Assessment of thyroid function during pregnancy: the advantage of self-sequential longitudinal reference intervals

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Abstract

Introduction: To evaluate clinical value of a new self-sequential longitudinal reference intervals of thyroid function during pregnancy.

Material and methods: We established two different series of reference intervals: self-sequential longitudinal reference intervals (SLRI) and general gestation-specific reference intervals (GSRI). For SLRI, the serum of 301 cases were collected five times in every case throughout the gestation. For GSRI, A total of 1455 subjects included in the study. We collected the serum respectively at various trimesters. We used TSH of both reference intervals to screen 1744 pregnant women, and compared the percentage of potential misclassification.

Results: Both SLRI and GSRI differed substantially from that for non-pregnant women ($p < 0.05$). There are similar fluctuations of serum TSH, FT4 and TPO-Ab during normal pregnancy. Although there were no significant differences in most reference intervals between SLRI and GSRI. But the IQR of SLRI were usually smaller than GSRI , especially in 1st trimester. Two hundred and fifty two women (14.4%) at various trimesters whose serum TSH concentration was within SLRI would be misclassified, while 23 women (1.3%) with a TSH concentration outside limit would not be identified. 0.11-3.84% women would got thyroid diseases during pregnancy. Subclinical hypothyroidism is most common maternal thyroid disorders.

Conclusions: The SLRI can reflected the changes of thyroid function realistically, and can be used to decrease the percentage of potential misclassification of thyroid dysfunction during pregnancy. Screening for thyroid dysfunction of pregnant women is recommended and important.

Key words: pregnancy, thyroid hormones, reference intervals, self-sequential, outcome.

Introduction

It is well known that it is very important to evaluate thyroid function accurately during pregnancy [1-3]. Maternal thyroid dysfunction or only the presence of thyroid-specific antibodies is associated with increased risk for early abortion, preterm delivery, neonatal morbidity [1, 4] and some obstetrical complications [5, 6], such as premature delivery, gestational hypertension, pre-eclampsia, and placental abruption. Benhadi *et al.* have reported that higher maternal TSH levels in pregnancy would increase miscarriage by 60% [7]. Männistö *et al.* found

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that women with thyroid dysfunction and antibodies during pregnancy were more prone to get thyroid disease later [8]. Maternal thyroid function is important not only to the parent but also to the fetus. For example, maternal hypothyroidism, even very mild, can have adverse effects on cognitive and neurological development of the fetus [2]. Both mild and severe maternal hypothyroxinaemia are a higher risk factor for cognitive delay in early childhood [9]. So recently researchers of perinatal medicine reached an agreement that screening thyroid dysfunction of pregnant women is recommended and important.

During a normal pregnancy, great physiological changes in the mother induce complex endocrine and immune responses. At the same time, thyroid gland volume may increase, the thyroid is hyperstimulated, and thyroid hormone concentrations change [10-13]. Recently, researchers recommended the use of gestation-specific reference intervals to evaluate maternal thyroid function. If a non-pregnant reference range is used, many maternal thyroid diseases could be potentially misclassified [14-16]. Some researchers have attempted to establish the gestation-specific reference intervals for a local population, such as Malaysian [16], Chinese [17, 18], Spanish [19], Indian [20], Australian [21], English [22], Czech [23], and so on. However, most studies are based on cross-sectional studies from different women in different stages of gestation. In our last study, we established new self-sequential longitudinal reference intervals based on the same group of pregnant women [24]. The new ranges could reflect the changes of thyroid function in pregnant women more realistically and reduce the variation caused by sampling error from different groups. Maybe it can also give a more accurate value for clinical diagnosis and therapy.

The aim of this study was to evaluate the clinical value of the self-sequential longitudinal reference intervals of thyroid function during pregnancy. First, we established two different series of reference intervals for serum thyroid stimulating hormone (TSH), free thyroxine (FT4) and thyroid peroxidase antibodies (TPO-Ab) during pregnancy: self-sequential longitudinal reference intervals (SLRI) and general gestation-specific reference intervals (GSRI). Then we used both reference intervals to clinically evaluate the status of thyroid function of pregnant women, and observed the percentage of potential misclassification and the relationship with pregnancy outcome. So we can know the advantage of the self-sequential longitudinal reference intervals for the diagnosis of thyroid dysfunction during pregnancy.

Material and methods

Study design

This study was designed and undertaken in the Changzhou Women and Children Health Hospital of Nanjing Medical University, Jiangsu Province, China. The cases of this study comprised the pregnant women who regularly checked in to our hospital for early pregnancy prenatal care from June 2008 to December 2009.

Target population and inclusion criteria

Based on the research of Teng [25], we established selection criteria as follows: 1) no past history of thyroid disease, 2) no life history in the areas of endemic goitre, 3) no other history of autoimmune diseases, 4) no medical history affecting thyroid function, 5) normal urinary iodine, 6) singleton pregnancies, 7) TPO-Ab negative (< 5.0 IU/ml), 8) after follow-up, no hyperemesis gravidarum, pregnancy-induced hypertension, gestational diabetes, premature birth or other adverse maternal-fetal outcomes.

For SLRI, 301 cases were included in the study [24]. For GSRI, a total of 1455 subjects were included in the study: 406 in the 1st trimester, 388 in the 2nd trimester, and 661 in the 3rd trimester were selected to establish general gestation-specific reference intervals. Their age was 26.21 ±6.35 years and the height was 157.4 ±11.2 cm.

Samples collection

For SLRI, samples of every case were collected five times throughout the gestation [24]. For GSRI, we collected the sera respectively from 1455 women at various trimesters of pregnancy. Three ml of blood of all the cases were collected by simple needle aspiration. The samples were centrifuged at 3000 rpm for 5 min to remove cells. The serum was stored at –86°C until assays.

Test

The levels of TSH, FT4 and TPO-Ab were quantified by electrochemistry immunoassay (ECL) using a COBAS e601 automated analyser (Roche Diagnostics, Germany). We observed the dynamic change of thyroid hormone over all gestation. Then we established two reference intervals. All cases were followed by a survey of hospital medical record and telephone interview. The detection range of serum TSH is 0.005-100 mIU/l, and 0.3-100 pmol/l for FT4, 5-600 IU/ml for TPO-Ab.

Clinical evaluation

In the study, we explored initially the value of clinical evaluation and diagnosis. Serum TSH

provides the most sensitive index to reliably detect thyroid function abnormalities [26]. At first, we used TSH of both reference intervals to screen 1744 pregnant women (458 in the 1st trimester, 449 in the 2nd trimester, and 837 in the 3rd trimester), and compared the percentage of potential misclassification. According to their gestational weeks, we selected the best reference intervals as the standard of diagnosis. After following up all cases, we observed the occurrences of pregnancy complication, including pre-eclampsia, hypertensive disorder complicating pregnancy, gestational diabetes mellitus, premature birth, intrahepatic cholestasis of pregnancy, and so on. Then, we analysed the relationship between misclassification and pregnancy outcome.

Statistical analysis

All data were collected and statistically analysed by SPSS 13.0 software. Results of parameters were expressed as median (M), interquartile range (IQR), 2.5th percentile (P2.5) and 97.5th percentile (P97.5). The limits of the reference intervals were calculated as P2.5 to P97.5. Non-parametric tests were employed to compare differences in thyroid hormones (the median level); comparisons among groups with different gestational ages were performed with Kruskal-Wallis test (H test) and comparisons between two different groups were compared with Wilcoxon test. Mann-Whitney U test was used to compare the difference between SLRI and GSRI. Positive ratios were calculated by χ^2-test. A value of p of less than 0.05 was considered to be statistically significant.

Details of ethics approval

The study design and protocol were reviewed and approved by the ethics committee of Changzhou Women and Children Health Hospital affiliated to Nanjing Medical University.

Results

Table I shows the median, P2.5-P97.5, IQR of serum TSH, FT4 and TPO-Ab. There are similar fluctuations of serum TSH, FT4 and TPO-Ab in SLRI and GSRI during normal pregnancy.

Compared with normal non-pregnant women, serum TSH was at a low level during the 1st trimester ($p < 0.05$), and began to rise, but always at a lower level ($p < 0.05$) (Figure 1). The level of FT4 was consistently lower than the non-pregnant group ($p < 0.05$), and tended to a steady state (Figure 2). TPO-Ab rose continuously all over gestation. It was significantly higher in the 3rd trimester ($p < 0.05$) (Figure 3). There were no significant differences in most reference intervals between SLRI and GSRI. But the IQR of SLRI was usually smaller than GSRI, especially in the 1st trimester.

Based on the results of 1744 pregnant women, SLRI had the best clinical specificity among three reference intervals in the 1st trimester and 2nd trimester. Women whose TSH level was outside SLRI would have higher risk of pregnancy complications ($p < 0.01$). The difference of risk was greatest in the 2nd trimester (34.6% vs. 9.5%, $p < 0.001$). For GSRI, it was also satisfied results in the 2nd trimester and 3rd trimester ($p < 0.05$). However, there were no significant differences in non-pregnant reference interval throughout the gestation ($p > 0.05$).

In order to evaluate the value of clinical diagnosis, we investigated the thyroid function of 1744 pregnant women with SLRI. The number and percentage of potentially misclassification if non-pregnant reference intervals were used are summarized in Table II. Two hundred and fifty-two women (14.4%) at various trimesters whose serum TSH concentration was within SLRI would be misclassified, while 23 women (1.3%) with a TSH concentration outside the limit would not be

Table I. Reference intervals of thyroid function in normal pregnant women

Gestation	Group	n	TSH [mIU/l]		FT4 [pmol/l]		TPOAb [IU/ml]	
			$M(P_{2.5}-P_{97.5})$	IQR	$M(P_{2.5}-P_{97.5})$	IQR	$M(P_{2.5}-P_{97.5})$	IQR
1st trimester	SLRI[24]	301	1.00 (0.02-3.65)#	1.18	15.48 (11.85-21.51)#	3.05	6.35 (5.00-19.69)*	4.03
	GSRI	406	1.08 (0.19-3.54)#	1.26	16.04 (12.01-24.62)#	3.06	8.63 (5.00-20.45)#	6.83
2nd trimester	SLRI[24]	301	1.26 (0.36-3.46)#	0.93	12.08 (9.45-16.26)*#	2.12	7.05 (5.00-19.92)*	5.13
	GSRI	388	1.35 (0.38-3.29)#	0.94	12.64 (9.53-16.91)#	2.46	9.57 (5.00-31.05)#	8.38
3rd trimester	SLRI[24]	301	1.50 (0.44-5.04)*#	1.10	12.62 (9.30-17.14)#	2.59	8.63 (5.00-21.96)*#	4.97
	GSRI	661	1.72 (0.51-5.43)#	1.36	12.86 (9.37-17.14)#	2.52	9.71 (5.00-25.68)#	6.95
Non-pregnancy[24]		150	2.10 (0.75-5.28)	1.35	17.71 (14.35-21.69)	2.22	6.78 (5.00-22.42)	2.92

*Compared with GSRI group, p < 0.05, #Compared with non-pregnancy group, p < 0.05
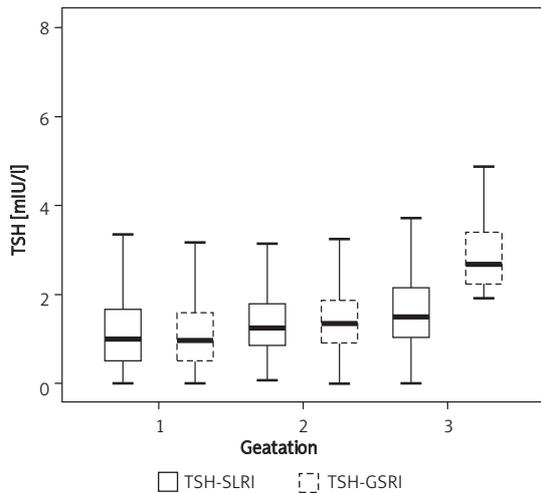
Figure 1. The fluctuations of both reference intervals of TSH in normal pregnancy
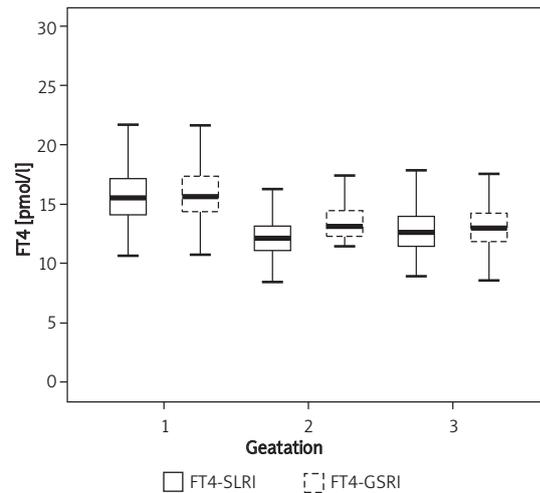

Figure 2. The fluctuations of both reference intervals of FT4 in normal pregnancy
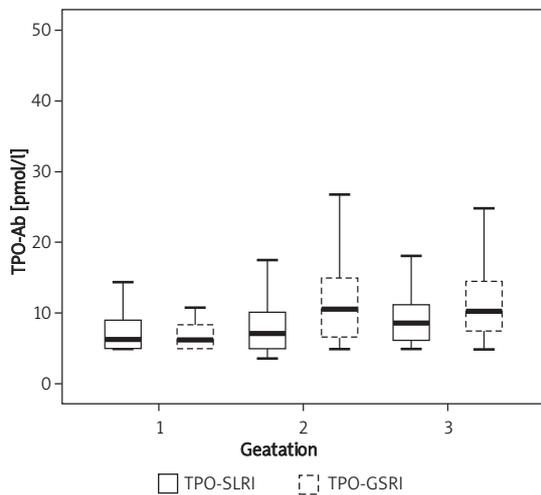

Figure 3. The fluctuations of both reference intervals of TPO-Ab in normal pregnancy

identified. The potential for misclassification of TSH results was greatest in the 1st trimester (31.0%). During the later pregnancy, 34.8% of missed diagnosis cases would have had some pregnancy complications. However, only 9.1% of the misdiagnoses might have occurred. If the GSRI was applied, a total of 40 women (2.3%) would have

been incorrectly classified, and only 3 women (0.1%) would not have been identified. Both SLRI and GSRI were quite consistent in clinical diagnosis.

Table III shows the prevalence of thyroid hormone during pregnancy clinically evaluated with SLRI. 0.11-3.84% of women would have got thyroid diseases during pregnancy, including hypothyroidism, subclinical hypothyroidism, and hyperthyroxinaemia. Subclinical hypothyroidism is the most common maternal thyroid disorder. The positive rate of TPO-Ab was 8.66% throughout the gestation.

Discussion

The basic premise for clinical management of thyroid disease in pregnancy is in accurate laboratory measurement of thyroid hormones. Because of complicated physiological changes during pregnancy, it is urged to establish the normal gestational-specific reference values of thyroid function. Recently, several studies have already established the normal gestational-specific reference intervals of thyroid function [16-23]. However, most of the present reference ranges were based on the cross-sectional survey from different populations. Andersen *et al.* [27] have reported that the intra-individual variation

Table II. Potential for misclassification of TSH in pregnant women using non-pregnant reference intervals

Gestation	N	Misdiagnosis		Missed diagnosis		Overall	
		n (%)	Pregnancy complication, n (%)	n (%)	Pregnancy complication, n (%)	n (%)	Pregnancy complication, n (%)
1st trimester	458	134 (29.3)	13 (9.7)	8 (1.7)	2 (25.0)	142 (31.0)	15 (10.6)
2nd trimester	449	69 (15.4)	4 (5.8)	13 (2.9)	6 (46.2)	82 (18.3)	10 (12.2)
3rd trimester	837	49 (5.9)	6 (12.2)	2 (0.2)	0	51 (6.1)	6 (11.8)
Total	1744	252 (14.4)	23 (9.1)	23 (1.3)	8 (34.8)	275 (15.8)	31 (11.3)

Table III. Prevalence of thyroid disorders during pregnancy using SLRI

Gestation	N	Hypothyroidism	Subclinical hypothyroidism	Hypothyro-xinaemia	Hyperthyro-xinaemia	Subclinical hyperthyro-xinaemia	TPO-Ab positive
1st trimester	458	1 (0.22)	12 (2.62)	6 (1.31)	10 (2.18)	3 (0.66)	31 (6.77)
2nd trimester	449	1 (0.22)	15 (3.34)	7 (1.56)	2 (0.15)	8 (1.78)	51 (11.36)
3rd trimester	837	0	40 (4.78)	17 (2.03)	1 (0.12)	16 (1.91)	69 (8.24)
Total	1744	2 (0.11)	67 (3.84)	30 (1.72)	13 (0.75)	27 (1.55)	151 (8.66)

of thyroid function tests in non-pregnant individuals is smaller than the inter-individual variation. In our study, we established respectively two different series of reference intervals during pregnancy: self-sequential longitudinal reference intervals (SLRI) and general gestation-specific reference intervals (GSRI). Our study showed that both of them differed substantially from that for non-pregnant women. There were no significant differences in most reference intervals between SLRI and GSRI. But the IQR of SLRI was usually smaller than GSRI, especially in the 1st trimester. So we considered that intra-individual variations of thyroid hormones were smaller than inter-individual variations also during pregnancy. Boas *et al.* [28] also drew a similar conclusion. The SLRI could reduce the variation caused by sampling error from different groups and reflected the changes of thyroid function in pregnant women more realistically, resulting in a more important value for clinical diagnosis and therapy.

More and more researchers are aware of the importance of evaluating maternal thyroid function during pregnancy by gestation-specific reference intervals. If a non-pregnant reference interval is used, a number of maternal thyroid diseases could be potentially misclassified. Stricker *et al.* [14] have reported that 5.6-18.3% of misdiagnoses and missed diagnoses likely occur in clinical practice due to the use of the non-pregnant normal population reference values as a basis for diagnosis. The potential for misclassification was greatest in the first trimester (10.4%) for TSH [14]. In China, the percentage of potentially misclassified cases of subclinical hypothyroidism and hypothyroxinaemia in pregnant women was decreased by using the gestational age-specific reference intervals [15]. Similar results were obtained in Malaysia [16] and Australia [21]. As a single hormone determination, serum TSH provides the most sensitive index to reliably detect thyroid function abnormalities [26]. So we explored the clinical value of TSH of SLRI at first. We investigated the status of thyroid hormone by screening 1744 pregnant women with three kinds of reference intervals: SLRI, GSRI and non-pregnant reference intervals. After comparing the results and pregnancy outcome, we found that SLRI had the best clinical specificity among the three reference

intervals. Women whose TSH level was outside SLRI would have higher risk of pregnancy complications, especially in the 1st trimester and 2nd trimester. Applying the non-pregnant reference intervals to pregnant women results in misclassification of thyroid status for 6.1-31.0% of women. Use of non-pregnant reference intervals has the potential to result in a large number of misclassified results. The SLRI can be used to decrease the percentage of potential misclassification of thyroid dysfunction.

Thyroid disorders are common in pregnancy and affect maternal and fetal outcome. Our data showed that 0.11-3.90% of women would have got thyroid diseases during pregnancy. Subclinical hypothyroidism is the most common maternal thyroid disorder. Some studies have reported that thyroid insufficiency may be associated with adverse obstetric outcome and fetal neurodevelopmental deficits [1, 29]. So it is important to find thyroid hormone deficiency during the early stage of pregnancy. At the same time, TPO-Ab is used to predict some autoimmune thyroid diseases, such as post-partum thyroiditis [30]. Higher TPO-Ab level during pregnancy is also a marker of the danger of the development of postpartum thyroiditis [31]; about 50% of TPO-Ab positive women will get thyroid dysfunction after delivery [30-32]. In the present study, TPO-Ab was detectable in 8.66% of pregnant women. Evaluating the TPO-Ab levels may be helpful in later pregnancy. Overall, we think screening for thyroid dysfunction of pregnant women is recommended and important.

Acknowledgments

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