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Thyroid function profile in cord blood and postnatal changes at 24 and 72hours in healthy term Nigerian neonates

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Abstract: *Background:* Studying the acute postnatal changes of newborn thyroid function is essential for determining the best timing of screening for congenital hypothyroidism. There is paucity of literature on neonatal thyroid function and particularly the postnatal changes in Nigeria.
Objectives: To describe the profile of thyroid function in cord blood and the postnatal changes at 24hours and 72hours in healthy term neonates delivered in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto.
Subjects and methods: This was a prospective, descriptive and cross sectional study conducted over a six month period (between July-December 2009). Forty seven consecutively delivered healthy term neonates had their serum T_3, T_4, TSH assayed at birth, 24 and 72hours using the Enzyme linked immunosorbent assay (ELISA) technique. Data obtained are presented as mean, median and standard deviation (SD). Paired- t- test was used for statistical inference.
Results: The mean (SD) of the serum hormone concentrations in cord blood, and venous blood at 24hours and 72hours, respectively were as follows: T_3, 0.58 (0.56) nmol/l, 1.15 (0.77)nmol/l, 0.83 (0.74)nmol/l; T_4, 91 (83.1)nmol/l, 121.5 (106.4)nmol/l, 104.2 (84.2) nmol/l; and TSH, 5.95 (5.81)mU/l, 8.61 (8.34)mU/l, 2.52 (2.61) mU/l.. The mean serum T_3, T_4 and TSH at 24hours were higher than cord blood levels ($p<0.001$, 0.03, 0.05 respectively). The mean serum T_3 and T_4 at 72hours, were higher than cord blood levels ($p = 0.07$, 0.44), whereas TSH at 72hours was significantly lower than cord blood levels; ($p<0.001$).
Conclusions: There was a rise, above cord level, of T_3, T_4 and TSH at 24hours, and a decline at 72hours, the latter being most marked in TSH. It is recommended that serum TSH taken at or greater than 72hours of life may be utilized for screening for congenital hypothyroidism in term babies, using postnatal age appropriate reference ranges. Serum T_3, T_4 should then be assayed for confirmation in all neonates with a positive TSH screening.

Key words: Thyroid profile, postnatal changes, Term, Healthy neonates.

Introduction

Thyroid hormones in normal quantities are vital for physical growth and mental development during fetal and postnatal life[1]. In the developing human fetus, thyroid hormone deficiency is associated with severe retardation of growth and maturation of almost all organ systems[2]. However, the sensitivity of different organs to thyroid hormone deficiency varies. The brain is particularly susceptible to damage during the fetal and early postnatal period[3]. It has been established that nerve cells require thyroid hormone for their development. Thyroid hormone is therefore, essential for normal brain development[2-4].

Most infants with congenital hypothyroidism are asymptomatic at birth[5]. This may probably be because of an ectopic thyroid gland with clinically significant thyroid function, partial defect in thyroid hormone synthesis, or to the moderate amount of maternal T_4 that crosses the placenta and is able to boost fetal levels within 25-50% of normal levels at birth[5]. The clinical picture is fully developed by 3 – 6 months of age[5], by which time therapy may not prove 100% useful in restoring neurodevelopment to normal[6]. Prognosis for neurodevelopment is excellent if therapy is instituted at the postnatal age of

one month or less.[7,8] Less than 5% of neonates with congenital hypothyroidism are diagnosed clinically first, before laboratory confirmation.[5] The clinician is, therefore, dependent on neonatal screening tests for the early diagnosis.[7] Routine screening is, however, not yet universal in some countries such as Nigeria, and may be responsible for the lower prevalence and/or poor documentation of congenital hypothyroidism in the literature.

The American Academy of Paediatrics advocates that blood specimen for screening should be collected optimally by 2-4 days of age[7], but there are situations in which this is virtually impossible. In infants discharged before 48 hours of life following delivery, blood should be obtained before discharge[7]. In most screening programs blood is collected for screening between 5-6 days of age[9]. Some researchers have documented the use of cord blood TSH in term neonates as a screening tool for congenital hypothyroidism[9,10]. They argue that serum TSH at birth does give reliable and good result. To buttress their argument, they opined that the acute changes take a few hours post-delivery to develop hence it does not affect cord levels of TSH. Furthermore, the level of TSH in the cord reflects the concentration of this hormone in the fetus and not early neonatal period that is fraught with acute changes in TSH levels. Lastly, quantity of TSH in the cord is not affected by maternal levels as compared to T_4 levels because TSH does not cross the placenta because of its high molecular weight [9,10,11.]

Thyroid profiles have been described in neonates in North America and Europe[12,13] with the establishment of reference values which are utilized by neonatal screening centers[12,13]. However, in Africa, limited studies have been documented[14,15]. It was, therefore, considered that since no study has been done describing thyroid profile in term neonates in Sokoto, and little is known about its postnatal changes in Nigerian neonates, particularly in this part of the country, it was pertinent to undertake this study. Moreover, studying the thyroid profile of Nigerian neonates is a pre-requisite for the establishment of neonatal screening centers for congenital hypothyroidism in Nigeria.

Subjects and Methods

Study location

This study was carried out in the Labour Ward and Special Care Baby Unit (SCBU) of UDUTH, Sokoto, Sokoto State, Nigeria. The SCBU of the hospital adjoins the Labour ward and admits all babies that are high risk; this is inclusive of babies with abnormal birth weight, those who experienced perinatal complications, post caesarean section and other instrumental deliveries and sick newborns (the latter are kept in a demarcated section of the SCBU).

UDUTH is sited at No. 1 Nadama Road within the Sokoto Metropolis, the Seat of the Caliphate and the State capital. It has a 554 bed capacity and an annual delivery rate of approximately 2,500 babies. It also serves two neighboring states (Zamfara and Kebbi) and a contiguous neighboring country, Niger Republic.

Sokoto State is located in the Northwestern geopolitical zone of Nigeria with a population of 3.69million people and annual growth rate of 3%[16]. It is situated at 900m above sea level[16]. It lies between latitude $10^0$and 14^0N, and longitude 3^03^1N and 7^07^1E of the equator[17]. The climate is semi-arid with a hot dry season that spans from October to April, a rainy season which starts in May and lasts till September[17], the cold harmattan months spanning from December to February. Annual rainfall ranges between 550mm and 600mm[17]. The temperature fluctuates within wide limits from about 15^0C during the cold night to over 40^0C during the hot days[17]. The low ambient temperature during the cold season could predispose to higher TSH surge[18]. Most of the ethnic groups are represented, but the majorities are the indigenous Hausas/Fulani population. The society is basically agrarian with majority living in the rural areas. Ethical permission to carry out this study was obtained from the UDUTH Ethics Committee. Informed verbal and written consent was obtained from the parents of the babies recruited for the study, during Labour

Study Design

This was a prospective, descriptive and cross sectional study conducted over a six (6) month period between1st July 2009 - 31st December 2009.

Inclusion criteria: These were healthy term neonates, delivered in UDUTH, Sokoto and informed written parental consent.

Exclusion criteria: They include maternal history/ family history of thyroid disease or ante-partum ingestion of anti-thyroid drugs[19,20]. Maternal goiter, sick newborns (because of the effect of illness on thyroid function)[21, 22], probable sepsis[21], severe respiratory distress or cardiopulmonary illness[22], perinatal asphyxia/ seizures[23], history of blood transfusion in the neonates.[7]

Recruitment Procedure: The parturient mothers and some expectant fathers were addressed during labour for informed/written consent. Forty seven (47) consecutive babies delivered between 5am and 1pm, who met the inclusion criteria, were recruited for the study. This specific delivery time preference was chosen to allow for uniformity in the time of blood sample collection at 24hours and 72hours, as well as to remove any circadian bias (i.e. Circadian rhythm of TSH secretion characterized by low concentrations during the daytime, increase in the evening and peak shortly before sleep)[24].

A thorough physical examination (general and systemic) was carried out on each neonate after birth and on subsequent follow up visits, to ensure they were healthy.

Apgar scores, birth weights, gestational age by dates or ultrasound, gender, maternal complication (medical or obstetric), parity and others were documented in the proforma data sheet. The importance and implication of outcome of the study was further explained to the mothers after delivery. The mothers were also examined for goiter by palpation of their anterior neck while asking

them to swallow, the investigator standing behind the mothers during palpation. History of ingestion of anti-thyroid medication or family history of thyroid disease was taken and documented in the pro-forma data sheet. Follow up written appointments were subsequently given to the mothers to bring their babies at 24hours, and 72hours. Blood samples were obtained from enrolled neonates for the assay of T_3, T_4 and TSH. Mothers were reassured and given incentives for transportation during follow up visits.

Blood Sampling: Two milliliters (2mls) of umbilical venous and arterial mixed blood was obtained by gently milking a 15-20cm length of the fetal side of the severed umbilical cord within five minutes of birth of each baby.[9] Two milliliters (2mls) of venous blood was also obtained by venipuncture of a peripheral vein at 24hours, and 72hours using a 21G needle without syringe, after cleaning the site with a cotton wool wet with methylated spirit. The blood samples were collected into a plain sample bottle without anticoagulant and allowed to clot. It was subsequently spun in a centrifuge at 4000 revolution per minute for five minutes. The serum was decanted into small tubes using a pipette and kept frozen at -20^0C in the freezer of the Chemical Pathology Department of UDUTH until required for analysis.

Blood Analysis Technique: A solid phase enzyme immunoassay utilizing the competitive binding principle (ELISA) was used for the assay of Triiodothyronine, thyroxine, and thyroid stimulating hormone[25]. The values of T_3, T_4 and TSH were expressed in, ng/dl, µg/dl and µIU/ml, respectively. To facilitate comparison with other studies, these units were converted to their corresponding SI units as follows: *T_4 µg/dl×12.87=nmol/l, T_3 ng/dl×0.01503= nmol/l and TSH µIU/ml= mU/L.*[26]

The commercial test kits were obtained from Syntron Bioresearch, Inc California, USA. The test procedure for triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) were as contained in the Syntron Bioresearch, Inc. Micro well T_3 EIA catalogue 3810-96, T_4 EIA ref #2210-96 and TSH EIA ref #2211-96, respectively.

Data Analysis: The data from pro-forma sheets and results of serum T_3, T_4 and TSH were entered into a microcomputer (Microsoft excel 2003). A double check entry approach was utilized to ensure accuracy of data entered. Measures of statistical location like mean, standard deviation, median and range were generated using the Statistical Package for Social Sciences (SPSS) version 20. Statistical comparison involved the paired- t-test. Probability (p) value less than or equal to 0.05 were interpreted as statistically significant.

Results

General characteristics of the study population

Forty seven apparently healthy term neonates were enrolled for the study. Eighteen (38%) were males and twenty nine (62%) were females. (M: F =1:1.61). All subjects had Apgar scores between 8 and 10 at 1 and 5 minutes, respectively.

Birth weights and gestational ages of the studied Neonates

The birth weights of the neonates ranged from 2,150g to 4,900g {mean (SD) 3,230g (2.141)g}. There were thirty eight (81%), Appropriate for Gestational Age (AGA), three (6%), Small Gestational Age (SGA), and six (13%), Large for Gestational Age (LGA) neonates. Gestational ages ranged from 37 to 42 weeks [Mean (SD) 39 (20) weeks]

Mode of delivery

Thirty (64%) of the neonates were delivered via caesarean section (CS), sixteen (34%) by spontaneous vertex delivery (SVD) and one (2%) by vacuum extraction.

Age, parity and antenatal clinic attendance of mothers: Forty four (93%) of the mothers were booked and three (7%) were un-booked. Thirty eight (80%) of the mothers were multiparous and nine (20%) were primiparous. Maternal ages ranged from 16 to 39 years. (Mean (SD) age 26 (14) years).

Serum concentrations of T_3, T_4, and TSH: The mean and median serum concentration of serum T_3, T_4, and TSH in cord blood, at 24hours and 72hours are as shown in Table 1.Comparison between two means was done using the paired- t- test. There was a rise above cord blood levels in all the hormones at 24hours ($p = < 0.001$, 0.03, 0.05 respectively) and a fall at 72hours ($p= 0.05$, 0.29, 0.001 respectively), the fall being most marked in TSH. At 24 hours, the mean T_3 was about twice the cord blood value: the mean T_4 level increased by about 30% of the cord levels while that of TSH increased by about one and a half times of the cord levels.

At 72 hours, the mean T_3, T_4 and TSH all fell below the 24-hour levels. However, while the values of T_3 and T_4 remained above the cord levels that of TSH fell to about half the cord value.

Table 1: The mean, standard deviation and the median of serum T_3, T_4, and TSH in cord blood of term neonates, and venous blood at 24hours and 72hours.

Hormone	Cord blood (CB) Mean(SD) Median	24hrs Mean(SD) Median	72hrs Mean(SD) Median
T_3 (nmol/l)	0.58(0.56)[a]	1.16(0.77)[b]	0.83(0.74)[c]
	0.45	1.14	0.80
T_4 (nmol/l)	91.0(83.1)[d]	121.5(106.4)[e]	104(84.2)[f]
	51.1	81.6	71.6
TSH (mu/l)	5.95(5.81)[g]	8.61(8.34)[h]	2.52(2.61)[i]
	4.66	7.08	1.71

CB: Cord blood vs Versus
T_3: [a] vs [b] p=<0.001; [a] vs [c] p=0.05
T_4: [d] vs [e] p=0.03; [d] vs [f] p=0.29
TSH: [g] vs [h] p=0.05; [g] vs i p=0.001

Thyroid profile of term AGA neonates in Cord blood and venous blood at 72hours as seen in this study compared with a similar study in Benin are shown in Table 2. Similar pattern of a higher mean serum T_3 and

T_4 and lower TSH at 72hours compared to cord blood levels was demonstrated.
The neonates in Benin had a lower mean serum T_4 and a higher mean serum T_3 and TSH than was demonstrated in this study both in cord blood and at 72hours.

Table 2: Neonatal thyroid profile of term Appropriate for Gestational Age (AGA) neonates in Cord blood and venous blood at 72hours in Sokoto (present study) compared with that reported in Benin (Nigeria)

	Sokoto (Present Study) Mean (Range) n=38		Benin Study Mean (Range) n=114	
Hormones	Birth	72hrs	Birth	72hrs
T_3nmol/L	0.62 (0.01-2.78)	0.83(0.01-3.69)	0.89(0.65-1.52)	1.06(0.71-1.61)
T_4nmol/L	91.3 (0.4-265.9)	104(1.0-274.6)	75.48(47-120)	101.38(62.2-130.5)
TSH mU/L	5.89 (0.45-35.76)	2.52(0.04-11.97)	13.59(6.2-26.1)	10.25(5.0-22.1)

Discussion

The present study has demonstrated higher mean levels of serum T_3 and T_4 at 24 hours in term neonates compared to cord blood levels of these hormones(<0.001, 0.03). This is consistent with the findings of Jacobsen *et al*[27] in Copenhagen, Denmark.
The reason for the higher levels of serum T_3 and T_4 at 24 hours is most likely due to adaptive changes in the newborn, characterized by early rapid increase in serum TSH in the first few hours of life post-delivery, in all groups of newborn[18]. This surge of TSH is called the physiologic surge, and is as a result of extra-uterine cooling[18]. The TSH surge stimulates the thyroid gland to increase production of thyroid hormones (T_3 and T_4)[18, 28] in order to adapt to extra uterine life via synergistic action with catecholamine to increase non shivering thermogenesis. The finding of higher relative increases of serum T_3 above cord levels, at 24 hours, compared to T_4, confirms earlier reports that the increased thyroid hormone secretion following the TSH surge is due more to T_3 than T_4[27,29].

The finding of low T_3 at birth confirms the earlier reports that the fetus is T_3 deficient most likely due to the reduced capacity to de-iodinate T_4 to T_3 in the extra thyroidal tissues.[29] The rapid increase in serum T_3 after delivery at a time when the capacity to convert T_4 to T_3 is reduced suggests that the increment in serum T_3 is due predominantly to increased T_3 secretion from the thyroid gland stimulated by the TSH surge rather than increased conversion of T_4 to T_3 in the peripheral tissues.[29]
The subsequent decline in T_3 to levels above cord blood at 72hours, is consistent with earlier reports[27,29], and is due to the waning of the physiologic TSH surge usually at 48-72 hours[18]. The declining levels of T_3 coinciding with falling TSH seems to agree with this explanation. The decline in serum T_4 at 72 hours is consistent with earlier report by Jacobsen *et* al[27,7] The fact that mean TSH at 72 hours fell to about half the cord level is a reflection of the fact that necessary homeostatic temperature adjustments are complete and the stimulus for physiologic surge of TSH, removed. Therefore, newborn screening at 72 hours will likely give reliable results applicable to the neonate in stable state.

A similar study in Benin[15] was conducted in the year 2005 on term AGA neonates. Their serum T_3, T_4 and TSH were assayed in cord blood and at 72hours.Comparison with this study, therefore, was only possible with the term AGA group which were thirty eight in number. Coincidentally a similar method of assay was used (ELISA technique), though the test kits were different. The Benin study[15] showed lower mean serum T_4 and higher mean serum T_3 and TSH than this present study. This observation may be due to the differences in location, Sokoto being a mild goiter zone while Benin is in an area of moderate goiter.
Previous work done has shown that newborns from areas of iodine deficiency as seen in the goiter belts have a higher frequency of elevated TSH levels and low T_4 values than is found in areas where iodine intake is normal even though the mothers may be clinically and biochemically euthyroid[30]. It may be reasonable to proffer that this would occur more profoundly in areas with moderate to severe iodine deficiency than the mildly deficient areas. One of the possible explanations given is that in the presence of iodine deficiency, the newborn thyroid is unable to obtain an adequate iodine supply because of the competition by the mother's thyroid, also eager for iodine. This may result in more severe reduction in the iodine content in the newborn thyroid and consequently reduction in its functional capacities. The demonstrated pattern of postnatal changes at 72hours in this study (higher mean T_3, T_4 and lower TSH) compared with mean cord blood levels is similar to that reported in Benin[15]. This suggest that the postnatal changes in neonatal thyroid function are qualitatively the same irrespective of geographical location but there may be slight quantitative differences.

In the same study done in Benin,[15] the researchers found no significant differences between the mean cord blood T_3, T_4, and TSH and the mean serum values of these hormones at 72hours (p=0.36, 0.08, 0.33 respectively), meaning that it makes no difference whether screening for congenital hypothyroidism is done with cord blood or venous blood at 72hours, consequently the same reference values may be utilized. This is however, contrary to this present study which demonstrated differences between the cord blood and 72hours mean concentrations of T_3 and TSH (p=0.05, 0.001 respectively). The mean T_4 however was not significantly different from the cord blood levels (p=0.29). The reasons for the differences in findings of the two studies (present study and Benin study) is not quite clear, but may be related to the smaller number of AGA neonates (38) recruited in this study, compared to the larger number in the Benin study (114), the varying modes of delivery in this study (CS, SVD, vacuum). All the subjects enrolled in the Benin study were delivered by SVD. Conflicting reports have earlier been documented by various researchers on the effect of mode of delivery on thyroid function[31-33].

Conclusion

mean T_3 levels peaked at 24 hours and decreased at 72 hours in term healthy neonates, while T_4 levels increased slightly but progressively from birth to 24hours and declined at 72 hours. However, the mean TSH levels peaked at 24 hours before declining at 72 hours to lower than cord blood levels. It is therefore recommended that serum TSH assayed at or greater than 72hours may be utilized for screening for congenital hypothyroidism, particularly where cord TSH is missed (e.g. home deliveries), and the results should be interpreted using post natal age appropriate reference ranges. Serum T_3, T_4 and repeat TSH should then be assayed for confirmation in all neonates with a positive TSH screening. There should be vigorous pursuit of further multicenter collaborative evaluations of neonatal thyroid function in Nigeria.

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