



## Original Research Article

## Comparison of hematological parameters in congenital hypothyroidism in neonates: A case controls study

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## ABSTRACT

**Background:** Thyroid hormones play an important role in human metabolism. Red blood cells abnormalities are mostly associated with thyroid hormones. However, they are very rarely investigated and associated to the permanent and transient congenital hypothyroidism in Tamil Nadu's Government Territory care centre in Chennai. In this study, an attempt was made to study the haematological status in permanent and transient hypothyroidism neonates at birth.

**Materials and Methods:** This retrospective study included 135 subjects, among which were permanent hypothyroidism (n=9), transient hypothyroidism (n=18), and euthyroid neonates (108). This study was carried out at department of biochemistry, Regenix super speciality laboratories and sample collection were done at Government Institute of Child's health and hospital for children and Government institute of obstetrics and Gynaecology and hospital for Women under Madras Medical college, Chennai. The haematological parameters and thyroid profile of the subjects were assessed by sysmex hematology analyser, Roche Cobas e411 ECLIA and Neonatal screening by BIORAD QUANTASE ELISA. Mean and standard deviation, analysis of variance (two way anova) with  $p < 0.05$  considered as statistically significant.

**Results:** In this study group, we compared the homological status in these groups, permanent, transient, and euthyroid as controls neonates. We found that haematological status like Hb, RBC, MCV, and RDW were significantly increased at birth in both permanent and transient hypothyroidism were compared with euthyroid neonates, results are significant. The results reported in these are Mean  $\pm$  S.D., were statistically tested by ANOVA. In permanent Congenital Hypothyroidism (CH), MCV (79.97-87.58 fl) and RDW (13.98-16.6%), whereas in Transient Congenital Hypothyroidism, MCV (72.52-87.05 fl) and RDW (13.41-15.68%), and controls MCV (78.69-82.12 fl) and RDW (12.4 – 14.3%), suggesting that these patients were at risk of anemia and other RBS abnormalities. Mean corpuscular volume is an important to view the change in RBCs destruction, production, loss and morphology.

**Conclusion:** The thyroid dysmorphogenesis is frequently associated with mothers' complications like anemia, hypo/hyperthyroid mother, siblings developmental delay, and goitre. Permanent CH is associated with serious complication with erythrocyte abnormalities. The risk of congenital hypothyroidism could be getting into developmental delay and mentally retarded children, Such conditions should be detect early and corrected.

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### 1. Introduction

Congenital hypothyroidism is a partial or total loss of thyroid gland function (hypothyroidism) that affects birth-time (congenital) babies. The thyroid gland inside the lower

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neck is a butterfly-shaped tissue. In people with congenital hypothyroidism, the levels of these substantial hormones are lower than usual. Congenital hypothyroidism takes place when the thyroid gland cannot grow or function effectively. The thyroid gland is absent, significantly decreased in size (hypoplastic), or unusually positioned in 80 to 85 per cent of cases. These cases are classified as Dysgenesis of the Thyroid. In the rest of the cases, there is a normal-sized or swollen thyroid gland (goitre), but thyroid hormone production is either decreased or absent. Most of these cases occur when one of the phases in the process of hormone synthesis is impaired; these are known as thyroid dysmorphogenesis. Less commonly, the reduction or absence of thyroid hormone synthesis is triggered by an impaired stimulation of the production process (which is usually achieved by a system at the base of the brain called the pituitary gland), whereas the process itself is unimpaired. These cases are known as being core (or pituitary) hypothyroidism. A deficiency of thyroid hormones leads to signs of congenital hypothyroidism and symptoms. While some babies with congenital hypothyroidism are less active and sleep more than normal, the affected babies do not show any signs of the condition. They will have digestive problems, and may have constipation. Congenital hypothyroidism can lead to slow growth and intellectual disability if left untreated. Typically, babies develop normal if treatment begins within the first two weeks after birth. The prevalence of thyroid dysmorphogenesis is increasing gradually, particularly in women.<sup>1</sup> They regulate human haematopoiesis in the bone marrow.<sup>2</sup> Defects of erythrocytes are also associated with thyroid dysmorphogenesis. They are, however, rarely examined and linked to thyroid.<sup>3</sup> Thyroid hormones have a major influence on erythropoiesis in that different forms of anaemia (normocytic, hypochromic-microcytic or macrocytic) have been associated with declines in thyroid function.<sup>1</sup> However, the association between another haematological parameter, namely, Hemoglobin, RBC, Red Blood Cell Distribution Width (RDW) and other haematological parameters has been established. The RDW is the width of the RBC volume (one SD) frequency distribution curve, separated by the mean RBC volume. An elevated RDW, which is red blood cells of unequal size, is known as anisocytosis.<sup>4</sup> In this research, an attempt is made to determine RDW values in neonates with congenital hypothyroidism along with Mean corpuscular volume (MCV).

## 2. Materials and Methods

### 2.1. Subject and recruitment process

This study retrospective study was a hospital-based study was conducted at the department of biochemistry, Regenix super speciality laboratories and sample collection were

done at Government Institute of child's health and hospital for children and Government. Institute of obstetrics and gynaecology and hospital for Women from June 2018 to June 2019- 1-year study. All patients referred from out-patient and In -patient of government hospital for thyroid screening at out-patient centre and through child hospital for evaluation of thyroid function and performing complete blood count. All the patients were examined by an Endocrinologist. The study was approved by Institutional Ethics Committee, Madras Medical college, Chennai, Tamil Nadu. All the subjects recruited through selection process for matched controls and positive cases for this study. A total of 135 subjects were selected for the study. These included the euthyroid as controls (n=108), Transient Congenital hypothyroidism (n=18) and permanent congenital hypothyroidism(n=9).

### 2.2. Exclusion criteria

1. Neonates with neurological diseases
2. Severe Infection
3. kidney failure
4. Respiratory distress

### 2.3. Inclusion criteria

1. Maternal complications with hypothyroid/anemia/goiter
2. Down's Phenotype babies
3. Coarse Cry at birth
4. Low birth weight <2.5 kg
5. Abdominal distension
6. Macrosomia
7. Normal neonates with no abnormalities as controls

### 2.4. Sample collection

The neonatal heel prick or Guthrie test is a screening test done on newborns. Heel prick capillary blood collected on S&S 903 filter paper, air dried and transported along with ice pack in cold storage. All neonates aged (0-28) days after birth, normal newborns and including low birth weight neonates and that admitted to Neonatal Intensive Care Unit (NICU), Institute of obstetrics and Gynecology and Govt. hospital for Women and Children, will be systematically enrolled into the study.

### 2.5. Investigation of thyroid hormones

For TSH-ELISA method for dried blood spots-neonatal TSH screening by BIORAD QUANTASE method. About 2ml of whole blood sample were collected for complete blood count and serum TSH and Free T4 values were measured by serum aliquots were stored at 4C° to be run in batch. The samples were processed by electro chemiluminescence method by fully automated ECLIA -

COBAS e411 immunology analyzer.

### 2.6. Investigation of haematological parameters

The 2ml of peripheral venous blood was taken in sterilized EDTA vials. The complete blood count and hemogram comprised of (Hb, RBC, PLT, MCH, MCV, RDW, MCHC, neutrophils, lymphs, absolute neutrophil count). Blood samples were processed for various hematological status the complete blood count (CBC) were analysed in SYSMEX (Italy) by wintrobe's method.

### 2.7. Statistical analysis

Data were extracted and analysed by XLSTAT. The results were expressed as mean  $\pm$  standard deviation (SD). Differences in variables were analysed by an analysis of variance (ANOVA), two-way ANOVA and Chi square test. The differences were considered to be significant at  $p < 0.05$  or  $p < 0.01$ .

### 3. Results

In this study, the total of 135 subjects (neonates) participated in this research among which 44.5% were males and 64.5% females in the age group from 0 – 28 days of life after birth. They were 108 euthyroid, congenital hypothyroidism 27 (both transient and permanent), through new-born screening. Mean  $\pm$  Standard deviation values of Hb, RBC, WBC, MCV, RDW, MCH, MCV, with TSH, F4 values were accessed and data are presented as P value. The value of  $P < 0.05$ , denotes as results as statistically significant (Tables 1, 2, 3 and 4). After birth from 3- 7 days at early neonatal period, new-born screening were done, for each neonates, for each positive samples four control samples were taken as matched healthy controls without any abnormalities. The values were obtained as euthyroid values were TSH  $4.23 \pm 1.8$ , transient congenital hypothyroidism (TCH)  $17.8 \pm 10.7$ , and permanent congenital hypothyroidism (PCH)  $86.4 \pm 28.4$ , which supportively the neonates with RBC abnormalities, risk of iron deficiency anemia and anemia (normocytic) and also they heel -prick were used for TSH screening euthyroid values were DBS-TSH  $7.7 \pm 3.4$ , transient congenital hypothyroidism (TCH)  $22.7 \pm 3.2$ , and permanent congenital hypothyroidism (PCH)  $34.2 \pm 11.2$ . The samples were processed for complete blood count and thyroid values. As compared with euthyroid RDW are significantly high in both transient as well as permanent congenital hypothyroidism. MCV values showed as statistically significant high among neonates with congenital hypothyroidism. MCV values were significantly decreased in hyperthyroidism and significantly increased in hypothyroidism. It shows increased RDW in thyroid dysmorphogenesis, it implies that abnormal thyroid hormone levels may significantly influence circulating RBCs' size variability. For other RBC indices, however, no

difference was observed, including platelets, RBC range, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentrate (MCHC), neutrophils, white blood cells, lymphs, and absolute neutrophil count ( $p$ -value  $< 0.05$ ).

### 4. Discussion

This retrospective study conducted at Govt children's hospital and Govt. Women's hospital at Madras medical college. New-born Screening for congenital hypothyroidism, were conducted a high risk screening pilot study from Regenix super speciality laboratories in neonates after 3 days with 7 days of life at early neonatal period on the one hand, hypothyroidism may cause certain types of anaemia or on the other, hyperproliferation of immature progenitors. Anemia is typically hypochromic macrocytic and or normocytic anaemia, and hypothyroidism raises MCV. Anemia would be of moderate severity.<sup>5,6</sup> MCV is positively correlated with TSH serum levels, hypothesising that the premature ageing of erythrocytes in the blood stream, the increased lipolytic potency of the hypothyroid characteristic of RBC, or the distribution of lipids in the erythrocyte membranes may play a role in deciding this association.<sup>7</sup>

The present study showed increased MCV values in congenital hypothyroidism, both transient and permanent. The cause of MCV rise and mild decrease in Hb in hypothyroidism may be different.<sup>8,9</sup> Hypothyroidism anaemia has been due to a physiological mechanism for the reduced need for oxygen tissues. This theory is in line with the low plasma erythropoietin levels observed in hypothyroid anaemia. The increase in MCV in conjunction with the congenital hypothyroidism can grow rapidly. The MCV was observed to decline steadily on thyroxine replacement therapy, even though the initial value was within the normal range. In hypothyroidism the present study showed increased values of MCV and RDW. Although no conclusive mechanism (s) can be proposed to explain the greater prevalence of increased RDW in neonates with thyroid dysmorphogenesis, the findings of this case - control study indicates that abnormal levels of thyroid hormones may significantly influence the size variability of circulating RBCs.<sup>10</sup>

### 5. Conclusion

Thyroid hormones affect RBC's production suggestively. Given the increased RDW of thyroid problems, it indicates that elevated thyroid hormone levels may have a significant impact on the variable size of RBC circulation. These anomalies should be examined and remedied. Their involvement may lead to clinically significant thyroid disorders that can be managed early.

**Table 1:** General characteristics of study population in this study

Characteristics	Category	CH Cases with permanent CH and Transient CH (n=27) %	Controls(n= 108)	P value
Age at clinical suspicion	0-15 days	19(70.4)	79(73.1)	0.39
	16- 28 days	8(29.6)	22(20.4)	
Gender	Male	5(18.6)	28(25.9)	0.42
	Female	22(81.5)	80(74.0)	
Mother's age	<25	10(37.0)	67(62.0)	0.08
	26- 35	8(29.6)	21(19.5)	
	>35	9(33.4)	20(18.6)	
Socioeconomic	Educated	12(44.5)	70(64.9)	0.05*
	Non- educated	15(55.6)	38(35.1)	
Maternal complications	Euthyroid	1(3.7)	97(89.9)	0.0001*
	Hypo/hyperthyroid mother	20(74.0)	11(10.9)	
	Goiter	4(14.8)	0	
	History with development delay	5(18.5)	0	
Geographical	Urban	11(40.7)	32(29.7)	0.27
	Rural	16(59.2)	76(70.4)	

\*Maternal complications and Socio-economic demographics shows high significant

**Table 2:** Comparison of haematological parameters and Thyroid hormone levels in Transient and Permanent congenital hypothyroidism neonates (Mean±S.D.)

Newborn Range	Parameters	Controls (n=108)	PCH(9)	TCH(18)
(11-17.5) g/dl	Hb	13.03 ± 0.4262	10.29 ± 0.6403	11.81 ± 0.5313
(3.5-6.0)*10 <sup>3</sup> /ul	RBC	5.57 ± 0.0924	3.89 ± 0.3463	4.58 ± 0.3003
3.5- 10*10 <sup>6</sup> /ul	WBC	7.38 ± 0.1867	2.12 ± 0.3659	2.63 ± 0.3973
35-54%	PCV	39.52 ± 0.0792	30.68 ± 2.0122	35.72 ± 1.8617
80-100 fl	MCV	80.41 ± 1.7117*	79.97 ± 7.6177*	79.8 ± 10.264*
26-34 pg	MCH	27.35 ± 0.0132	26.85 ± 2.6264	26.39 ± 2.3745
31.5- 36.0 g/dl	MCHC	34.53 ± 0.0528	32.64 ± 0.7448	32.37 ± 0.4527
100- 350 *10 <sup>3</sup> /ul	Plts	38.42 ± 0.0264	18.86 ± 1.2609	21.05 ± 1.7324
11-16%	RDW	13.51 ± 0.911*	15.34 ± 1.3589*	14.53 ± 1.118*
% (40-60)	Neutrophils	42.54 ± 0.0792	42.67 ± 1.7901	55.39 ± 3.9129
%(20-40)	Lymphs	32.59 ± 0.1339	30.89 ± 3.9395	29.39 ± 2.6147
1.5- 8.0 *10 <sup>3</sup> mm	ANC	5.46 ± 0.0528	4.12 ± 0.9277	3.76 ± 0.6791

\*MCV and RDW values were highly significant with PCH and TCH.

**Table 3:** Comparison of Thyroid hormone levels in Transient and Permanent congenital hypothyroidism neonates (Mean±S.D.)

Reference Range-Neonates(0-28 days)	Parameters	Euthyroid Neonates(n=108) (Controls)	Permanent CH (n=9)	Transient CH (n=18)
<20 uIU/ml	DBS-TSH	7.7 ± 3.4	34.2 ± 11.2	22.7 ± 3.2
0.8-2 ng/dL	FT4	1.01 ± 0.4	2.67 ± 3.4	1.24 ± 0.3
1.7 to 9.1 uIU/ml	Serum TSH	4.23 ± 1.8	86.4 ± 28.4	17.8 ± 10.7

**Table 4:** Euthyroid Vs TCH Vs PCH significance (using Two Way Anova)

Parameters	Euthyroid Vs Permanent CH (p value)	Euthyroid Vs Transient CH (p value)	Transient Vs Permanent CH (p value)
DBS-TSH( $\mu$ IU/ml)	<0.023*	0.0211*	0.0001*
FT4(ng/dl)	<0.05*	0.05	0.05*
Serum TSH( $\mu$ IU/ml)	<0.021*	0.021	0.0021*
Hb(g/dl)	<0.05*	<0.001	0.0023*
RBC (*10 <sup>3</sup> /ul)	<0.003*	<0.003	0.0109*
WBC(*10 <sup>6</sup> /ul)	<0.04*	<0.04	0.1274
PCV%	<0.05*	<0.05	0.0030*
MCV (fl)	<0.002*	<0.07*	0.055*
MCH (pg)	<0.05	<0.0022	0.8167
MCHC (g/dl)	<0.05	<0.05	0.5282
Plts (*10 <sup>9</sup> /ul)	<0.05	<0.05	0.0419*
RDW (%)	<0.0001	<0.0001	0.004
Neutrophils	<0.023	<0.023	0.0002*
Lymphs	<0.021	<0.021	0.5308
ANC	<0.05	<0.05	0.3614

\*p<0.05 represents significance and correlation between the analytes.

## 6. Competing Interests

The authors declare that they have no competing interests.

## 7. Source of Funding

None.

## 8. Authors Contributions

Dr. Samu Subramaniam and Dr. Shyama Subramaniam, planned the design of the study, and drafted the manuscript. CRS participated in the design of the study and carried out biochemical analysis, performed the statistical analysis. Dr. A.T. Arasar Seeralar participated as external coordinator under Madras Medical college, Chennai. All authors read and approved the final manuscript.

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