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ABSTRACT:

Neonatal Thyroid Stimulating Hormone (TSH) level in blood is one of the indicators recommended for assessing iodine deficiency control programs in a population. This study evaluates the TSH level in cord blood as a way of assessing the iodine status of neonates in the National Capital District, PNG.

Assay of TSH in 150 cord sera was by enzyme immunoassay (EIA 96 Microwell plates) using the sensitive EIA kit provided by LiNEAR Chemicals, S.L. The median TSH level in the sera for all the neonates was 2.17mIU/L, the interquartile range (IQR) was 1.53 – 3.48mIU/L. The TSH level in only 2 (1.3%) cord serum samples was greater than 10.0mIU/L. The lower limit (2.5th) and upper limit (99.0th) of the TSH percentile cut-off levels in all the cord sera were 0.76mIU/L and 11.16mIU/L. The median TSH level in the cord sera of the male neonates was 1.98mIU/L and the IQR was 1.55 – 3.38mIU/L. For the female neonates the median TSH level was 2.22mIU/L and the IQR was 1.52 – 3.81mIU/L.

The data indicates normal iodine and thyroid status and zero prevalence of congenital hypothyroidism among the neonates in NCD.

Key Words: Cord Serum, Neonates, TSH, Iodine deficiency, Papua New Guinea

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INTRODUCTION:

Hypothyroidism caused by inadequate intake and proper utilization of iodine can result in a spectrum of diseases collectively referred to as Iodine Deficiency Disorders (IDD) [1-3]. Maternal iodine deficiency during pregnancy and lactation may compromise the thyroid status of the foetus and neonate [1 – 6]. Maternal T4 is particularly required for neurodevelopment during the first half of gestation [1, 3, 5 – 7]. Maternal iodine deficiency affects neurophysiologic development and functional abnormalities of the foetus and also indirectly impairs foetal brain development by causing hypothyroidism in both mother and foetus [1, 7]. Maternal iodine deficiency also impairs foetal thyroid function, causing increase TSH level in the neonate [1, 5 – 7].

Increase in neonatal TSH level indicates sub-optimal supply of thyroid hormones to the developing foetus and the neonate [7 – 10]. Neonatal TSH level in blood is one of the indicators recommended for assessing IDD control programs in a population [1, 4, 7 – 11]. Iodine deficiency in a population is indicated if the frequency of neonatal TSH level is above 5.0mIU/L in whole blood or above 10.0mIU/L in serum is found in more than 3% of the blood samples obtained from Cord Blood or 3 days old neonates [1, 7 –

11]. The cut-off points for mild, moderate and severe IDD are indicated by neonatal TSH frequencies of 3 – 19.9%, 20 – 39.9% and greater than 40%, respectively [1, 7 – 11]. Congenital Hypothyroidism (CH) has been recognized as a preventable cause of mental impairment, thus routine screening programs have been implemented in most developed countries [1].

According to several experts there are difficulties in the diagnosis of CH at birth by physical examination of neonates, in addition CH is usually asymptomatic for several months, thus if neonatal screening is not carried out, most cases of hypothyroidism may be missed at birth and treatment may be delayed [7 – 14]. CH is a relatively common condition with a frequency of about 1:400 in Southern Australia and other countries [7 – 14].

Early diagnosis and treatment is vital for normal physical and mental development of affected neonates. The use of TSH as the primary screening test for neonates is recommended because it can also detect compensated or transient primary hypothyroidism caused by iodine deficiency with a frequency of about 1 in 10 neonates in some countries [12 – 14].

In some countries blood samples for screening are collected from 3 to 5 days old neonates [7 – 13]. Because of logistical

reasons cord blood samples have been recommended as the most practical specimens for neonatal screening in most developing countries [12 – 17].

According to recent reports by WHO/UNICEF/ICCIDD [4, 15], there is growing evidence that iodine deficiency may be reappearing in some countries, where it was previously under control. This statement underscores the need for continued monitoring and evaluation of the iodine status and thyroid function of populations that have been at risk in the past.

Recent data indicates prevalence of mild to moderate status of iodine deficiency in some areas in Papua New Guinea [18 – 20]. However, there are limited data on iodine status of neonates in Papua New Guinea (PNG). There are no indications of neonatal screening for CH in any of the hospitals in PNG. No data is available on the prevalence of compensated or transient primary hypothyroidism, which can be caused by maternal iodine deficiency, and whose incidence can be as high as 1 in 10 neonates.

The principal aims of this study were to determine the thyroid stimulating hormone (TSH) levels in cord blood as a way of assessing the iodine status of neonates and to assess the prevalence of Congenital

Hypothyroidism among the neonates in the National Capital District (NCD), PNG.

SUBJECT AND METHODS:

This cross-sectional study was conducted in the NCD, which is the incorporated area around Port Moresby the capital of PNG. The Obstetrics and Gynaecology unit in Port Moresby General Hospital (PMGH) was selected as the sampling site because of the difficulty in obtaining blood samples from healthy neonates in NCD.

The study population included consecutive deliveries at the PMGH from May to September, 2009 of women resident in the NCD. In this period of time, 5000 deliveries occurred. Two hundred (200) of the deliveries were randomly selected for study. The sample size was based on a design effect of one, relative precision of 10%, confidence level of 95%, predicted non-response rate of 25% and assumed prevalence rate of 10% [1]. Selection of the women was by simple random sampling. Cord blood was collected from cords of neonates whose mothers had given informed written consent before delivery. Cord blood samples were collected into sterile properly labelled containers. The neonates' side of the cord was clamped and cut, blood was collected with a plain vacutainer from the placental cord just before delivery of the placenta.

Serum was prepared from each blood sample and stored frozen at -20°C till required for assay. Before delivery the following information was recorded for each consented pregnant mother, age, parity, thyroid status, blood pressure, diabetic status and use of iodine antiseptics. After delivery the gender, weight and length of the neonate were recorded.

Consenting mothers with history of thyroid disease or medications that affect thyroid status including those with systemic illness were excluded from the study.

Assay of TSH was by enzyme immunoassay (EIA 96 Microwell plates) using the sensitive EIA kit provided LiNEAR Chemicals, S. L. [21]. Assay of TSH in cord sera, standard sera and quality control sera were carried out as indicated in the instructional protocol of the manufacturer [21].

Statistical analysis of data was by the SPSS-PC software (version 11). Kolmogorov-Smirnov and Shapiro-Wilks tests were used to assess normality of the data. Mann Whitney U test, Kruskal-Wallis and Friedman were used as appropriate. Scheffe test was used for post-hoc analysis. $P < 0.05$ was considered as statistically significant.

In the present study the data were interpreted using the WHO/UNICEF/ICCIDD recommended criteria [1, 7, 11, 22]. Iodine deficiency is not indicated if less than 3.0% of all the cord sera have TSH level greater than 10.0mIU/L. In addition, the severity of IDD was classified using the proportion of cord sera with frequency of TSH $> 10.0\text{mIU/L}$: frequency of 3% – 19.9% indicates mild IDD; Frequency of 20% – 39.9% indicates moderate IDD and Frequency above 40% indicate severe IDD [1, 7, 11, 22]. Cord Serum TSH level $> 20.0\text{mIU/L}$ indicates Congenital Hypothyroidism [1].

Ethical clearance and approval for the study was obtained from the SMHS Ethics and Research Grant Committee and the PNG Medical Research Advisory Committee (PNG MRAC). Permission for the study was also obtained from the appropriate authorities in PMGH.

RESULTS:

A total of 200 pregnant mothers were randomly selected, but enrolment occurred just before they were prepared for delivery. Verbal consent was obtained from 172 of them, but signed informed consent was obtained from only 150, which gave a total response rate of 75.0%. Assay for TSH was carried out in the 150 sera obtained from the 150 cord blood samples collected.

The mean (\pm standard deviation) age of all the pregnant mothers was 25.2 ± 5.3 years and the median age was 25.0 years. The diabetic status and thyroid status of all the mothers were normal. The blood pressures of the women that participated in the study were all normal. The gestational age was within the range 38 – 42 weeks. All the mothers had normal vaginal delivery. Gender distribution of the 150 neonates delivered indicated 81 (54%) males and 69 (46%) females.

The mean and median birth weights of the neonates were 3.1 ± 0.46 kg and 3.1kg respectively. The range was 2.0 – 4.2kg, and the 95% CI was 3.0 – 3.2kg. The mean and median birth weights for the male neonates were 3.1 ± 0.43 kg and 3.2kg respectively, with a range of 2.0 to 4.2kg and 95% CI was 3.0 – 3.2kg.

For the female neonates the mean birth weight was 3.1 ± 0.49 kg, the median was 3.0kg, the range was 2.1 – 4.0kg and the 95% CI was 2.9 – 3.2kg. There was no statistically significantly different ($p = 0.21$) between the birth weights of the male and female neonates. Distribution of the neonates according to the cut-off points for classification of birth weights is presented in Table 1. The birth weight was ≥ 2.5 kg in 139 (92.7%) of all neonates, among 78 (96.3%) male and 61 (88.4%) female

neonates. None of the neonates was classified as having very low birth weight.

The Kolmogorov-Smirnov test for normality indicated that the TSH levels (m IU/L) in all the cord sera and in the male and female cord sera were not normally distributed. This is confirmed by the several outliers shown on the Box-plots (Fig. 1) of the TSH levels in the cord sera of all the neonates and of the male and female neonates. Thus further analysis of the TSH data was by non-parametric statistics. Table 2 shows the TSH levels in the cord sera for all the neonates and for the male and female neonates. The median TSH level in the sera for all the neonates was 2.17mIU/L the interquartile range (IQR) was 1.53 – 3.48mIU/L. The TSH level in only 2 (1.3%) cord serum samples of all the neonates was greater than 10.0mIU/L. The TSH level in all the cord serum samples of all the neonates was less than 20.0mIU/L.

The percentile cut-offs of the TSH levels in the cord sera of all the neonates are presented in Table 3. The lower limit (2.5th) and upper limit (99.0th) of the TSH percentile cut-off levels in the cord serum samples for all the neonates were 0.76mIU/L and 11.16mIU/L.

The median TSH level in the cord sera of the male neonates was 1.98mIU/L and the

IQR was 1.55 – 3.38mIU/L. For the female neonates the median was 2.22mIU/L and the IQR was 1.52 – 3.81mIU/L. The Kruskal Wallis ($p = 0.55$) and Chi-Square ($p = 0.42$) tests indicated that there was no statistically significant difference between the TSH levels in the cord sera of the male and female neonates. The Mann-Whitney and Wilcoxon tests also indicated no statistically significant ($p = 0.55$) difference between the mean TSH levels in the cord sera of the male and female neonates. The TSH level was greater than 10.0mIU/L in 1.2% and 1.4% of the cord serum of the male and female neonates respectively.

The Percentile cut-offs for the TSH levels in the cord sera of both male and female neonates are presented in Table 3. The

lower limits (2.5th) of the TSH percentile cut-offs in the cord sera for the male and female neonates were 0.83mIU/L and 0.71mIU/L respectively. The upper limits (99.0th) TSH percentile cut-offs for male and female neonates were 9.28mIU/L and 10.70mIU/L respectively. The Spearman's rho coefficient of correlation ($r = - 0.048$, $p = 0.668$) indicated a weak inverse non-significant relationship between the TSH level in the cord serum of the male neonates and the birth weights of the male neonates.

For the female neonates the Spearman's rho coefficient of correlation ($r = 0.069$, $p = 0.575$) indicated a weak non-statistically significant linear relationship between the TSH level in the cord serum samples and the birth weights.

Table 1: Distribution (%) of neonates according to classification of birth weights

Birth Weights	Classification	All neonates (n = 150)	Males (n = 81)	Females (n = 69)
< 2.0kg	Very Low Birth Weight	0	0	0
2.0 – 2.49kg	Low Birth Weight	11 (7.3%)	3 (3.7%)	8 (11.6%)
≥ 2.5kg	Normal Birth Weight	139 (92.7%)	78 (96.3%)	61 (88.4%)

Figure 1: Box-plots for TSH levels (m IU/L) in cord sera of all neonates and of the male and female neonates

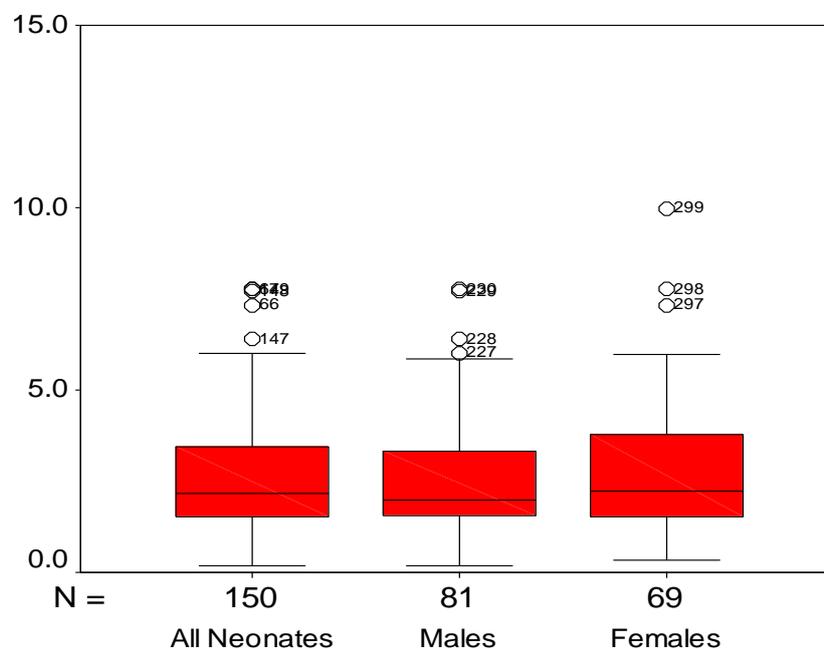


Table 2: TSH levels (m IU/L) in cord sera of all neonates and for male and female neonates

Parameters (TSH m IU/L)	All Neonates (n = 150)	Males (n = 81)	Females (n = 69)
Median	2.17	1.98	2.22
Interquartile Range (IQR)	1.53 – 3.48	1.55 – 3.38	1.52 – 3.81
Mean	2.83	2.75	2.93
95% CI	2.49 – 3.18	2.28 – 3.22	2.42 – 3.44
Std Dev	2.13	2.13	2.13
Range	0.19 – 15.3	0.19 – 15.30	0.35 – 12.3
TSH >10.0mIU/L	2 (1.3%)	1 (1.2%)	1 (1.4%)
TSH ≥ 20.0mIU/L	0	0	0

Table 3: Percentile cut-offs for TSH levels (m IU/L) in cord sera of all neonates and of male and female neonates

Percentile	All Neonates	Males	Females
2.5 th	0.76	0.83	0.71
5 th	0.9	0.94	0.86
10 th	1.23	1.11	1.25
20 th	1.45	1.46	1.41
25 th	1.53	1.55	1.52
50 th	2.17	1.98	2.22
75 th	3.48	3.38	3.81
90 th	5.45	5.43	5.62
95 th	6.81	6.36	7.55
97.5 th	7.77	7.72	8.43
97.8 th	7.77	7.72	8.88
99.0 th	11.16	9.28	10.70

DISCUSSION:

In most developing countries the use of TSH in cord serum for assessing the thyroid status of neonates is well documented and supported by WHO/UNICEF/ICCIDD [1, 12 – 16, 22]. In addition, mixed cord blood is a good sampling technique that has proved to be an attractive neonatal TSH screening tool for CH, because it is easier to obtain from consented mothers [11 – 14]. Sera from mixed cord blood samples were used in the present study. The non-response rate of 25% was similar to the predicted non-response rate used in the calculation of the sample size. Some authors have reported

non-response rates over 25.0% in research studies involving collection of biological samples from “apparently” healthy infants [20, 23].

The proportion of female neonates (11.6%) with low birth weight was higher than that of the male neonates (3.7%). This difference was not statistically significant ($p = 0.43$). After routine examinations each of the pregnant mothers was satisfied as “apparently” healthy before delivery. The gestational age of between 38 to 42 weeks was satisfied as normal in each case.

The non-Gaussian distribution of the TSH levels for cord sera obtained in the present

study is similar to that reported by others [1, 10 – 14]. In the present study, the mean TSH level in the cord sera of all the neonates (2.83 ± 2.13 mIU/L) was within the reference ranges of 1.0 to 20.0 mIU/L and 2.4 – 20.6 mIU/L reported in the literature [22, 24]. Our mean TSH level was lower than the mean TSH levels of 6.13 ± 5.29 mIU/L and 9.6 ± 7.8 mIU/L reported by Manglik et al [13] and Feleke et al [10] in the cord sera of neonates in India and Ethiopia respectively.

The TSH level was greater than 10.0 mIU/L in only two (1.3%) cord serum samples. This according to the WHO/UNICEF/ICCIDD criteria indicates normal iodine status among the neonates. The TSH levels in the two cord serum samples were 12.3 mIU/L and 15.3 mIU/L, both of which are lower than 20.0 mIU/L that represents the lower limit of the cut-off point indicating CH. Thus, none of the neonates in our study population was recalled for repeat testing. Our data indicates zero prevalence rate of CH among the neonates that participated in our present study. This indicates normal thyroid status among the neonates. This finding should, however be interpreted with care because of the small sample size (150 cord sera) used in our present study.

The 97.8th and 99.0th percentiles TSH levels obtained for all the neonates were 7.77 mIU/L and 11.16 mIU/L respectively. These TSH levels were lower than the 97.8th

(14.98 mIU/L) and 99.0th (25.8 mIU/L) percentiles reported for neonates in India [13]. The median TSH level in the cord sera of the male (1.98 mIU/L) and female (2.22 mIU/L) neonates were similar to the TSH values reported by Mekonnen et al [14] for male (1.94 mIU/L) and female (1.45 mIU/L) neonates in Ethiopia. The mean TSH levels for both male (2.75 ± 2.13 mIU/L) and female (2.93 ± 2.13 mIU/L) neonates were also similar to the TSH levels for male (2.71 ± 2.42 mIU/L) and female (2.34 ± 2.26 mIU/L) neonates in Ethiopia [14].

Our mean cord sera TSH values were however lower than the 6.48 ± 5.2 mIU/L and 5.75 ± 4.16 mIU/L reported by Manglik et al [13] for male and female neonates respectively in India. TSH level was greater than 10.0 mIU/L in only one (1.2%) of the male cord serum samples and one (1.4%) of the female cord serum samples. The TSH levels in the two cord serum samples were below 20.0 mIU/L as indicated earlier.

Our data indicate normal iodine status and normal thyroid status among the male and female neonates in our study population. The 2.5th and 97.5th percentile cord sera TSH cut-off levels obtained for both the male and female neonates can be used tentatively as reference ranges for apparently healthy male and female neonates in NCD, until a larger and more extensive study is conducted.

In conclusion, our data indicates normal iodine and thyroid status and zero prevalence of congenital hypothyroidism among the NCD neonates in the study population. Despite these findings there is need to advocate for the implementation of routine screening test for primary hypothyroidism among neonates in PNG.

The use of cord blood TSH level for screening of neonates is more practical, cost-effective and relatively simple. It has the highest detection rate compared to other methods.

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