Original Article

Comparison of the Reference Intervals Used for the Evaluation of Maternal Thyroid Function During Pregnancy Using Sequential and Nonsequential Methods

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Abstract

Background: Maternal thyroid dysfunction is common during pregnancy, and physiological changes during pregnancy can lead to the overdiagnosis of hyperthyroidism and misdiagnosis of hypothyroidism with nongestation-specific reference intervals. Our aim was to compare sequential with nonsequential methods for the evaluation of thyroid function in pregnant women.

Methods: We tested pregnant women who underwent their trimester prenatal screening at our hospital from February 2011 to September 2012 for serum thyroid stimulating hormone (TSH) and free thyroxine (FT4) using the Abbott and Roche kits. There were 447 and 200 patients enrolled in the nonsequential and sequential groups, respectively. The central 95% range between the 2.5th and the 97.5th percentiles was used as the reference interval for the thyroid function parameter.

Results: The nonsequential group exhibited a significantly larger degree of dispersion in the TSH reference interval during the 2nd and 3rd trimesters as measured using both the Abbott and Roche kits (all $P < 0.05$). The TSH reference intervals were significantly larger in the nonsequential group than in the sequential group during the 3rd trimester as measured with both the Abbott (4.95 vs. 3.77 mU/L, $P < 0.001$) and Roche kits (6.62 vs. 5.01 mU/L, $P = 0.004$). The nonsequential group had a significantly larger FT4 reference interval as measured with the Abbott kit during all trimesters (12.64 vs. 5.82 pmol/L; 7.96 vs. 4.77 pmol/L; 8.10 vs. 4.77 pmol/L, respectively, all $P < 0.05$), whereas a significantly larger FT4 reference interval was only observed during the 2nd trimester with the Roche kit (7.76 vs. 5.52 pmol/L, $P = 0.002$).

Conclusions: It was more reasonable to establish reference intervals for the evaluation of maternal thyroid function using the sequential method during each trimester of pregnancy. Moreover, the exclusion of pregnancy-related complications should be considered in the inclusion criteria for thyroid function tests.

Key words: Nonsequential; Pregnancy Trimester; Reference Interval; Sequential; Thyroid Function Tests

Introduction

During pregnancy, thyroid physiology often exhibits large changes due to the influence of varying hormone levels.[1] Maternal thyroid dysfunction is common during pregnancy[2] and may be associated with many adverse outcomes in both the mother and the fetus.[3,4] For example, human maternal thyroid hormones are important for fetal brain development, and low concentrations of thyroid hormones in patients with hypothyroidism during early pregnancy can be potentially damaging to the neurodevelopment of the fetus.[5]

Given the prevalence and adverse outcomes related to maternal thyroid dysfunction, a considerable number of studies have been conducted in the past few years that focused on improving pregnancy outcomes by screening pregnant women for thyroid disease.[6] However, only 30–80% of women with hypothyroidism are identified by screening based on symptoms/risk factors.[7-10] Thyroid gland physiology changes during pregnancy because of the effects of increased levels of thyroid-binding globulin and human

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chorionic gonadotropin and enhanced iodine metabolism.[11] This can lead to the overdiagnosis of hyperthyroidism and misdiagnosis of hypothyroidism with nongestation-specific reference intervals. Thus, it is important to establish gestation-specific reference intervals for thyroid function tests (TFTs) during pregnancy.

Recently, a number of studies on the establishment of gestation-specific reference intervals have been published;[12,13] however, these reference intervals varied due to regional and ethnic differences[14-16] and due to the different detection methods and kits used.[17-19] One study examining a mixed ethnic population of pregnant women in the United Arab Emirates (UAE) showed that free thyroxine (FT4) levels differ significantly between UAE nationals and Asians during the 1st and 2nd trimesters while there was no significant difference in thyroid stimulating hormone (TSH) levels between the various ethnic groups.[20] Therefore, the International Federation of Clinical Chemistry and the International Committee for Standardization Hematology recommend that each laboratory should establish its own reference range.[21] Correct establishment of reference intervals will directly affect the accuracy of disease diagnosis, and the TSH upper limit and the FT4 lower limit play key roles in the diagnosis of clinical/subclinical hypothyroidism or hypothyroxinemia.

There are two methods of blood sample collection, the nonsequential[20] and sequential methods.[22] However, few studies have been performed to deciding more stable and reasonable method for establishing reference intervals. In this study, we aimed to establish a gestation-specific reference interval for TFTs by analyzing data derived from a Chinese population of pregnant women, and we compared the nonsequential and sequential methods for the evaluation of maternal thyroid function.

Methods

This study was performed in accordance with the relevant guidelines and regulations. Ethical approval for this project was granted by the Ethics Committee of the International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University. Written informed consent was obtained from each subject before sample collection.

The study group consisted of pregnant women undergoing their trimester prenatal screenings at the International Peace Maternity and Child Health Hospital. According to the recommendations of the National Academy of Clinical Biochemistry (NACB) for determining TFT reference intervals,[23] the inclusion criteria were as follows: single birth; Han Chinese women; no history of thyropathy or autoimmune disease; no goiter; thyroid peroxidase antibody (TPOAb) negative; and no use of medicine affecting the thyroid hormone. The study area (Shanghai) is an iodine-stable and adequate area, and the median level of urinary iodine in the population is 231.01 μg/L.[24]

From February 2011 to June 2011, a total of 447 pregnant women were enrolled in the nonsequential group, including 140 patients in their 9th–13th week of pregnancy (1st trimester [T1]), 184 in their 16th–20th week of pregnancy (2nd trimester [T2]), and 123 in their 37th–40th week of pregnancy (3rd trimester [T3]). Eligible pregnant women who consented to enroll in the study had blood collected when presenting to the antenatal clinic. For each woman, all data used in this study relate only to a single set of results from blood collected at their visit to the antenatal clinic. No women were included in more than one trimester.

From June 2011 to September 2012, a total of 381 pregnant women undergoing their 1st trimester prenatal screening in their 9th–13th week of pregnancy were included in the sequential group. Thirty individuals with no blood drawn were excluded. Moreover, all those with pregnancy-related complications, such as hyperemesis gravidarum, gestational hypertension, and gestational diabetes mellitus (GDM), were excluded from the sequential group as described previously.[25] Therefore, 71 patients were excluded during T1 for TPOAb (+) and thyroid dysfunction, eighteen were excluded during T2 for taking medicines influencing thyroid function, and 62 patients were excluded during T3 for GDM, anemia, and gestational hypertension. A total of 200 pregnant women were enrolled in the sequential group, and the study population is shown in a flow chart [Figure 1]. Blood samples from the sequential group were collected 3 times (T1, T2, and T3) from every individual during gestation.

Fasting blood samples (3 ml) were drawn from the median cubital vein. The serum was separated by centrifugation within 6 h and was then divided into two aliquots. TSH and FT4 levels were measured from one aliquot of serum using Abbott (ARCHITECT i2000; Abbott, Chicago, USA) kits according to the manufacturer's protocol, while in parallel, TSH and FT4 levels were measured from another aliquot of serum from the same time point using Roche kits (Cobas E Systems; Roche Diagnostics GmbH, Mannheim, Germany). The kit parameters are shown in Table 1, and quality controls were provided by the manufacturer. Both of the two

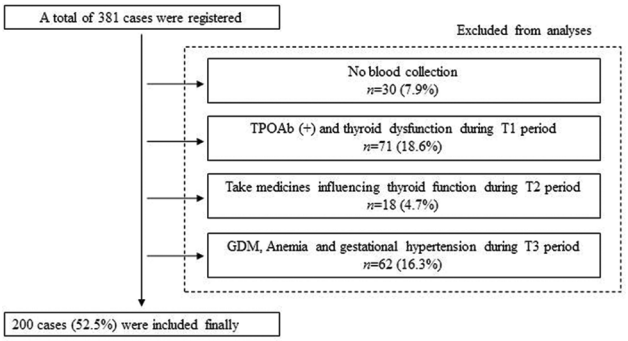

Figure 1: Flow chart of study population selection in the sequential group. T1: 1st trimester, the 9th–13th week of pregnancy; T2: 2nd trimester, the 16th–20th week of pregnancy; T3: 3rd trimester, the 37th–40th week of pregnancy. GDM: Gestational diabetes mellitus; TPOAb: Thyroid peroxidase antibody.

companies performed pretests with their instruments before the examinations, and the quality control was performed by a third party.

All statistical analyses were performed using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). TSH and FT4 did not exhibit normal distributions and were, therefore, expressed as the median (range). The central 95% range between the 2.5th and the 97.5th percentiles was used as the reference interval for the thyroid function parameter during each trimester of pregnancy. Bootstrap methods were used to compare the reference intervals established using the Abbott and Roche kits. Comparisons between the nonsequential and sequential groups were analyzed using the Mann–Whitney *U*-test. Differences were considered to be statistically significant with a $P < 0.05$.

Results

Degree of dispersion in the reference interval for the thyroid function parameter using the Abbott and Roche kits

No difference in age was observed between the nonsequential and sequential groups (30 [28–32] vs. 30 [28–33] years, $P = 0.400$). The degree of dispersion in the reference intervals for the thyroid function parameter as measured using the Abbott and Roche kits is shown in Figure 2. A similar degree of dispersion in the TSH reference interval as measured using the Abbott kit was observed between the nonsequential and sequential groups during T1 [Figure 2a]. However, the nonsequential group exhibited a significantly larger degree of dispersion than the sequential group during T2 and T3 (both $P < 0.05$). For FT4, the degree of dispersion was slightly higher in the sequential group than in the nonsequential group during T1 [Figure 2b, $P < 0.05$], and it was significantly higher in the nonsequential group than in the sequential group during T2 and T3 (both $P < 0.05$).

With the Roche kits, the degree of dispersion in the TSH reference interval was slightly lower in the nonsequential group than in the sequential group during T1 [Figure 2c, $P < 0.05$]. However, the nonsequential group exhibited a larger degree of dispersion than the sequential group during T2 and T3 (both $P < 0.05$). As shown in Figure 2d, the nonsequential group exhibited a larger degree of dispersion in the FT4 reference interval than the sequential group during all three trimesters (all $P < 0.05$).

Thyroid stimulating hormone reference intervals using the Abbott and Roche kits

The TSH reference intervals measured using the Abbott kit are shown in Table 2. The use of the 2.5th and 97.5th percentiles for the calculation of the reference intervals resulted in a reference interval of 0.03–3.47 mU/L and 0.10–3.88 mU/L in the nonsequential group and 0.06–3.01 mU/L and 0.29–2.93 mU/L in the sequential group during T1 and T2, respectively. The TSH reference intervals were significantly

Table 1: Parameters of the Abbott and Roche kits

Parameters	TSH (mU/L)		FT4 (pmol/L)	
	Abbott	Roche	Abbott	Roche
Normal range	0.34–5.60	0.27–4.20	7.50–21.10	12.00–22.00
Inter-assay variation (%)	3.59	3.44	4.01	3.94
Intra-assay variation (%)	1.60	1.47	1.90	1.76
Sensitivity	0.00	0.01	0.62	0.30

TSH: Thyroid stimulating hormone; FT4: Free thyroxine.

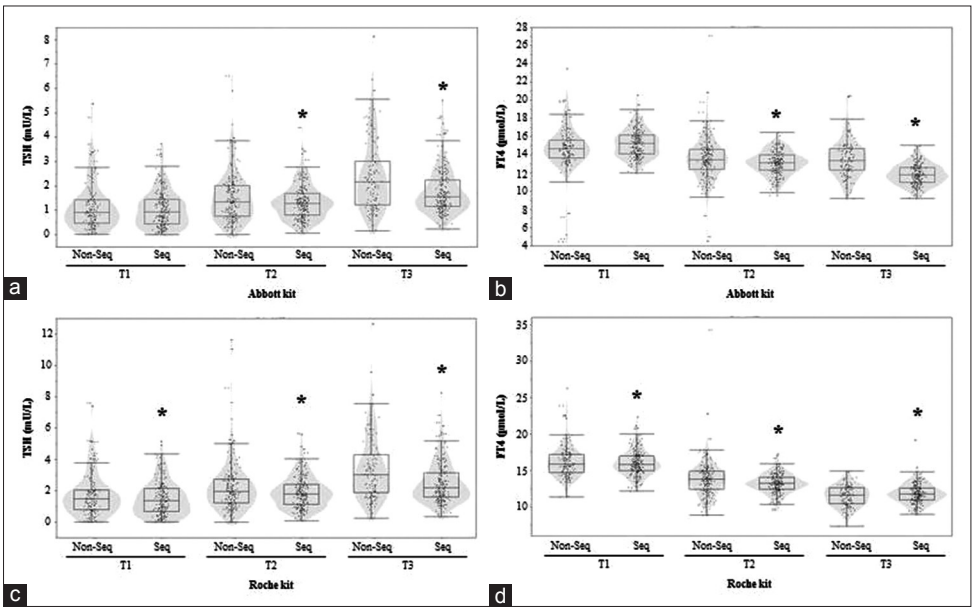

Figure 2: Degree of dispersion in the TSH (a and c) and FT4 (b and d) reference intervals using Abbott and Roche kits, respectively. TSH: Thyroid stimulating hormone; FT4: Free thyroxine. Nonseq: Nonsequential group; Seq: Sequential group. T1: 1st trimester, the 9th–13th week of pregnancy; T2: 2nd trimester, the 16th–20th week of pregnancy; T3: 3rd trimester, the 37th–40th week of pregnancy. *Means within the same trimester sequential group differ from the nonsequential group ($P < 0.05$).

wider in the nonsequential group than in the sequential group during T3 (4.95 vs. 3.77 mU/L, $P < 0.001$). The TSH reference intervals measured using the Roche kit are shown in Table 3. Significantly higher TSH reference intervals were observed in the nonsequential group compared with the sequential group during T3 (6.62 vs. 5.01 mU/L, $P = 0.004$).

Free thyroxine reference intervals using the Abbott and Roche kits

The FT4 reference intervals measured using the Abbott kit are shown in Table 4. Significantly higher FT4 reference intervals were observed in the nonsequential group compared with the sequential group during all three trimesters (T1: 12.64 vs. 5.82 pmol/L, T2: 7.96 vs. 4.77 pmol/L, and T3: 8.10 vs. 4.77 pmol/L, all $P < 0.05$).

For the Roche kit, the use of the 2.5th and 97.5th percentiles to calculate the reference interval resulted in similar FT4 reference intervals for the nonsequential and sequential groups during T1 and T3 [Table 5]. However, significant differences in the reference interval were observed during T2. Specifically, during T2, the reference interval was wider in the nonsequential group compared with that in the sequential group (7.76 vs. 5.52 pmol/L, $P = 0.002$).

Discussion

Thyroid disease during pregnancy has been discussed a great deal in recent years, and clinical guidelines often devote attention to this problem. However, screening for thyroid dysfunction in early pregnancy using the reference intervals for nonpregnant women is difficult[1] and is not reliable considering the influence of pregnancy. Therefore, it is necessary to establish specific reference intervals for pregnant women to reduce misdiagnoses and missed diagnoses.[26] In this study, the reference values for TFTs were analyzed using data derived from a Chinese regional population of pregnant women.

Previous studies have been conducted on specific reference intervals for the evaluation of maternal thyroid function during pregnancy. For example, Silvio *et al*.[27] used Elecsys E170 and ARCHITECT i2000SR methods to determine the TSH and FT4 reference intervals during the mid-trimester of pregnancy. Despite having a large sample size, their study did not consider regional iodine levels in the study area and only excluded pregnant women with both TPOAb (+) and thyroglobulin antibody (+). Meanwhile, they only determined a reference interval for the mid-trimester of

Table 2: TSH reference intervals (mU/L) during pregnancy for the nonsequential and sequential groups using the Abbott kit

Trimester	2.5th percentile	97.5th percentile	Reference interval	Difference in reference interval	*Z*	*P*	95% *CI*
T1							
Nonsequential	0.03	3.47	3.44	0.75	0.02	0.981	−0.077–2.367
Sequential	0.06	3.01	2.95				
T2							
Nonsequential	0.10	3.88	3.78	1.25	1.27	0.202	0.436–3.151
Sequential	0.29	2.93	2.64				
T3							
Nonsequential	0.61	5.56	4.95	1.30	3.67	<0.001	0.151–3.030
Sequential	0.47	4.24	3.77				

TSH: Thyroid stimulating hormone; T1: 1st trimester, the 9th–13th week of pregnancy; T2: 2nd trimester, the 16th–20th week of pregnancy; T3: 3rd trimester, the 37th–40th week of pregnancy; *CI*: Confidence interval. *P* value was from the comparison of reference intervals between the nonsequential and sequential groups.

Table 3: TSH reference intervals (mU/L) during pregnancy for the nonsequential and sequential groups using the Roche kit

Trimester	2.5th percentile	97.5th percentile	Reference interval	Difference in reference interval	*Z*	*P*	95% *CI*
T1							
Nonsequential	0.04	5.17	5.13	1.36	1.17	0.242	0.106–3.707
Sequential	0.09	4.13	4.04				
T2							
Nonsequential	0.13	6.57	6.44	2.69	1.49	0.137	0.419–7.076
Sequential	0.44	4.24	3.80				
T3							
Nonsequential	0.97	7.58	6.62	1.91	2.29	0.004	0.190–4.681
Sequential	0.69	5.70	5.01				

TSH: Thyroid stimulating hormone; T1: 1st trimester, the 9th–13th week of pregnancy; T2: 2nd trimester, the 16th–20th week of pregnancy; T3: 3rd trimester, the 37th–40th week of pregnancy; *CI*: Confidence interval. *P* value was from the comparison of reference intervals between the nonsequential and sequential groups.

Table 4: FT4 reference intervals (pmol/L) during pregnancy for the nonsequential and sequential groups using the Abbott kit

Trimester	2.5th percentile	97.5th percentile	Reference interval	Difference in reference interval	*Z*	*P*	95% *CI*
T1							
Nonsequential	7.16	19.80	12.64	6.13	3.06	0.002	0.588–9.995
Sequential	12.81	18.63	5.82				
T2							
Nonsequential	9.76	17.72	7.96	4.15	2.21	0.027	1.870–8.500
Sequential	10.57	15.34	4.77				
T3							
Nonsequential	9.83	17.93	8.10	3.27	7.90	<0.001	1.308–6.363
Sequential	9.63	14.39	4.77				

FT4: Free thyroxine; T1: 1st trimester, the 9th–13th week of pregnancy; T2: 2nd trimester, the 16th–20th week of pregnancy; T3: 3rd trimester, the 37th–40th week of pregnancy; *CI*: Confidence interval. *P* value was from the comparison of reference intervals between the nonsequential and sequential groups.

Table 5: FT4 reference intervals (pmol/L) during pregnancy for the nonsequential and sequential groups using the Roche kit

Trimester	2.5th percentile	97.5th percentile	Reference interval	Difference in reference interval	*Z*	*P*	95% *CI*
T1							
Nonsequential	13.41	22.50	9.09	1.99	0.46	0.647	−1.545–5.303
Sequential	12.96	19.96	7.00				
T2							
Nonsequential	10.04	17.80	7.76	2.65	3.12	0.002	0.930–7.255
Sequential	10.41	15.92	5.52				
T3							
Nonsequential	8.70	14.80	6.10	0.87	1.44	0.150	−0.473–2.440
Sequential	9.47	14.56	5.10				

FT4: Free thyroxine; T1: 1st trimester, the 9th–13th week of pregnancy; T2: 2nd trimester, the 16th–20th week of pregnancy; T3: 3rd trimester, the 37th–40th week of pregnancy; *CI*: Confidence interval. *P* value was from the comparison of reference intervals between the nonsequential and sequential group.

pregnancy. Boas *et al*.[28] also used the sequential method using Roche kits (Roche Modular E170) to establish six reference intervals for different gestational weeks (10th, 14th, 21th, 28th, 34th, and 40th weeks of pregnancy). During their study, pregnant women with gestational hypertension and GDM were also excluded. Although dynamic variance in hormonal levels was detected, the sample size was small (104 patients). In our study, both nonsequential and sequential methods were used to determine TSH and FT4 reference intervals during all three trimesters of pregnancy.

Springer *et al*.[29] previously determined the TSH reference interval during the 1st trimester of pregnancy to be 0.06–3.67 mU/L in a group of pregnant women who were selected in accordance with the recommendations of the NACB. Their TSH reference interval was similar to ours of 0.03–3.47 mU/L measured using the nonsequential method. During our use of the nonsequential method, we found that thyroid function was associated with pregnancy-related complications. Therefore, in the sequential group, patients with pregnancy-related complications were excluded apart from the NACB criteria for TFT. The sequential procedure was conducted to assemble cases to rule out the inter-individual variation. We cannot rule out the possibility that these exclusion criteria may affect the results.

Moreover, considering the regional and ethnic differences as well as the influence of the different kit parameters, we evaluated maternal thyroid function using kits made by two different manufacturers (Abbott and Roche). Therefore, we obtained more reliable reference intervals. In our study, the nonsequential group exhibited a larger degree of dispersion in the TSH and FT4 reference intervals compared with the sequential group during pregnancy, especially in T2 and T3, regardless of whether the Abbott or Roche kit was used. This was in accordance with our result showing that the nonsequential group had higher reference intervals for TSH than the sequential group during T3 as measured using both the Abbott and Roche kits. We also observed a larger FT4 reference interval in the nonsequential group compared with the sequential group during all three trimesters using the Abbott kit, while we observed a higher FT4 reference only in T3 with the Roche kit. This could be explained by the following observations: First, the sequential method was used in the same population during three different trimesters, reducing the sampling error, and the coefficient of variation in the reference interval; and second, pregnant women with hyperemesis gravidarum, gestational hypertension, or GDM were excluded from the sequential group. Therefore, it was more reasonable to establish the reference interval using the sequential method, and the exclusion of pregnancy-related

complications should be considered in the inclusion criteria for TFT.

Several limitations to this study must be addressed. First, the number of cases of pregnant women enrolled in the nonsequential and sequential groups were insufficient, and this might affect the statistical accuracy of the results. Second, our single-hospital experience may not be generalized to the broader community. Therefore, further studies including a larger number of pregnant women with design verification testing from several hospitals would be warranted.

In summary, it was more reasonable to establish reference intervals for the evaluation of maternal thyroid function using the sequential method during each trimester of pregnancy. Moreover, the exclusion of pregnancy-related complications should be considered in the inclusion criteria for TFT.

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Conflicts of interest

There are no conflicts of interest.

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