Establishment of reference range for thyroid hormones in normal pregnant women in China's coastal area

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Summary

Purpose: The current study aims to establish reference ranges for thyroid hormones in normal pregnant women during their pregnancy period. *Materials and Methods:* A one-time cross-sectional survey was conducted on 490 normal pregnant women and 51 non-pregnant women (control). The serum thyroid stimulating hormone (TSH), free triiodothyronine (FT3), and free tetraiodothyronine (FT4) levels were measured. *Results:* The serum FT3 and FT4 levels in pregnant women decreased gradually from the first to the last three months of pregnancy ($p < 0.01$). The serum TSH level increased gradually during the whole pregnancy ($p < 0.01$), and was significantly lower than the control ($p < 0.01$) in the first three months. However, in the middle and last three months of pregnancy, TSH was higher than the control ($p < 0.01$). *Conclusions:* The thyroid hormone levels in normal pregnant women are different from those in non-pregnant women; significant differences exist among the three stages of pregnancy.

Key words: Pregnancy; Stage of pregnancy; Thyroid hormone; Reference range.

Introduction

Thyroid diseases, such as hypothyroidism and hyperthyroidism, are the most common endocrine diseases in women of childbearing age, with an incidence from one to two percent in pregnant women [1]. In the past ten years, studies on thyroid diseases and pregnancy have developed rapidly. The studies focused on the optimized treatment of pregnant women with thyroid diseases, effects of maternal thyroid diseases, and therapeutic strategies on fetal development and pregnancy outcome. Pregnancy causes great changes in thyroid function; maternal thyroid diseases adversely affects pregnancy and fetal growth [2]. Therefore, the proper management of gestational thyroid diseases, which depends on the accurate measurement of thyroid hormone levels for the establishment of proper therapy strategies, can ensure a normal pregnancy and avoid adverse pregnancy outcomes.

The clinical assessment of thyroid function is more difficult for pregnant women because of their high metabolic state. The physiological changes in pregnancy (increase of plasma volume and thyroid binding globulin amount), relative lack of iodine, and difference of iodine nutritional status in different areas implies that the generally uniform reference ranges of thyroid hormones for non-pregnant women are not suitable for pregnant women. During pregnancy, the free triiodothyronine (FT3) and free tetraiodothyronine (FT4) levels gradually decrease, and the thyroid stimulating hormone (TSH) level gradually increases. Therefore, using the normal serum TSH level in non-pregnant women as reference may misdiagnose normal pregnant women with reduced TSH to have hyperthyroidism. Likewise, pregnant women with subclinical hypothyroidism, of whom the TSH level is mildly elevated but less than the high limit of reference range, may be misdiagnosed as normal, which is not an advisable approach to the management of thyroid diseases during pregnancy.

The current evaluation of thyroid function during pregnancy is performed following the manufacturer-provided reference values for non-pregnant women. Studies on reference ranges for thyroid hormones during pregnancy have been reported; however, these established reference ranges are unreliable because the research subjects were from areas with different iodine nutritional status, the sample size was small, the data were restricted to certain three months in pregnancy, and the statistical method used was inappropriate. Mean ± 2SD is used for calculating reference ranges. This study avoids the methodological defects on reports about reference ranges for thyroid hormones during pregnancy using larger sample size and more precise cross-sectional data during different stages of pregnancy. Moreover, the subjects in this study were from the same area (Quanzhou, China), in which there was no report about established reference ranges for thyroid hormones in pregnancy.

In the current study, a one-time cross-sectional survey was conducted on pregnant women in China's coastal area (Quanzhou, China), with appropriate urinary iodine level (100 g/l to 200 g/l) in the population. This study aims to establish specific reference ranges for thyroid hormones in the first, middle, and last three months of pregnancy. The result would be helpful for objective and accurate evaluation of changes in thyroid function for pregnant women in this area, and correct establishment of prevention and treatment measures to ensure normal pregnancy.

Revised manuscript accepted for publication December 27, 2012

Materials and Methods

Exactly 490 healthy pregnant women (22- to 36-years-old), who were admitted from October 2010 to December 2011 in the maternal and child health hospitals in Shishi, Dehua, and Anxi (Quanzhou, China), as well as in the Second Affiliated Hospital of Fujian Medical University (Quanzhou, China), were enrolled in this study. There were 122 cases in first three months of pregnancy (four to 12 weeks), 240 cases in middle three months (13 to 27 weeks), and 128 cases in last three months (≥28 weeks). Pregnancy was confirmed via ultrasound scanning and pregnancy test in the first three months. The cases with hyperemesis gravidarum, multiple pregnancy, thyroid diseases and other endocrine diseases before pregnancy, preeclampsia, and administration of drugs affecting thyroid function were excluded. Fifty-one healthy non-pregnant women (25- to 42-years-old) in the same area were selected as controls. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Shishi (Overseas Chinese) Hospital. Written informed consent was also obtained from all participants.

Approximately five ml of fasting venous blood was drawn and centrifuged and the serum was separated and stored at -70°C for future testing. The serum FT3, FT4, TSH, thyroid peroxide enzyme antibody (TPOAb), and human chorionic gonadotropin (hCG) levels were measured. The manufacturer-provided reference ranges for non-pregnant women (manufacturer's value) were attached to each test results. The gestational week of blood sample collection was also recorded.

Inter-laboratory quality control was performed in this study. The thyroid hormone concentrations of each sample were detected. The samples were mixed to obtain the high and low quality control samples (QC) for every 100 samples. The expected and measured values of QC and the manufacturer's values are shown in Table 1. The instant method (Grubbs method) [3] was used for the assessment of inter-laboratory quality control (five batches). According to the quality control international system (SI) value table (n = 5: n2s = 1.67, n3s=1.75), the low and high limits of SI were less than 2s, indicating a controllable range. In addition, the measured values were within the range of the expected values (X ± 2S), suggesting that the random error of this batch of sample was small and the data were reliable.

Table 1. — *Expected values, measured values, and SI values of quality control samples.*

Index		FT3 (pmol/l)	FT4 (pmol/l)	TSH (mIU/l)
n		5	5	5
QC_{high}	Expected value	10.97±2.9	35.54±6.95	40.3±36.8
	Measured value	12.29±0.37	34.87±1.54	36.65±1.69
QC_{low}	Expected value	4.21±2.23	11.06±6.97	1.38±0.87
	Measured value	3.9±0.01	13.5±1.07	1.82±0.06
	CV_{high}	3.08	4.42	4.55
	CV_{low}	0.2	7.8	3.13
SI (QC_{high})	High limit	1.349	1.376	0.79
	Low limit	1.2	1.271	1.613
SI (QC_{low})	High limit	1.0	1.5657	0.8032
	Low limit	1.0	1.1438	1.6413

Nadults)ote: $SI_{high\ limit} = (X_{max} - Mean)/S$, $SI_{low\ limit} = (Mean - X_{min})/S$.

Table 2. — *Reference ranges for thyroid hormones of pregnancy, control, and manufacturers's values (percentile).*

Index	Group	n	2.5th	50th	97.5th	Manufacturers's Values
FT3 (pmol/l)	First 3 months	122	3.75	4.59	7.23	3.5-6.5 (95% confidence interval)
	Middle 3 months	240	3.31	4.06	4.93	
	Last 3 months	128	3.16	3.87	4.48	
	Control	51	3.82	4.66	5.61	
FT4 (pmol/l)	First 3 months	122	12.85	18.07	25.3	11.5-22.7
	Middle 3 months	240	12.03	16.68	20.14	
	Last 3 months	128	11.02	15.89	19.43	
	Control	51	11.72	16.05	20.61	
TSH (mIU/l)	First 3 months	122	0.01	1.08	3.79	0.55-4.78 (2.5^{th} and 97.5^{th} percentile, adults)
	Middle 3 months	240	1.09	2.13	4.17	
	Last 3 months	128	1.08	2.39	5.95	
	Control	51	0.62	1.68	3.74	

Statistical analysis

Statistical analysis was performed using SPSS 16.0. Histogram generation and tests of data normality and difference among groups were performed. The ranges of middle 95% (2.5^{th}, 50^{th}, and 97.5^{th} percentiles) for FT3, FT4, and TSH in the three stages of pregnancy were calculated as references. A correlation analysis on the changes of thyroid hormones and hCG was also performed. The thyroid hormone levels of first, middle, and last three months of pregnancy, as well as the control and manufacturer's value, were compared; the differences between the pregnant and non-pregnant patients were analyzed.

The serum FT3 and FT4 levels were calculated from the corresponding mean values. A normal distribution of FT3 and FT4 was observed in the middle and last three months of pregnancy as well as in the control ($p > 0.05$), with normal distribution for FT4 and positively skewed distribution for FT3 in first three months, respectively ($p < 0.05$). The serum TSH level was also calculated from the median. A positively skewed distribution of TSH was noted in each stages of pregnancy ($p < 0.05$). The histograms of thyroid hormones are shown in Figure 1. The homogeneity of variance test showed a heterogeneity of variance for each group (FFT3 = 11.669, $p < 0.01$; FFT4 = 6.064, $p < 0.01$; FTSH = 19.853, $p < 0.01$). Therefore, the Kruskal-Wallis test was used for compare the overall difference among different groups; the Dunnett's T3 test was used for multiple comparisons. Bilateral $p < 0.05$ was considered as statistically significant.

Results

Reference ranges of thyroid hormones in different stages of pregnancy

The 2.5^{th} and 97.5^{th} percentiles of serum FT3, FT4, and TSH concentrations are shown in Table 2. In the first three months of pregnancy, the low and high limits of TSH were 0.01 mIU/l and 3.79 mIU/l, respectively. These values were the lowest among the three stages of pregnancy; lower than the manufacturer's values. The low limit of TSH was lower and the high limit was almost the same compared with the control. During the middle and last three months of pregnancy, the low limit of TSH was higher than the manufacturer's value and the control, respectively. The high limit of TSH during the middle three months was lower than the manufacturer's value, but higher than the control. However, the high limit of TSH during the last three months was higher than the manufacturer's value and control.

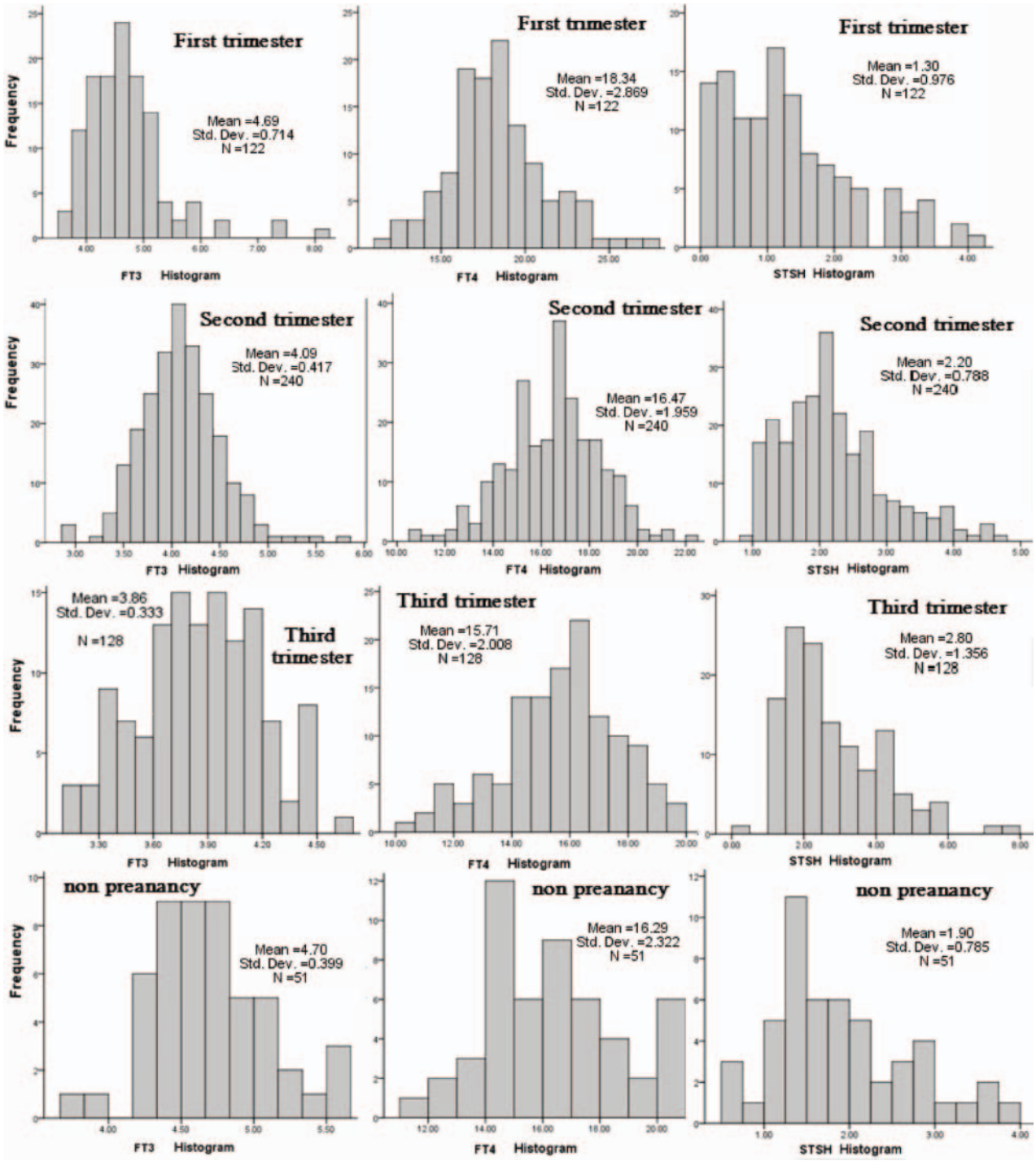

Figure 1. — Histograms of FT3, FT4, and TSH in pregnancy and non-pregnancy.

During the first three months of pregnancy, the low and high limits of FT3 were 3.75 pmol/l and 7.23 pmol/l, respectively, which were both higher than the manufacturer's values. However, the low and high limits during the middle and last three months were lower than the manufacturer's values. In addition, the low limit at each stage of pregnancy was lower than the control. The high limit during the first three months was higher than the control, whereas that during the middle and last three months was lower than the control.

The low and high limits of FT4 during the first three months of pregnancy were higher than the manufacturer's values. The low limit during the middle three months was

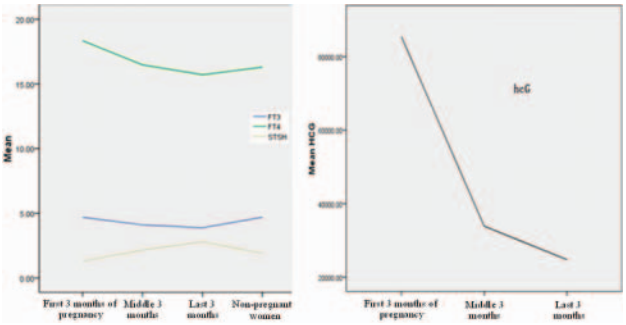

Figure 2. — Variations of thyroid hormones and hCG levels during different stages of pregnancy.

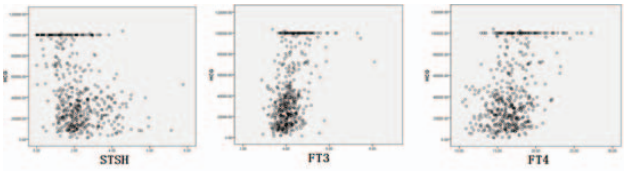

Figure 3. — Scatter diagrams of hCG with thyroid hormones.

Table 3. — *Thyroid hormones, TPOAb, and hCG levels in different stages of pregnancy (Mean ± SD).*

Group	n	FT3 (pmol/l)	FT4 (pmol/l)	TSH (mIU/l)	TPOAb	hCG
First 3 months	122	4.69 ± 0.71	18.34 ± 2.87	1.08	48.45	100000
Middle 3 months	240	4.09 ± 0.42	16.47 ± 1.96	2.13	36.15	28305
Last 3 months	128	3.86 ± 0.33	15.71 ± 2.01	2.39	37.70	20559
Control	51	4.69 ± 0.39	16.29 ± 2.32	1.89 ± 0.78	47.08 ± 13.7	
p		< 0.05	< 0.05	< 0.05	< 0.05	

higher than the manufacturer's value, whereas that during the last three months was slightly lower than the manufacturer's value. The high limit during the middle and last three months was lower than the manufacturer's value. During the first three months of pregnancy, the low and high limits were higher than the control. The low limit during the middle three months was higher than the control, but lower than the high limit. The low and high limits during the last three months were lower than the control. If the FT4, FT3, and TSH levels during the first three months and the TSH level during the last three months were evaluated according to manufacturer's values and control, the thyroid function of pregnant women may be misdiagnosed.

Variations of thyroid hormones and hCG levels in different stages of pregnancy

As shown in Figure 2, the serum FT3, FT4, and hCG levels decreased gradually throughout the entire pregnancy. During the first three months of pregnancy, the serum FT3 level was almost similar to the control; the FT4 level significantly higher. During the middle and last three months, the serum FT3 level was significantly lower than the control, whereas the FT4 level was almost similar or slightly lower. During pregnancy, the serum TSH level increased gradually, which was significantly lower than the control during the first three months and higher than the control during the middle and last three months of pregnancy.

The measurement results of thyroid hormones, TPOAb, and hCG levels are shown in Table 3. The overall differences among FT3, FT4, and TSH levels in the four groups were statistically significant (HFT3 = 193.58, HFT4 = 66.918, HTSH = 114.92; $p < 0.01$). The differences among FT3, FT4, and TSH levels between any two of the three stages of pregnancy were also statistically significant ($p < 0.01$). No significant difference of FT3 levels was noted between the control and first three months of pregnancy ($p = 1$), with significant difference from control to middle and last three months of pregnancy ($p < 0.01$). The difference of FT4 levels between the control and the first three months of pregnancy was significant ($p < 0.01$), whereas the differences from control to middle and last three months of pregnancy were not ($p = 0.996$, $p = 0.52$). A significant difference of TSH levels was observed from the control to the first and last three months of pregnancy ($p < 0.01$), with no significant difference from the control to the middle three months ($p = 0.08$). The overall differences of hCG levels among the three stages of pregnancy were significant (HhCG = 203.4, $p < 0.01$), with significant difference between any two of the three groups (HhCG = 203.4, $p < 0.01$). The TPOAb medians at each stage of pregnancy were within the reference ranges of the manufacturer's values (negative).

Correlation between hCG and thyroid hormones in pregnant woman

The scatter diagrams of hCG with thyroid hormones are shown in Figure 3. A negative correlation was observed between HCG and TSH (r = -0.367, $p < 0.01$), whereas a positive linear correlation was noted between hCG and FT3, as well as hCG and FT4, respectively (r = 0.431, $p < 0.01$; r = 0.35, $p < 0.01$).

Discussion

During pregnancy, a series of physiological changes occur in maternal thyroid, including thyromegaly and changes in the thyroid hormone level and autoimmune system, which are often caused by the TSH-similar regulatory effect of hCG and increased serum thyroid binding globulin. These changes cause the TSH level to decrease during and then increase after the early stage of pregnancy. The FT4 level increases during the early stage of pregnancy, followed by a decrease during the late stage [2]. This change has caused a perplexity in gestational diagnosis, treatment of thyroid diseases, and perinatal management. Stricker *et al*. [3]. used normal reference ranges of thyroid function for non-pregnant population to assess pregnant women, and found that approximately 3.6% of pregnant women with elevated TSH level are misdiagnosed and

Table 4. — *Reference ranges of FT3 (pmol/l), FT4 (pmol/l) and TSH (mIU/l) for pregnant women in different literatures.*

Authors	Index	First 3 months	Middle 3 months	Last 3 months	Indicating parameter
Gong *et al.*[12]	FT4	11-19	9.7-17.5	8.1-15.3	2.5th, 97.5 th
	n	224	240	211	
Lambert-Messerlian	TSH	1.05	1.23		Median
et al.[13]	FT4	14.16	13		Median
	n	300	300		
Haddw *et al.*[14]	TSH	1.0	1.29		Median
	n	1126	1126		
		378	375		
Yan *et al.*[15]	TSH	0.05-4.5	0.03-4.5	0.5-4.5	2.5th, 97.5 th
	FT3	3.6-5.6	3.65-5.2	3.5-5.2	2.5th, 97.5 th
	FT4	11.8-21.0	10.6-17.6	9.2-16.7	2.5th, 97.5 th
	n	168	168	169	
Panesar *et al.*[16]	TSH	0.8	1.09	1.56	Median
	FT3	3-5.7	2.5-4.1	2.1-4.2	2.5th, 97.5 th
	FT4	11.1-22.9	8.7-15.1	9.1-15.6	2.5th, 97.5 th
	n	343	343	343	
Springer *et al.*[17]	TSH	0.06-3.67			2.5th, 97.5 th
	n	4337			
Mannisto *et al.*[18]	TSH	0.07-3.5			2.5th, 97.5 th
	FT4	11-22			
	FT3	3.4-7	3.4-7	3.4-7	
	n	5805			
Karakosta *et al.*[19]	TSH	1.02	1.14		Median
	FT4	12.36-20.59	10.81-18.5		2.5th, 97.5 th
	FT3	2.83-8.28	1.99-8.14		2.5th, 97.5 th
	n	403	403		
Cotzias *et al.*[20]	TSH	0-1.6	1-1.8	0.7-7.3	2.5th, 97.5 th
	FT4	11-22	11-19	7-15	2.5th, 97.5 th
	FT3	4-8	4-7	3-5	2.5th, 97.5 th
	n	335	335	335	
Moleti *et al.*[21]	TSH	0.71	1.0	1.2	Median
	FT4	16.7±2.4	14.8±1.9	13.5±1.8	
		(11.1-26.2)	(10.9-19.4)	(13.5-18.3)	Mean
	FT3	5.69±0.77	5.84±0.92	5.69±0.92	
		(3.85-8.31)	(3.23-7.54)	(2.92-7.69)	Mean
	n	143	215	137	
Marwaha *et al.*[22]	TSH	0.6-5	0.44-5.78	0.74-5.7	5th, 95 th
	FT4	12-19.45	9.49-19.58	11.32-17.7	
	FT3	1.92-5.86	3.2-5.73	3.3-5.18	
	n	107	137	87	
Stricker *et al.*[23]	TSH	0.946	1.021	1.14	Median
	FT4	10.48-18.49	9.53-15.68	8.63-13.61	5th, 95 th
	FT3	3.52-6.22	3.41-5.78	3.33-5.59	5th, 95 th
	n	575	528	501	

3.7% are diagnosed with decreased TSH level. Assessing maternal thyroid function based on reference values for specific thyroid function in pregnancy has become a consensus. However, this process is seldom conducted in clinical practice because of the lack of specific regional reference ranges with particular thyroid hormone level. Previous studies [4-12] found that the hCG level reaches the highest value during the middle and late period of the first three months of pregnancy, stimulating the TSH receptors, and promoting the increase of thyroxine level and decrease of TSH level. The TSH level during this period was 0.2 mIU/l to 2.5 mIU/l, lower than that in non-pregnant women. However, the TSH level is over 3.5 mIU/l during late pregnancy. In early pregnancy, the total triiodothyronine and total thyroxine levels increase. The FT3 and FT4 levels correspondingly increase, followed by a gradual decrease. The 95% confidence interval of the FT4 level was 30% lower than the reference range for non-pregnant women. Yan *et al.* [13]. reported on the specific reference ranges of thyroid function for pregnant women in an area with sufficient iodine nutrition in northern China. The results of their study are generally consistent with the present study.

Iodine nutrition level is an important factor that affects gestational thyroid function. The iodine nutrition levels in different regions are not the same. The reference ranges for thyroid hormones in normal pregnant women in China's coastal area was investigated in the current study. The results showed that the serum FT3 and FT4 levels in pregnant women decreases gradually from the first to the last three months of pregnancy. During the first three months, the FT3 level was almost the same with the control, whereas the FT4 level was significantly higher. During the middle and last three months, the FT3 level was significantly lower than the control, whereas the FT4 level was almost the similar or slightly lower. The serum TSH level increased gradually during the whole pregnancy. The TSH level during the first three months was significantly lower than the control, but higher during the middle and last three months. These results show that the serum FT4 and TSH levels significantly vary in the first three months of pregnancy, but with little changes for the FT3 level. During the middle and last three months, the changes for FT3 and TSH levels become more significant, but with little changes for FT4 level. Therefore, the variations of thyroid hormones reflect the overall trend of change in thyroid function during pregnancy. However, the changes in trends of the three thyroid hormones were not similar. This condition is helpful in making a correct determination on the test results for pregnant women at different stages.

This study showed that the low and high limits of FT3 during the first three months of pregnancy were higher than the manufacturer's value. The low and high limits of FT4 were higher than the manufacturer's value and the control; however, the low and high limits of TSH were significantly lower than the manufacturer's values and control. If the reference ranges for non-pregnancy provided by the laboratory or manufacturers were used for normal pregnant women, they may be misdiagnosed with hyperthyroidism. Likewise, when pregnant women with subclinical hypothyroidism, of whom the serum TSH level is mildly elevated but less than the high limit of reference range, may be misdiagnosed as normal. According to "Management of Thyroid Dysfunction during Pregnancy and Postpartum: an Endocrine Society Clinical Practice Guideline" [2], the less than normal serum TSH level during the early stage of pregnancy should not be used as basis for the diagnosis of hyperthyroidism. During first-half period of pregnancy, the TSH median was about 0.8 μU/ml, with low limit of 95% confidence interval, 0.03 μU/ml. The serum FT4 level in first three months of pregnancy was higher than the control, but decreased during the last three months of pregnancy, which was consistent in this study. The low and high limits of FT3 and FT4 during the middle and last three months of pregnancy are within the range of control and manufacturer's values. During the

last three months, the low and high limits of TSH were higher than the control and manufacturer's values. If the reference range of the control and manufacturer's values are used in normal pregnant women, they may be misdiagnosed with hypothyroidism.

In this study, the serum hCG level decreased gradually throughout the entire pregnancy, with a variation similar with TSH, but opposite with that of FT3 and FT4. This result was consistent with the physiological change basis of normal pregnancy. The correlation between hCG and TSH was very strong; however, correlations of hCG with FT3 and FT4 may be the indirect results of TSH variation, resulting to similar clinical manifestations, to a certain degree, of hyperthyroidism and hyperemesis gravidarum.

The reported reference ranges of thyroid hormones [13-23] are shown in Table 4. Slight differences among the reference ranges were noted, which may be due to the difference in race, iodine nutritional state, region, and research method. However, the variations of FT3, FT4, and TSH levels during the three stages of pregnancy were consistent, similar to the current study. The subjects of this study were from Quanzhou, a coastal city in Fujian, China. According to "iodine content of salt (draft)" (national standards of food safety, Ministry of Health of China, 2010), the urinary iodine content in Fujian has an appropriate level (100 g/l to 200 g/l): however, the data for Quanzhou were not found. The study lacked the iodine nutrition status survey, which will be conducted in the authors' further studies on the characteristics of thyroid function changes in pregnancy.

The thyroid hormone levels in normal pregnant women are different from those in non-pregnant women; significant differences were observed among the three different stages of pregnancy. Thus, it is not an advisable strategy to manage thyroid diseases during pregnancy according to the reference ranges of thyroid hormones for non-pregnant women. The establishment of specific reference range of thyroid hormones in pregnancy is helpful for the evaluation of the thyroid function change during pregnancy, as well as for an effective management of thyroid diseases, thereby ensuring a normal pregnancy. This study has established reference ranges of thyroid hormones in normal pregnant women in one of China's coastal areas, which were not only suitable to for the clinical practice for this area, but also for other areas with similar iodine nutritional status in the population.

References

[1] Glinoer D.: "Management of hypo-and hyperthyroidism during pregnancy". *Growth. Horm. IGF. Res.,* 2003, *13*, 45.

[2] Abalovich M., Amino N., Barbour L.A., Cobin R.H., De Groot L.J., Glinoer D. *et al.*: "Management of Thyroid Dysfunction during Pregnancy and Postpartum: An Endocrine Society Clinical Practice Guideline". *J. Clin. Endocrinol. Metab.,* 2007, *92*, 1.

[3] Stricker R., Echenard M., Eberhar R., Chevailler M.C., Perez V., Quinn F.A. *et al.*: "Evaluation of maternal thyroid function during pregnancy: the importance of using gestational age-specific reference intervals". *Europ. J. Endocrinol.,* 2007,*157*, 509.

[4] Aggarwal K., Choe L.H., Lee K.H.: "Quantitative analysis of protein expression using amine-specific isobaric tags in Escherichia coli cells expressing rhsA elements". *Proteomics.,* 2005, *5*, 2297.

[5] Smallridge R.C., Glinoer D., Hollowell J.G., Brent G.: "Thyroid function inside and outside of pregnancy: What do we know and what don't we know?". *Thyroid.,* 2005, *15*, 54.

[6] Lee R.H., Spencer C.A., Mestman J.H., Miller E.A., Braverman L.E., *et al.*: "Free T4 immunoassays are flawed during pregnancy". *Am. J. Obstet. Gynecol.,* 2009, *260*, 1.

[7] Kahric-Janicic N., Soldin S.J., Soldin O.P., Gu J., Jonklaas J.: "Tandem mass spectrometry improves the accuracy of free thyroxine measurements during pregnancy". *Thyroid.,* 2007, *17*, 303.

[8] Virtanen A., Kairisto V., Uusipaikka E.: "Regression-based reference limits: determination of sufficient sample size". *Clin. Chem.,* 1998, *44*, 2353.

[9] Glinoer D.: "Management of hypo-and hyperthyroidism during pregnancy". *Growth. Horm. IGF. Res.,* 2003, *13*, 45.

[10] ACOG Practice Bulletin.: "Clinical management guidelines for obstetrician-gynecologists. Number 37, August 2002. (Replaces Practice Bulletin Number 32, November 2001). Thyroid disease in pregnancy". *Obstet. Gynecol.,* 2002, *100*, 387.

[11] Ecker J.L., Musci T.J.: "Treatment of thyroid disease in pregnancy". *Obstet. Gynecol. Clin. North. Am.,* 1997, *24*, 575.

[12] Alkafajei A., Amarin Z., Alazaizeh W., Khader Y., Marji M.: "Prevalence and risk factors for hypothyroidism in Jordanian women: comparison between different reference ranges". *East. Mediterr. Health. J.,* 2012, *18*, 132.

[13] Yan Y.Q., Dong Z.L., Dong L., Wang F.R., Yang X.M., Jin X.Y. *et al.*: "Trimester- and method-specific reference intervals for thyroid tests in pregnant Chinese women: methodology, euthyroid definition and iodine status can influence the setting of reference intervals". *Clin. Endocrinol.,* 2011, *74*, 262.

[14] Gong Y., Hoffman B.R.: "Free thyroxine reference interval in each trimester of pregnancy determined with the Roche Modular E-170 electrochemiluminescent immunoassay". *Clin. Biochem.,* 2008, *41*, 902.

[15] Lambert-Messerlian G., McClain M., Haddow J.E., Palomaki G.E., Canick J.A., Cleary-Goldman J. *et al.*: "First- and second-trimester thyroid hormone reference data in pregnant women: a FaSTER (First- and Second-Trimester Evaluation of Risk for aneuploidy) Research Consortium study". *Am. J. Obstet. Gynecol.,* 2008, *199*, 62.

[16] Haddw J.E., Knight G.J., Palomaki G.E., McClain M.R., Pulkkinen A.J.: "The reference range and within-person variability of thyroid stimulating hormone during the first and second trimesters of pregnancy". *J. Med. Screen.,* 2004, *11*, 170.

[17] Panesar N.S., Li C.Y., Rogers M.S.: "Reference intervals for thyroid hormones in pregnant Chinese women". *Ann. Clin. Biochem.,* 2001, *38*, 329.

[18] Springer D., Zima T., Limanova Z.: "Reference intervals in evaluation of maternal thyroid function during the first trimester of pregnancy". *Europ. J. Endocrinol.,* 2009, *160*, 791.

[19] Mannisto T., Surcel H.M., Ruokonen A.,Vaarasmaki M., Pouta A., Bloigu A. *et al.*: "Early pregnancy reference intervals of thyroid hormone concentrations in a thyroid antibody-negative pregnant population". *Thyroid.,* 2011, *21*, 1.

[20] Karakosta P., Chatzi P., Bagkeris E., Daraki V., Alegakis D., Castanas E. *et al.*: "First- and Second-Trimester Reference Intervals for Thyroid Hormones during Pregnancy in "Rhea" Mother-Child Cohort, Crete, Greece". *J. Thyroid. Res.,* 2011, *2011*, 490783.

[21] Cotzias C., Wong S.J., Taylor E., Seed P., Girling J.: "A study to establish gestation-specific reference intervals for thyroid function tests in normal singleton pregnancy". *Europ. J. Obstet. Gynecol. Reprod. Biol.,* 2008, *137*, 61.

[22] Moleti M., Presti V.P., Campolo M.C., Mattina F., Galletti M., Mandolfino M. *et al.*: "Iodine prophylaxis using iodized salt and risk of maternal thyroid failure in conditions of mild iodine deficiency". *J. Clin. Endocrinol. Metab.,* 2008, *93*, 2616.

[23] Marwaha R.K., Chopra S., Gopalakrishnan S., Sharma B., Kanwar R.S., Sastry A. *et al.*: "Establishment of reference range for thyroid hormones in normal pregnant Indian women". *BJOG.,* 2008, *115*,602.

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