Trimester-Specific Reference Intervals of Thyroid Function in Healthy Pregnant Women in Macau

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Abstract—

Background: *To establish the trimester-specific reference intervals of thyrotropin (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) in healthy pregnant women in Macau.*

Methods: *Serum samples were collected from 166 healthy pregnant Macau women since early pregnancy until the third trimester. The study was performed in Macau Kiang Wu Hospital from July 2020 to October 2021.Basic clinical and obstetrics data were gathered using questionnaires. Blood samples were sequentially collected from the pregnant women at the first (≤12weeks), the second (12-28 weeks) and the third (>28 weeks) trimesters, respectively.*

Result: *Reference intervals of TSH were 0.02~3.30mIU/L, 0.45~3.80mIU/L and 0.18~3.43mIU/L in three trimesters. For FT4, Reference intervals were 12.82~22.0pmol/L, 9.86~15.58pmol/L and 10.10~15.30pmol/L in three trimesters. For FT3, Reference intervals were 3.53~6.0pmol/L, 2.74~4.65pmol/L and 2.62~4.45pmol/L in three trimesters. The concentration of TSH was significant lower in the first trimester compared to the second and third trimesters of pregnancy (median o.89, 1.66, 1.41mIU/L); TSH concentration differences between trimesters are significantly different ($p<0.05$).FT4 values decreased with the progression of gestational period (median 16.60, 12.40, 12.15pmol/L). FT3 values decreased with the progression of gestational period (median 4.56, 3.65, 3.57pmol/L). FT4 and FT3 concentration had significantly differences between first and second trimesters ($p<0.05$), but had no significantly differences between second and third trimester ($p>0.05$).*

Conclusion: *This study is the first to establish trimester-specific reference intervals of TSH, FT4 and FT3 in healthy pregnant Macau women. Trimester-specific reference intervals may help in diagnosis and management of thyroid dysfunction during pregnancy which will prevent both maternal and fetal outcomes.*

Keywords— pregnancy, reference-intervals, thyroid hormone.

I. INTRODUCTION

Disease of the thyroid gland affects about 5% of the general population, and predominantly affects females [1]. During pregnancy, the thyroid gland increase in size by 10% in iodine-sufficient countries but by 20% to 40% in areas of iodine deficiency. Production of the thyroid hormones, thyroxine (T4), and triiodothyronine (T3), increases by nearly 50%, in conjunction with a separate 50% increase in daily iodine requirement. Normal thyroid function is essential for fetal development. The fetus is totally dependent on maternal thyroxine supply during the first trimester and second trimester for normal development and nerves system maturation. Because the progression of pregnancy and fetal, neonatal and child health are dependent on adequate thyroid hormones supplementation throughout pregnancy.A deficiency or an excess of thyroid hormone can occur in pregnancy. Thyroid dysfunction during pregnancy is common,with a prevalence of 2-4%. The prevalence of overt hyperthyroidism is approximately 0.1-0.4%, subclinical hyperthyroidism about 3.3% [2], overt hypothyroidism about 0.3%, and subclinical hypothyroidism may reach 2.5% or more, thyroid nodules about 3-21%[3]. Maternal thyroid dysfunction is association with an increased risk of various adverse maternal and child outcomes, including miscarriages , intrauterine growth retardation, anemia, abruption placenta, hypertensive disorders, preterm delivery,

postpartum hemorrhage, fetal distress, decreased child IQ and cognitive impairment in the offspring [4-6].Overt thyroid dysfunction is related with adverse obstetric outcomes and needs to be treated in pregnant women[7]. A large prospective cohort study suggested potential harmful effects of levothyroxine therapy on the child neurodevelopmental outcomes in pregnant women with subclinical hypothyroidism[8]. Because of a lack of specific clinical symptoms, subclinical hypothyroidism has to be defined by serum TSH concentration. Thus, the reference range of TSH is critical for diagnosis and treatment. Thyroid function reference ranges vary among different populations, with might be explained by variation in ethnicity, iodine intake, body mass index, and as well as assay methodology.It is important to determine reference intervals for normal thyroid function during pregnancy. Although several studies from different global regions,define reference ranges for thyroid hormones during pregnancy[9-14].Recent years, reference intervals of serum TSH reported for pregnant women in China have been reported[15-19], but China is a big country with large population, multi-ethnic and a variety of eating habits, peoples in south and the north are different in size, the reference intervals of serum TSH may be difference .For this reason, international guidelines for the diagnosis and management of thyroid disease during pregnancy recommend that trimester-specific reference intervals for TSH be calculated locally for each center in a population with optimal iodine intake.

II. MATERIALS AND METHODS

2.1 Study population

From July 2020 to October 2021, pregnant women who were attending Macau Kiang Wu Hospital for antenatal care since early pregnancy were enrolled. Analyses of thyroid function and thyroid antibodies were performed as part of their routine.

After explaining the purpose of the study, informed consent was obtained from each participants and the study was approved by the Research Ethics committee of the Macau Kiang Wu Hospital. On enrolment of participants, detailed history was enquired and participants were subjected to relevant general physical examination and findings were record on a predesigned form. Physical examination included the presence or absence of goiter and general and systemic examination.Salt has been iodized since 1994 and as a result, iodine intake in China is likely adequate Recruitment criteria included Chinese women residing in Macau for more than 10 years, age 19-40 years old, and single pregnancy at 7 to 12 weeks of gestation. Exclusion criteria applied to multiple pregnancies, patients with thyroid disease history or any other chronic diseases, diabetes, body mass index greater than 25 Kg/m^2, goiter on physical examination, positive of anti-thyroid peroxidase antibodies (TPOAb), TSH>10mIU/L, poor obstetrics history included 3 or more abortions, use of medications that can affect thyroid function, such as glucocorticoids, dopamine, or antiepileptic drugs, a history of thyroid surgery or radioactive iodine treatment.

Gestational age was determined by the last period and confirmed by ultrasound examination. When a major discrepancy between these two dates was found, the date of gestation was ultimately defined by ultrasound examination. Gestational age ≤12, 12-28 and >28 weeks comprised the first, second and third trimester of pregnancy. All the participants accepted sequential blood sampling once each trimesters.

2.2 Methods of sampling and laboratory testing

A morning fasting venous blood sample was obtained and isolated within 3 h of collection. Reference population was identified to calculate serum free triiodothyronine (FT3), free thyroxine (FT4), and TSH for each trimester of pregnancy, and autoantibodies (TPOAb) for first trimester of pregnancy only. The concentration of serum FT3, FT4, TSH and TPOAb were detected by an electrochemiluminescence immunoassay diagnostic kit (Roche Diagnostics Ltd, Basel, Switzerland) and analysed on Cobas E801 Module immunology analyser (Roche Diagnostics Ltd). Laboratory reference range for FT3, FT4, and TSH were 2.8-7.8pmol/L, 12-22pmol/L, and 0.27-4.2mIU/L, respectively. Normal range for TPOAb was <34 IU/ml and value greater than or equal to indicate elevated TPOAb in serum.

2.3 Statistical analysis

According to the aims and objectives of the study, the data were compiled and entered into MS Excel and analysed, using appropriate statistical test in SPSS software (version 22; SPSS Inc., Chicago, IL, USA). For descriptive statistics, frequencies, percentages, mean with standard deviations, and median of different variables were calculated. Data for TSH, FT3, and FT4 were expressed as median 2.5^{th}-97.5^{th} percentiles. One-Way analysis of variance (one-way ANOVA) was used to compare mean.

III. RESULTS

3.1 Baseline characteristics

From July 2020 to October 2021, 221 pregnant women were invited to participate in the study. We excluded 14 women who had positive thyroid autoantibodies, 7 women whose thyroid hormones were increased, 6 women who were abortion, 2 women whose foetuses had congenital deformities and needed abortion, 1 woman who refused to participate , 16 women who did not completed the study and 9 women who diagnosed of GDM in second trimester. Thus, a total of 166 participants were enrolled in the final study population. Flowchart of included/excluded pregnant women was showed in Fig. 1.

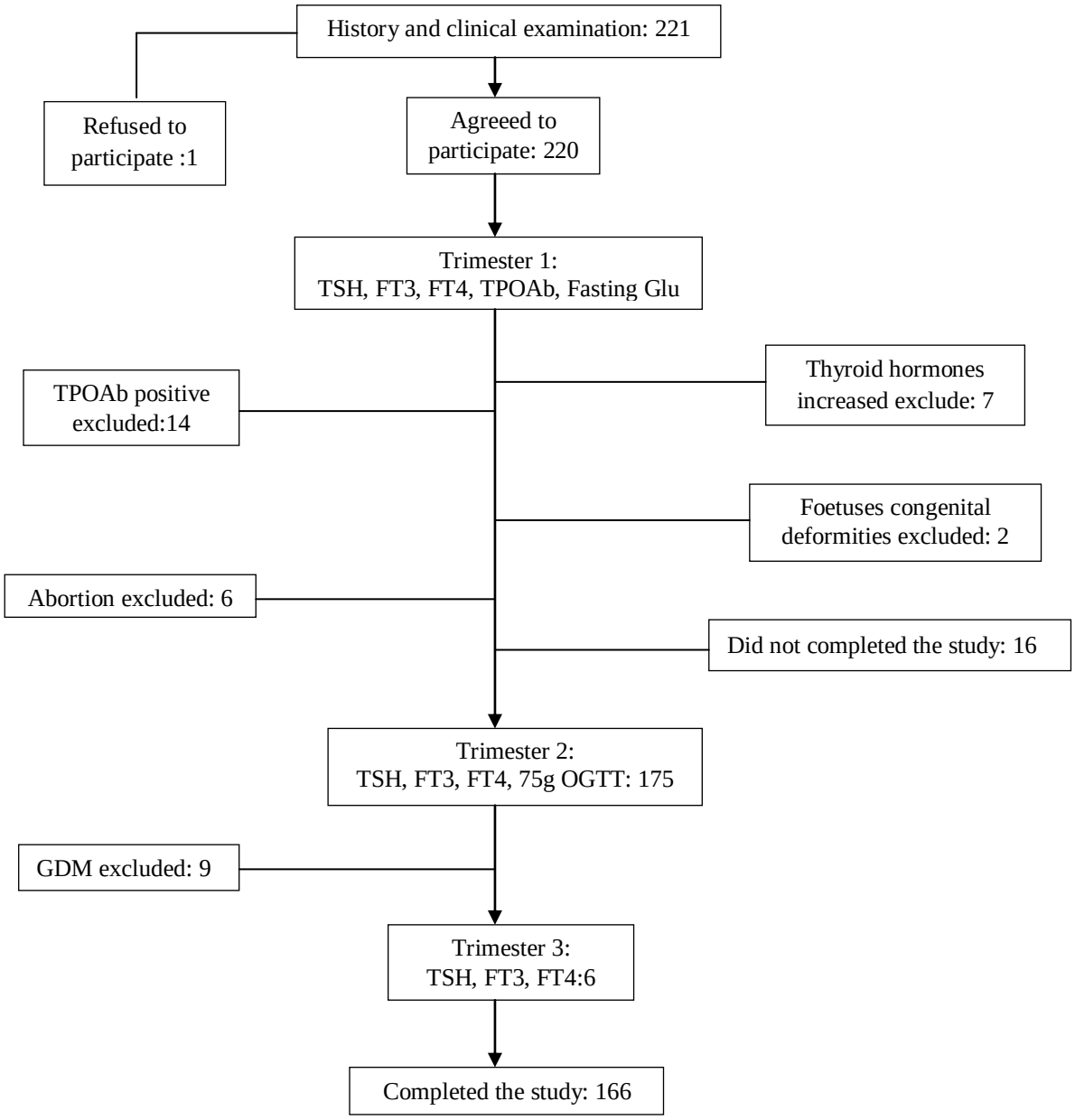

FIGURE 1: Flow chart of included/excluded pregnant women

The demographic, clinic, and obstetric characteristics of the 166 participants are described in Table 1. The mean age at study enrolment was 30.22(30±3.32) years, the range from 22~40 years old. Mean BMI was 20.14(19.95±2.37)Kg/m^2. Median TSH was 0.85, 1.66, 1.41mIU/L in each trimester. Median FT4 was 16.6, 12.4, 12.15pmol/L. Median FT3 was 4.57, 3.65, 3.57pmol/L.

TABLE 1
CLINICAL AND OBSTETRICAL CHARACTERISTICS OF THE STUDY POPULATION (n= 166)

Mean maternal age ± SD, years	30.22 ± 3.33
Maternal age	
20-25	11(6.63%)
26-30	84(50.60%)
31-35	60(36.14)
36-40	11(6.63%)
History of previous pregnancies, n (%)	
Nulliparous	82(49.40)
Parity 1	71(42.77%)
Parity ≥ 2	13(7.83%)
Smoker, n(%)	0
Mean BMI ± SD Kg/m2	20.14 ± 2.31
BMI range	15.7-28.9
Initial weight, n(%)	
Underweight(BMI< 18)	83(50%)
Normal weight (BMI: 18-24.9)	79(47.6%)
Overweight (BMI: 25-29)	4(2.4%)
Obesity (BMI ≥ 30)	0

Reference intervals of TSH, FT4, FT3 in each trimester

Reference intervals of TSH, FT4 and FT3 were defined as the range between 2.5th and 97.5th percentiles. Reference intervals of TSH were between 0.02 to 3.30mIU/L in the first trimester, 0.45 to 3.80mIU/L in second trimester, and 0.18 to 3.43 in third trimester. For FT4, Reference intervals were between12.82 to 22.0pmol/L in first trimester, 9.86 to 15.58pmol/L in second trimester, and 10.10 to 15.30pmol/L in third trimester. For FT3, Reference intervals were between 3.53 to 6.0mol/L in first trimester, 2.74 to 4.65pmol/L in second trimester, and 2.63 to 4.45pmol/L in third trimester. The reference interval (mean±standard deviation, median, 2.5th and 97.5th percentiles) for thyroidhormone levels in healthy pregnant Macau women was showed in Table 2.

TABLE 2
REFERENCE INTERVALS (mean±standard deviation, median, 2.5th and 97.5th percentiles) FOR THYROID HORMONE LEVELS IN HEALTHY PREGNANT MACAU WOMEN

Trimester	Hormones	Mean±SD	2.5th	5th	Median	95th	97.5th
First	TSH(mIU/L)	0.88±0.92	0.02	0.05	0.89	2.94	3.3
	FT4(pmol/L)	16.60±2.29	12.82	13.21	16.6	20.8	22
	FT3(pmol/L)	4.58±0.56	3.53	3.7	4.58	5.6	6
Second	TSH(mIU/L)	1.66±0.92	0.44	0.53	1.66	3.22	3.8
	FT4(pmol/L)	12.40±1.42	9.86	10.34	12.4	14.9	15.58
	FT3(pmol/L)	3.65±0.52	2.74	3	3.65	4.5	4.65
Third	TSH(mIU/L)	1.41±0.76	0.18	0.34	1.41	2.98	3.43
	FT4(pmol/L)	12.15±1.39	10.1	10.44	12.15	14.9	15.3
	FT3(pmol/L)	3.57±0.45	2.63	2.79	3.57	4.28	4.45

AS shown in Fig. 2, TSH values were suppressed in the first trimester, rose following the second trimester and supressed again in the third trimester. The concentration of TSH was significant lower in the first trimester compared to the second and third trimesters of pregnancy (median o.89, 1.66, 1.41mIU/L); TSH concentration differences between trimesters are significantly different ($p<0.05$). FT3 values decreased with the progression of gestational period (median 4.56, 3.65, 3.57pmol/L). FT3 concentration had significantly differences between first and second trimesters ($p<0.05$), but had no significantly differences between second and third trimester ($p>0.05$). FT4 values decreased with the progression of gestational period (median 16.60, 12.40, 12.15pmol/L). FT4 contraction decreased significantly from first trimester to second trimester ($p<0.05$), but the decreased from second trimester to third trimester was nonsignificant ($p>0.05$).

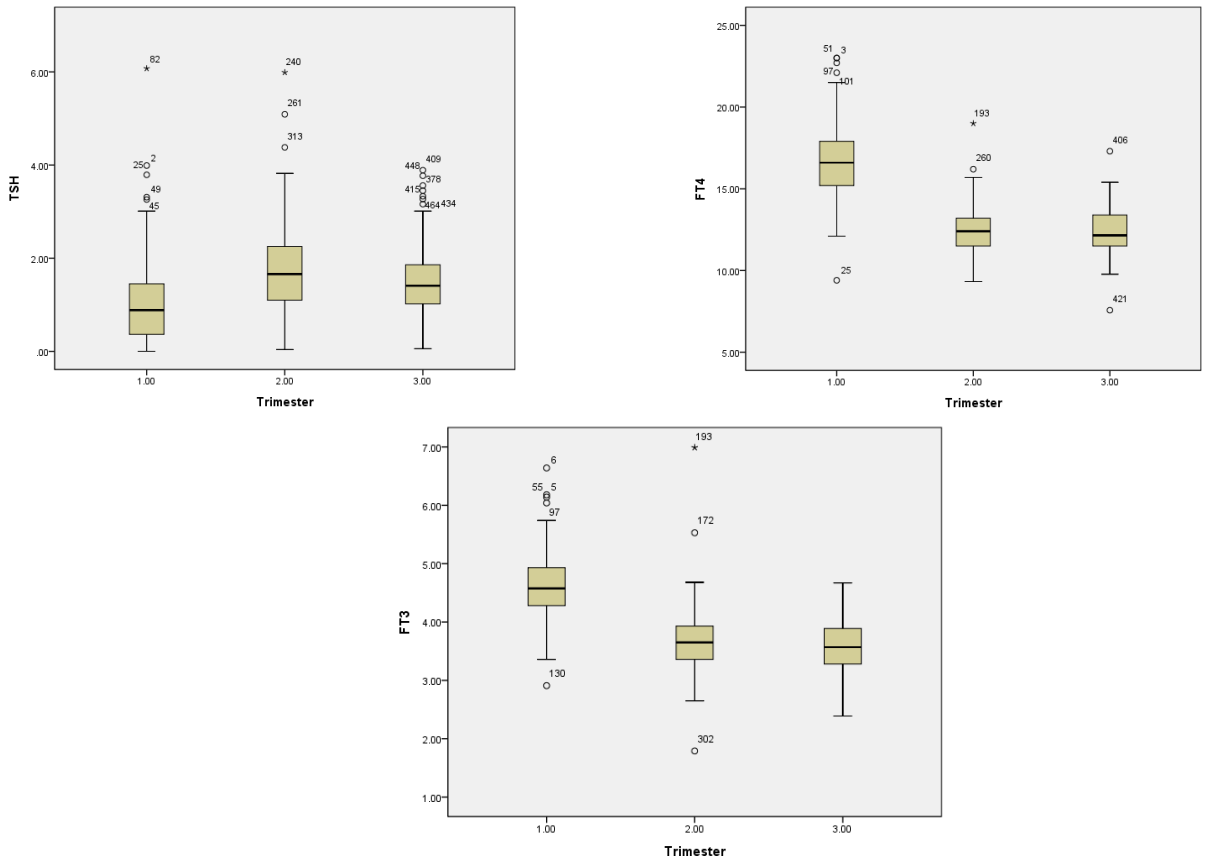

FIGURE 2: The concentrations of TSH, FT4, FT3. Data is presented as medians, interquartile range (box), non-outlier range (whiskers), and outliers (dots)

3.2 Prevalence of thyroid dysfunction

TPOAb was positive in 6.34% (14/220) of the participants. When the trimester-wise 2.5th to 97.5th percentile of TSH derived from the reference population in this study was applied to the total population with TPOAb(-), the number of women with subclinical hypothyroidism were 2(0,97%, 2/206) , hypothyroidism were 1(0.48%, 1/206), isolated hypothyroxinaemia were 3(1.36%, 3/206) in first trimester. The number of women with subclinical hypothyroidism were 5(2.86%, 5/175), isolated hypothyroxinaemia were 5(2.86%, 5/175) in second trimester. The number of women with subclinical hypothyroidism were 4(2.41%, 4/166), isolated hypothyroxinaemia were 2(1.20%, 2/166) in third trimester. There was no hypothyroidism case in second and third trimester.

IV. DISCUSSION

We recognized that pregnancy affects thyroid physiology, thereby leading to changes in thyroid parameters. As not only overt thyroid disease but also more subtle difference in thyroid hormone levels can lead to a wide range of complications in the mother and new-born. In pregnancy, a number of factors affect the determination of reference intervals for thyroid parameters. The most important definition is who healthy pregnant women are. Moreover, iodine saturation, different ethnics and analytical methods play a role. Clinical heterogeneity, test assay platform, thresholds used and quality features of the studies are likely to have contributed to the statistical heterogeneity observed.

2011 ATA guideline has recommended that when available, population- and trimester-specific reference ranges for serum TSH during pregnancy should be defined by a provider's institute of laboratory and should represent the typical population for whom care is provided. Reference ranges should be defined in healthy TPOAb-negative women with optimal iodine intake and without thyroid illness.

Macau is an iodine-sufficient city. In this study, we examined the thyroid parameters of same pregnant woman in ≤12, 12-28 and >28 weeks. Reference intervals of TSH in the present study were 0.02-3.30mIU/L during first trimester. As shown in Table 2, TSH reference in our study are comparable with some Western countries and India [9, 14, 25, 27], but lower compared with some other countries [10-13, 23, 24](Table 3).

TABLE 3
PREVIOUS REPORTS FROM OTHER COUNTRIES

Reference	Country	n	2.5th- 97.5th percentile
Bestwick et al(2014)	UK[9]	16334	0.06-3.50
Bestwick et al(2014)	Italy[9]	5505	0.04-3.19
Medici et al(2012)	Netherland[10]	5393	0.03-4.04
Marta et al(2017)	Poland[11]	172	0.009-3.177
Lorena et al(2016)	Chilean[12]	720	0.11-5.96
Kim et al(2016)	Korea[13]	417	0.03-4.24
Rajesh et al(2016)	India[14]	1430	0.37-3.69
Shrook et al(2019)	Egypt[23]	150	0.6-4.3
Fereidoun et al(2013)	Iran[24]	466	0.2-3.9
Joosen et al(2016)	Dutch[25]	60	~3.39
Almomin et al(2016)	Iraq[27]	893	0.04-3.77

If trimester-specific reference intervals for TSH are not available, 2011 ATA recommended the following reference intervals; 0.1-2.5mIU/L, 0.2-3.0mIU/L, 0.3-3.0mIU/L for first, second and third trimester. If using the upper limit reference of TSH, about 14(6.80%), 17(9.71%), 8(4.82%) pregnant women in the first, second and third trimester would be confirmed to be subclinical hypothyroidism. According to 2017 ATA guideline, the upper limit reference of TSH was 4mIU/L for first, second and third trimester. If using the new criteria, about 1 (6.80%) pregnant woman in the first trimester would be confirmed to be subclinical hypothyroidism. On the other hand, about 3(1.81%) pregnant women in the second trimester would be confirmed to be subclinical hypothyroidism. But, no pregnant woman was diagnosis of subclinical hypothyroidism in third trimester. The results of second-trimester reference range are closer to the values suggested by 2017 ATA for pregnant women than the TSH range in the first and third trimesters.

Since neither over diagnosis nor missed diagnosis is unacceptable, ATA propose that the local-specific reference is the best choice. If such reference is unavailable, than reference ranges from similar population using similar TSH assays will be the second choice. As shown in Table 4, in other Chinese Studies, the upper-limit reference of TSH in the first trimester is from 3.13 to 5.23mIU/L[15, 16, 18-22, 26, 28].TSH reference in our study within trimesters are comparable with Zhejiang, Shanghai and Sichuan [16, 19,22, 26], but significantly lower compared with some other provinces[15, 18, 20-21](Table 4).In this study, we tracking the TSH mean value for each trimester: we notice that the TSH value in the first trimester is lower than in the second trimester while the third trimester decreases again.

FT4 levels decrease as pregnancy progressed. This downward sloping curve in FT4 was also observed in many studies in China. In first trimester, the normal reference range of FT4 in our study is higher than studies of other coastal area of China. The reference intervals of FT4 in our study are consistent with Shenyang, Sichuan and Southwest [15, 26, 28], but higher than some other studies in China. Studies showed that hypothyroxinaemia in the early stages of pregnancy, is a predictor of lower IQ, language delay, worsened motor function, small head circumference, and an increased risk of autism [29-32]. In second trimester, the reference intervals of FT4 in our study are actually nearer to the values reported in Zhejiang and Sichuan, but different from some other studies in China

FT3 concentrations were significantly highest in the first trimester compared to the second and third trimester. Our data are consistent with the observations of Shen's study in Zhejiang [16].In our study, a maximum decrease in FT3 and FT4 was achieved in the second trimester, with no significant changes up to the end of pregnancy.

The populations of Zhang's study were living in Shenzhen where is very near Macau. But the reference ranges for thyroid hormones in normal pregnant women were significantly different [21].We therefore believe that even in women from the same geographical area, using the different methodological approach to calculating reference ranges, such reference ranges were highly laboratory-dependent and not applicable outside of its own clinical setting. In addition, it is known that, thyroid function is also affected by living habits, diet, and geographical location. In our population, people were in coastal area of China, while in Yang's , Shen's and Yang H's studies, the population were also from the coastal area of China and he reference range of thyroid hormone in healthy pregnant women had no significantly differences. The population of Ying's , Xing's and Chens studies were mainly from South-West of China, and in Li's study, the population was mainly from the North-East of China.

TABLE 4

TRIMESTER REFERENCE RANGE FROM DIFFERENT AREA OF CHINA

	Area	n	TSH(mIU/L)			FT4(pmol/L)			FT3(pmol/L)		
			Trimester1	Trimester 2	Trimester 3	Trimester 1	Trimester 2	Trimester 3	Trimester1	Trimester2	Trimester 3
Li	Shenyang[15]	640	0.10-4.34			12.3-20.88					
Shen	Zhejiang[16]	1409	0.16-3.78	0.34-3.51	0.34-4.32	10.9-17.7	9.3-15.2	7.9-14.1	2.9-5.0	2.9-4.6	2.9-4.5
Chen	Chengdu[18]	579	0.02-4.03	0.02-4.05	0.24-5.41	11.93-21.04	11.23-19.22	11.10-17.0	3.85-6.27	3.51-5.82	3.18-4.97
Yang H	Zhejiang[19]	3882	0.09-3.41	0.02-3.81		7.74-15.8	5.55-12.56				
Xing	Zhengzhou[20]	3314	0.07-3.96	0.07-3.96	0.27-4.53	9.16-18.12	8.67-16.21	7.80-13.90			
Zhang	Shenzhen[21]	2743	0.06-3.13	0.07-4.13	0.15-5.02	8.72-15.22	7.10-13.55	6.16-12.3			
Yang	Shanghai[22]	52027	0.03-3.52		0.39-3.67	11.7-19.7		9.1-14.4			
Wei	Sichuan[26]	150	0.08-3.29		0.81-4.33	11.88-20.06	9.89-15.8	9.22-15.77			
Ying	Southwest[28]	33040	0.02-5.23	0.03-5.24	0.37-5.68	11.66-20.69	10.1-18.59	9.85-16.86			
Our study	Macau	166	0.02-3.30	0.45-3.80	0.18-3.43	12.82-22.0	9.86-15.58	10.10-15.30	3.53-6.0	2.74-4.65	2.63-4.45

We performed a longitudinal study and the pregnant women were followed since early pregnancy until the third trimester. The trimester-specific ranges of TSH, FT4 and FT3 in our study are comparable with the studies from Dutch and China [19, 25,26]. But the sample size of our studies was limit and the same problems with the studies of Dutch and Wei et al. On the other hand, other studies use three different populations in three trimesters.

V. CONCLUSIONS

We found that the upper limit of serum TSH reference in the first trimester and second trimester were much higher than 2.5mIU/L and 3.0mIU/L in our population. On the other hand, the upper limit of serum TSH references in trimesters of our study was lower than 4mIU/L which proposed by ATA 2017 guideline. The trimester-specific reference ranges for serum TSH during pregnancy obtained from similar populations and maternal iodine status, but using different laboratory assay method were inconsistent. In conclusion, we established the trimester-specific reference intervals of TSH, FT4 and FT3 in TPOAb negative pregnant women from a tertiary care centre in Macau. In our opinion, measurement of TSH, FT4 and TPOAb in the systematic screening for thyroid dysfunctions in pregnancy is beneficial and it can help decide whether women should be treated with levothyroxine or not.

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DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical clearance was approved by the Research Ethics committee of the Macau Kiang Wu Hospital. All enrolled participates signed a written consent form before they joined the study.

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