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Influence of Perinatal Factors and Sampling Methods on TSH and Thyroid Hormone Levels in Cord Blood

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Abstract. To evaluate the effect of perinatal factors and sampling methods on thyroid stimulating hormone (TSH) and thyroid hormone levels in cord blood, serum TSH, free thyroxine (FT_4) and free triiodothyronine (FT_3) concentrations were measured in 124 healthy term neonates. Eighty-eight infants were born in normal vaginal deliveries, 25 were delivered by vacuum extractor and 11 by Cesarean section. There was no significant difference among the three infant groups in the mean TSH levels. Birth weight, the infant's sex, duration of labor and uterotonic agents had no effect on cord serum TSH and free thyroid hormone levels in the neonates born by normal vaginal delivery. To assess the adequacy of specimen collection, mixed cord blood samples, obtained by a direct application of cord on a filter paper, and venous blood withdrawn with a plastic syringe were collected in another 200 infants. There was a significant linear correlation in the TSH concentration in mixed cord blood and cord venous serum from the same individuals, while a poor correlation was found in T_4 values from two specimens. Our results suggest that the TSH value in cord blood is less influenced by perinatal factors, including the sampling method, and the mixed cord blood collected by this technique might be a feasible alternative specimen for a TSH screening program with cord blood which is useful in countries where neonatal blood is not available.

Key words: TSH, T_4, T_3, Cord blood, Perinatal factors. (*Endocrinol Japon* **38**: 297–302, 1991)

SCREENING programs for congenital hypothyroidism have been carried out in Europe [1], North America [2], Australia [3], New Zealand [4], Japan [5] and expanding throughout South America and Asia-Oceania [6]. Thyroid stimulating hormone (TSH) and/or thyroxine (T_4) concentrations are measured in blood-spotted filter paper specimens collected from newborn infants by a heel prick during the first week of life. However, in some regions blood sampling from neonates is difficult for a variety of reasons such as early discharge of babies from hospital. To solve this problem cord blood has been used in the screening program [7–10] and the effectiveness of primary cord blood TSH screening has been recognized [10]. In the perinatal period, marked changes in thyroid function have been reported [11]. We evaluated the effect of various perinatal factors on cord TSH and thyroid hormone concentrations in Experiment 1, and assessed the adequacy of using the mixed cord blood obtained by direct application of umbilical cord on a filter paper for neonatal thyroid screening in Experiment 2.

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Subjects and Methods

Experiment 1

One hundred twenty-four infants, born at Toho

University Ohmori Hospital between March and May, 1985, were randomly enrolled in the study. Infants with major congenital malformations, severe birth asphyxia, intracranial hemorrhage and systemic infection were excluded from the study. Informed consent for the study was obtained from one or both parents. The following situations were not reasons for exclusion: asymptomatic hypoglycemia or hypocalcemia and physiologic jaundice. Infants were divided into three groups according to the mode of delivery and there were no significant differences in gestational age and birth weight among these groups (Table 1). Eighty-eight infants were spontaneously born vaginally with no medical intervention except episiotomy or augmentation of labor with intravenous prostaglandin F_2 (PG) and/or oxytocin (Group 1). In this group, 38 infants were delivered by infusing uterotonic agents. Twenty-five infants were delivered with a vacuum extractor for weak pain, malrotation or fetal distress and their APGAR score was more than 7 (Group 2). Eleven infants were delivered by elective Cesarean section for cephalopelvic disproportion, breech presentation or previous Cesarean section (Group 3).

The cord was ligated and cut within two minutes after birth and 10 m*l* of blood was drawn from the umbilical vein on the maternal side. From the newborn infants in Group 1, one blood sample was obtained from a peripheral vein on the 5th day of age. The sera were immediately separated and frozen at −20°C.

TSH concentrations were measured in cord and postnatal blood samples from all infants. Free thyroid hormone concentrations were determined only in cord blood samples from Group 1 infants. TSH was measured by a immunoradiometric assay (IRMA) with the Sucrosep TSH IRMA of Boots-Celltech Diagnostics, Berkshire, UK [12]. The detection limit of this assay was 0.1 μU/m*l*. The interassay and intraassay coefficients of variation were 3.8–8.8% and 5.4–8.8%, respectively. Free T_4 and free T_3 were measured with the Gamma Coat ^{125}I Free/Total T_4 RIA kit of Clinical Assays, Division of Travenol Labs., USA, and Immophase Free T_3 ^{125}I RIA kit of Corning Medical, USA. The interassay coefficients of variation for free T_4 and free T_3 were 12% and 10%, respectively. The corresponding intraassay coefficients of variation were 5% and 6%.

Table 1. Gestational ages and birth weights of study groups

Group	n	Gestational Age (wk)	Birth Weight (g)
1	88	39.2 ± 1.1	3167.9 ± 324.7
2	25	39.3 ± 1.2	3145.6 ± 315.3
3	11	38.1 ± 1.4	2967.1 ± 665.8

Values represent mean ± SD.

Experiment 2

From February to April, 1987 cord blood was obtained in 200 healthy term newborn infants by two different methods; 1. applying the cut end of the cord directly onto a filter paper (mixed cord blood sample), 2. withdrawing blood from the umbilical vein by a plastic syringe (cord venous blood sample). The venous blood was subsequently applied on a filter paper and the rest of the blood was centrifuged and the sera were stored. Neonatal dried heel blood was collected on the 5th day of age. TSH and T_4 concentrations were determined in filter paper specimens and serum samples. Two 0.3 cm discs were punched from dried blood spots on filter paper and the Immophase T_4 ^{125}I-Radioimmunoassay Test and Immophase Neonatal TSH Assay of Corning Medical, USA, were used for the assays. All assays were performed in duplicate. The interassay coefficients of variation for TSH and T_4 were 15% and 12%, respectively. The corresponding intraassay coefficients of variation were 6% and 8%.

Statistical analysis

Data were expressed as the mean ± SD and analysed by Student's *t* test. The correlation between two variances was calculated by the least square method and the significance of the correlation coefficient(r) was read from a probability table. A probability value of less than 0.05 was used as the limit of significance.

Results

Experiment 1: Influences of perinatal factors on TSH and thyroid hormone levels in cord venous blood

The cord serum TSH concentrations (mean ± SD) were 11.4±9.2 μU/m*l* for Group 1 (normal delivery), 14.7±11.5 μU/m*l* for Group 2 (vacuum extraction), and 7.3 ± 3.4 μU/m*l* for Group 3 (Cesarean section) (Fig. 1). The mean TSH value for Group 3 was relatively lower than those in the

other two groups but the differences were not statistically significant.

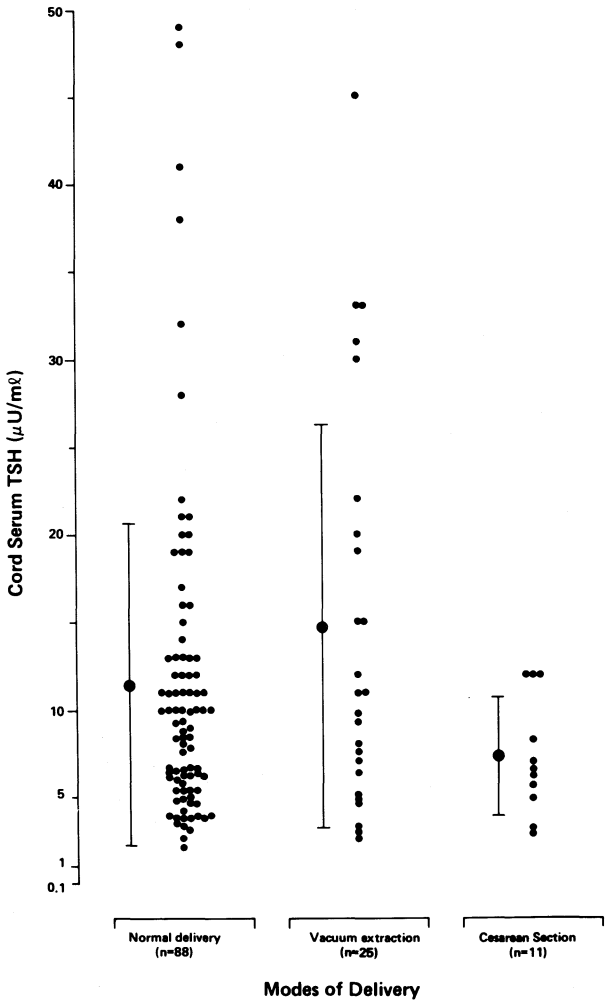

Fig. 1. Distribution of serum TSH concentrations in cord blood according to the mode of delivery. Mean ± SD.

Cord serum TSH and free thyroid hormone concentrations in the infants in Group 1, which is regarded as the normal delivery group, are shown in Table 2. There was no linear relation between these hormone values and either birth weight or the duration of labor (the time interval between the onset of labor and the delivery). The mean TSH, FT_4 or FT_3 levels in the infants delivered with uterotonic agents were not significantly different from those in infants without these drugs. There was also no significant difference in the mean values for TSH or free thyroid hormone according to the infant's sex (Table 2).

The cord TSH concentrations in the infants in Group 1 were not linearly correlated with either free thyroid hormone levels of the same serum sample (r=0.16 for FT_3, r=0.22 for FT_4) or the serum TSH levels on the 5th day of age (r=0.28).

Experiment 2: Studies on cord blood sampling methods

The TSH concentrations in the mixed cord blood, collected by direct application of cord on a filter paper, were positively correlated with those in cord venous blood serum (r=0.81) or the elutes from a filter paper specimen (r=0.79) (Figs. 2 and 3). The TSH values in the cord serum were also correlated with those measured in the filter paper blood sample (r=0.85). However, there were poor linear correlations in TSH concentrations between the mixed cord blood or two venous cord blood samples and neonatal capillary blood samples on the 5th day (r=0.05–0.38).

The T_4 concentrations in the mixed cord blood, cord venous blood and neonatal capillary blood were poorly correlated with each other (r=0.13–0.52).

Table 2. Cord serum TSH and free thyroid hormone concentrations in the infants of Group 1 (normal delivery group) according to perinatal factors

	TSH (μU/ml)	Free T_4 (ng/dl)	Free T_3 (pg/ml)
Birth weight (g)	r=0.158	r=0.141	r=0.092
Male (n=45)	11.9 ± 9.7	1.85 ± 0.8	1.52 ± 1.25
Female (n=43)	11.4 ± 9.2	1.60 ± 0.3	1.54 ± 0.63
Duration of labor (h)	r=0.106	r=−0.079	r=−0.148
PG/Oxytocin (+)	13.8 ± 11.6	1.46 ± 0.39	1.40 ± 1.05
PG/Oxytocin (−)	9.9 ± 7.1	1.75 ± 0.78	1.53 ± 0.71

Values are mean ± SD. r: correlation coefficients.

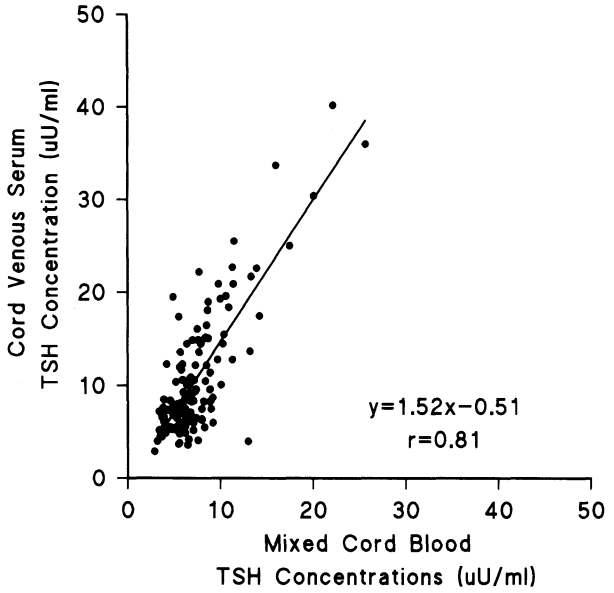

Fig. 2. Correlation between TSH concentrations in mixed cord blood and cord venous serum. y=1.52x −0.51, r=0.81.

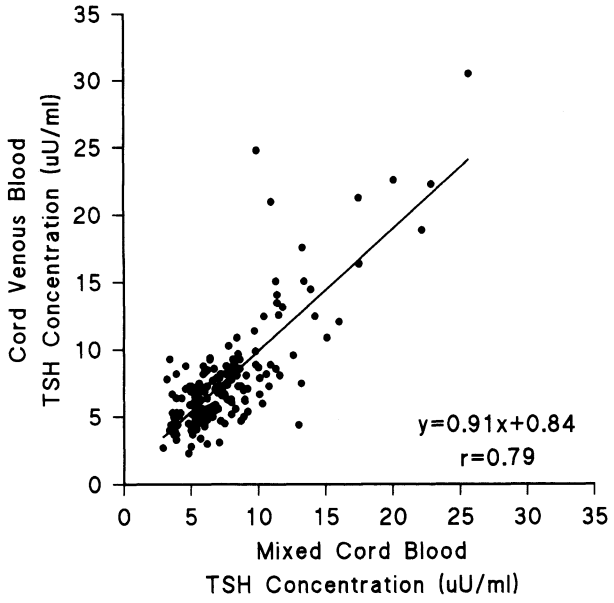

Fig. 3. Correlation between TSH concentrations in mixed cord blood and cord venous blood measured in the filter paper specimen. y=0.91x + 0.84, r=0.79.

Discussion

There are several earlier studies on the perinatal factors influencing TSH and thyroid hormone concentrations in cord blood [13–15]. We observed in the Experiment 1 that cord serum TSH and free thyroid hormone levels in healthy term infants are not influenced by birth weight, the infant's sex, mode of delivery, duration of labor or uterotonic agents, although the effect of the mode of delivery on free thyroid hormones was not analysed. These results are generally consistent with previous reports. Franklin *et al.* [13] analysed the effects of the perinatal factors on neonatal thyroid function in healthy term infants during the first 15 days of age. The factors included maternal diabetes mellitus, toxemia, fetal distress, duration of labor, method of delivery, asphyxia, infant's race, sex, birthweight, height, head circumference and method of feeding. These factors had no effects on cord serum TSH concentrations and only the method of delivery influenced cord serum T_4 values and the free thyroxine index, which were significantly lower in the babies delivered by forceps or elective Caesarean section. Cord T_4 and FT_4 concentrations positively correlated with birthweight. The same authors reported the influence of nonthyroidal illness such as birth asphyxia, hypoglycemia, meconium aspiration, sepsis and surgery in term infants during the early neonatal period [16]. The mean cord serum T_4 concentration in the infants with sepsis was lower than in the healthy control, whereas none of these factors affected the cord serum TSH concentration. Erenberg [17] showed that maternal diabetes mellitus, prolonged rupture of membranes, Cesarean section, asphyxia, meconium staining, twins and respiratory distress syndrome had no effect on cord serum T_4 concentrations, but that lower mean cord T_4 values were observed in the term SFD (small for gestational age) infants. Thus, the TSH concentrations in cord blood of mature infants might be less influenced by perinatal factors than the T_4 values.

We also observed poor correlations between cord blood TSH values and either cord free thyroid hormone or neonatal blood TSH values. The feedback control of TSH release by thyroid hormone is already mature at birth [18]. Lack of a linear correlation between TSH and free thyroid hormone in cord blood may reflect variations in the blood level of these hormones within their physiological range. Oddie *et al.* [15] reported a positive correlation between cord and postnatal serum TSH values in healthy term infants. The exact reason for this discrepancy between the two studies is unclear, but the infants in their study

were much older than those in ours (8–94 vs 5 postnatal days).

In Experiment 2, TSH values in the mixed cord blood collected by direct application of the cord stump on filter paper highly correlated to those of cord serum in the same individuals in spite of possible contamination with amniotic fluid or maternal blood. In contrast to TSH, there was poor correlation between T_4 values in the mixed cord blood and the serum sample. Walfish *et al.* [10] has reported that primary TSH screening by cord and by neonatal heel dried blood sampling have identical primary hypothyroidism detection incidences. Our observations suggest that the mixed cord blood collected by this method is a feasible alternative specimen for primary TSH screening program which might be useful in the area where neonatal blood is not available if appropriate recall/cut-off criteria are utilized.

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