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Reference Range for Thyroid Function during Twin Pregnancies

ZHANG Chao，LONG Yan，FU Xina*

【**Abstract**】 **Background** The correct reference range for maternal thyroid function during pregnancy is essential for making an accurate diagnosis of thyroid disease and delivering proper interventions in pregnant women. But there is still no universal standard for this in women with a twin pregnancy. **Objective** To determine a rational reference range for maternal thyroid function during twin pregnancies. **Methods** Healthy pregnant women who underwent an antenatal examination in Obstetric Clinic，Beijing Friendship Hospital，Capital Medical University from January 2009 to September 2019 were retrospectively selected，including 352 with a twin pregnancy（twin group），and 988 with a singleton pregnancy（singleton group）. Clinical and laboratory data were collected. The lower and upper limits for determining normal maternal thyroid function during twin pregnancies were the 2.5（P2.5）and 97.5 （P97.5） percentiles of TSH and FT4. Clinical hyperthyroidism was defined as TSH<P2.5（total TSH）and FT4>P97.5（total FT4）. Clinical hypothyroidism was defined as TSH>P97.5（total TSH） and FT4<P2.5（total FT4）. Subclinical hypothyroidism was diagnosed by TSH>P97.5 and P2.5 ≤ FT4 ≤ P97.5. Low T4 syndrome was diagnosed by P2.5（total TSH） ≤ TSH ≤ P97.5 （total TSH） and FT4<P2.5 （total FT4）. FT4 and TSH levels in the 1st，2nd, and 3rd trimester were compared between singleton and twin groups. The prevalence of thyroid function abnormalities in the 1st，2nd, and 3rd trimester in the twin group was recorded and analyzed. **Results** Three hundred and fifty-two pregnant women with a twin pregnancy and 988 with a singleton pregnancy were finally included. The average FT4 level in the twin group was higher than that of the singleton group regardless of the stage of pregnancy（P<0.05）. The average TSH level in the twin group was lower in 1st trimesterbut was higher in 3rd trimester compared with that of the singleton group（P<0.05）. For maternal thyroid function during a twin pregnancy，the determined normal FT4 in the early，2nd and 3rd trimester expressed as median and interquartile range M（P2.5，P97.5） was 〔11.84（7.95，26.73）〕，〔8.24（5.53，18.58）〕，〔8.37（5.80，15.79）〕pmol/L，respectively，and the determined normal TSH in the three stages of pregnancy was〔0.67（0.03，3.99）〕，〔1.44（0.06，4.79）〕，〔2.43（0.41，6.92）〕mU/L，respectively. In the twin group，the prevalence of clinical hyperthyroidism，clinical hypothyroidism，subclinical hypothyroidism，and low T4 syndrome was 0，0.28% （1/352），4.83% （17/352）and 3.98% （14/352），respectively，by the above-mentioned criteria for diagnosing thyroid disease in a twin

pregnancy，and that of the four diseases was 8.24% （29/352），0，15.91% （56/352） and 1.99% （7/352），respectively，by the criteria for diagnosing thyroid disease in a singleton pregnancy. **Conclusion** In this study, the recommended reference ranges of FT4 in the 1st, 2nd, and 3rd stages of pregnancy were 7.95-26.73, 5.53-18.58, and 5.80-15.79 pmol/L, respectively, and the reference ranges of TSH were 0.03-3.99, 0.06-4.79 and 0.41-6.92 mU/L, respectively. Based on the FT4 and TSH standards of the pregnant women with twin pregnancies obtained in our laboratory as the reference standards, the incidence of thyroid dysfunction detected in the pregnant women with twin pregnancies is low, which is consistent with relevant literature reports. The FT4 and TSH standard range of single pregnancies obtained in our laboratory may lead to overdiagnosis of clinical hyperthyroidism and subclinical hypothyroidism in pregnant women of twin pregnancies. So it is necessary to establish specific reference intervals for pregnant women with twin pregnancies based on the FT4and TSH standard ranges obtained in our laboratory.

【Key words】 Thyroid diseases；Pregnancy，twin；Thyrotropin；Free thyroxine；Reference values

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Pregnancy combined with thyroid dysfunction may cause abortion, premature delivery, placental abruption, and other adverse pregnancy outcomes[1], affect the development of the fetal nervous system, and lead to the intellectual and physical development of the offspring[2]. The thyroid function of women in a twin pregnancy is affected by placental hormone secretion. The thyroid stimulating hormone (TSH) level of women in a twin pregnancy is lower than that of women in a singleton pregnancy, while the free thyroxine (FT4) level is higher than that of women in a singleton pregnancy[3]. If the laboratory tests for singleton pregnancies are used to determine the thyroid function of women with twin pregnancies, it may lead to misdiagnosis and missed diagnosis of thyroid disease during pregnancy. Therefore, it is necessary to establish diagnostic criteria for thyroid function in women with twin pregnancies. In this study, thyroid function and thyroid peroxidase antibody (TPOAb) were detected in healthy pregnant women with twins during pregnancy, to analyze the changing characteristics of thyroid function during pregnancy, establish the specific reference range of thyroid function indicators, and explore the prevalence of thyroid dysfunction in twin pregnancy, aiming to provide a reference for the clinical diagnosis and treatment of thyroid diseases in pregnant women with twins during pregnancy.

1 Data and methods

1.1 General data Healthy pregnant women with twin pregnancies who underwent an antenatal examination in the

Obstetric Clinic of Beijing Friendship Hospital Affiliated to Capital Medical University from January 2009 to September 2019 were selected retrospectively as the twin group. Twin Pregnancy Healthy Pregnant Women Subject to National Institute of Clinical Biochemistry(National Academy of Clinical Biochemistry, NACB) recommends the conditions of "standard population" during pregnancy[3], including (1) one or more thyroid function screening during pregnancy; (2)TPOAb levels are within the reference range provided by the manufacturer of the test reagent; (3)Age 20~40 years old; (4) Twin pregnancy is confirmed through ultrasound for the first time in the 6–9 weeks of gestation until the delivery of double live births. Exclusion criteria: A history of thyroid disease (thyromegaly, hyperthyroidism or hypothyroidism, thyroid cancer, thyroid surgery or radioiodine treatment history) and a family history of thyroid disease; Taking medicines for treating thyroid diseases during pregnancy; Using medications that may affect thyroid function (such as anticonvulsants, antipsychotics, glucocorticoids, dopamine, etc.); History of heart disease, chronic hypertension, diabetes, asthma, inflammatory bowel disease, cancer, kidney disease, liver disease, autoimmune disease, connective tissue disease, and other chronic diseases; History of blood-borne diseases; Pregnancy-related complications (including gestational hypertension, gestational diabetes mellitus, premature birth or other adverse pregnancy outcomes, gestational trophoblastic disease, or late pregnancy bleeding); Chromosomal abnormalities or genetic syndromes; Post-assisted reproductive surgery; History of repeated abortions; Fetal chromosome or genetic abnormalities, etc.The retrospective study was conducted at Beijing Friendship Hospital Affiliated to Capital Medical University during the same period. Healthy pregnant women with single pregnancy who underwent an antenatal examination in the obstetrics outpatient department were taken as the singleton pregnancies group.Inclusion criteria: (1)Age: 20–40 years old; (2)The pregnancy outcome is good; (3)Singleton pregnanciesare confirmed for the first time through ultrasound after 6–9 weeks of gestation; (4)Screen for thyroid function once or more times. Exclusion criteria were the same as those in the twin group.

This study was approved by the Ethics Committee of Beijing Friendship Hospital Affiliated to Capital Medical University（2001-1002-04）.

1.2 Research methods

1.2.1 Data collection The age, height, body weight, pre-pregnancy body mass index (BMI), and whether was primiparityof the pregnant women were recorded.

1.2.2 Laboratory examination indexes 3 ml fasting elbow venous blood of pregnant women in the early morning

was collected and sent to the clinical laboratory of our hospital for detection. The chemiluminescence methodis adopted on the same day.

Thyroid function indicators (including serum TSH, FT4, and TPOAb levels) were determined by photoacoustic immunoassay. TSH and FT4 were determined using the UnicelDxl 800 immunoassay system and kit from Beckman, the US. TPOAb levels were determined using the ADVID Centaur immunoassay analyzer and kit from Siemens, Germany. The inter-batch and intra-batch variations of each indicator were all < 6%. The reference range of TPOAb provided by the manufacturer is 0–60 and 0–1 300 mu/L (the reference range of TPOAb provided by the manufacturer of the former test kit in 2017 is 0–60 mu/L; Kit replaced in 2017, the reference range provided by the manufacturer is 0–1 300 MU/L).

Determination of urine iodine: 5 ml urine samples of pregnant women in two groups were collected in the morning and determined with the hydrogen peroxide tetramethylbenzidine oxidation color method. The pregnant women were asked to avoid consuming foods with high iodine content such as kelp and laver one day before the urine sample was taken, and the results were measured continuously for three days and the average value was recorded. The reference range of urine iodine for pregnant women was 100–300 g/L.

1.2.3 Diagnostic criteria In this study, the 2.5th percentile (P2.5) and 97.5th percentile (P97.5) were used as the lower and upper limits of TSH and FT4 reference ranges to determine the reference interval of thyroid function during pregnancy. According to the pregnancy cycle at the time of detection, 1sttrimester: less than 12+6 weeks, 2nd trimester: 13+0~27+6 weeks, and 3rd trimester: 28+0 weeks to delivery. If TSH<P2.5 (total TSH) and FT4>P97.5 (total FT4), it is diagnosed as clinical hyperthyroidism. TSH>P97.5 (total TSH), FT4<P2.5 (total FT4) is diagnosed as clinical hypothyroidism. TSH>P97.5 (total TSH), P2.5 (total FT4) ≤ FT4 ≤ P97.5 (total FT4) was diagnosed as subclinical hypothyroidism. P2.5 (total TSH)≤ TSH ≤ P97.5 (total TSH), FT4<P2.5 (total FT4) was diagnosed as clinical hypothyroidism (T4). TPOAb positivity is defined asTPOAb> 60 mu/l (before 2017) and TPOAb> 1 300 mu/l (after 2017).

1.3 Statistical analysis Statistical analysis was performed using SPSS 24.0 statistical software. The reference range was determined using 95%CI, the normality test using the Kolmogorov-Smirnov method, and the measurement data subject to normal distribution were expressed as (x±s). The comparison between the two groups was performed using a group t-test. Measurement data of non-normal distribution were expressed as M(P2.5, P97.5), and the rank

sum test was used for comparison between groups. The count data were expressed as the relative number, and the comparison between groups was performed using the $\chi 2$ test. $P<0.05$ was considered as the difference with statistical significance.

2 Results

2.1 Basic information In the twin pregnancy group, 6 cases with a history of thyroid disease were excluded, 20 cases were treated with drugs for thyroid disease during pregnancy, 39 cases had a TPOAb level higher than the upper limit of the reference range, and 69 cases had other disease history and no data on thyroid function test. Finally, 352 pregnant women were included. 988 pregnant women were finally included in the single fetus group. There was no significant difference in age, urine iodine, BMI before pregnancy, and the proportion of primiparous women between the singleton group and the twin group ($P>0.05$), as shown in Table 1.

Table 1 Comparison of general data between the singleton and twin groups

Group	Cases	Age(years)	Urinary iodine（μg/L）	Pre-pregnancyBMI (kg/m^2)	Primipara〔n（%）〕
twin	352	32.0±4.6	175.5±6.88	22.1±2.5	304（86.36）
singleton	988	30.6±3.6	179.03±8.36	219±3.4	879（89.00）
t（X^2）value		0.983	-0.451	0.052	0.478[a]
P value		0.341	0.635	0.933	0.655

Note: [a] represents $\chi 2$ value; BMI= body mass index

2.2 Comparison of TSH and FT4 between singleton group and twin group in different pregnancy cycles TSH of pregnant women in the twin group was lower in 1sttrimester than that in the singleton group, and higher in 3rd trimester than that in the singleton group ($P < 0.05$). There was no significant difference in TSH between the two groups in the 2nd trimester of pregnancy ($P > 0.05$). In the 1st, 2nd, and 3rd trimesters of pregnancy, FT4 in the twin group was higher than that in the singleton group, and the difference was statistically significant ($P<0.05$), as shown in Table 2.

Table 2 Comparison of fT4 and TSH levels in the first, second, and third trimesters of pregnancy between the singleton and twin groups

Group	fT4（pmol/L）						TSH（mU/L）					
	Cases	1st trimester [a]	Cases	2nd trimester	Cases	3rd trimester	Cases	1st trimester	Cases	2nd trimester	Cases	3rd trimester
Twin	217	11.84（7.95,26.73）	344	8.24（5.53，18.58）	177	8.37（5.80，15.79）	217	0.67（0.03，3.99）	344	1.44（0.06，4.79）	177	2.43（0.41，6.92）
Singleton	988	10.68（7.20，18.66）	988	7.98（5.53，12.74）	988	7.72（5.40，10.40）	988	1.12（0.03，3.67）	988	1.60（0.60，3.74）	988	2.33（0.58，4.45）
Z value		2.195		2.201		2.878		-2.973		-1.893		2.365
P value		0.034		0.031		0.018		0.003		0.068		0.021

Note: a indicates missing data; TSH= thyroid-stimulating hormone, fT4= free thyroxine

2.3 Distribution of thyroid dysfunction in twin pregnant women in different pregnancy periods P2.5 and P97.5 of the overall data of TSH and FT4 in both twin and singleton pregnancy were used as lower and upper limits, respectively, and the prevalence of thyroid dysfunction in the first, second and third trimesters of twin pregnant women was counted. The prevalence rate and cumulative prevalence rate of thyroid dysfunction in twin pregnant women in different pregnancy periods are shown in table 3~6.

3 Discussion

3.1 Physiological changes of thyroid function during pregnancy characteristics The maternal thyroid function during pregnancy undergoes complex changes under the influence of hormones secreted by the placenta, hypothalamus, and pituitary, mainly manifested in the following aspects:(1) The placenta secretes a large amount of estrogen, which stimulates the liver to synthesize globulin, and the serum thyroid-binding globulin is significantly increased, increasing serum total thyroxine (TT4) in the 1st trimester, which can be up to 1.5 times that of non-pregnant women; (2) human chorionic gonadotrophin (hCG) secreted by the placenta can inhibit the secretion of TSH by the pituitary gland[4], and TSH drops to the lowest in the 8–10 weeks of pregnancy, and gradually returns to normal after 12 weeks of pregnancy[3]. (3)FT4 is generally within the reference range, and it slightly increases in the 1st trimester of pregnancy and decreases in the 2nd trimester of pregnancy[2]. In this study, 352 pregnant women with twin pregnancies were tested for thyroid function during the whole pregnancy, and the results showed that the changes in TSH and FT4 were consistent with the above characteristics.

3.2 Establishment of the reference range for thyroid function during pregnancy There has been controversy over the reference range for thyroid function during pregnancy in academic circles for many years. As more evidence-based medical evidence has been provided by relevant clinical studies, the standards for the establishment of a reference range for thyroid function during pregnancy have been continuously revised by relevant international endocrine academic groups. In 2007, the Endocrine Society (TES) recommended the reference range of TSH to be ≤ 2.5 μ U/L in the 1sttrimester of pregnancy, ≤ 3.0 μ U/L in the 2ndtrimester of pregnancy, and ≤ 3.0 μ U/L in the 3rd trimester of pregnancy[5]. It is recommended in the 2011 guidelines issued by the American Association of Clinical Endocrinologists (AACE) and the 2017 guidelines issued by the American Thyroid Association (ATA). The pregnancy-specific TSH reference range should be defined as follows: the pregnancy-specific TSH reference range, if possible, should be determined by the laboratory conducting the study (high-quality evidence) based on optimal iodine intake in healthy TPOAb-negative pregnant women and data from pregnant women without thyroid disease[1]. When this goal is not achieved, pregnancy-specific TSH reference ranges should be obtained from similar patient populations and similar TSH assays should be used. If there is no internal or loanable pregnancy-specific TSH reference range, 4.0 μ U/L can be used as the upper limit of the reference range in the first and second trimesters of pregnancy[6]. At present, there are few high-quality large-scale studies on the reference range of thyroid function in twin pregnancy in China, and the reference range of twin pregnancy is not mentioned in the domestic guidelines[6]. Therefore, we conducted relevant studies on pregnant women with twin pregnancies in our hospital to obtain a reasonable reference range of thyroid function in pregnant women with twin pregnancies.

After the trophoblasts began to secrete hCG on the sixth day of pregnancy, serum hCG level increased exponentially, peaked at 8–10 weeks of pregnancy, and then decreased rapidly after about 10 days. In the 2nd and 3rd trimester, serum hCG level was only 10% of its peak[7]. The peak values of both intact and free β-hCG were significantly increased and persisted for an extended period during the 8th to 16th weeks of gestation in both fetuses compared with singleton pregnancies; Generally, for every 10 000 U/L increase in serum hCG level, the serum TSH level will decrease by 0.1 μ U/L, and there is a correlation between the two during the whole gestation period[8]. Since a sharp and persistent increase in hCG may result in a decrease in TSH level, studies have found that a significant decrease in TSH level in pregnant women with twins is three times that in pregnant women with a single fetus[3]. These findings explain why median TSH is lower in women with twin pregnancies than in women with single pregnancies. In clinical practice, the use of reference ranges for single fetuses to assess thyroid function in twin

pregnancies may lead to an overdiagnosis of clinical hyperthyroidism and subclinical hypothyroidism. In addition, improper antithyroid drug treatment may lead to leukopenia and hepatic dysfunction in pregnant women as well as malformations in the offspring.

Therefore, it is necessary to establish a specific reference range for the evaluation of thyroid function in pregnant women with twin pregnancies[6]. The results of this study showed that the median TSH of pregnant women in the first trimester of twin pregnancies was significantly lower than that of pregnant women in the first trimester of a singleton pregnancy, which was consistent with the relevant literature reports[3].

3.3 Assessment of the prevalence of thyroid dysfunction during pregnancy According to the reference interval of thyroid function indicators during twin pregnancy, the prevalence of clinical hyperthyroidism during pregnancy in twin pregnancy was 0 in our hospital. Some studies reported the prevalence of clinical hyperthyroidism during pregnancy in twin pregnancies to be 0.02%–0.50%[2,6]. The results of this study were lower than those reported in relevant studies; In this study, the prevalence rate of clinical hypothyroidism during pregnancy in twin pregnancy was 0.28%, and the prevalence rate of clinical hypothyroidism during pregnancy in the United States was 0.3%–0.5%[2]. In the 2019 Guideline for Diagnosis and Treatment of Thyroid Diseases during Pregnancy and Postpartum (Version 2)[6], it was reported that the prevalence rate of clinical hypothyroidism during pregnancy in China was 1.0%. The results of this study were similar to the above results. In this study, the prevalence rate of subclinical hypothyroidism in pregnant women with twins was 4.83%, while some studies reported the prevalence rate of subclinical hypothyroidism in pregnant women to be 2%–3%[2]. The results of this study were slightly higher than the research results mentioned above. According to the reference interval of thyroid function in singleton pregnancies, the prevalence of clinical hyperthyroidism in the pregnancy of twins in our hospital was 8.24%, which was significantly higher than that in the literature.

It has been reported that the prevalence rate of clinical hypothyroidism is 0, and the cumulative prevalence rate of subclinical hypothyroidism is 15.91%, which is significantly higher than that reported in the literature. If 4.0 mU/L is used as the upper limit of the TSH reference interval, the total prevalence of clinical hypothyroidism and subclinical hypothyroidism is 11.36%(40/352), which is significantly higher than that reported in the literature[2]. It can be seen that whether the single-fetus reference interval or the upper limit of TSH 4.0 mU/L as the reference interval is used as the standard for the diagnosis of thyroid function in pregnant women with twin pregnancies, it will lead to an

increase in the number of pregnant women with subclinical hypothyroidism, which will lead to the over-diagnosis in some pregnant women, and increase the ideological burden on pregnant women, so that pregnant women may receive unnecessary drug treatment. In addition, differences in results may arise from different analytical methods used in different laboratory tests, and the international standardization of analytical methods will help to reduce such differences[9].

3.4 Potential Bias Due to the limited number of cases of twin pregnancy pregnant women in this study and the existence of certain missing data, which may cause certain biases, further and larger researches are needed.

To sum up, FT4 in the 1st, 2nd, and 3rd trimesters of twin pregnancies are higher than those in the singleton pregnancies, while TSH is different. TSH in the 1st trimester of pregnancy is lower than that in singleton pregnancies. TSH level increases along with the growth of the pregnancy cycle. In the 3rd trimester of pregnancy, TSH in twin pregnancies is higher than that in singleton pregnancies. With the reference range of specific thyroid function indicators of twin pregnancy as the standard, subclinical hypothyroidism is the most common thyroid function indicator of twin pregnancy. If the reference range of singleton pregnancies is used as the evaluation standard, it will cause thyroid function abnormalities of twin pregnancy pregnant women, especially the excessive diagnosis of clinical hyperthyroidism and subclinical hypothyroidism. Therefore, it is recommended that a qualified laboratory establish the specific reference range of thyroid function indicators of twin pregnancy in our laboratory.

Author's contributions: Zhang Chao was responsible for data collection, paper drafting, statistical analysis, and chart drawing, and was responsible for the revision of the final version of the paper as a whole. Long Yan is responsible for the investigation and design of the study; Fu Xina assists with data collection.

There is no conflict of interest in this article.

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