14] (iodine sufficient), Belgium[3] (moderate iodine deficient) and Zaire[3] (severe iodine deficient) is shown in Table III. Our mean T_4 level was lower than those of USA and Belgium and was higher than that of Zaire. However, mean TSH level was lower than all studies. A comparison of our reference ranges of both T_4 and TSH to USA[15] is shown in Table IV. Our normal limits for both T_4 and TSH were smaller than USA.

During gestation, maternal and fetal thyroid functions are autonomously regulated yet they are not independent of one another. Fetal thyroid activity depends entirely on the availability of iodine transferred from the maternal circulation and is hypersensitive to fluctuations in the iodine supply from the mother[16]. There is some evidence to the effect that maternal T_4 is transferred from mother to fetus in order to protect the fetus from hypothyroidism.[11,16] Levels of cord serum T_4 and TSH reflect the iodine availability from mother to fetus[3]. In moderate iodine deficiency, fetal thyroid stimulation is increased in order to have the required amount of T_4, which is reflected by hyperthyrotropinemia (normal T_4 with higher TSH levels) at birth and significantly higher TSH and thyroglobulin (Tg) levels in cord serum.[16] In severe iodine deficient localities low levels of T_4 and high levels of TSH are observed in cord serum of neonates of iodine deficient mothers and many neonates exhibit chemical hypothyroidism.[13,16]

Elevated serum TSH in the neonates indicates insufficient supply of thyroid hormones to the developing brain. International organizations like World Health Organization (WHO), United Nations International Children's Emergency Fund (UNICEF) and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) has included neonatal TSH as one of the indicators for assessing iodine deficiency disorders (IDD) and their control in a population[17]. In the absence of iodine deficiency, the frequency of neonatal serum TSH above 10 mU/L is less than 3%. A frequency of 3%-19.9% indicates mild IDD. Frequencies of 20%-39.9% and above 40% indicate moderate and severe IDD respectively[17]. According to our data 11.4% neonates had TSH levels above 10 mU/L and 3% neonates exhibited hyper-thyrotropenemia. This indicates mild degree of IDD in Lahore. It means a

substantial number of fetuses were iodine deficient and hence were victims of utero hypothyroxenemia. However, our results need confirmation from maternal thyroid status and iodine intake data because cord serum is affected by such factors like birth-weight, gestational age and health of neonate[18]. Low-birth weight, premature and sick neonates are reported to have lower T_4 and TSH levels as compared to healthy full term infants.[18,19] In Pakistan birth of low-weight babies is very high i.e., 25% according to official source.[20] Both T_4 and TSH levels below the 5th percentile in ten neonates in this study may be due to above mentioned factors. It seems that besides iodine deficiency, these factors had contributed in lowering mean T_4 and TSH and their reference ranges.

Two features of this data are noteworthy: First, this data indicates a high recall rate for the re-testing of neonates for neonatal hypothyroidism[1-3] i.e. in 58 neonates T_4 levels were below 5th percentile. Among them four neonates had TSH levels above 20mIU/L including two neonates with TSH levels above 50mIU/L. Overall 20 neonates had TSH levels above 20mIU/L. They were all suspected cases of congenital or at least transient hypothyroidism which require monitoring and follow up. Second, an unusual finding of this study was the detection of ten neonates with both T_4 and TSH levels characteristic of hyperthyroidism. This may be due to trans-placental passage of thyroid stimulating antibodies (TSAb) from mother to fetus[21] and requires further investigation. However, keeping in view the importance of the role of thyroid hormones in fetal brain development, all the aspects of thyroid hormones regulation during pregnancy require elaborate studies.

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