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Reference Ranges Of Thyroid Function Tests For Infants Under One Year Age in Nineveh province, Iraq

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Background: *The reference ranges(RRs) in pediatric age group is difficult because of the continual physiological changes that occur during childhood period of life.*

Aims: *The main objective of this study is to establish the RRs for Thyroid Stimulating Hormone(TSH) and Free Thyroxin (FT4) for healthy infants below age of one year in Nineveh province, Iraq, based on their sex and age.*

Materials and Methods: *A cross-sectional study was conducted on one hundred twenty-four healthy infants (62males,62 females, age range from 2 days to 1 year) .The blood samples were collected at Ibn Al-Atheer Teaching Hospital, from October 2023 to April 2024 after obtaining ethical approval from the committee of Ethics at Nineveh Health Directorate, Nineveh, Iraq with the consent of their parents . They were divided into three groups: G1=0–14 days,G2= 15–30 days, and G3=1–12 months .Serum TSH and FT4 were analyzed using enzyme linked fluorescent assay method by Mini Vidas .*

Results: *The RRs were established using a non-parametric method of statistical analysis of TSH and FT4. The RRs were determined using the 2.5% and 97.5 % percentiles, median, range and 95% CI of mean. The mean TSH (µIU/ml) values of females G1 =7.19±6.81, G 2 =2.16±1.58 and G 3 =2.23±1.39 ($p<0.01$)and that for males groups G1=6.50±7.51, G 2=3.09±1.52 and G 3 =2.72±1.72 ,($p<0.01$). The mean FT4(pmol/L) values of females G1=25.01±5.44, G 2=21.00±2.46 and G 3 =18.70±3.42 , ($p<0.0001$) and that for males G1=22.75±5.40, G 2 = 18.66±3.09 and G 3=19.42±3.53 , ($p<0.009$) for males groups.*

***Conclusions**. Higher mean values of FT4 and TSH are noted in the (0-14 days) age group for both sex. No sex difference of mean TSH and FT4 across the three age groups. The 2.5th%-97.5th% percentile for TSH of males obviously higher than that of females for similar age groups, while the reverse of Ft4 is found.*

INTRODUCTION

The present study represents the first study performed on pediatric in Nineveh province, Iraq which is a well-known area of iodine deficiency (1). This study enrolled infants under one years of age, since they were the most vulnerable group that showed an alteration in FT4 and TSH levels, which can impair growth and more particularly brain development(2). Insufficient iodine in diet is a community problem. The pregnant women are the main sectors of community affected by iodine status, as iodine is a main microelement share in the production of thyroid hormones (3). In the first trimester of pregnancy, fetal brain development is entirely dependent on maternal thyroid function(4). Severe iodine deficiency during pregnancy leads to insufficient thyroid hormones production and permanent brain damage manifested as cretinism, which is still prevalent globally (5). Several studies in the pediatric population have established RRs for thyroid hormones . There is a significant heterogeneity between studies due to differences in child age, ethnicity, anthropometric characteristics, and iodine status (6,7). Age-specific RRs for clinical laboratories are important because the physiological development of a healthy child from birth to adolescence results in varying concentrations of thyroid hormones in their blood. The European Thyroid Association guidelines (ETA) recommended the use of age-related reference values for the management of thyroid disorders since this age group carries a vital sequelae when delayed in diagnosis (8).

MATERIALS & METHODS

1. Study design

A cross sectional study was conducted on one hundred twenty-four healthy infants (62males,62 females, age range from 2 days to 1 year) in Nineveh province at Ibn Al-Atheer Teaching Hospital from October 2023 to April 2024 After getting verbal and written consent of their parents. The infants were categorized into three age groups (G 1:2–14 days, G2:15–30 days andG3: 1–12 months).

The enrolled infants should have normal thyroid function with no family of any thyroid disorders or medications that affect thyroid functions. Premature babies, gestation below 37 weeks and babies who is obese or undersized are excluded from this study.

1. **Samples collection:**

Serum TSH and FT4 were measured using the enzyme-linked fluorescent immunoassay (ELFA) by mini vidas (Biomerieux), .Approximately 4 mL of venous blood was drawn, centrifuged for 10 min at 3000 rpm. Serum samples were stored at -20 °C until analyzed. The detection limit of TSH kit is 0.05 µIU/ml with RR (0.24-5.4 µIU/ml)and that for FT4 is (1pmol/L) with RR (9-20 pmol/I).

Statistical Analysis

All data are analyzed in a computer using IBM SPSS for Windows version 26.0 (SPSS software, Chicago, ILL). The Kolmogorov-Smirnov test was performed to assess the distribution of serum TSH and FT4 concentrations and their variance between groups of children, by age and sex. Because the non-normally distributed data , non-parametric Kruskal-Wallis test and Mann–Whitney were performed to show the difference when ever needed. P values ≤ 0.05 were considered significant(9) .

Results

Table (1) shows that no significant difference between the number of females and males for G1,G 2 and G3. Similarly , no significant difference between the mean age of males and females for all groups. Table (2) shows a highly significant difference ($p<0.01$) among mean TSH(µIU/ml) of G 1 (6.50±7.51), G 2 (3.09±1.52) and G 3 (2.72±1.72) for males and ($p<0.01$) among mean TSH of G 1 (7.19±6.81), G 2 (2.16±1.58) and G 3 (2.23±1.39)for females. No significant difference of mean TSH shown between males and females of each age group. The G 1 had a significantly higher (2.5th% - 97.5th%) percentile for each sex than the other age groups. The level of TSH decreases as age increases.

Table (3) shows a highly significant difference ($p<0.009$) among mean FT4(pmol/L) of G 1(22.75±5.40), G 2 (18.66±3.09) and G 3 (19.42±3.53) for males and ($p<0.0001$) among mean FT4(pmol/L) of G 1(25.02±5.44), G 2 (21.00±2.45) and G 3 (19.03±3.46)for females. No significant difference of mean FT4 shown between males and females of each age group. The G 1 had a significantly higher (2.5th% - 97.5th%) percentile for each sex than other age groups moreover, the (2.5th% - 97.5th%) percentiles of females are higher than that of males for each age group. The level of FT4 decreases as age increases.

Table (1) Gender distribution according to age groups

Parameters		G 1	G 2	G3
no.(%)	Male	25 (38.5%)	10 (16.1%)	27 (43.5%)
	Female	16 (25.0%	13 (21.0%)	33 (53.2%
	Total	41 (31.8%)	23 (18.5%)	60 (48.4%)
P-value*		0.3	0.7	0.4
Mean age ±SD (month)	Male	0.19±0.12	0.80±0.21	5.40±4.15
	Female	0.13±0.08	0.72±0.16	4.57±2.62
	Total	0.14±0.11	0.75±0.20	4.96±3.43
P-value**		0.1	0.4	0.3
Range (month)	Male	0.07-0.46	0.50-1.00	2-11
	Female	0.07-0.33	0.53-1.00	1-11
	Total	0.07-0.46	0.50-1.00	1-11

P-value* by Z-test.

P-value** by Mann–Whitney test.

Table (2) Different parameters of TSH (µIU/ml) of both gender for age groups

TSH Parameters		Group 1	Group 2	Group3	P-value*
Mean TSH (µIU/ml) ±SD	Male	6.50±7.51	3.09±1.52	2.72±1.72	0.01

	Female	7.19±6.81	2.16±1.58	2.23±1.39	0.01
	Total	6.77±7.16	2.57±1.59	2.45±1.55	0.0001
P-value**		0.7	0.1	0.2	
Median	Male	3.99	3.02	2.07	
	Female	4.60	1.81	2.03	
	Total	4.50	3.02	2.05	
Range	Male	1.78-38.17	1.12-4.91	0.80-6.30	
	Female	1.06-25.41	0.26-4.26	0.57-5.85	
	Total	1.06-38.17	0.26-4.91	0.57-6.30	
2.5^{th} % -97.5^{th} % percentile	Male	1.78-31.25	1.12-4.91	0.82-6.29	
	Female	1.06-20.52	0.26-4.26	0.61-5.78	
	Total	1.06-24.71	0.26-4.91	0.80-6.00	
95% CI for mean	Male	3.40-9.60	2.00-4.17	2.04-3.40	
	Female	3.56-10.82	1.21-3.12	1.74-2.73	
	Total	4.51-9.03	1.88-3.25	2.05-2.85	

P-value * by Kruskal -Wallis test. P-value** by Mann–Whitney test.

Table (2) Different parameters of FT4 (pmol/L) of both gender for age groups

FT4 Parameters		Group 1	Group 2	Group3	P-value*
Mean FT4 (pmol/L). ±SD	Male	22.75±5.40	18.66±3.09	19.42±3.53	0.009
	Female	25.02±5.44	21.00±2.45	18.70±3.42	0.0001
	Total	23.64±5.46	19.98±2.93	19.03±3.46	0.0001

P-value**		0.1	0.06	0.4
Median	Male	23.07	19.38	20.39
	Female	25.24	19.80	17.71
	Total	24.15	19.80	18.52
Range	Male	13.57-34-87	13.91-22.68	12.95-25.51
	Female	14.20-36.80	18.28-24.60	13.43-27.25
	Total	13.57-36.80	13.91-24.60	12.95-27.25
2.5^{th} % -97.5^{th} % percentile	Male	13.68-34.85	13.91-22.64	13.06-25.28
	Female	14.20-31.62	18.28-24.53	13.96-25.79
	Total	13.98-34.86	13.91-24.56	13.47-25.16
95% CI for mean	Male	20.52-24.98	16.45-20.87	18.03-20.82
	Female	22.12-27.92	19.52-22.49	17.48-19.92
	Total	21.91-25.36	18.71-21.25	18.13-19.92

P-value * by Kruskal -Wallis test**.** P-value** by Mann–Whitney test.

Discussion

Primary hypothyroidism is identified as elevated serum TSH with low FT4 (10). TSH surge shortly after birth as a response to the detachment from the placenta and the change in thermal environment (11–14). Consequently, establishing the RRs for thyroid hormones during neonatal period of life is essential. The American Academy of Pediatrics (AAP) recommends measuring serum TSH and free T4 as a screening work for early diagnosis and monitoring primary hypothyroidism (15).

Thyroid function RRs in the pediatric population have been determined by several researches from a long time . In the 2012 up to this time the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has established age-sex specific RRs for thyroid hormones using different immunoassay methods (16–21) . Chemiluminescence immunoassay (CLIA) method is the principle in the (Beckman Coulter, Abbott Architect i2000, Siemens Atellica and Ortho Vitros) and electrochemiluminescence immunoassay (ECLIA) by Roche cobas.

The range for TSH (µIU/ml) in this study exhibited much more fluctuation in males than females specially in the first month of life. The male range variable from (1.78-38.17) to (1.12-4.91) and that of females from (1.06-25.41) to (0.26-4.26) Table(2) . Such variation can be explained by the slower neural development occurring in males or to unknown biological or environmental factors (22,23). This result was in accordance with Omuse *et al*. *2023* who collected TSH and FT4 data between March 2020 and June 2021 in Aga Khan University Hospital, Nairobi for neonates aged 2-30 days . Samples were analyzed by using chemiluminescence immunoassay method by Siemens Atellica® IM analyzer (24).They reported median for TSH(μIU/ml) of males was 2.308 and for females was 1.976. A similar conclusion has been described in Western Australia, by Hadlow *et al*.(25). They collected TSH and FT4 data between January 1, 2000–December 31, 2011. The median TSH was higher in males than in females though children below 1 year of age

A similar conclusions were stated by Kapelari *et al* , 2008 who conducted a study in Innsbruck, Austria showed that the level of thyroid hormones changed significantly during childhood, and the adult RRs are not universally applicable to children(26) , the reported (2.5th -97.5th)% percentile of TSH(μIU/ml) for 0 – 1 months were (0.70-18.10), 1-12 months were (1.12-8.21) and for 1-5 years were (0.80-6.26) . The reported (2.5th -97.5th)% percentile of FT4(pmol/L) for 0-1 month,1-12 months and 1-5 years were (8.50-30.50), (9.17-25.28)and(10.45-22.35) respectively.

As shown in Table (2 and 3) no significant difference of mean TSH and FT4 of males and females of each age group .This was in agreement with Wong *et al*(27) from Electronic medical records from September 2016 -March 2017 were retrospectively reviewed 633 infant's data of TSH and FT4 aged 14–30 days. TSH and FT4 were analyzed using immunoassay analyzer Dxl 800, Beckman Coulter. Mean FT4 (SD) for females was 15.7(2.50) (pmol/L) and for males was 15.6(2.41) (pmol/L), Median TSH for males 4.60(μIU/ml) and for females was 4.67 (μIU/ml).

Conclusions

Higher mean values of FT4 and TSH are noted in the (0-14 days) age group for both sex with a decline of the mean TSH and FT4 as the age increased . No sex mean difference of FT4 and TSH are seen across the three age groups. The 2.5th%-97.5th% percentile for TSH of males obviously higher than that of females for similar age groups, while the 2.5th%-97.5th% percentile of FT4 for females are higher than that of males for similar age groups.

REFERENCES:

1. Marrwa M.Naif, Hazim A.Allawi, Sura Khairialdeen Mohialdeen. Trimester-Specific Free Thyroid Hormones Levels among Pregnant Women in Nineveh Governorate,Iraq. Iraqi J Pharm Sci P-ISSN 1683 - 3597 E-ISSN 2521 - 3512). 2024 Jul 3;33(2):79–85. https://bijps.uobaghdad.edu.iq/index.php/bijps/article/view/2283,doi :10.31351/vol33iss2pp79-85
2. Schiera G, Di Liegro CM, Di Liegro I. Involvement of thyroid hormones in brain development and cancer. Cancers (Basel). 2021;13(11). https://www.mdpi.com/2072-6694/13/11/2693,doi :10.3390/cancers13112693
3. Portulano C, Paroder-Belenitsky M, Carrasco N. The Na+/I− Symporter (NIS): Mechanism and Medical Impact. Endocr Rev. 2014 Feb 1;35(1):106–49. https://doi.org/10.1210/er.2012-1036,doi :10.1210/er.2012-1036

4. Eng L, Lam L. Thyroid function during the fetal and neonatal periods. Neoreviews. 2020 Jan;21(1):e30–6. http://www.ncbi.nlm.nih.gov/pubmed/31894080,doi :10.1542/neo.21-1-e30

5. Segni M. Disorders of the Thyroid Gland in Infancy, Childhood and Adolescence [Internet]. Endotext. 2000. http://www.ncbi.nlm.nih.gov/pubmed/25905261

6. Boucai L, Surks MI. Reference limits of serum TSH and free T4 are significantly influenced by race and age in an urban outpatient medical practice. Clin Endocrinol (Oxf). 2009 May 1;70(5):788–93. https://doi.org/10.1111/j.1365-2265.2008.03390.x,doi :10.1111/j.1365-2265.2008.03390.x

7. Kratzsch J, Schubert G, Pulzer F, Pfaeffle R, Koerner A, Dietz A, et al. Reference intervals for TSH and thyroid hormones are mainly affected by age, body mass index and number of blood leucocytes, but hardly by gender and thyroid autoantibodies during the first decades of life. Clin Biochem. 2008 Sep;41(13):1091–8. https://linkinghub.elsevier.com/retrieve/pii/S0009912008001604,doi :10.1016/j.clinbiochem.2008.04.007

8. Fadeyev VV. Comments to the European Thyroid Association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. Clin Exp Thyroidol. 2014 Sep 15;10(3):20. https://www.cet-endojournals.ru/jour/article/view/6882,doi :10.14341/ket2014320-26

9. Berry KJ, Johnston JE. Two-Sample Tests. In: Statistical Methods: Connections, Equivalencies, and Relationships. Springer; 2023. p. 91–169.

10. Hegedüs L, Bianco AC, Jonklaas J, Pearce SH, Weetman AP, Perros P. Primary hypothyroidism and quality of life. Nat Rev Endocrinol. 2022;18(4):230–42. ,doi :10.1038/s41574-021-00625-8

11. Knobel RB. Thyroid hormone levels in term and preterm neonates. Vol. 26, Neonatal network : NN. 2007. p. 253–9. ,doi :10.1891/0730-0832.26.4.253

12. Walsh JP. Thyroid Function across the Lifespan: Do Age-Related Changes Matter? Endocrinol Metab. 2022 Apr 30;37(2):208–19. http://e-enm.org/journal/view.php?doi=10.3803/EnM.2022.1463,doi :10.3803/EnM.2022.1463

13. Eng L, Lam L. Thyroid Function During the Fetal and Neonatal Periods. Neoreviews. 2020 Jan 1;21(1):e30–6. https://publications.aap.org/neoreviews/article/21/1/e30/92158/Thyroid-Function-During-the-Fetal-and-Neonatal,doi :10.1542/neo.21-1-e30

14. Aktas S. Evaluation of Thyrotropin and Thyroxine Levels in The First Month of Life. Cyprus J Med Sci. 2019;4(2):99–102. ,doi :10.5152/cjms.2019.698

15. Rose SR, Brown RS, Foley T, Kaplowitz PB, Kaye CI, Sundararajan S, et al. Update of newborn screening and therapy for congenital hypothyroidism. Pediatrics. 2006;117(6):2290–303. ,doi :10.1542/peds.2006-0915

16. Karbasy K, Lin DCCC, Stoianov A, Chan MK, Bevilacqua V, Chen Y, et al. Pediatric reference value distributions and covariate-stratified reference intervals for 29 endocrine and special chemistry biomarkers on the Beckman Coulter Immunoassay Systems: a CALIPER study of healthy community children. Clin Chem Lab Med. 2016 Apr 1;54(4):643–57.

https://www.degruyter.com/document/doi/10.1515/cclm-2015-0558/html,doi :10.1515/cclm-2015-0558

1. Colantonio DA, Kyriakopoulou L, Chan MK, Daly CH, Brinc D, Venner AA, et al. Closing the gaps in pediatric laboratory reference intervals: A caliper database of 40 biochemical markers in a healthy and multiethnic population of children. Clin Chem. 2012;58(5):854–68. ,doi :10.1373/clinchem.2011.177741
2. Bailey D, Colantonio D, Kyriakopoulou L, Cohen AH, Chan MK, Armbruster D, et al. Marked biological variance in endocrine and biochemical markers in childhood: establishment of pediatric reference intervals using healthy community children from the CALIPER cohort. Clin Chem. 2013 Sep 1;59(9):1393–403. https://academic.oup.com/clinchem/article/59/9/1393/5621904,doi :10.1373/clinchem.2013.204222
3. Higgins V, Fung AWS, Chan MK, Macri J, Adeli K. Pediatric reference intervals for 29 Ortho VITROS 5600 immunoassays using the CALIPER cohort of healthy children and adolescents. Clin Chem Lab Med. 2018 Jan 26;56(2):327–40. https://www.degruyter.com/document/doi/10.1515/cclm-2017-0349/html,doi :10.1515/cclm-2017-0349
4. Bohn MK, Higgins V, Asgari S, Leung F, Hoffman B, Macri J, et al. Paediatric reference intervals for 17 Roche cobas 8000 e602 immunoassays in the CALIPER cohort of healthy children and adolescents. Clin Chem Lab Med. 2019;57(12):1968–79. ,doi :10.1515/cclm-2019-0707
5. Bohn MK, Horn P, League D, Steele P, Hall A, Adeli K. Pediatric reference intervals for endocrine markers and fertility hormones in healthy children and adolescents on the Siemens Healthineers Atellica immunoassay system. Vol. 59, Clinical Chemistry and Laboratory Medicine. 2021. p. 1421–30. ,doi :10.1515/cclm-2021-0050
6. Tanner JM (James M. Foetus into man : physical growth from conception to maturity [Internet]. Harvard University Press; 1978. https://cir.nii.ac.jp/crid/1130282272750624512.bib?lang=en
7. de Onis M, Garza C, Onyango AW, Rolland-Cachera MF. Les standards de croissance de l'Organisation mondiale de la santé pour les nourrissons et les jeunes enfants. Arch Pédiatrie. 2009 Jan;16(1):47–53. https://linkinghub.elsevier.com/retrieve/pii/S0929693X08005101,doi :10.1016/j.arcped.2008.10.010
8. Omuse G, Kawalya D, Mugaine P, Chege A, Maina D. Neonatal reference intervals for thyroid stimulating hormone and free thyroxine assayed on a Siemens Atellica® IM analyzer: a cross sectional study. BMC Endocr Disord. 2023 May 19;23(1):112. https://bmcendocrdisord.biomedcentral.com/articles/10.1186/s12902-023-01367-6,doi :10.1186/s12902-023-01367-6
9. Hadlow NC, Rothacker KM, Wardrop R, Brown SJ, Lim EM, Walsh JP. The Relationship Between TSH and Free T4 in a Large Population Is Complex and Nonlinear and Differs by Age and Sex. J Clin Endocrinol Metab. 2013 Jul 1;98(7):2936–43. https://academic.oup.com/jcem/article/98/7/2936/2537137,doi :10.1210/jc.2012-4223
10. Kapelari K, Kirchlechner C, Högler W, Schweitzer K, Virgolini I, Moncayo R. Pediatric reference

intervals for thyroid hormone levels from birth to adulthood: a retrospective study. BMC Endocr Disord. 2008 Dec 27;8(1):15. https://doi.org/10.1186/1472-6823-8-15,doi :10.1186/1472-6823-8-15

11. Wong JSL, Selveindran NM, Mohamed RZ, Zain FM, Anas SS, Hong JYH. Reference intervals for thyroid-stimulating hormone (TSH) and free thyroxine (FT4) in infants' day 14–30 of life and a comparison with other studies. J Pediatr Endocrinol Metab. 2020 Sep 25;33(9):1125–32. https://www.degruyter.com/document/doi/10.1515/jpem-2020-0148/html,doi :10.1515/jpem-2020-0148