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Thyroid hormone levels during pregnancy: a hospital-based cross-sectional survey of the healthy gestational population living in Kinshasa, DRC

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Abstract

Background: Thyroid hormones for fetal growth and development are mainly dependent on the stage of pregnancy, especially in the first trimester, requiring the proper maternal thyroid function.

Objectives: To describe the thyroid profile of a hospital-based cross-sectional survey of 100 healthy women living in Kinshasa.

Methods: Mindray chemiluminescence analyzer in detecting Thyroid Stimulating Hormone (TSH), free and total triiodothyronine (FT3, TT3), and free and total thyroxine (FT4, TT4).

Results: The Reference Intervals (RIs) in plasma were proposed as follows: TSH 0.09 to 4.34 μIU/mL, FT3 1.46 to 3.35 pg/mL, FT4 0.62 to 1.25 ng/dL, TT3 0.71 to 2.04 ng/mL, and TT4 7.09 to 17.77 μg/dL. The specific RIs of TSH vs FT4 vs FT3 were respectively 0.22 - 2.66 μIU/mL vs 0.71-1.25 ng/dL vs 0.71-1.59 pg/mL in the first trimester, 0.09 - 4.34 μIU/mL vs 0.62-1.22 ng/dL vs 0.86-2.04 pg/mL in the second trimester, and 0.71-3.40 μIU/mL vs 0.72-0.99 ng/dL vs 0.86-1.66 pg/mL in the third trimester. No subject had hypothyroidism risk, but 6% of subclinical hyperthyroidism prevalence was found.

Conclusions: The findings of this study suggest to undertake a national program extending thyroid function screening as recommended by the American Thyroid Association as well as the European Thyroid Association.

Keywords: Thyroid stimulating hormone (TSH); Free and total triiodothyronine (FT3, TT3); Free and total thyroxine (FT4, TT4); Gestational population; Kinshasa

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Background

Thyroid Hormones (TH) are signaling molecules produced and released by the thyroid gland which is the largest endocrine gland in the body. The major form of TH in the blood is Free and Total triiodothyronine (FT3, TT3) and thyroxine (FT4, TT4), as well as Thyroid Stimulating Hormone (TSH), which play crucial roles in the process of physiological metabolism, reproduction, and neurodevelopment, especially in the early stage of brain development when the central nervous system is highly sensitive to thyroid hormones.[1,2]

During pregnancy, the developing fetus is completely dependent on its mother for thyroid hormones especially for brain and nervous system development in the first trimester, requiring the proper maternal thyroid function.[3,4] However, several studies revealed that a high proportion of pregnant women suffer from thyroid disorders in their first trimester and Thyroid dysfunction has been shown to be associated with adverse pregnancy outcomes, including preeclampsia, miscarriage, gestational diabetes, neonatal death, and intrauterine growth restriction.[4,5,6,7] In addition, Thyroid dysfunction is a common complication during pregnancy, with some pregnant women do not always develop symptoms, and the prevalence of subclinical hypothyroidism (4%-17%), and gestational thyroid dysfunction (2%-4%).[4,8]

To reduce the risks of these adverse pregnancy outcomes, the International Endocrine Society (IES), American Thyroid Association (ATA) and European Thyroid Association (ETA), recommend universal thyroid function screening as well as trimester-specific reference intervals for the entire gestational population.[6,7]

Given these facts with the lack of the reliable database involving the gestational population in Kinshasa, this study was undertaken to describe the thyroid profile of a hospital-based cross-sectional survey of 100 healthy women living Kinshasa, DR Congo generating reference ranges (RIs) for TSH, FT3, TT3, FT4 and TT4.

Methods

Study site and subjects

A hospital-based cross-sectional study was conducted in Kinshasa city which was stratified to have prenatal clinics according to the four districts: Funa (Bondeko health and maternity center), Lukunga (Binza Saint-Sacrement Health and Maternity Center), Mont-Amba (Kinshasa University Clinics) and Tshiangu (Bomoyi Health and Maternity Center). Participants (n = 113) were recruited from pregnant women during the pregnancy hospital visit between May to July 2024. Pregnancy information collected in the questionnaires were clinics, socio-demographics, Anthropometrics, current and previous pregnancies, current and previous preeclampsia, diabetes mellitus or thyroid disorders, smoking during pregnancy, and lifestyle. The pregnant women suffered from these above pregnancy outcomes were excluded (n =13). The research protocol was approved by the Bio-ethics Committee of the School of Public Health at the University of Kinshasa.

Data collection

During their routine hospital checkup, pregnant women provided venous blood samples in 10 ml metal free tubes containing lithium heparin which were immediately centrifuged (10 minutes, 3000 g) and the plasma fraction was transferred into 2.5 ml pre-cleaned glass vials (Supelco) and stored at -80°C (Clinics of Kinshasa University). The plasma samples were transported to the Clinical laboratory of Marie Biamba-Mutombo Hospital for the hormone measures.

Analytical methods

The description of assays used to measure various thyroid variables are provided the Mindray CL-1200i which is a fully automated Chemiluminescence Immunoassay (CLIA) system designed for high-throughput testing in clinical laboratories and offers a wide range of immunoassays, including TT3, FT3, TT4, FT4, and TSH.[5,9,10,11,12] For quality control purposes, the reagent kits and calibrators used in this study were the Mindray CL-1200i. Each sample was tested according to the manufacturer's instructions with supplied reagents and the results were analyzed statistically performing precision, linearity, accuracy, and reference interval verification of 4 hormones.

Statistical analysis

Statistical data analysis was completed using Prism GraphPad 9.41 (GraphPad Soft - ware, San Diego, CA, USA). The normality of residuals (TT3, FT3, TT4, FT4, TSH) was evaluated using Kolmogorov-Smirnov test for continuous variables. For the descriptive statistics, results are presented as percentage for categorical variables and as means (± standard deviation) and percentiles (P5, P50, P75, P95) for continuous variables. All results were assessed for data quality based on the Coefficient of Variation (CV). The level below the Limit of Detection (LOD) was assigned a value of LOD/2 for statistical calculations as described elsewhere.[13]

Results

Characteristics of the subjects: a total of 100 pregnant women were recruited, as follows: 87% had lower or middle school degree, 58% were married, 94% earned none or less than 500$ USD monthly, 36% were multiparous, 33% consumed alcohol during pregnancy, and none of them smoked during pregnancy (Table 1).

Table 1: Sociodemographic characteristics of the Kinshasa study subjects (n = 100)

Maternal characteristics	AM ± SD (Min-Max)	Proportion (%)
Weight (kg)	67.88 ± 12.74 (43.00-105.00)	
Height (m)	1.63 ± 0.06 (1.45-1.84)	
BMI (kgxm^2)	25.57 ± 4.19 (17.30-39.25)	
Amenorrhea period (weeks)	22.47 ± 7.91 (10.00-41.00)	
PAS (mm Hg)	105.95 ± 12.45 (80.00-144.00)	
PAD (mm Hg)	65.86 ± 9.79 (40.00-93.00)	
Education		Lower school or none, 71%
		Middle school, 16%
		High school or university degree, 13%
Marital status		Married or living as married, 58%
Family income (month)		None, 21%
		100$ - 500$, 73%
		≥ 500$, 6%
Smoking during pregnancy		Yes, 0%
Alcohol use during pregnancy		Yes, 33%
Parity		≥1 (multiparous), 36%

AM ± SD (Min-Max)

Table 1 lists also the means (±SD) of the continuous variables including, weight (67.88 ± 12.74 Kg), height (1.63 ± 0.06 m), amenorrhea period (22.47 ± 7.91 weeks), BMI (25.57 ± 4.19 kg/m2), SBP (105.95 ± 12.45 mm Hg), and DBP (65.86 ± 9.79 mm Hg).

Table 2: Arithmetic means with standard deviations and selected percentiles for thyroid stimulating hormone (TSH) in µIU/mL, free triiodothyronine (FT3) in pg/mL, free thyroxine (FT4) in ng/dL, total triiodothyronine (TT3) in ng/dL, and total thyroxine (TT4) in µg/mL for the healthy gestational population from Kinshasa.

Parameters	LOQ (n)	P5	AM ± SD	P50	P75	P95
FT3 (pg/mL)	0.88 (n = 100)	1.46	2.23 ± 0.30	2.18	3.27	3.35
FT4 (ng/dL)	0.30 (n = 100)	0.62	0.84 ± 0.12	0.83	1.24	1.25
TT3 (ng/mL)	0.36 (n = 100)	0.71	1.22 ± 0.24	1.21	1.81	2.04
TT4 (µg/dL)	1.30 (n = 100)	7.09	11.75 ± 1.99	11.86	16.8	17.77
TSH (µIU/mL)	0.02 (n =100)	0.09	1.27 ± 0.76	1.13	1.69	4.34

Table 2 lists the LOQ, means (±SD) and percentiles (P5, P50, P75 and P95) of the continuous variables. The means (±SD) were 1.22 ± 024 ng/mL for TT3, 2.23 ± 0.30 pg/mL FT3, 11.75 ± 1.99 µg/dL TT4, 0.84 ± 0.12 ng/dL for FT4, and 1.27 ± 0.76 µIU/mL for TSH.

Table 3: Arithmetic means with standard deviations and selected percentiles for thyroid stimulating hormone (TSH) in µIU/mL, free triiodothyronine (FT3) in pg/mL, free thyroxine (FT4) in ng/dL, total triiodothyronine (TT3) in ng/dL, total thyroxine (TT4) in µg/mL by amenorrhea weeks versus Mindray Specific Reference Ranges.

Parameters	Amenorrhea weeks (n)	P5	AM ± SD	P50	P75	P95
FT3 (pg/mL)	10 – 15 (n =18)	1.87	2.25 ± 0.32	2.13	3.01	3.17
	16 – 28 (n = 60)	1.46	2.24 ± 0.30	2.21	3.13	3.35
	29 -41 (n = 22)	1.75	2.18 ± 0.28	2.15	2.32	2.88
FT4 (ng/dL)	10 – 15 (n =18)	0.71	0.92 ± 0.30	0.93	1.39	1.25
	16 – 28 (n = 60)	0.62	0.82 ± 0.13	0.81	1.02	1.22
	29 -41 (n = 22)	0.72	0.85 ± 0.07	0.84	0.93	0.99
TT3 (ng/mL)	10 – 15 (n =18)	0.71	1.08 ± 0.23	1.02	1.53	1.59
	16 – 28 (n = 60)	0.86	1.27 ± 0.23	1.23	1.84	2.04
	29 -41 (n = 22)	0.86	1.18 ± 0.22	1.16	1.47	1.66
TT4 (µg/dL)	10 – 15 (n =18)	7.14	10,78 ± 2.11	9.92	12.88	13.89
	16 – 28 (n = 60)	7.09	12.01 ± 0.27	11.87	16.08	17.77
	29 -41 (n = 22)	7.83	11.81 ± 1.39	12	13.03	14.32
TSH (µIU/mL)	10 – 15 (n =18)	0.22	0.87 ± 0.57	0.6	1.03	2.66
	16 – 28 (n = 60)	0.09	1.35 ± 0.82	1.21	1.81	4.34
	29 -41 (n = 22)	0.71	1.37 ± 0.63	1.32	1.98	3.4

Regarding differences between amenorrhea periods (Table 3), levels of plasma FT3 were 2.25 ± 0.32 pg/mL in 10-19 weeks, 2.24 ± 0.30 pg/mL in 20-36 weeks, and 2.18 ± 0.28 pg/mL in ≥37 weeks. Levels of plasma FT4 were 0.92 ± 0.30 ng/dL in 10-19 weeks, 0.82 ± 0.13 ng/dL in 20-36 weeks, and 0.85 ± 0.07 ng/dL in ≥37 weeks. Levels of plasma TT3 were 1.08 ± 0.23 ng/mL in 10-19 weeks, 1.27 ± 0.23 ng/mL in 20-36 weeks, and 1.18 ± 0.22 ng/mL in ≥37 weeks. Levels of plasma TT4 were 10,78 ± 2.11 µg/dL in 10-19 weeks, 12.01 ± 0.27 µg/dL in 20-36 weeks, and 11.81 ± 1.39 µg/dL in ≥37 weeks. Levels of plasma TSH were 0.87 ± 0.57 µIU/mL in 10-19 weeks, 1.35 ± 0.82 µIU/mL in 20-36 weeks, and 1.37 ± 0.63 µIU/mL in ≥37 weeks. with t-test, there was no significant difference observed.

Discussion

Thyroid function during pregnancy plays a vital role in pregnancy outcomes and fetal growth. Thus, accurate diagnosis is particularly important. In addition, the most majority of the thyroid hormones circulating in the

blood is bound to transport proteins, and the remaining THs is unbound and biologically active. Therefore, free THs measuring is important for thyroid function screening.

The use of isotope dilution- Liquid Chromatography/tandem Mass Spectrometry (LC/MS-MS) for measuring THs in the dialysate or ultrafiltrate using online solid phase extraction is helpful to obtain a gold-standard reference measurement procedure.[14,15] However, this procedure is currently not widely available due to high instrument and operating costs. Thus, the automated immunoassays are still widely used as the best way for serum/plasma THs analyses. In this study, data was measured using a chemiluminescence immunometric assay from Mindray CL-1200i with a normal reference range of 0.09 to 5.40 µIU/mL for TSH, 0.54 to 1.54 ng/dL for FT4, 1.88 to 3.21 pg/mL for FT3, 6.59 to 17.54 µg/dL for TT4, and 0.71 to 1.95 ng/mL for TT3.[5,10,11,12,16] The specific RIs of plasma TSH were 0.22 - 2.66 µIU/mL in the first trimester, 0.09 - 4.34 µIU/mL in the second trimester and 0.71-3.40 µIU/mL in the third trimester which are consist in the specific reference ranges from the assay used in this study (Table 4). During pregnancy, TT4 levels increase and reach a peak by week 7 to week 16 of amenorrhea remaining high until delivery.[6,17] In the first trimester, maternal human Chorionic Gonadotropin (hCG) directly stimulates the TSH receptor, increasing thyroid hormone production and resulting in a subsequent reduction in plasma TSH levels.[4,6,7] Therefore, pregnant women have lower plasma TSH levels than before pregnancy, and a lower limit of 0.4 µIU/mL is observed in several healthy pregnant women during the first trimester.[4] However, the TSH upper specific reference limit in this study was higher than those recommended in pregnant women in the United States and Europe, 2.5 µIU/mL in the first trimester, 3.0 µIU/mL in the second and 1.15 µIU/mL in the third trimester.[6,7,15,16,18] Probably due to the inconsistency of test results from different laboratories.[5] The specific RIs of plasma FT4 vs FT3 were 0.71-1.25 ng/dL vs 0.71-1.59 pg/mL in the first trimester, 0.62-1.22 ng/dL vs 0.86-2.04 pg/mL in the second, and 0.72-0.99 ng/dL vs 0.86-1.66 pg/mL in the third trimester respectively which are within the acceptable ranges from the assay used in this study (Table 4) as well as in the China gestational women.[6,18]

Table 4: Comparison of specific reference ranges for thyroid stimulating hormone (TSH) in µIU/mL, free triiodothyronine (FT3) in pg/mL, free thyroxine (FT4) in ng/dL, total triiodothyronine (TT3) in ng/dL, total thyroxine (TT4) in µg/mL by trimesters.

Parameters	Trimesters	Specific Reference intervals (P5-P95)[a]	Mindray Specific Reference Ranges[b]	Trimester-specific reference range [c]
FT3 (pg/mL)	First	1.87-3.17	2.11-3.21	2.48-4.20
	Second	1.46-3.35	1.91-3.13	2.29-3.26
	Thirst	1.75-2.88	1.88-2.75	2.07-3.13
FT4 (ng/dL)	First	0.71-1.25	0.82-1.54	1.08-2.05
	Second	0.62-1.22	0.56-1.32	0.95-1.50
	Thirst	0.72-0.99	0.54-1.04	0.88-1.49
TT3 (ng/mL)	First	0.71-1.59	0.71-1.60	0.91-2.65
	Second	0.86-2.04	0.84-1.95	0.85-2.60
	Thirst	0.86-1.66	0.78-1.85	0.93-2.33
TT4 (µg/dL)	First	7.14-13.89	6.98-16.37	8.02-24.78
	Second	7.09-17.77	7.68-17.54	7.16-18.22
	Thirst	7.83-14.32	6.59-16.92	6.48-20.03
TSH (µIU/mL)	First	0.22-2.66	0.09-4.87	0.02-3.78
	Second	0.09-4.34	0.47-4.79	0.47-3.89
	Thirst	0.71-3.40	0.50-5.40	0.55-4.91

a: This study

b: Mindray [11]

c: Zang et al. [19]

Physiological changes of TSH/FT4 are used to assess the prevalence of hypo-and hyperthyroidism according to some criteria, such as those defined by Jain (2015) [16] or Surks (2004) [19]: subclinical hyperthyroidism [TSH < 0.45 μIU/mL and 0.6 ≤ FT4 ≤ 1.6 ng/dL], subclinical hypothyroidism [TSH > 4.5 μIU/mL and 0.6 <= FT4 <= 1.6 ng/dL], and clinical hypothyroidism [TSH > 4.5 μIU/mL and FT4 < 0.6 ng/dL]. In this study, although no subject had no hypothyroidism risk, high prevalence (6%) of subclinical hyperthyroidism was found. This should be of concern since subclinical hyperthyroidism has been associated with some outcomes including major risks of dementia and cardiovascular diseases.[4,6,7,19,20]

Reference range determinations include pregnant women with no known thyroid disease, optimal iodine intake, and negative thyroid peroxidase antibody (TPOAb) status.[4,16] The RIs for thyroid function were proposed as follows: TSH 0.09 to 4.34 μIU/mL, FT3 1.46 to 3.35 pg/mL, FT4 0.62 to 1.25 ng/dL, TT3 0.71 to 2.04 ng/mL, and TT4 7.09 to 17.77 μg/dL (Table 5). These RIs are similar as that established for the China gestational women TSH 0.55 to 4.78 μIU/mL, FT3 2.27 to 4.23 pg/mL, FT4 0.89-1.76 ng/dL, TT3 0.59-1.81 ng/mL, TT4 1.90-13.31 μg/dL [18] as well as ATA.[6,7]

Table 5: Comparison of reference ranges for thyroid stimulating hormone (TSH) in μIU/mL, free triiodothyronine (FT3) in pg/mL, free thyroxine (FT4) in ng/dL, total triiodothyronine (TT3) in ng/dL, total thyroxine (TT4) in μg/mL by between Kinshasa and others databases.

Parameters	Reference Ranges		
	This study[a]	Mindray[b]	Traditional Chinese and Western Medicine[c]
FT3 (pg/mL)	1.46 – 3.35	1.88-3.21	2.27 – 4.23
FT4 (ng/dL)	0.62 – 1.25	0.54-1.54	0.89-1.76
TT3 (ng/mL)	0.71 – 2.04	0.71-1.95	0.59-1.81
TT4 (μg/dL)	7.09 – 17.77	6.59-17.54	1.90-13.31
TSH (μIU/mL)	0.09 - 4.34	0.09-5.40	0.55 -4.78

a: This study

b: Mindray [11]

c: Zang et al. [19]

A major limitation should be considered in evaluating present results. With regard to study population, data collection and analytical methods, the relatively small number of pregnant women cohorts studied. The sample collection methods (excluding criteria) used here were not robust but by chance, which were practically inevitable under present survey conditions and susceptible to errors associated with sample collection. Although their essential role in thyroid metabolism and function, as well as their link to thyroid autoimmunity, iodine (I2) and Selenium (Se) were not assessed in this work as well as the TPOAb which is positivity adversely modulates the impact of maternal thyroid status (especially hypothyroidism) on the pregnancy and the developing fetus.[4,16,21]

Conclusion

Thyroid function during pregnancy plays a vital role in pregnant outcomes and fetal growth. Thus, accurate screening is important to define the population-based trimester-specific reference ranges (RIs) through

assessment of local population data representative of a health care provider's practice. This study presented the first database on thyroid profile of the gestational population in Kinshasa from the DR Congo generating RIs for TSH, FT3, TT3, FT4 and TT4. The findings of this study suggest to undertake a national program extending thyroid function screening as recommended by the American Thyroid Association and the European Thyroid Association.

Declarations

Ethical Approval

The research protocol was approved by the Bio-ethics Committee of the School of Public Health at the University of Kinshasa. Kinshasa, DR Congo.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions

The first draft of this manuscript has been written by the first author A.M.K. The co-authors Y.M.T. prepared Tables 1, 2, 3, 4 and 5. The co-authors M.M.K. and M.S.N-N reviewed equally the manuscript. The co-authors JP.E-M. and A.M.M. supervised equally all the manuscript. The J.K.T contributed to supervise all the work and to correspond with the Journal.

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Availability of data and materials

Not applicable. However, the study results will report to individuals sample donors with proper explanations.

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